DETERMINATION OF SOIL AND PLANT NUTRIENT SUFFICIENCY LEVELS FOR HASKAP (*LONICERA CAERULEA* L.)

by

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Dedication

This thesis is dedicated to my late father, Mr. Hillary Alike and to my entire family members for their supports and prayers.

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ABSTRACT

Balanced nutrition is crucial for haskap (*Lonicera caerulea* L.) growth, productivity, and economically viable commercial production. However, there are no clearly established soil fertility and leaf tissue nutrient sufficiency levels. A field survey was conducted in 2015 and 2016 on 19 farms in Nova Scotia to identify optimal soil fertility and leaf tissue nutrient levels from 148 paired samples. Plant growth rate, leaf size and chlorophyll content were determined for the variety Indigo Gem after berry harvest in 2016. Using a boundary line approach, nutrient sufficiency levels in soil by Mehlich III extraction were 80-280 kg P_2O_5 ha^{-1}, 260-570 kg K_2O ha^{-1}, 1300-4000 kg Ca ha^{-1}, and 250-510 kg Mg ha^{-1}, while leaf nutrient sufficiency ranges were 2.23-2.96.0% for N, 0.22-0.28% for P, 0.84-1.32% for K, 1.63-2.10% for Ca, and 0.14-0.50% for Mg. Further research is needed to validate fertility and leaf nutrient sufficiency ranges in relation to haskap yield.

LIST OF ABBREVIATIONS AND SYMBOLS USED

A	Aurora
ANOVA	analysis of variance
B	boron
BB	Berry Blue
BLA	boundary-line approach
BO	Borealis
C	carbon
^{o}C	degrees Celsius
Ca	calcium
CEC	cation exchange capacity
CLT	central limit theorem
cm	centimeter
CND	compositional nutrient diagnosis
Cu	copper
CV	coefficient of variation
cv.	cultivar
CVA	critical value approach
DRIS	diagnosis and recommendation integrated system
E	Erin
Fe	iron
g	gram
ha	hectare

HG	Happy Giant
kg	kilogram
kg ha^{-1}	kilogram per hectare
H_a	alternate hypothesis
H_o	null hypothesis
IG	Indigo Gem
K	potassium
L	Larisa
lb	pounds
LSD	least significant difference
m	meter
Meq	milliequivalent
Mg	magnesium
Mn	manganese
N	nitrogen
n/a	not applicable/available
NB	New Brunswick
NSDA	Nova Scotia Department of Agriculture
NH_4	ammonium-nitrogen
NO_3	nitrate-nitrogen
NS	Nova Scotia
P	phosphorus
PCA	principal component analysis

ppm	parts per million
p-value	probability > F
R	Ruth
R^2	correlation coefficient
SAS	statistical analysis system
SE	standard error
SD	standard deviation
SOM	soil organic matter
SPAD	soil-plant analysis development
T	Tundra
Zn	zinc
$ZnSO_4$	zinc sulfate
α	alpha level
%	percentage
<	less than
>	greater than
≤	less or equal
≥	greater or equal

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CHAPTER 1.

INTRODUCTION

1.0. Introduction

Haskap (*Lonicera caerulea* L.) known as blue honeysuckle or honeyberries is relatively novel crop in Canada and is regarded as a new super fruit due to its health benefits (Bors 2009; Bors et al. 2012). The functional foods and nutraceutical industry in Canada has potential to grow to $50 billion US dollars (Basu et al. 2007) and the increasing demand for powerful antioxidants such as anthocyanin due to its roles in health related issues (Valko et al. 2007; He and Giusti 2010; de Pascual-Teresa et al. 2010; Wallace 2011). Haskap could be a highly sought-after berry crop as it contains high levels of anthocyanin, vitamin C, potassium, phenolic compounds and other antioxidants that is approximately three times the level of antioxidants as in wild blueberry (Arus and Kask 2007; Bakowska-Barczak et al. 2007; Rupasinghe et al. 2012). Haskap is believed to have therapeutic properties and it has been used traditionally by Japanese Ainu aborigines to treat malaria, decrease the effects of glaucoma and risk for heart attack, gastrointestinal diseases, inhibition of anemia and reducing aging process (Anikina et al. 1989; Thompson 2006; Lefol 2007). Also, the emergence of a whole industry extracting antioxidants from fruits to be used in cosmetics, food supplements and fortified foods (Castañeda-Ovando et al. 2009), has given the haskap industry a better grounding to thrive among other industries.

Due to the aforementioned health benefits of haskap berry, the haskap industry in Atlantic Canada is rapidly growing, especially in Nova Scotia (NS) though relatively young, and has been estimated to become approximately a $500 million a year business in the next five years (O'Connor 2015). The only hindrance to achieving this may be tied to slow plant establishment which would,

in turn, affect fruit availability and supply, thereby making the targeted evaluation of the industry unattainable in Atlantic Canada. The major limitation to plant establishment and productivity are the soil conditions, which depend largely on plant root growth and rhizosphere processes such chemical (nutrient availability) and biological processes (plant-microbial interactions). Also, newly cleared forest for orchard establishment is likely to be at a higher risk of failure if adequate measures are not taken to enhance the soil environment. Therefore, there is a need to consider the nutrient management practice for haskap to aid proper plant establishment and fruit production.

Nutrient application is a standard practice of growing crops (Plaster 2009) and the aim of fertilization is to eliminate limitations to yield and quality (Hart et al. 2006) by supplying nutrition in sufficient quantities to sustain maximum crop productivity and profitability while minimizing environmental impacts of nutrient use (Havlin et al. 2014). However, the amount of nutrient required depends on plant characteristics, environmental conditions, soil characteristics, soil and crop management (Havlin et al. 2014). Given this knowledge, there is a need to understand the interactions between the soil, plant, and environment to ensure optimum nutrient availability through effective nutrient management practices (Havlin et al. 2014).

Plants require several nutrients for growth and development. However, nitrogen (N), phosphorus (P) and potassium (K) are particularly essential nutrients for fruit crops (Yadong et al. 2009). Deficiency in any of the essential nutrients will disrupt either the vegetative or reproductive growth cycles in plants (Fuqua et al. 2005). So, improving haskap vegetative growth may improve future berry yield as reported in black currants (Rhodes 1986; McCarthy and Stoker 1988). Therefore, effective fertilizer management requires a good understanding of plant nutritional needs; both in nutrient amount and the time of application of each nutrient (Santos 2011).

The usage of fertilizer should be part of a comprehensive management program. Nutrient application should not be a substitute for poorly timed irrigation, late harvest, or failure to control insects, diseases, or weeds (Hart et al. 2006). Soil characteristics such as high and low pH and/or poor drainage can be important limiting factors to obtaining optimal yields. Also, increasing the rates of fertilizer application or supplementing nutrients already sufficient available will not correct these limiting factors. Therefore, the question of how much to apply, time of application, sources of fertilizer, and method of application need to be addressed prior to fertilizer applications in relation to efficient nutrient management (Hart et al. 2006).

To optimize haskap establishment and productivity, there are no clear established soil fertility recommendation or tissue nutrient standards for haskap (Bors 2009). Phosphorus and K could be significant constraints in bush growth and yield potential for black currants which are similar in stature and fruit yields (Hobson et al. 2013). Therefore, it is important to understand the interaction between soil available nutrient and plant nutrient uptake. This project would seek to find answers to some of these questions but first, would start with the studying and understanding the problems associated with plant establishment, and further with the study of supplementing fertility to enhance establishment and productivity in NS.

1.1. Need for Research

Haskap production is a relatively young but then, rapidly growing industry in NS. For the industry to thrive, sustainable soil management practices need to be assessed in NS orchards. No recommended ranges for soil or leaf tissue nutrient levels are presently available for haskap growers. This information is essential for informing proper soil fertility management practices. Haskap growers spend thousands of dollars (approximately $ 10-15,000/acre) to establish an

orchard and bring it to maturity in four to six years. Therefore, studies are required on soil fertility management in relation to establishment and productivity. In addition, there is no documentation of the level of variability in leaf tissue nutrient levels among haskap varieties.

1.2. Project Goal

The overall goal of this project is to identify optimal soil fertility and leaf tissue nutrient standards for the NS haskap industry.

1.3. Objectives

The specific objectives and hypotheses of this project include:

1. To determine the relationship between soil fertility status and leaf tissue nutrient concentration of haskap.

Ho: There will be no relationship between soil fertility status and leaf tissue nutrient concentrations.

Ha: There will be a significant positive relationship between soil fertility status and leaf tissue concentration.

2. To determine the levels of variability among haskap varieties commonly grown in NS.

Ho: There will be high variability among haskap varieties in terms of leaf tissue nutrient concentrations.

Ha: There will be low variability among haskap varieties in terms of leaf tissue nutrient concentrations

3. To establish the relationship between soil fertility status and leaf tissue nutrient concentration with Indigo Gem physiological characteristics.

Ho: There will be no correlation between soil fertility status and leaf tissue nutrient content with Indigo Gem physiological characteristics physiological characteristics.

Ha: There will be a significant positive relationship between soil fertility status and leaf tissue concentration with Indigo Gem physiological characteristics.

1.4. Thesis Organization

This thesis is written and organized in a manuscript format consisting of five chapters including this present chapter (Introduction). Chapter 2 is the literature review, and this reviews the literature on the origin and distribution, botany of haskap, the role of nutrient management for a sister crop of haskap, the role of soil and plant tissue testing in evaluating the nutritional needs of perennial fruit, and finally, the approaches used in developing nutritional standards. Chapters 3 and 4 will be addressing the objectives; then the thesis will be concluded in chapter 5 (synthesis), followed by the combined references of all the chapters and appendix in different sections.

CHAPTER 2.

LITERATURE REVIEW

2.1. Origin and Distribution of Haskap

Haskap berry, blue honeysuckle, or honeyberry - *Lonicera caerulea* L. is native to Siberia and northeastern Asia, where it is mostly found in low-lying wet or mountainous areas (Bors et al. 2012). Haskap was reported as a horticultural plant in 1894 and domestication attempts started from 1913 in Russia (Hummer 2006). From the early 1950s, Russian breeding programs focused on new cultivar development with the characteristics of higher production, improved fertility, improved nutritional content, larger fruits and even ripening to ease mechanical harvesting (Thompson 2006). Breeding programs started in North America more recently (Bors et al. 2012) and presently, some varieties and cultivars are now accessible in USA and Canada. Canadian growers are using a minimum of five different Canadian cultivars and over 35 Russian, and 70 Japanese varieties (Bors et al. 2012). The Japanese Ainu aborigines uses the plant traditionally (Thompson 2006) due to its therapeutic and medicinal properties to cure malaria, decrease the effects of glaucoma and risk for heart attack, gastrointestinal diseases, inhibition of anemia and reducing aging process and it is regarded by Hokkaido Island as a "gold remedy for the eternal youth and longevity" (Lefol 2007).

2.2. Botany of Haskap

Haskap is a perennial shrub (Arus and Kask 2007) belongs to the genus *Lonicera,* and family of *Caprifoliaceae*, which consist of about 200 species (Thompson 2008). Haskap grows about 2 m or more in height; leaves are simple, opposite, oval to elongated of between 3 and 5 cm (Hummer et al. 2012). Haskap are either diploids (2n = 18) or tetraploid forms (2n = 36) (Miyashita

et al. 2009). Flowers are small, pale yellow to cream in color, about 2 cm long, tubular with flared lobes. The pairs of flowers are usually borne at the lowest one to four nodes of shoots. The flower consists of two tubular corollas and two ovaries surrounded by fleshy bracts (Hummer et al. 2012). Blooming occurs early in spring and bumble bees are the principal pollinators. Blue orchard bees (*Osmia* sp.) also are used in Japanese plantings. Fruits are dark blue to purple berries, with varying amounts of white waxy covering. The shape of the fruit varies from oval to long and thin with a size that ranges from 0.3 g to over 2.0 g (Hummer et al. 2012). According to Thompson (2008), haskap shrubs can withstand -46°C, flowers can survive temperatures between -8º and -10°C, and can tolerate a wider range of pH between 5-8 (Retamales and Hancock 2012). Due to self-incompatibility, haskap requires different cultivars and pollinators such as bumblebees to pollinate and successfully produce fruits (Hummer et al. 2012). Ninety percent of the fruit produced is due to access of pollinators to the flowers while the fruit of isolated haskap plants are smaller and lighter with reduced number of seeds (Bożek 2012). The shrubs of highbush blueberry (*Vaccinium corymbosum*) and Saskatoon berry (*Amelanchier alnifolia*) are similar to haskap in terms of shape and size. Therefore, similar space distribution and harvesting could be used in haskap cultivation (Retamales and Hancock 2012) but need to be investigated. Haskap may be resistant to pests, diseases and requires less attention compared to other small fruit crops making haskap an alternative crop to organic growers (Hummer et al. 2012; Szot and Wieniarska 2012).

2.3. Nutrient Management in Perennial Small Fruit Crops

To achieve the full potential of high yielding crop, balanced plant nutrition is critically essential to ensure acceptable growth and fruit production (Pormale et al. 2009). Plants require several nutrients for growth and development. However, nitrogen (N), phosphorus (P) and

potassium (K) are particularly essential nutrients for fruit crops (Yadong et al. 2009). Deficiency in any of the essential nutrients will disrupt either the vegetative or reproductive growth cycles in plants (Marschner 1995; Fuqua et al. 2005). Therefore, effective fertilizer management requires a good understanding of plant nutritional needs both in nutrient amount and time of application of each nutrient (Mattson and Van Iersel 2011; Santos 2011). The amount of nutrient to apply depends on the age of the plant and soil available nutrients. For instance, mature bushes of highbush blueberry (7 or 8 years plantings on the field) require 160-180 kg N ha^{-1} (145-165 lb N/acre), while young bushes (3-4 years) need 60-68 kg N ha^{-1} (54-60 lb N/acre) (Hanson and Hancock 1996; Hart et al. 2006). In black currant, mature bushes required 100 kg N ha^{-1}, 20 kg P_2O_5 ha^{-1}, and 40 kg K_2O ha^{-1} (Harmat et al. 1990; Barney and Hummer 2005). This could be applicable to haskap as they tend to have similar growth structure i.e. in shape and size, especially to black currant when considering soil pH.

2.3.1. Macronutrients – Roles in Fruit Crop Production

Nitrogen is a key factor that promotes plant growth and yield, also influencing fruit quality (Yadong et al. 2009). The highest demand of N is in the early spring and bloom which is required at the growth and development stages of production (Patrick et al. 2004). In general N application in the spring promotes vegetative growth, while summer applications promotes vegetative and reproductive growth the succeeding year (Christensen et al. 1994). Nitrogen fertilization should be based on tissue N concentration, cane vigor, yield, plant age, soil types and irrigation practices (Barney et al. 2007). In fruit trees, N taken up is sent to aerial organs (bud and bark) in the spring and early summer while in late summer and early fall, N taken up is placed by the roots (Tagliavini and Millard 2005).

The predominant nutrient that is applied for commercial blueberry production is N (Stiles and Reid 1991) and is mostly required during the growth of shoot and fruit (Throop and Hanson 1997). Suitable N fertilizer rates result in improved yield and quality of fruit (Yadong et al. 2009). Too little N can reduce vegetative growth, yield and quality of fruit by reducing fruit- set, berry growth and maturation (Kliewer et al. 1991; Bell and Robson 1999; Schreiner et al. 2013). It was reported that 50 kg N ha^{-1} $year^{-1}$ will stimulate more growth and yield than no N fertilizer during the establishment of highbush blueberry (Bryla et al. 2012), while 100 kg N ha^{-1} or greater in young plants is excessive and will lead to salt stress and plant mortality (Bañados et al. 2012). Harmat et al. (1990) recommended 100 kg N ha^{-1} for black currant production. Similarly, Nova Scotia Department of Agriculture - NSDA (2010) recommended 135 kg N ha^{-1} for small fruit production. Cane growth is an initial indicator of N sufficiency (Barney et al. 2007). Excessive N application can be unfavourable for yield but can also promote vigorous vegetative growth (Spayd et al. 2002; Wheeler and Pickering 2003; Barney et al. 2007). Excessive N supply also results in excessive vine vigor and incomplete vine hardening (Winkler et al. 1974) and poor berry quality due to increased shading clusters thus decreasing color development (Spayd et al. 2002). In addition, several studies have reported a negative influence of increased rate of N fertilization on disease incidence such as powdery mildew in wheat - *Triticum aestivum* L. (Chen et al. 2007), hop – *Humulus lupulus* (Iskra et al. 2016)) and *Botrytis cinerea of* tomato - *Solanum lycopersicum* (Abro et al. 2012).

Phosphorus contributes to yield by participating in metabolism (Lu 2003). Phosphorus promotes early maturation of fruit and enhanced root growth (Spectrum Analytical 2011a). Phosphorus is needed for germination, root development, fruit maturity and quality, also to improve N absorption (NSDA 2010a). Phosphorus plays a key role in energy transfer of the vine

as its necessary for photosynthesis and transforming of sugar to starch and starch to sugar (Winkler et al. 1974; Spectrum Analytical 2011a). Phosphorus deficiency results in reduced flower production and poorly developed root systems, leading to heavily-stressed plants during droughts (NSDA 2010a). Also, P deficiency results in stunted growth, dull gray-green leaves, and premature defoliation and fruit ripening in grapevine (Winkler et al. 1974; Spectrum Analytical 2011a).

In strawberry, P deficiency resulted in small, yellowish green leaves becoming uniformly yellow with reduced fruit size (Domoto 2011; Trejo-Téllez and Gómez-Merino 2014). Harmat et al. (1990) recommended 20 kg ha^{-1} of P_2O_5 for black currant production. NSDA (2010a) also recommended 232-360 kg ha^{-1} of P_2O_5 for small fruit production. However, the rate to apply is dependent on soil test result and the ratings for small fruit presented in Appendix (Table A-1). Phosphorus can be limiting in fruit crop production. Soil pH plays a significant role in nutrient uptake as pH close to 6.5 will aid in maintaining the optimal uptake of P but also depends on the interaction and availability of other nutrients in the soil (May and Pritts 1993; Trejo-Téllez and Gómez-Merino 2014; Havlin et al. 2014).

Potassium plays a key role in the plant development. It is required in relatively high amounts for growth, longevity, over-wintering ability, disease resistance, and cell elongation (NSDA 2010a; Trejo-Téllez and Gómez-Merino 2014). It improves fruit yield and quality attributes such as fruit number, fruit weight, fruit chemical properties, and external fruit color (Lester et al. 2010; Ebrahimi et al. 2012; Lázaro Rodas et al. 2013) and the ability to tolerate stressful environments (Lu 2003). It also influences plant growth and root elongation (Ebrahimi et al. 2012).

Potassium is needed for transportation, production, and storage of carbohydrate in grapevines (Winkler et al. 1974; Spectrum Analytical 2011b). An excessive supply of K resulting

from excessive fertilizer use can lead to high berry K concentrations and often high pH (Conradie and Saayman 1989a). Deficiency of K will result in increased disease problems and dead plant tissues (NSDA 2010a). Deficiency of K also leads to poor vine growth, low yield, premature leaf fall, delayed ripening and low fruit K concentration (Conradie and Saayman 1989b; Kudo et al. 1998; Schreiner et al. 2013). Winkler et al. (1974) reported that K deficiency resulted in a small tight cluster of unevenly ripened various sized grapes and chlorosis in older plants. Harmat et al. (1990) recommended 40 kg ha^{-1} of K_2O for black currant production. NSDA (2010a) also recommended 122-236 kg ha^{-1} of K_2O for small fruit production. However, these rates are dependent on soil test ratings for small fruit presented in Appendix (Table A-1).

