Relating nutrient availability to yield: an examination of lowbush blueberry (*Vaccinium anustifolium*) resource limitation in Prince Edward Island

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ABSTRACT

This study investigates the role of nutrient availability within the lowbush blueberry (*Vacciunium Angustifolium*) to add to the current body of knowledge on the role of resource limitation in predicting final yield. The relative concentrations of ten nutrients (N, P, K, Ca, Mg, Fe, B, Cu, Mn, and Zn) are compared to previously established levels and to final yield. The nutrient levels are tracked throughout the growing season across five fields in eastern Prince Edward Island. Four experimental treatments were set up to limit the amount of pollinator exposure in an attempt to compare the nutrient levels and seasonal nutrient changes across a range of yields. Percent fruit set was used as an indication of how successful the treatment plots were, but the observed fruit set did not align with the predicted results. The concentrations of most elements were consistent with at least one of the previously established optimum ranges, while the concentrations of Mg, Cu, and Zn fell outside these ranges. Throughout the growing season the primary macronutrients (N, P, K) all decreased in concentration, along with Cu. The concentrations of secondary macronutrients (Ca, Mg) all increased from the sprout to harvest period, as did Mn, Zn, and Fe. Boron fell from sprout to harvest, then rose again by harvest. Additionally, there appears to be no trend with final yield and any given nutrient concentration within stem tissue. These conclusions may help inform blueberry crop management and our understanding of the importance of nutrients in yield increases.

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Chapter 1: INTRODUCTION

1.1 Overview

In this study I will investigate the relative concentrations of micro- and macronutrients in lowbush blueberries, *Vaccinum angustifolium*, under varying levels of pollination exposure. Field managers of lowbush blueberry fields tend to purchase managed honeybees *(Apis mellifera*) to pollinate their fields in an attempt to maximize yield, as blueberry plants require cross-pollination to fertilize. While it is true that no berries will set in the absence of pollinators, the possible yield from any given blueberry plant does not increase proportionally with the number of managed bees that are employed. Recent studies have tried to identify how resource limitation functions to limit the yield potential in crop plants. In the lowbush blueberry industry it is often assumed that pollination is responsible for all yield increases, and little is understood of the role of plant nutrients in limiting yield. Nutrient studies tend to look at nutrient inputs only, and not at fluctuating nutrient levels within plant tissue throughout the growing season. A better understanding of relative micro- and macronutrient concentrations throughout the growing season and their relationship to final blueberry yield will provide information for effective management of the crop. This research will further establish recommended soil nutrient concentrations, and inform pollination limitation.

1.2 Background and context

The lowbush blueberry industry provides an interesting case study of an agricultural ecosystem because of the infrequent interference and minimal management employed. They are considered to be a 'wild' crop because they occur naturally in areas of production (Yarbourough et al. 1986). Truly wild lowbush blueberries occur in smaller patches in forested areas, but once the trees are removed the berries are allowed to dominate, a process that may take several years.

The lowbush blueberry has a two-year pruning cycle with one year of production and one year in sprout. The nature of the management of these fields means that nutrients available to the soil are nearly always a recycling of the nutrients from plant tissue in this monoculture. This differs from the neighbouring highbush blueberry (*Vaccinium corymbosum*) industry where industry standard recommends a considerable amount of fertilizer (Strik, 2014). Lowbush blueberries rely on insect pollination for cross-fertilization in order to reproduce. In recent years, wild blueberry crop management has focused on the purchase of honeybee (*Apis mellifera*) colonies for supplemental pollination in order increase the number of possible flowers that will set into berries, and therefore maximize berry yield. This trend is becoming more common amongst pollinator-dependent crops. Current management strategies assume that inadequate pollination resulting in below-optimal fertilization is the primary limitation in berry production. This conclusion is ignorant of the restraints in berry production that are due to limited nutrient availability (Bos et al., 2007). So far, there have been no studies attempting to account for nutrient requirements in lowbush blueberries as it relates to varying levels of fruit set and final fruit yield. This study will investigate nutrient levels in the stem tissue of lowbush blueberries at different points in the growing season and under varying levels of pollination exposure in an attempt to add to the body of knowledge around resource limitation. This research addresses three specific questions:

(1) How do current levels of nutrients in lowbush blueberry stem and leaf tissue in PEI compare to previously established levels?

(2) How do macro- and micronutrient concentrations change throughout the growing cycle, and are there any variations with yield?

(3) Does pollinator input level alter the nutrient concentrations within plant tissue or the uptake of nutrients throughout the growing season?

1.3 Commercial benefits

The lowbush blueberry, *Vacciunium angustifolium,* is produced commercially throughout Atlantic Canada, Quebec and Maine (Yarbourough et al., 1986) and the industry is expanding rapidly. A Quebec study reports an increase in blueberry yields by over 500% from 1980 to 2000 (Lafond, 2008). Prince Edward Island is a province that is economically sustained through agricultural export, of which the blueberry industry plays a major role. Previous studies have established threshold concentrations for optimum leaf concentrations of *V. angustifoliums* for both Atlantic Canada and the Sagueney-Lac-Saint-Jean area of Quebec (Lafond, 2008) and a 1972 study is currently used as a baseline for recommended nutrient concentrations (Sanderson et al., 2007). Most studies carried out in Atlantic Canada took place during a period of blueberry crop management that involved burning the sprout crop in the "off" year of the two-year rotation cycle (Lafond, 2008). Blueberry management practices have progressed and now typically involve mowing the field after harvest, allowing the sprout field to lie fallow for a year during which nutrients are added back to the soil. The transition away from the burning practice means that the soil nutrient concentrations have a different base threshold, and further studies of optimal tissue nutrient concentrations would be advantageous.

1.4 Summary of Literature and Knowledge Gaps

Lowbush blueberry management practices over the past 50 years have targeted perceived and actual limitations on yield. The most significant changes in practice have been to combat competition with weeds and to control both the spread of disease and of pests like fruit flies (*Rhagoletis mendax*). In the past decade or so, as fields have become larger, wild bees are not able to sufficiently pollinate all the blueberry flowers and use of managed bees has become commonplace. Studies that investigate the relative benefit of additional bee colonies in blueberry

fields have shown a strong positive relationship between increased pollination and final yield. These studies often do not suggest that there is an upper limit to this positive correlation. More recent studies in other pollinator-dependent or pollinator-mediated crops (e.g. in cucumber, cacao, and pumpkin) have suggested that the focus on pollinator-limitation is often ignorant of other factors that are synergistic with increased fertilization.

Many studies have overestimated the marginal benefit of pollination on yield by using percent fruit set as a parameter of increase. This is not representative of final yield because many of the set fruit will not be carried to maturity due to resource limitations, especially limits in nutrients. There has so far been little research on resource limitation in conjunction with pollinator limitation in any crop, and especially in lowbush blueberry. Recommended tissue concentrations have been established for this species in some areas, but no one has explored how the nutrient levels may vary with restricted access to plantation. There is also a lack of knowledge on how nutrient levels in plants change throughout the growing cycle.

1.5 Introduction to study

In this study the exposure of blueberry clones to pollinators will be manipulated to produce clones with a range of fruit set while all other environmental conditions held constant. Based on the experimental design for a related project I will have five levels of pollination in each of five fields in eastern Prince Edward Island. The levels of pollination have been labeled: Least Pollination (1), Low Pollination (2), Intermediate Pollination (3), High Pollination (4), and Most Pollination (5). The varying access to pollinators is expected to result in a range of levels of fruit set and therefore final yield in each field. Stems will be sampled from each treatment at the end of the season, with replicates of each treatment averaged amongst all the fields. The stems will be analysed for the concentrations of five macronutrients and five micronutrients.

The control in the study will be the background or "base" rate of nutrient transfer, averaged across the five fields. Sampling of the base concentrations of stem nutrients in the sprout season of 2013 will be compared with the base concentrations of stem nutrients during bloom and at harvest in 2014 (when berries are ripe). The open plots (most pollination) will also serve as a control when monitoring fruit set. The change in relative proportion of nutrients in the treatment plots compared to the background rate of change will reveal how lowbush blueberry plants distribute their resources under varying levels of fruit set. Comparing final yield with the relative proportions of tissue nutrients will reveal which combination of nutrients are most associated with positive yield increases.

1.6 Contribution to Environmental Science

Environmental science is the study of human activity and our interactions with the natural systems that support us. These interactions include how we may take advantage of natural systems for our purposes, often termed "ecosystem services". Pollination is a natural mutualistic relationship that has become very important for the global agricultural industry. It is increasingly important to understand our reliance on pollination so we can make better management decisions. Furthermore, elements, in the form of nutrients, are integral to connecting the abiotic world to the biotic world. The uptake of nutrients by plants is what allows all other organisms within ecosystems to obtain what they need to survive. Understanding the flow of nutrients within ecosystems and within plants will therefore help us better understand the world around us. Efficient management in agriculture more broadly is an essential way to reduce the negative impacts of our practices on the surrounding ecosystems and to reduce waste.

Chapter 2: Literature Review

2.1 Overview of Lowbush blueberry production

The lowbush blueberry industry is isolated to the eastern side of North America, with substantial commercial fields in the Canadian provinces of Quebec, Prince Edward Island, New Brunswick, Nova Scotia, and Newfoundland and the state of Maine. In 2003 there were 70,000 hectares in production, and this was expected to rise ten percent by 2013 (Bell et al., 2010). This crop is considered to be "wild" blueberries because the crops are not planted. Lowbush blueberry fields are created by clear cutting a forest patch and over time removing the competing vegetation to allow the bush to spread on its own (Bell et al., 2010). The blueberries grow through underground rhizomes in genetically distinct clones with a wide range of characteristics, such that there is significant natural variability in yield and berry size between clones and the berries of each tend to ripen at different times within the harvest period (Farooque et al., 2012; Hepler & Yarborough, 1991). A recent study of clone genetics found that close neighbouring clones are no more genetically similar than far neighbours (Bell et al., 2010). Unlike most other crops, there had been little work in plant breeding or genetic selection of certain traits within the lowbush blueberry due to an industry-wide fear of losing the "wild" designation on their product (Bell et al., 2010). The crop requires minimal management, though the application of pesticides and introduction of bee colonies has significantly increased yields. The species is xenogamic, meaning that they require cross-pollination, and they are reliant on outside organisms for reproduction. Wild blueberries have a mutualistic relationship with several wild bee species, the most significant of which is the *Andrenidae* family, ground-nesting bees that are evolutionarily adapted to pollinate the wild blueberry very efficiently. Since the 1990s many managers have

found wild pollinators to be unable to reach maximum fertilization levels, and have noted an increase in yield after the introduction of supplementary pollinators.

