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Nutrient Sufficiency Levels for Haskap (*Lonicera caerulea* L.) Using the Boundary-Line Approach

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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 (*Lonicera caerulea* L. cv. Indigo Gem) plant characteristics in relation to soil and leaf tissue nutrient status. A total of 19 composite soil samples and corresponding paired with plant leaf tissue samples were collected in 2016 from 12 locations in Nova Scotia. Plant parameters 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 and correlation analysis suggested a possible antagonistic interaction between leaf K and Mg. Negative associations were observed most frequently between Ca and Mg and other nutrients, especially K. Plant parameters such as bush volume, leaf size and growth rate 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 on representative farms in Nova Scotia.

Key words: boundary-line approach, haskap, Indigo Gem, *Lonicera caerulea* L., leaf tissue nutrients, nutrient sufficiency, soil fertility

INTRODUCTION

Haskap is a relatively new crop of rapidly growing interest in Canada and Nova Scotia. As such, sustainable management strategies including agronomic and postharvest practices need to be developed for the industry to thrive. However, soil nutrient management and tissue nutrient standards for haskap are not clearly established (Bors 2009). 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 and height of 2 m or more (Plekhanova 1992; Hummer et al. 2012). Haskap fruit yield increases as the plant increase in size (Plekhanova 1992), suggesting that management strategies that increase bush growth will potentially increase fruit yield. According to McCarthy and Stoker (1988), management practices should be aimed at improving number and length of shoots in order to optimize vegetative growth and maximize the first commercial harvest. Cultivated haskap plants begin to produce significant amount of fruits after four years and reach full bearing after seven to eight years (Plekhanova 1992).

The appropriate nutrient composition is required to achieve potential growth, development and yield of all cultivated plants including haskap. For instance, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) are essential macronutrients of crops (Yadong and Shuang 2009; Havlin et al. 2014). A deficiency or excess amount in any of the essential nutrients will result in tissue nutrient imbalance that may disrupt either the vegetative or reproductive growth cycles in plants and alter tissue nutrients 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 plants. The imbalance of essential nutrients might contribute to slow haskap growth after establishment. Therefore, the interactions of these essential nutrients and other micronutrients should be considered in ensuring haskap growth and productivity.

Nutrients applications could have a synergistic effect, antagonistic effect, or no effect on plant growth and productivity (Fageria 2001). Nutrient interactions are said to be synergistic when the application of two nutrients increased 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 reduced the availability and uptake of the other nutrient elements (Dibb and Thompson 1985). For instance, increasing soil K beyond an acceptable level may reduce uptake of P, Ca and Mg, or increased Ca level may inhibit P, K and Mg availability and uptake (Chou et al. 2011; Sun et al. 2013; Havlin et al. 2014). A balance in plant 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 to understanding the importance of balanced supply of nutrients (Fageria 2001; Mattson and Van Iersel 2011; Santos 2011), and its subsequent enhancement of haskap growth and yield.

Nutrients interactions are usually measured in terms of plant growth response and changes in nutrients concentrations (Fageria 2001). Many researchers have used different agronomic 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 et al. 2006) and tree growth rate (René et al. 2013) to diagnose and develop nutritional standards. As it is a relatively new crop, the growth response of haskap to different levels of nutrients is in itself not well documented and thus it is not known how important plant nutrition is for haskap, or which nutrients are most important. We hypothesize that the relationship between the concentration of individual leaf tissue nutrients and plant parameters such as growth rate and leaf size will be positive and statistically significant. The study of leaf tissue nutrient content and plant characteristics will further help in understanding the variability observed among haskap orchards. The objectives of this study were to i) investigate the relationships between soil fertility, tissue nutrient contents, and some plant characteristics such as bush volume, growth rate, leaf size and chlorophyll content; and ii) derive nutrient sufficiency levels for haskap using cv. Indigo Gem as the test plant.