Calcium (Ca) is essential for cell division and elongation (Havlin et al. 2014) and vital in protein formation and carbohydrate movement in plants (Plaster 2009). Calcium increases cell wall strength and thickness, thus being a key nutrient for fruit firmness (Easterwood 2002; Trejo-Téllez and Gómez-Merino 2014). Calcium has also been shown to trigger signaling pathways associated with plant growth and development. It also enhances N uptake (Easterwood 2002). Calcium deficiency inhibits the development of shoot terminal buds and apical root tips, resulting in deformed tissues and/or death of young points such as buds, blossoms and root tips (Havlin et al. 2014). Motamedi et al. (2013) established that Ca application had significantly influence plant growth and improve the post-harvest life of berry fruits. NSDA (2010a) recommended 1188-3083 kg Ca ha^{-1} for small fruit production (Appendix, Table A-1).

Magnesium (Mg) ions are found in the center of chlorophyll molecules (Trejo-Téllez and Gómez-Merino 2014). Chlorophyll is an important constituent in photosynthesis, producing energy for plant growth, thus making Mg crucial for plant survival. Magnesium functions considerably in P transportation and support in protein synthesis, and initiation enzymatic activities

(Marschner 1995; Plaster 2009; Trejo-Téllez and Gómez-Merino 2014). However, Mg-deficient plants show marginal and interveinal chlorosis, browning, and burning of leaf blades (Trejo-Téllez and Gómez-Merino 2014). Calcium and Mg deficiencies are rare since these nutrients are contained in dolomitic or calcitic limestone applications used to adjust soil pH (Naugler and Wright 2006). Cations such as K contend with Mg for root uptake, and thus, must be checked to avert one from dominating the other (Trejo-Téllez and Gómez-Merino 2014). NSDA (2010a) recommended 81-432 kg Mg ha^{-1} for small fruit production (Appendix, Table A-1).

2.3.2. Micronutrients - Role in Fruit Crop Production

Iron (Fe) is a micronutrient needed by plants in minor quantities. Nonetheless, it forms part of many important compounds and plays a vital role in plant physiological processes. For example, Fe is involved in the process of chlorophyll production, and it is needed for certain enzymatic purposes. Due to iron's involvement in chlorophyll synthesis, shortage of Fe might lead to chlorosis in young leaves (Trejo-Téllez and Gómez-Merino 2014). Chlorosis caused by Fe shortages is mostly noticed in young leaf blades (Havlin et al. 2014). Domoto (2011) reported that Fe deficiency could result in a slight decrease in fruit size and fruit number produced per plant. However, Fe shortage might not indicate inadequate Fe supply from the soil solely. It may be associated with several conditions such as soil carbonate levels, salinity, moisture, low temperature, and concentration of other elements (e.g. P, Ca), could influence Fe availability (Trejo-Téllez and Gómez-Merino 2014).

Boron (B) is important for plant root growth (Havlin et al. 2014) and flower pollination (Plaster 2009). It can be leached easily from the soil and is very often deficient (Trejo-Téllez and Gómez-Merino 2014). Although B is often recommended as a supplemental nutrient for fruit

crops, extreme levels can be toxic to the plants, so a sufficient amount is required (Handley 2007). Boron deficiency results in marginal yellowing and crinkling of young leaf blades, tip-burn, and interveinal chlorotic areas of leaf blades (Plaster 2009; Havlin et al. 2014). According to Domoto (2011), B shortage leads to reduced flower size and declined pollen production, which results in small fruits of inferior quality. May and Pritts (1993) also reported a positive interaction between B and Ca application on growth and yield of strawberry. They also reported that increasing B at a high P level increases the branch crowns per plant and yield while aboveground biomass weight and fruit weight were also significantly influenced.

Similarly, it has been reported that the application of Ca + B prior to harvest resulted in improved berry firmness, concentration of soluble solids, and fruits that are resilient to Botrytis rot (Wójcik and Lewandowski 2003; Singh et al. 2007). Therefore, nutrient application at the right timing will improve root growth as well as increase fruit quality and could also be beneficial to haskap.

Manganese (Mn) is a crucial micronutrient for numerous plant functions (Trejo-Téllez and Gómez-Merino 2014). During photosynthesis, Mn partakes in carbon dioxide assimilation (Havlin et al. 2014). It helps in chlorophyll synthesis and nitrate assimilation (Plaster 2009). Its shortage may result in yellowing of young developing leaves (Plaster 2009; Domoto 2011; Havlin et al. 2014). Also, Domoto (2011) and Trejo-Téllez and Gómez-Merino (2014) reported that plants with Mn shortage may also show dark green main veining, with interveinal chlorosis, resulting in decreased fruit size. An adequate amount of Mn will significantly improve fruit number, fruit weight, and fruit quality while excess will reduce the number of flowers, which could lead to a reduction in fruit yield (Mehdi et al. 2008; Shahrokhi et al. 2008).

Zinc (Zn) is involved in numerous physiological roles in plants (Trejo-Téllez and Gómez-Merino 2014) and the shortage will reduce crop yields (Hafeez et al. 2013). Likewise, Zn regulates and maintains some genes that are needed for the tolerance of environmental stresses (Cakmak 2000). Zinc shortages result in stunted growth, increased maturity, and poor fruit quality (Hafeez et al. 2013; Havlin et al. 2014). Also, Ullio (2010) found that yellowing and green-veining are common in Zn deficient strawberry plants. Hart et al. (2006) reported that Zn deficiency can be seen in blueberry plants as short internodes and small leaves with the young leaves folding upwards along the midribs.

May and Pritts (1993) reported that yield performance improved while increasing Zn at a high P-rate, but at a low P-rate, yield reduces, showing an interaction of P and Zn. They also reported that tissue Zn content was influenced positively by the P level applied, but not by the Zn level applied. It can be deduced that application of $ZnSO_4$ significantly increases plant height, leaf number, flowers, fruit set, fruits and fruit yield per plant. In strawberry, it has also been reported that adequate amount of Zn significantly influences growth characteristics (like petiole length, leaf area) and yield qualities such as fruit set, fruit weight, and total soluble solids (Abdollahi et al. 2010; Lolaei et al. 2012).

Copper (Cu) plays a part in N-fixation, uptake of Ca and it is a significant component of chloroplasts (Trejo-Téllez and Gómez-Merino 2014). It is also involved in photosynthesis, respiration, lignin formation of cell walls, carbohydrate and lipid metabolism (Plaster 2009; Havlin et al. 2014). Havlin et al. (2014) stated that fruit crops such as apples, blueberries, and strawberries have a mild sensitivity to Cu shortage. Hart et al. (2006) also stated that Cu shortage symptoms include yellowing between veins of young leaves, and in severe cases, young shoots die-back. They also reported that Cu deficiency is more severe on soils with more than 25% organic matter.

In addition, Plaster (2009) stated that Cu deficiency could result in distorted new growth and leaf bleaching, inhibited pollen formation, poor fruiting, and reduced fruit yield. However, excessive Cu fertilization leads to reduced shoot vigor, poorly developed root system and leaf chlorosis (Havlin et al. 2014).

2.4. Soil and Tissue Testing – Role in Nutrient Management

Plant and soil analysis are proven and effective means of predicting fertilizer needs for many crops; particularly perennial crops (Mylavarapu 2010). Soil and plant diagnostics are complementary and serve different purposes. Plant tissue tests aid in monitoring plant nutrient status during the growing season to determine whether each nutrient is present in sufficient concentrations for optimum growth characteristics (Hanson and Hancock 1996; Hart et al. 2006; Mylavarapu 2010). Tissue tests help to ratify hidden and/or nutrient deficiency symptoms and verify toxic phytochemical levels. Tissue tests also indirectly aid in assessing the efficiency of applied fertilizers (Mylavarapu 2010). Plants may not show any visible symptoms, but the nutrient content could be insufficient to reduce yields. In contrast, tissue test results may not be very useful for predicting current-season fertilizer needs of perennial crops such as blueberries (Hanson and Hancock 1996; Hart et al. 2006). This is due to the minimal short-term effect of fertilizer on yield in perennial crops. In blueberry production tissue testing is best used for end-of-season assessment of a fertilizer program. For problems such as poor growth or discoloration of shoots during the growing period, tissue testing can be used to check for nutrient deficiencies. Tissue testing is based on sampling at the proper time; sampling the appropriate plant part, and using standards for comparison (Hart et al. 2006).

Soil testing indirectly evaluates the amount of nutrients available and the percentage of the soil nutrients that will be available during the crop growing period (Hanson and Hancock 1996; Mylavarapu 2010). Leaf analysis shows the plant nutrient status at the specific time of sampling. Conversely, leaf analysis alone may not give a precise representation of nutrient requirements. Soil nutrient levels are one of several factors that control the nutritional status of a plant (Mylavarapu 2010). Factors like temperature, water availability, and management factors also influence soil fertility status and nutrient balance. Due to such complexity, leaf analysis must be integrated with soil analysis (Mylavarapu 2010). However, due to lack of consistent field calibrations of recommendations for new varieties, new crop species and altered management practices, the viability of soil testing will be limited (Mylavarapu 2010).

2.5. Approaches in Nutrient Sufficiency Range Determination

In developing optimum nutritional standards, several methods or approaches has evolved over the years. The critical value approach (CVA) involves looking at individual nutrient concentrations by comparing the nutrient concentrations with reference values. When the nutrient concentrations fall below the reference value, a deficiency is assumed (Bates 1971). This approach does not account for nutrient interactions resulting in criticism from several authors (Wilkinson et al. 2000; Barker and Pilbeam 2007; Marschner 2011). The diagnosis and recommendation integrated system (DRIS) is based on dual ratios (Beaufils and Sumner 1976; Walworth and Sumner 1987), and have also been criticized for not providing a generic approach to support diagnosis of nutrient imbalance or a well-defined covariance matrix for conducting multivariate statistical analysis (Parent et al. 1994). Standard regression methods involving crop yield and tissue

nutrient levels have been used also but are frequently limited to controlled conditions, which can have limited usefulness to growers (McCray et al. 2010).

The plant ionomes diagnosis using sound balances were proposed by Parent et al. (2013) and is based on novel binary classification techniques which is based on a receiver operating characteristics technique. Parent et al. (2013) criticized the statistics on concentration or dual ratios as biased. They proposed the use of isometric log ratios which avoids biases resulting from redundancy of information, incoherence and non-normal distribution. The compositional nutrient diagnosis (CND) is based on multi-nutrient ratios (Parent and Dafir 1992). This method proffer solutions to the limitations of both CVA and DRIS by having a well-defined covariance matrix and also, computes nutrient ratios from concentration values (Aitchinson 1986). The CND-generated nutrient standards are comparable to those of the boundary-line approach (BLA) (Walworth et al. 1986; Vizcayno-Soto and Cote 2004; Quesnel et al. 2006; Blanco-Macías et al. 2009).

The BLA is an alternative to the aforementioned methods and have shown to be an important and effective tool in nutrient norm determinations. The BLA approach was suggested by Walworth et al. (1986) and have been used by several authors to establish optimum nutrient standards for different crops (Vizcayno-Soto and Cote 2004; Quesnel 2004; Blanco-Macías et al. 2009; Bhat and Sujatha 2013). Walworth et al. (1986) and Bhat and Sujatha (2013) used the BLA to establish leaf diagnostic norms by linking tissue nutrient values to crop yield. Furthermore, Vizcayno-Soto and Cote (2004) generated foliar nutrient standards for sugar maple from foliar nutrient values and spatial variation of growth. Casanova et al. (2002) successfully used the BLA in analyzing data that have multiple yield-limiting growth factors. This approach can be used to

quantify the level of a given factor at which optimal performance is found (Webb 1972). The steps involved in using BLA are clearly illustrated in Appendix (Fig. A-1).

2.5. Conclusion

The nutrient application rates of fruit crop similar to haskap, such black currant and highbush blueberry, depend largely on plant age, soil types, management practices, and environmental factors. Also, the rates of applications should be based on soil and tissue testing results. Application of fertilizers without soil and tissue testing could lead to insufficient or excessive supply of nutrient. However, the insufficient or excessive supply of nutrient elements might have adverse effects on growth, fruit yield, and quality, and/or negative impact on the environment. Quantification of the soil and tissue nutrient sufficiency ranges for haskap is needed in addition to a good understanding of how shortage or excess supply affects haskap growth and development.

To achieve the optimum performance of haskap, efficient nutrient management is critical to guarantee not only plant growth and establishment, but also fruit production and responses to environmental indicators. This review has demonstrated that both macro- and micronutrients could have a positive influence as well as negative influence on fruit crop production.

Soil and leaf tissue analysis can aid in assessing the nutrient status of small fruit crops and more precisely determine fertilizer needs, develop or modify fertilizer programs. Finally, an accurate balance in the ratios of nutrients is vital for a balanced uptake of these nutrient elements in plants. As soil and plant tissue recommendations for haskap due not exist, this project seeks to establish these recommendations through soil and plant tissue testing.

To develop nutritional standards for haskap, the adoption of similar approach like the BLA will be an ideal start as it has shown to be an effective tool in this study.

CHAPTER 3.

RELATIONSHIP BETWEEN SOIL AND LEAF TISSUE NUTRIENT CONCENTRATIONS OF HASKAP VARIETIES

3.0 Abstract

Haskap (*Lonicera caerulea* L.) is a rapidly emerging and promising berry crop. However, there is no clear recommendation of soil fertility or tissue nutrient level ranges for haskap. A multi-locational field survey in Nova Scotia was carried out in 2015 and 2016 to evaluate soil and leaf tissue nutrient contents of different varieties of haskap. Soil samples were collected in mid-May, and leaf tissue samples were collected starting from late June to early July in both years during which 50% of the berries had turned color. The results revealed significant difference ($p<0.05$) among the three most common varieties (Indigo Gem, Tundra, and Berry Blue) in tissue P, Ca and Cu. Indigo Gem and Tundra had similar response in nutrient uptake. Correlation analysis revealed significant ($p<0.05$) negative relationships between leaf Mg content with soil and leaf tissue P, K and Ca. The boundary-line approach (BLA) was adopted to show the response trend of haskap to soil available nutrient levels since it could not be related to haskap yield at this point. At 90% maximum leaf tissue levels, the determined BLA for soil fertility levels were 80-280 kg P_2O_5 ha^{-1}, 260-570 kg K_2O ha^{-1}, 1300-4000 kg Ca ha^{-1}, and 250-510 kg Mg ha^{-1}. More than 50% of the locations were deficient in soil K, 71% had adequate Ca, 51% had adequate P, and 46% were deficient and 46% adequate in Mg. Future research should consider plant growth performance in validating the determined BLA soil fertility levels.

Keywords: Berry crop, haskap, leaf tissue, *Lonicera caerulea* L., nutrient status, soil fertility, boundary-line approach.

3.1 Introduction

Haskap (*Lonicera caerulea* L.) is a perennial shrub native to Siberia and northeastern Asia where it is mostly found in low-lying wet or mountainous areas (Bors et al. 2012). Haskap was first reported as a horticultural plant in 1894 in Russia (Hummer 2006). Haskap commonly known as haskap berries, blue honeysuckle or honeyberries is a relatively new fruit crop for Canada and is presently not well known in North America (Bors 2009; Bors et al. 2012). It is believed that haskap berries may possibly substitute blueberries as a new super fruit (Bors et al. 2012) due to its high levels of anthocyanin, phenolic compounds and other antioxidants (Arus and Kask 2007; Bakowska-Barczak et al. 2007; Rupasinghe et al. 2012). For instance, it was reported that antioxidant content of haskap is nearly three times that of wild blueberry (Rupasinghe et al. 2012).

Plant growth and establishment vary widely among orchards in Nova Scotia (NS), which could be attributed to variability in environmental conditions and management practices as reported for other plants (Mylavarapu 2010; Khan et al. 2011). Plant nutrient uptake is dependent on crop species, cultivar or genotypic variations within species (Kowalenko 2005; Fageria 2016). In addition to genetic characteristics, soil factors such as type, nutrient composition, moisture and temperature influence soil fertility status (Mylavarapu 2010) and tissue nutrient status (Dresler et al. 2015). These factors also influence plant growth, productivity and quality (Khan et al. 2011). Other soil factors that influence phytoavailability of mineral nutrients include pH, organic carbon (OC), cation exchange capacity (CEC), (Kabata-Pendias 2004; Bhat and Sujatha 2014). Buskienė and Uselis (2008) stated that only fertile soil with a regulated pH and optimal mineral nutrient supply can ensure adequate yield and quality. Therefore, adequate fertility is of utmost importance for haskap growth and yield.

It is important to determine the nutritional status of perennial crops (Stellacci et al. 2010) in order to minimize environmental impact and optimize fertilizers use efficiency. In diagnosing plant nutritional status, tissue testing is considered to be a useful and practical approach (Kelling et al. 2000; Memon et al. 2005; Self 2005). However, the combination of both plant tissue and soil analysis would be a more effective and useful tool not only in predicting and determining nutritional status but also help in management decisions for improving nutritional requirements for perennial crops (Porro et al. 2001; Niskanen 2002; Niskanen and Dris 2002; Rashid 2005; Mylavarapu 2010; Stellacci et al. 2010). Therefore, the study of the relationships between parameters such as soil characteristics and leaf tissue nutrient concentrations provides a better understanding of synergistic and antagonistic phenomena which aid in defining standards for plant analysis interpretation (Stellacci et al. 2010). Hence, it appears quite suitable for haskap as a perennial crop also.

Soil and plant diagnostics are complementary and serve different purposes. Soil testing estimates the fertility status and potential supply and balance of nutrients that will be available during the crop growing period (Mylavarapu, 2010). Plant tissue analysis shows nutrient status at the time of sampling and can also be used to identify hidden hunger of plants (Kelling et al. 2000; Tisdale et al. 2002; Rashid 2005; Mylavarapu 2010; Self 2014). Conversely, plant analysis alone may not give a precise representation of nutrient requirements (Mylavarapu, 2010).

The assessment of leaf nutrient status (both macro- and micronutrient) is crucial for attaining expected yield (Kowalenko 2005). However, there is no published literature describing the relationship between soil and leaf tissue nutrient content relationship in respect to haskap nor clear recommendations for soil fertility or tissue nutrient level ranges. It is therefore hypothesized that haskap varieties would respond differently to soil fertility status in terms of nutrient uptake,

and soil fertility status will correlate positively with leaf tissue nutrient content. Thus, this study seeks to: 1) examine variability in leaf tissue nutrient concentrations of haskap varieties, and 2) investigate the influence of selected soil properties on leaf tissue nutrient content of haskap growing under different environmental conditions and nutrient management practices.

3.2 Material and Methods

3.2.1 Experimental Design and Location

The relationship between soil fertility and leaf tissue nutrient status of multiple varieties of haskap was carried out as a survey of haskap on multiple farms with a range of soil conditions primarily in Nova Scotia. The farms are located in Kings County (5 farms), Colchester County (5 farms), Lunenburg County (8 farms), and Pictou County (1 farm) in NS, and one farm in New Brunswick (NB). The orchards in Pictou County and NB were only sampled in 2016. The ages of the plants ranged from 2 to 5 years old; sampling occurred only on plants that were in the field for at least one full growing season after transplanting to ensure that tissue nutrient levels reflected soil conditions. Multiple varieties at each site were sampled, and sometimes from several locations/fields with different fertility management histories on the same farm. Each unique soil-tissue-variety combination comprised of soil and leaf tissue samples collected from the same section of a row of one variety. A total of 148 soil-leaf tissue combinations were collected from 17 commercial farms from 2015 to 2016. From 2 to 30 samples were collected from a total of 10 varieties depending upon availability on the farms. The varieties Indigo Gem (IG), Tundra (T), and Berry Blue (BB) were all present on almost all the farms and thus were selected for more detailed comparison and IG was selected as a key reference variety. The nutrient management history for each farm was documented.