2.2 Impacts on blueberry yield

Wallace and Wallace think that we have a long way to go before reaching the theoretical yield potential for most crop plants (1993). While some argue that we have reached a plateau in food production that can only be expanded with breakthroughs in genetic engineering or even plant breeding (1993), Wallace and Wallace argue that there is still enormous potential for yield improvement by better researching the limiting factors. They describe Leibig's Law of the Minimum, which states "only an increase in the factor most limiting will result in an increase in yield" (Wallace & Wallace, 1993, p417). This law is applied by blueberry crop mangers, who consistently look for ways to improve yield to bring in more revenue from the same amount of land.

As Yarborough (2004) describes, lowbush blueberry yields across North America have increased drastically in the past few decades. While some of that increase has been due to field expansion, a more significant portion of the increase is due to a concerted shift in management practices. Previous ecological research has investigated many possible limits to yield and recommended such practices to field managers as weed and pest control, fertilizers, supplementary pollination, and irrigation. When terbacil and hexazinone were introduced in the 1980s to control weeds, the yields in these fields immediately doubled because the crops were no longer competing for nutrients, especially nitrogen (Yarbourough, 2004; Penney & Mcrae, 2000) The introduction of herbicides, fungicides, and other pesticides has become relatively widespread across the lowbush blueberry industry, but a more recent standard is the introduction of supplementary pollination. Various studies have shown that introducing managed honeybee (*Apis*

mellifera) colonies into blueberry fields will significantly increase yields. Other species such as leafcutter bees and varieties of bumblebees are also employed in blueberry fields, but to a much lesser extent. According to Yarborough (2004), growers have known about yield increases associated with pollinator increase since the 1960s. The recommended stocking rates for honeybee colonies vary from 2-5 colonies for every hectare of field, and they are most effective when implemented during peak bloom, when the majority of clones are in flower. In this way, managers can help to ensure that the vast majority of clones are pollinated within the same time frame, they will ripen within the same narrow time frame, and subsequently only one harvest will be required.

2.3 Pollination limitation

There is a lot of focus on pollinators as the most significant limiting factor in yield, especially for crops like the lowbush blueberry, which are dependent on pollination to reproduce. The problem with such an assumption is that all marginal increase in yield is attributed to an increased number of pollinators (Melathopoulos et al., 2014). It is commonly acknowledged that the number of flowers a plant produces is much higher than the number of mature fruit it will bare. The reasons for excess flowers are generally not well understood; one possible explanation is that the extra flowers serve as "insurance" for variations in pollinator levels and nutrient availability (Bos et al., 2007). The percentage of set fruit (or fertilized flowers) will increase with the presence of additional pollinators, but eventually will reach a plateau where additional colonies added to a field will not increase the percentage of either set fruit or mature fruit. This is evidenced in a study on cacao trees, where after testing different pollination levels, there was found to be no added benefit after pollination in excess of 40% (Groeneveld, 2010). Many studies on pollinator effectiveness do not take this property into account.

While investigating the *Relationships of Pollinator Numbers in Blueberry Fields to Fruit Development and Yields*, Eaton and Murray conclude that there is a positive correlation between an increased number of pollinators and final fruit yield (1997). Their study was fairly broad, incorporating 107 fields across Prince Edward Island, Nova Scotia, New Brunswick, and Maine. After tracking the number of wild and managed bees under various manipulated conditions, they conclude that the optimum management practice for honey bee colonies in lowbush blueberry fields be 5 or more colonies per hectare of field (Eaton & Murray, 1997). Other studies have recommended stocking densities of 2-4 colonies per hectare (Yarborough, 2004). Yarbourough further notes that each additional colony inputted can be associated with a corresponding yield increase of 785kg/ hectare (2004). None of these recommendations set any upper limit for additional colonies, suggesting that the positive correlation seen between final yield and number of pollinators will increase forever.

There is certainly conflicting evidence on pollination limitation in several pollinator-dependent and pollinator-mediated crops and not enough research has been conducted to quantify the upper limits of pollination benefits, or how such benefits interact with other variables. Pumpkins are a crop that similarly relies on cross-pollination from insect pollinators. Stocking recommendations for pumpkin fields are 7.5-10 *Bombus impatiens* (Eastern bumblebee) colonies per hectare (Stubbs and Drummond 2001, qtd in Patterson et al, 2014). This recommendation is not representative of all pumpkin fields, however, because in their 2014 study Patterson et al. reached opposing conclusions. Testing both hand- and wild pollination versus various stocking densities of *B. impatiens* colonies, they found no significant difference in fruit set or seed set between the treatments (Peterson et al, 2014). In cucumbers, pollination is the most important driver of yield, but even still, cannot be considered in isolation from the other variables that affect

yield. Motzke et al. (2014) tested the interaction of weed control, fertilization, and herbivore control practices with varying levels of pollination to determine the effect on fruit set and yield in the cucumber crop. They found that the treatment that incorporated all management practices in conjunction with insect pollination had the highest fruit set and yield. Though milkweed is not pollinator-dependent, it was found that fruit yield was determined primarily by energy and nutrient limitations and not by pollinator activity (Wilson & Price, 1979). Bos et al., 2007 looked at the importance of pollination levels for three tropical fruits: passion fruit, cacao, and coffee. They note that increasing the pollination rates is superflouous above a certain level because the increased fertilization means that the plant only has to abort more fruit than it normally would, the act of abortion could also use some energy. In this study they found that rates of abortion were highest in fruits that received highest levels of pollination. For both cacao and coffee the high abortion rates overshadowed the benefits of increased pollination (Bos et al., 2007).

With this overemphasis on pollinator-reliance many people have drummed up concern for world food supply due to a global decline in bees (Bos et al, 2007; Melathopoulos et al, 2014). A further contributor to the overestimation of yield due to pollinators can be traced to a misrepresentation of data. Knight et al. (2006) suggest that a publication bias for favourable results has influenced how some researchers present their findings. They further discuss how the influence of pollination limitation is often overestimated due to a bias of response indicators used (Knight et al., 2006). Whether a conscious bias exists or not, it is apparent that estimating the effectiveness of pollination on yield by only measuring the percent fruit set is a premature estimation that does not take fruit abortion into account (Bos et al., 2007). If plants are limited primarily by access to resources, the percentage of flowers that are fertilized will not reflect that, and it important to instead observe how many fruit are carried until maturity (Bos et al., 2007). In

cacao plants, only 5% of flowers will result in mature fruit (Bos et al., 2007), while in commercial blueberry fields the number improves a bit, with 60-70% fruit set considered a good crop.

In lowbush blueberries, specifically, few studies have attempted to account for resource limitations when pollination has reached capacity. Melathopoulos et al. (2014) identify that after pollination limitation has been overcome in lowbush blueberry, other factors are likely influencing the yield. To help clarify the relationship between management practices, pollination, and resource limitation, they test different pollinator treatment levels against recommended fungicide and pesticide use (Melathopoulos et al., 2014). They conclude that pollination and pest suppression interact synergistically to increase fruit yield, indicating that there is further room for research on other variables that affect yield. No study thus far has investigated the synergistic effect of plant nutrient availability with varying pollination levels on final fruit yield.

2.4 Optimal nutrient concentrations in lowbush blueberries

It is widely understood that all plants require a minimum amount of minerals in order to grow and reproduce. The majority (~90%) of dry mass in terrestrial plants is made up of carbon, hyrdrogen, and oxygen which are relatively constant among plant species, but the remaining 10% of essential nutrients vary significantly in amount and relative proportion amongst species and individuals depending on environmental conditions (Knecht & Goransson, 2004). Essential macronutrients include nitrogen (N), phosphorous (P) and potassium (K), calcium (Ca) and magnesium (Mg) and the recognized essential micronutrients, or trace elements are boron (B), sulfur (S), chlorine (Cl), manganese (Mn), iron (Fe), zinc (Zn), molybdenum (Mo), nickel (Ni) and copper (Cu) (Welch & Shuman, 1995; Gupta & Gupta, 2005). Several studies have tried to determine the optimum concentration of these nutrients within the stem and leaf tissue of

lowbush blueberry crops by carefully correlating the yield with different levels of nutrient inputs through fertilization. A 1972 study by Trevett established optimal nutrient concentrations that have been used as a baseline for comparison in further studies (Penney & Mcrae, 2000; Lafond, 2009; Sanderson & Percival, 2008). In 2009 Jean Lafond tested optimal concentrations of all five macronutrients across lowbush blueberry fields in Quebec and compared his optimal results with the recommended concentrations of three other studies (Lockhart & Langille, 1972; Townsend & Hall, 1970; Trevett, 1972). The optimal concentrations determined in 2009 all fell within the ranges designated by Trevett in 1972 ([N]:16.00-20.00, [P]:1.25-2.22, [K]:4.00-9.00, [Ca]:2.70-5.20, and [Mg]:1.30-2.50 mg/g), though they differed slightly from the other two studies (Lafond, 2009). These results show that although there is a wide variation of nutrient concentrations amongst clones, from year to year, and between fields, the optimum concentrations of macronutrients in lowbush blueberries is relatively constant, and Trevett's 1972 study is still representative today, at least in some areas.

