An Ecological Study on Red Sorrel (*Rumex acetosella* L.) in Wild Blueberry Fields in Nova Scotia

by

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Table of Contents

List of Tables

List of Figures

Abstract

Red sorrel is a perennial weed in wild blueberry fields that decreases yield. Multiple experiments were conducted to evaluate its impact on blueberry pollination, Botrytis blight incidence, and berry yield. Kerb applications did not significantly impact blueberry stem or floral bud formation. Removal of red sorrel with Kerb increased blueberry yield at both sites. However a double application had no difference than one application. Blueberry and red sorrel flowering overlapped and red sorrel pollen grains were found on blueberry flowers in both years at all sites. Red sorrel pollen grains increased the incidence of germinating spores in Petri dishes and this relationship was adequately modeled with a three parameter, exponential rise to a maximum. Red sorrel pollen significantly increased disease incidence on immature blueberry flowers. Honey bees foraged from blueberry and red sorrel flowers, but there was no evidence to suggest that they favored red sorrel flowers over blueberry flowers.

List of Abbreviations and Symbols Used

°C	Degree Celsius
CO_2	Carbon dioxide
cm	Centimetre
F4	Immature blueberry flower
F7	Mature blueberry flower
Ha	Hectare
kg	Kilogram
LS	Least Square
L ha^{-1}	Litre per hectare
mm	Millimeter
m	Metre
m^2	Metre square
mL	Millilitre
psi	Pounds per square inch
%	Percent
µL	Microlitre

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Chapter 1 Introduction

1.1 Introduction to the Problem

Wild blueberry (*Vaccinium augustifolium* Ait.) is the number one fruit crop in Nova Scotia in terms of total acreage, export sales, and total value to the province's economy (Robichaud 2006; Strik and Yarborough 2005). In 2010, the wild blueberry industry produced 16, 500 tones of blueberries and contributed over $13 million dollars to the annual farm-value in Nova Scotia alone (Stats Canada 2010).

Wild blueberries are grown in six Canadian provinces and 36 U.S. states (Moore 1993) and are continuing to expand. They are a unique horticultural crop as they are not planted but developed from native stands. They are an increasing popular crop grown in many parts of the world (including Canada, United States, Norway and China) for their antioxidant properties and health benefits (Howatt 2008). In 1996, blueberries were found to be the highest in antioxidant activity out of 41 fruits and vegetables tested (Prior et al. 1996).

Weed control is the most important and most common challenge in commercial production. Weeds compete with the wild blueberry for nutrients, light and moisture. They also impede harvest activities, decrease berry quality, and may be a crop contaminant. There are many problematic weeds that occur in the blueberry fields (Esser 1995; Sampson et al. 1990), but red sorrel (*Rumex acetosella* L.) is one of the most common. In a survey from 1984-1985 it was found that red sorrel was the third most common weed in wild blueberry fields (McCully et al. 1991). In 2000-2001 sorrel was the most abundant weed out of 125 weed species recorded and it had increased 43% since the 1984-1985 survey (Jensen and Sampson 2001, unpublished data). It is a

perennial plant that is hard to control due to its rapid spread, perennial life cycle, and copious seed production. It is a concern due to its impact on yields, interaction with the disease Botrytis blight and possible interaction with honey bees.

There are several diseases that impact blueberry growth and yields. Botrytis blight caused by *Botrytis cinerea* Pres.:Fr. is an occasional but destructive fungal disease (Hildebrand et al. 2001) that overwinters as a dormant mycelium or sclerotia on blueberry and other plant debris including red sorrel (Lambert 1990; Hildebrand et al. 2001). On blueberries, *B. cinerea* appears mostly on expanded corollas under favorable conditions (Hildebrand et al. 2001) and outbreaks of Botrytis blight tend to occur in coastal areas where fog is prevalent.

Wild blueberry pollination is essential for increased fruit and larger berry formation. There are numerous species of native pollinators which are associated with pollination of the wild blueberry (Finnamore and Neary 1978; Morrissette et al. 1985) and their efficiency is well documented (McGregor 1976; Fisher et al. 1993). However, these native pollinators are not sufficiently abundant to ensure adequate pollination of all flowers in blueberry fields (Boulanger et al. 1967; Mohr and Kevan 1987). Thus, introduced bees such as bumble bees (*Bombus impatiens* Cresson.), alfalfa leafcutter bees (*Megachile rotundata* F.), and honey bees (*Apis millifera* L.) are frequently used to enhance blueberry fruit set.

There is a lack of published peer-reviewed literature on the interaction between red sorrel, Botrytis blight and honey bee activity in wild blueberry fields. I hope to broaden our understanding of the ecological impacts of these interactions and determine if they are detrimental or beneficial to the crop.

1.2 Wild Blueberry History

Wild blueberries are native to northeast North America and are an important food source for birds and other wild animals. Native Americans enjoyed them long before the first Europeans discovered them in North America (Wood 2004). Early explorers such as Samuel de Champlain documented that Native Americans gathered and dried wild blueberries for use in the winter months and would add them to meals (Wood 2004). They encouraged the growth of blueberries by periodically burning fields which would quickly regenerate new shoots (Howatt 2008). The first European settlers found them to be similar to types of berries that grew in their homeland such as the blueberry in Scotland, whortleberries in Ireland, bilberries in Denmark, blabar in Sweden, and bickberren and blauberren in Germany (Howatt 2008).

The commercial development of today's wild blueberry industry began in the late nineteen forty's and early nineteen fifties (Kinsman 1993). In Yarmouth County Nova Scotia during the early 1800's specific areas were repeatedly burned due to forest fires and the land soon filled in with blueberry and other plants that thrive in acidic soils (Wood 2004). Early records of harvesting and selling wild blueberries date back to the1800's. At that time, berries were handpicked and sold in baskets in nearby towns and shipped in barrels or cans to the Boston market (Kinsman 1986).

Today, commercial production of wild blueberries is limited to a rather small area in eastern Canada and the north-eastern United States, in the provinces of Nova Scotia, New Brunswick, Prince Edward Island, Newfoundland & Labrador, Quebec and Maine (Howatt 2008). Nova Scotia is the third largest producer of wild blueberries in the world and frozen blueberries are one of Canada's major exports (USDA 2012). In recent years,

wild blueberries have received much attention due to their health attributes. The fruit is rich in antioxidant compounds that fight free radicals that are associated with cancer, heart disease and premature aging (Howatt 2008).The industry has seen many changes along the way and continues to evolve today.

1.2.1 Species

The blueberry plant is a member of the *Ericaceae* or heath family, genus *Vaccinium*, subgenus *Cyanococcus.* There are three main blueberry species of economic importance: (1) the highbush blueberry, *V. corymbosum* L., (2) the wild 'lowbush' blueberry, *V. angustifolium,* Aiton, and (3) the rabbiteye blueberry, *V. ashei* Reade (Gough and Korcak 1995). Commercial wild blueberry fields consist of native clones of V. *angustifolium* and/or V. *myrtilloides* (Strik and Yarbourough 2005.) The plant is a perennial that spreads vegetatively by roots (Vander Kloet 1998) and by seed. Shoots are low growing reaching 10 to 60 cm in height. New shoots of maturing plants develop from dormant buds on underground stems called roots (Kinsman 1993).

1.2.2 Blueberry Management

Wild blueberry plants are native to North America and are adapted to temperate climates. They are found on a wide range of soil types. They are most common, on coarse textured, well-drained, infertile podzols with low pH of glacial or alluvial origin (Jensen and Yarborough 2004) with acidity levels of 4.2-5.0 that are generally unsuitable for other types of agricultural crops (Howatt 2008).

Commercial blueberry fields are not planted but are developed from native stands on deforested or abandoned farmland by removing competing vegetation. The blueberry

population generally consists of numerous distinct and variable clones that slowly expand to provide complete cover (Jensen and Yarborough 2004). Wild blueberries primarily spread by roots, which give rise to new shoots and stems from the same rhizome system (McIsaac 1997). Fruit production is influenced by both management and environmental factors over the unique 2-year production cycle (Eaton 1994). In year one, the plants are pruned in either the fall or spring by burning, or flail mowing to near ground level. Pruning enhances new upright shoot growth from the underground stems (Kinsman 1993) and increases flower bud development (Jensen and Specht 2002). New shoots grow during year one and in the second year the shoots bloom and produce fruit in clusters. Blueberry blossoms are bell-shaped and are white or pinkish white (Kinsman 1993). Flowers usually open in June and if pollination does not occur within two to three days after a flower opens fruit set is less likely and by 7 to 8 days becomes improbable (Drummond and Yarborough 2002) making the window for pollination very short. At bloom it is essential to introduce pollinators such as honey bees to help increase berry formation. Herbicides, insecticides, fungicides, fertilizer application and commercial pollinators are the main management inputs added to blueberry fields throughout the year. Some or all may be used depending on weed species, insect presence, disease incidences, soil type and blueberry yield.

1.3 Weeds in Blueberries

1.3.1 General Weed Management

Weeds are the major yield limiting factor in commercial blueberry production (Jensen 1985). They compete with the wild blueberry plant for light, moisture and soil

nutrients (McCully et al. 1991). This may lead to competition with the blueberry plant, reduced yield, impede harvest and lower berry quality. There are numerous weeds that infest wild blueberry fields for which there are only a few control options. Weeds and weed seeds spread via wind, animals and human activities (Boyd and White 2009). The many weed species which prove problematic to wild blueberry include but are not limited to: black bulrush (*Scirpus atrovirens* Wild), bunchberry (*Cornus canadensis* L.), fern (*Pteridium aquilinum* L. Kuhn), goldenrod (*Solidago canadenis* L.), hair fescue (*Festuca tenuifolia*), lambkill (*Kalmia angustifolia* L.), red sorrel (*Rumex acetosella* L.), tickle grass (*Agrostis hyemalis*), and vetch (*Vicia cracca* L.).

Herbicides have been the primary means of weed control in wild blueberry fields for the last 50 years due to their fast acting and continuous control of weeds from year to year (Jensen and Yarborough 2004). There are a number of widely used herbicides that fit into three categories, (1) non-selective post emergence treatments which are applied only to the top growth, (2) pre-emergence soil applied treatments which are principally active by root uptake, and (3) selective post emergence treatments applied broadcast to foliage (Jensen and Yarborough 2004). Management of the competing vegetation is primarily achieved with the broad spectrum, pre-emergence herbicide hexazinone (Velpar DF). It is a systemic herbicide that was first introduced in the 1970's and is still applied every other year in most commercial fields to control broadleaf weed populations. Upon discovering that Velpar decreases weed density and increases blueberry yields farmers became increasingly reliant on this herbicide to solve all their weed problems. In recent years, plant species that are not controlled effectively by Velpar have become more prominent (Jensen and Yarborough 2004). Cultural and

physical weed management practices are used in blueberry fields but since blueberry plants remain established in the field from year to year there are only certain practices which are beneficial. Practices such as pruning by means of mowing reduce above ground weed growth. Red sorrel is just one weed species that is not effectively controlled by Velpar, and new management practices to control red sorrel are needed.

1.4. Red Sorrel

1.4.1 Species

Red sorrel is a very common weed species in wild blueberry fields in Nova Scotia (Sampson et al. 1990). Red sorrel is a member of the family Polygonaceae and is also called sheep sorrel, field sorrel and horse sorrel. It is a dioecious, perennial herb with a creeping rhizome system (Uva et al. 1997; Kennedy 2009) having male and female organs on separate plants. Flowers are generally thought to occur from May to September, but recent field studies recorded flowers opening in early December (White personnel Communication 2010). Senescence of the male flowering ramets occurs immediately after flowering, but the female flowering ramets do not senesce until after fruit ripening (Fujitaka and Sakai 2007). The fruit is dispersed by gravity and wind acts as a vector for pollen (Fujitaka and Sakai 2007). Male flowers are yellowish green and female flowers are reddish brown (Uva et al. 1997). The seed is enclosed in a triangular achene 1-1.5 mm long (Uva et al. 1997). Red sorrel produces a copious amount of pollen, is wind pollinated and its seeds are dispersed by wind, insects and humans (Escarré & Houssard 1991). The plant also grows as an annual and can germinate, produce flowers and seeds within one growing season (Alex 2001). Red sorrel exhibits a very plastic

morphology (Korpelainen 1993) as it inhabits a variety of habitats. Plasticity is the ability of an organism to change its phenotype in response to changes in the environment.

Red sorrel reproduces sexually by setting seed and vegetatively by reproducing from creeping roots. (Uva et al. 1997). Shoots develop from stem buds that arise adventitiously at irregular intervals on horizontal roots (Esser 1995). Adventitious buds are usually found in the top 20 cm of soil (Kiltz 1930) and in blueberry fields they tend to be near the soil surface. The buds are capable of sprouting immediately after development (Vodolazksky 1979). Kennedy et al. (2010) found that Velpar applied in the sprout year reduced red sorrel density and increased yields, while hexazione applied in the crop year decreased sorrel densities in some fields but did not increase yields. Fertilizer and Velpar applied in combination had no effect on sorrel density while fertilizer applied alone increased sorrel density (Kennedy et al. 2010). Red sorrel is a concern to the blueberry industry because of its competitive life cycle, limited herbicide control options, possible interaction with the disease Botrytis blight and possible impact on pollination.

1.4.2 Management

Velpar as a treatment to control red sorrel tends to decrease population density but does not eliminate the weed completely and it continues to grow and produce a large number of seeds (McCully et al. 1991, Kennedy et al. 2010, Kennedy et al. 2011). Pronamide (Kerb) is a registered herbicide for use in blueberry that decreases red sorrel roots (Boyd, personal communication 2010) but it does not eliminate the weed. Both herbicides Velpar and Kerb have inconsistent efficacy but are the only registered herbicides available for blueberry growers to try to control sorrel in their fields.

Pronamide is the herbicide of interest which was used to suppress red sorrel in this study. It has been used to suppress red sorrel and hair fescue in blueberry fields but it does not provide complete red sorrel control. Pronamide is a Dow Agro Sciences product and it is in the chemical family benzamide. Pronamide should be applied to blueberry fields in late fall of the sprout or crop year. It is a soil active herbicide taken up through the roots of weeds and is stored in the roots during the winter months and becomes active in the spring. While pronamide may be generally effective it is problematic to apply as it is a selective herbicide which needs to be carried into the root zone by rainfall or irrigation to be effective.

Synthetic fertilizers are applied at relatively low rates to commercial fields. They increase yields but also impact weed populations. Adding fertilizer will have little effect on yield unless the competing weeds are controlled before application (Penney and McRae 2000). Kennedy et al. (2010) found that fertilizer inputs increased red sorrel density in the absence of herbicides but had no effect on weed density in the presence of herbicides. Thus, fertilizer applications may be contributing to the rise in weed problems in blueberry fields.

Pruning practices such as periodic burning regenerates the crop and is also beneficial as it provides limited disease and insect control (Lathrop 1952). However, burning is not a common practice today due to the rising cost of oil. Mowing as a means of pruning is a more feasible method and is safer but may help stimulate the growth of the underground roots of weed species and increase weed density in the coming year (MacIssac 1997).

1.5 Botrytis Blight

Botrytis species affect the vegetative and flowering structures of many crops throughout the growing season often causing reduction in quality and yield (Coley-Smith et al. 1980). They are important pathogens of nursery plants, vegetables, ornamentals, field and orchard crops (Elad et al. 2007). The genus consists of some two hundred or more species with several of them of great economic importance (Stevens 1913). Botrytis blight, caused by *B. cinerea*, is a major disease in wild blueberry fields (Strik & Yarborough 2005; Kinsman 1993). *Botrytis* spp. are able to produce masses of windborne spores on infected and dead tissues under humid conditions, epidemics can develop very quickly in comparison with most other diseases (Coley-Smith 1980).

