Bumble Bees & Blueberry Fields: Landscape Characteristics that Affect Growth of *Bombus impatiens* Colonies

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Abstract

Development of bumble bee colonies can be affected by landscape composition and configuration. My study measured relationships between landscape metrics and development of bumble bee *Bombus impatiens* (Cresson 1863) (Hymenoptera: Apidae) colonies placed for one month near blueberry fields within landscapes that spanned a gradient of natural land coverage. The results showed that proportion of natural land cover did not correlate with colony development, but that landscapes with a greater proportion of abandoned farmland produced larger bumble bee colonies. Colonies varied significantly amongst fields in terms of weight of the hive, brood production, and the dry weight of the workers as well as all castes measured together. Landscape configuration did not correlate with colony growth or reproduction.

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Chapter 1: Introduction

1.1 Importance of Native Pollinators

Animal pollination, most often by insects, and to a much lesser extent by bats, birds, and other vertebrates, helps approximately 87% of all flowering plants achieve reproductive success (Ollerton, Winfree, & Tarrant, 2011). Globally approximately 30% of all crops rely on insects for pollination (Klein et al., 2007), which amount to 3-8% of global crop production by weight (Aizen, Garibaldi, Cunningham, & Klein, 2009). Some crops can be effectively pollinated by a range of insect species, whereas others receive specialized pollination service by a single insect species. In agricultural landscapes within temperate regions, most insect-mediated pollination is provided by domestic honey bees (*Apis mellifera* L.), bumble bees (*Bombus spp.),* managed solitary bees (e.g. several *Osmia* species*, Megachile rotundata* F.), as well as many species of wild solitary bees and hoverflies (Diptera: Syrphidae) (Klein et al., 2007). Several species (*B. terrestris* L.*, B. impatiens* Cresson*, M. rotundata*) are reared at an industrial scale to augment existing pollinator populations, or to provide pollination for systems where pollinators are absent (i.e. greenhouse production).

Despite the wide availability of honey bee colonies for crop pollination, it is clear that wild bees are also a crucial component of crop pollination. For example, flower visitation by wild insects was positively associated with fruit set in all of the 41 crop systems studied worldwide by Garibaldi et al. (2013). Wild bees were the dominant flower visitors in crops within deciduous forests (Winfree, Williams, Dushoff, & Kremen, 2007) and likely contribute greatly to pollination of many crops (Goulson, 2003). Although honey bees are excellent generalist pollinators, they can have limitations. For example, they are unreliable pollinators in cold, wet environments whereas many wild bees, bumble bees included, fly in damp conditions and are likely a crucial component of crop success (Goulson, 2003). There are at least 87 species of bees present in Canadian lowbush blueberry fields (Cutler, Nams, Craig, Sproule, & Sheffield, 2015). Even non-bee insects are important contributors to crop pollination (Rader et al., 2016). Not all pollinators are equally effective, in north-eastern North American lowbush blueberry (*Vaccinium angustifolium* Ait.) fields, native bumble bee queens deposited nearly four times as many pollen tetrads per visit as honey bees (Javorek, Mackenzie, & Vander Kloet, 2002). Wild pollinators are an important component of crop pollination, which means that the habitats that support them are valuable. The stability of flower-visitor richness, fruit set, and the visitation rate of wild pollinators all decrease with distance from natural and semi-natural habitats, suggesting that habitat plays an important role in determining the prominence of crop pollination by wild insects (Garibaldi et al., 2011).

1.2 Landscape Effects on Native Bee Abundance and Diversity

The quality of a given landscape is herein referred to in terms of its character, which can be divided into two distinct categories; composition (proportions of different land cover classes) and configuration (measures of heterogeneity and shape). Typically, abundance and richness of bee species are related to the composition of the landscape in which they are observed. Ricketts et al. (2008) synthesized results of 23 studies to demonstrate that pollinator richness and visitation rates decline with increasing distance from semi-natural habitats (e.g. meadows, abandoned land). Another global quantitative synthesis modelled composition and configuration of landscape against abundance and richness of native pollinators in 39 crop systems (Kennedy et al., 2013). Bee abundance and richness were highest in diversified organic landscapes and, of the measures of landscape quality, composition (proportion of land cover likely to offer floral and nesting resources) had the most significant positive influence on bee richness (Kennedy et al., 2013). Measures of configuration (perimeter area ratio and heterogeneity) were at best weakly correlated with abundance and diversity of native pollinators (Kennedy et al., 2013).

Within blueberry fields, greater distance from natural habitat is consistently negatively correlated with native bee abundance (Cutler et al., 2015; Moisan-DeSerres, Chagnon, & Fournier, 2015; Vieli, Davis, Kendall, & Altieri, 2016). In Quebec, native bee abundance and richness were sampled in lowbush blueberry fields with either windbreaks or forest borders (Moisan-DeSerres et al., 2015); samples taken near forest borders had higher abundance than all three types of windbreaks assessed, supporting the hypothesis that pollinators are supported by natural habitats. In New Brunswick, forested edges were also shown to have a greater diversity of bees, and the amount of forest surrounding a field was positively correlated with the abundance of native pollinators (McKechnie, Thomsen, & Sargent, 2017). The same study showed that parasitic bees were particularly abundant in forested edges, whereas pollen foraging bees were more abundant along deforested edges (McKechnie, 2017).

This is also consistent with other crops, for example, in highbush blueberry (*Vaccinium corymbosum L.*) fields of central Chile, the abundance of the main ‘wild’ pollinator, *B. terrestris* queens (a species that was inadvertently released while being used to pollinate greenhouse crops (Torretta, Medan, & Arahamovich, 2006)), was measured in fields surrounded by varying proportions of natural forests and agricultural land (Vieli et al., 2016). There the abundance of queens was found to be positively correlated with natural forest cover, and negatively correlated with high-food output (intensive) agricultural land (Vieli et al., 2016).

Proportion of forested habitat has also been found to positively correlate with pollinator diversity and abundance when measured in the landscapes surrounding apple orchards in Wisconsin, USA (Watson, Wolf, & Ascher, 2011), Nova Scotia, Canada (Sheffield, Kevan, Pindar, & Packer, 2013) and in the Carolinian forests of Ontario, Canada (Taki, Kevan, & Ascher, 2007). In agricultural landscapes in central Europe, Hopfenmüller, Steffan-Dewenter, & Holzschuh (2014) determined that large generalists such as bumble bees are affected by the composition of the landscape. Whereas habitat specialists were dependant on local-scale characteristics (e.g. patch density & connectivity), generalists were positively correlated with the percentage of semi-natural habitats in the landscape (Hopfenmüller et al., 2014). Bumble bees, being large generalists, were positively affected by the composition of the landscape, and their abundance and richness increased with increasing percentage of semi-natural habitats (Hopfenmüller et al., 2014). This could be because bumble bees can travel relatively far when foraging resources are widely dispersed across landscapes. Even in systems dominated by semi-natural habitats, only a few patches of habitat drive the relationship between pollinators and the landscape (Martins, Gonzalez, & Lechowicz, 2015). A diversity of forests and meadows complement each other by supporting flowering plants that bloom at different times, thereby providing forage throughout the growing season (Martins et al., 2015).

The relationship between type of farming system (conventional vs. organic) and bee abundance and richness was tested against bumble bee abundance and richness on the border of cereal fields in Sweden (Rundlöf, Nilsson, & Smith, 2008). They found that bee richness and abundance were significantly higher on organic farms in homogeneous landscapes and hypothesized that it was likely the result of higher floral abundance along the edge of the organic fields (Rundlöf et al., 2008). This hypothesis was corroborated by a study conducted in agricultural regions across Europe that compared landscape complexity to richness of bees as well as birds, plants, and spiders (Concepción et al., 2012). Concepción et al. (2012) found that length of the boundary between arable fields and semi-natural areas was positively correlated with the abundance and diversity of bees, and further hypothesized that this relationship was the result of the abundance of floral resources in these margins. These studies indicate that the configuration of the landscape does influence pollinators, whereas in Canadian cranberry fields, the diversity of the bee community did not differ based on adjacent habitat character (Gervais, Fournier, Sheffield, & Chagnon, 2017).

1.3 Landscape Effects on Bumble Bee Colony Growth and Reproduction

Bumble bees are highly dispersive pollinators that forage across large spatial scales (Darvill, Knight, & Goulson, 2004). Even amongst species, bumble bees use the landscape at different spatial scales depending on the size of the colony and individual workers (Catrin Westphal, Steffan-Dewenter, & Tscharntke, 2006). By placing colonies within agricultural landscapes of varying quality, researchers can assess the importance of landscape characteristics on the growth of bumble bee colonies (Williams, Regetz, & Kremen, 2012). Focusing bumble bee landscape ecology research on colony development is helping to explain the effects that habitat at the local and landscape level have on their communities.

While there are several studies that deal with bee abundance and richness as it relates to landscape character, there are far fewer studies that consider the influence of landscape character on the growth rate or reproduction of bumble bee colonies. Williams et al. (2012) measured the availability of floral resource in 20 different land use cover types throughout the bumble bee flight season and then compared these data to bumble bee colony growth. They found that the proportion of the total area available that hosts floral resources suitable for bees strongly correlates with the natural landscapes in the study area. They also demonstrated that floral resource at the landscape level, but not the local level, positively affected the number of workers and drones produced in a colony (Williams et al., 2012).

Crone & Williams (2016) compared the growth rate of 59 hand reared *B. vosnesenskii* colonies amongst 39 field sites of varying habitat quality, the sites selected were all within the same mixed agricultural-natural region but spanned a gradient of land use, ranging from those with a low proportion of semi-natural land cover, to those dominated by intensive agriculture. Colony growth increased with floral resource available in the landscape, and local land cover had no significant effect on colony growth, but queen production was higher when the local landscape was a semi-natural area (Crone & Williams, 2016).

Spiesman, Bennett, Isaacs, & Gratton (2017) measured the relative growth rate and reproduction of 76 *B. impatiens* colonies across a gradient of natural habitat in the Midwestern United States. Each colony was located within grassland habitats, and the effects of local floral abundance and proportion of natural habitat in the surrounding landscape (2 km scale) were tested against colony mass and queen production (Spiesman et al., 2017). Colony growth depended on landscape-scale measures of resource availability, and bumble bees responded negatively when natural habitats were a high proportion of the landscape (Spiesman et al., 2017). Floral dominance, not abundance or richness, was an important predictor of colony growth, suggesting that the landscape scale factors that influence growth may not be simple measures like land cover proportion (Spiesman et al., 2017).

1.4 Bumble Bee Biology

Bumble bees are large, social, generalist pollen-foragers, with most species forming colonies that grow to contain 70-1800 individuals (Cueva del Castillo, Sanabria-Urbán, & Serrano-Meneses, 2015). Size of both individual bees and the whole colony depends on the location of the hive, length of the growing season, as well as the species (Cueva del Castillo et al., 2015). In most regions, colonies grow for a single season, after which all workers and drones die, and mated queens find a safe place to hibernate (Goulson, 2010). In north-eastern North America queens emerge in the early spring and begin to forage immediately, although most queens that emerge from hibernation will not successfully establish a new colony (Goulson, 2010). A successful queen will find enough forage to fuel her in finding a safe space for her brood, where she will build a honey pot and a single clutch of eggs (Goulson, 2010). Eggs are laid together in a single distensible cell, and as the communally fed larvae grow, the cell grows with them (Goulson, 2010). While the larvae mature, advancing through four larval stages, they separate and are fed individually (Heinrich, 2004). The queen will continue to lay additional eggs on top of developing larvae (Heinrich, 2004). When they are no longer fed each larva spins itself in a silken cocoon, thereby isolating itself, pupates, and emerges as a worker (Heinrich, 2004). Total development from egg to adult takes approximately three weeks. Emergent workers are fed for an additional two days before they can begin foraging (Heinrich, 2004).