Most of the studies of nutrient concentration in plant tissue have been conducted in order to provide crop managers with recommended fertilizer application. As Percival and Sanderson (2008) attest, there is a lack of consistency in nutrient management within the lowbush blueberry industry and confusion about best practice. It is fairly widely understood that *Vaccinium angustifolium* is a hearty species. It grows best in acidic soils and relative to other plants, does not require a large nutrient input. Various studies have shown that the berry grows well in low P environments, can withstand high levels of Mn, and has a high absorptive capacity for Ca (Eaton et al., 1997; Hall et al., 1964; Bohner et al., 2014). Further studies have demonstrated the importance of nitrogen for optimal growth, and N fertilizer application has resulted in higher yields and is recommended for growers (Santiago & Smagula, 2010; Hall et al., 1972; Penney

and Mcrae, 2000). Nitrogen was also determined to be the only nutrient with significant changes in concentration in plant tissue between sprout and bloom periods (Hall, 1982). To add to the body of knowledge on the effects of individual nutrient applications, Percival and Sanderson (2008) found that there is an interactive effect between N, P, and K, though they recommend further research in this area.

Studies of nutrients in lowbush blueberries have helped to inform fertilizer management, but there is still discrepancy among managers. Because lowbush blueberries have comparatively low nutrient requirements, many managers choose not to use fertilizer and instead focus on other limiting factors to growth such as plant competition (with herbicides) and pest management. Optimal nutrient concentrations have been set to allow for maximum yield, but these concentrations have not been tested against the effects of pollination limitation.

2.5 The roles of macronutrients in lowbush blueberry physiology

Few have directly investigated the roles of specific nutrients on the physiology of the lowbush blueberry. Reports that recommend fertilizer application or state the optimum nutrient levels in plant tissue tend to focus only on the correlation between nutrients and yield rather than their relative concentrations. The concentrations and relative proportions of nutrients within plant tissue are affected by a range of factors including rate of photosynthesis, mineral availability in soil, rainfall, and pH. Some minerals (like Ca) are passively taken up into plant roots when they are dissolved in aqueous solutions, while other ions, such as N, P, and K require the help of enzymes to be actively transport into plant roots because of their typically low soil concentration **(**Schlesinger & Bernhardt, 2013). Nitrogen and phosphorous are generally immobile in soil but are necessary macronutrients for plant growth, and are therefore commonly the limiting nutrients in plant development **(**Schlesinger & Bernhardt, 2013). In typical leafy plants N, P and K are

initially allocated to new leaves and their concentration within the leaf tissue is gradually diluted as photosynthetic products accumulate and decline further as the leaf ages and senesces (Schlesinger & Bernhardt, 2013). In contrast, the concentrations of Ca, Mg, and Fe tend to build up over the lifespan of a leaf due processes such as, "calcium pectate deposition in cell walls, and from increasing storage of calcium in cell vacuoles" which are a part of leaf thickening (Schlesinger & Bernhardt, 2013, p186). The balance of elements within plant tissue is important because sometimes the addition of one element can have unexpected consequences on the concentrations of other required elements. There are many established interactions between nutrients within plants, though their precise mechanisms are not well understood. For instance, high levels of P correspond to lower Zn, increased levels of Cu corresponds to lower B, and increased Mn is linked with lower Zn (Stafne, 2013).

Nitrogen is connected to lush leaf growth and improved water use and is often the limiting factor in plant growth. It is essential for building amino acids and nucleotides in cells (Maathuis, 2009). Phosphorous is important for regulating energy within plants and interacts with trace elements to build cell walls and compounds used by plants. Deficiency in phosphorous leads to immediate reductions in photosynthesis, and affects the reproductive ability of plants (Maathuis, 2009). Potassium plays an important role in gas exchange among plants and is important for forming starch and chlorophyll (Rajaselvam, 2015). It also has a key role in the activation of many enzymes (Maathuis, 2009). The essential macronutrients may all be in the soil, but due to the form they're in, the pH, or the amount of water around plant roots, the plants may be deficient in any one of them.

Chapter 3: Research methodology

3.1 Overview

This study records how the relative proportions of ten essential nutrients change over time when exposed to varying levels of pollination, and how they relate to final yield. The ten nutrients to be analyzed are N, P, K, Mg, Ca, Cu, B, Mn, Fe and Zn. Representative control plots are integral to establishing the present nutrient levels and tracking the levels over three periods: sprout, bloom, and harvest. The 25 control plots (1m x 1m) placed across five fields will provide a baseline of the relative proportions of nutrients. To address my third research question, a method is needed to restrict the inflow of pollination to the plants. I will utilize experimental treatments that were set up for a related study, which is expected to result in a range of fruit set and yields. Each field will have three replicates of each treatment. Establishing multiple replicates is important to account for natural variation between clones and between fields. Three stems in each plot will be randomly tagged at the start of the crop season, and their flower formation, fruit set, and final mature berry numbers will be recorded.

3.2 Study location

The location for this study is within five different commercial lowbush blueberry fields on the eastern side of Prince Edward Island. PhD candidate Andony Melathopoulos established the five fields in the summer of 2013 as part of his thesis work. The five fields are located outside the town of Bridgetown, northeast of Souris (Greenvale), south of Bridgetown off highway 311 (Seven Mile), just north of Bridgetown (Shaw) and at Iris (see figure 1). Across the northern edge of each field a 100m transect was established, and five 1m x 1m quadrants were laid out at 0m, 25m, 50m, 75m, and 100m. These open plots will serve as sampling sites for stems that are

exposed to all pollinators during the study. Background rates of nutrient concentrations will be averaged from transect data across five commercial blueberry fields.

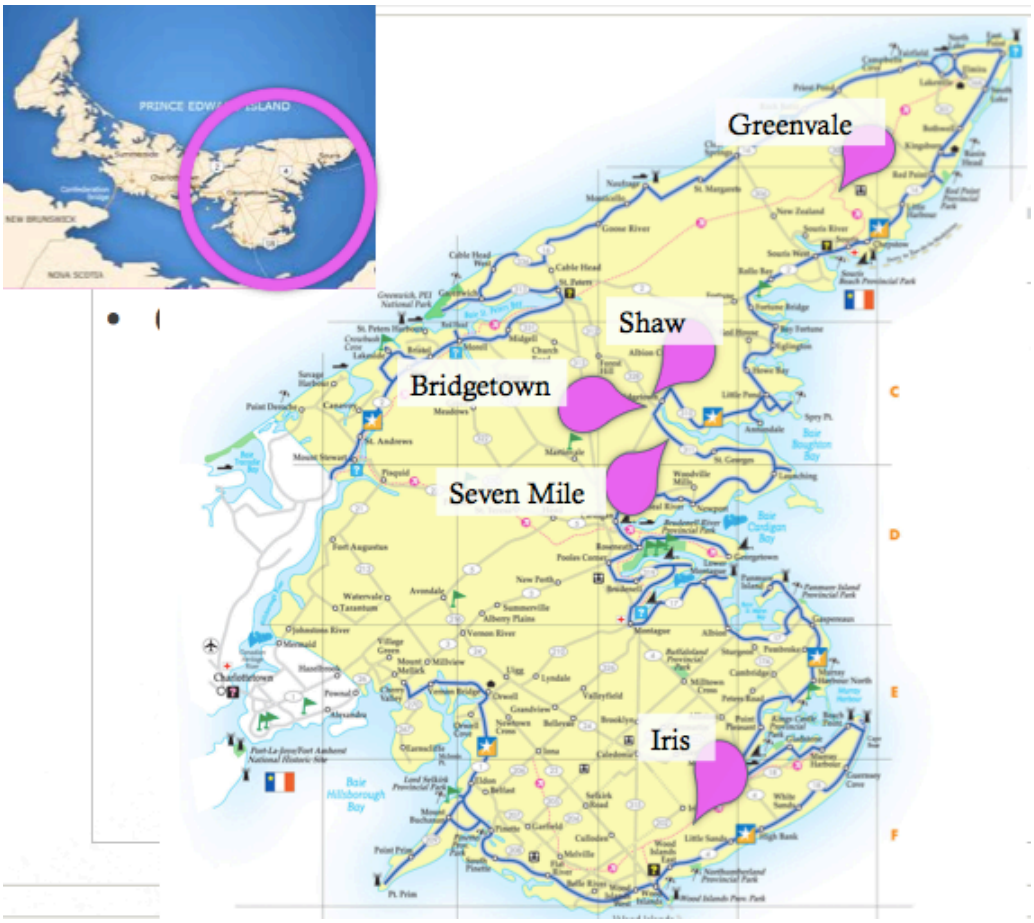


Figure 1: Site locations of five fields used in this study located within the eastern side of Prince Edward Island, Canada. Fields are labeled: Greenvale, Shaw, Bridgetown, Seven Mile, and Iris. These fields were selected by PhD candidate Andony Melathopoulos as part of his thesis project.

3.3 Description of study design

To determine the background or "base" rate of change in nutrient proportions within stems, 50 stems will be clipped along the transect of each field at three intervals: during the sprout season (2013), during bloom (mid-July 2014), and just before harvest (mid-August 2014). A report on highbush blueberry management practices recommends that tissue samples for nutrient analysis be taken during harvest, or when berries are ripe, because this is a period of stability for the plant (Hart et al., 2006). It can be assumed that the timing of nutrient transfer

would be similar for the lowbush variety. During the summer when nutrients are being drawn up from the soil and through the plant, the concentrations of each element are in fluctuation, so measuring stem nutrients when berries are ripe will be the most stable time for the plant. Despite this, the nutrient concentrations that are followed as recommended standards tend to be based on levels during the sprout period, so comparisons will be made based on sprout levels (Trevett, 1972; Sanderson et al., 2007).