MATERIALS AND METHODS

Experimental Design and Location

A survey on multiple farm locations with a range of soil conditions in Nova Scotia was performed between May and August 2016. Indigo Gem is the most commonly grown variety present on all sites. A total of 19 paired soil and leaf tissue samples were collected in 2016 from farm fields throughout Nova Scotia. Each paired sample represented a site with a unique management history or soil type. Thus, the leaf samples are collected from plants immediately within the soil sampling zone. Plant characteristics were collected from the same soil and plant sampling locations as described below. The history of agronomic practices of individual fields and age of plants were documented (Table 1). The age of the plants sampled ranged from two to five years and growing under field conditions for at least one growing season. The weed management practices across the farms varied from cultivation at the base of the plant, use of coconut fiber mats at plant base, wood-chip mulch to no-weeding at all.

Soil Sampling and Analysis

Soil samples were collected from the within the plant rows beginning from the 10th 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 rows to a depth of approximately 15 cm using a sampling probe. Samples were stored in a cooler and refrigerated until submission to the Nova Scotia Agricultural Laboratory Services, Truro for standard soil test analysis including Mehlich III determination of mineral nutrients. The analytical method omitted soil mineral N testing as this is not possible using the Mehlich III extract. Soil pH was determined in water (1:1 soil-to-water). 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 10-g soil samples were weighed into 50 mL test tubes and 25 mL of Mehlich III extracting solution was added before shaking for 5 min using a reciprocating shaker. The mixture was filtered through Whatman #42 filter paper and the resulting filtrate was used to determine mineral nutrients concentrations.

Plant Tissue Sampling, Preparation and Analysis

A composite of whole leaf samples was collected from 20 plants within the same row where the soil samples were collected per location. The leaf samples were collected in June 2016 when approximately 50% of the berry had color turn. Leaves were collected from new stem growth, typically three nodes down from the tip of the branch, which were immediately placed in a cooler for transportation to the Nova Scotia Agricultural Laboratory Services for nutrient determination. Briefly, leaf samples were cleaned with distilled water; air-dried and then packed in paper bags. The packed leaf samples were oven dried at 60^{o}C for 48 hrs to a constant weight before grinding to powder. Total N was determined by combustion method (AOAC-990.03) [Association of Official Analytical Chemists (AOAC) 2003a] using LECO-Spec Analyzer (TruSpec® Micro, LECO, MI USA), while P, K, Ca, Mg, Cu, Mn, B, Zn, Al, and Fe was determined using the inductively-coupled plasma spectrometer method (AOAC-968.08) (AOAC 2003b).

Plant Parameters and Observations

Bush volume was estimated as the cylindrical volume using the formula: [3.142 × height × width × 0.5 × breadth × 0.5] as used to calculate the bush volume for blackcurrant by Erb et al. (1993) and modified by Hobson et al. (2013). A meter stick was used to measure the plant height from the collar of the stem to the apex while breadth and width was measured from the sides of the plant. The chlorophyll content or leaf greenness was measured using a 502 plus SPAD meter (Spectrum Technologies, Inc., Aurora, IL, USA). 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® (Ehab M. Bakr, Cairo, Egypt). Plant growth rate was estimated by dividing the bush volume by the age of the plants on the field. Visual observation was made to determine the presence or absence of nutrient deficiency symptoms based on common nutrient deficiency symptoms of plant as indicated by Havlin et al. 2014. All data were collected from 20 plants per location in summer from late July to early August 2016 after berry harvest.