The first emergent worker bees take over the foraging responsibility for the colony after which the queen no longer leaves the colony (Heinrich, 2004). These foragers spend the daylight hours gathering pollen, fuelling themselves with nectar, and returning to the hive with pollen for the brood (Goulson, 2010). Only rarely do bumble bee colonies store pollen (Goulson, 2010). Pollen that is brought back to the hive is fed to the queen and the growing brood cells (Heinrich, 2004). Nectar however is regularly stored, wax collars (broad rims made of wax) are added to each empty cocoon so that it can be used to store honey (Heinrich, 2004). The queen continues to lay eggs whenever the workers foraging provides enough pollen to support further growth (Heinrich, 2004). Each new clutch is laid on the existent hive structure, typically near larvae (Heinrich, 2004). When the hive becomes large enough, some emergent workers will stay in the hive to help the queen keep her brood clean and warm (Goulson, 2010). Worker bees typically take on the role of foragers, or guard bees, but rarely will a worker bee switch its role. Workers typically live for two weeks. (Heinrich, 2004)

1.5 Bumble Bees in Blueberry Fields

Lowbush blueberry (*Vaccinium angustifolium*) fields are cultivated in the Atlantic Provinces and Quebec in Canada, and the north-eastern United States (Strik & Yarborough, 2005). In the study area on Prince Edward Island, lowbush blueberry production is of significant economic importance with more than 5,500 acres in production (Yarborough, 2012). Lowbush blueberry production requires an intensive management regime, which often includes the use of honey bees to facilitate cross pollination. This mode of pollination occurs when pollen from the anther of one flower is delivered to the stigma of another flower. Since blueberry flowers release pollen through small pores in the anthers, which severely limits access, some bees have evolved a behaviour of using their indirect flight muscles to release pollen (Goulson, 2010). This method of pollination is called sonication, or buzz-pollination, and is essential for blueberry flower pollination (Usui, Kevan, & Obbard, 2005). Since bumble bees are more effective at buzz pollination than honey bees (Javorek et al., 2002), blueberry farmers often use commercial bumble bees as well. A bumble bee will effectively pollinate at least 85% of the blueberry flowers that it visits, whereas a honeybee will adequately pollinate only 31% of flowers she visits (Javorek et al., 2002). Native bumble bees often thrive within and around lowbush blueberry fields (Javorek et al., 2002; Stubbs & Drummond, 2001). It is likely that bumble bees already provide a considerable proportion of lowbush blueberry pollination, but the extent of their contribution has not been well quantified.

1.6 Hypothesis & Experimental Design

My experiment primarily examined how the characteristics of land cover within the landscape surrounding *B. impatiens* colonies influences colony growth and reproduction. Each landscape consisted of a focal lowbush blueberry field surrounded by habitat of varying proportion of semi-natural land cover. The quality of a habitat as it relates to bee abundance and diversity is often defined in terms of the proportion of natural land cover available. In this study, I selected fields to span a range of composition, and considered the configuration of habitat, and their effects on bumble bee development. My definition of habitat quality was informed by studies which determined that semi-natural land cover was an important driver of bee abundance and diversity (Kennedy et al., 2013; Ricketts et al., 2008), as well as the definitions of habitat quality in Williams et al. (2016), and Spiesman et al. (2017) as they related to colony development. Herein natural landscapes were assumed to better support colony growth and reproduction than agricultural landscapes.

I hypothesized that the growth rate and reproductive potential of colonies placed adjacent to blueberry fields during the crop's bloom period would not vary significantly amongst fields. This hypothesis assumed that the abundance of lowbush blueberry pollen in the local landscape would provide each colony with adequate forage. I then tested if differences among colony development would vary at these sites after blueberry bloom, and if so, were the differences a function of the surrounding landscape.

Chapter 2: Materials & Methods

2.1 Colony Maintenance

Bumble bee colonies (*B. impatiens*) were provided by a commercial bumble bee producer (BioBest, Leamington, Ontario). Typically, a single commercial colony used for pollination contains hundreds of workers when purchased. I instead procured colonies at the initial stages of development, with each colony having a single queen and fewer than 50 workers. Colonies were received 2 and 14 days prior to being placed in the field for the spring and summer introductions, respectively. All colonies were held under ambient conditions in a laboratory and provided sugar water (1 g white sugar:1 mL water), water, and fed freeze-dried pollen (O'Healthy Market, Truro, Nova Scotia) each day before being moved to the field. While in the lab, each colony was photographed once (Figure 1), so that adult bees could be accurately counted, and each hive was weighed to the nearest gram. These measures were used to assess initial colony size. Colonies placed near the blueberry fields during the spring introduction (see explanation below) had 46-90 individual workers prior to their field placement. To ensure that each field received colonies of the same relative size, three categorized groups of colonies were established: colonies with 46-70 workers, colonies with 71-82 workers, and colonies with 83-90 workers. Two colonies from each grouping were placed at each field. The colonies used for the summer introduction (see explanation below) had 10-31 workers when received from BioBest. As before, three categories of colonies were established in the lab, with varying numbers of workers (10-15, 16-25, and 26-31). When these colonies were brought to fields, I placed 8 colonies in each field: three colonies from each of the 10-15 worker and 16-25 worker categories, and two colonies from the 26-31 worker category.

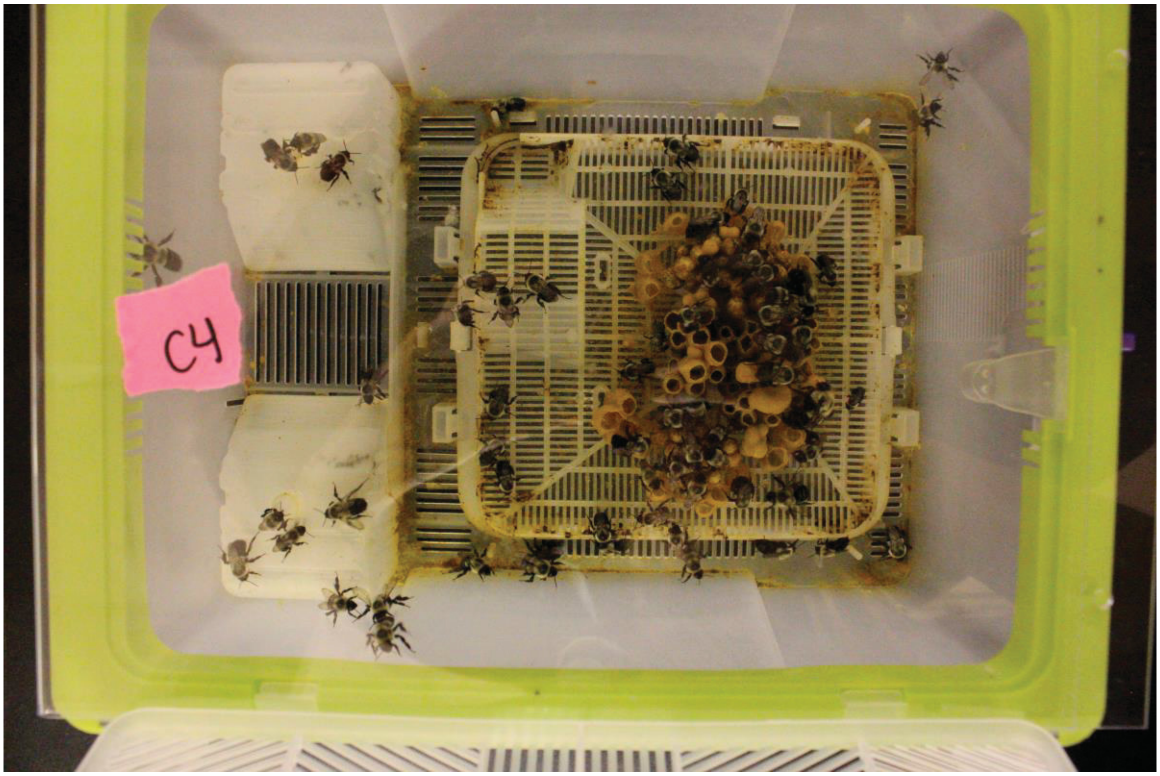

Figure 1: Image of a *B. impatiens* hive box used to estimate the size of the colony prior to introduction.

Colonies were transported to the field sites in their original cardboard shipping containers. All the colonies were placed in fields on either 26 May 2016 (spring introduction) and 16 August 2016 (summer introduction), the start date of each respective trial. The entrance to each hive was opened after placement on a wooden pallet which elevated hive boxes approximately 10 cm off the ground surface. Colonies where then watched to ensure worker bees could freely exit and return to hives. Hive entrances were designed with three modes: 1) two-way, allowing bees to move into and out of the hive, 2) one-way, which held bees in the hive while still allowing those outside to return, and 3) completely closed. The spring introduction of hives to the fields started on 26 May 2016 when the blueberry fields were at 50% bloom, and ended on 27 June 2016 (32 days later) when colonies were retrieved. Another introduction of hives happened on 26 July

2016 while blueberry fields were fruiting. During the first week in the field of this second introduction, the colonies were exposed to applications of Imidan® 70WP (phosmet, Gowan Company LLC, Yuma, Arizona) sprayed for blueberry fruit fly in the blueberry fields. Most colonies died thereafter within 7 days of exposure, and all were severely affected. The following week, all colonies were dead, and were therefore removed from their fields. The deceased colonies were not used in my analyses, instead a third introduction (hereafter referred to as the summer introduction) of colonies occurred on 16 August 2016 and lasted 30 days, with colonies being retrieved on 15 September 2016. Each hive entrance was set to the one-way entrance 24 h prior to hives being collected for transport back to the laboratory.

2.2 Colony Assessments

During each introduction, each colony, including the plastic box it was contained in, was weighed weekly to the nearest 0.5 g on a mobile scale (Digital Kitchen Scale, Taylor Precision Products, Oak Brook, Illinois). The scale was set on a pallet and each hive was weighed. Hives were weighed between 10:00 h and 16:00 h.

After the final hive weight was taken in the field, colonies were transported back to the laboratory. Colonies were then killed by freezing at -20°C. Colonies were subsequently dissected, and the following endpoints were measured: number of workers, drones, queens, dead bees, brood cells (further separated into eggs, larvae, pupae, and empty brood cells), pollen pots, and honey pots; dry weight of bees from each caste; and the weight, max height, max length, and max width of each colony. These endpoints were selected to measure the growth rate and reproductive potential of each colony (Heinrich, 2004).

2.3 Site Selection

Six lowbush blueberry field sites in Prince Edward Island, all managed by Jasper Wyman's and Sons, were selected based on the landscape characteristics within 2,000 m of each field. I selected fields to span a broad spectrum of 'natural' land cover. The proportion of natural land cover was determined using corporate land use data, provided by Steve Javorek, Agriculture and Agri-Food Canada (AAFC Kentville Research and Development Centre). The PEI Department of Agriculture and Forestry created a series of orthophotographs (high resolution aerial images) and converted them to a dataset categorized by land use at a 0.75 m resolution using the heads-up digitization method (tracing images thereby converting them from raster to vector data), paired with Javorek's ground-truthing. In the dataset, patches of landscape were defined based on the dominant cover class. The predominant land cover classes surrounding the fields selected were upland natural forest, agricultural land, and wetlands (Table 1). I used this dataset to estimate the proportion of landscape covered by each class of land cover, e.g., forest cover (Table 1). Using ArcGIS 10.3 (ESRI, 2017) I created a map of the cover types within a circle with a 2,000 m radius of each potential blueberry field. These maps were used to assess the proportion of natural land cover in the landscape surrounding each field site. Wetland and forest land cover types were considered natural, as was abandoned farmland, given the natural regrowth and succession of vegetation at these sites. Agricultural land and other anthropogenic land covers were considered not to be natural lands. These classifications were made according to the PEI government's State of the Forest Report (Prince Edward Island Agriculture and Forestry, 2013) (Appendix). Fields were then selected along a gradient from those with the least amount of surrounding natural land cover (53.6%) to those with the most (81.3%) (Table 1).

Table 1: Area (ha) of distinct natural land use classes within 2,000 m of each selected lowbush blueberry field site on Prince Edward Island, Canada. Data was generated using shapefiles that were recorded and characterised by the Government of Prince Edward Island in 2010 (PEI Department of Agriculture and Forestry, PEI Corporate Land Use Inventory 2013).