This study establishes four treatment types based on the experimental design set out by Master's candidate, Laurel Schut (School for Resource and Environmental Studies, Dalhousie) that took place during the same time period. Schut's research is comparing the effects of wild and managed pollinators on blueberry quality and quantity. Her research design involved four treatment types that excluded wild or managed bees using mesh pollinator exclusion tents and also discriminated between early and late blueberry clones using 1m x 1m plots. I predicted that these treatments, along with the open plots, would lead to five different levels of pollination with correlated levels of yield in the following way:

(1) Least pollination: late clone excluded from honeybees

(2) Low pollination: early clone excluded from honeybees

(3) Intermediate pollination: early clone excluded from wild bees

(4) High pollination: late clone excluded from wild bees

(5) Most pollination: open plot

Table 1 shows the data that is available for each of the fields. Due to human error, two treatments in each of Bridgetown and Greenvale were not recorded. Data from each of the treatments in all

the fields will be amalgamated to maximize the number of replicates. This results in 12 replicates of each treatment type (3 replicates per field times 4 full fields).

Table 1: The available data from five fields, resulting in the equivalent of a full data set from four fields in total ("Yes" refers to data that is available and "No" refers to unavailable data).

Field	Least Pollination	Low Pollination	Intermediate Pollination	High Pollination	Most Pollination
Iris	Yes	Yes	Yes	Yes	Yes
Seven Mile	Yes	Yes	Yes	Yes	Yes
Shaw	Yes	Yes	Yes	Yes	Yes
Bridgetown	Yes	No	Yes	No	Yes
Greenvale	No	Yes	No	Yes	No

In order to estimate the fruit set, three stems in each 1m by 1m plot will be randomly tagged and tracked throughout the season. The number of buds and flowers will be recorded, and the subsequent number of set fruit and then mature fruit will be counted. During harvest in late August, 20 stems per treatment type per field will be clipped and analysed for nutrient content. These stem samples along with the base rate stem samples will be dried and sent to the PEI Analytical Laboratory, PEI department of Agriculture and Forestry for analysis (as in Perrin, 1999). Therefore the final data for each treatment will be: average # flowers/ stem, average fruit set / stem, average # ripe berries/ stem, and average concentration of 10 nutrients in stem tissue. The average stem data will be based on 36 stems (3 tagged stems in each of 12 replicates) and the average nutrient concentrations based on 80 clipped stems per treatment (20 stems in each of four fields). During harvest, each of the plots will be hand-raked and weighed to give a final plot yield. Yield for each treatment per field will be presented as the mean of three replicates. The discrimination in treatment types is intended to produce a range of fertilization levels, which I expect will be reflected in the fruit set and yield results.

3.4 Analysis of Data

Percent fruit set is the proportion of berries that set per stem relative to the number of flowers the stem had. Even with regular monitoring percent fruit set is difficult to establish because flowers bloom at different times, some flowers fall off, and berries set and ripen at different times. In this study, percent fruit set will be estimated using data from two time periods: peak bloom and harvest. At peak bloom I will add together the number of open flowers, closed flowers, dropped flowers, and set fruit recorded on each tagged stem to give a 'Total per plot'. At harvest the total number of berries observed on each tagged stem (both green and blue) will be combined to give 'Total berries at harvest'. The percent fruit set will be calculated by dividing 'Total berries at harvest' by 'Total per plot' during bloom.

Data analysis will aim to answer the three research questions previously established for this study.

(1) How do current levels of nutrients in lowbush blueberry stem and leaf tissue in PEI compare to previously established levels?

The control plots will serve as the basis for evaluating the tissue nutrient levels in lowbush blueberry fields across eastern PEI. The mean nutrient levels (during the sprout period) found across the sampled fields will be compared to Trevett's (1972) study and the reports released by Agriculture and Agrifood Canada that show measured levels of nutrients in Prince Edward Island blueberry fields over the past three years (Sanderson et al., 2007). If any field is outside the recommended ranges I will compare the final recorded yields to look for trends or correlations. This analysis will reveal if there are any strong trends between yield and nutrient level. For this

portion of the study, I will perform multiple linear regressions to determine which nutrient or combination of nutrients has the highest positive correlation with final yield.

(2) How do macro- and micronutrient concentrations change throughout the growing cycle, and are there any variations with yield?

The change in base nutrient concentration will be determined by comparing transect nutrient data from the three time periods: sprout, bloom, and harvest. This background rate of change will be compared with the rates of nutrient change in each of the treatments. If nutrient limitation operates independently of pollination limitation, we would expect to see no significant difference in nutrient concentrations for each of the treatments. Results will be presented visually in graphs. This data is not being compared to any previous studies and therefore will stand on their own. Based on the descriptions of plant nutrient cycling described by Schlesinger and Bernhardt (2013) I expect the concentrations of N, P and K to decrease throughout the season, and the concentrations of Ca, Fe, and Mg to increase.

(3) Does pollinator input level alter the nutrient concentrations within plant tissue or the uptake of nutrients throughout the growing season?

I will first determine whether the treatments were successful in limiting pollination by comparing the percent fruit set amongst the five treatment types. I will also compare the final yield harvested from each treatment. If there is a significant correlation between pollination exposure and fruit set or with final yield then I will be able to examine the relationships between nutrient levels and different treatment types.

Sampling of the base concentrations of stem nutrients that took place in the bloom period will be compared to the concentrations at harvest (when berries are ripe). These concentrations will be compared with the optimum concentrations that have been established in previous studies (i.e.Trevett et al., 1972). The change in relative proportion of nutrients in the treatment plots compared to the background rate of change will reveal how lowbush blueberry plants distribute their resources under varying levels of fruit set.

3.6 Limitations

Due to the limited time period available for this study (the nature of undergraduate honours projects) and because my research is based off study designs intended for other projects, there are some limitations to my study. Firstly I will assume that the 100m transect on each field is representative of the conditions of the entire blueberry field. It is assumed that the 50 stem clippings across the transect are representative of the broader field, and that the five open quadrants, placed at regular intervals along the transect, are further representative of this sample pool. Secondly, in the design of separate "pollination bins" it is assumed that there will be a range in levels of fruit set. If this is not the case, the analysis of my study may change slightly. The data available limits what I can analyse. For instance, there is no available data on tissue nutrients in the treatment plots during the "fruit set" period. This would be ideal to properly track how nutrient concentrations change over time. As such, I will assume that the only significant changes in plant tissue nutrients under different pollination treatments will be captured in the measurements at final harvest. Finally, my study is limited by the number of replicates. To make more conclusive results, a larger sample size and more stems sampled for each treatment would be necessary.

3.7 Delimitations

This research aims to only cover the commercial lowbush industry in eastern PEI, and covers five fields from across this side of the province. The number of fields and replicates were chosen to account for the wide variability that is found between clones and between fields in lowbush blueberry. To ensure representative sampling, the open plots were selected using systematic random sampling, and the stem samples along the quadrat were selected using simple random sampling.

Chapter 4: Results

4.1 Observed nutrient levels

The 1972 Trevett standard lowbush blueberry tissue concentrations are used by blueberry growers across North America as an established 'optimum' level to strive towards, and are shown in the left-hand column of Table 2 (Sanderson et al., 2007). Agriculture and Agrifood Canada sampled lowbush blueberry tissue nutrients across PEI during the sprout period over a three-year period, the mean results of which are presented in the centre column of Table 1 (Sanderson et al., 2007). The results from this experiment are shown in Table 1 in the right-hand column. All tissue measurements were taken during the sprout period, or "die back" stage.

Table 2: Mean concentration of nutrients in blueberry tissue compared to previously measured nutrient levels and to Trevett's 1972 standard for lowbush blueberry nutrient requirements. Results highlighted in yellow fall only within Trevett's ranges, those in blue fall only within Sanderson et al.'s ranges, and those highlighted in green fall within both ranges.

Nutrient	Trevett standard (1972)	PEI ranges established by Sanderson et al. (2007)	Mean open plot measurements, n=25, PEI (2014)
N	1.60 – 2.00 %	1.3 – 1.7%	1.74 %
P	0.13 – 0.22 %	0.112 – 0.142 %	0.13 %
K	0.40 – 0.90 %	0.43 – 0.58 %	0.42 %
Ca	0.27 – 0.52 %	0.51 – 0.67 %	0.32 %
Mg	0.13 – 0.25 %	0.13 – 0.19 %	0.12%
Cu	7 – 14 ppm	2.1 – 3.4 ppm	4.20 ppm
B	24 – 60 ppm	22 – 41 ppm	35.6 ppm
Zn	25 – 50 ppm	7 – 16 ppm	20.0 ppm
Fe	50 – 100 ppm	7 – 36 ppm	27.6 ppm
Mn	750 – 1490 ppm	486 – 2217 ppm	803.6 ppm

Nutrient levels that fall within Trevett's established optimum levels include N, P, K, Ca, B and Mn. Of these elements, K, B, and Mn also fall within Sanderson et al.'s established ranges. The Fe level falls outside Trevett's range, but within the range established by Sanderson et al. Measurements of Mg, Cu and Zn do not fall within either range with Mg being below both established ranges and Cu and Zn both fall between the two ranges. Looking at the nutrient levels

broken down by field (see Table 3, below) shows the variation across the region. Colours used are the same as Table 2.

Table 3: Measured tissue nutrient levels (sprout year) over the five study fields and their corresponding observed yields. Numbers highlighted yellow fall within Trevett's range (1972), those highlighted blue fall within Sanderson et al.'s range (2007) and those highlighted green fall within both ranges.