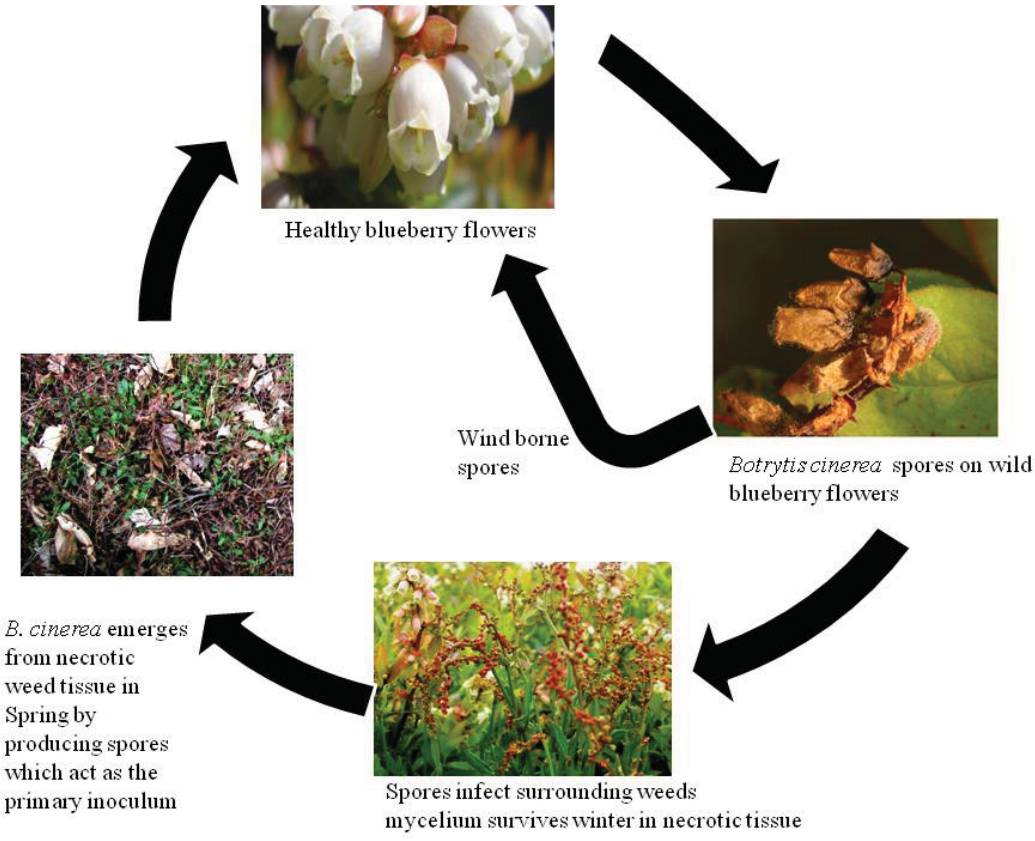

Figure 1.1 Infection cycle of *B. cinerea* in a wild blueberry field.

Blueberry flowers become susceptible just prior to opening (Delbridge et al. 2007) and Botrytis blight mostly attacks expanded corollas (Hildebrand et al. 2001) but can also attack the leaves, blossoms and fruit (Kinsman 1993) and can also work its way down the stem. *B. cinerea* usually is active after the blueberry crop is at about 50 % bloom and it continues to infect as flowers become mature (Hildebrand, personnel communication 2010). Thus, the majority of losses occur from mid-bloom until several weeks after fruit set (Kinsman 1993). The fungus overwinters on weed and blueberry debris as mycelium and sclerotia (Lambert 1990; Hildebrand et al. 2001). Under favorable weather conditions in spring, conidiophores emerge on the debris and produce conidia which are windblown and infect the developing flowers. Once the fungus has established on early flowering clones the infected tissue can serve as a source for spores to infect the later flowering clones (Hildebrand 2010).

A few days after infection, blossoms turn light brown and develop a greyish-brown mold which is easy to see under low magnification (Bell et al. 1999). Outbreaks of Botrytis blight are infrequent but they can be severe in blueberry (Hildebrand et al. 2001) with 30-35% losses recorded (Delbridge & Hildebrand 1995). Blueberry growers in coastal areas often apply protectant fungicides every year regardless of the actual disease threat (Hildebrand et al. 2001).

1.5.1 Pruning

Pruning in wild blueberry fields has largely changed from burning to mowing due to the increase in oil costs in recent years. Burning in the past has been a valuable method of controlling some weed species, as well as a few species of noxious insects and disease (Wood 2004). Increasing fuel costs and realization of the damage burning does to

the blueberry soil surface organic layer brought about this change to the industry (Lambert 1990). However, a shift from burning to mowing was attributed to a 90% increase of mummy berry (*Monilinia vaccinii corymbosi*) incidence in wild blueberry fields in Maine over a 12 year span (Lambert 1990). Apple orchards were also noted to produce apples free from scab caused by *Venturia inaequalis* the following year after the leaf litter was burned (Hardison 1976).

1.5.2 Impact of Weeds on Disease

A plant may be classified as a pest in a crop due to its competitive nature or its ability to function as a vector of a pathogen, or a reservoir of a pathogen or its vector (Wisler and Norris 2005). There are many weed species in agricultural crops that may significantly influence disease incidence and spread (Wisler and Norris 2005). Hairy nightshade (*Solanum sarrachoides*) is a common weed found in potato rotations and hosts a vast array of potato nematodes, diseases and insect pests including, potato leaf roll virus (*Polerovirus*), late blight (*Phytophthora infestans*), corky ring spot (*Paratrichodorus minor*), powdery scab (*Spongospora subterranea*) and green peach aphid (*Myzus persicae*) (Boydston et al. 2008). Dodder, *Cuscuta* spp., is not only an important parasitic weed but it also is a vector for pathogens such as cucumber mosaic virus (Bromoviridae) (Wisler and Norris 2005). In many cases, a weed or the nutrients from a weed may act as a stimulus to activate a disease. Weed presence has a large impact on crop management and strategies need to be looked at in detail to prevent pathogens from decreasing crop yields.

Strawberry pollen (Chu and Preece 1968) and blueberry pollen (Hildebrand et al. 2001) have been shown to stimulate spore germination of *B. cinerea* on plant material.

This is likely from nutrients that are supplied by the pollen (Hildebrand et al. 2001). Red sorrel produces large amounts of wind-borne pollen within the blueberry field which may in turn coat blueberry leaves and flowers. Thus, red sorrel pollen may be an abundant source of nutrients for *B. cinerea* and thereby may contribute to increased disease severity. Immature blueberry flowers appear to be resistant to infection because spores do not germinate on them (Hildebrand et al. 2001). This may occur because there is not enough nutrient leakage onto the immature flowers to stimulate germination. Red sorrel pollen, however, has not been examined for its impact on disease severity and this will be further studied in my thesis.

1.6 Honey bee

1.6.1 Species

Honey bees are insects belonging to the order Hymenoptera, superfamily Apoidea, family Apidae, tribe Apini that contains only one genus *Apis* (Kevan 2007). All species are characterized by building combs from the wax that they secrete from glands on the underside of their abdomens (Kevan 2007). The honey bee (*Apis mellifera* L.) is native to Africa, western Asia, and south-east Europe (Michener 1974), but has become naturalized throughout much of the rest of the world and is now among the most widespread and abundant insects on earth (Hanley and Goulson 2003). The European honey bee is still considered the most versatile workhorse and most commonly managed bee in the world used for pollination of agricultural crops although there are a dozen other bee species which have been commercialized for use as pollinators (James and Pitts-Singer 2008). There are at least 17, 000 other described bee species globally

(Michener 2000). It is believed that one-third of our total diet is dependent, directly or indirectly, upon insect-pollinated plants (McGregor 1976). The importance of honey bees to increase fruit set on wild blueberry is well documented (Lee 1958; Wood 1969; Lomond and Larson 1983). Honey bees are dependent on plant resources for their food. Both climate and soil determine what plants are able to grow and flower within the foraging range of their colonies. The diet of all bee species consists more or less exclusively of pollen and nectar collected from flowers (occasionally supplemented by honeydew, plant sap, waxes, resins and water (Michener 1974; Goulsan 2003). In social bees such as honey bees, stingless bees and bumble bee foraging is organized hierarchal by integrating behaviors of individual worker bees with colony requirements. Foraging is a part of the social structure and its ultimate benefit is for the colony rather than the individual (Kevan 2007). Honey bees are known for their highly social organized system in which a very distinct worker caste is found. There are three types of bees in a colony, divided into female workers, queens, and male drones. The workers in a bee colony are the most numerous caste and they are a sterile female caste incapable of laying fertile eggs. The workers are smaller than the drones and have shorter abdomens than the queen (Sammataro and Avitabile 1998). Bee colonies are usually monogynous- having only one egg producer, the queen (Afshar et al. 2007). The queen has lost the pollen collecting apparatus, wax and pharyngeal salivary glands, and among other modifications becomes extraordinary fertile and capable of producing enormous numbers of eggs (Butler 1949). The only function of the drone is to mate with virgin or newly mated queens (Afshar et al. 2007). Worker bees have many jobs in and outside of the hive but the needs of the colony and the age of the worker bee dictate what her duties will entail. Workers from 1

to 3 weeks old remain in the hive where they rest, feed and clean larvae cells, tend to the queen, build new comb and guard the entrance (Sammataro and Avitabile 1998). After three weeks, the workers move onto brood less comb where they come in contact with returning foragers and are then recruited to forage food sources (Sammataro and Avitabile 1998).

1.6.2 Foraging

Honey bees mainly forage for pollen and nectar from open flowers. Nectar is rich in sugar and provides fuel for the working bee. It is converted into honey which provides the energy source for the colony by supplying fuel for flight, heat production and other activities of the adult workers (Camazine 1993; Dreller & Tarpy 2000). Pollen is the only source of protein for the hive which plays a critical role in both adult and larval development (Camazine 1993; Dreller & Tarpy 2000; Sagili and Pankiw 2007). Pollen is collected by honey bees from many sources but when one source becomes especially abundant, foragers may gather from that source almost exclusively (Campana and Moeller 1977).

Free (1963) observed that when the pollen that foraging honey bees were accustomed to collecting was unavailable for a day, most bees foraged for nectar or remained in the hive. This demonstrates that female workers are extremely loyal to the plant species from which they originally collected pollen. Hives are frequently transported and moved into crops for cross-pollination. Honey bees will continue to forage from the flower they were conditioned to forage on unless the crop they are moved to is more attractive (Free 1963). Wetherwax (1986) found that honey bees approach a flower and then decide to accept or reject that flower on the basis of how the

flower smells. This decision is made by either hovering over the flower or by briefly touching it with their antennae or feet. Flight costs were found to be largely determined by plant density (Levin and Kerster 1969). Pollinator energy intake increases with flowering plant density, and thus visitation to flowers should be higher in denser stands (Schmitt 1983). It also has been shown that a richer foraging source that is farther from the colony creates the same profitability rating (as measured by number of waggle dances) as a source that is closer but not as rich, when they have the same net energetic efficiency (Seeley et al. 1996).

1.6.3 Foraging on Non-Native Weeds

Honey bees ultimately forage on weed species growing in or nearby agricultural crops which are in season at the time of hive placement. It was found in a study by Hanley and Goulson (2003) that introduced bees favored foraging on introduced plant species and in some cases the bees depended entirely on these plants for sources of pollen and nectar. A study by Barthell et al. (2001) examined the relationship between honey bees and seed set in yellow star thistle (*Ceutavrea solstitialis*). Honey bees were the most frequent visitors to thistle and had a significant correlation between visit rates and the average number of viable seeds produced. Honey bees were found to be important to the reproductive ecology of the plant. There have been no long term studies attempting to study the role pollinators play on the fecundity and population dynamics in introduced plant species.

1.6.4 Management

Honey bees are the most economically valuable crop pollinators of monocultures worldwide (Klein et al. 2007) and the use of honey bees has increased significantly over the last 40 years in the wild blueberry industry (Drummond and Yarborough 2002). For adequate pollination of wild blueberry flowers 2.5-5 hives per hectare are recommended (Aras et al. 1996). Importation of honey bees brought into Maine for wild blueberry pollination has increased from 500 to 60, 000 bee colonies annually between 1965 and 2000 (Drummond and Yarborough 2002).

Many flowers including wild blueberry flowers must be insect pollinated to ensure berry formation (Bell et al. 1999; McGregor 1976). There are 78 bee species that are associated with wild blueberry and apple in Nova Scotia (Sheffield et al. 2003). Two known native species of bumble bees have been recorded to be declining in the last few years throughout Nova Scotia (Cutler, personal communication 2010) which may be due to habitat fragmentation and pesticide use. Thus, many growers supplement the native pollinators with managed bees such as bumble bees, honey bees or alfalfa leafcutter bees in their fields during the bloom period (Drummond and Yarborough 2002; McIsaac 1997; McGregor 1976).

Herbicides registered for use in blueberries are ineffective at controlling red sorrel and red sorrel has increased in fields due to limited control options. Blueberry growers are concerned that red sorrel may interfere with honey bee pollination of wild blueberry flowers. Dense red sorrel patches may act as a barrier for pollinators in the blueberry fields. Bees may also prefer red sorrel flowers over blueberry flowers since male red sorrel flowers produce an abundance of wind dispersed pollen. This may result

in decreased berry yields. Red sorrel pollen may also act as a nutrient source and contribute to the increased severity of Botrytis blight in the blueberry field.

There is limited published research on the interaction of red sorrel, honey bees and Botrytis blight in wild blueberry fields and it is hoped that through this study these concerns and problems can be addressed and recommendation can be made to benefit blueberry growers and the industry.

The objectives of my M.Sc. research are to:

1) determine the impact of annual red sorrel control on blueberry growth,

2) determine the efficacy of annual pronamide applications on red sorrel ramet density and cover,

3) determine if blueberry and red sorrel flowering overlap,

4) determine if the presence of red sorrel pollen enhances infection on blueberry flowers by *B. cinerea*,

5) estimate the amount of red sorrel pollen that is transferred by wind onto blueberry flowers in a blueberry field,

6) estimate the amount of time honey bees spend foraging on red sorrel versus pollinating blueberry flowers in the blueberry field,

7) estimate the impact of flowering male red sorrel plants on blueberry pollination success and pollinator selection.

My hypotheses are as follows:

1) Control of annual red sorrel will increase blueberry yield.

2) Two applications of pronamide will decrease red sorrel ramet density and cover.

3) Timing of flowering of the blueberry plant and the red sorrel plant will coincide.

4) Red sorrel pollen will increase disease severity of Botrytis blight on immature blueberry flowers.

5) When red sorrel pollen is in abundance in the blueberry field, a large percentage of the sorrel pollen grains will be found on blueberry flowers.

6) Assuming flowering of blueberry and red sorrel coincides, it is hypothesized that the honey bees will forage on red sorrel more than blueberry flowers.

7) Flowering male red sorrel plants will have an effect on foraging and pollinating honey bees in the blueberry field and red sorrel will deter honey bees from pollinating blueberry flowers.

Chapter 2 Red Sorrel Management with Pronamide

2.1 Introduction

Wild blueberries, a native fruit crop to North America, is managed under a 2 year production cycle where the shoots are pruned in the first year (prune or vegetative) and harvested in the second year (crop). Flowering occurs in late May or June with the onset of fruit in mid July (Wood 2004). Flowers must be cross pollinated by insects to ensure adequate fruit set (Chiasson and Argall 1996). Farmers are reliant on herbicides for weed control because traditional weed management practices such as tillage cannot be applied in a perennial crop as the crop is not planted annually and regenerates each year from a complex rhizome system. Growing wild blueberries has become a highly managed system which includes pruning, irrigation, applying fertilizer and herbicides as well as introduced pollinators to all help increase the growth and yield of the crop.

Weeds are one of the major yield limiting factors in wild blueberry production (McCully at al. 1991). They compete with the crop for light, nutrients and moisture (Hildebrand 1946, McCully et al. 1991). Predominant weeds found in commercial fields include grasses, broadleaves and woody shrubs (McCully et al. 1991). Weeds impede harvest practices, may act as a host for diseases, and reduce yields.

Red sorrel (*Rumex acetosella* L.) is a perennial weed found in turf grass, pastures, (Ito 1988) landscapes, nursery crops, (Uva et al. 1997), roadsides and most importantly in Canadian blueberry fields (Sampson et al. 1990). It is highly competitive within the crop and is hard to control due to its creeping roots and ability to thrive in similar conditions as the blueberry plant. Red sorrel is dioecious, (Uva et al. 1997) meaning male and female flowers are produced on separate plants. It is capable of reproducing by seed

or vegetatively by buds on creeping roots. Plants typically flower in early spring over several days (Fujitaka and Sakai 2007). Red sorrel has a very plastic morphology as it is capable of adapting to the many growing conditions (Korpelainen 1993).