Field Site	Forested	Wetland	Abandoned	% Natural Land Cover
Belle River	512.7	109.8	46.5	53.6
Bridgetown	561.7	57.9	79.8	56.1
Commercial Cross	713.3	58.4	22.3	63.2
French Village	445.4	248.5	35.9	58.7
Iris	981.5	27.8	12.0	81.3
Mt. Vernon	897.9	24.2	18.1	74.8

In addition to the description of natural land cover, the polygons defining land cover were used to create a diversity index (Table 2), measures of patch shape and size, and measures of edge distance, all of which were measured using the landscape 2,000 m from each of the potential blueberry fields. The selected fields contained a variety of natural land cover classes including upland natural forests, wetlands, and abandoned farmlands. The diversity of land cover was measured using Shannon's Indices of Diversity and Evenness, which accounts for both the abundance and evenness of a given set of values (Nagendra, 2002). Land cover classes are herein referred to as land uses. Some of the potential field sites were grouped together as complexes of fields, where this was the case only one of the potential fields from a given complex was selected, so that no two neighbouring fields were analyzed. Although individual fields from the same complex often varied with respect to landscape character in the surrounding area, only one of from each complex was selected in order to reduce the risk of spatial autocorrelation. Each field site was separated from its nearest neighbouring field site by a minimum of 3 km (Figure 2).

Table 2: Shannon's diversity index, and Shannon's evenness index of the landscape cover classes within 2,000 m of each selected lowbush blueberry field site on Prince Edward Island, Canada. Data was generated using shapefiles that were recorded and characterised by the Government of Prince Edward Island in 2010 (PEI Department of Agriculture and Forestry, PEI Corporate Land Use Inventory 2013).

Fields	Shannon's Diversity Index	Shannon's Evenness Index
Belle River	1.3	0.7
Bridgetown	1.3	0.6
Commercial Cross	1.2	0.6
French Village	1.4	0.6
Iris	0.7	0.4
Mt Vernon	1.0	0.5

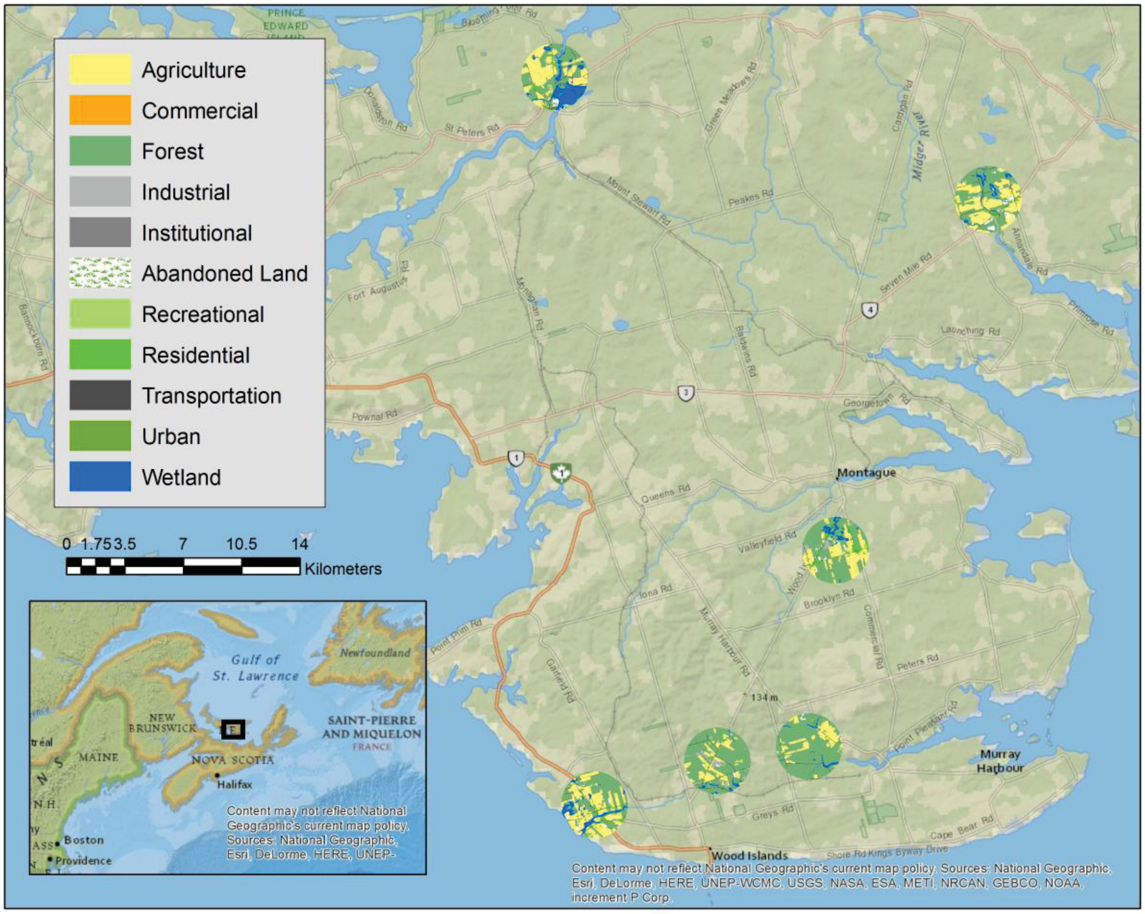

Figure 2: Map of study region on eastern Prince Edward Island, Canada, marking the location of lowbush blueberry field sites selected during the 2016 field season. Predominant land cover types within a 2,000 m radius of each site are characterized. Land cover classes were recorded and characterised by the Government of Prince Edward Island in 2010 (PEI Department of Agriculture and Forestry, PEI Corporate Land Use Inventory 2013). Inset map displays the Maritimes and indicates the area of interest with a black border.

Once the field sites were selected, a location for the placement of the colonies at the field edge was determined. Colonies were placed 5-15 m from the field edge at a site that offered protection from sun, rain, and high wind. All field sites were subjected to similar agricultural practices since they were all managed by Jasper Wyman's & Sons. As noted above, when blueberry fruit began to ripen (25 July 2016), all fields were sprayed twice with Imidan. The second introduction of hives unfortunately occurred during the application of this insecticide and many colonies died soon after application. I was able to place another set of colonies at the sites later in the season during the harvest.

2.4 Wild Bumble Bee Diversity & Abundance

The abundance and diversity of native *Bombus* species were measured in each of the six selected fields using by capturing bees off blueberry flowers using timed transect walks in blooming blueberry fields. In each field a 50 m transect was set in the Northern most section of the field, approximately 100 m from the field edge. Transects were walked for a combined total of 30 min on each of three separate days during the crop bloom period when winds were less than 15 km/h, the temperature was above 10°C, and cloud cover was minimal (after LeBuhn et al., 2003).

Only *Bombus* species were netted for identification. Common eastern bumble bees (*Bombus impatiens*) and tri-coloured bumble bees (*Bombus ternarius*) were identified in the field using the pictorial section of the "Bumble Bees of North America" (BBoNA) identification guide (Williams et al., 2014) and released on site. All other specimens were kept, euthanized using ethyl acetate, and later pinned in the lab for more careful identification using the BBoNA dichotomous key (Williams et al., 2014).

2.5 Pollen Collection

To understand the diversity of pollen used by bumble bees at each site, and the degree to which commercial bumble bees used different pollen from all around blueberry fields during and after the crop bloom, pollen was collected from foraging workers returning to hives enclosed in artificial hive boxes. This was done by first closing the hive entrances so that returning foragers could not enter their respective hives. Then, bees with visible pollen loads were collected, using a sweep net, and then euthanized in a killing jar prepared with ethyl acetate. Dead bees were handled with forceps so that each pollen load could be removed from their corbicula (‘pollen baskets’), located on the tibia of each hind leg. A total of 20 pollen loads were collected from each of the six fields, on each of three collection periods. No more than three bees were taken from any individual hive, and each corbicular pollen ball was considered a single load. If a bee returned with a single pollen load, or the load was lost through poor handling, additional pollen loads were collected. Pollen samples were collected 13-17 June 2016, 23-24 August 2016, and 7 September 2016. Pollen loads from bees in each field on each sampling day were pooled and stored in individual Eppendorf tubes. After transport to the laboratory, pollen samples were kept in a freezer at -20°C.

The frozen pollen samples were sent to Johanne Parent at Laboratoire BSL (Rimouski, Quebec), who identified subsamples of the pollen according to their genus or “type” using light microscopy. Pollen was identified to the genus level wherever possible, but in many cases, could only be classified according to the type of pollen within the Family level. As a result, identification to the genus was not always possible and genera with the same palynological features were grouped together and termed a “type”.

This consisted first of measuring a quantity of distilled water with a pipet to achieve a 25 x dilution factor. The pollen was then washed into a 15 mL centrifuge tube, until no grains remained in the Eppendorf tube, and vortexed for 2 minutes. A drop of the resultant pollen preparation was put on a slide that had been warmed on a histology plate (≤70°C) with a small cube of fuchsia stained glycerin jelly. The cube was stirred gently until completely melted and homogenized, a cover slip was applied over the preparation, and a drop of paraffin wax with clear nail polish was added to seal the slide. A second slide was prepared the same way. Slides were left to dry at room temperature and the clearer of the two was used for microscopic analysis.

Once dry, a vertical line passing through the centre of the drop was drawn on the underside of each slide. The analysis began at this centre line and moved outward towards one side of the slide to reduce the possibility of discrimination based on size (smaller pollen grains may move further away from the centre due to the pressure from the application of the cover slide). For each sample, 500 pollen grains were identified at 1000 X.

2.6 Landscape Analysis

Analyses of the landscape surrounding each field site was completed using data provided by AAFC (PEI Department of Agriculture and Forestry, PEI Corporate Land Use Inventory 2013), as well as data from the federal governments open source database (GeoGratis, 2017). The files described the primary land use of each patch (polygons), climate and weather (points), watershed attribute (lines), and the network of roadways (lines) within the eastern half of Prince Edward Island. The land use data, used for site selection and subsequent analysis of patch metrics, was created using colour infrared aerial photography converted to digital maps. A clear film displaying land use data from 2000 was overlaid manually allowing for interpretation of the changes. Heads-up digitizing was used to accommodate changes indicated by the interpreters of the aerial photos. The source photos were taken at a scale of 1:17,500 and the data were precise to 0.75 m.

Bumble bees are thought to forage on resources close to their hive. *Bombus impatiens* workers typically travel *c.* 500 m per foraging trip (Westphal, Steffan-Dewenter, & Tscharntke, 2006; Osborne et al., 1999). Long distance foraging (>1,500 m) is common only in larger species of bumble bees that live at higher latitudes, although most species are capable of doing so (Rao & Strange, 2012). Consequently, landscape data analysis was undertaken at two spatial scales: within 500 m, and 2,000 m of the colonies. Landscape characteristic data were analysed with a precision of +/- 1 m.

The latitude and longitude of each placement location of colonies was uploaded to ArcGIS 10.3.1 (ESRI, 2017). These coordinates were used as the centre point for the analysis of the landscape surrounding each field. The area of interest was defined using the “Buffer” command in the spatial analysis toolbar. Using each of the imported points, a set of two circles, with radii of 500 m and 2,000 m respectively, were built around the location of each set of colonies. Each buffer was built using a double stereographic projection, the same projection used by the government of P.E.I. to build the Corporate Land Use (CLU) maps. A double stereographic projection is effective at preserving the true direction from a single point because at small scales on a plane, any two concurrent lines have the same angle as corresponding lines on a sphere. This means that shapes are locally preserved, an ideal projection for a small area like eastern P.E.I. All shapefiles produced for the analysis were assigned a double stereographic projection. The buffers were then used to extract landscape data from around each field so that each site could be analyzed independently.

Unique areas of interest were created using the “Clip” command in the spatial analysis toolbar. This tool operates as a ‘cookie cutter’ allowing the user to create a new shapefile by defining a new extent and assigning the attribute data from a larger data set to it. I used the 500 m and 2,000 m buffers to clip new shapefiles from each of the data sets describing the landscapes surrounding each commercial colony site. The outputs were a set of circles with the attribute data from data sets that spanned the full breadth of the island, limited to 500 m and 2,000 m from each set of colonies. This allowed me to consider only the attribute data that described the area workers were most likely to be foraging within. The result of each clip (performed on the shapefiles describing land use, roadway network, and hydrological network) was a unique shapefile that could be analyzed separately.