3.2.2 Soil Sampling and Analysis

A total of 148 soil samples were collected from mid May 2015 and 2016 from a 15 to 20 m section of row for each variety beginning from the 10^{th} plant. Each soil sample consisted of a composite of at least 10 subsamples for each variety to a depth of 15 cm using a sampling probe. Soil samples were stored in a cooler during transportation and refrigerated until analysis at the NS Agricultural Laboratory Services for standard soil test analysis including Mehlich III mineral nutrient concentrations (Mehlich 1984). Mineral nutrient concentrations were determined using Mehlich III solution (0.2 M acetic acid (CH_3COOH) + 0.25 M ammonium nitrate (NH_4NO_3) + 0.015 M ammonium fluoride (NH_4F) + 0.013 M nitric acid (HNO_3) + 0.001 M ethylene diamine tetra-acetic acid (EDTA)), according to Mehlich (1984). Air dried soil samples (10 g) were weighed in 50 mL test tubes, 25 mL of Mehlich III extracting solution were added and shaken for 5 min. using a reciprocating shaker. The solutions were filtered through Whatman #42 filter paper and the resulting filtrate were used to determine mineral nutrient concentrations.

3.2.3 Leaf Tissue Sampling and Analysis

A composite of whole leaf samples was collected for each variety from approximately 20 plants in the row length where the soil samples were collected. Leaf tissue samples were collected when 50% of the berries had turned color in late June - early July 2015 and 2016. Leaves were collected from new stem growth three to five nodes down from the tip of the branch. The leaf samples were stored in a cooler during transportation and refrigerated until submission to the NS Analytical Laboratory for elemental determination by inductively-coupled plasma spectrometer (ICP, Thermo Fisher Scientific Inc., Waltham, MA). Leaf samples were cleaned with distilled water, air dried and then packed in paper bags. The packed leaf samples were oven dried at 60 ^oC

for 48 hours to a constant weight and ground. The grounded sample were digested using sulfuric acid-hydrogen peroxide (Wolf 1982) and analyzed for N, P, K, Ca, Mg, Cu, Mn, B, Zn, Al, and Fe using inductively-coupled plasma spectrometer (ICP, Thermo Fisher Scientific Inc., Waltham, MA).

3.2.4 Statistical Analysis

Analysis of variance (ANOVA) was used to test for variety effects among Indigo Gem (IG), Tundra (T), and Berry Blue (BB) growing under the same environment, soil conditions, and management practices. The analysis was done using PROC GLM procedure in SAS (9.2, SAS Institute, Cary, NC) at $\alpha = 0.05$. Considering that the sample size was large enough ($n > 30$), central limit theorem (CLT) was applied since various transformation methods could not satisfy ANOVA assumptions (Hogg et al. 2015). Where there was a significant difference, means were separated using Fisher's protected least significant difference (LSD) test at 5% probability level ($\alpha = 0.05$). Futhermore, correlation analysis was also performed to study the relationships among nutrients levels in the soil and leaf tissue.

3.2.5 Boundary-line Approach

The BLA approach was suggested by Walworth et al. (1986), used by Blanco-Macías et al. (2009), Vizcayno-Soto and Cote (2004) and Bhat and Sujatha (2013) to establish optimum nutrient norms for prickly pear (*Opuntia ficus-indica* L.), sugar maple (*Acer saccharum* Marsh.), and arecanut (*Areca catechu* L.), respectively. Traditionally, the points relating nutritional status to crop yield or relative crop yield are plotted and the line relating the maximum yield observed over a range of nutrient values measured is regarded as the optimum nutrient level for optimum crop

productivity while points below this line relate to plants where other factor hinders response to nutrient (Schnug et al. 1996; Vizcayno-Soto and Cote 2004; Blanco-Macías et al. 2009; Bhat and Sujatha 2013). However, in this study, it is only adopted to show the response trend of haskap to soil available nutrient levels since it could not be related to haskap yield at this point.

The first steps involved using scatterplots in plotting data of leaf tissue nutrient (on Y-axis) vs. soil available nutrient levels (on X-axis) for one nutrient at a time (bivariate relations) to analyze the patterns of distribution. This would help in analyzing the potential and suitability for use (Fig. A-1a) and identify obvious outliers. The second step involved using an iterative procedure to select the highest points of the scatter of points. This was done by splitting the soil available nutrient levels (independent variable) into 10-20 intervals (Fig. A-1b) and the highest points for each interval were selected (Fig. A-1c). The basis for using 10-20 intervals as stated by Vizcayno-Soto and Cote (2004), is to use < 25% of the observations to develop a model. This would help to limit the selection of points to the superior boundary of the scatterplot and to maximize the possibility of developing statistically significant models by increasing the number of observation.

The third step consisted of applying the new data subset of leaf tissue and soil available nutrient levels to a fitted second-degree polynomials regression (Fig. A-1d). Together with the BLA and line of best fit regression, a graph was produced showing nutrient levels in Fig. 3.3. Prior to boundary-line approach (BLA), box and whisker plot were used to screen the data set from outliers. All outlier points found were removed from the data set (Fig. A-2 and A-3). Scatter plots and boundary-line regression was done using Minitab software (version 17.3.1).

3.2.6 Determination of Nutrient Ratios and Range

Nutrient ratios are important for small fruit nutrition. A similar procedure used by Bhat and Sujatha (2013) in estimating nutrient ratios for arecanut (*Areca catechu* L.) was followed. The nutrient ratios were estimated by dividing the concentrations of nutrients in haskap leaves. In addition, the mean, range (min. and max.), and coefficient variations were also determined. Black currant nutrient ratios used in this study were calculated from Barney and Hummer (2005) nutrient sufficiency ranges and was also used for comparison purposes, considering its similarity with haskap.

3.3 Results and Discussion

3.3.1 Soil Chemical Composition

A wide range of soil chemical properties was observed in the test haskap orchards (Table 3.1). This is desirable as this permitted evaluation of the plant tissue nutrient response across a wide range of conditions. Soil pH varied from 5.11 to 7.04. While haskap can tolerate a wider range of pH between 5-8 (Retamales and Hancock 2012), a recommended pH range has not been established. For instance, approximately 8% of the soils from the sampled locations had pH below the optimum range of 5.5-7.0 recommended for black currant – *Ribes nigrum* (Barney and Hummer 2005). Therefore, it is not very different from the above findings for the various locations.

Extreme levels of macronutrients were observed among soils of the sampled locations. For instance, based on soil fertility recommendation for small fruit crops (Nova Scotia Department of Agriculture - NSDA 2010a), approximately 40% of the locations were extremely high in soil P levels and 45% were lower than the recommended range of 232-360 kg ha^{-1} (Table 3.1). Similar trends were also observed for soil K and Ca. These findings could be attributed to variability in

soil properties, climatic factor and/or soil management practices such as fertilization and/or manure application (Prive and Sullivan 1994; Hargreaves et al. 2008) in the sampled orchards.

In addition, P-availability in acidic soil is controlled by soil pH and Al + Fe contents therefore, making it challenging to estimate how much P that would be available during the growing season (Government of Prince Edward Island - PEI 2017). This is due to the fact that Al and Fe oxides are the major components of P fixation in acidic soils. According to Beauchemin and Simard (1999), P saturation indices could be a reliably estimated using P, Al, and Fe Mehlich III test values. The P/Al has been used as a basis for P fertilization recommendations for potato crop in New Brunswick (Government of New Brunswick 2010) and Quebec (CRAAQ 2010). In PEI, the critical P saturation (P/Al) is 14% at soil pH >5.5 while at pH <5.5 is 19% (Government of PEI 2017). In this study, the minimum and maximum value of P/Al with soil pH range of 5.11-7.04 seems to below and above the P/Al recommended for PEI soil (Table 3.1). However, this could not be said of Nova Scotia soils but could be applicable. Therefore, there is need to further understand how this affects Nova Scotia soils and its impact on haskap productivity.

3.3.2 Leaf Tissue Nutrient Composition

Leaf tissue nutrient content was compared with other small bush crops since recommended ranges of nutrients in haskap are not available. Black currant, another small bush plant with similar size and growth habit as haskap, already has leaf tissue nutrient recommendations established (Barney and Hummer 2005). Based on black currant sufficiency levels (Barney and Hummer 2005), the average N, P and K values are below the sufficiency range for optimum productivity. Also, the highest values measured exceeded levels recommended for black currant. The average

values for Ca and Mg content were within or exceeded recommended levels for black currants (Table 3.2; Barney and Hummer 2005).

The average leaf tissue micronutrients are within the recommended sufficiency range for black currant (Barney and Hummer 2005) and highbush blueberry - *Vaccinium corymbosum* (NSDA 2010b). Highbush blueberry is another small bush crop cultivated under acidic soil conditions with tissue nutrient sufficiency levels. Using highbush blueberry sufficiency ranges (NSDA 2010b), 62% of the locations were low in leaf P content while 68% had low leaf K content. It has been reported that nutrient uptake by plants is influenced by management practices, plant genetic variation, climatic conditions and soil characteristics (Prive and Sullivan 1994; Reickenberg and Pritts 1996; Daugaard 2001; Rakshit et al. 2015). The differences between location in terms of leaf tissue nutrient levels could be related to environmental and soil conditions.

In addition, the weed management practices across the orchards in this study could have a negative or positive influence on nutrient availability and nutrient uptake as reported for Blackberry - *Rubus* L. subgenus *Rubus Watson* (Harkins et al. 2014; Dixon et al. 2016). For instance, leaf N and Mg have been found to be significantly low in no-weeded management compared to the use of weed mat (Harkins et al. 2014). According to Dixon et al. (2016), no-weeded management reduced nutrient content of nutrients in primocanes, floricanes, and fruit, whereas the use of weed mat resulted in higher nutrient content accumulation. They all concluded that weed management had the largest impact on plant nutrient content and biomass. The use of wood-chip could also have a negative influence on N availability. Wood-chips are known to have high carbon to nitrogen ratio which may result to N deficiency. This is due to use of N by soil microbes thereby immobilizing N (University of Massachusette Extenion Fact-sheet: https://ag.umass.edu/sites/ag.umass.edu/files/fact-sheets/pdf/mulching_fruit.pdf). This could be

the case of haskap in the study locations where weed management varied from cultivation at the base of plant, use of coconut fiber mats at plant base, wood-chip mulch to no-weeding at all.

Overall, most average of macronutrient levels seem to be below or above the nutrient sufficiency ranges of black currant and/or blueberry, but the micronutrients all seem to be within the sufficiency range. Leaf Ca and Mg content could be said to be very high. The high leaf tissue Ca and Mg could be attributed to high soil N. High soil N has been reported to decrease leaf tissue P and K and increase leaf Ca and Mg (Boynton and Compton 1944). However, this could not be ascertained in this study considering that the soil N status on the sites could not be confirmed.

3.3.4 Berry Blue, Indigo Gem, and Tundra Comparison

Haskap varieties Berry Blue (BB), Indigo Gem (IG), and Tundra (T) were among the earliest planted in NS and were present on almost all sites visited. Thus, the differences among these varieties could be compared in more detail. The results of leaf tissue nutrient contents of the three haskap varieties are presented in Fig. 3.1.

The percentage N, K and Mg contents in the leaf tissue among BB, IG, and T was not significantly different ($p>0.05$). The observed N and K levels among varieties were below the N sufficiency range of 2.7-2.9% for black currant (Barney and Hummer 2005) and are within highbush blueberry sufficiency range (NSDA 2010b) while Mg content of the test varieties was higher than both highbush blueberry and black currant. However, the time of leaf tissue sampling could affect the N content levels. According to Daugaard (2001), tissue N content is lower during fruit formation and development stage than other vegetative growth stages in strawberry plant *(Fragaria × ananassa)*. Using blackcurrant recommendation (Barney and Hummer 2005), the observed levels of N and K tend to be deficient and could impede growth and yield of haskap. The K deficiency observed points towards nutrient imbalance between N:K, K:Ca and K:Mg ratios.

The average leaf tissue P content was significantly higher in BB than in T and IG, indicating that BB might respond differently to P nutrition or has a better ability to extract P. It has been reported by several authors that the colonization of plant roots by arbuscular mycorrhizal promotes improves P acquisition (Roy-Bulduc and Hijri 2011; Smith et al. 2011; Nouri et al. 2014). This may be the case in BB but it could be ascertained. Also, the average Ca content (%) was significantly higher in IG than in T and BB (Fig. 3.1a). This suggest that IG might have higher Ca nutrition than T and BB.

In terms of micronutrient content, no statistically significant differences ($p>0.05$) were found in B, Zn, Mn and Fe contents of leaf tissues among IG, BB, and T but Cu content was significantly ($p<0.05$) higher in BB than in T and IG (Fig. 3.1b). In a similar study, variations in nutrient uptake mechanism among different variety of raspberry (*Rubus idaeus* L.) growing in the same soils under same growing conditions have also been reported (Daugaard 2001; Horuz et al. 2013)**.** However, the levels of the aforementioned leaf tissue nutrients are within the sufficiency range for other small fruit crops such as black currant (Barney and Hummer 2005) and highbush blueberry (NSDA 2010b) except for BB and IG, which tend to have higher leaf B and Mn levels respectively than both crops (Table 3.2).

In general, it can be inferred that IG and T are similar in terms of leaf tissue nutrient response, but slightly different from BB. According to Bors (2011), IG and T are closely related genetically having been progeny from the same parental cross. Therefore, it is expected for both variety to response in a similar manner due to the similarity in their genetic characteristics.

3.3.5 Varietal Comparison to Indigo Gem

Indigo Gem is the most commonly grown variety and since IG was present on all sites, it is being used as a reference value to which all other varieties are compared. The relative comparisons of the nutrient contents of haskap varieties in relation to IG growing in the same field are presented in Fig. 3.2. In general, there were wide variations in leaf tissue nutrient contents among varieties. For instance, the Erin variety had 36% less tissue N content, Aurora 48% more leaf P content, and Borealis 39% more Mn content than IG (Fig. 3.2a). Similarly, in terms of micronutrients, Borealis had 72% more Mg content, Ruth had 62% more Fe content, and Blue Perfection had 38% more tissue Zn content than IG (Fig. 3.2b). For the aforementioned varieties, there could be a need for individual leaf tissue nutrient calibration.

Indigo Gem had lower P, K and Fe than most other varieties but was higher than most varieties in Ca, B, Cu and Mn with a few exceptions. The least overall variability among varieties not including IG was in K, Ca and B and the greatest variation was in Mg. However, in most cases all varieties were within 40% of the IG value and in many cases within 20%. These findings are similar to those of Horuz et al. (2013) in raspberry varieties. They reported that plant of the same species but different cultivars growing in same soils under same conditions varies in terms of nutrient uptake mechanism. Kabata-Pendias (2004) and Bhat and Sujatha (2014) also reported that apart from soil characteristics, plant variety also influence nutrient elements uptake, which is likely to be the same for haskap.

With regards to variability in nutrient uptake among varieties, growers could either sample only IG as a reference, or collect samples evenly from across all varieties in the field. Indigo Gem can be used as a reference variety for assessing haskap nutritional status, but it should be

recognized that it is lower than other varieties in P and K, and higher than other varieties in leaf Ca.

3.3.6 Nutrient Ratios and Soil - Plant Relationships

Based on Wilding (1994) criteria, haskap N:P ratio within sites showed medium variability with CV of 26.03%. However, high levels of variability were observed in N:K, N:Mg, P:K, Ca:Mg, K:Ca, and other nutrient ratios with coefficient of variability ranging from 37 to 72%. This could be due to variety, soil characteristics, and nutrient management practices.

The N:K ratio ranged from 1.09-9.43 with an average that is higher than the N:K ratio of raspberry and black currant (Table 3.3). This could mean that haskap had sufficient to excess N relative to other nutrients. Du et al. (2010) reported that N intake is limited when N:P is less <14.5 and N:K <2.1. This could be different for haskap plants. Leaf N had a significant positive correlation with soil available nutrients, negative association with soil Al, and no correlation was found with soil Mg, S and Mn (Table 3.4). Also, a significant negative association was observed between leaf N and Mg, while no relationship was found with leaf Fe and Mn (Table 3.5).

The N:P ratio in haskap leaves varies from 4.08–16.08 (Table 3.3). The average N:P ratio of haskap exceeded that for raspberry (9.0) (Horuz et al. 2013) and was below that for black currant (10.0) (Barney and Hummer 2005). This could be an indication of P deficiency resulting from excess N nutrition and/or shortage of P (Tessier and Raynal 2003; Güsewell 2004; Horuz et al. 2013; Dresler et al. 2015). However, Piao et al. (2005) reported that P intake could also be limiting when N:P is >10.

The average P:K ratio of haskap was higher than the calculated reference value of 0.22 for black currant (Barney and Hummer 2005). This suggest that K might be deficient or haskap could

have a higher P:K ratio and/or a possible imbalance among the two nutrients. A similar finding in P:K ratio was also reported by Horuz et al. (2013) in raspberry fields in Turkey. A significant positive correlation was observed between leaf P and leaf N, K, Cu and Zn while no significant correlation was found between leaf P and leaf Ca, Mg, B, Fe and Mn (Table 3.5).

The N:K ratio varies from 1.09-9.43 in haskap with an average N:K ratio higher than the calculated reference value for black currant (Table 3.3; Barney and Hummer 2005) and 2.5 for raspberry (Horuz et al. 2013). According to Delgado et al. (2004) and Horuz et al. (2013), this indicates K deficiency due to relatively high N availability compared with K; which may also be the case in haskap. Also, It has been reported that excess N uptake can disrupt the interaction between N and K, resulting in K deficiency (Spiers 1993; Horuz et al. 2013; Dresler et al. 2015). However, the tissue K content can vary with time of sampling. Chen et al. (1999) stated that leaf tissue K decreases during the fruiting period when compared with other stages of growth, this is also likely to be the case in this study.

The average K:Ca ratios in haskap was below the calculated value for black currant (Barney and Hummer 2005) and critical value (1.16) for raspberry (Horuz et al. 2013). This suggests that haskap plants could be having too much Ca and a risk of K deficiency. It is also likely that haskap could be deficient in K since the average K:Mg ratio was four times below that of black currant (Table 3.3). This also an indication of a possible antagonistic relationship between K and Mg (Spiers 1993). The leaf tissue K content was significantly ($p<0.05$) correlated positively with leaf N, P and Zn but there was no significant relationship between leaf K and Ca (Table 3.5). A significant negative correlation between leaf K with leaf Mg (Table 3.5) also signifies antagonistic association between these nutrients.