Red sorrel is a concern to blueberry growers because of its competitive life cycle and limited registered herbicide control options. There is a lack of peer reviewed research on red sorrel in wild blueberry fields. Red sorrel studies have been predominately focused in abandoned fields, pastures (Escarré and Houssard 1991) and in greenhouse settings (van Andel and Jager 1981, Harris 1972).

Pronamide (Kerb 50 WSP) and hexazinone (Velpar) are two herbicides registered for use in the blueberry crop that have activity on red sorrel (Boyd, personal communication 2010). Kennedy et al. (2010) found that hexazinone and fertilizer inputs in a sprout year blueberry field reduced red sorrel shoot density and increased blueberry yields. Velpar is applied in early spring to most sprout year fields and tends to suppress red sorrel but does not adequately control it. Therefore, many growers apply pronamide in the fall of the crop year after harvest or in the fall of the sprout year.

Kerb controls weeds by entering the plant via the roots. It is a selective, soil active herbicide that controls perennial grasses such as quackgrass, annual grasses (Dow 2010), plus many broadleaved weeds (Fisher 1974). It is applied from late September to early November of either the sprout or the crop year blueberry field at rates of 3.25 - 4.50 kg of product per hectare. For optimal results it should be applied when soil temperatures are low but above freezing and when soil moisture is high (Dow 2010) since rainfall or irrigation is required to move Kerb into the soil where it is active. Kerb can be applied pre and post emergence to effectively control weeds in lettuce (Lavalleye et al. 1969),

alfalfa, legumes (Viste et al. 1970) and in southern turf grasses (Burt 1970). Kerb is applied preemergence to the weeds in blueberry fields for the control of red sorrel and perennial grasses. Its use is limited due to the high cost of the product, application timing, and inconsistent efficacy. Techniques to control or limit population spread of troublesome weeds such as red sorrel are needed to increase the overall blueberry yield.

The objectives of this experiment were to: 1) determine the impact of annual red sorrel control on blueberry growth, and 2) determine the efficacy of annual pronamide applications on red sorrel ramet density and cover. I hypothesized that a double application would decrease red sorrel shoot density in the wild blueberry field and increase blueberry yields. A double application of pronamide is not typically a recommended management option but was used as a tool to remove as much red sorrel as possible in order to evaluate its impact on blueberry growth and yield.

2.2 Material & Methods

2.2.1 Study Sites

Two experimental field sites were chosen in October 2008 and maintained for 2 consecutive years. The Mt. Thom, NS site, ($45^0$29' N, $63^0$59' W) was a crop year field in 2009 during the first year of data collection and Kemptown, NS site ($43^0$29' N, $63^0$59' W) was a sprout year field. Data were collected in 2009 and 2010 at both sites.

2.2.2 Experimental Design

The experimental design was a randomized complete block design with 8 blocks and 2 treatments established at each site. Plots were 4 m x 6 m, with a 1 meter buffer between replications. Pronamide was applied on November 17, 2008 and November 9,

2009 at both sites, Mt. Thom and Kemptown at a rate of 4.5 kg of product per ha $^{-1}$ in a water volume of 300 L ha^{-1}. Herbicide applications were made using a CO_2 pressurized backpack sprayer equipped with XR8002VS Teejet nozzles at a pressure of 40 psi. Air temperature at application date in 2008 was 3.8°C at Mt. Thom and 4.2°C in Kemptown while in 2009 it was 7.7°C at Mt. Thom and 10.2°C at Kemptown. Treatments were: (1) fall application of Kerb in each year, and (2) an untreated control. Red sorrel and blueberry stem densities were counted on June 19 and July 30, 2009 and July 6 and 29, 2010 at Mt. Thom and on June 18 and July 30, 2009 and July 6 and 29, 2010 at Kemptown using 3, 25 x 25 cm randomly placed quadrats in each plot. Ground cover in each plot was visually rated on a percent basis at Kemptown on June 18, and July 30, 2009 and at Mt. Thom on June 10, 19, and July 30, 2009, where 0% was no cover and 100% was complete cover of blueberry, red sorrel, other weed species or bare ground. Visual herbicide damage ratings of red sorrel were also completed where 0 was no effect by the herbicide and 10 was complete plant death. Above ground biomass was collected by cutting red sorrel and blueberry plant material at the soil surface on August 13, 2009 and August 10, 2010 from 6, 25 x 25 cm quadrats per plot at Kemptown and 4 quadrats per plot at Mt. Thom. Plant material was separated and dried for 48 hours at 50°C and then weighed to determine the dry biomass. Blueberry fruit was harvested and weighed in late August in 2009 from 3, 1 x 0.3 m quadrats per plot at Mt. Thom and 5, 1 x 0.3 m quadrats per plot at Kemptown in August 2010. Blueberry stem heights were measured and floral buds per stem were counted on 30 randomly selected stems per plot in late September of 2009 and 2010 in the sprout year fields.

2.3 Statistical Analysis

Blueberry and red sorrel plant densities, red sorrel damage ratings and blueberry harvest were statistically analyzed using the PROC MIXED procedure with LSMEANS means comparison in the SAS system for Windows, version 9.2 (SAS Institute 2003). Ratings and densities were analyzed with repeated measures. Percent ground cover was analyzed using PROC MIXED with LSMEANS comparison. The sites were analyzed separately since the plants were in opposite production cycles at each site. Density and harvest data were transformed to achieve normality and constant variance.

2.4 Results & Discussion

2.4.1 Impact of Kerb Applications on Blueberries

Kerb applications did not significantly reduce blueberry stem height or number of floral buds per stem at either site or year (Table 2.1). These results indicate that red sorrel competition in the crop and sprout year of the production cycle did not reduce stem heights or number of floral buds produced per area.

Table 2.1 Average blueberry stem height and floral bud counts at Mt. Thom and Kemptown Nova Scotia in 2009 and 2010.

	Site			Site		
	Kemptown			Mt. Thom		
Treatment	2009	SE	DF	2010	SE	DF
	Average blueberry stem height (cm)			Average blueberry stem height (cm)		
No kerb	23.2a	1.3	11	19.3a	1.2	11
Kerb	25.2a	1.3	11	19.1a	1.2	11
p-value	0.1517			0.9029		
	Average floral bud per stem			Average floral bud per stem		
No kerb	4.0a	0.54	11	3.0a	0.26	11
Kerb	4.0a	0.54	11	3.0a	0.26	11
p-value	0.7980			0.5357		

[a]Means within a column with the same letter are not significantly different ($P \leq 0.05$)
SE = Standard error, DF = Degrees of freedom

Red sorrel damage ratings were significantly higher where Kerb was applied at Kemptown and Mt. Thom in 2009 and 2010 ($P < 0.001$) than where it was not applied. Ratings were averaged over time since there was no significant time by treatment interaction ($P = 0.8670$). Average ratings at Kemptown for the control and Kerb plots were 0.53 and 7.5, respectively ($P = 0.001$) at Mt. Thom the average ratings for the control and Kerb plots were 2.4 and 7.5, respectively ($P = 0.001$). Thus, Kerb suppressed but did not eliminate red sorrel.

Red sorrel density counts were significantly reduced at Kemptown in 2009 ($P = 0.0005$) and 2010 ($P = 0.0001$) and at Mt. Thom in 2009 ($P = 0.0140$). Red sorrel and blueberry densities were variable at all sites possibly due to the different growing parameters (temperatures, rainfall, and slope) at each location, yet Kerb decreased red sorrel densities at both sites (Table 2.2). At Kemptown in 2009, there was a 74% decrease in red sorrel density and 88 % decrease in 2010. At Mt. Thom in 2009 there was a 33% decrease in red sorrel density.

Table 2.2 Density and biomass of red sorrel ramets and blueberry stems at two sites for the 2-year blueberry production cycle in Nova Scotia
No plant material in plot where (.)

Site	**Kemptown**		**Mt. Thom**	
	2009	2010	2009	2010
	----------Blueberry stem density #/m^2----------			
Control	422 (167)	456 (104.9)	363 (72)	635 (147.2)
Kerb	515 (167)	519 (104.9)	365 (72)	623 (147.2)
p-value	0.2404	0.0623	0.5102	0.5102
	----------Red sorrel ramet density #/m^2----------			
Control	665 (398)	443(257.3)	442 (191)	56 (53.5)
Kerb	174 (398)	54(257.3)	303 (191)	26 (53.3)
p-value	0.0005	0.0001	0.0140	0.0942
	----------Blueberry biomass kg/ha----------			
Control	1,602 (1216)	6,252 (1452)	2,913 (1030)	3,331 (977)
Kerb	3,364 (1216)	3,364 (1452)	3,570 (1030)	4,144 (977)
p-value	0.0021	0.0672	0.2272	0.0838
	----------Red sorrel biomass kg/ha----------			
Control	1732 (776)	745 (448)	319 (266)	196 (304)
Kerb	549 (776)	357 (448)	294 (266)	144 (304)
p-value	0.0003	0.095	0.8707	0.7569
	----------Other biomass----------			
Control	.	102 (268)	.	270 (429)
Kerb	.	145 (268)	.	128 (429)
p-value	.	0.7013	.	0.5136

() represents standard deviation.

Biomass was collected to determine if the growth of blueberry and red sorrel was affected by Kerb applications. For each year and site where Kerb was applied there tended to be an increase in blueberry biomass and a decrease in red sorrel biomass (Table 2.2) Based on these observations, red sorrel appears to negatively impact blueberry biomass which may decrease the blueberry plant's photosynthetic capabilities and decreases the overall growth and success of the plant.

Visual ground cover ratings were taken to determine what percent of the field was covered by blueberry, red sorrel, other weed species or bare ground. There was a significantly greater percent blueberry ground cover where Kerb was applied for count 1

at Kemptown in 2009 and 2010 and Mt. Thom 2009 (Table 2.3). There was a significantly greater percent blueberry ground cover in Kerb applied plots for rating 2 in Kemptown 2010. The percent red sorrel ground cover was significantly greater in control plots except for Mt. Thom rating 1, 2010 and rating 2, 2009 and 2010.

Table 2.3 Percent ground cover of blueberry and red sorrel in two Nova Scotia fields on June 19 2009 for rating one and July 30, 2010 for rating 2.

Site	Kemptown		Mt. Thom	
	2009	2010	2009	2010
Rating 1	---------- **% Blueberry cover**----------			
Control	40 (24.6)	64 (16.1)	64 (14.9)	75 (10.6)
Kerb	85 (24.6)	90 (16.1)	81 (14.9)	84 (10.6)
P-value	0.0182	<0.001	0.0175	0.0615
	---------- **% Red sorrel cover**----------			
Control	60 (24.6)	36 (16.4)	31 (13.4)	14 (8.4)
Kerb	15 (24.6)	9 (16.4)	10 (13.4)	8 (8.4)
P-value	0.0182	<0.001	0.0005	0.1405
Rating 2	---------- **% Blueberry cover**----------			
Control	.	68 (12.2)	58 (10.1)	81 (8.5)
Kerb	.	86 (12.2)	59 (10.1)	86 (8.5)
P-value	.	0.0017	0.8244	0.0862
	---------- **% Red sorrel cover**----------			
Control	.	23 (8.5)	34 (8.5)	8 (5.5)
Kerb	.	10 (8.5)	26 (8.5)	4 (5.5)
P-value	.	0.0004	0.0651	0.0891

No data collected where ‘ .’ () represents standard deviation.

It is possible that the blueberry plant provided shelter for young sorrel ramets when the plant was increasing its growth and nutrient uptake. Sorrel is found underneath the blueberry canopy at the beginning of the season but by mid season during berry set sorrel is taller than the crop. The crop evidently also allows an adequate amount of sunlight for the weed to continue to photosynthesize. Sorrel may take advantage of the blueberry crop by coexisting beneath the crop and then increases its biomass at floral initiation. Blueberry stem height did not differ following Kerb applications but blueberry plant cover and leaf area decreased with increased density of red sorrel ramets. Therefore red

sorrel has a negative impact on the photosynthetic capabilities of the blueberry plant and decreased blueberry biomass. Data collected in chapter 3 also showed that there were fewer blueberry stems where sorrel was growing. Although sorrel reduced blueberry plant density it is not clear how red sorrel decreased blueberry plant biomass. Further studies are needed to determine what factors are responsible.

Floral buds per stem were counted to determine the yield potential for the following year. In 2009, Kerb applications at Mt. Thom resulted in a 32 % blueberry yield increase but this was not significant at $P = 0.0611$ (Table 2.4). In 2010 at Kemptown, Kerb applications resulted in a 16 % increase in blueberry yield and this was significant ($P < 0.0001$). Kemptown clearly had a greater harvestable yield due to the two applications of Kerb compared to Mt. Thom which received only one application. These yield increases can be attributed to the application of Kerb which reduced the density of red sorrel. Red sorrel biomass was much reduced in the Kerb plots evidently allowing more light to reach the blueberry plants resulting in more floral buds and more berries. The yield differences between sites may be attributed to many variables including the tree cover and well sheltered field at Kemptown compared to the Mt. Thom site which is located on the top of a hill and it is open to the elements, differences in blueberry clones, or management practices.

The labeled application rate of Kerb per hectare is 3.25-4.50 kg/ha and the application costs are approximately $500/ha when applied at full rate (Burgess, personal communication 2011). In 2010 growers received between $1.32/kg ($0.60/lb) and $1.43/kg ($0.65/lb) for blueberries (Burgess, personal communication 2011). At Mt. Thom in 2009, the control plots yielded a profit of $7,290.36/ha whereas Kerb plots

obtained a profit of $10,247/ha. At Kemptwon in 2010, the control plots yielded a profit of $20,915.40/ha while the Kerb plots yielded a profit of $23,762/ha. The differences in yield between the two sites was considerable and this can be possibly attributed to many factors including how long the fields have been in production, weather conditions, pollination factors, and previous herbicide and fertilizer applications. Additionally, yields at Kemptown were unreasonably high and are likely due to experimental error. Kemptown yields were consistently too high and the difference between treatments is likely real and therefore valid.

Table 2.4 Blueberry yield and profit after applications of Kerb in the fall and sprout year at two crop year fields in Nova Scotia.

Site	Year	Control	Kerb	p-value
		---------Blueberry yield kg/ha------		
Kemptown	2010	15,845 (8248)	18,790 (8248)	0.0001
Mt. Thom	2009	5,523 (2630)	8,157 (2630)	0.0611
		----------Profit $/ha----------		
Kemptown	2010	20,915	23,762[1]	
Mt. Thom	2009	7,290	10,247[1]	

[1] Based on the blueberry price growers received in 2010 $1.32/kg, Kerb application at full rate $500 /ha and labor costs $20/ha the profit value was calculated.
() represents standard deviation.

2.5 Conclusions

Kerb applications did not affect blueberry plant height or floral buds. However, two applications of Kerb decreased the above ground shoot growth of red sorrel. The double application of Kerb decreased the growth of red sorrel until the end of July as observed from the herbicide ratings. A double application of Kerb is not a common practice in the blueberry field due to cost effectiveness however; removal of red sorrel with Kerb increased the blueberry yield at both sites substantially but a double application had no difference than one application.

Chapter 3 The Impact of Red Sorrel (*Rumex acetosella* L.) on Honey Bee (*Apis mellifera* L.) Activity and *Botrytis cinerea* Spore Germination in a Wild Blueberry Field

3.1 Introduction

Wild blueberry production is one of Nova Scotia's largest agricultural industries. In 2010, the wild blueberry industry produced 16, 500 tonnes of blueberries and contributed over $13 million dollars to the annual farm-value in Nova Scotia (Stats Canada 2010). Blueberry fields are managed on a 2-year production cycle where they are pruned biannually by burning or mowing and harvested every alternate year. Flowering occurs in mid spring from May to June and pollinators are introduced to the blueberry fields at this time to ensure adequate fruit set. Blueberry flowers have limited pollen present in their anthers, and nearly 45% of blueberry plants produce little or no pollen, thereby limiting the pollen available for pollination (Chiasson and Argall 1996).