2.6.1 Matrices of Configuration

Subsequent analysis of these new shapefiles clipped from the CLU data was completed using the Patch Analyst © tool; an extension of the spatial analyst toolbar created by the Centre for Northern Forest Ecosystem Research (Rempel, 2012). Patch Analyst facilitates the analysis of either vector or raster data through use of "Patch Analyst" or "Patch Grid", respectively. The tool was used to assess landscapes using Shannon's Diversity Index and Shannon's Evenness Index, the prevalence of edges (total edge, and mean patch edge), and the number and shape of patches (number of patches, perimeter-area ratio, patch size, and patch index) with respect to the land use classes.

2.6.2 Measures of Composition

Patch Analyst can also be used to measure the composition of landscapes. Using the clips of the CLU, the composition of the landscape surrounding each field site was uniquely considered. As with the metrics of configuration, the composition of the landscape was measured for areas within 500 m and 2000 m from each site. The CLU data was divided into distinct landscape cover classes, each of which is further divided into multiple subclasses. I measured the total area covered by each of the dominant landscape classes as well as each individual subclass within each clip. The former was used as part of the statistical analysis, while the latter offered a more detailed description of each landscape for the purpose of discussion.

2.7 Data Analysis

To test the hypothesis that a bumble bee colony's growth is influenced by characteristics of its surrounding environment, each individual measure of growth from commercial colonies was compared across fields using an analysis of variance (Minitab, 2017). The direction and groupings of these differences was measured using Fisher's least significant difference to assign letter groupings (Minitab, 2017). Assumptions of normality and constant variance of the residuals of the error term were tested for each colony growth metric. The assumption of normality was tested using both the Anderson-Darling ($p > 0.10$) and the pen test (Montgomery, 2013). All measures included in the analysis demonstrated normality after a $\log_{10}$ transformation. Constant variance was tested by plotting the residuals against fitted values and observing their distribution (Montgomery, 2013), and independence was assumed to result from the territorial nature of bumble bees (Heinrich, 2004). Although bumble bees can recognize intruding bumble bees from their scent alone and large colonies will have guard bees to defend the hive entrance, independence could have been violated by bees from neighbouring colonies invading a given colony (Goulson, 2010).

For each endpoint, the average of all colonies at a given field was calculated and tested amongst fields in an ANOVA. These values were once again used to discern which of the measures of landscape configuration and composition might result in any differences in the growth rate in a series of correlations. Pearson's correlation coefficient was used to compare the means of each of the metrics of colony growth at each field with all the measures of landscape configuration and composition. This method was chosen because there were too few degrees of freedom in the treatment type to support the analysis of each regression line. Comparing the correlation coefficient of each linear association explained which measure of landscape might be resulting in the difference amongst fields.

Shannon's Diversity Index was used to measure the diversity of each pollen sample, and the diversity of the landscape class and subclass. This index makes no assumptions about the distribution of species (Magurran, 2004), confounds both richness and evenness while prioritizing the former, and is sensitive to actual site differences (DeJong, 1975). It has also been noted that Shannon's index is more appropriate for landscape level analysis since it emphasizes the values of small patches (Nagendra, 2002). These indices of diversity were compared against the individual metrics of colony growth along with the measures of landscape configuration and composition.

Chapter 3: Results

3.1 Field Site Selection, Location, and Descriptions

Site descriptions were based on the character of landscape within a 2,000 m radius of where the colonies were placed, which amounted to a total landscape area of 1257 hectares (ha). The selected field sites were all within predominantly semi-natural landscapes dominated by forests (53.6-83.1%), and to a lesser extent agricultural lands (16.2-40.1%) (Table 1). The field with the highest proportion of natural habitat, the Iris site, also had the highest proportion of forested land, with 78.1% of its land cover represented as forest. Iris also had the least diverse surrounding habitat (Table 2), having relatively little abandoned farmland, built up, or wetland cover (Figure 3). The Mt Vernon site had the next highest proportion of natural land cover which was also predominantly forest, although the field was adjacent to some mining and restoration operations (Table 3) (Figure 3). The Commercial Cross site had the third highest proportion of natural land cover, which was again mostly forest. The Commercial Cross site had a moderate proportion of agricultural land, and a high proportion of built up land cover relative to the other field sites (Table 3) (Figure 3). The other 3 field sites had comparatively similar proportions of natural land cover, though the land cover classes were varied (Table 3).

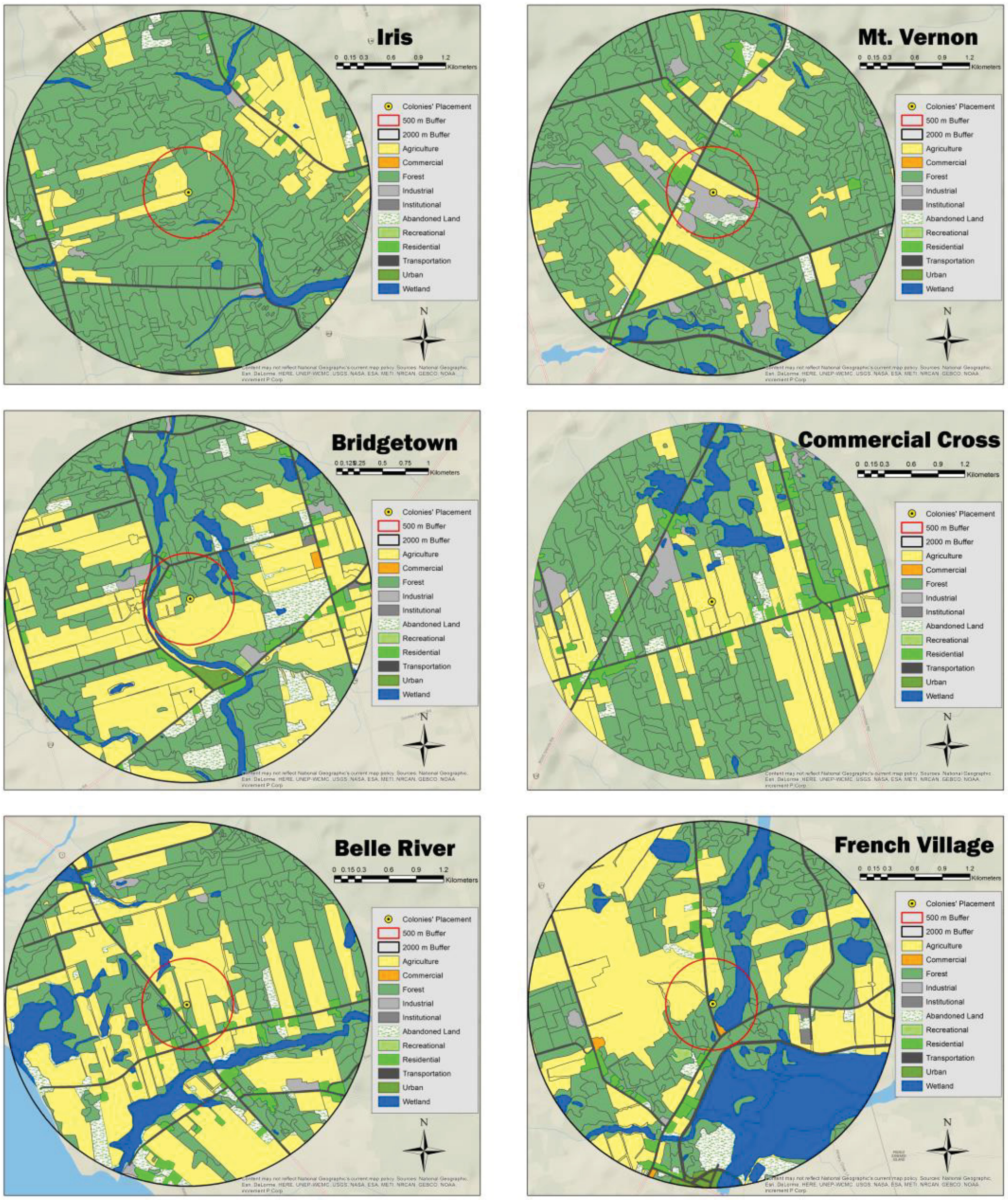

Figure 3: Maps of the land use classes within a 2,000 m radius of a lowbush blueberry field selected for commercial colony placement. Land cover classes were recorded and characterised by the Government of Prince Edward Island in 2010 (PEI Department of Agriculture and Forestry, PEI Corporate Land Use Inventory 2013). Each field is on Prince Edward Island and was selected for the 2016 field season.

Table 3: Area of land (ha) covered by each land use categorizations within 2,000 m of lowbush blueberry fields on Prince Edward Island. Generated using shapefiles created by Government of Prince Edward Island in 2010 (PEI Department of Agriculture and Forestry, PEI Corporate Land Use Inventory 2013).

Field Site	Agriculture	Forested	Wetland	Abandoned Farmland	Built Up Landscape
Belle River	500.8	512.7	109.8	46.5	79.0
Bridgetown	464.9	561.7	57.9	79.8	82.1
Commercial Cross	356.0	713.3	58.4	22.3	106.5
French Village	435.9	445.4	248.5	35.9	73.6
Iris	203.5	981.5	27.8	12.0	31.8
Mt. Vernon	203.6	897.9	24.2	18.1	112.8

The Belle River site, where agriculture constituted 39.9% of the surrounding landscape, had the most agricultural land of the six field sites (Table 3). Its surrounding landscape also consisted of 40.7% forested lands (Table 3), making it the site with the highest eveness at this scale (Table 4). Belle River also had a relatively large proportion of wetlands (8.74%), most of which were open water wetlands (Table 3). The Bridgetown site had a larger proportion of both built up and abandoned landscapes than Belle River (Table 3). Although there was less wetland at this site, the wetlands that were adjacent to the field were within a matrix of what is classed as wet forest (Figure 3). The French Village site had the highest proportion of wetland landscape, with 19.7% of the surrounding landscape belonging to this landscape class (Table 3) (Figure 2). The agricultural and forested landscapes neighbouring French Village were also similar in their proportion of total landscape at 34.6% and 35.4% respectively (Table 3).

The selected sites also varied in terms of habitat heterogeniety. Mt. Vernon was the most diverse site at the 500 m scale, whereas French Village was the most diverse field at the 2,000 m scale (Table 4). Belle River, Bridgetown, and Iris were all moderately diverse at the 500 m scale, and Commercial Cross was more diverse at the 2,000 m scale, though still moderate relative to the other sites at that scale. Belle River and Bridgetown were nearly as diverse at 2,000 m as French Village. Iris was the least diverse at both landscape scales (Table 4).

Table 4: Shannon's indices of diversity and evenness at both 500 m and 2,000 m from lowbush blueberry field sites on Prince Edward Island. Generated using shapefiles created by Government of Prince Edward Island in 2010 (PEI Department of Agriculture and Forestry, PEI Corporate Land Use Inventory 2013).

Field Sites	Shannon's Diversity Index		Shannon's Evenness Index	
	500 m	2,000 m	500 m	2,000 m
Belle River	1.1	1.3	0.6	0.7
Bridgetown	1.1	1.3	0.6	0.6
Commercial Cross	1.0	1.2	0.5	0.6
French Village	1.2	1.4	0.7	0.6
Iris	0.6	0.7	0.5	0.4
Mt Vernon	1.5	1.0	0.8	0.5

The difference in diversity of cover classes was not reflected in the size or shape of the patches. At 500 m, Iris, which was the least diverse field, had the fewest patches and the largest mean and median patch size (Table 5). Within 2,000 m however, there were more distinct patches surrounding Iris than most other sites. French Village, which was the most diverse field, had relatively few patches within 500 m, and the fewest patches within 2,000 m (Table 5).

Table 5: Patch size and number of patches within 500 m and 2,000 m from lowbush blueberry field sites on Prince Edward Island. Generated using shapefiles created by Government of Prince Edward Island in 2010 (PEI Department of Agriculture and Forestry, PEI Corporate Land Use Inventory 2013).