The average N:Mg ratio (5.85) was below the critical value (15) for raspberry (Dresler et al. 2015) and the calculated black currant value (21.54). According to Horuz et al. (2013); this further confirm that haskap had sufficient to excess Mg supply. Meanwhile, since the average Ca:Mg ratio was higher than the calculated value for black currant, this also suggests sufficient to excess Ca supply (Horuz et al. 2013; Dresler et al. 2015). Several authors have also reported that regardless of the crop type, increase in K, Ca, or Mg levels can lead to low uptake of any of the other two nutrient, and the uptake of K and Ca is influenced by the antagonistic effect of NH_4 (Fageria 2001; Kacar and Katkat 2009; Horuz et al. 2013). The correlation coefficients revealed that leaf Mg levels in haskap was negatively correlated with soil P, K and Ca content (Table 3.4) and also, with leaf N, K and Ca content (Table 3.5), which further confirms the antagonistic interaction between K, Ca and Mg (Fageria 2001; Dresler et al. 2015).

The average Ca:B ratio was also higher than raspberry critical value (450) (Horuz et al. 2013) indicating a possible risk of B deficiency. This is further supported by the fact that the average soil available B content was low (Table 3.1). The leaf B content had a significant positive correlation with K content in soil (Table 3.4) as well as with leaf N content (Table 3.5).

Considering that there is no clearly established soil fertility or leaf nutrient sufficiency levels for haskap, it could not be ascertained if the above findings are applicable to haskap. It is also possible that haskap will be needing lower and/or higher nutrient ratios than that of black currants and raspberry used as references in this study.

3.3.3 Boundary-Line Approach

The boundary-line for soil P, K, Ca, and Mg with 10, 16, 12, and 14 intervals, respectively, produced a curvilinear response typical of a nutrient response curve for controlled conditions (Fig. 3.3). The BLA models for soil P, K, and Mg were significant with R^2 values ranging from 0.51-

0.70 ($p \leq 0.10$), while no significant model was produced for soil Ca ($R^2 = 0.31$; $p \geq 0.10$). At 90% maximum leaf tissue P, K, Ca, and Mg, soil levels appear to be in the range of 80-280 kg ha^{-1} for P, 260-570 kg ha^{-1} for K, 1300-4000 kg ha^{-1} for Ca, and 250-510 kg ha^{-1} for Mg in soil (Fig. 3.3). However, to be more economical and nutrient efficient, the ranges 80-190 kg P_2O_5 ha^{-1}, 260-420 kg K_2O ha^{-1}, 1300-2700 kg Ca ha^{-1} and 250-390 kg Mg ha^{-1} should not be exceeded as the derived BLA upper limit did not increase nutrient uptake. The minimum and maximum limit of the BLA derived soil fertility ranges for haskap appeared to be higher than the NSDA (2010a) soil fertility recommendations for small fruits crops, except for soil P with minimum and maximum limits.

Using the generated BLA soil fertility levels in assessing locations on the level of soil available nutrients, 51% of the locations were deficient in soil K, while more than 71% were adequate in Ca. Excessive levels of soil available nutrients were found in 41% of the cases for soil P. The BLA revealed that soil P and Mg were in adequate amount in 51% and 46% of the locations, respectively.

3.4 Conclusion

The study of the relationships between soil and leaf tissue nutrient content carried out in haskap orchards characterized with high spatial variability, revealed a clear influence of soil fertility status on mineral nutrient absorption and build-up. Due to the variability in nutrient uptake among varieties, growers should either sample only IG as a reference, or collect samples evenly from across all varieties in the field. Indigo Gem can be used as a reference variety for assessing leaf tissue nutrient status, but should be recognized that it is lower than other varieties in P and K, and higher than other varieties in Ca. From the varietal comparison results, IG and T have similar response to fertility status which is due to similar genetic characteristics.

Correlation analysis revealed significant negative relationships between leaf Mg content with soil and leaf tissue P, K, and Ca. The results indicate that K is deficient due to imbalance or K shortage and it most likely be related to Mg. In addition, the BLA was adopted in this study to show the response trend of haskap to soil available nutrient levels since it could not be related to haskap yield at this point. At 90% maximum leaf tissue levels, the determined BLA soil fertility levels were 75-280 kg P_2O_5 ha^{-1}, 270-580 kg K_2O ha^{-1}, 1250-4000 kg Ca ha^{-1}, and 245-510 kg Mg ha^{-1}. However, more studies will be needed to verify and explain the nutrient uptake mechanism of haskap in a controlled growth conditions, especially relating the determined BLA soil fertility levels to haskap growth and fruit yields.

Table 3.1. Soil pH and extractable nutrients of haskap orchards in 2015 and 2016: mean, standard deviation (SD), minimum and maximum levels as observed and compared with recommended ranges for small fruit production (values represent the sufficient range for crop requirements).

Parameters	Units	Mean	SD	Minimum	Maximum	Recommended range for small fruits [a]
pH	-	5.95	0.46	5.11	7.04	5.5-7.0 [b]
SOM	%	5.15	1.58	2.40	9.50	n/a [c]
P_2O_5	kg ha^{-1}	709	879	39.0	3989	232-360
K_2O	kg ha^{-1}	290	164	65.0	753	122-236
Ca	kg ha^{-1}	2499	1540	784	9291	1188-3083
Mg	kg ha^{-1}	306	167	91.0	933	81-329
S	kg ha^{-1}	34.92	12.89	15.0	87	n/a
Na	kg ha^{-1}	33.16	12.76	16.0	103	n/a
P/Al [d]	%	28.44	50.13	0.90	354.75	n/a
P/(Al + Fe)	-	0.23	0.36	0.01	2.46	n/a
Al	mg kg^{-1}	1466	262	469	1885	n/a
B	mg kg^{-1}	0.67	0.21	0.50	1.13	n/a
Cu	mg kg^{-1}	4.14	3.70	0.48	20.23	n/a
Fe	mg kg^{-1}	187	59.86	84.0	335	n/a
Mn	mg kg^{-1}	39.36	25.45	11.0	150	n/a
Zn	mg kg^{-1}	5.90	6.89	0.91	37.60	n/a
CEC	cmol kg^{-1}	11.5	3.8	4.50	31.10	12-25

[a] Nova Scotia Department of Agriculture (NSDA) (2010a) soil interpretation ratings for small fruit crops in Nova Scotia
[b] Barney and Hummer (2005) soil fertility recommendations for black currants.
[c] No available recommendation
[d] P were determined by converting P_2O_5 to P by multiplying with 0.436.

Table 3.2. Leaf tissue nutrient content from haskap orchards in 2015 and 2016: mean, standard deviation (SD) and range as observed with recommended levels for small fruit crops (values represent the sufficient range for crop requirements).

Nutrient(s)	Mean	SD	Range	Recommendations for small fruit crops	
	Macronutrients (%)			Black currant [a]	Highbush blueberry [b]
N	2.11	0.40	1.20-3.19	2.70-2.90	1.50-2.50
P	0.25	0.08	0.09-0.52	0.26-0.30	0.10-0.40
K	0.83	0.33	0.23-1.69	1.00-1.60	0.30-0.80
Ca	1.76	0.38	1.03-3.19	1.00-1.50	0.20-0.70
Mg	0.43	0.15	0.15-0.94	0.10-0.15	0.10-0.25
	Micronutrients (ppm)				
Na	0.02	0.00	0.02-0.03	n/a [c]	n/a
B	37.14	14.46	10.37-96.88	20-40	20-70
Cu	8.46	2.04	5.05-14.64	5-20	5-20
Fe	76.87	36.98	37.01-393	n/a	40-150
Mn	59.95	38.72	14.97-284	20-70	50-350
Zn	17.83	6.28	6.39-38.04	20-50	10-50

[a] Barney and Hummer (2005) nutrient sufficiency ranges for black currants

[b] Nova Scotia Department of Agriculture - NSDA (2010b) sufficiency range for highbush blueberry

[c] No available recommendation.

Table 3.3. Leaf tissue nutrient ratios of all 148 haskap samples from orchards in Nova Scotia in 2015 and 2016.

Nutrient ratios	Mean	Range	CV (%)	Black currant [a]
N:P	9.28	4.08-16.08	26.03	10.0
N:K	2.99	1.09-9.43	48.28	2.15
P:K	0.34	0.12-1.12	54.40	0.22
N:Mg	5.85	1.90-19.93	51.93	21.54
Ca:Mg	4.67	1.96-13.28	47.14	9.60
Fe:Mn	1.96	0.36-6.77	68.31	-
Fe:Zn	4.76	1.94-22.45	57.87	-
Ca:B	536	147-1818	43.54	-
K:Ca	0.52	0.09-1.68	52.86	1.04
K:Mg	2.43	0.24-12.00	72.04	10.0
Ca:P	7.88	3.26-16.38	36.92	4.46

[a] Ratios were calculated from black currant sufficiency levels recommended by Barney and Hummer (2005)

-: No available recommendation.

Table 3.4. Significant Pearson correlation coefficients (R^2) between Mehlich III extractable soil and leaf tissue nutrient contents (LNC) in haskap from 2015 and 2016 at $\alpha = 0.05$.

LNC	Mehlich III extractable soil nutrient content									
	P_2O_5	K_2O	Ca	Mg	S	Al	Cu	Fe	Mn	Zn
N	0.41	0.56	0.40	.	.	-0.37	0.31	0.50	.	0.36
P	0.31	0.25	0.40	0.49	-0.34	-0.54	0.46	0.50	.	.
K	0.55	0.64	0.45	0.30		.	0.34	0.42	.	0.27
Ca	.	.	.	.	.	.	.	.	.	0.35
Mg	-0.34	-0.52	-0.36	0.24	-0.30	.	-0.31		0.34	.
B	.	0.28	.	.	.	.	.	.	.	.
Cu	.	0.25	.	.	.	-0.25	0.25	0.28	.	.
Fe	.	.	.	.	.	.	-0.30	.	0.34	.
Mn	.	.	.	.	.	.	.	0.26	0.39	.
Zn	.	.	.	.	.	-0.35	.	0.34	.	.

"." - Not statistically significant at $\alpha = 0.05$.

Table 3.5. Significant Pearson correlation coefficients (R^2) between leaf tissue nutrient contents in haskap from 2015 and 2016 at $\alpha = 0.05$.

	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
N										
P	0.53									
K	0.40	0.41								
Ca	0.30	.	.							
Mg	-0.31	.	-0.53	-0.36						
B	0.28	.	.	.	.					
Cu	0.65	0.41	.	0.34	.	.				
Fe	.	.	.	.	0.33	.	.			
Mn	.	.	.	.	.	.	.	.		
Zn	0.58	0.43	0.38	.	.	.	0.51	.	0.45	

"." - Not statistically significant at $\alpha = 0.05$.

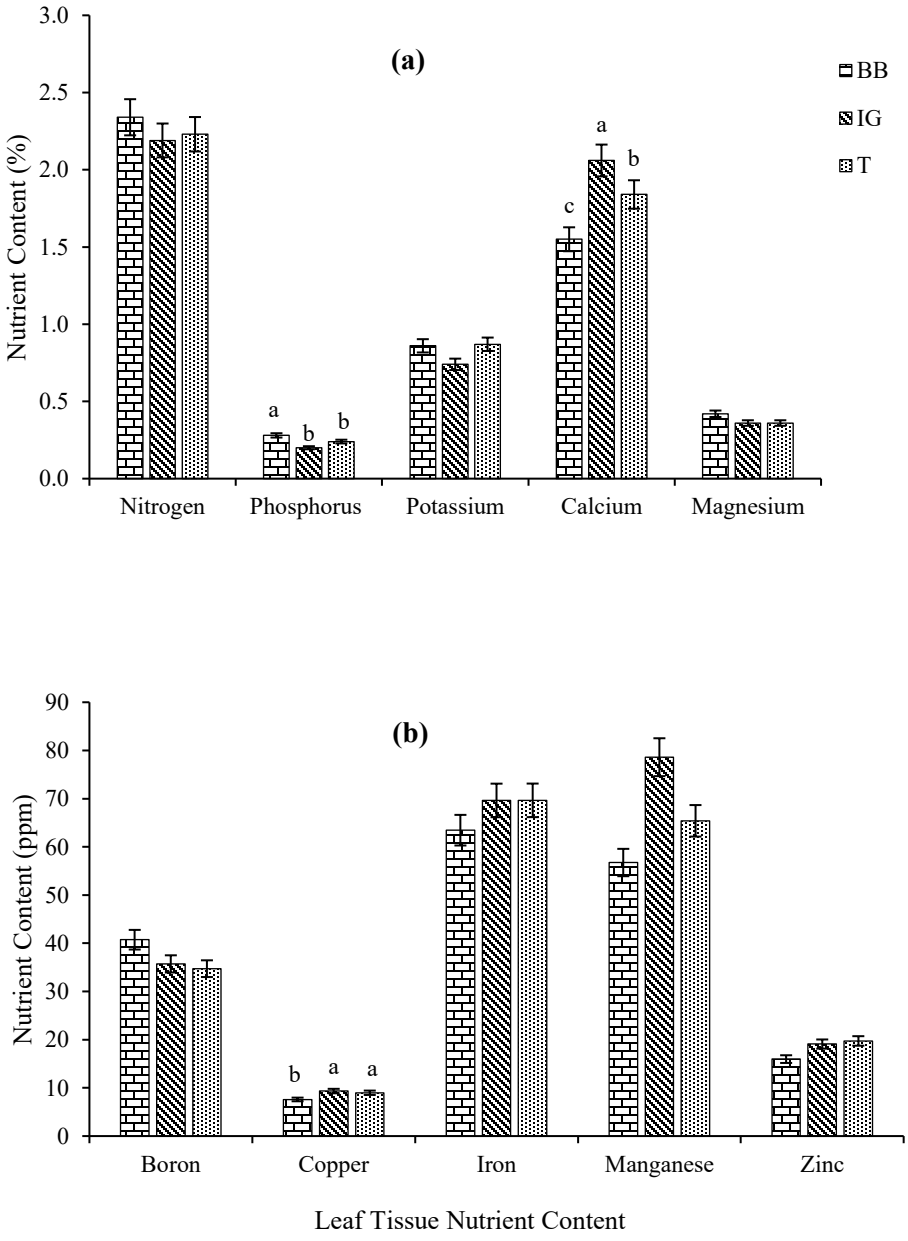

Figure 3.1. Leaf tissue nutrient content levels of the three common varieties growing on the same soil conditions across sampled haskap orchards in 2015 - 2016. Berry Blue (BB); Indigo Gem (IG) and Tundra (T); means followed by the same letter are not significantly different within each nutrient ($p \geq 0.05$); bars represent standard errors.

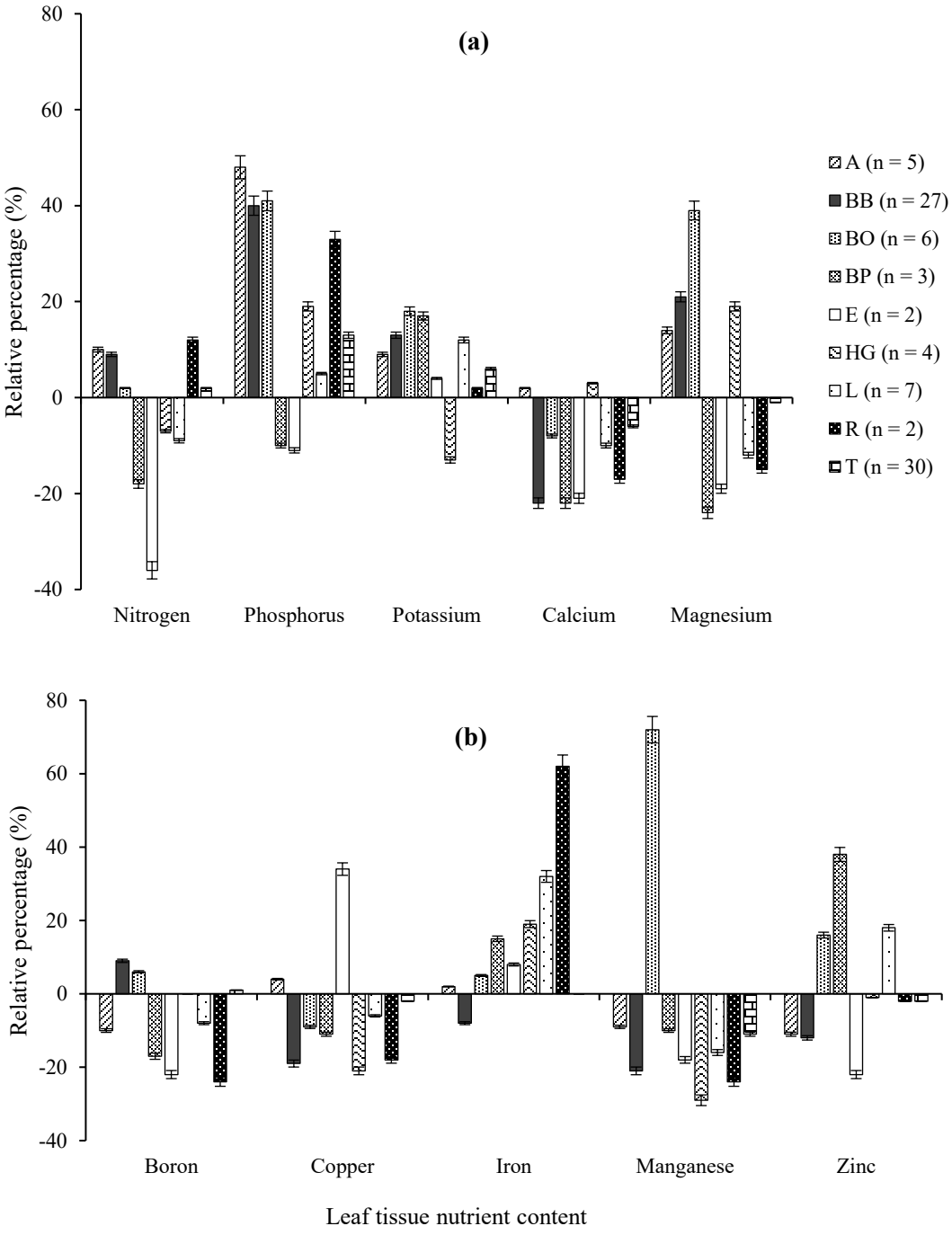

Figure 3.2. Relative comparison of leaf tissue nutrient content levels of other haskap varieties to Indigo Gem (100%) from across sampled haskap orchards in 2015 - 2016. Aurora (A), Berry Blue (BB), Borealis (BO), Erin (E), Happy Giant (HG), Larisa (L), Ruth (R), and Tundra (T); n (numbers of samples); bars indicate standard error.