Red sorrel (*Rumex acetosella* L.) is a creeping perennial herb that has become increasingly prolific in wild blueberry (*Vaccinium angustifolium* Ait.) fields in Atlantic Canada. Weed surveys taken in 1984-1985 and 2000-2001in commercial blueberry fields suggest that there has been an increase in red sorrel abundance from 85 to 90 percent (McCully et al. 1991, Jensen and Sampson unpublished data). In 2000 and 2001, red sorrel was the most abundant of 125 weed species found in Nova Scotia blueberry fields (Jensen and Sampson 2001, unpublished data).

Red sorrel is dioecious and flowers in early spring (Uva et al. 1997). Little is known about the timing of sorrel flowering but visual observations by growers suggest that it flowers at a similar time as the blueberry. During flowering male plants produce copious amounts of wind dispersed pollen which can coat surrounding vegetation. Sorrel

also reproduces vegetatively via creeping roots that produce buds at random intervals along its length (Putwin et al. 1968). Emerging ramets predominately grow beneath the blueberry canopy and then overtop the crop when flowering. This growth habit complicates weed control since chemical or physical means are limited as they may injure the crop.

Leaf and floral diseases are another common problem of wild blueberry fields. Growers regularly spray fungicides in the spring to decrease the rate of disease development. Botrytis blight, caused by *Botrytis cinerea* Pers.:Fr, is an airborne plant pathogen with a necrotrophic lifestyle which attacks over 200 crop hosts worldwide (Williamson et al. 2007). The fungus is common in blueberry fields especially near coastal areas where high humidity and fog are more common (Hildebrand et al. 2001). Botrytis blight spores overwinter on plant debris as dormant mycelia and sclerotia (Lambert 1990). New infections begin in the spring as soon as weather conditions are favorable for disease development. Once the fungus has established on early flowering clones the infected tissue serves as a source for spores to infect later flowering clones (Hildebrand, personal communication 2010). Blueberry flowers become susceptible to blight just prior to opening (Delbridge et al. 2007) with infection primarily occurring on expanded corollas (Hildebrand et al. 2001) but may also occur on leaves, blossoms, fruit (Kinsmen 1993) and even may work its way down the stem. Infection is most likely to occur when the blueberry crop is around 50 % bloom and the fungus continues to infect as flowers become mature (Hildebrand, personal communication 2010). A few days after infection, blossoms turn light brown and develop a greyish-brown mold which is easy to see under low magnification (Bell et al.1999). Outbreaks of Botrytis blight are infrequent

but can be severe (Hildebrand et al. 2001), with losses of 30-35 % having been recorded in wild blueberries in Nova Scotia (Delbridge and Hildebrand 1995). Pollen from multiple flowering species can stimulate spore germination and germ tube growth of *B. cinerea* (Chu and Preece 1968; Fourie and Holz 1998). It is unknown if spores of *B. cinerea* in the presence of red sorrel pollen are stimulated to germinate and infect wild blueberry flowers.

Cross-pollination by insects is essential to maximize wild blueberry fruit set and yield (Drummond and Yarborough 2002). Cross pollination by honey bees in wild blueberry fields increases fruit size (Drummond and Yarborough 2002). Honey bees (*Apis millifera* L.) as well as alfalfa leafcutter bees (*Megachile rotundata* F.), and bumble bees (*Bombus impatiens* Cr.) are commonly used pollinators in many crops including wild blueberries. Growers rely predominately on honey bees in the Atlantic region. For adequate pollination of wild blueberry flowers 2.5-5 hives per hectare are recommended (Aras et al. 1996). Given that red sorrel produces copious amounts of pollen, it has the potential to impact pollinator activity and distract honey bees from pollinating blueberry flowers.

The possibility of important ecological interactions between wild blueberries, red sorrel, *B. cinerea*, and honey bees has not been previously studied. Potential interactions include the overlap of red sorrel and blueberry flowering that may result in preferential selection of red sorrel flowers by honey bees and, red sorrel pollen functioning as a nutrient source for spores of *B. cinerea* leading to an increased severity of blight. Thus in this study I hope to determine: 1) if blueberry flowering coincides with red sorrel flowering, 2) the amount of red sorrel pollen that is naturally transferred on to blueberry

flowers in a fruiting field, 3) if red sorrel pollen enhances *in vitro* spore germination of *B. cinerea,* 4) if disease caused by *B. cinerea* is increased on immature blueberry flowers in the presence of red sorrel pollen, and 5) if red sorrel presence has an effect on blueberry pollinator activity.

3.2 Materials & Methods

3.2.1 Study Sites

Experiments were set up in two fruiting fields in 2009 and two different fruiting fields in 2010 in Debert, NS ($45^0$25' N, $63^0$30' W) and Collingwood, NS ($45^0$33' N, $63^0$51' W). All fields had dense blueberry and red sorrel cover and honey bee hives were placed around the field perimeter or adjacent to the fields. In each field, five 1 m^2 quadrats were placed in areas with dense red sorrel and blueberry coverage and five quadrats were placed in areas with dense blueberry coverage but no sorrel.

3.2.2 Timing of Blueberry & Red Sorrel Flowering and Sorrel Pollen Release

At each site on May 26, 2009 and June 15, 2010 red sorrel and blueberry densities were counted in ¼ of all 1 m^2 quadrats. An 'x'pattern in each quadrat was used to randomly select twenty blueberry stems which were tagged with twist ties and water proof tags placed at the bottom of each stem. Closed and open flower buds per stem were counted in Debert on May 27, 29, 31, and June 3, and 9 in 2009. The same data were collected in Collingwood on May 26, and June 1, 5, 9, in 2009. In 2010, counts at Debert occurred on May 19, 23, 26 and June 4, 10, 15, 18 and counts at Collingwood occurred on May 19, 23, 26 and June 2, 4, 8, 15, and 18.

Male red sorrel and wild blueberry flowers were collected at two sites in 2009 and 2010 by walking in 3 separate zig zag formations across the fields and collecting plant samples every five steps to ensure collection of a representative sample. Each zig zag formation was considered a replicate. In 2009, red sorrel samples were collected in Debert on June 3, 9, 13, 15, 22, 26 and on July 1, 6, 16, 19, 24 and blueberry flowers were collected on each date until July 1st. In Collingwood red sorrel samples were collected on June 3, 9, 13, 15, 22, 26 and July 1, 6, 16, 24, blueberry flowers were collected until July 1st. In 2010 red sorrel samples were collected at Debert on May 26 and 28 and June 1, 4, 5, 10, 17, 23, 25 and 30, blueberry flowers were collected on all days until June 20th. At Collingwood red sorrel samples were collected on June 1, 4, 8, 15, 17, 23, 25 and 30, blueberry flowers were collected until June 20th. A total of twenty male red sorrel stems per replication per sample date and site were randomly collected by walking in zig zag formation and collecting a stem every five steps. Stems were cut off at ground level and were brought back to the lab and the number of opened, closed and spent flowers (flowers with open or released stamens) was recorded to determine the flowering stage. The red sorrel stems were placed into a flask with 30 mL of distilled water and the solution was shaken for 1 minute. From each sample, five subsamples of 1 mL were taken to determine the number of pollen grains released using a haemocytometer (Hildebrand et al. 2001). The pollen grains per mL of solution were estimated to determine the potential number of sorrel pollen being released in the field at each sampling date. Blueberry flowers were also collected on the same dates as the sorrel flowers in 2009 and 2010 near areas with red sorrel flowers. Forceps were used to randomly collect 50 blueberry flowers per sample from 25 stems. Flowers were then

brought back to the laboratory in flasks, where 10 mL of distilled water was added to each flask and shaken for 1 minute. From each sample, five subsamples of 1 mL were taken and any pollen grains present were counted microscopically with the help of a haemocytometer (Hildebrand et al. 2001). The number of pollen grains per flower was estimated to determine if red sorrel pollen is transferred to blueberry flowers in commercial fields.

3.2.3 Impact of Red Sorrel Density on Pollinator Activity

In 2009 honey bee activity was observed in the same fields at Collingwood and Debert used for the evaluation of floral timing of blueberry and red sorrel. Bees were observed at Debert on May 29, 31 and on June 1, 3, 15 and at Collingwood on June 5, 9, 13, 16, 22 until colonies were removed from the fields. In 2010, weather conditions were unfavorable for bee activity with above normal precipitation, thus honey bees were observed only twice at Debert. No observations occurred at Collingwood as the bee hives were removed by the producer without notice. The pollinator observations were a complete randomized design. Honey bees were monitored in ten 1 m^2 quadrats at each site, with five quadrats with red sorrel and five without red sorrel. Collection periods coincided with placement of bee hives in blueberry fields by growers and continued until bees were removed in 2009. The number of bees that entered each quadrat over 60 seconds and a bee's position on blueberry or red sorrel flowers was recorded (Javorek et al. 2002). Six bees at each quadrat were also followed for 3 minutes and time spent on red sorrel versus blueberry plants was recorded.

3.2.4 Effect of Red Sorrel Pollen on *B. cinerea* Spore Germination and Floral Infection

The experiment was completed in the summer of 2009 and consisted of three replications of eleven treatments. *B. cinerea* spores were collected and co-incubated with increasing concentrations of red sorrel pollen in water droplets and microscopically examined for spore germination. Red sorrel flowers near maturity were collected from crop year fields in July 2009 and were brought to the lab where they were put into a flask of water to allow the flowers to open for 2 to 4 days. When flowers began releasing pollen, the pollen was shaken off and collected into a vial where it was allowed to dry for 2-3 days. The pollen was then put into a Mason jar containing a small amount of Dri-rite (a desiccant made from calcium sulfate) and stored at 4° C for 4-6 weeks.

An isolate of *B. cinerea* (B-94) was grown on *Pseudomonas* Agar F in Petri dishes supplemented with glucose (Hildebrand et al. 2001) for one week at 22°C in the laboratory. This medium produced abundant spores in 5 to 7 days. Spores from 2-3 Petri dishes were aspirated into distilled water with a vacuum device and the resulting heavy suspension was shaken vigorously to break up clumps of spores. Spores were then filtered through several layers of sterile cheesecloth. The stock suspension of *B. cinerea* spores was prepared by diluting the suspension to 300, 000 spores mL^{-1} with the aid of a haemocytometer. A stock suspension of red sorrel pollen was also prepared to a concentration of 32, 000 grains mL^{-1}. A dilution series of 11 red sorrel pollen concentrations (16, 000, 8,000, 4,000, 2,000, 1,000, 500, 250, 125, 62.5, 31.5 grains mL^{-1}) and a constant spore concentration of *B. cinerea* (150, 000 spores mL^{-1}) was prepared. For the dilution series, 0.5 mL of spore suspension was pipetted into 11 micro

centrifuge tubes. Then 0.5 mL of the red sorrel solution was pipetted into tube number one, then 0.5mL from tube one was pipetted into tube two and so on until all eleven tubes were filled. Three 0.75µL droplets of each spore/pollen suspension and a control (spores but no pollen) were transferred to plastic Petri dishes and incubated at 22°C for 20 hours. The dishes were then frozen to stop germination and subsequently thawed to microscopically assess incidence of spore germination. A cover slip was placed over each droplet and up to 100 spores were counted to obtain percentage of germinated spores.

3.2.4.2 Effect of Red Sorrel Pollen on Infection of Wild Blueberry Flowers by *B. cinerea*

The experiment was a 2 x 2 factorial with four greenhouse grown blueberry plants per treatment with 3 replications. Factor one was the presence or absence of red sorrel pollen grains and factor two was floral stage (F4-immature and F7-mature) (Hildebrand et al. 2001). The plants were removed from cold storage and allowed to develop in a greenhouse to the F4 flower stage (corolla beginning to protrude from the calyx) and to the F7 flower stage (full bloom) (Hildebrand et al. 2001). Two replications of the experiment were completed in June of 2009 and one replication in September 2010 at the Atlantic Food & Horticulture Research Centre in Kentville, NS. Blueberry plants for this experiment were purchased from Briar Patch Nursery (South Berwick NS) in late winter and kept dormant in cold storage until needed. A stock suspension of *B. cinerea* spores at 200,000 spores per mL^{-1} and a stock suspension of red sorrel pollen at 16, 000 grains mL^{-1} were prepared. The spore and pollen suspensions were then mixed 1:1 to obtain suspensions of 100,000 spores mL^{-1} and 8,000 grains mL^{-1}. A suspension of 100,000 spores mL^{-1} (200,000 spores diluted 1:1 with water) served as a control.

Approximately 4 mL of spore/pollen suspension was sprayed onto each plant using an atomizer. The plants then were incubated at 20°C in darkness in a moist chamber for 72 hours to encourage spore germination and infection. Flowers were then assessed for incidence of disease. Flowers were scored by presence or absence of disease and flowers showing any sign of brown discoloration were scored as diseased those without brown were not diseased.

3.3 Statistical Methods

Analysis of variance (ANOVA) with a least square means comparison was done to determine if red sorrel pollen had an effect on the germination of *B. cinerea* spores and infection on blueberry flowers (SAS Institute 2003). The response of spore germination versus pollen concentration was modeled using nonlinear regression in Sigma Plot. The data were fitted to a 3 parameter exponential rise to maximum nonlinear regression curve. Blueberry density in plots with and without sorrel was compared using a paired t-test. Bee counts within quadrats were analyzed using Proc Mixed with repeated measures with least square means comparison (SAS Institute 2003). The level of significance was set at $P < 0.05$ and the data were tested for normality and constant variance. Data were transformed when necessary to achieve normality and constant variance.

3.4 Results and Discussion

3.4.1 Timing of Blueberry and Red Sorrel Flowering and Sorrel Pollen Release

Blueberry flowering occurred at similar times in both years but in 2009 on the first sample day at Collingwood 65 % of blueberry flowers were open while at Debert

only 30 % of the flowers were open (Figure 3.1). Differences between sites may be attributed to the variability in blueberry clones present in each field. Peak blueberry bloom could not be determined in 2009 but in 2010 peak bloom occurred at approximately day 170 at both sites. Red sorrel flowering occurred over a wider time frame without a distinct peak.

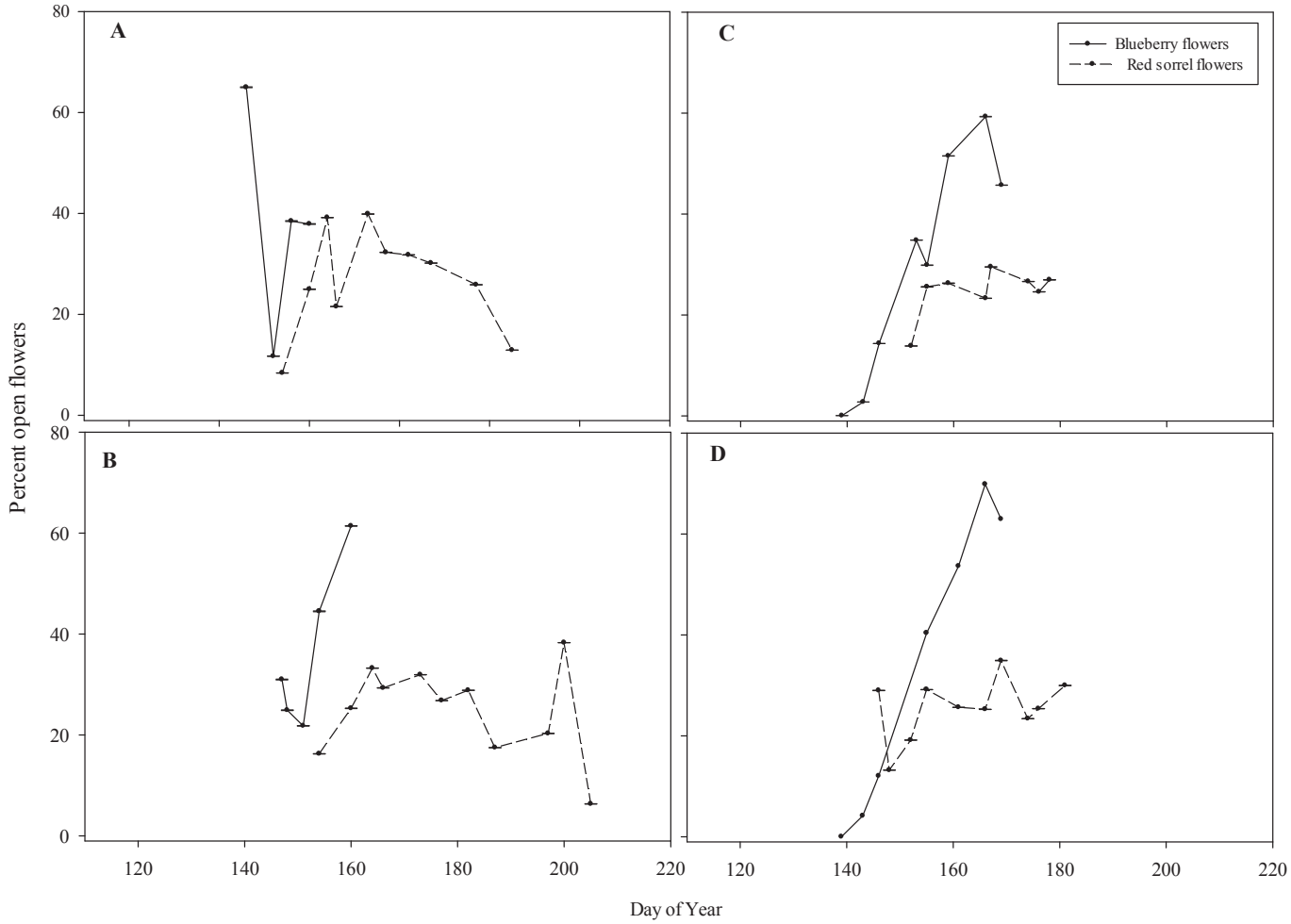

Figure 3.1 Percent open blueberry and red sorrel flowers in blueberry fields in A) Collingwood 2009 B) Debert 2009 C) Collingwood 2010 and D) Debert 2010. Error bars represent standard errors.