Statistical Analysis

Boundary-Line Approach and Averaging Approach

The boundary-line approach (BLA) was proposed by Walworth et al. (1986) and has been adopted by many researchers such as Schnug et al. (1996), Vizcayno-Soto and Cote (2004), Blanco-Macías et al. (2009) Bhat and Sujatha (2013) and Ali (2018). They used the BLA to investigate 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 type. Thirdly, a second-degree polynomial function was generated from the selected points at $\alpha = 0.10$ significant level. Finally, the optimum nutrient concentrations were determined by solving the second-degree function as described by Ali (2018). The corresponding values of up to 90% of the highest growth rate was used to define the minimum and maximum nutrient sufficiency ranges. Prior to the BLA steps, it is important to remove outliers from the data set (Ali 2018). Thus, outlier test was carried out to detect and remove outliers from the leaf tissue nutrient concentrations data set using box and whiskers plots (Fig. 1). An outlier was found only for Mg. The outlier point observed was 0.93%, which was the upper limit for leaf Mg and 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. 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 leaf tissue nutrient concentration of the high growth rate subpopulation was recorded as the optimum nutrient level for each nutrient element.

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 plant 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. Pearson correlation analysis was done to determine the relationship between soil and leaf tissue nutrients. Data analysis was performed using Minitab version 18.1 (Minitab Inc., State College, Pa., USA).

RESULTS AND DISCUSSION

Soil Fertility Status

The soil fertility status of the study locations is presented in Table 2. The pH values of the soils were moderately acidic to neutral (i.e. pH values ranged from 5.2 - 7.0). The pH ranges observed were within the tolerable levels for haskap (Retamales and Hancock 2012), and the variability was small among the different sites. However, a recommended pH range has not been clearly established for haskap.

Based on soil fertility recommendations for small fruit crops (NSDA 2010a), soil P and K across locations were found to range from very low to extremely high i.e. 65 - 2320 kg P_2O_5 ha^{-1} and 65 - 753 kg K_2O ha^{-1}. Soil Ca and Mg tended to be adequate and ranged from 1225 - 6497 kg ha^{-1} and 127 - 713 kg ha^{-1} respectively. However, the upper limit of the observed ranges for Ca and Mg was twice the NSDA (2010a) recommended upper limits for small fruit crops. In general, the variability observed within the various soil parameters measured was low i.e. 0.99 - 12.28%, according to the criterion established by Wilding et al. (1994).

Plant Growth and Leaf Tissue Nutrient Concentrations

A wide range of variability in leaf tissue nutrient concentrations was observed within the study locations (Table 3). Wilding et al. (1994) criteria were used to determine the magnitude of variability; a coefficient of variation (CV) of 0 - 15% as low, 15 - 35% as medium, and 35 - 100% as high. There was high variability in growth rate and bush volume, 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 3). Haskap cv. Indigo Gem leaf tissue concentrations of N, P, Ca, Cu, Zn and Fe within locations showed medium variability with a CV ranging

from 16.7 – 34.0%. However, high levels of variability were observed in leaf K, Mg, B and Mn with the CV ranging from 39.5 - 91.3%.

As this was not a controlled experiment, there could be several explanations for variability in bush growth aside from leaf tissue nutrient concentration including variability in age of the plant, fertility management practices, or weed management practices (Table 1). The levels of variability observed in growth and leaf nutrient concentrations could be due to variations in plant age (Table 1). Preliminary observations suggested that haskap vegetative growth rate may slowly decrease after the fourth year (data not presented). According to Plekhanova (1992), yearly bush growth rate decreased after the eight year. However, this could not be confirmed since the bushes sampled were between two to five years old. Additionally, soil properties and farmers' nutrient management practices could also attribute to the variations (Prive and Sullivan 1994; Kabata-Pendias 2004; Ali 2018) across the different study locations.

In addition, the weed management practices across the orchards in this study could influence nutrient availability in the soil and henceplant 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 lower in non-weeded management compared to the use of weed mat (Harkins et al. 2014). According to Dixon et al. (2016), non-weeded treatments reduced nutrient content of primocanes, floricanes, and fruits, whereas the use of weed mat resulted in higher nutrient accumulation in blackberry. They all concluded that weed management had the largest impact on plant nutrient content and biomass. The use of wood-chip mulch could also have a negative influence on N availability. Wood-chips are known to have high C to N ratio, which may result in N deficiency under low N supply when incorporated. This is due to use of N by soil microbes and thereby, immobilizing N (Gallardo and Merino 1998; Idol et al. 2003; Homyak et al. 2008). This could be the case of haskap in the study locations where weed management varied across locations, and vary from cultivation at the base of plant, use of coconut fiber mats at plant base to wood-chip mulch and no-weeding fields.