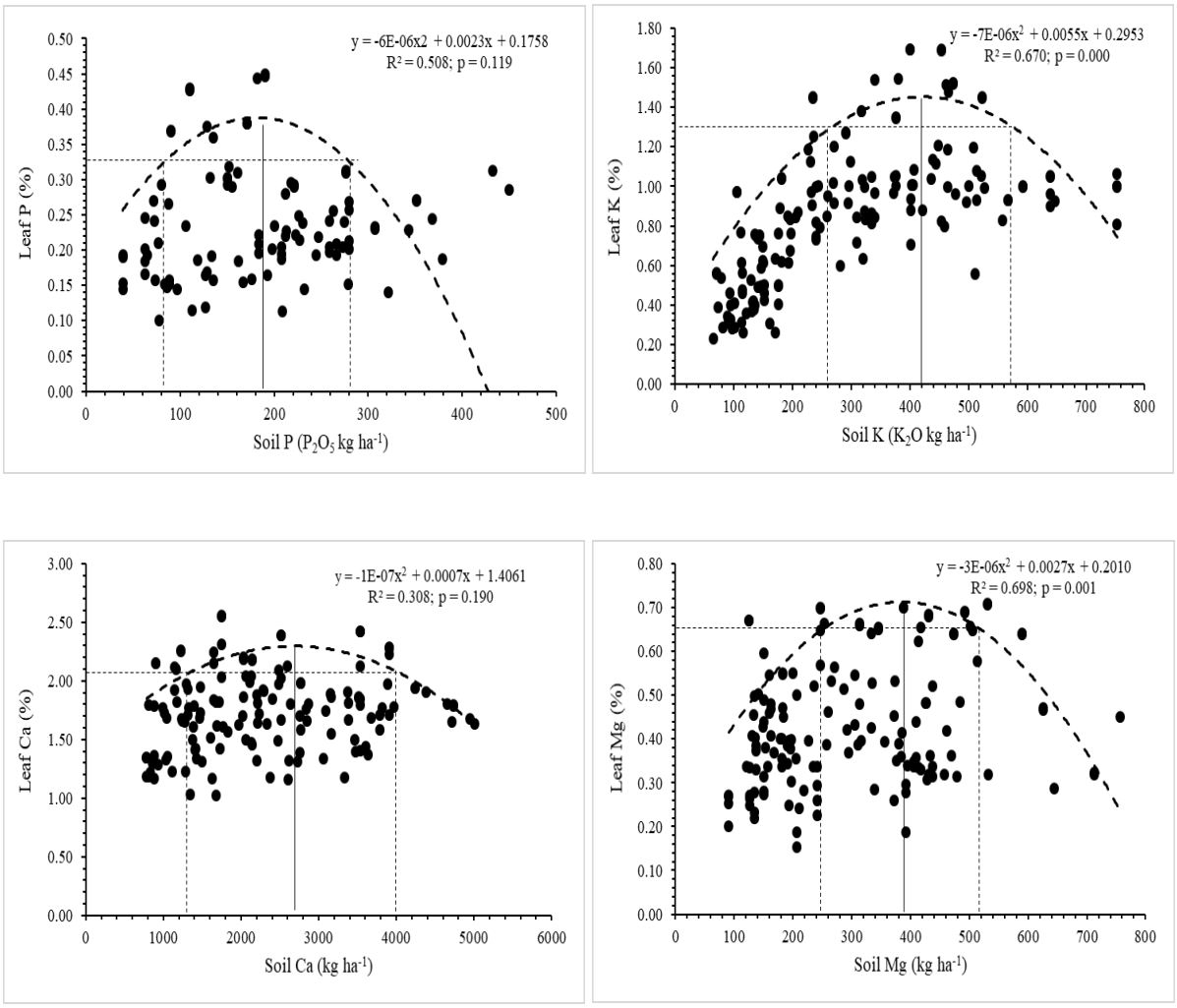

Figure 3.3. The relationship between soil available nutrients and leaf tissue nutrient concentrations of haskap varieties sampled in 2015 and 2016 showing boundary-line approach and best fitted regression described by second-degree function ($p \leq 0.10$).

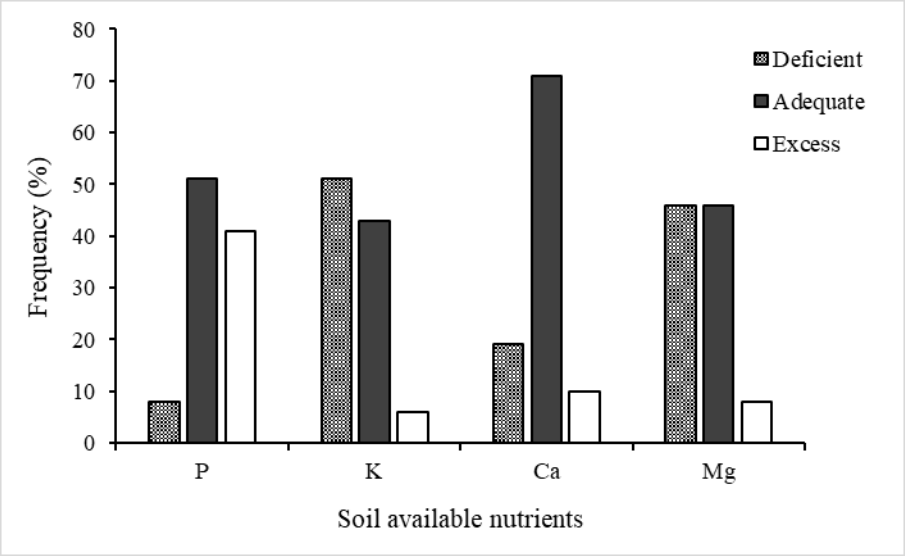

Figure 3.4. Frequency of occurrence of soil available nutrients disorder from 148 locations across Nova Scotia sampled in 2015 and 2016. Diagnoses are based on the BLA generated soil fertility levels.

CHAPTER 4.

RELATIONSHIP BETWEEN SOIL NUTRIENT STATUS AND NUTRIENT UPTAKE, AND ITS IMPACT ON HASKAP CV. INDIGO GEM PLANT PHYSIOLOGY

4.0 Abstract

Adequate supply of plant nutrients is crucial for haskap plant growth and increased productivity. A study was carried out to determine the variability in haskap cv. Indigo Gem physiological characteristics in relation to soil and leaf tissue nutrient status. A total of 19 composite soil samples and the corresponding plant leaf tissue samples were collected in 2016 from 12 locations in Nova Scotia. Plant physiological characteristics measured include growth rate, leaf size, leaf chlorophyll content, and visual observations. A boundary line approach was used to determine nutrient sufficiency ranges in leaf tissue of 2.23-2.96% for N, 0.22-0.28% for P, 0.84-1.32% for K, 1.63-2.10% for Ca, and 0.14-0.50% for Mg. Principal Component Analysis revealed antagonistic interactions among soil nutrients leading to decreased availability and uptake of one or more essential nutrients. Negative associations were observed most frequently between Ca and Mg and other nutrients, especially K. Plant physiological characteristics were closely related to soil and leaf K. Deficiencies in leaf tissue K and P were identified as potentially important factors limiting growth. Therefore, there is a need to adjust or balance the application of these nutrients. In conclusion, the sufficiency ranges derived can be used as guiding principle in diagnosing nutritional status of haskap cv. Indigo Gem.

Keywords: Haskap (*Lonicera caerulea*), boundary-line approach, Indigo Gem, nutrient antagonism, nutrient sufficiency levels, soil-plant relationships, leaf tissue nutrients.

4.1. Introduction

Haskap is a relatively new crop of rapidly growing interest in Nova Scotia (NS). For the industry to thrive, sustainable management practices need to be developed. However, soil nutrient management and tissue nutrient standards or norms for haskap are not clearly established (Bors 2009). Haskap grows about 2 m or more in height, leaves are simple, opposite, oval to elongated leaves of between 3 and 5 cm long (Arus and Kask 2007; Hummer et al. 2012). A fully matured haskap plant produces a dense and erect bushy plant of rounded shape with a diameter of between 1.5 and 2 m (Plekhanova 1992; Hummer et al. 2012). The fruit yields of haskap increase as the plants increase in size (Plekhanova 1992) suggesting that management that increased bush size could result in increased yield. The cultivated haskap plants begin to produce significant amounts of fruit after four years in the field and reach full bearing after seven to eight years (Plekhanova 1992).

Management practices should be aimed at improving number and length of shoots in order to optimize vegetative growth and maximize the first commercial harvest (McCarthy and Stoker 1988). The appropriate nutrient composition is required to achieve potential growth, development and yield of all cultivated plants including haskap. Nitrogen (N), phosphorus (P), and potassium (K), calcium (Ca), and magnesium (Mg) are particularly essential nutrients of crops (Yadong and Shuang 2009; Havlin et al. 2014). The deficiency or excess amount in any of the essential nutrients will disrupt either the vegetative or reproductive growth cycles in plants and/or alter nutrient composition (Marschner 1995; Fageria 2001; Fuqua et al. 2005). Therefore, adequate supply of plant nutrition is crucial for haskap establishment and production (Wrona 2011) as it is for most plant.

The imbalance of essential nutrients might contribute to slow haskap growth after establishment. Therefore, the interactions of these essential nutrients and other minor nutrients should be considered in ensuring haskap growth and productivity. Nutrient applications could have a synergistic (positive), antagonistic (negative) effect, or no effect on plant growth and productivity (Fageria 2001). Nutrient interactions are said to be positive (synergistic) when the application of two nutrients increases productivity and antagonistic when yield is reduced as a result of an adverse effect of one nutrient (Fageria 2001). This could be because of an excess of one nutrient which reduces the availability and uptake of the other nutrient elements (Dibb and Thompson 1985). For instance, increasing K levels in the soil would decrease availability and uptake of P, Ca, and Mg while increasing Ca levels would also inhibit P, K, and Mg availability and uptake (Chou et al. 2011; Sun et al. 2013; Havlin et al. 2014). Balanced nutrition is essential for achieving the full potential of bush growth and fruit yield (Fageria 2001; Pormale et al. 2009). A good understanding of plant nutritional needs and nutrient interactions could be beneficial in understanding the importance of a balanced supply of nutrients (Fageria 2001; Mattson and Van Iersel 2011; Santos 2011) and subsequently enhance haskap growth and yield.

Nutrient interactions are usually measured in terms of growth response and change in nutrient concentrations (Fageria 2001). Several authors have used different growth response parameters such as yield (Bhat and Sujatha 2013; Ali 2018), dry matter content (Blanco-Macías et al. 2009), basal-area growth for trees (Vizcayno-Soto and Cote 2004; Quesnel 2004; Quesnel et al. 2006), and tree growth rate (René et al. 2013) to diagnose and develop nutritional standards. Therefore, the study of leaf tissue nutrient content and physiological characteristics will further help in understanding the variability observed among haskap orchards in NS. The objectives of this study were to i) investigate the relationships between soil fertility, tissue nutrient contents, and

plant physiological characteristics; and ii) derive nutrient sufficiency levels for haskap cv. Indigo Gem. Emphasis was placed on macronutrients.

4.2. Materials and Method

4.2.1. Experimental Design and Location

The study of the relationship between soil fertility and leaf tissue nutrient status of haskap cv. Indigo Gem and how it affects the physiological performance was carried out as a survey on multiple farms with a range of soil conditions in Nova Scotia, Canada. Indigo Gem is the most commonly grown variety present on all sites. A total of 19 soil and tissue samples were collected in 2016 from 12 farms. Each sample represented a unique soil-leaf tissue combination. Plant physiological characteristics were collected from the same soil-plant sampling locations as described below. The history of agronomic practices of various fields and age of plants were documented. The age of the plants sampled ranged from two to five years old plants growing under field conditions for at least one growing season. The weed management practices across the farms varied from cultivation at the base of plant, use of coconut fiber mats at plant base, wood-chip mulch to no-weeding at all.

4.2.2. Soil Sampling and Analysis

Soil samples were collected in rows planted with Indigo Gem beginning from the 10^{th} plant (to avoid a field edge effect) over an approximate 20 m length in May 2016. A composite of approximately 10 subsamples were collected from between plants within a row to a depth of 15 cm using a sampling probe. Samples were stored in a cooler and refrigerated until submission to the Nova Scotia Agricultural Laboratory Services for standard soil test analysis including Mehlich III determination of mineral nutrients, but which omitted soil mineral N testing as this is not a

standard practice. Mineral nutrient concentrations were determined using Mehlich III solution as described in Chapter 3, section 3.2.2.

4.2.3. Plant Tissue Sampling and Analysis

A composite of whole leaf samples were collected from approximately 20 plants in the same row length where the soil samples were collected at each location. A composite leaf tissue sample was collected at approximately 50% color turn on the berry in June 2016. Leaves were collected from new stem growth three to five nodes down from the tip of the branch. Leaf tissue samples were stored in a cooler during transport and submitted to Nova Scotia Agricultural Laboratory Services for elemental determination by inductively-coupled plasma spectrometer (ICP, Thermo Fisher Scientific Inc., Waltham, MA). Leaf nutrient concentrations were determined as described in Chapter 3, section 3.2.3.

4.2.4. Plant Physiological Characteristics and Observations

Bush volume was estimated as the cylindrical volume using the formula provided by Erb et al. (1993) and modified by Hobson et al. (2013) (3.142 × height × width × 0.5 × breadth × 0.5) to calculate the bush volume for blackcurrant. This measurement was done with a meter stick. The chlorophyll content or greenness of leaves was measured using a SPAD meter (SPAD 502 plus, Spectrum Technologies, Inc.). The leaves used for chlorophyll measurements were collected and scanned to determine average leaf size with the aid of Compueye Leaf and Symptom Area software. Plant growth rates were estimated by dividing the bush size by the age of the plants on the field. Plant observations were also carried out for any visual nutrient deficiency symptoms. Nutrient deficiency symptoms where based on common symptoms observed for other crops, no

chart was used. All data were collected only in the summer of 2016, from late July to early August after berry harvest. These measurements were collected from 20 plants in each location.

4.2.5. Statistical Analysis

4.2.5.1. Boundary-Line Approach and Averaging Approach

The boundary-line approach (BLA) was proposed by Walworth et al. (1986) and has been adopted by several authors (Schnug et al. 1996; Vizcayno-Soto and Cote 2004; Blanco-Macías et al. 2009; Bhat and Sujatha 2013; Ali 2018) to determine and/or study nutrient sufficiency ranges for several crops. The first step was to plot scatter diagrams of relative growth rate as a dependent variable against leaf tissue nutrient concentrations as the independent variable. Secondly, the scatter diagram was divided into 5-7 intervals, and only the maximum points were selected from each interval for each nutrient. Thirdly, a second-degree polynomial function was generated from the selected points. Optimum nutrient concentrations were obtained by solving the second-degree function as reported by Ali (2018). The corresponding values to 90% of the highest growth rate defines the minimum and maximum nutrient sufficiency ranges.

Prior to boundary line steps, it is important to remove outliers from the data set (Ali 2018). Outlier test was carried out to detect and remove outliers from the leaf tissue nutrient concentrations data set using box and whiskers plots (Fig. 4.1). There was no outlier point observed for Leaf N, P, K, and Ca, except for Mg. The outlier point observed was 0.93% (the upper limit for leaf Mg), which was excluded in the selection process.

The averaging approach is the mean of high growth rate subpopulation and were used in this study for comparative purposes. In order to achieve this, the growth rate data were divided into low (<0.10 m^3 yr^{-1}) and high growth rate (>0.10 m^3 yr^{-1}) subpopulations. The mean of leaf

tissue nutrient concentration of high growth rate subpopulation were recorded as the optimum nutrient level for each nutrient element.

4.2.5.2. Principal Component Analysis

Principal Component Analysis (PCA) was used to synthesize the information derived from the multivariate data set. The first step to a PCA is to standardize the variables, followed by analysis to extract the principal components (Bowley 2008). PCA was applied to the selected variables to ascertain how soil fertility and nutrient absorption affects physiological parameters. PCA was performed on standardized data set (a mean equal to 0 and variance equal to 1) and Kaiser's rule and percentage of total variance explained were used in selecting and retaining components. The data analysis was performed using Minitab (version 17, Minitab Inc., State College, Pa.).

4.3. Results and Discussion

4.3.1. Soil Fertility Status

The soil fertility status of the study locations is presented in Table 4.1. The pH of the soils was moderately acidic to neutral (pH values ranged from 5.22-7.04). The pH ranges observed were within the tolerable levels for haskap (Retamales and Hancock 2012) and the variability was low among the different sites. However, a recommended pH range has not been established for haskap and therefore, could not confirm this finding with literature values for haskap.

Based on the soil fertility recommendation for small fruit crops (Nova Scotia Department of Agriculture - NSDA 2010a), soil P and K ranged across locations from very low to extremely high (65-2320 kg $ha^{-1}$and 65-753 kg ha^{-1}, respectively). Soil Ca and Mg tends to be adequate and ranged from 1225-6497 kg ha^{-1} for Ca and 127-713 kg ha^{-1} for Mg. However, the upper limit of

the observed ranges for Ca and Mg was twice the recommended upper limits for small fruit crops (NSDA 2010). In general, the variability observed within the various soil parameters measured was low according to the criterion established by Wilding et al. (1994) i.e. 0.99-12.28%.

4.3.2. Growth and Leaf Tissue Nutrient Concentrations

A wide range of variability in leaf tissue nutrient concentrations was observed within the study locations (Table 4.2). Wilding et al. (1994) criteria was used to determine the magnitude of variability. They characterized coefficient of variation (CV) of 0-15% to be low, 15-35% as medium, and 35-100% as high variability. There was high variability in growth rate and bush growth, which ranged from 0.01-0.19 m^3 yr^{-1} and 0.01-0.75 m^3, respectively; while low variability was observed in leaf size (Table 4.2).

Haskap cv. Indigo Gem leaf tissue concentrations of N, P, Ca, Cu, Zn, and Fe within locations showed medium variability with CV between 16.69-33.95%. However, high levels of variability were observed in leaf K, Mg, B, and Mn with CV ranging from 39.48-91.31%. The levels of variability observed in growth and leaf nutrient concentrations could be due to variations in plant age, soil properties and farmers' nutrient management practices (Prive and Sullivan 1994; Hargreaves et al. 2008; Kabata-Pendias 2004; Ali 2018) in the different study locations.

4.3.3. BLA Optimum Nutrient, Sufficiency Range(s) and Nutrient Ratio(s)

In the scatterplot diagrams, the data points were mostly grouped at lower growth rates (Fig. 4.2). The BLA second-degree polynomial regression functions for haskap cv. Indigo Gem were generated with 5-7 interval points for leaf tissue nutrient concentrations (Fig. 4.2). Traditionally, >10 interval points are normally used in developing BLA regression functions for large data sets (Vizcayno-Soto and Cote 2004; Blanco-Macías et al. 2009; Bhat and Sujatha 2014; Ali 2018).

However, intervals <10 has also been used (Bhat and Sujatha 2014) and therefore, the number of intervals used in this study could be suitable for the small data set to reduce the likelihood of including haskap plants that are not growing in optimum conditions as reported by Vizcayno-Soto and Cote (2004).

The application of BLA produced significant ($p<0.10$) second-degree functions with high R^2 values ranging from 0.84-0.91 for leaf N, K, and Mg, and from 0.77-0.81 for N:P and K:Ca ratios. For leaf P and Ca concentrations, the models produced were not significant ($p>0.10$) indicating the need to significantly increase the data set (Quesnel 2004; Blanco-Macías et al. 2009). The optimal nutrient concentration, sufficiency ranges, and optimal nutrient ratios corresponding to 90% maximum growth rate were obtained from the regression coefficient and are presented in Table 4.3. The optimum values derived through the averaging approach (i.e. mean high growth rate >0.10 m^3 yr^{-1} subpopulation) population is also presented in Table 4.3.

The optimal leaf N and Ca derived from BLA tend to vary a little from that of the averaging method, while for leaf P, K, and Mg, the optimum concentrations were comparable (Table 4.3). The averaging method has been used to generate nutrient norms (Bhat and Sujatha 2014), therefore, the methods were comparable in this study. This suggests that maximum bush growth is possible within the same optimum nutrient levels derived from both methods (Bhat and Sujatha 2014). However, the most realistic approach would be to keep haskap nutrient levels at or very close to the optimal leaf nutrient levels. The nutrient sufficiency ranges developed may be use as guiding principle in assessing haskap nutritional status as reported by Bhat and Sujatha (2014) for arecanut (*Areca catechu* L.).