Blueberry and male red sorrel flowering overlapped at both sites and in both years (Figure 3.1). In 2009, at Debert and Collingwood I observed an overlap in flowering from day 154 (June 3) to day 160 (June 9) and the overlap extended beyond this point (Figure 3.1 A & B) due to incomplete data. In 2010, I observed an overlap from day 145 (May 25) to day 170 (June 19) (Figure 3.1 C & D).

At both sites and in both years there was a similar, gradual red sorrel pollen release pattern with flowers open on approximately day 150 in both years and dropping off in 2009 at approximately day 210 (Figure 3.2). Complete sorrel pollen release data from 2010 was not obtained due to time constraints. Pollen release began around day 160 at both sites and years and continued throughout the measurement period. The extended release period increased the likelihood of overlap with blueberry bloom. Sorrel pollen release timing and level varied between years but was relatively consistent across sites. When comparing the blueberry flowering lifecycle (Figure 3.1 A-D) to the red sorrel pollen release (Figure 3.2 A & B) I conclude that in both years red sorrel pollen was dispersed when blueberry flowers were open.

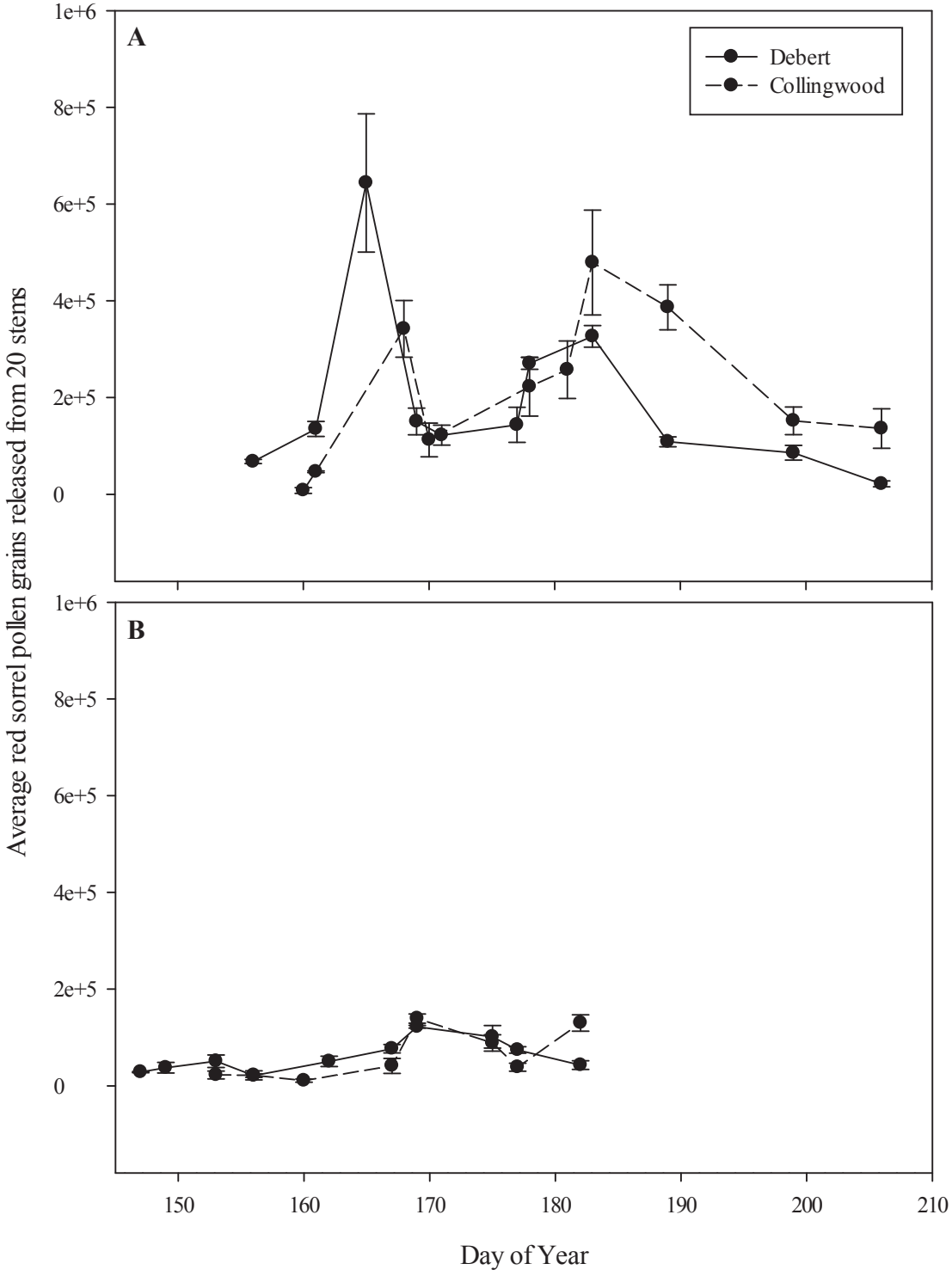

Figure 3.2 Red sorrel pollen grain release from 20 red sorrel stems in A) 2009 and B) 2010 in two Nova Scotia blueberry fields. Error bars represent standard errors.

Red sorrel pollen grains were also removed from blueberry flowers with 100 - 900 sorrel pollen grains per mL in 2009 (Figure 3.3 A) and 250 - 2300 grains per mL in 2010 (Figure 3.3 B). Sorrel pollen release patterns were similar in 2010 but in 2009 very little pollen was removed from blueberry blossoms collected in Collingwood (Figure 3.2 A). When comparing the time frame between days 150 to 170 in Figures 3.2 to Figure 3.3 the patterns are very similar. In 2010 at both sites there was a gradual increase of red sorrel pollen being released as well as an increase on blueberry flowers. It is not clear why in 2009 there was a greater red sorrel pollen release but then less deposition on the blueberry flowers. This may have been due to rainfall events throughout the bloom period washing pollen grains from the blueberry flowers.

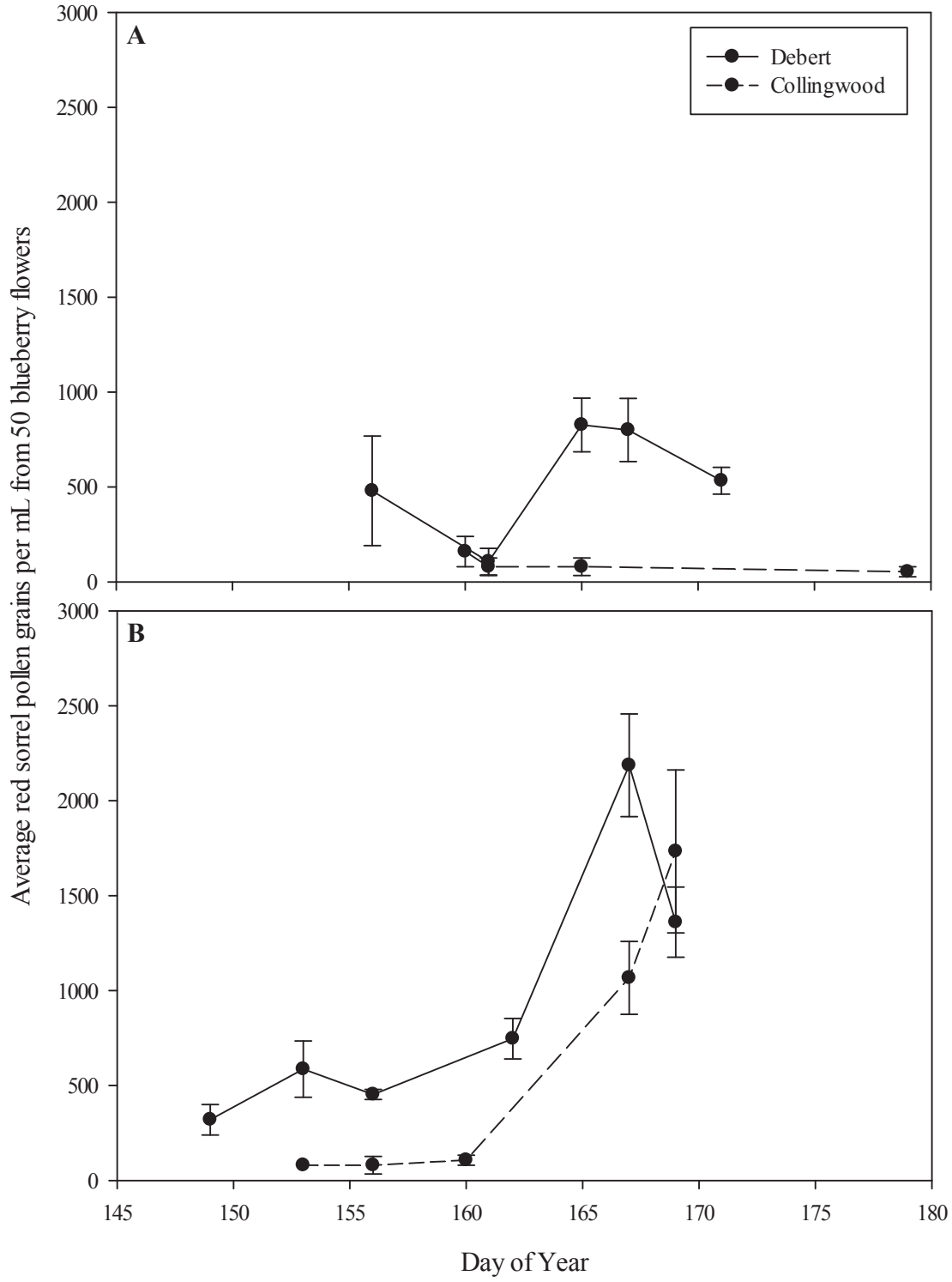

Figure 3.3 Red sorrel pollen grains per 50 blueberry flowers in Nova Scotia in A) 2009 and B) 2010. Error bars represent standard errors.

Nevertheless, these results confirm that red sorrel pollen is present and is dispersed at the same time blueberry plants are flowering, and that sorrel pollen is deposited on blueberry blossoms.

3.4.3 In Vitro Germination of *Botrytis cinerea* Spores and Red Sorrel Pollen

Increasing concentrations of red sorrel pollen suspended with spores of *B. cinerea* in water droplets were found to markedly increase the incidence of germinating spores (Figure 3.4). This relationship was best described by a three parameter single exponential rise to a maximum model in the form:

$$f = y0 + a(1 - \exp(-bx))$$

Where *f* is the estimated percent germination, *y0* is the intercept, *a* is the best fit value, and *b* is the concentration of red sorrel pollen grains. The adjusted R squared value of fit was 0.99 and the root mean squared corrected error was 72.3. As the concentrations of red sorrel pollen increased there was a gradual increase in spore germination in the water droplets to a maximum. For example, there was a 65 % germination of *B. cinerea* spores when a concentration of 2, 500 red sorrel pollen grains per mL was suspended with 150, 000 mL of *B. cinerea* spores and this approached 100 % germination when greater than 9,000 red sorrel pollen grains were used (Figure 3.4). These results are similar to other studies that have found evidence that pollen increases incidence of spore germination of *B. cinerea* (Chu and Preece 1968; Fourie and Holz 1998).

Since red sorrel is abundant in many blueberry fields in Nova Scotia throughout the blueberry bloom period, these results suggest that red sorrel pollen could increase Botrytis blight incidence when weather conditions are appropriate, and with a greater

amount of pollen released there will be a greater probability of infection.

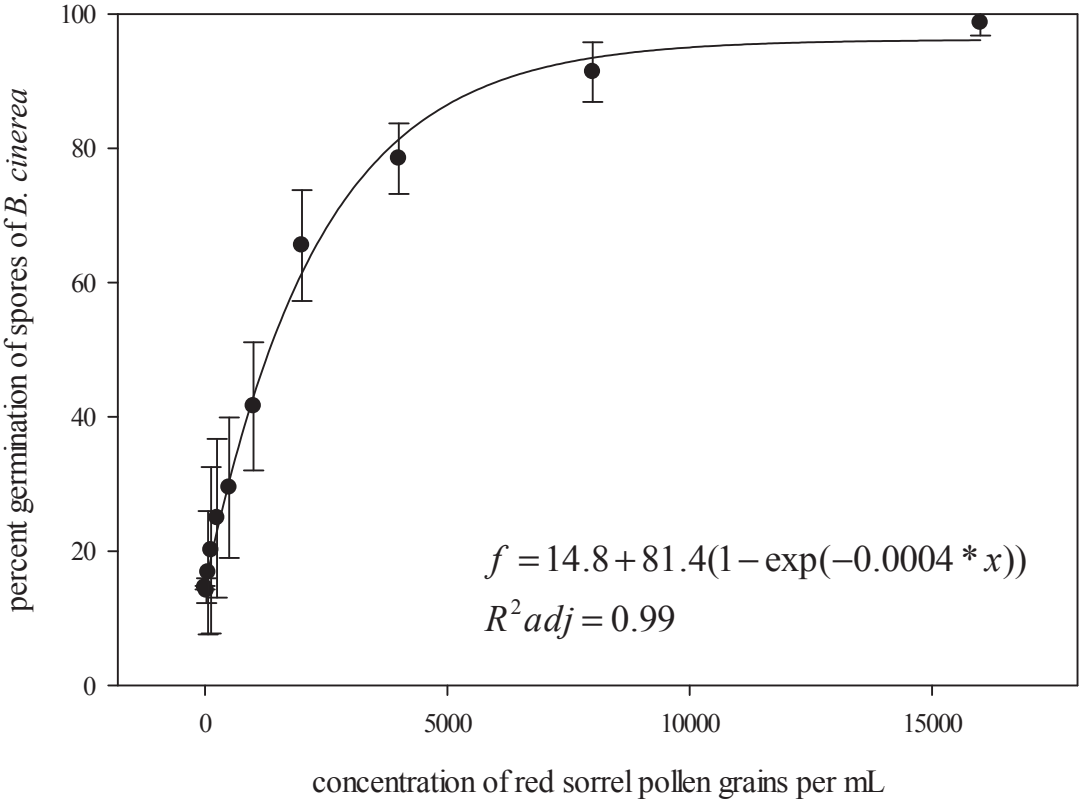

Figure 3.4 Percent germination of *B. cinerea* spores in the presence of increasing concentrations of red sorrel pollen. Error bars represent standard errors.