Nutrient	Iris	Seven Mile	Greenvale	Bridgetown	Shaw
N	1.86 %	1.8	1.56 %	1.66 %	1.8
P	0.14%	0.13%	0.14 %	0.12 %	0.12%
K	0.57%	0.47%	0.14 %	0.50 %	0.44%
Ca	0.31 %	0.35%	0.34 %	0.31 %	0.3%
Mg	0.10%	0.13%	0.11 %	0.11 %	0.13%
Cu	3.23 ppm	5.17	4.26 ppm	3.80 ppm	4.53
B	35 ppm	33.9	37.7 ppm	32.1 ppm	29.2
Zn	19 ppm	23 ppm	21.5 ppm	17.7 ppm	24.92
Fe	25 ppm	27 ppm	23 ppm	29 ppm	34 ppm
Mn	760 ppm	824 ppm	944 ppm	704 ppm	786 ppm
Average yield/ plot	**1.345 kg** (n=5, *s*=0.42)	**1.032 kg** (n=5, *s*=0.18)	**0.633 kg** (n=5, *s*=0.63)	**0.580 kg** (n=5, *s*=0.46)	**0.707 kg** (n=5, *s*=0.55)

Iris, the field with the lowest highest yield per plot, had a yield that is 2.3 times greater than the average yield per plot in Bridgetown, which had the lowest average (1.345 kg/m^2 compared to 0.580 kg/m^2).

4.2 Control plots - changes throughout growing period

Further analysis on the open, control plots shows how the nutrient levels within stem tissue change throughout the growing season. Figure 2 shows the average change in macronutrient levels. Nitrogen, phosphorous and potassium levels all drop throughout the growing season and calcium and magnesium increase in concentration. Figure 3 shows the average change in micronutrient levels. Manganese, zinc, and iron all rise considerably throughout the growing season, the concentration of copper drops, and the concentration of boron drops before bloom (from 35.6% to 24.1%) and then rises again at harvest (to 33.3%)

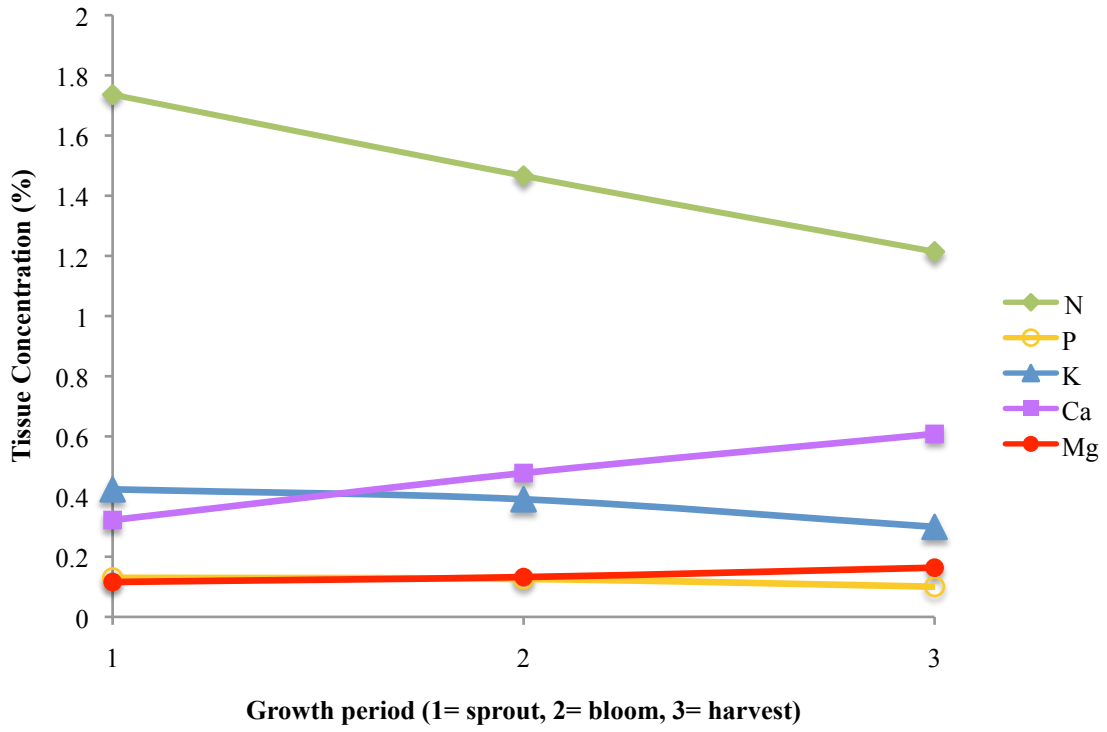
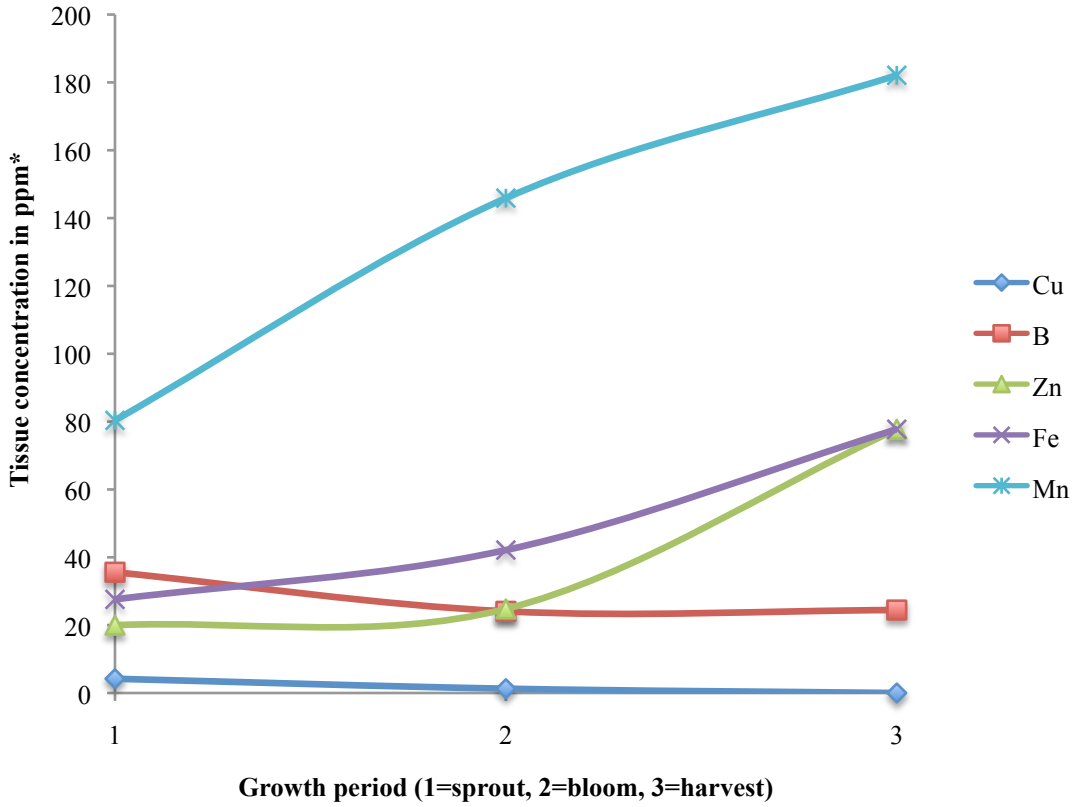


Figure 2 Relative concentrations of macronutrients within blueberry stem tissue measured at three periods throughout the growing cycle. Stems were sampled randomly along the 100m transect at each field. N=150 (50 stems x 5 fields) for each period.



* Mn is measured in parts per 100,000

Figure 3 Relative concentrations of micronutrients within blueberry stem tissue measured at three periods throughout the growing cycle. All concentrations are measured in ppm except for Mn which is presented in parts per 100, 000. Stems were sampled randomly along the 100m transect at each field. N=150 (50 stems x 5 fields) for each period.

It is important to establish whether the samples sizes are adequate representation of the plot, and whether the plots used in this study are representative of both the field, and of lowbush blueberries in general. Figure 4 (below) shows how the yield of tagged stems within plots (total number of both green and blue berries counted on the stems at time of harvest) compares with the total yield per plot that was harvested. There is a positive correlation between the two variables, with an R^2 value of 0.16473. Because the correlation is relatively weak, the stem samples cannot be considered entirely representative of the plots.

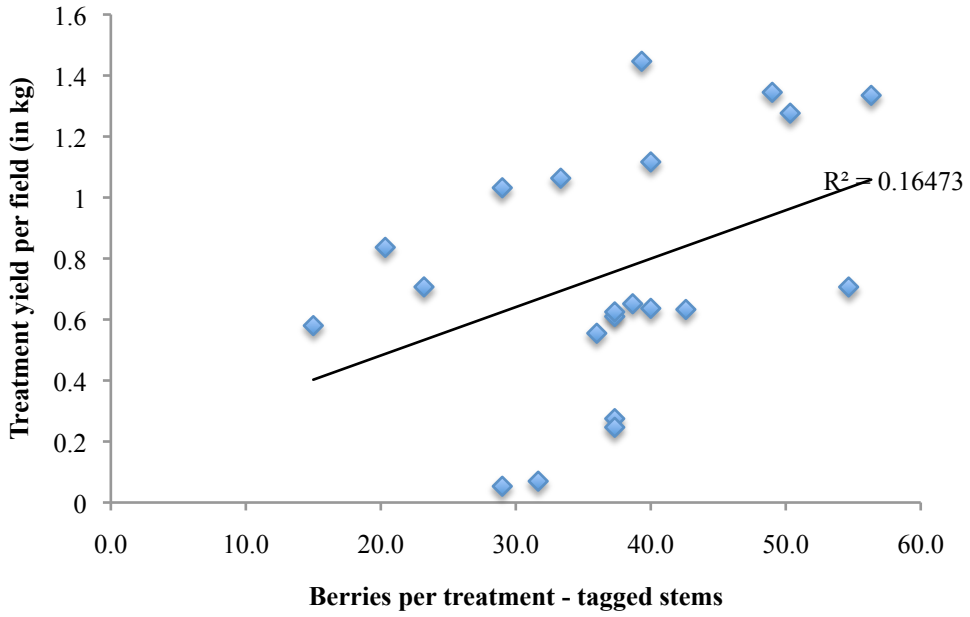


Figure 4: Berries on tagged stems (N= 9 for each data point: 3 tagged stems/ treatment x 3 treatments/ field)

4.3 Treatment plot results

The treatments set up in this experiment were intended to limit the pollinator access to flowers and thereby result in a range of fruit set and yield across the plots. I predicted that the treatments would result in five levels of pollination exposure: (1) Least pollination, (2) Low pollination (3) Intermediate pollination (4) High pollination and (5) Most pollination. As can be seen in figure 5, there is a weak positive correlation between pollination level and final yield in the predicted direction, with an R^2 value of only 0.14039. This correlation is strongly influenced by the data points in category 1. Figure 5 shows the average percent fruit set measured for each treatment within each field compared to the average yield for those plots.