On the other hand, high proportion of locations were diagnosed as having deficient or excess nutrition, with few locations having adequate nutrition (Fig. 4.3a). Based on the N, P, and

K concentration models, 53% of the locations were diagnosed as been deficient in N and P, while 58% were also deficient in K. However, 42% of the locations were diagnosed to have adequate leaf N concentrations. High proportion of the locations were identified as having adequate leaf Ca (58%) and Mg (74%) concentrations (Fig.4.3a). The % of locations diagnosed as having adequate (74%) or excess (26%) Mg seems realistic when considering the widespread occurrence of Mg-rich soil parent materials in eastern Canada as reported by Quesnel (2004). In general, majority of the locations were identified as having deficient and/or adequate in one nutrient or the other. This suggests that nutrient imbalance is the major problem slowing haskap bush growth in the studied locations.

A balanced nutrition is vital in maximizing haskap growth and productivity. This involved the use of nutrient ratios as reported for other plants (Bhat and Sujatha 2013; Horuz et al. 2013; Dresler et al. 2015). Haskap nutrient ratios derived from BLA are also presented in Table 4.3. The BLA nutrient ratios of P:K and K:Ca was identical to that of the averaging approach, while N:P, N:K, K:Mg, and Ca:Mg ratios tend to vary widely between the two methods. However, any variations in the derived nutrient ratios can result in reduced bush growth as reported for other crops (Bhat and Sujatha 2013; Horuz et al. 2013; Dresler et al. 2015). The nutrient ratios derived would be helpful in highlighting antagonistic relationships between nutrients and also, can be a useful advance warning tool for overcoming insufficient nutrition in haskap.

The soil and leaf tissue data sets used in this study were collated from different locations with different soil and climatic conditions. This might reduce the influence of seasonal variations and climatic factors such as temperature and rainfall on haskap establishment and productivity as expressed for other crops (Daugaard 2001; Bhat and Sujatha 2013). So, the nutrient sufficiency ranges and ratios derived for haskap could be reliable.

With regards to haskap nutrient ratios, the nutrient imbalance could be the major cause of slow bush growth in the studied locations (Fig. 4.3b). Based on the nutrient ratio models, majority of the locations were diagnosed as having below or within the BLA-nutrient ratios. The locations with growth rate >0.10 m^3 yr^{-1} (high-growth rate subpopulations) tend to have balanced nutrient ratios, expect for N:K ratio that appears to be relatively low. More than half of the locations (74%) were identified as having P:K ratio within the derived BLA nutrient ratio. For N:P ratio, 42% of locations were diagnosed as having below the BLA nutrient ratios (Fig. 4.3b). Also, 53% of the locations were identified as deficient in K:Ca and Ca:Mg. This suggests an imbalance among the nutrients due to deficiency or excess of one nutrient or the other as reported in other crops (Chou et al. 2011; Sun et al. 2013).

4.3.4. Comparison of BLA Derived Haskap Sufficiency Range(s) to Other Small Fruits

The BLA sufficiency nutrient ranges of haskap was compared to other small fruit crops such as highbush blueberry and black currants as farmers tend to use sufficiency ranges for these crops to evaluate the nutritional need of haskap. This could be attributed to the similar growth stature and fruit type of these crops.

The BLA sufficiency levels for haskap were fairly comparable to black currants sufficiency ranges than that of highbush blueberry. Haskap leaf Ca and Mg sufficiency levels were poorly matched to that of black currant and highbush blueberry (Table 4.4). However, the approach used to determine black currant (Barney and Hummer 2005) and highbush blueberry sufficiency levels (NSDA 2010b) cannot be ascertained. The differences between the minimum and maximum sufficiency levels for haskap were in general, wider than that for both other crops. This suggests that the nutritional standards for black currant and highbush blueberry can not be used to determine nutritional status for haskap. Haskap tended to have lower minimum and maximum sufficiency

levels for N, P and K than black currant except for leaf Ca and Mg (Table 4.4). This also suggests that haskap might have higher Ca and Mg requirements.

4.3.5. Relationship between Soil, Tissue, and Plant Physiological Characteristics

The principal component analysis (PCA) applied to selected soil available nutrients and leaf tissue nutrients clearly identified the relationships among the variables, and how soil fertility and nutrient absorption affected Indigo Gem physiological characteristics (Table 4.5).

Following Kaiser's criteria (Bowley 2008), four principal components (PCs) were retained that explained 80.0% of the total variance (Table 4.5). The first PC (PC1) explained 41.10% of the total variance, which showed a positive relationship among leaf N, P, K, and plant health variables and negatively related to soil organic matter (S.O.M), leaf Ca, and Mg (Table 4.5). The second PC (PC2) accounted for 17.40% of the total variance; leaf K, Mg, and P were positively related to leaf N, including plant physiological characteristics, while soil P, Ca, and Mg were negatively associated to soil K (Table 4.4).

The negative grouping observed between soil P, Ca, and Mg versus soil K in PC2 suggests a possible imbalance in nutrient supply. This is further revealed in the nutrient uptake pattern, where leaf Ca and Mg were negatively related to leaf N, P and K in PC1 (Table 4.5). This disturbance may be due to various nutrients competing for functional sites near the root surface or within plant tissues (Fageria 2001; Bhat and Sujatha 2014). Nitrogen is an integral part of chlorophyll, which converts light energy into chemical energy required for photosynthesis (Havlin et al. 2014). The overlapping of N and SPAD (chlorophyll content) vectors was considered a clear indication of the relationship between N and chlorophyll content as previously reported (Peterson et al. 1993; Amaliotis et al. 2004; Bojović and Marković 2009). Cabrera (2004) also reported that leaf N content is significantly correlated with chlorophyll content and color attributes in the rose

plant (*Rosa hybrida)*. This finding also confirms the relationship between the two variables (leaf N and chlorophyll content).

Magnesium as a primary constituent of chlorophyll is essential for photosynthesis, and the chlorophyll content accounts for 15-20% of Mg in plants (White and Broadley 2009; Havlin et al. 2014). It is therefore expected that leaf Mg to have a close association with leaf N and chlorophyll content. However, the reverse was observed in both PCs (Table 4.5). Huang and Grunes (1992) reported that increasing NO_3^- levels would increase Mg uptake but would also decrease Mg translocation. Similarly, the uptake of NH_4^+ would reduce the uptake of Ca, Mg, and K (Havlin et al. 2014). The negative association of leaf Mg and Ca with soil available nutrients such as P and K (Table 4.5) confirms the antagonistic nutrient interaction. Similar observations were made for other crops by several authors (Chou et al. 2011; Sun et al. 2013; Horuz et al. 2013; Havlin et al. 2014; Dresler et al. 2015). They all concluded that an increase in any of K, Ca and Mg levels would affect the uptake of the other nutrients.

Furthermore, the opposite grouping of the leaf tissue nutrients in the PCs (Table 4.5) supports antagonistic nutrient interactions. Several authors have reported that excessive levels of any of the cations would inhibit availability and uptake of the others (Chou et al. 2011; Sun et al. 2013; Havlin et al. 2014). Also, the positive grouping of leaf N, P and K versus plant physiological characteristics indicates the importance of these nutrients in haskap growth and establishment. It has been reported that P and K are needed to stimulate growth, which leads to increased plant growth and enhanced uptake of both nutrients (Havlin et al. 2014; Ali 2018).

Nutrient deficiency symptoms such as P and K were visible on Indigo Gem leaves (Fig. 4.4). This could be attributed to the nutrient imbalance (antagonistic interaction). These deficiency symptoms conform with descriptions for P and K deficiencies in plants (Havlin et al. 2014).

According to the present findings, the deficiencies in Indigo Gem may be the result of complex interactions between nutrients in the soil. Fageria (2001) stated that interaction of ions with similar chemical properties compete for site of adsorption, absorption, transport, and functions on root surfaces or within tissues; and this is common among Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}. The negative correlation of leaf Mg with selected leaf nutrients confirms the role of antagonistic interactions leading to nutrient deficiencies observed (Fig. 4.5) because of impaired nutrient uptake. Therefore, it can be inferred that the deficiencies of P and K might be the major cause of growth variabilities observed among the selected locations.

4.4. Conclusion

The BLA have been proved to be a reliable method in diagnosing and developing nutritional standards in this study. The BLA sufficiency ranges for haskap cv. Indigo Gem leaf were determined to be 2.2-3.0% for N, 0.2-0.3% for P, 0.8-1.3% for K, 1.6-2.1% for Ca and 0.1-0.5% for Mg. These sufficiency ranges may be used to guide diagnosis of nutritional status of haskap. Deficiencies in leaf tissue K and P were identified as potentially important factors limiting growth of haskap in NS. Principal component analysis also clearly illustrated how nutrient imbalance could hinder Indigo Gem growth and productivity because of antagonistic interactions among soil nutrients. Fertilization should not be based on leaf tissue analysis alone; the incorporation of both soil and tissue testing would give a better understanding of a balanced nutrient supply. Finally, soil and plant tissue testing need to be considered before haskap develops visual deficiency symptoms. This would help in detecting and averting nutrient deficiency or excess during the growing season. More studies are needed to further elucidate nutrient interactions and validate optimum nutrient concentrations in haskap leaf.

Table 4.1. Descriptive statistics of soil pH, soil organic matter (S.O.M), and soil available nutrients observed from 19 selected haskap locations growing Indigo Gem in Nova Scotia at a depth of 0-15 cm.

Parameter(s)	Units	Mean	Minimum	Maximum	CV [a] (%)
pH	-	6.0	5.22	7.04	12.28
S.O.M	%	4.92	2.80	7.70	3.24
P_2O_5	kg ha^{-1}	813.68	65.0	2320.0	1.16
K_2O	kg ha^{-1}	316.79	65.0	753.0	1.85
Ca	kg ha^{-1}	2919.68	1225.0	6497.0	2.01
Mg	kg ha^{-1}	343.68	127.0	713.0	2.25
Na	kg ha^{-1}	30.83	16.0	59.0	2.60
S	kg ha^{-1}	34.74	15.0	87.0	2.21
Al	ppm	1387.42	745.0	1874.0	5.16
B	ppm	0.56	0.50	1.10	4.0
Cu	ppm	5.11	0.56	17.24	1.17
Fe	ppm	186.42	122.0	297.0	3.73
Mn	ppm	40.47	19.0	150.0	1.26
Zn	ppm	6.87	1.09	32.01	0.99

[a] Coefficient of variation

Table 4.2. Descriptive statistics of haskap cv. Indigo Gem growth characteristics and leaf tissue nutrient composition from 19 selected haskap locations in Nova Scotia.

Parameter(s)	Units	Mean	Minimum	Maximum	CV [a] (%)
Plant growth characteristic					
Bush size	m^3	0.21	0.01	0.75	110.07
Growth rate	$m^3\ yr^{-1}$	0.06	0.01	0.19	98.56
Leaf size	cm^2	9.23	2.24	14.39	34.28
SPAD [b]	-	33.53	26.60	40.0	9.96
Leaf tissue nutrient concentrations					
N	%	2.19	1.59	3.05	16.69
P	%	0.23	0.16	0.33	22.58
K	%	0.85	0.23	1.54	39.48
Ca	%	1.91	1.42	2.61	18.28
Mg	%	0.42	0.15	0.93	44.59
B	ppm	36.99	16.93	96.60	45.71
Cu	ppm	8.63	5.11	11.52	18.70
Fe	ppm	80.85	51.73	151.60	33.95
Mn	ppm	65.68	18.23	284.22	91.31
Zn	ppm	18.23	11.62	34.69	33.28

[a] Coefficient of variation
[b] Chlorophyll content.

Table 4.3. Second-degree polynomial function(s), sufficiency range(s), and nutrient ratio(s) for leaf tissue nutrient concentrations in haskap cv. Indigo Gem generated using boundary-line approach.

Nutrient(s) and ratio(s)	Second-degree polynomial function(s)	R^2	Sufficiency range(s)			Averaging approach
			Optimum	Minimum	Maximum	Optimum [a]
N (%)	$y = -0.1962x^2 + 1.0181x - 1.1475$	0.84 *	2.60	2.23	2.96	2.48
P (%)	$y = -16.075x^2 + 8.1452x - 0.8611$	0.30 [ns]	0.25	0.22	0.28	0.27
K (%)	$y = -0.2375x^2 + 0.5132x - 0.1154$	0.84 *	1.08	0.84	1.32	1.09
Ca (%)	$y = -0.2388x^2 + 0.8884x - 0.7026$	0.67 [ns]	1.86	1.63	2.10	1.93
Mg (%)	$y = -0.4161x^2 + 0.2613x + 0.1235$	0.91 *	0.32	0.14	0.50	0.32
	Nutrient ratio(s)					
N:P	$y = -0.0055x^2 + 0.1176x - 0.4385$	0.81*	10.80	8.90	12.60	9.13
N:K	$y = -0.0519x^2 + 0.3008x - 0.2511$	0.84 [ns]	2.90	2.35	3.45	2.28
P:K	$y = -3.165x^2 + 1.561x + 0.0324$	0.49 [ns]	0.25	0.18	0.32	0.25
K:Ca	$y = -0.7565x^2 + 0.8645x - 0.0664$	0.77*	0.57	0.43	0.72	0.56
K:Mg	$y = -0.0304x^2 + 0.1745x - 0.0713$	0.57 [ns]	2.90	2.18	3.60	3.34
Ca:Mg	$y = -0.0095x^2 + 0.1216x - 0.2237$	0.80 [ns]	6.40	5.10	7.70	5.94

* Regression coefficients are statistically significant ($p \leq 0.10$).
[a] Calculated from high-growth rate subpopulation (growth rate >0.10 m^3 yr^{-1})
[ns] Not significant.

Table 4.4. Comparison of haskap cv. Indigo Gem estimated boundary-lines sufficiency range(s) and optimum nutrient ratio(s) to NSDA (2010b) nutrient recommendations for small fruit crops.

Nutrient(s)	Haskap [a]	Black currant [b]	Highbush blueberry [c]
N (%)	2.23-2.96	2.70-2.90	1.50-2.50
P (%)	0.22-0.28	0.26-0.30	0.10-0.40
K (%)	0.84-1.32	1.0-1.60	0.30-0.80
Ca (%)	1.63-2.10	1.0-1.50	0.20-0.70
Mg (%)	0.14-0.50	0.10-0.15	0.10-0.25
	Nutrient ratio(s) [d]		
N:P	10.80	10.0	8.00
N:K	2.90	2.15	2.86
P:K	0.25	0.22	0.36
K:Ca	0.57	1.04	1.56
K:Mg	2.9	10.40	4.00
Ca:Mg	6.40	10.0	2.57

[a] Nutrient sufficiency ranges and ratios derived from boundary-line approach
[b] Barney and Hummer (2005) nutrient sufficiency ranges for black currants
[c] Nova Scotia Department of Agriculture - NSDA (2010b) nutrient sufficiency ranges for highbush blueberry
[d] Nutrient ratios for black currant and highbush blueberry were calculated from recommended sufficiency ranges.

Table 4.5. Eigenvalues, cumulative variance (%), and loading scores between the first four principal components of haskap cv. Indigo Gem.

Variable	PC1 [a]	PC2	PC3	PC4
Soil parameters				
pH	0.30	0.31	0.16	-0.08
S.O.M [b]	-0.09	-0.20	0.53	-0.31
P_2O_5	0.29	0.10	-0.38	0.20
K_2O	0.33	-0.16	0.04	-0.10
Ca	0.33	0.20	0.06	-0.00
Mg	0.13	0.52	0.06	0.004
Leaf tissue nutrients				
N	0.20	-0.34	0.00	0.541
P	0.21	0.39	0.03	0.24
K	0.29	0.03	-0.32	-0.29
Ca	-0.01	0.07	0.47	0.42
Mg	-0.26	0.28	0.16	0.29
Plant physiological characteristics				
Leaf size	0.29	-0.09	0.16	-0.25
Bush size	0.32	-0.08	0.29	-0.02
Growth rate	0.34	-0.11	0.26	-0.02
SPAD [c]	0.21	-0.39	-0.12	0.29
Eigenvalue	6.17	2.60	2.06	1.12
Cumulative Variance (%)	41.10	58.50	72.20	80.0

[a] First principal component
[b] Soil organic matter
[c] Chlorophyll content.

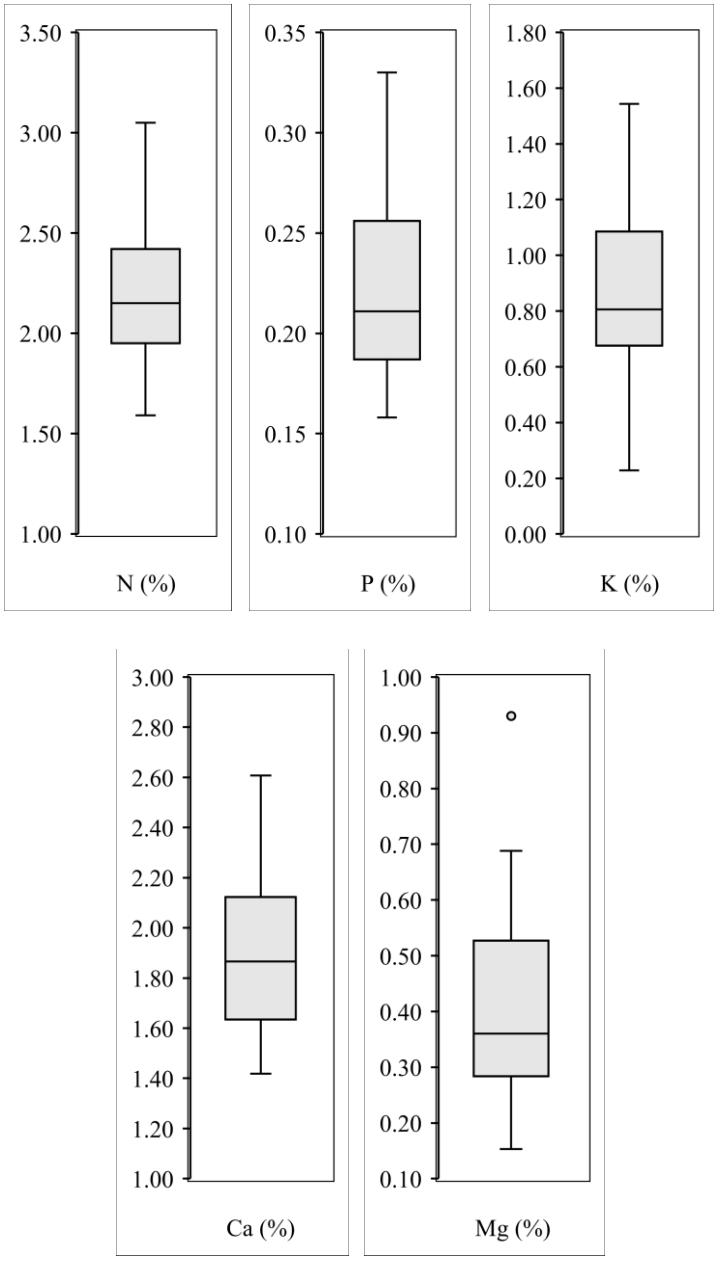

Figure 4.1. Box and whiskers plot of haskap cv. Indigo Gem leaf tissue nutrient content. The box indicates interquartile range, line within the box indicates median, the whiskers represent 1.5 times the interquartile range, and the dotted points represent outliers.