3.4.4 Infection of Mature and Immature Blueberry Flowers by *B. cinerea* Spores and Red Sorrel Pollen

Floral stage ($P = 0.0001$), presence of sorrel pollen ($P = 0.0214$) and the interaction of the two effects ($P = 0.0400$) significantly increased disease incidence (Figure 3.5). Immature blueberry flowers inoculated with *B. cinerea* spores and red sorrel pollen had a significant increase of disease incidence compared with immature flowers inoculated with just *B. cinerea* spores. Mature blueberry flowers reached a maximum

rate of infection with and without the addition of red sorrel pollen. Optimal conditions in a blueberry field result when *B. cinerea* spores are present, blueberry flowers are at a mature stage and when weather conditions are warm and humid for several consecutive days. Thus, in a blueberry field if *B. cinerea* spores in combination with red sorrel pollen are present under optimal conditions immature blueberry flowers will also have an increased incidence of *B. cinerea* infection.

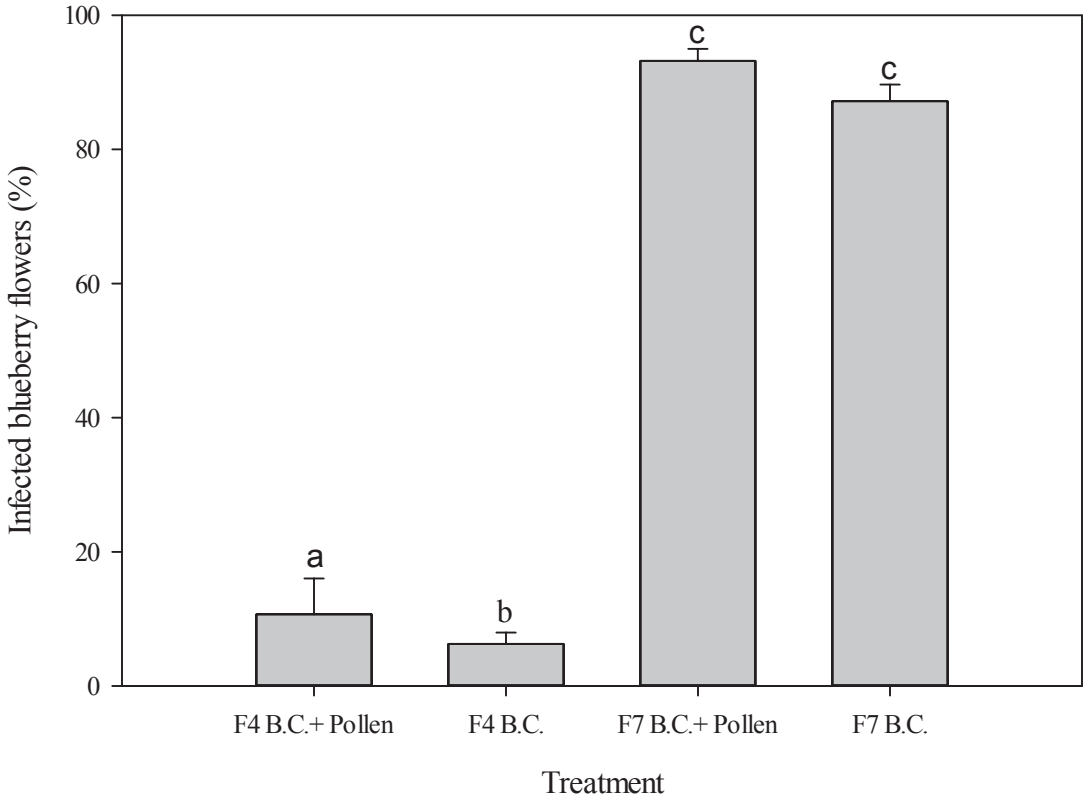

Figure 3.5 Percent infected blueberry flowers following inoculation at the F4 or F7 stage of development with suspensions of *B. cinerea* (B.C.) alone or with red sorrel pollen. Means with the same letter are not significantly different ($P \leq 0.05$). Error bars represent standard errors.

3.4.5 Honey Bee Observations

In 2009, greater honey bee activity was observed at Collingwood than at Debert. This can likely be attributed to differences in the number of hives at each site. For adequate pollination of wild blueberry flowers, 2.5-5 hives per hectare are recommended (Aras et al. 1996). At the Debert site in 2009 a total of four hives used for pollination of approximately four hectares, while at the Collingwood site of approximately 20 hectares, more than 140 hives were located adjacent to the experimental plots. No honey bee activity observations were taken in Debert or Collingwood in 2010 as the hives were relocated to new fields early in the season due to lack of warm sunny weather.

Data analyzed by repeated measures found a positive significant interaction between time and treatment at Collingwood ($P = 0.0006$) however the interaction was not significant at Debert ($P = 0.8958$) (Figure 3.6). In 2009 at Collingwood, on the first and second sample days there were significantly more honey bees observed in plots with red sorrel than in those without red sorrel (Day 156: $P = 0.041$; Day 160: $P = 0.048$). However thereafter, more bees were found in plots without sorrel (Day 167: $P = 0.003$) and this decrease was due almost exclusively to a decrease in the number of bees on red sorrel (Figure 3.6),

At Collingwood and Debert in 2009 more honey bees were observed foraging in plots without red sorrel. At Collingwood there was significantly more honey bees on Day 156: $P = 0.041$; Day 164: $P = 0.048$; and Day 168: $P = 0.0025$). At Debert honey bees were not significantly different in plots with or without sorrel (0.4711) (Figure 3.7). The number of honey bees foraging on blueberry flowers per minute was always greater in plots without red sorrel (Figure 3.7 A and B). This may have occurred due to a greater

number of blueberry flowers. Also honey bees at this time in the season may have been foraging predominately for nectar if stores were low, and since blueberry flowers have more nectar than red sorrel flowers honey bees may prefer the blueberry flowers. Data also showed that when red sorrel is present honey bees made fewer visits to blueberry flowers (Figure 3.6) possible due to the abundance of pollen on sorrel and this could affect the crop.

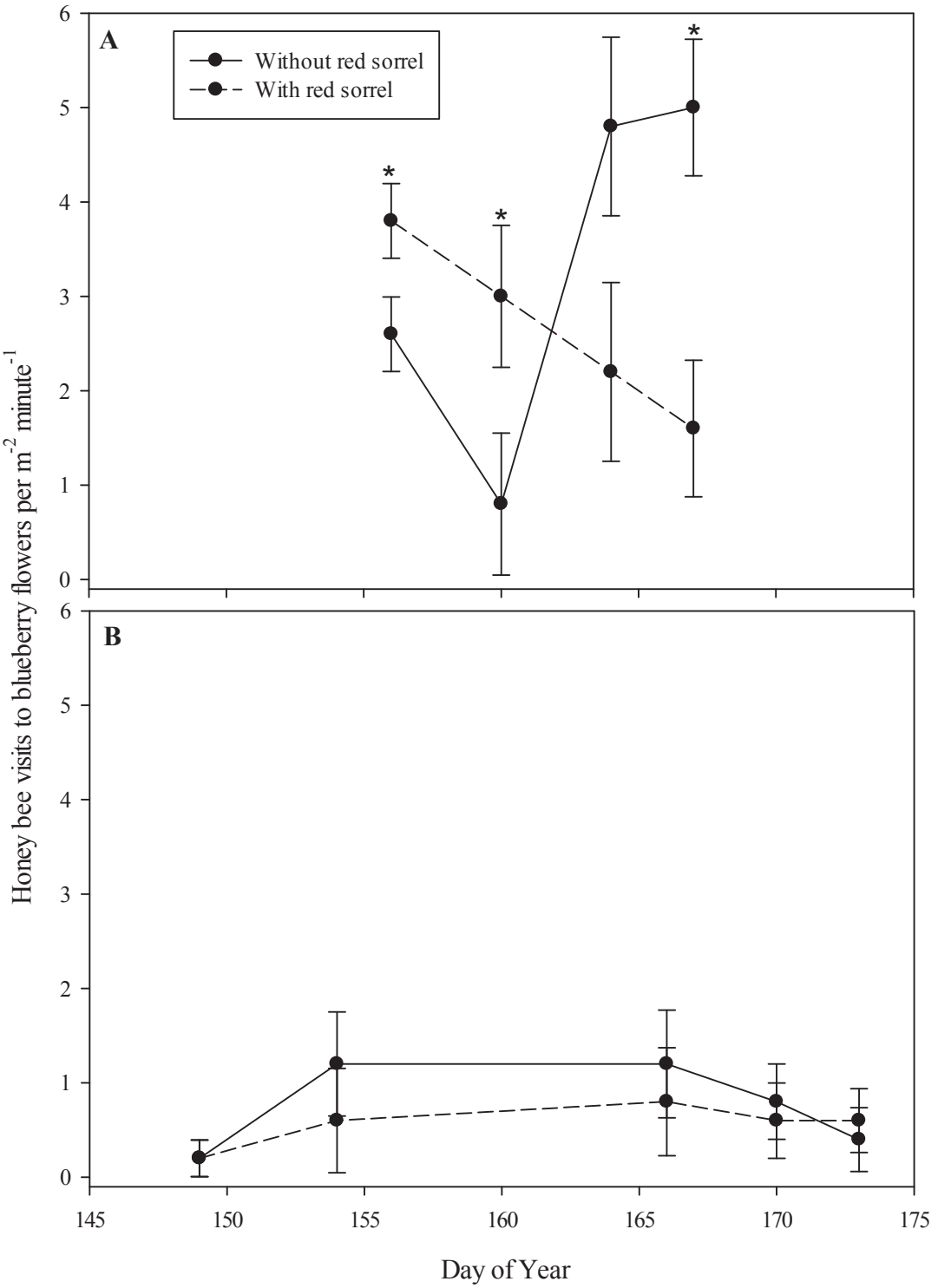

Figure 3.6 Total honey bee visits per minute to 1 m^2 quadrats in wild blueberry fields at A) Collingwood and B) Debert Nova Scotia in 2009, * indicates a significant treatment effect on a given day ($P \leq 0.05$). Error bars represent standard errors.

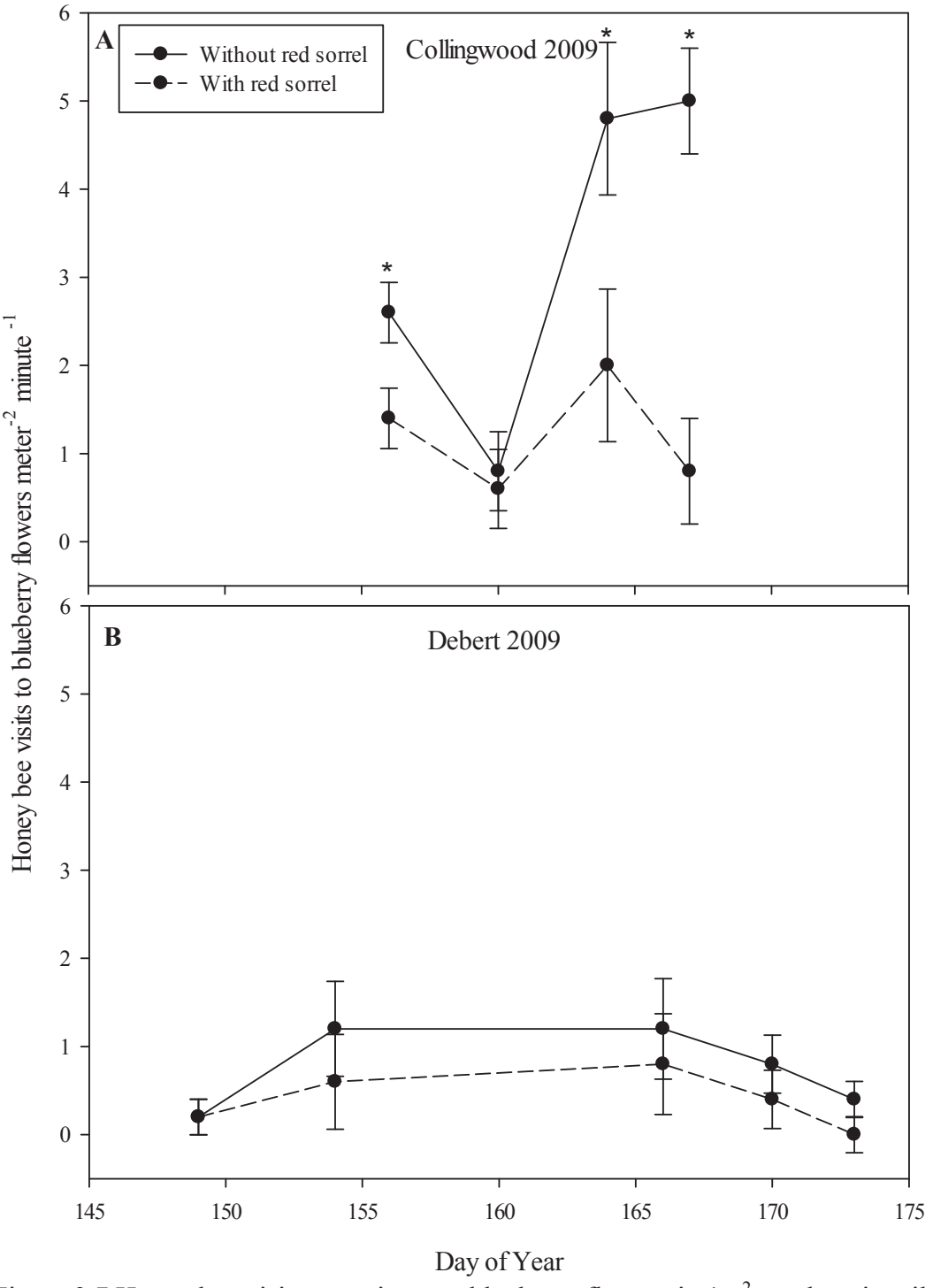

Figure 3.7 Honey bee visits per minute to blueberry flowers in $1m^2$ quadrats in wild blueberry fields at A) Collingwood and B) Debert, Nova Scotia in 2009. * indicates a significant treatment effect on a given day ($P \leq 0.05$). Error bars represent standard errors.

When quadrats with red sorrel were analyzed on their own, the number of honey bees foraging on blueberry versus red sorrel flowers was greater on red sorrel stems than on blueberry stems on Day 156 P = ≤0.001and Day 160 P = ≤0.001 at Collingwood (Figure 3.8 A). After day 162, more honey bees were observed foraging on blueberry flowers, which coincided with a decrease in red sorrel flowers (Figure 3.1 A). At Debert, more honey bees tended to forage on blueberry flowers until day 167. After day 167, there was more honey bees foraging on red sorrel flowers (Figure 3.8 B).