Field Sites	Number of Patches		Median Patch Size (ha)	
	500 m	2,000 m	500 m	2,000 m
Belle River	53	461	0.54	1.44
Bridgetown	41	456	0.84	1.59
Commercial Cross	40	434	0.70	1.77
French Village	32	382	0.97	1.49
Iris	28	450	2.09	1.99
Mt Vernon	40	436	0.86	2.19

The shape of the patches was consistent across sites according to the mean shape index (Table 6). There was a marked difference in the mean perimeter area ratio between fields, but these differences were not consistent across scales. Mt. Vernon had more than twice the mean perimeter-area ratio as the next leading site at 500 m, but at 2,000 m it had one of the lowest ratios (Table 6). French Village switched in the opposite direction, having one of the smallest ratios at 500 m, and the highest ratio at 2,000 m.

Table 6: Patch shape metrics within 500 m and 2,000 m from lowbush blueberry field sites on Prince Edward Island. Generated using shapefiles created by Government of Prince Edward Island in 2010 (PEI Department of Agriculture and Forestry, PEI Corporate Land Use Inventory 2013).

Field Sites	Mean Shape Index		Mean Perimeter-Area Ratio	
	500 m	2,000 m	500 m	2,000 m
Belle River	1.7	1.7	989.4	754.5
Bridgetown	1.8	1.7	1021	941.7
Commercial Cross	1.8	1.7	1306	570.2
French Village	1.6	1.7	948.2	2330
Iris	1.7	1.7	627.0	644.8
Mt Vernon	1.6	1.6	3522	652.3

The edge metrics varied dramatically amongst fields and across scales. There was nearly an 8,000 m difference between the total edge measured within 500 m of the landscape surrounding Iris and Belle River (Table 7). On the larger scale, there was more than a 60,000 m difference between the total edge value at French Village and Commercial Cross. The relationship between fields with respect to total edge was inconsistent across fields (Table 7).

Table 7: Edge metrics within 500 m and 2,000 m from lowbush blueberry field sites on Prince Edward Island. Generated using shapefiles created by Government of Prince Edward Island in 2010 (PEI Department of Agriculture and Forestry, PEI Corporate Land Use Inventory 2013). Total edge is measured in metres, mean patch edge is measures in metres/patch, and edge density is measured in metres/hectare.

Field Sites	Total Edge		Mean Patch Edge	
	500 m	2,000 m	500 m	2,000 m
Belle River	30,465	391,907	574.8	850.1
Bridgetown	27,601	400,579	673.2	878.5
Commercial Cross	27,265	405,026	681.6	933.2
French Village	24,554	340,959	767.3	892.6
Iris	22,927	398,139	818.8	884.8
Mt Vernon	26,988	402,409	674.7	923.0

3.2 Bumble Bee Diversity and Abundance

Blueberry plants across the sites reached 50% dehiscence from 13-17 June 2016. Three *Bombus* collection days using aerial netting took place at each field between 13 June and 22 June 2016. The transect walks yielded too few observations to be used in any meaningful statistical analysis (Table 8). Wild *Bombus* were most abundant at the Belle River site, followed by the Iris, and Bridgetown sites. The French Village and Mt.

Vernon sites had comparatively few wild individuals, with only three wild bees caught at Mt. Vernon compared to the 24 caught at Belle River. Most of the individuals caught were *B. impatiens*, however three species were recorded at each site with the exception of the Mt. Vernon site, where only two species were found. Some of the individuals recorded may have come from my commercial colonies, but the fact that the majority of the individuals identified were queens implies that there are many wild bees in the system.

Table 8: *Bombus* caught by aerial netting in transects. Transects were conducted 13-17 June 2016 within lowbush blueberry fields on Prince Edward Island. Species listed in the top row, "q" denotes queen, "w" denotes worker.

Field Sites	*B. impatiens*	*B. ternarius*	*B. vagans*	*B. insularis*	Total
Belle River	9 q, 9 w	3 w	3 w	-	24
Bridgetown	10 q, 5 w	1 q, 2 w	-	1 q	19
Commercial Cross	8 q, 3 w	4 q	-	1 q	16
French Village	1 q, 1 w	1 w	-	1 q	9
Iris	8 q, 6 w	6 q, 1 w	1 q	-	22
Mt Vernon	1 q, 1w	-	1 w	-	3

3.3 Pollen Diversity

Of the bees caught returning to their hive, approximately 50% were carrying pollen loads. The size of the loads was quite variable (Table 9) but did not seem to vary according to size of the returning worker. There was no clear trend in the differences between cumulative weight of pollen collected at each field during the spring and summer introductions (Tables 9).

Table 9: Cumulative mass (g) and Shannon's Diversity Index of 20 pollen loads collected off *B. impatiens* workers returning to the hive located near lowbush blueberry fields on Prince Edward Island during the 2016 field season.

Sampling Dates	Field Site	Weight (g) Pollen Loads	Shannon's Diversity Index
13-17 June	Belle River	0.14	1.21
13-17 June	Bridgetown	0.17	0.98
13-17 June	Commercial Cross	0.24	1.16
13-17 June	French Village	0.24	0.85
13-17 June	Iris	0.20	1.09
13-17 June	Mt. Vernon	0.18	1.24
23-24 August	Belle River	0.22	0.64
23-24 August	Bridgetown	0.32	1.51
23-24 August	Commercial Cross	0.23	1.26
23-24 August	French Village	0.29	1.48
23-24 August	Iris	0.32	1.33
23-24 August	Mt. Vernon	0.16	0.32
7 September	Belle River	0.17	0.51
7 September	Bridgetown	0.30	1.37
7 September	Commercial Cross	0.19	1.11
7 September	French Village	0.30	0.78
7 September	Iris	0.18	1.22
7 September	Mt. Vernon	0.16	0.93

During the spring introduction, heath (Ericaceae) pollen was present in the samples from each field, making up 16-66% of pollen grains counted (Figure 4). At Bridgetown, French Village, and Mt. Vernon, Ericaceae pollen was the most abundant type. At all other sites, Ericaceous pollen was either the second or third most abundant pollen type (Figure 4). Despite the difference in reliance on Ericaceae pollen, pollen diversity was relatively consistent across fields during the spring introduction (Shannon's Diversity Index = 0.846-1.24) when compared to the variability of diversity found across both sampling periods during the summer introduction (Shannon's Diversity Index = 0.323-1.51) (Table 9).

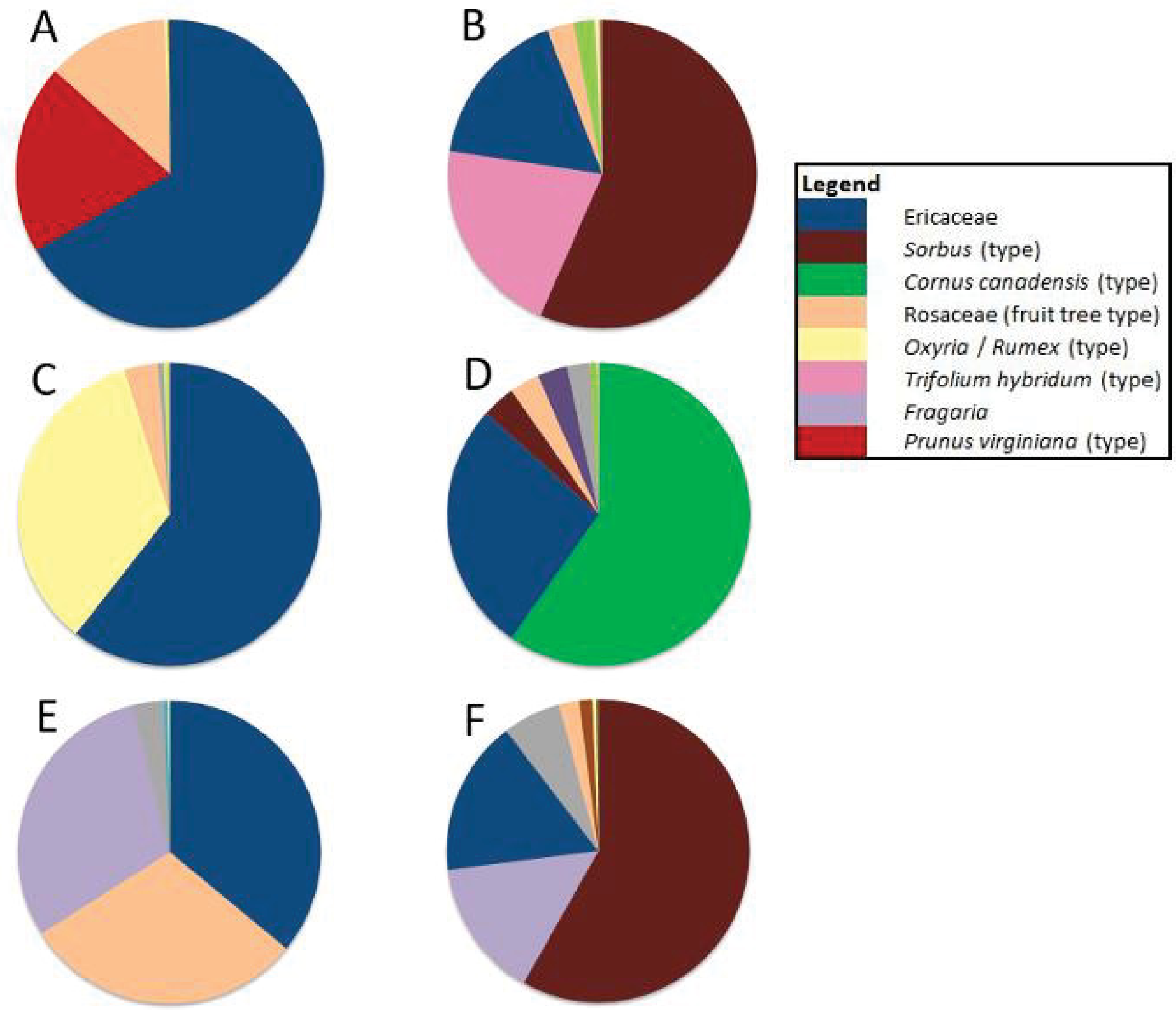

Figure 4: Diversity of pollen collected from *B. impatiens* foragers returning to hives in lowbush blueberry fields on Prince Edward Island, 13-17 June 2016. Letters refer to field sites: A. Bridgetown; B. Commercial Cross; C. French Village; D. Iris; E. Mt. Vernon; F. Belle River.

Between the two sampling periods during the summer introduction, except for the case of Mt. Vernon, fields had a greater diversity of pollen during the August sampling period (Table 10). Bridgetown had the greatest pollen diversity collected from bees in both August and September. The only field that had a similar diversity of pollen during the summer introduction was French Village, but this field had relatively low diversity during the September sampling period (Table 9). Commercial Cross and Iris has consistently high relative pollen diversity. Belle River had a low diversity of pollen across sampling periods (Table 9).

In total 42 different pollen types were identified; 18 during the spring introduction, and 28 during the summer introduction. Individual samples taken during the spring introduction contained 4-8 kinds of pollen (Figure 4), whereas samples during the summer introduction contained 2-11 types of pollen (Figure 5 & 6). The variety of pollen types varied across sites and sampling days, however the three most abundant pollen types on each sampling day made up >75% of the sample (500 grains). In most cases, most of the pollen types identified in a sample were a small proportion (<5%) of the grains counted. Pollen that accounted for a large proportion (>5-30%) of the total grains counted was often unique to the field, whereas *Aster* / *Solidago* pollen was abundant at every field (Figures 5 & 6).