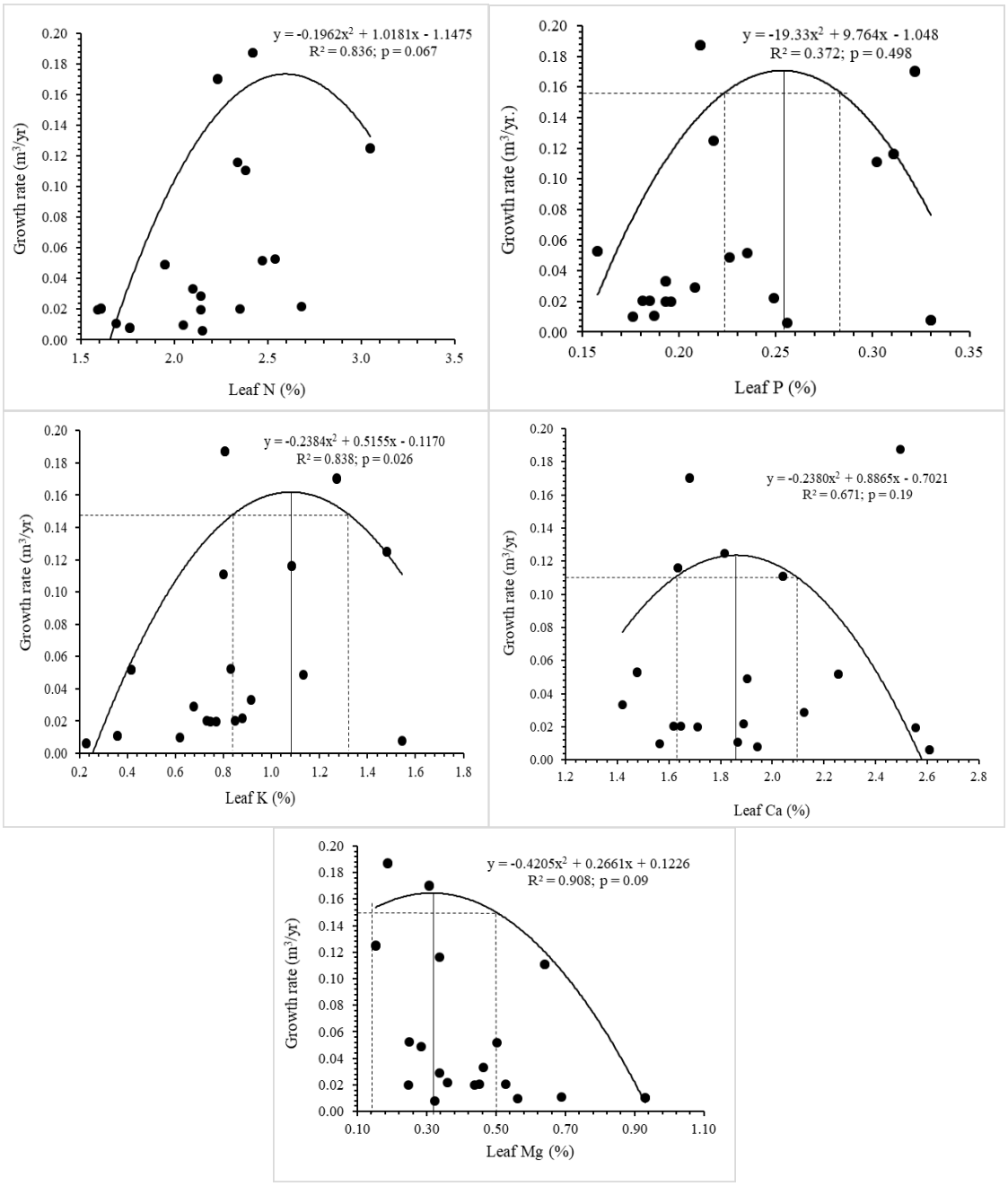

Figure 4.2. Scatter diagram of bush growth rate vs. leaf N, P, K, Ca, and Mg nutrient concentrations of haskap cv. Indigo Gem showing boundary lines approach described by second-degree polynomial regression functions ($p \leq 0.10$).

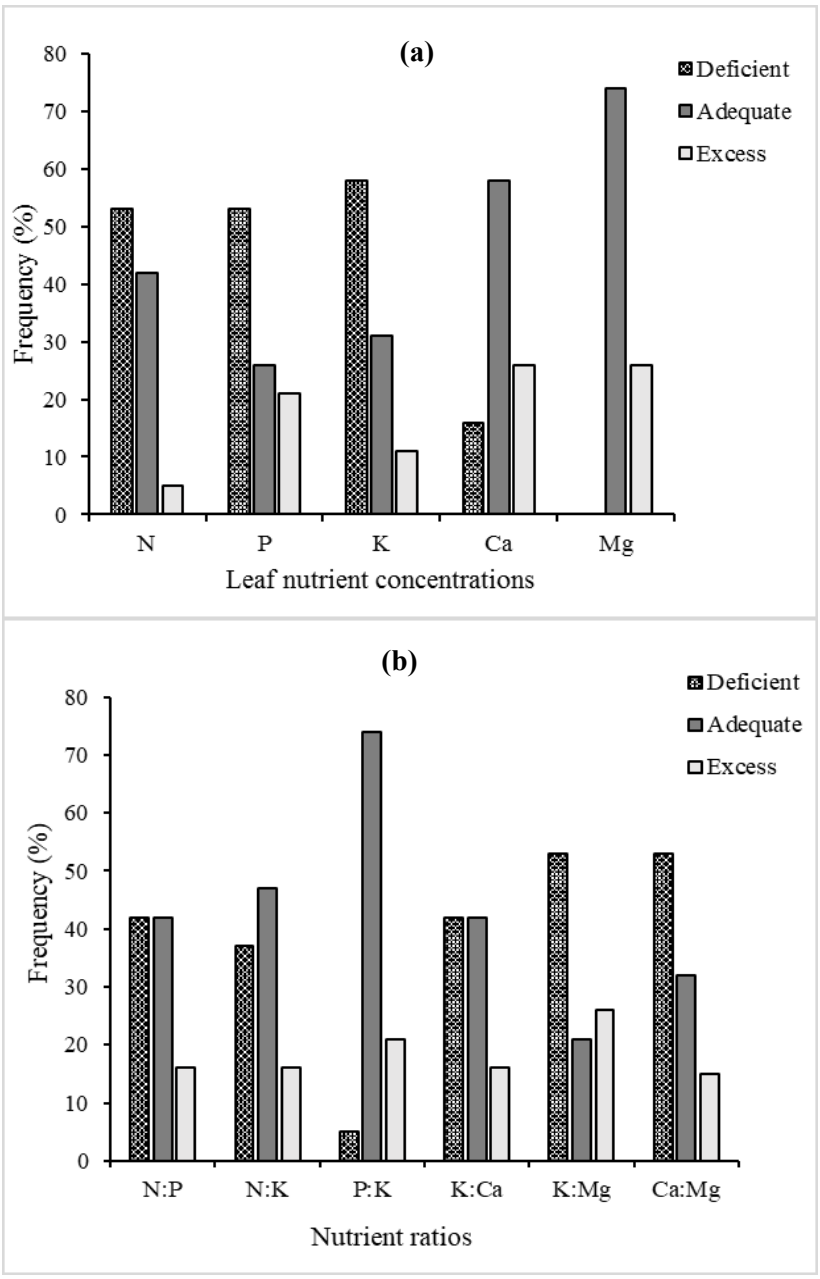

Figure 4.3. Frequency of occurrence of nutritional status (a) and nutrient ratios (b) in haskap cv. Indigo Gem from 19 locations across Nova Scotia. Diagnoses are based on the BLA generated nutrient sufficiency ranges.

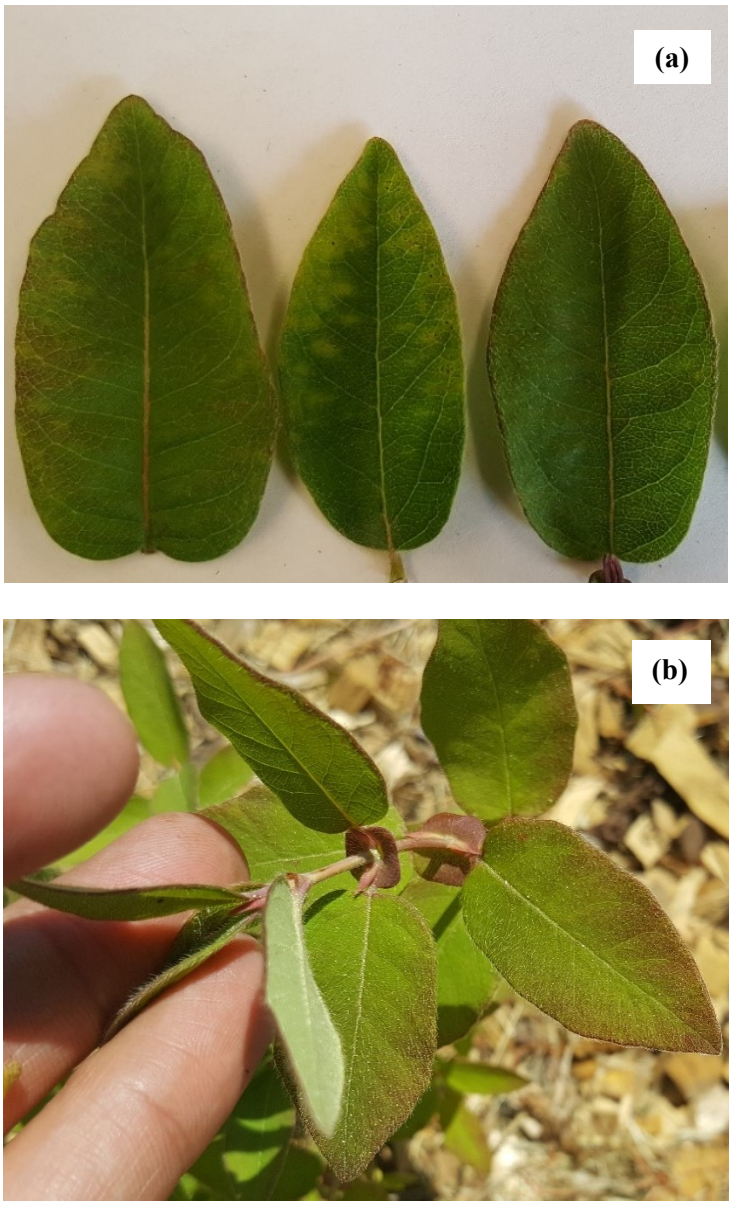

Figure 4.4. Visual nutrient deficiency symptoms observed on haskap leaf cv. Indigo Gem. a) K-deficient leaves showing chlorosis of leaf edges where tissue along veins and leaf base remain green; b) P-deficient leaves showing purple coloration of leaf tips which progresses until the entire leaf is purple.

CHAPTER 5.

CONCLUSION

5.1 Overview of Problem and Research Objectives

Haskap (*Lonicera caerulea* L.) production in Canada is still nascent, especially in Nova Scotia (NS) where for instance, optimal nutrient management practices are yet to be assessed. In Atlantic Canada, it is anticipated that the haskap industry will be worth millions of dollars in the next five years (O'Connor 2015). However, the inherent slow rate of plant growth could affect the targeted valuation making it unattainable. Also, mechanical harvesting will be required for commercial production, suggesting that haskap bushes will have to attain a minimum size to warrant mechanization. Appropriate nutrient application can help reduce limitations to haskap bush growth, yield and quality through the supply of adequate amount and type of nutrient applied at the critical growth stage. However, the quantity of nutrients to apply is yet to be clearly determined for haskap plants.

The amount of nutrient required by haskap depends on plant growth stage and characteristics, environmental conditions, soil properties and management practices as reported for other crops (Havlin et al. 2014). Moreover, the understanding of how different haskap varieties respond to the aforementioned factors (especially soil-plant interaction) are important information for nutrient management decisions. Typically, plant nutrient status for bush plants is determined by leaf nutrient status. Nonetheless, there is no documentation on the level of variability in leaf tissue nutrient levels among haskap varieties and/or how haskap respond to soil fertility levels.

To optimize bush growth and minimizing environmental impacts of nutrient use, nutrient sufficiency ranges are helpful in diagnosing and correcting haskap nutritional status. But for haskap, there is also no clear established leaf tissue nutrient sufficiency ranges (Bors 2012) making

it difficult to assess nutritional status of haskap. Therefore, the development of soil and leaf tissue nutrient standards would enable haskap growers to properly assess and/or apply the right amount of nutrient needed for maximum bush growth and productivity. Also, the appropriate recommendation will help minimize fertilizer misapplication and nutrient wastage.

The research presented in this thesis seeks to identify optimal standards for soil fertility and leaf tissue nutrients for haskap production. This was achieved by addressing the following gaps: i) studying the relationship between soil fertility status and leaf tissue nutrient contents of haskap; ii) identifying the level of variability in leaf tissue nutrient content among haskap varieties; and iii) studying the relationship between soil, leaf tissue nutrient status and plant physiological characteristics using Indigo Gem as a model variety.

5.2 Approach to Project and Conclusions

The first study was to i) determine the relationship between soil fertility status and leaf tissue nutrient content of haskap and ii) determine the levels of variability among haskap varieties commonly grown in NS. A multi-locational field survey was conducted, and 148 soil samples were collected in mid-May, and leaf tissue samples were collected starting from late June to early July in 2015 and 2016 during which time 50% of the berries had turned color (Chapter 3). The result revealed significant antagonistic relationships between leaf Mg content with soil and leaf tissue P, K, and Ca. This suggests an imbalance in nutrient uptake pattern. Owing to the nutrient imbalance, P and K deficiencies are the most visible symptoms observed in the fields. The P and K deficiencies may be related to either low availability in the soil or an imbalance with competing nutrients such as Ca and Mg, which were higher in the soil.

A varietal comparison was conducted to determine the level of variability among varieties. Firstly, the three most commonly grown varieties in NS, namely: Berry Blue (BB), Tundra (T), and Indigo Gem (IG) were tested. The result revealed that the three varieties had similar leaf tissue contents of N, K, Mg, and micronutrients except in P, Ca and Cu. IG and T had similar compositions of nutrient uptake, which could be attributed to their similar genetic characteristics. Secondly, IG was used as a reference to which all other varieties are compared because it was widely grown and present in all the studied locations. The results also revealed wide variations in leaf tissue nutrient contents among varieties. The greatest variation of IG from other varieties was in P content. The least overall variability among varieties not including IG was in K, Ca and B and the greatest variation was in Mg. From the result, it can be concluded that in most cases all varieties were within 40% of the IG value.

The boundary-line approach (BLA) was applied to the data set to further understand the relationship between soil fertility status and leaf tissue nutrient content, and to identify optimal soil fertility levels. The BLA produced a curvilinear response with significant R^2 values for soil P, K and Mg. This result revealed that at 90% of maximum leaf P, K, Ca, and Mg, the soil fertility levels ranged from 80-280 kg P_2O_5 ha^{-1}, 260-570 kg K_2O ha^{-1}, 1300-4000 kg Ca ha^{-1}, and 250-510 kg Mg ha^{-1}. With regards to the soil levels observed in the studied locations and the BLA generated soil fertility ranges, it can be concluded that more than 50% of the locations were deficient in soil K, 8% were deficient in P_2O_5, 19% deficient in Ca and 46% deficient in Mg.

The final study (Chapter 4) determined the relationship between soil fertility and IG leaf tissue nutrient content with physiological characteristics. Nineteen (19) soil samples were collected in mid-May and leaf tissue samples were collected from the same locations as soil sampling starting from late June to early July in 2016 during which time 50% of the berries had turned color, while

physiological characteristics such as SPAD value (chlorophyll content), bush size, leaf size and bush growth rate were measured after berry harvest between late July to early August of 2016. The results revealed negative associations among soil available nutrients and leaf tissue nutrient concentrations. A negative association was observed between soil P, Ca and Mg versus soil K, and in leaf tissue nutrients between leaf Ca and Mg versus leaf N, P, and K. This result further confirmed the antagonistic relationship (nutrient imbalance) observed in Chapter 3. The result also revealed a positive relationship between leaf N, P and K versus plant physiological characteristics showing the importance of these nutrients in haskap plant growth and establishment. Most importantly, there was a close association between leaf K and plant physiological characteristics, especially bush size, growth rate, and leaf size. This suggests that K is an important nutrient in improving haskap bush growth. It has been reported that P and K are needed to stimulate plant growth, which led to increased plant growth and enhanced uptake of both nutrients (Havlin et al. 2014; Ali 2018).

In order to further understand the variability in leaf tissue nutrient content and to identify nutrient sufficiency ranges for haskap, BLA was also applied to study the relationship between leaf tissue nutrient content and bush growth rate. Bush growth rate was used since yield data cannot be obtained at this stage of growth. Moreover, yield data which is dependent on the level of bee pollination and plant age make yield data unreliable. Therefore, bush growth seems to be the better option to yield data. The application of BLA produced significant ($p \leq 0.10$) second-degree models with high R^2 values ranging from 0.84 to 0.91 for leaf N, K, and Mg and 0.77 to 0.81 for N:P, N:K, and K:Ca ratios. The result revealed that at 90% of maximum growth rate, the leaf tissue nutrient sufficiency levels were 2.23-2.96% for N, 0.22-0.28% for P, 0.84-1.32% for K, 1.63-2.10% for Ca, and 0.14-0.50% for Mg. This suggests that maximum bush growth could be obtained within

the nutrient sufficiency ranges above. Considering the leaf tissue nutrient levels observed in the studied locations and the BLA nutrient sufficiency ranges, it can be concluded that 53% of the locations were diagnosed as being deficient in leaf N and P, while 58% were deficient in leaf K.

Considering the varietal comparison (Chapter 3) and the generated BLA nutrient sufficiency levels (Chapter 4), the leaf tissue nutrient content of BB and T were within the IG BLA derived nutrient sufficiency levels. Most of the other varieties such as Borealis, Larisa, Erin, Happy Giant, Aurora, Ruth, and Blue Perfection tended to have slightly lower minimum and higher maximum limit than the IG nutrient sufficiency levels. However, the number of samples of these varieties were inadequate to conclude if these varieties would have a lower or higher nutrient sufficiency levels than IG.

5.3 Recommendations

A balanced nutrition is crucial to achieving the full potential of bush growth. However, imbalance or shortage of essential nutrients is the major factor to slow haskap growth after establishment in NS. For the observed nutrient imbalance, there is a need for growers to periodically carry out soil and plant tissue testing before haskap develops visual deficiency symptoms. This would help in detecting and averting nutrient deficiency or imbalance during the growing season. Also, the nutrient management strategies should be based on soil and leaf tissue tests as the results will help growers to better understand balanced nutrient supply and adopt the best nutrient management practices.

Owing to the variability in nutrient uptake among varieties and the financial involvement in carrying out separate leaf tissue analysis for the different varieties, growers could either sample only IG as a reference, or collect samples evenly from across all varieties in the field. IG can be

used as a reference variety for assessing leaf tissue nutrient status, but should be recognized that it is lower than other varieties in P and K, and higher in Ca.

The BLA sufficiency ranges for haskap cv. Indigo Gem leaf tissue was determined to be 2.23-2.96% for N, 0.22-0.28% for P, 0.84-1.32% for K, 1.64-2.10% for Ca, and 0.14-0.50% for Mg. The nutrient sufficiency ranges and ratios can be used to guide diagnosis of nutritional status of haskap and to optimize haskap growth and productivity. Also, the established nutrient ratios would be helpful in highlighting antagonistic relationships and in overcoming insufficient nutrition in haskap production.