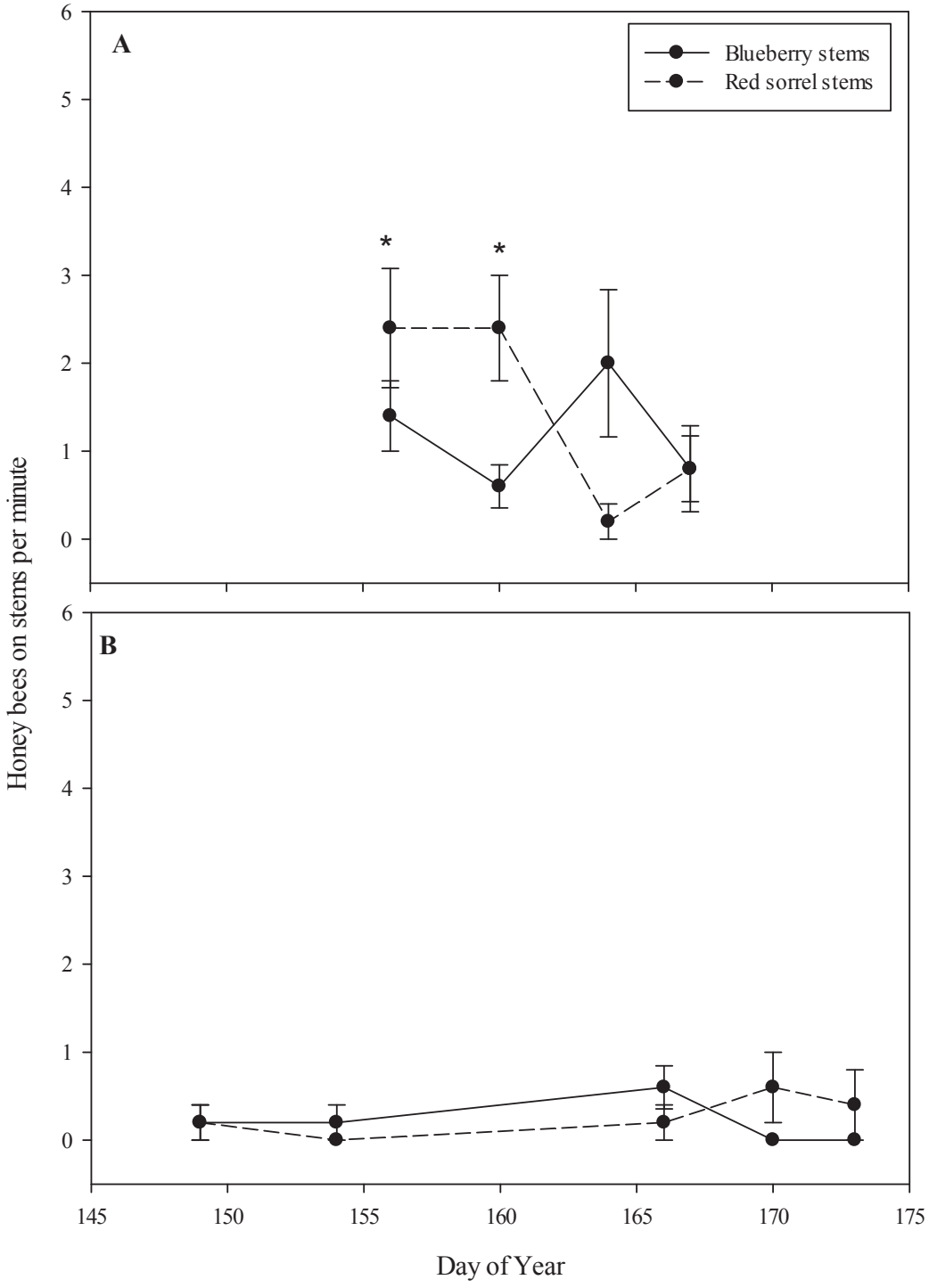

Figure 3.8 Average number of honey bees counted per minute in a $1m^2$ quadrat containing red sorrel, in wild blueberry fields in A) Collingwood and B) Debert, Nova Scotia 2009. * indicates a significant treatment effect on a given day ($P \leq 0.05$). Error bars represent standard errors.

Blueberry stems were counted in ¼ of 1m^2 plots with and without red sorrel where bees were also observed to determine if there was a difference in blueberry densities between the two treatments. Blueberry stem density was lower where red sorrel occurred (Table 3.1). In 2009, at Debert and Collingwood, respectively, there was 35 and 45 % fewer blueberry stems in plots with red sorrel. In 2010 there was a 23 and 32 % decrease in blueberry stems at Debert and Collingwoood, respectively (Table 3.1). Although not significant, at each site and year there were always more blueberry flowers/m^2 in plots without red sorrel. Lack of significance is due to variability of blueberry flowers per quadrat.

Table 3.1 Crop year blueberry stem density counts in plots with and without red sorrel in a Nova Scotia blueberry field.

Site	Debert 2009	Collingwood 2009	Debert 2010	Collingwood 2010
	----------Blueberry stem density #/m^2 ----------			
Without sorrel	532	712	240	240
With sorrel	348	392	184	164
P-value	0.031	≤0.001	0.050	0.001
	----------Blueberry flowers/m^2----------			
Without sorrel	3485	4439	1302	970
With sorrel	1993	2862	847	710
P-value	0.336	0.142	0.153	0.297

A p-value of 0.05 was used for level of significance.

Honey bees remained completely loyal to one flower species at all sites during the three minute observations and did not inter-forage among blueberry, red sorrel or other weed flowers. Free (1970) and Kevan (2007) also noted that honey bee foragers are strictly faithful to one source of pollen, at least on a single trip or a set of foraging trips. However, as blueberry and red sorrel floral timing overlapped honey bees were observed

foraging individually from blueberry and red sorrel flowers in the blueberry field at similar time but in different quadrats. In 2009, at Collingwood once the male red sorrel flowers had opened (on approximately day 150 at each site) there was an increase in honey bees foraging on the sorrel flowers.

In a wild blueberry field Shaw et al. (1954) found that only 4 % of the honey bees observed were collecting pollen from wild blueberry flowers. A study by Lesaffre et al. (1975) found that less than 10 % of the pollen collected by 16 honey bee colonies placed in the centre of a 200-ha blueberry crop in Quebec came from blueberry flowers. It was also found that 50 % of the pollen loads came from *Taxacum officinale* and *Hieracium* spp., while 77 % of honey bees foraging on blueberry flowers collected nectar only and 23% collected pollen (Lesaffre et al.1975). This shows that honey bees are indeed foraging from other resources to obtain food stores. This may occur because blueberry flowers are known to have a small amount of pollen present in their anthers, and nearly 45 % of blueberry plants produce little or no pollen, thereby limiting the pollen available for pollination (Chiasson and Argall 1996). Nectar is more abundant in the blueberry flowers and thus would provide a greater reward for foragers.

During my observational study, honey bees were observed foraging on red sorrel flowers growing in wild blueberry fields. This may suggest that honey bees allocate energy into foraging on resources that are more plentiful and readily available such as red sorrel. Honey bees were observed foraging from male red sorrel flowers but these bees may only be designated to forage for pollen while other bees foraged for nectar supplies as the colony is reliant on both pollen and nectar foragers. If honey bees are in fact foraging for both resources at the same time it would make sense to supply honey

bee colonies upon arriving to the blueberry field with enough pollen stores to ensure foragers will pollinate blueberry flowers and forage for nectar instead of foraging for pollen from red sorrel flowers.

3.5 Conclusions

Blueberry and red sorrel flowering coincided at both sites and years. Results indicate that blueberry and red sorrel flowers are open at similar times in the wild blueberry field although the period of overlap varied with each year. Red sorrel pollen was found on blueberry flowers with the amount of pollen ranging from 100 to 900 grains per mL in 2009 and 250 to 2, 300 grains per mL in 2010. My data confirms that male red sorrel pollen is transferred to blueberry flowers in commercial blueberry fields. In addition, red sorrel pollen enhanced *in vitro* spore germination of *B. cinerea* in Petri dishes. This suggests that sorrel pollen functions as a nutrient source for *B. cinerea* spores. When *B. cinerea* spores and red sorrel pollen suspensions were inoculated on immature and mature blueberry flowers, red sorrel pollen increased the incidence of infected flowers at the immature floral stage (F4) only. Immature blueberry flowers inoculated with *B. cinerea* spores and red sorrel pollen had a significant increase in disease incidence compared with immature flowers inoculated with just *B. cinerea* spores. Honey bees were found foraging from red sorrel flowers when blueberry flowers were in bloom. Honey bees preferred plots without sorrel but moved to other areas including red sorrel plots once the preferred flowers were visited. A greater number of honey bees were observed foraging in plots with red sorrel flowers than from blueberry flowers in 2009 at Collingwood. This occurred when there was a large amount of red

sorrel flowers open and once red sorrel flowering decreased honey bees shifted back to foraging on blueberry flowers.

Chapter 4 Conclusions

4.1 Overall Conclusions

Red sorrel decreases blueberry yields and interferes with harvest practices once established in the fruiting year (Kennedy 2009, Kennedy et al. 2011). Kerb applications did not affect blueberry plant heights or floral bud numbers. However, two applications of Kerb decreased red sorrel above ground shoot growth and ramet density. A double application of Kerb is not a common practice for blueberry growers but removal of red sorrel with Kerb increased the blueberry yield. However a double application did not prove to have an increased yield benefit compared to one application. In Kerb applied plots at both sites the blueberry profit was higher than in the control plots. Differences in profit between the two sites that were studied can possibly be attributed to many factors including the different year, locations, how long the fields have been in production, weather conditions, pollination, and previous herbicide and fertilizer applications. As well, blueberry yields were obtained from 3 or 5 square meter quadrats in each plot, so it is possible that these were areas with unusually dense blueberry cover.

We conclude that blueberry and male red sorrel flowering overlaps. Our results indicate that red sorrel flowering occurs over a wider time frame than blueberry without a distinct peak. Once sorrel is mature, bolting stems may negatively affect the crop by competing for light thereby reducing photosynthesis of the blueberry leaves and ability to produce flowers or vegetative buds. Red sorrel may also compete for nutrients and the bolting stems tend to lie on top of the crop making harvesting the berries a challenge. Red sorrel pollen was dispersed within the blueberry field and pollen grains were transferred on to blueberry flowers.

Male red sorrel flowers do not all flower at the same time. The wide pollen release time frame likely ensures successful pollination of female flowers. It also gives honey bees a longer time to forage for red sorrel pollen after they have ceased foraging for nectar from blueberry flowers. Apple (*Mallus* Mill) and big leaf lupine (*Lupinus polyphullus* Lindl) have also been shown to have slight floral overlap (Sheffield et al. 2008). Apples opened prior to lupins and overlap occurred for approximately 2 weeks, with lupin flowering continuing for almost 4 weeks beyond the life span of apple flowers. Lupins in this study were used to entice honey bees to forage in the orchards. In my research red sorrel may or may not have the potential to entice honey bees to stay in the surrounding areas of a blooming blueberry field.

Increasing concentrations of red sorrel pollen suspended with spores of *B. cinerea* in water droplets increased the incidence of spore germination markedly. This relationship was best described by a three parameter single exponential rise to a maximum model. Previous studies have demonstrated that pollen stimulated spore germination on broad bean leaves as well as strawberry fruit (Chu Chou & Preece 1968) as the pollen acts as a nutrient source for the spores.

In controlled experiments, red sorrel pollen mixed with *B. cinerea* spores significantly increased disease incidence on immature blueberry flowers compared to mature flowers inoculated with *B. cinerea* only. Mature blueberry flowers reached a maximum rate of infection with and without the addition of red sorrel pollen. If red sorrel is present, there is a good likelihood that pollen transfer onto blueberry flowers will occur. Fields should be monitored for *B. cinerea* spores after wet periods during

blueberry bloom. Typically fields are sprayed with fungicides during blueberry bloom when *B. cinerea* sporulation is detected or when symptoms are visible.

The amount of red sorrel pollen grains sprayed onto the mature and immature blueberry flowers was 8, 000 grains per mL in 4 mL of solution per plant equaling a total of 32,000 red sorrel pollen grains per plant. This contrasts to the amount of sorrel pollen grains found on the blueberry flowers in the field which was at most 700, 000 grains per mL in 2009 and 140, 0000 grains per mL in 2010. Thus the amount of pollen grains found on the blueberry flowers collected in the field well exceeded the amount of pollen grains which were applied to the blueberry flowers in the controlled experiment. Accordingly, the potential for disease may actually be higher in a blueberry field severely infested with red sorrel than what was simulated in the experiments carried out.

Honey bee activity corresponded with flowering and red sorrel pollen release. The average number of honey bees foraging on blueberry flowers in both treatments were compared. In Collingwood honey bees were always found foraging on blueberry. This difference can be attributed at least in part to the increased density of blueberry flowers in these areas. Honey bees were observed to forage from blueberry plants for nectar but once blueberry flowers started to decrease honey bees foraged from red sorrel flowers. In this study the presence of red sorrel did not have a significant impact on foraging honey bees. However, honey bees forage from red sorrel flowers when blueberries were still flowering. Honey bees were loyal to one flower species at a time throughout my study and also seen in many other studies (Free 1963 and Kevan 2007). Honey bees placed in a monoculture crop are driven to forage from the species most plentiful.

From my observations honey bees did forage for pollen when blueberries were in bloom. If a hive was potentially starved of pollen these bees may look for a pollen source only. From a study by Camazine (unpublished) a colony can adaptively modulate its pollen collection so that it maintains an appropriate reserve of pollen inside the hive. So realistically a hive will always maintain smaller pollen reserves than nectar. In a perfect situation one would expect to have more blueberry flowers in a field than red sorrel and so there should not be a problem. However if a hive became deprived of resources it would try to fill the reserves even if the flowers of the opposite resource are plentiful. A colony stops gathering nectar only after it has completely filled its combs with honey, and stops collecting pollen as soon as it has accumulated a modest reserve (Seeley 1996).

Weed species in a horticulture crop are not always seen as a negative resource in the field. Sheffield et al. (2008) demonstrated that bigleaf lupine *(Lupinus polyphyllus* Lindl.) (Fabaceae) provided a secondary source of food for *Osmia* Panzer (Hymenoptera: Megachilidae) when grown amongst apples *Malus* Mill. (Rosaceae) thus resulting in a larger bee population. Further studies on the percent of honey bees in a colony which forage on blueberry versus red sorrel flowers would be an asset as it is uncertain what percent of honey bees are foraging from other pollen sources and possibly reducing blueberry yield.

4.2 Recommendations

The first step in pest management should always be frequent monitoring of pest pressures. A survey of weed presence in a crop or sprout field is a good first step toward the implementation of an integrated weed management plan. Scouting for weeds may provide some indication for potential disease. The detection of sorrel in fields with a

history of Botrytis blight may also lead to preventive fungicide sprays. By scouting for weeds not only are you compiling a list of weeds present in your fields but you are taking precautions in disease and pest management. By understanding basic information about the weeds growing in your fields such as the name of the weed, when it flowers, when it senesces will help in managing and controlling the weed. Once some basic phenology of the weeds are known herbicides can be chosen more effectively and a management plan for the next few years can be made.

Controlling red sorrel thus far has proven to be a challenge in the wild blueberry field. It is important to treat red sorrel as soon as patches are detected before it becomes established and spreads throughout the entire field. This is important because sorrel decreases blueberry density (Hughes et al. 2012), and reduces stand and yield (Kennedy 2009). Decreasing red sorrel in the blueberry field is not a cheap investment although Kerb and Velpar have been used and have been shown to decrease sorrel density.

Honey bees are a valuable resource in the blueberry crop. It is important that honey bees are used efficiently and adequately to increase fruit set. Honey bees should be placed in the field during early blueberry bloom. We speculate that supplying pollen to the hive may be advantageous to decrease time the bees spend foraging for pollen in the field and increase their demand for nectar and consequently increase pollination success.

4.3 Management recommendations

Year 1(Sprout year field) If dense red sorrel is present in your field my management strategy would be to mow or burn old growth and then apply Kerb in the fall of the sprout year. Though difficult, scouting for *B. cinerea* on red sorrel may give an indication of the potential for disease in the following crop year.

Year 2 (Crop year field) If red sorrel is still present apply Kerb in the fall of the crop year to achieve suppression of red sorrel. Scout for Botrytis blight on blueberry flowers and apply fungicides if needed. When introducing honey bee hives to the blueberry field ensure hives have a good store of pollen in the hive to prevent bees from foraging for pollen stores.

References

Afshar A., O. Bozorg Haddada, M. A. Mariñob, and B. J. Adamsd. 2007. Honey-bee mating optimization (HBMO) algorithm for optimal reservoir operation. Journal of Franklin Insitute 344:452-462.

Alex J. F. 2001. Ontario Weeds: Descriptions, Illustrations and Keys to their Identification. Ontario, Canada: Ontario Ministry of Agriculture, Food and Rural Affairs.

Aras P., D. De Oliveira, and L. Savoie. 1996. Effect of a honey bee (*Hymenoptera: Apidae*) gradient on the pollination and yield of lowbush blueberry. Journal of Economic Entomology 89:1080-1083.

Barthell J.F., J.M. Randall, R.W. Thorp, and A.M. Wenner. 2001. Promotion of seed set in yellow star-thistle by honey bees: Evidence of an invasive mutualism. Ecological Applications. 11:1870-1883.

Bell D. K., D. E. Yarborough, and J. Dill. 1999. Crop Profile for Blueberries (Wild) in Maine. Orono, ME: Wild Blueberry Commission of Maine. [Online] Available:http://www.ipmcenters.org/cropprofiles/docs/meblueberries-wild.pdf

Boulanger L. W., G. W. Wood, E. A. Osgood, and C. O. Dirks. 1967. Native Bees Associated with the Lowbush Blueberry in Maine and Eastern Canada. Orono, ME: Maine Agriculture Experiment Station. Technical Bulletin No. 26.

Boyd N. and S. White. 2009. Impact of wild blueberry harvesters on weed seed dispersal within and between fields. Weed Science 57:541-546.