Optimum Nutrient, Sufficiency Ranges and Nutrient Ratio

In the scatterplot diagrams, the data points were mostly grouped at lower growth rates (Fig. 2). The BLA second-degree polynomial regression functions for haskap cv. Indigo Gem were generated with at least 5 interval points for leaf tissue nutrient concentrations. 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 have also been used (Bhat and Sujatha 2014) and therefore, the number of intervals used in this study is considered suitable for small data set to reduce the likelihood of including haskap plants that are not growing under 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 nutrient ratios ranging from 0.77 - 0.81 for N:P and K:Ca. For leaf P and Ca concentrations, the generated models were not significant ($p > 0.10$). 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. The optimum values derived through the averaging approach (i.e. mean high growth rate > 0.10 m^3 yr^{-1} subpopulation) are also presented in Table 4. Comparatively, the optimal leaf N and Ca derived from the BLA tended to vary a little from that of the averaging method, while for leaf P, K and Mg, the optimum concentrations were comparable. The averaging method has been used to generate nutrient standards (Bhat and Sujatha 2014) and therefore, both methods were comparable in this study. This suggested 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 status at or close to the generated optimal leaf nutrient levels for optimum plant growth. The nutrient sufficiency ranges developed may be used as guiding principle in assessing haskap nutritional status as reported by Bhat and Sujatha (2014) for arecanut (*Areca catechu* L.).

On the other hand, many of the study locations were diagnosed as having deficient or excess soil nutrients with few locations having adequate nutrition (Fig. 3a). For instance, based on the derived nutrient sufficiency ranges, and visual deficiency symptoms recorded, 53% of the locations were diagnosed as being

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. Many of the study locations were identified as having adequate leaf Ca (58%) and Mg (74%) concentrations (Fig.3a). The percentage number of locations diagnosed as having adequate (74%) or excess (26%) Mg seemed realistic when considering the widespread occurrence of Mg-rich soil parent materials in eastern Canada (Quesnel et al. 2006). In general, majority of the locations were identified as having deficient or adequate in one nutrient or the other. This suggested that nutrient imbalance could be the major problem causing slow growth of haskap bush in the studied locations.

Balancing nutrition to maximize haskap growth and productivity, requires the use of nutrient ratios (Bhat and Sujatha 2013; Horuz et al. 2013). Haskap nutrient ratios derived from BLA are also presented in Table 4. 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 from the derived nutrient ratios could result in reduced bush growth as reported for other crops (Bhat and Sujatha 2013; Horuz et al. 2013). The nutrient ratios derived (Table 4) would be helpful in highlighting antagonistic relationships between nutrients and also, can be a useful advance warning tool for overcoming insufficient nutrition in haskap.

With regards to haskap nutrient ratios, the nutrient imbalance could be the major cause of slow bush growth in the studied locations (Fig. 3b). Based on the nutrient ratio models, a majority of the locations were diagnosed as having below or within the BLA-based optimum range. The locations with growth rate > 0.10 m^3 yr^{-1} (high-growth rate subpopulations) tended to have balanced nutrient ratios, except for N:K ratio that appeared to be relatively low. More than half of the locations (74%) were identified as having a P:K ratio within the derived BLA nutrient ratio. For N:P ratio, 42% of the locations were diagnosed as having below the BLA nutrient ratios (Fig. 3b). Also, 53% of the locations were identified as deficient in K:Ca and Ca:Mg. This suggested 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).

The soil and leaf tissue data sets used in this study were collated from different locations with different soil and climatic conditions (data not presented). 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 (Bhat and Sujatha 2013). So, the nutrient sufficiency ranges and ratios derived for haskap may be reliable.