The following soil fertility ranges: 80-280 kg P_2O_5 ha^{-1}, 260-580 kg K_2O ha^{-1}, 1300-4000 kg Ca ha^{-1}, and 250-510 kg Mg ha^{-1} seem to be adequate for haskap production. However, to be more efficient and economical, soil fertility levels: 80-190 kg P_2O_5 ha^{-1}, 260-420 kg K_2O ha^{-1}, 1300-2700 kg Ca ha^{-1}, and 250-390 kg Mg ha^{-1} would be adequate for haskap growth and productivity as the derived BLA upper limits does not increase nutrient uptake.

5.4 Future Research

The studies provided insights on the response of haskap to soil fertility status as well as into optimal soil fertility and leaf tissue nutrient ranges. However, several limitations were encountered which can be addressed in the following way.

- Unable to collect yield data was one of the major limitations to this study. Therefore, studying the relationship between soil fertility status and leaf tissue nutrient concentrations in relation to haskap yield will be required. This will further provide better understanding on the pattern of haskap response to soil fertility status.

- The locations under this study varied in terms of nutrient management practices, which made it difficult to ascertain the actual amount of nutrient applied. Therefore, the derived BLA soil fertility levels will have to be evaluated in a controlled growth conditions in relation to haskap growth and productivity.
- The data set used in developing leaf tissue nutrient sufficiency levels seemed to be inadequate for this study. Therefore, future study needs to significantly increase the data set. At least, 2-3 years of data set on leaf nutrient concentrations, growth and yield from locations with a well-documented nutrient management practices would be ideal.
- In some locations, high levels of soil Ca and Mg could be interfering with plant uptake of K. Therefore, further research is needed to identify optimum K fertilization relative to soil Ca and Mg levels.
- Finally, field experiments will be needed to validate the derived BLA soil fertility levels under varying climatic conditions using different haskap varieties. This would help in validating both the BLA soil fertility and leaf nutrient sufficiency levels obtained in this study.

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APPENDICES

Table A-1. Soil fertility recommendation for small fruit crops in Nova Scotia (NSDA 2010a)

Soil rating	Soil test levels (kg ha^{-1})			
	P_2O_5	K_2O	Ca	Mg
Low (L-, L, L+)	0-231	0-121	0-1187	0-80
Medium (M-, M, M+)	232-360	122–236	1188-3083	81-432
High (H-, H, H+)	361-558	237-514	3084-5434	330-563
Excessive (E)	559+	515+	5435+	564+

Table A-2. Leaf tissue nutrient sufficiency ranges (NSDA 2010b)

Crop	N	P	K	Ca	Mg	B	Zn	Cu	Mn	Fe
	---------- % ----------					---------- ppm ----------				
Black currants	2.7-2.9	0.26-0.30	1.0-1.6	1.0-1.5	0.1-0.15	20-40	20-50	5-20	20-70	-
Highbush blueberries	1.5-2.5	0.1-0.4	0.3-0.8	0.2-0.7	0.1-0.25	20-70	10-50	5-20	50-350	40-150
Raspberries	2.0-3.5	0.2-0.5	1.0-2.0	0.8-2.5	0.25-0.5	20-60	15-100	5-20	20-200	25-200
Strawberries	2.0-3.0	0.2-0.5	1.5-2.5	0.5-1.5	0.25-0.5	20-60	15-100	-	20-200	25-200

"-": No available recommendation.

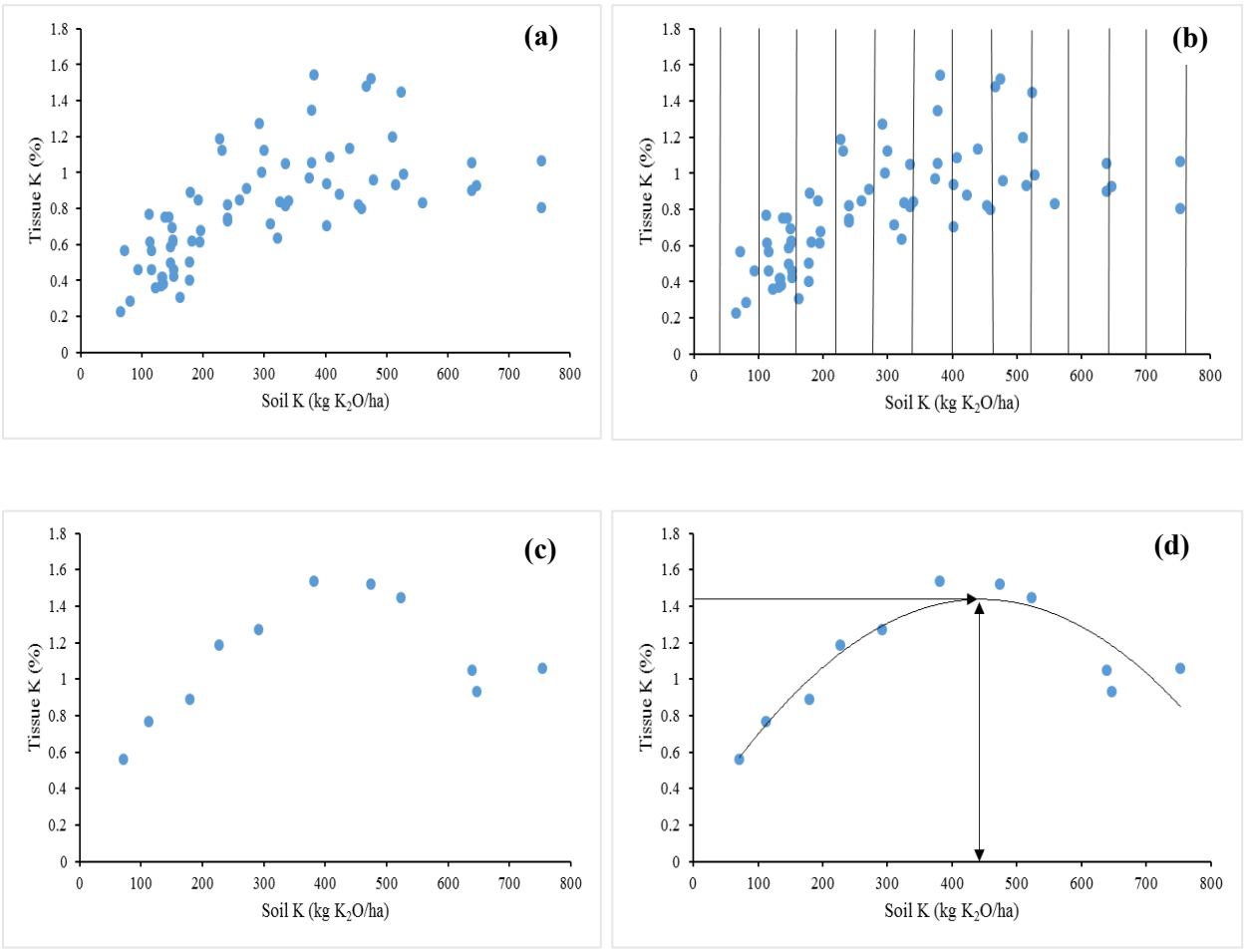

Figure A-1. The different steps of the boundary-line regression approach to leaf tissue and soil nutrient concentration relationship for all haskap varieties sampled in 2015 and 2016.

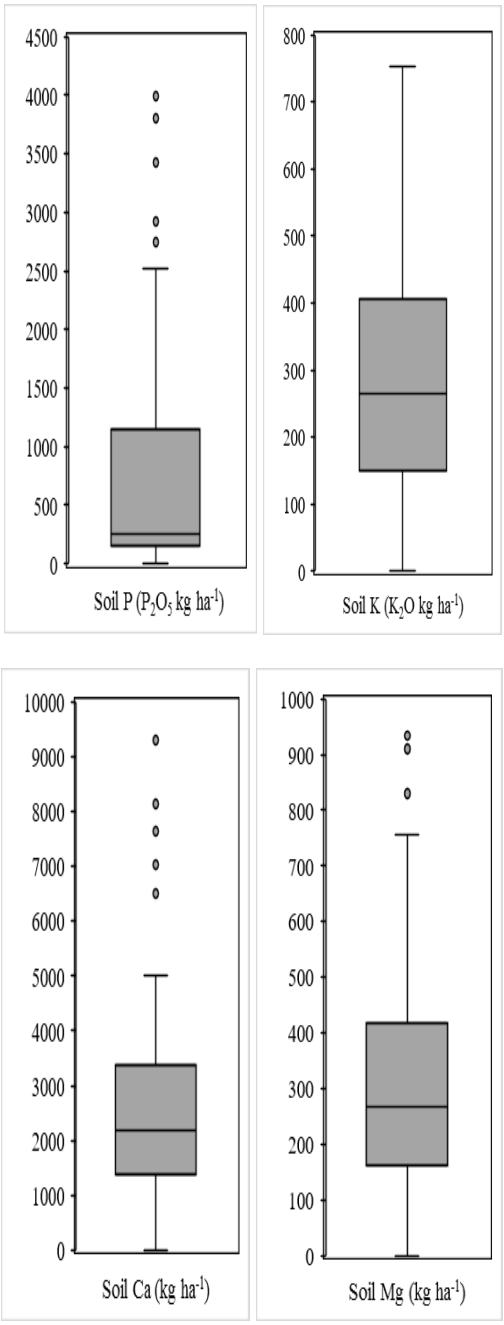

Figure A-2. Box- and whiskers plot of soil P, K, Ca, and Mg nutrient concentrations in 2015 and 2016. The box indicates interquartile range, line within the box indicates median, the whiskers represent 1.5 times the interquartile range, and the dotted points represent outliers.

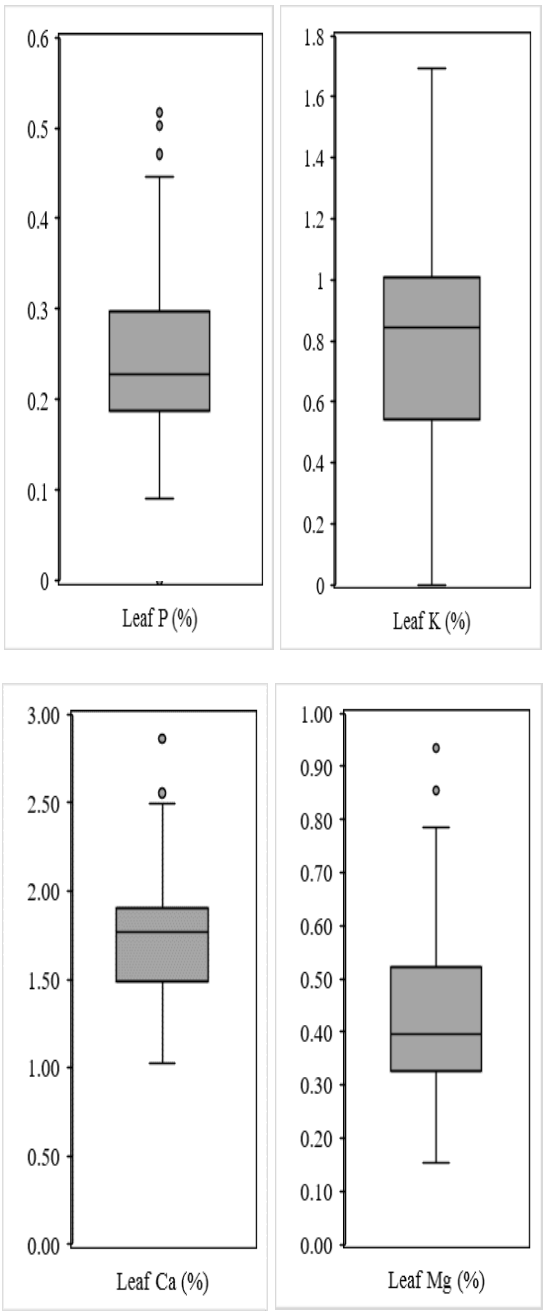

Figure A-3. Box- and whiskers plot of haskap leaf tissue nutrient concentrations sampled in 2015 and 2016. The box indicates interquartile range, line within the box indicates median, the whiskers represent 1.5 times the interquartile range, and the dotted points represent outliers.

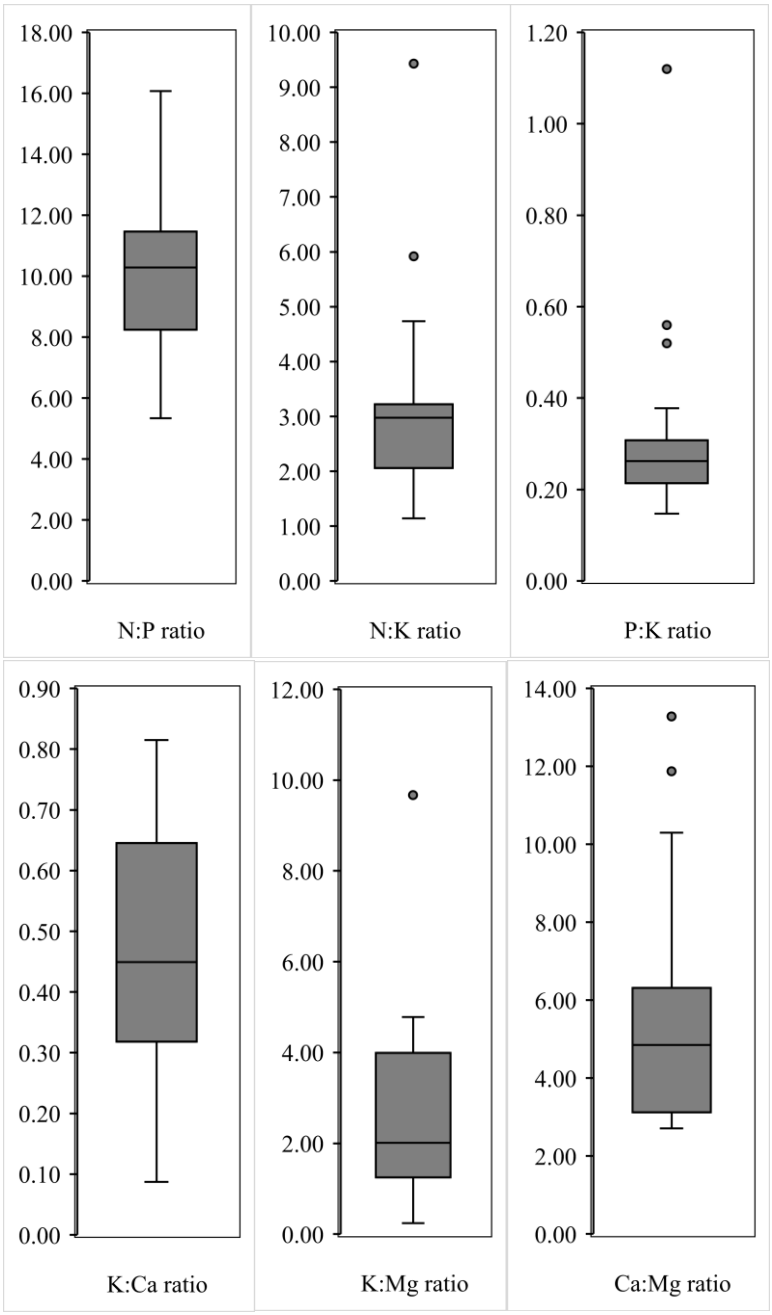

Figure A-4. Box- and whiskers plot of haskap leaf tissue nutrient ratios sampled in 2015 and 2016. The box indicates interquartile range, line within the box indicates median, the whiskers represent 1.5 times the interquartile range, and the dotted points represent outliers.

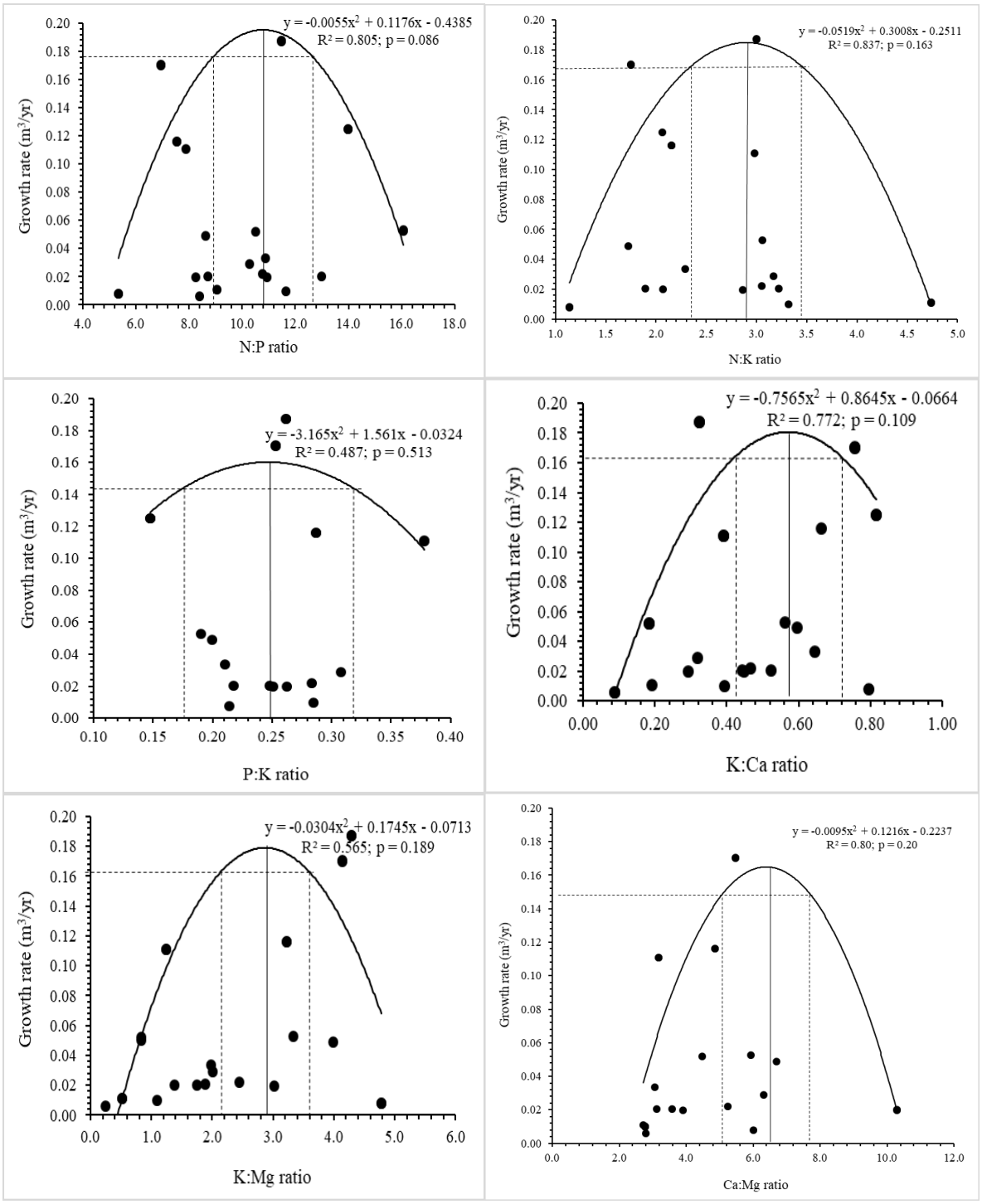

Figure A-5. Scatter diagram of bush growth rate vs. nutrient ratios of haskap cv. Indigo Gem showing boundary lines approach described by second-degree polynomial regression functions ($p \leq 0.10$).