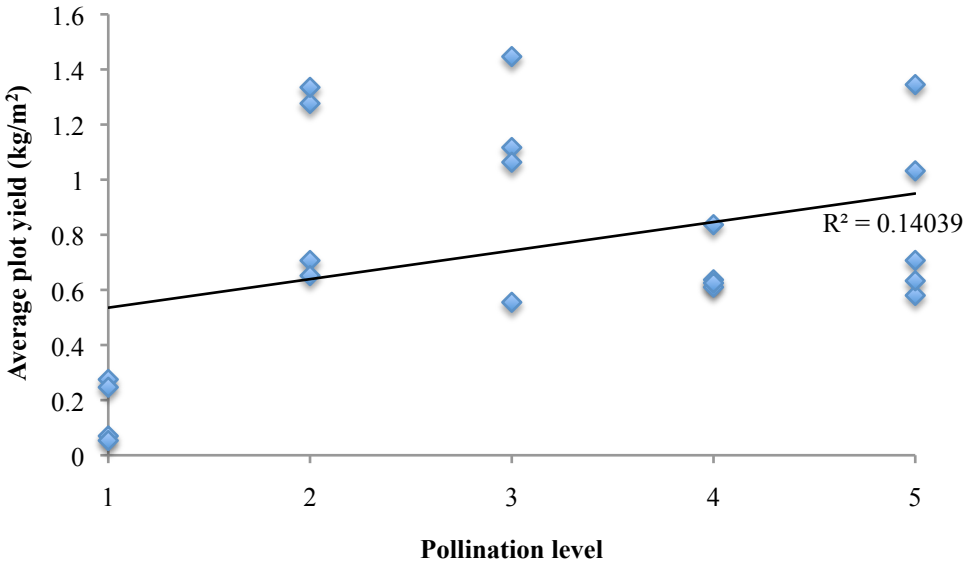


Figure 5: Pollination level (based on treatment predictions) and the corresponding mean yield of all plots of that treatment across all five fields (N=12).

Tables 4 and 5 (below) demonstrate the change in macro- and micronutrient concentrations within plant tissue in each of the treatments. Due to incomplete data, changes in copper concentrations within treatment plots could not be measured. In these tables it is notable that there is no clear trend in the way nutrient levels change depending on the associated pollination level. It is unclear whether this is evidence that nutrient changes are unrelated to level of pollination exposure or whether the treatments were ineffective at limiting pollination.

Table 4: Changes in macronutrient concentrations within the stem tissue of five treatment levels from sprout to harvest period.

Pollination level	Δ N	Δ P	Δ K	Δ Ca	Δ Mg
Least	- 32.3%	- 23.0%	- 27.2%	+ 86.2%	+ 44.0%
Low	- 27.5%	- 20.1%	- 28.6%	+ 90.9%	+ 38.4%
Intermediate	- 32.7%	- 25.1%	- 30.5%	+ 91.1%	+ 53.1%
High	- 27.1%	- 20.3%	- 28.8%	+ 94.9%	+ 50.2%
Most	- 29.5%	- 22.5%	- 39.3%	+ 89.2%	+ 40.8%

Table 5: Changes in micronutrient concentrations within the stem tissue of five treatment levels from sprout to harvest period.

Pollination level	Δ B	Δ Zn	Δ Fe	Δ Mn
Least	- 3.7 %	+ 16.3 %	+ 51.2%	+ 114.6%
Low	+ 2.1 %	+ 9.6 %	+ 51.2%	+ 113.2%
Intermediate	+ 5.1%	+ 27.1%	+ 52.8%	+ 112.6%
High	- 0.2%	+ 16.8%	+ 61.4%	+ 126%
Most	0 %	+ 23%	+ 150%	+ 114%

Figure 6 (below) compares the percent fruit set (calculated as an average of tagged stems) to the yield that was harvested from the corresponding plot. If the method of calculating fruit set is an accurate representation of the fruit set within the entire plot we would expect to see a strong positive correlation between fruit set and plot yield. This correlation is not seen below. This could either mean that there is no relationship between fruit set and final yield (highly unlikely), or that the calculated percent fruit set (based on three stems) is not really reflective of the fruit set in the plot it was supposed to represent. Furthermore, it is interesting to note that fruit set is clustered relatively close together (all within 41 and 71% fruit set). Because some plots had almost no access to pollinators and others were completely exposed for the entire season, a wider range was expected.

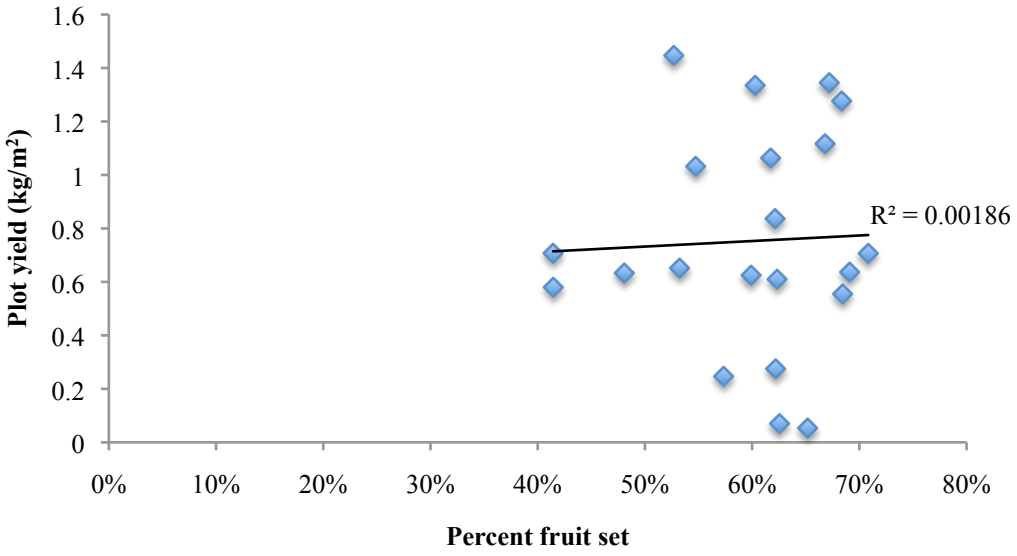


Figure 6: Percent fruit set per plot compared to the plot yield. Surprisingly, there appears to be no relationship between fruit set and yield, suggesting that the method of calculating percent fruit set may not actually be representative of the whole plot.

Although the treatments do not seem to have effectively limited pollinator input in the expected trend, the experiment did succeed in creating a range of yields across plots. This range of yields provides a good base for investigating the relative concentrations of nutrients within stem tissues. If high concentrations of a certain element were integral to a high yield, we would expect to see the concentration within stem tissue rise as yield increases, and that trend would be visible in figures 7 and 8. The levels of nutrients in stem tissue appear to be relatively steady across plots of different yields. As can be seen from Table 4, above, there is no distinct trend in nutrient changes amongst the treatments, and the numbers do not differ greatly from one another.

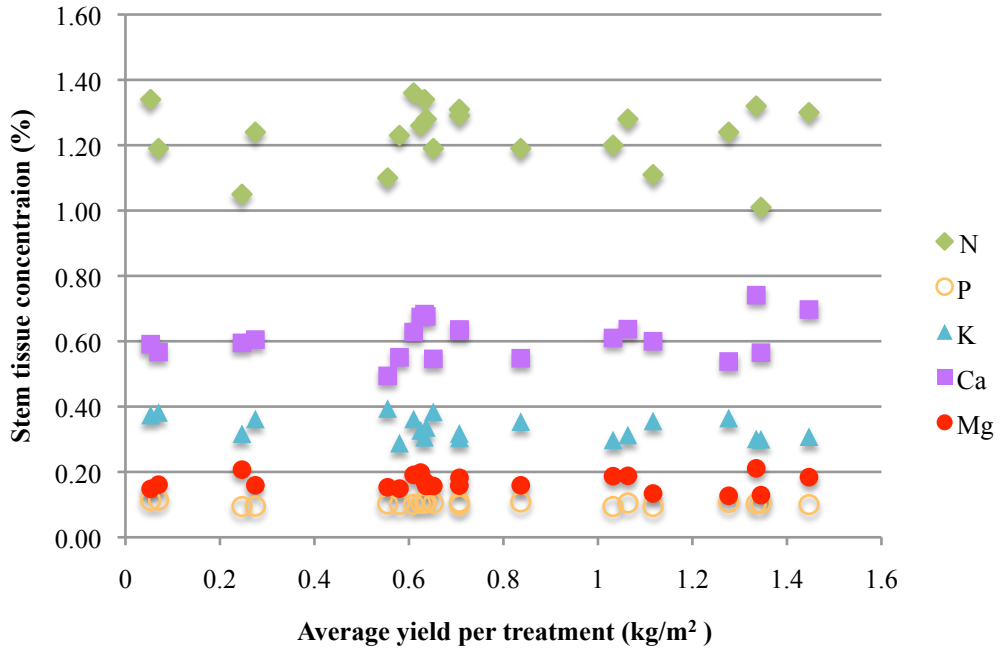
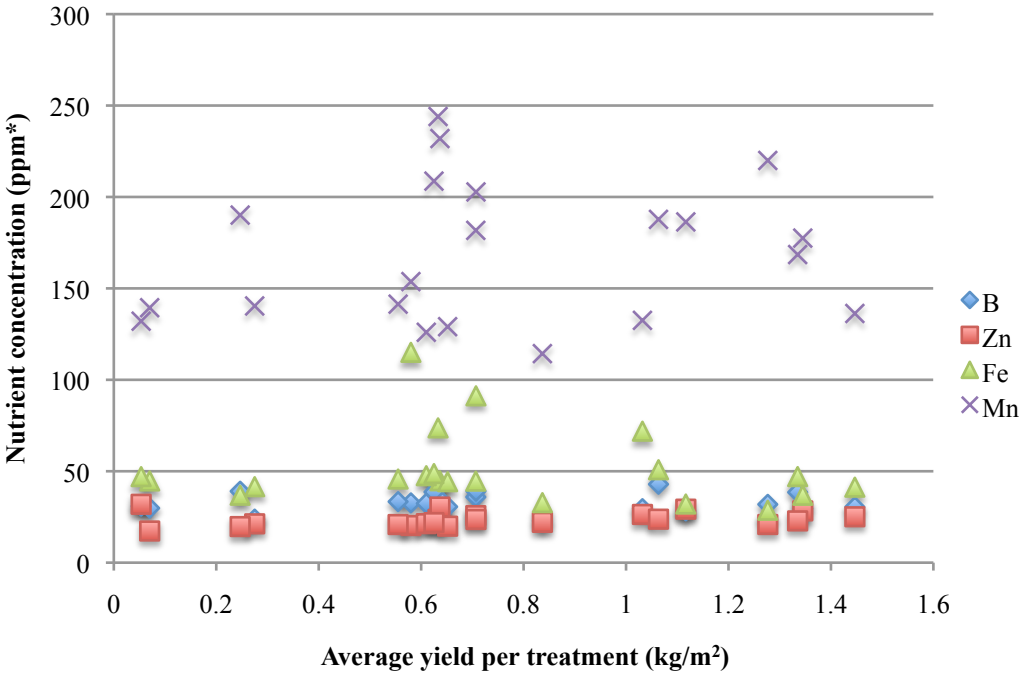