Boydston R. A., H. Mojtahedi, J. M. Crosslin, R. Brown, and T. Anderson. 2008. Effect of hairy nightshade (*Solanum sarrachoides*) presence on potato nematodes, diseases, and insect pests. Weed Science 56:151-154.

Burt E. O. and N. R. Gerhold. 1970. *Poa annua* control in bermuda turf with kerb. Proceedings of the Southern Weed Science Society 23:122-126.

Butler C. G. 1949. The Honeybee, an Introduction to Her Sense-Physiology and Behaviour. London: Clarendon Press.

Camazine S. 1993. The regulation of pollen foraging by honey bees: How foragers assess the colony's need for pollen. Behavioral Ecology and Sociobiology 32:265-272.

Campana B. J. and F. E. Moeller. 1977. Honey bees: Preference for and nutritive value of pollen from five plant sources. Journal of Economic Entomology 70:39-41.

Chiasson G. and J. Argall. 1996. Pollination of Wild Blueberries. Wild blueberry Fact Sheet Number B.1.0. Fredericton, New Brunswick: New Brunswick Department of Agriculture and Rural Development

Chu Chou C. M. and T. F. Preece. 1968. The effect of pollen grains on infections caused by *Botrytis cinerea* Fr. Annals of Applied Biology 62:22.

Coley-Smith J. R., K. Verhoeff, and W. R. Jarvis. 1980. The Biology of Botrytis. London: Academic Press Inc.

Delbridge R. and P. Hildebrand. 1995. Botrytis Blight of Lowbush Blueberry. Nova Scotia: Province of NS. [Online] Available: http://nsac.ca/wildblue/facts/disease/botrybli.asp

Delbridge R., P. Hildebrand, D. Yarborough, and S. Annis. 2007. Botrytis Blight Control for Wild Blueberries. The University of Maine. Fact sheet No. 212. Orono, ME.

Dow. 2010. Kerb 50WSP. Dow AgroScience Canada Inc. Calgary, Alberta.

Dreller C. and D. R. Tarpy. 2000. Perception of the pollen need by foragers in a honeybee colony. Animal Behaviour 59:91-96.

Drummond F. A., J. Smagula, S. Annis, and D. Yarborough. 2009. Organic Wild Blueberry Production. Maine Agriculture Forest & Experment Station Orono ME. Bulletin 852. 1-49 p.

Drummond F. A. and E. D. Yarborough. 2002. Honey Bees and Wild Blueberry Production. Fact Sheet No.629. [Online] Available: http:www.wildblueberries.maine.edu/FactSheets/

Eaton L. J. 1994. Long-term effects of herbicide and fertilizers on lowbush blueberry growth and production. Canadian Journal of Plant Science 74:341-345.

Escarré & Houssard 1991.The effects of seed weight on growth and competitive ability of *Rumex acetosella* from two successional old-fields. Oecologia. 2:86 236-242.

Elad Y., B. Williamson , P. Tudzynski , and N. Delen. 2007. Botrytis: Biology, Pathology and Control. The Netherlands: Springer. pp. 403.

Esser L. L. 1995. *Rumex acetosella* in: Fire Effects Information System, Index of Species Information. Rocky Mountain Research Station, Fire Sciences Laboratory: U.S. Department of Agriculture, Forest Service.

Finnamore A. T. and M. E. Neary. 1978. Blueberry pollinators of Nova Scotia, with a checklist of blueberry pollinators in eastern Canada and northeastern United States. Annanls of the Entomological Society of Quebec 23:168-181.

Fisher J. D. 1974. Metabolism of the herbicide pronamide in soil. Journal of Agriculture and Food Chemistry Pg. 22.

Fisher R. M., B. J. Sampson, and S. K. Javorek. 1993. Improving Lowbush Blueberry Pollination in Nova Scotia. Nova Scotia: Acadia University.

Fourie J.F. and G. Holz. 1998. Effects of fruit and pollen exudates on growth of *Botrytis cinerea* and infection of plum and nectarine fruit. Plant Disease. 82:165-170.

Free J. B. 1963. The flower constancy of honeybees. Journal of Animal Ecology 32:119-131.

Fujitaka T. and S. Sakai. 2007. Sexual dimorphism in clonal forms and ramet distribution patterns in *Rumex acetosella* (Polygonaceae). Ecological Research 22:248-254.

Gough R. E. and R. F. Korcak. 1995. Blueberries: A Century of Research. Binghamton, NY: Food Products Press.

Goulson D. 2003. Effects of introduced bees on native ecosystems. Annual Review of Ecology, Evolution & Systematics 34:1-26.

Hanley M. E. and D. Goulson. 2003. Introduced weeds pollinated by introduced bees: Cause or effect? Weed Biology & Management 3:204.

Hardison J. 1976. Fire and flame for plant disease control. Annual Review of Phytopathology 14:355-379.

Harris W. 1972. Shading, defoliation, temperature, growth stage, and reduce fertility effects on competition between *Rumex acetosella*, *Trifolium repens*, and *Lolium* (*multifolum X perenne*). New Zealand Journal of Agriculture Research 15:687-705.

Hildebrand E. M. 1946. War on weeds. Science 19:465-492.

Hildebrand P., K. B. McRae, and X. Lu. 2001. Factors affecting flower infection and disease severity of lowbush blueberry by *Botrytis cinerea*. Canadian Journal of Plant Pathology 21:23-32.

Howatt S. 2008. Crop Profile for Wild Blueberry in Canada. Ottawa, Ontario: Pest Management Centre. Agriculture and Agri-Food Canada 1-61 p.

Hughes A., N. Boyd, C. Cutler, and P, Hildebrand. 2012. An Ecological Study on Red Sorrel (*Rumex acetosella* L.) in Wild Blueberry Fields in Nova Scotia. Msc. Thesis dissertation. Truro, NS.: Nova Scotia Agricultural College.71 pp.

Ito M. 1988. Biology of perennial weeds: As a basis for their control. Korean Journal of Weed Science 12:4:309-316.

James R. R. and T. L. Pitts-Singer. 2008. Bee Pollination in Agricultural Ecosystems. New York: Oxford University Press. Pp. 232.

Javorek S. K., K. E. MacKenzie, and S. P. Vander Kloet. 2002. Comparative pollination effectiveness among bees (Hymenoptera: Apoidea) on lowbush blueberry (Ericaceae: *Vaccinium angustifolium*). Annual Entomology Society of America 95:345-351.

Jensen K. I. N. 1985. Weed control in lowbush blueberries in eastern Canada. Acta Horticulturae 165:259-263.

Jensen K. I. N., and D. E. Yarborough. 2004. An overview of weed management in the wild lowbush blueberry-past and present. Small Fruits Review 3:229-255.

Jensen K. I. N. and M. G. Sampson. 2001. Unpublished data. The frequency, field uniformity, density and relative abundance of 125 species recorded in a survey of 128 fruiting fields of lowbush blueberry conducted in July and August of 2000 and 2001.

Jensen K. I. N. and E. G. Specht. 2002. Response of lowbush blueberry (*Vaccinium angustifolium*) to hexazinone applied early in the fruiting year. Canadian Journal of Plant Science 82:781-783.

Kennedy K. 2009. Combined Effect of Fertilizer and Hexazione on Sheep Sorrel (*Rumex acetosella* L.) Population in Lowbush Blueberry Fields. Msc. Thesis dissertation. Truro, NS.: Nova Scotia Agricultural College.116pp.

Kennedy K. J., N. S. Boyd, V. O. Nams, and R. A. Olson. 2011. The impacts of fertilizer and hexazinone on sheep sorrel (*Rumex acetosella*)
growth patterns in lowbush blueberry fields. Weed Science 59:335-340.

Kennedy K. J., N. S. Boyd, and V. O. Nams. 2010. Hexazinone and fertilizer impacts on sheep sorrel (*Rumex acetosella*) in wild blueberry. Weed Science 58:317-322.

Kevan P. G. 2007. Bees: Biology and Management. Ontario, Canada: Enviroquest Ltd.

Kiltz B. F. 1930. Perennial weeds which spread vegetatively. Journal of the American Society of Agronomy 22:216-234.

Kinsman G. 1993. The History of the Lowbush Blueberry Industry in Nova Scotia 1950-1990 . The Nova Scotia Department of Agriculture and Marketing: The Blueberry Producers' Association of Nova Scotia. Pp. 153.

Kinsman G. 1986. The History of the Lowbush Blueberry Industry in Nova Scotia 1880-1950. Truro, NS: Dept. of AgricultureF and Marketing. Pp. 104.

Klein A.M, Vaissière, B.E., Caine, J.H., Steffan-Dewenter, I, Cunningham, S.A. Kremen, C and Tscharnlke, T. 2007. Importance of pollinators in changing landscape for world crops. Proceedings of the Royal Society of Biological Sciences. 274:303-313.

Korpelainen H. 1993. Vegetative growth in *Rumex acetosella* (Polgonaceae) originating from different geographic regions. Plant Systematics and Evolution 118:115-123.

Lambert D. H. 1990. Effects of pruning method on the incidence of mummy berry and other lowbush blueberry diseases. Plant Disease 74:199-201.

Lathrop F. H. 1952. Fighting the Blueberry Fruit Fly in Maine. Maine Agriculture: Experimental Station Bulletin 500. 34 Pp.

Lavalleye M., H. Agamalianm, A. Lange, and R. Brendler. 1969. RH 315 a new herbicide with potential for weed control in lettuce. California Agriculture 23:14-15.

Lee W. R. 1958. Pollination studies in low-bush blueberries. Journal of Economic Entomology 51:544-545.

Lee J. A. 1960. A study of plant competition in relation to development. Evolution 14:18-28.

Lesaffre O., B. Dupont, and J. M. Perron. 1975. Méthode simple pour comparer l'activité la planche de vol de différentes ruches d'abeilles domestiques. Annales de la Societe Entomologique du Quebec 20:180–187.

Levin D. A. and H. W. Kerster. 1969. The dependence of bee mediated pollen and gene dispersal upon plant density. Evolution 23:560-571.

Lomond D. and D. J. Larson. 1983. Honey bees, *Apis mellifera* (Hymenoptera: Apidae), as pollinators of lowbush blueberry, Vaccinium angustifolium, on Newfoundland coastal barrens. The Canadian Entomologist 115:1647-1651.

McCully K., V. Sampson, M. Glen, and A. K. Watson. 1991. Weed survey of Nova Scotia lowbush blueberry (Vaccinium angustifolium) fields. Weed Science 39:180-185.

McGregor S. E. 1976. Insect Pollination of Cultivated Crop Plants: Economics of Plant Pollination. University of Minnesota: Agricultural Research Service, U.S. Dept. of Agriculture, U.S. Govt. Print Office. Pp. 411.

McIssac D. 1997. Growing Wild Lowbush Blueberries in Nova Scotia. Nappan, Nova Scotia: Nova Scotia Department of Agriculture and Marketing. [Online] Available: http://nsac.ca/wildblue/facts/grow.asp

Michener C. D. 2000. The Bees of the World. Baltimore, Maryland: The John Hopkins University Press.

Michener C. D. 1974. The Social Behaviour of Bees: A Comparative Study. University of Minnesota: Belknap Press of Harvard University Press. Pp. 404.

Mohr N. A. and P. G. Kevan. 1987. Pollinators and pollination requirements of lowbush blueberry (Vaccinium angustifolium ait. and V. myrtilloides michx.) and cranberry (V. macrocarpon ait.) in ontario with notes on highbush blueberry (V. corymbosum L.) and lingonberry (V. vitis-ideae L.). Proceedings of Entomological Society of Ontario 118.

Moore J. N. 1993. The blueberry industry of North America. ISHS Acta Horticulturae: V International Symposium on Vaccinium Culture 346:15-26.

Morrissette R., A. Francouer, and J. -M. Perron. 1985. Importance of native Apoidea in the pollination of lowbush blueberries *Vaccinium* spp. in Sagamie, Quebec. Revue d'Entomologie Du Quebec 30:44-53.

Penney B. G. and K. B. McRae. 2000. Herbicidal weed control and crop-year NPK fertilization improves lowbush blueberry (*Vaccinium angustifolium* ait.) production. Canadian Journal of Plant Science 82:351-361.

Putwain P. D., D. Machin, and J. L. Harper. 1968. Studies in the dynamics of plant populations: II. components and regulation of a natural population of *Rumex acetosella* L. J. Ecology 56:421-431.

Robichaud M. J. 2006.Vista on the Agri-Food Industry and the Farm Community. Statistic Canada Catalogue no.21-004-XIE.

Sagili R. R. and P. Pankiw. 2007. Effects of protien-constrained brood food on honey bee (*Apis mellifera* L.) pollen foraging and colony growth. Behavioral Ecology and Sociobiology 61:1471-1478.

Sammataro D. and A. Avitabile. 1998. The Beekeeper's Handbook. Third Edition ed. Ithaca, New York: Cornell University Press. Pp. 190.

Sampson M. G., K. V. McCully , and D. L. Sampson. 1990. Weeds of Eastern Canadian Blueberry Fields. Truro, NS: Nova Scotia Agricultural College Bookstore. Pp.229

SAS Institute. 2003. SAS (Statistical Software). SAS Institute: Cary, North Carolina.

Schmitt J. 1983. Flowering plant density and pollinator visitation in Senecio. Oecologia. 60:97-102.

Seeley T. D., S. Kühnholz, and A. Weidenmüller. 1996. The honey bee's tremble dance stimulates additional bees to function as nectar receiver bees. Behavioral Ecology and Sociobiology 39:419-427.

Shaw F. R., M. Savos , and W. M. Shaw. 1954. Some observations on collecting habits of bees. American Bee Journal 94:442.

Sheffield C. S., P. G. Kevan, and R. F. Smith. 2003. Bee species of Nova Scotia, Canada, with new records and notes on bionomics and floral relations (*Hymenoptera: Apoidea*). Journal of Kansas Entomological Society 76:357-384.

Sheffield C.S., S.M. Westby, R.F. Smith, P.G. Kevan. 2008. Potential of bigleaf lupine for building and sustaining *Osmia lignaria* populations for pollination of Apple. Canadian Entomology 140. 589-599.

Statistics Canada Agriculture Division Crops Section. February 2010. Fruit and Vegetable Production [Online] Available: http://publications.gc.ca/collections/collection_2010/statcan/22-003-X/22-003-x2009002-eng.pdf [April 2010].

Stevens F. L. 1913. The Fungi which Cause Plant Disease. New York, NY.: The MacMillian Company.

Strik B. C. and D. Yarborough. 2005. Blueberry production trends in North America, 1992 to 2003, and predictions for growth. HortTechnology 15:391-398.

USDA. 2012. New England Agricultural Statistics. Maine Wild Blueberries. [Online] Available :http://www.nass.usda.gov/Statistics_by_State/New_England_includes/Publications/blujul08.pdf

Uva R. H., J. C. Neal, and J. M. DiTomaso, eds. 1997. Weeds of the Northeast. Ithaca, NY: Cornell University Press. Pp. 367.

Van Andel J. and J. C. Jager. 1981. Analysis of growth and nutrition of six plant species of woodland clearings. Journal of Ecology 69:871-882.

Vander Kloet, S.P. 1988. The Genus Vaccium in North America. Agriculture Canada Branch. Publication 1828. Ottawa Canada.

Viste K. L., A. J. Crovetti, and B. W. Horrom. 1970. Dimethylpropynylbenzamides: A new group of herbicides. Science, New Series 167:280-281.

Vodolazksky L. E. 1979. Concerning variation of adventive buds and shoots from root-offspring of the herbaceous perennial *Chamaenerion angustifolium* (L.) scop. (Onagraceae). Botanical Journal of the Academy of Science, SSSR, 64:734-739.

Williamson B., B. Tudzynski, P. Tudzynski, and J. A. L. Van kan. 2007. Pathogen profile *Botrytis cinerea*: The cause of grey mould disease. Molecular Plant Pathology 8:561-580.

Wisler G. C. and R. F. Norris. 2005. Interactions between weeds and cultivated plants as related to management of plant pathogens. Weed Science 53:914-917.

Wood G. W. 1969. Evidence of fruit set in lowbush blueberry by using honeybees. HortScience 4:211-212.

Wood G. W. 2004. The wild blueberry industry - past. Small Fruits Review 3:11-18.