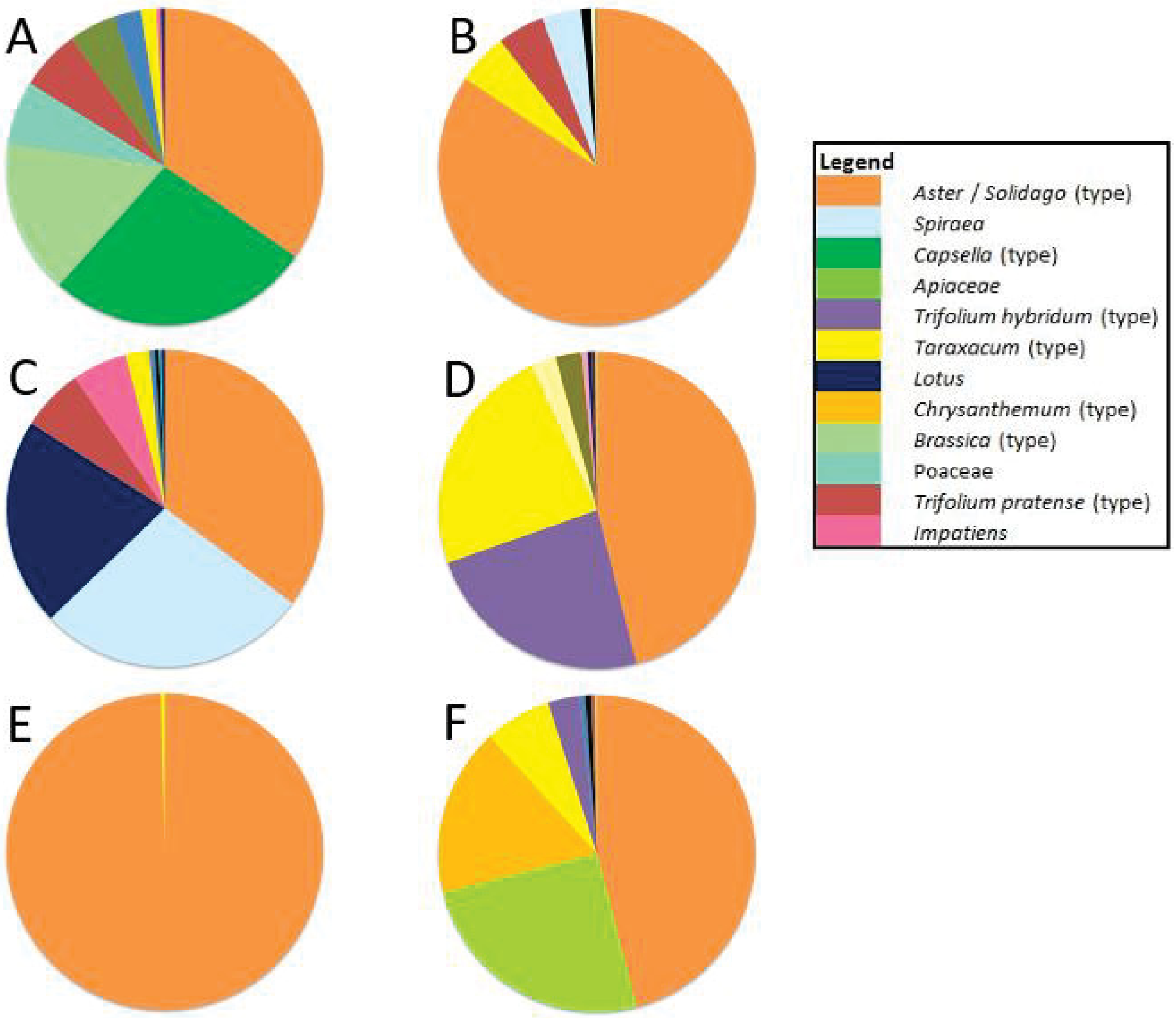

Figure 5: Diversity of pollen collected from *B. impatiens* foragers returning to hives in lowbush blueberry fields on Prince Edward Island, 23-24 August 2016. Letters refer to field sites: A. Bridgetown; B. Belle River; C. French Village; D. Commercial Cross; E. Mt. Vernon; F. Iris.

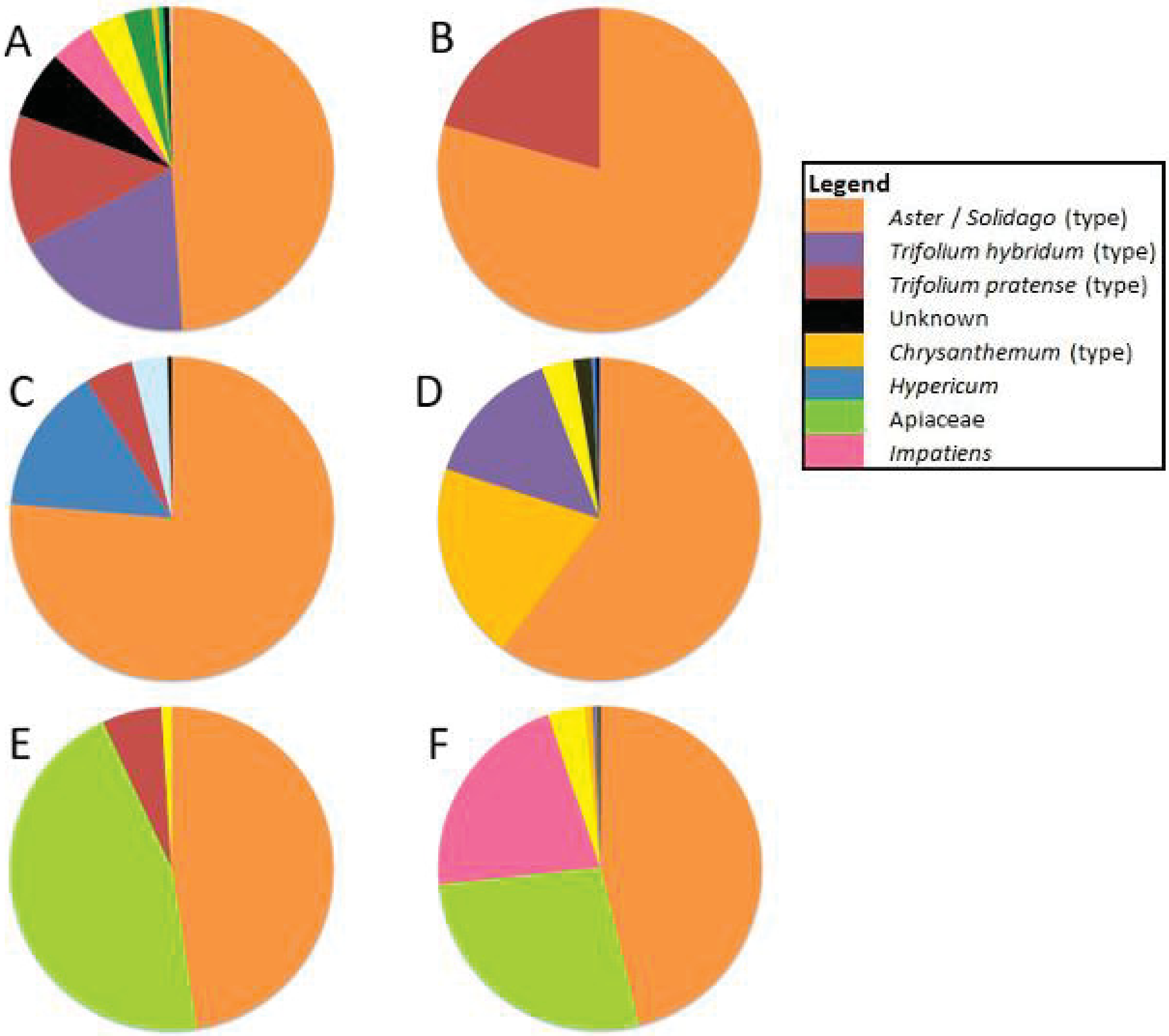

Figure 6: Diversity of pollen collected from *B. impatiens* foragers returning to hives in lowbush blueberry fields on Prince Edward Island, 7 September 2016. Letters refer to field sites: A. Bridgetown; B. Belle River; C. French Village; D. Commercial Cross; E. Mt. Vernon; F. Iris.

3.4 Commercial Colony Growth

During the blueberry bloom, all but a single colony had fewer workers after the growth period than when they were placed in the field. Although at each field some colonies produced fewer workers than others, each produced new brood cells, and each had a surviving queen upon collection. It was noted that nearly half of the colonies (n=14/36) were invaded by kleptoparasite bumble bee queens. For this first set of colonies that were introduced during the blueberry bloom, I observed no significant difference amongst fields between the means of any of the measures of growth and reproduction according to analysis of variance tests (Table 10).

Table 10: Descriptive statistics and results of ANOVA tests conducted to test for differences in the average growth metrics of *B. impatiens* colonies placed in lowbush blueberry fields on Prince Edward Island during the 2016 field season.

Introduction	Growth Metric	F-value	P-value	Mean +/- Standard Deviation
Spring	Weight of Hive (g)	0.45	0.809	82.9 (±14.6)
Spring	Weight of Bees (g)	0.69	0.633	2.9 (±1.3)
Spring	Weight of Workers (g)	0.60	0.701	2.0 (±1.0)
Spring	Weight of Drones (g)	0.69	0.640	0.0 (±0.1)
Spring	Weight of Queens (g)	2.36	0.078	0.8 (±0.7)
Spring	Total Brood (cells)	0.52	0.761	184 (±80.5)
Summer	Weight of Hive (g)	16.60	< 0.005	24.9 (±20.9)
Summer	Weight of Bees (g)	5.85	< 0.005	2.7 (±2.0)
Summer	Weight of Workers (g)	4.31	< 0.005	2.3 (±1.4)
Summer	Weight of Drones (g)	1.06	0.402	0.0 (±0.1)
Summer	Weight of Queens (g)	1.04	0.413	0.4 (±0.8)
Summer	Total Brood (cells)	3.87	0.007	135 (±81.6)

For colonies that were placed at sites in the summer, after the blueberry bloom, most (42/48) of the colonies produced new brood. Only colonies with less than 10 workers when they were placed in the field failed to produce new brood cells. Each field contained one such colony though none were included in my analyses because I assumed that their failure was the result of their poor starting condition. Colonies placed in the field after bloom also displayed uniform rates of parasitism by wax moths, though no kleptoparasitic bees were found in any of the colonies. There were significant differences amongst fields in most of the colony growth metrics (Table 10).

Mean hive weights, which included all food stores, brood cells, and wax structures, were 2.8-fold greater at Bridgetown than in the other five fields (Figure 7). Mean total weight of the drones, and queens did not differ significantly amongst fields, but weight of the workers, and the combined weight of all the bees in each hive (bee weight), did vary amongst fields (Figure 7). Mean bee weight was 2.2-fold higher at Bridgetown compared to the other fields (Figure 7). Number of brood cells in total was also significantly different amongst fields. Bridgetown and Belle River produced the most brood, Belle River was also grouped with Commercial Cross, and French Village, while Commercial Cross and French Village were also grouped with both Mt. Vernon and Iris. Total brood therefore displayed a gradient of colony growth from little to moderate growth (Figure 7).