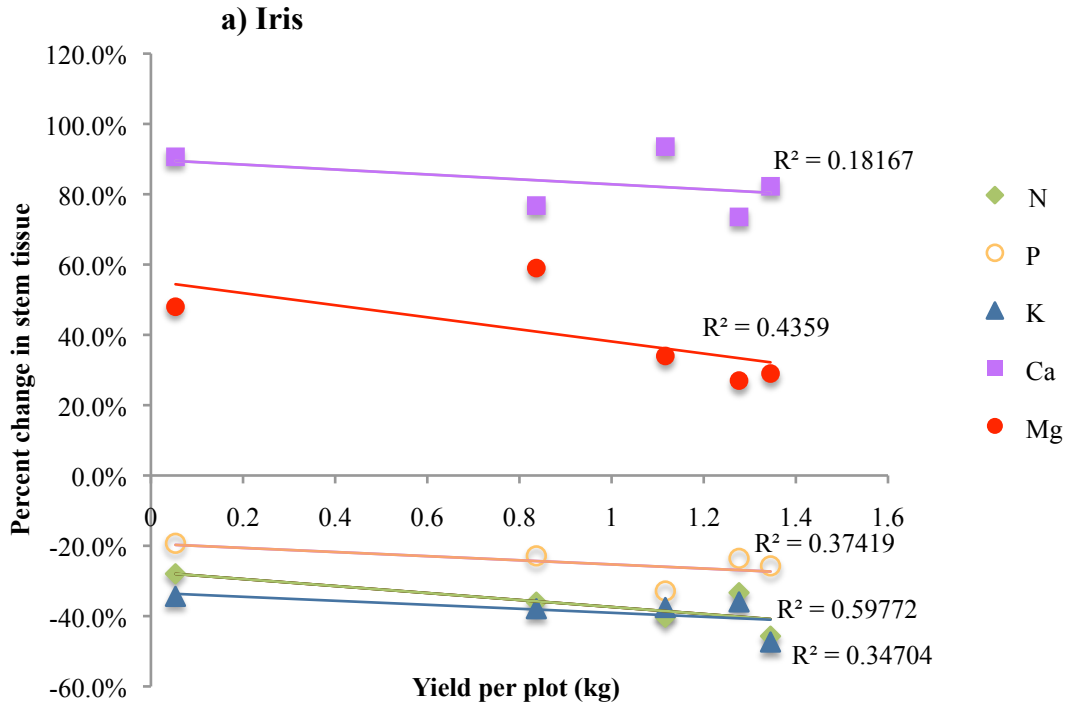
Figure 7 The concentrations of macronutrients within stem tissue at harvest across plots of varying yields.

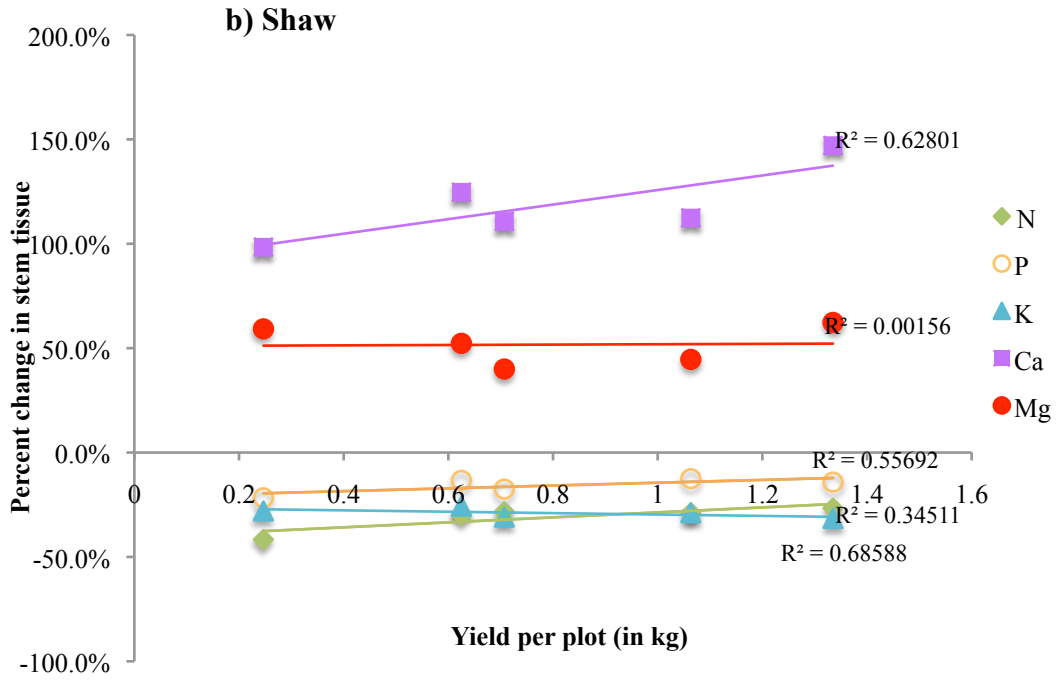


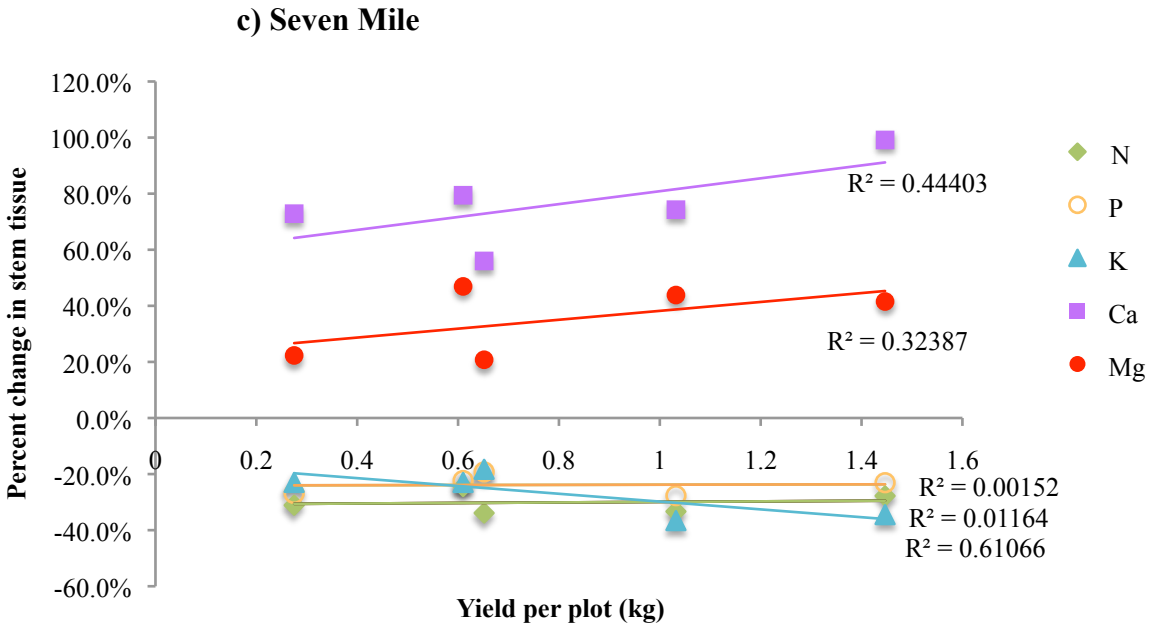
* Mn is measured in parts per 100,000

Figure 8 The concentrations of micronutrients within stem tissue at harvest across plots of varying yields.

There are three fields for which there was enough data to graph the yields against changes in nutrient levels: Iris, Shaw, and Seven Mile. As can be seen in Figures 9a, 9b, and 9c, the trends between yield and change in nutrient levels are inconsistent across fields.