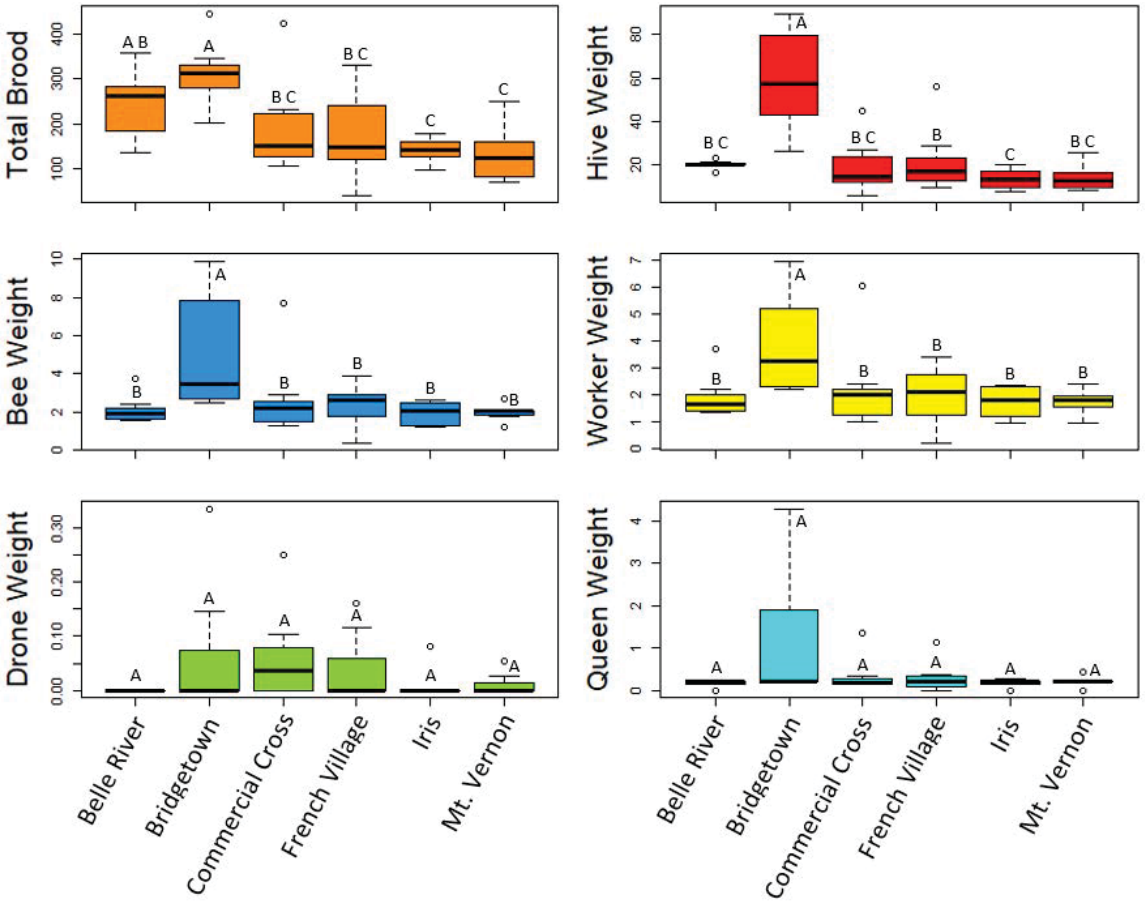

Figure 7: Boxplots displaying differences in the relative success of *B. impatiens* colonies at distinct lowbush blueberry fields on Prince Edward Island during the summer of 2016. The mean, standard deviation, and 1st and 3rd quartiles of growth metrics measured in 6 *B. impatiens* colonies at each field site. Endpoints are: number of brood cells (Total Brood) weight of the hives in g (Hive Weight), dry weight of all bees in the hives in g (Bee Weight), dry weight of the workers in g (Worker Weight), dry weight of the drones in g (Drone Weight), dry weight of the queens in g (Queen Weight).

3.5 Landscape Character Affects on Colony Growth

Each measure of landscape character was plotted against the mean of each measure of colony growth and reproduction for the colonies placed in the field after the blueberry bloom period. In Tables 11 & 12 metrics that were highly correlated ($p<0.05$) are bolded, and those moderately correlated ($0.10>p>0.05$) are underlined. The degree to which they correlated was measured using Pearson’s Correlation Analysis. Measures of landscape configuration within 500 m of the colonies did not correlate with measures of colony success (Table 11).

Table 11: Correlation coefficients and *p*-values (in brackets) of Pearson's correlation between the measures of *B. impatiens* colony growth and physical descriptions of the landscape within 500 m of their location. Colonies were placed on the edge of lowbush blueberry fields on Prince Edward Island in the summer of 2016.

	Hive Weight	Bee Weight	Worker Weight	Queen Weight	Total Brood
Total Edge	0.251 (0.631)	0.215 (0.683)	0.243 (0.643)	0.165 (0.755)	0.577 (0.231)
Mean Patch Edge	-0.208 (0.693)	-0.171 (0.746)	-0.198 (0.718)	-0.126 (0.812)	-0.525 (0.285)
Mean Perimeter-Area Ratio	-0.254 (0.627)	-0.224 (0.669)	-0.235 (0.654)	-0.183 (0.729)	-0.406 (0.424)
Mean Shape Index	0.545 (0.263)	0.637 (0.174)	0.643 (0.168)	0.569 (0.239)	0.624 (0.185)
Number of Patches	0.179 (0.734)	0.131 (0.805)	0.162 (0.759)	0.084 (0.874)	0.531 (0.278)
Median Patch Size	-0.267 (0.609)	-0.247 (0.637)	-0.255 (0.626)	-0.197 (0.708)	-0.487 (0.327)
Shannon's Diversity Index	0.011 (0.983)	-0.035 (0.948)	-0.046 (0.931)	-0.008 (0.988)	-0.054 (0.919)
Shannon's Evenness Index	-0.185 (0.726)	-0.252 (0.630)	-0.265 (0.612)	-0.183 (0.729)	-0.364 (0.478)

Measures of landscape configuration within 2,000 m of each colony did not correlate with measures of colony growth or reproduction except for in the case of mean shape index and total number of patches. Average total brood produced by the colonies at a site was slightly (*p*=0.073) positively correlated with the mean shape index of the patches within 2,000 m of the hive (Table 12). The type of land cover within 2,000 m demonstrated a clear relationship with average colony growth (Table 12). Area of land dedicated to agriculture, and area of natural land cover were moderately correlated with the average total brood produced by the hives at a field site (Table 12). Area of abandoned farmland, was highly correlated with all measures of colony growth and reproduction (Table 12).

Table 12: Correlation coefficient and *p*-values (in brackets) of Pearson's correlation between the measures of *B. impatiens* colony growth and physical descriptions of the landscape within 2,000 m of their location. Colonies were placed on the edge of lowbush blueberry fields on Prince Edward Island in the summer of 2016. Significant correlations are bolded and underlined, marginally significant correlations are underlined.

	Hive Weight	Bee Weight	Worker Weight	Queen Weight	Total Brood
Total Edge	0.088	0.184	0.204	0.171	0.125
	(0.868)	(0.727)	(0.698)	(0.746)	(0.813)
Mean Patch Edge	-0.283	-0.139	-0.167	-0.144	-0.488
	(0.587)	(0.793)	(0.752)	(0.785)	(0.326)
Mean Perimeter-Area Ratio	0.096	0.008	-0.016	0.026	-0.007
	(0.856)	(0.987)	(0.976)	(0.961)	(0.990)
Mean Shape Index	0.583	0.552	0.563	0.471	0.770
	(0.271)	(0.256)	(0.245)	(0.346)	(0.073)
Number of Patches	0.231	0.246	0.281	0.236	0.379
	(0.660)	(0.638)	(0.590)	(0.653)	(0.459)
Median Patch Size	-0.421	-0.331	-0.343	-0.278	-0.681
	(0.406)	(0.522)	(0.506)	(0.594)	(0.136)
Shannon's Diversity Index	0.487	0.422	0.420	0.379	0.638
	(0.327)	(0.405)	(0.407)	(0.459)	(0.173)
Shannon's Evenness Index	0.171	0.128	0.140	0.059	0.503
	(0.746)	(0.808)	(0.791)	(0.912)	(0.309)
Area of Wetlands	-0.007	-0.103	-0.117	-0.115	0.063
	(0.990)	(0.846)	(0.825)	(0.828)	(0.906)
Area of Agriculture	0.553	0.471	0.485	0.418	0.808
	(0.255)	(0.346)	(0.330)	(0.410)	(0.052)
Area of Forests	-0.459	-0.368	-0.371	-0.327	-0.646
	(0.360)	(0.473)	(0.469)	(0.527)	(0.166)
Area of Abandoned Land	**0.929**	**0.862**	**0.873**	**0.853**	**0.950**
	(0.008)	**(0.027)**	**(0.023)**	**(0.031)**	**(0.004)**
Area of Natural Land	-0.545	-0.474	-0.485	-0.420	-0.787
	(0.263)	(0.342)	(0.330)	(0.407)	(0.063)

Chapter 4: Discussion

Declining pollinator populations have been observed all over the world (Brown & Paxton, 2009; Goulson, Lye, & Darvill, 2008; Goulson, Nicholls, Botías, & Rotheray, 2015; Kosior et al., 2007; Williams & Osborne, 2009) and there is growing consensus that agricultural intensification may be an important driver of the declines (Potts et al., 2010). Brown et al. (2009) synthesized 12 reviews of pollinator decline and found that habitat loss was the most notable driver of decline in bee populations. The factors contributing to bee decline are likely to be unique to each species due to differences in life history traits (Hopfenmüller et al., 2014). However, large scale land use changes would likely have consistent impacts on most bee species (Persson, Rundlof, Clough, & Smith, 2015). Conservation efforts are more likely to be successful if causes of decline are correctly identified (Williams & Osborne, 2009). Focusing on bumble bees and determining how their populations are influenced by various threats will guide efforts to mitigate their decline (Williams & Osborne, 2009).

Not all *Bombus* species are in decline; although the majority are in varying levels of decline, some species exhibit increasing trends (Colla, Gadallah, Richardson, Wagner, & Gall, 2012). Changes in land use because of agricultural intensification are presumed to have substantially contributed to the decline in bumble bee populations (Williams & Osborne, 2009). A case study in Illinois showed this trend using specimen-level databases, correlating decline in *Bombus* abundance from historic levels to large-scale agricultural intensification (Grixti, Wong, Cameron, & Favret, 2009). Conversely, a comprehensive analysis of 64,866 bumble bee occurrence records in North America found that pesticide use and habitat loss, arguably the most severe impacts of agricultural intensification, are unlikely to be a major cause of decline in any *Bombus* species (Szabo, Colla, Wagner, Gall, & Kerr, 2012). Whether or not land use changes are the proximate cause of their decline, it is important that conservation efforts understand the landscapes that support bumble bee populations so that attempts to mitigate future decline are effective. Both good quality forage and nesting habitat are necessary for bumble bee conservation (Hopfenmüller et al., 2014), my study focused solely on the quality of forage provided by the landscape.

Since in most species only queens are active in the spring (Goulson, 2010), which is when the first set of colonies were introduced, the results of the spring introduction are not indicative of what would happen to wild colonies in the field. In the field the spring is when queens emerging from hibernation will find a suitable nesting site and lay their first clutch of eggs (Heinrich, 2004). My colonies were well established when placed in the field, and although they demonstrate the quality of forage available across sites in the spring, their success or failure is not necessarily indicative of what is happening to wild colonies.

4.1 Colonies in the Spring Introduction

My results indicate that the quality of habitat surrounding lowbush blueberry fields had no significant effect on bumble bee colony growth parameters during the spring introduction. Colonies were placed immediately adjacent to the blueberry fields during bloom, I predicted that these colonies would forage heavily on blueberries, which would buffer any potential landscape effect. Bumble bees are especially suited for foraging on blueberry plants due to their unique ability to dislodge the plants' pollen (Usui, 2005) so I assumed bumble bee colonies were likely to get a large portion of their forage from blueberry flowers during the spring introduction. These assumptions were verified using both the transect walks, and the pollen collection and identification.

The transect walks determined that native bumble bees were foraging in the blueberry fields during bloom. Although most of the bees caught were *B. impatiens*, many were native queens, which could be an indication that native bumble bees are more abundant than their managed counterparts. Queens from the managed colonies deployed by blueberry growers during the bloom, as well as those from my own colonies, would remain with their colonies, only native queens still searching for nesting sites would be foraging in the fields. Bumble bees are among the most effective pollinators of blueberry flowers (Javorek et al., 2002), which is why so many blueberry farmers choose to use them, but the results of my transect walks suggest that native bumble bees may be doing the work credited to native bees. The relative abundance of native species, as well as the rate of parasitism from cuckoo bees (Sheffield, Pindar, Packer, & Kevan, 2013) suggests that there is a community of native bumble bees thriving in these blueberry fields, and potentially out-performing their managed counterparts. That being said, work that focuses solely on the effectiveness of managed bumble bee colonies has determined that they are quite effective (Ings, Ward, & Chittka, 2006). Their fidelity to the blueberry fields was also supported by the results of my pollen samples and subsequent analysis.

Pollen samples collected from bees returning to commercial hives during blueberry bloom contained considerable pollen from ericaceous plants. Although it is not certain that this was *V. angustifolium* pollen, given that many *B. impatiens* workers were observed on blueberry flowers during the transect walks, and that *B. impatiens* is well known to pollinate blueberry (Javorek et al., 2002), it is reasonable to assume that much of the pollen collected was blueberry pollen. These results confirm the importance of local forage for supporting the bumble bee colony growth. When there is adequate forage within the local landscape, bumble bees will forage on this resource as opposed foraging on more distant resources, which have increased risk and energy requirements (Heinrich, 2004).

Although the landscape of each site was different, there was no difference in colony development across sites during blueberry bloom. Coupled with my transect walk observations that confirmed the presence of *B. impatiens* workers on blueberry flowers during bloom, and the results of the pollen samples, these results suggest the close proximity of a large blueberry field was sufficient to sustain colony growth. However, colonies placed in the same location after the blueberry bloom would demonstrate the potential effects of these landscapes on colony development.

4.2 Colonies in the Summer Introduction

Using the same field sites two months after the spring introduction, colonies were placed in the same location near fields during the summer introduction, and for the same duration. I found mean dry weight of the bees (both total, and workers alone), weight of the hive, and total brood produced by the colony differed significantly by field sites. There was no significant difference in the number of reproductives (queens and drones) produced amongst fields, which is a crucial measure of colony success, although very few drones and queens were produced across sites. This is the result of the relatively short growth period. Typically queens will lay only workers during the initial weeks of colony development (Shykoff & Muller, 1995). Increase in hive mass and amount of pollen ingested are consistently proportional to one another throughout the growth period of both worker and drone larvae (Ribeiro, 1994), making total weight of the hive a good indicator of the amount of pollen available to each colony. With respect to both weight of the bees and number of brood produced, the difference in the mean measured at each field was significant. Pollen availability also has a positive effect on worker production (Rotheray, Osborne, & Goulson, 2017), and both nectar and pollen availability affect brood production (Rotheray et al., 2017; Tasei & Aupinel, 2008).

That significant differences in colony growth endpoints across sites were not observed during the spring introduction while the blueberry fields were in bloom, but were observed during the summer introduction, suggests that the landscape had a discernible effect on colony growth during the summer. Colonies at the Bridgetown site grew, on average, to weigh more than twice as much as the colonies at all other fields. On average, the fastest growing colonies were found at the Bridgetown and Belle River sites. At the French Village and Commercial Cross sites, there were large colonies, but all measures of colony growth were lower than at the Bridgetown site. At the Mt. Vernon and Iris sites, the colonies that did not collapse grew very little and produced few brood cells. The fact that some fields produced large colonies, and other produce relatively small ones during the summer introduction suggests that landscape character may influence colony growth.

4.3 Influence of Landscape Character

For colonies in the summer introduction, there were significant differences in the mean hive weight, weight of the bees, and number of brood produced in the colonies amongst fields. These endpoints, particularly total brood produced, are significantly greater at the Bridgetown site than any other site. Comparing these values to the characteristics that defined the landscape using Pearson's correlation coefficient demonstrate that only landscape composition correlates with colony endpoints. None of the measures of configuration included in my analysis were shown to correlate with colony endpoints. Aside from a measurable edge effect in some systems (Moisan-DeSerres et al., 2015), there is little evidence that the configuration of a landscape correlates with native bee abundance or diversity (Kennedy et al., 2013). Bumble bees are highly dispersive relative to other bees, and can traverse landscapes uninhibited by patches that other animals may experience as barriers (Westphal et al., 2006). The composition of the landscape however did correlate with several recorded measures of colony growth.

Area covered by wetland cover classes, and area covered by natural cover classes, were both weakly positively correlated with average total brood produced. These results are consistent with my *a priori* assumption that natural land cover classes would benefit bumble bee development. In similar studies that examined the growth of bumble bee colonies, greater proportion of natural land cover resulted in larger colonies (Jha & Kremen, 2013; Williams et al., 2012). Natural land cover across my field sites ranged from 54-83% of the total land cover within 2 km of where colonies were placed, but my results show that only a small proportion of this land is behind the positive association. Abandoned farmland represented only 1.8-6.5% of total land cover, but positively correlated with most of the measured colony endpoints and correlated with all the measures that displayed a significant difference amongst fields. This finding is consistent with more detailed recent analyses which demonstrate that semi-natural landscapes provide bees with foraging resources (Kennedy et al., 2013; Williams et al., 2012). The landscape categorized as abandoned farmland matches the broad definition of semi-natural habitat defined in the literature. These land use data were identified using aerial imagery, making it unlikely that abandoned farmland would have been incorrectly identified. It is therefore likely that these patches of land would have been rich with early successional species, which are ideal for bumble bee foraging (Jha & Kremen, 2013)

Bumble bee foraging is adaptive such that they seek out floral-rich habitat patches regardless of habitat composition (Jha & Kremen, 2013). *Bombus impatiens* foragers will alter their foraging decisions in order to find optimal pollen sources (Vaudo, Patch, Mortensen, Tooker, & Grozinger, 2016). Workers from the summer introduction may have relied on few patches of landscape that contained a great diversity of flowering plants, making landscapes with more of these patches better able to support colony growth.

Pollen samples allowed me to determine which pollen sources were most used by the *B. impatiens* hives at the different field sites. Habitat composition has been shown to predict pollen load composition (Goulson, Hughes, Derwent, & Stout, 2002), and limited availability of pollen sources led to smaller colonies (Schmid-Hempel & Schmid-Hempel, 1998). In bumble bees, rare species are highly selective in their pollen foraging whereas common species forage on a broad range of flowers during pollen collection (Goulson & Darvill, 2004). Collecting many pollen species is likely advantageous for bumble bees as well since the nutritional quality varies greatly between plant species (Kitaoka & Nieh, 2009), and feeding larvae multiple pollen species is important for their growth (Tasei & Aupinel, 2008).

Here I used pollen diversity to sample the richness of foraging resources accessible to the colonies across fields. The pollen gathered by the bees at the Bridgetown site during the summer introduction was more diverse than at other sites. It appeared to me that bees returning to the hives at Bridgetown were carrying larger loads than their counterparts at other fields, and the cumulative weight of the two samples supports this field observation. There was trivial difference between the rest of the five field sites, in terms of both pollen diversity and cumulative weight of the loads. Although it appears that colonies at the Bridgetown site had a greater diversity of foraging resources in close range, with only two samples within each site no statistical comparison could be made. That being said, the richness and Shannon's Diversity Index value in the pollen samples collected at Bridgetown during the summer introduction was among the highest recorded. The Bridgetown site also had relatively high abundance of wild bees identified during the transect walks.

It is important to note that the Bridgetown site was also among the fields with the highest diversity of landscape cover classes. Although statistically I found that only the proportion of abandoned farmland was significantly correlated with colony endpoints, it is likely that many characteristics of the Bridgetown sites landscape contributed to the growth of the colonies placed there.

4.5 Uncertainties

There was no significant difference among fields in any of the in-hive endpoints measured on the colonies that were placed in the field during blueberry dehiscence. Though some colonies were greater than others in terms of the endpoints measured, each produced new brood cells, and each had a surviving queen upon their collection. There was a great degree of variability in the apparent success of a given hive; each field produced both relatively large and small colonies. Although the significant difference among sites supports a landscape effect, there are several reasons to suspect that my assumption about the adequacy of the available forage was not the only reason that there was no measurable difference of in-hive endpoints between fields.

Prior to their introduction, colonies that were set out in the spring introduction period had been housed in the lab for the first phase of their growth. Each colony was fed daily for two weeks prior to the placement in the field. This meant that each of the colonies placed in the field had grown to moderate size (50-80 workers) before the experiment took place, whereas the summer introduction colonies were introduced to the field immediately after their arrival from the commercial production facility and were significantly smaller (10-30 workers). The difference in initial size meant that the variation in the endpoints in the spring introduction was greater than the variation in the endpoints in the summer introduction. Each introduction contained colonies that were at separate phases of growth.

4.6 Future Research

Harnessing native pollinators to pollinate agricultural crops could be used as a precautionary measure to safeguard against potential shortages of honey bees as pollinators of lowbush blueberry. Using native species to pollinate agricultural crops is not a novel concept, but the landscape characteristics that support the successful growth of bumble bee colonies in the system are still poorly understood. In this study I found that the composition of the landscape has a measurable effect on bumble bee growth, and that a small proportion of the habitat may be driving this relationship. Further research into the strength and the limits of this relationship in lowbush blueberry fields, and other cropping systems where insect pollination is necessary are needed to further explain this trend.

My results are congruent with other research that strengthens the argument for extending agri-environment schemes to support native pollinators (Carvell et al., 2011). For example, native bee abundance and diversity was low in landscape dominated by apple orchards (Marini, Quaranta, Fontana, Biesmeijer, & Bommarco, 2012). Targeted agri-environment schemes in these systems could foster a greater community of wild bees that could in turn offer pollination services (Carvell et al., 2011). In some systems the groundcover plant community has the potential to provide these benefits (Rosa García & Miñarro, 2014). In blueberry systems the landscape in field margins could be modified to promote pollinator communities, to date field margins have only succeeded in providing pollination services along the fields edge (Evans & Spivak, 2006).

Landscape ecologists have demonstrated a clear relationship between habitat diversity and animal diversity (Tews et al., 2004). Here I have determined that the composition of the landscape around bumble bee colonies correlates significantly with colony weight, number of brood, number of workers, and total number of bees produced. In similar studies focusing on landscape composition, habitat specialists were only affected by local-scale characteristics, whereas generalists were positively influenced by landscape-scale characteristics (Hopfenmüller et al., 2014). Bumble bees, a generalist species, were positively affected by the percentage of semi-natural habitats (Hopfenmüller et al., 2014). I have also shown that it may be that a relatively small proportion of the landscape is resulting in a large shift in colony success. Further research should be directed towards the degree to which small scale land use changes can impact pollinator communities within the surrounding landscape. Slight changes in landscape management could yield disproportionate benefits if the effect of small landscape patches on native pollinators can be more precisely understood.

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Appendix

Land use classification defined by the Government of Prince Edward Island in 2010 (PEI Department of Agriculture and Forestry, PEI Corporate Land Use Inventory 2013).

Land use	**Sub-uses**
Agriculture	Farmstead, Christmas Tree, Feedlot, Fur Ranch, Hedgerow, Manure Storage, Nursery, Orchard, Strip Crop
Forestry	Plantation, Clear Cut, Wetland
Wetland	Reservoirs, Sewage, Forest
Abandoned Farmland	Abandoned Land
Residential	Cottage, Mobile Home Park, Multiple Units, Single Units
Industrial	Auto Salvage, Excavation Pits, Fertilization Plants, Food Processing, Land Fill, Tank Farm, Wind Farm, Sawmill/Lumberyard
Institutional	Church, Cemetery, Historic Site, Hospital, School
Recreational	Campground, Golf, Playing Field, Rink, Ski Slope
Transportation	Airport, Communications Structures, Lighthouse, Power Line, Railway Right of Way, Road, Wharf
Urban	City
Commercial	Accommodation, Food & Beverage, Motor Vehicle, Retail

Land use classifications were based on the interpretation of aerial imagery and categorized according to the following list and table. Definitions were provided by the AAFC Kentville Research Station.

Agricultural: "any land used in the production of livestock or crops including fields, farmsteads, hedgerows, etc.

Forestry: "any land in forest vegetation including clearcuts and plantations"

Wetland: "any land which permanently supports hydrophytic vegetation"

Abandoned Farmland: "any land where the use is not clearly discernible"

Built Up;

Residential: "any land outside of urban areas used for dwelling"

Industrial: "any land outside of urban areas used for large industrial purposes"

Institutional: “any land outside of urban areas used in an institutional manner such as schools, hospitals, etc.”

Recreational: “any land used for recreation such as golf courses, parks, etc.”

Transportation: “any land used to support transportation such as roads, wharfs, etc.”

Urban: “any land in a city, town, or village which is a contiguous area and is large enough to support community facilities such as buildings of worship”

Commercial: “any land outside of urban areas which is used for commercial purposes such as a gas station”