Figures 9 a-c: Tissue Concentrations vs. yield, sprout to harvest in three fields: Iris (a), Seven mile (b) and Shaw (c). Visible trends in macronutrients levels and corresponding yield are inconsistent across fields. For instance, in Iris smaller changes in magnesium levels across the growing cycle appear to correlate with a higher yield. The opposite trend in magnesium can be seen in Seven mile, and there is no notable correlation with magnesium in Shaw.

Chapter 5: Discussion

5.1 Addressing the research questions

1) How do current levels of nutrients in lowbush blueberry stem and leaf tissue in PEI compare to previously established levels?

Based on the measured nutrient levels, these study sites are reasonable representations of lowbush blueberry fields. Trace elements Cl, S, Mo and Ni were not included due to insufficient data, but these elements are rarely reported in literature, and likely make up a very small proportion of lowbush blueberry micronutrient content. The levels determined are mostly within previously established levels; all but three nutrients fall within one of the previously established ranges presented here. Of those three, Mg is only 0.01% low, while the concentrations of Cu and Zn fall between the two established ranges. I expected the nutrient levels in these fields to be closer to the levels previously measured in PEI fields, but found levels in general that were closer to Trevett's standards, which were established in Maine. The field variation seen in table 3 can likely be attributed to natural variation within and amongst blueberry fields. The average yields in open plots amongst fields sampled show a wide range, with the most plentiful field, Iris, having an average year that is more than two times larger than the two fields with lowest average yields (Greenvale and Bridgetown). Based only on table 3, it seems that fields that have a greater proportion of nutrients that fall within established 'optimum' ranges have a slightly higher yield than those that do not. For instance, the plants measured from Greenvale are outside established nutrient ranges in four of the ten elements measured. Greenvale's average yield per plot is relatively low, at 0.633 kg (n=5, s=0.63). There are many factors that influence yield which are beyond the scope of this study, so no accurate conclusions regarding the role of relative nutrient levels and yield can be made. Further research in this area could provide some useful results.

The primary macronutrients, N, P, and K are all within Trevett's ranges, but are on the high end of what would be expected for PEI. This could be connected to fertilizers that are used on the fields. In comparison to other plant species, blueberry plants are able to tolerate harsh soil conditions, and are especially adapted to grow in acidic conditions (Bell et al., 2010). In most leafy plants the N:P ratio is around 14 to 15, while one study of around 10,000 plant species found an average N:P ratio of 10.9 (Schlesinger & Bernhardt, 2013). The N:P ratio found in this study is about 13.4, which seems to be on the higher end of what is typical for lowbush blueberries (based on Trevett, 1972 and Sanderson et al., 2007). Because nitrogen is linked with tissue growth and phosphorous is linked with flower and berry production, a higher N:P ratio could result in lower yield within blueberry crops. While this does not appear to be a factor within this study, in a larger experiment such a trend could become apparent.

2) How do macro- and micronutrient concentrations change throughout the growing cycle, and are there any variations with yield?

Few studies track the change in nutrient levels over the production stages of a crop. Because the fields observed in this study are typical of the region and exhibit nutrient levels and yields consistent with previous studies, we can be reasonably certain that the data collected is accurate and trustworthy. Figures 2 and 3 show some distinct trends in nutrient levels throughout the growing season. The trends observed in the control plots are also carried through within the treatment plots (see Table 4 and 5). It is interesting to note that the three main macronutrients N, P, and K all decrease between the sprout period and harvest. For instance, nitrogen's drop from about 1.7% in sprout to a concentration of ~1.2% is a constant trend across treatments. The decreases in N, P, K and increases in Ca and Mg concentrations are consistent with Schlesinger and Bernhardt's descriptions of plant physiology. These changes are likely because as leaves

mature less nitrogen and phosphorous are allocated to them. Additionally, as photosynthetic products are deposited in leaf and stem tissue, the original macronutrients are diluted. We now know that nutrients within blueberry plants fluctuate in an expected manner.

Micronutrients make up a much smaller proportion of stem and leaf tissue, but as can be seen in Figure 3, there are some notable fluctuations in micronutrient levels throughout the growing season. The concentration of manganese rose to 112-126% of its original concentration, and displayed the greatest fluctuation amongst all the nutrients. Manganese helps to activate enzymes and plays a role in photosynthesis (Stafne, 2013). It is also known to be in relatively high concentrations within berries themselves (USDA, 2015). Although the function of Mn in berry production is unknown, it is clear that the plant uptakes more Mn in its leaves and tissues at the time it is producing berries. Iron concentrations increased by a factor of ~51-150% in plant tissue between sprout and bloom period. Iron is connected to respiration and metabolism within plants, and it's rise in concentration throughout the season is likely because the ability of plant leaves to store metabolic products increases with age (Schlesinger & Bernhardt, 2013).

Fluctuations in tissue concentrations from sprout to harvest are also influenced by which nutrients are directly pushed into blueberry fruit. The USDA reports that blueberry fruit contain Ca, Fe, Mg, P, K, Na, Zn, Cu, Mn and Se. The most notable macronutrients are K and P (77 and 12 mg/g respectively) and the more notable trace elements are Mn and Fe (0.37 and 0.28 mg/g respectively) (USDA, 2015). The nutrients that showed a rise in concentration throughout the growing season are also the nutrients that have a relatively large proportion within blueberry fruit. More research is needed to better understand the cycling of nutrients throughout the lifecycle of a blueberry plant, especially further analysis of nutrient concentrations within fruit.

3) Does pollinator input level alter the nutrient concentrations within plant tissue or the uptake of nutrients throughout the growing season?

This question cannot be adequately answered because of data limitations. Pollinator input levels were not restricted in the intended manner, and sample sizes are too small to draw significant conclusions. Figure 6 shows that the percent fruit set of all the plots, treatment and open, range from 41-71%. The upper limit is in line with literature that reports the fruit set of successful blueberry fields as 60 to 70%. The lower limit and narrow range is a surprising, however, because some plots had hardly an access to pollinators while they were in bloom and others had access to pollinators for the entire season. As stated earlier, it is likely that the numbers calculated for percent fruit set are not representative of what was happening within the plots. Figure 7 shows the proportions of macronutrients within stem tissue at harvest, and it is laid out in comparison to the resulting yields. This result, based on more reliable data than the previous figure, show some intriguing results. It appears that fruit yield is unaffected by the concentration of any given macronutrient at the time of harvest. This is potentially the first study that has investigated the question of nutrient relationship to yield in this way, so the results are novel. Further studies should attempt to perform this type of analysis with a larger sample size so that a proper statistical analysis can be applied to see if this trend continues.

5.2 Methods for determining pollinator input levels

In order to properly compare pollinator input levels, a better method is required to adequately assess how much pollen is reaching stigmas, and how much fertilization is taking place. Tracking individual stems across an entire growing season is tedious work, and it is impractical to count every stem within square meter plot. This study estimated the level of fruit set by counting the number of berries at the time of harvest and comparing this number to the

total number of flowers (open, closed, fallen) and buds during peak bloom. To quantify the level of pollinator input within a square meter plot, more that three stems need to be tracked, but this may not be possible. Though the exact level of input is hard to quantify, other methods could include a more general analysis. This could include visually estimating percent bloom, fruit set, and mature fruit over the whole plot more frequently throughout the season. Another method to determine the success rate of fertilization is to examine individual stigmas to see how many have been successfully fertilized.

Chapter 6: Conclusion

6.1 What was researched?

There is much to be learned about the effects and functions of nutrients in plants. Plants play an essential role in ecosystems, transferring inorganic forms of elements into usable organic compounds like amino acids that can be passed throughout the food web (Schlesinger & Bernhardt, 2013). It has been well-established that all plants require certain macronutrients (N, P, K, Ca, Mg) and micronutrients (Mn, Fe, B, Mo, Cu, Zi and Ni) in order to perform functions that are essential to their survival. Some nutrients are more closely related to high yield production than others because of their role in plant growth and flower or fruit production. This study intended to add to the body of research around nutrient limitation in lowbush blueberries to help determine what factors influence yield. This research is related to the debate on the importance pollinator limitation. The influence of pollination levels and yield is often overestimated, and the influence of nutrient levels in predicating final yield is not well understood.

6.2 Main findings of study

The data collected in this study further establishes what is known about variability within and amongst lowbush blueberry fields. Treatments were set out to compare nutrient levels in blueberry plants that were exposed to varying levels of pollination. There was no significant trend found in nutrient levels in plots, or in the changes in nutrient levels throughout the growing cycle. It is unclear whether there is no trend, or whether a better sampling system and higher sample size is needed to better account for the natural variation.

The fluctuations in nutrient levels investigated in this study provide potentially novel information in the field of lowbush blueberry research. Concentrations of primary macronutrients all decreased between sprout and harvest periods, while the concentrations of secondary

macronutrients and Fe, Zn, and Mn all increased during this period. Finally, based on available data, there is no notable correlation between levels of nutrient fluctuation and corresponding yields.

6.3 Implications and suggestions for future research

The fluctuations in nutrient levels found in this study should be further tested in future studies. To see whether these observations are standard across lowbush blueberry fields, samples should be taken more frequently during the growing season, and larger sample sizes should be collected. It would also be beneficial to establish new methodology to better test the effect of varying pollination success on nutrient levels. A better way to test this idea would be to systematically limit the amount of exposure various plots have to pollination. This could mean that during the possible season of blueberry flowering plots are covered for 100% of the time, 80% of the time, 60% of this time etc. This system would likely be more successful in creating a nuanced range of yield between treatments, which would be a great basis for nutrient analysis.

In her paper on pollination limitation Knight et al. (2006) notes that many researchers bias their work in order to present favourable result. In this study I attempted to avoid any bias by clearly outlining my limitations. My results are somewhat inconclusive due to low sample sizes and insufficient statistical analysis, yet I believe this project will be useful in informing future research on the topic of resource limitation in crop yield. This study was able to set a basis for nutrient fluctuations in lowbush blueberry plant tissue and provide relevant analysis on how best to monitor fruit set and yield in lowbush blueberry fields.

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