Comparison of Derived Haskap Sufficiency Ranges to Other Small Fruits

The BLA sufficiency nutrient range for haskap was compared to other small fruit crops such as highbush blueberry (*Vaccinium corymbosum*) and black currants (*Ribes nigrum*). This is important because farmers tend to use sufficiency ranges for these two crops to evaluate the nutritional need of haskap due to their similar growth stature and fruit type.

The BLA sufficiency levels for haskap were more comparable to black currants sufficiency ranges than that of highbush blueberry. Haskap leaf Ca and Mg sufficiency levels were poorly matched to those of black currant and highbush blueberry (Table 5). 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 those for both of the other crops. This suggested 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 but higher minimum and maximum sufficiency levels for leaf Ca and Mg (Table 5). This suggests that haskap might have higher Ca and Mg requirements than black currant and highbush blueberry.

Relationship between Soil, Tissue and Plant Parameters

The PCA applied to the 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 plant characteristics (Fig. 4). Following Kaiser's criteria (Bowley 2008), four PCs were retained that explained 80.0% of the total variance. The first PC (PC1) explained 41.1% of the total variance, which showed a positive and strong relationship among leaf N, P, K and plant variables. This implies that the

majority of the variation was explained by bush volume, growth rate, pH, soil K, and Ca. Soil organic matter, leaf Ca and Mg were negatively associated with plant variables, and leaf N, P, and K. The negative PCA values implies that these variables like soil organic matter, leaf Ca and Mg explained less of the variation in the overall dataset. The second PC (PC2) accounted for 17.4% of the total variance, and suggested that leaf K, Mg and P were positively related to leaf N including plant variables. Also, soil P, Ca and Mg were negatively associated to soil K (Fig. 4).

Soil pH had a moderate positive correlation with soil P and K and strong positive correlation with soil Ca and Mg. Also, soil P, K and Ca showed a significant positive relationship among each other; no significant relationship was observed between soil Mg versus soil P, K and Ca (Table 6). Fageria (2001) stated that increasing one nutrient will require the increase of the other in order to balance nutrient availabilty.

The negative relationship observed in PC2 between soil Mg and soil K which was highly correlated with plant growth indicators suggests that these two nutrients are vital in explaining haskap nutrient variation. From the correlation analysis, a very strong significant negative relationship was found between leaf K and Mg, suggesting a possible nutrient imbalance (Table 6). 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 (Fig. 4). This imbalance 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 during the photosynthesis process (Havlin et al. 2014). The positive association between N and SPAD value (chlorophyll content) was expected as it conformed to well documented reports (Amaliotis et al. 2004; Cabrera 2004; Bojović and Marković 2009).

Magnesium, as a primary constituent of chlorophyll, is essential for photosynthesis. 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 will have a strong positive association with leaf N and chlorophyll content. However, the reverse was observed in both PCs (Fig. 4). Huang and Grunes (1992) reported that increasing NO_3^- levels would increase Mg uptake but would also decrease Mg translocation in plants. 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 (Fig. 4) confirms antagonistic nutrient interaction as observed (Table 6). Similar observations were made for other crops by several researchers (Chou et al. 2011; Horuz et al. 2013; Sun et al. 2013; Dresler et al. 2015). These researchers concluded that increased K, Ca or Mg levels would affect the uptake of the other nutrients.

Furthermore, the opposite grouping of the leaf tissue nutrients in the PCs (Fig. 4) 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). The positive grouping of leaf N, P and K versus plant variables indicated 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 (data not presented). This could be attributed to the nutrient imbalance causing antagonistic interaction in the plants. These deficiency symptoms conformed 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 that might have led to the nutrient deficiencies that was observed. Therefore, it can be inferred that the deficiencies of P and K might possibly be the main cause of growth variabilities in haskap cv. Indigo Gem that was observed across the selected locations.

CONCLUSIONS

The BLA has been a useful method in diagnosing and developing nutritional standards in the present study. The BLA sufficiency ranges for haskap cv. Indigo Gem leaf tissues were determined to be

approximately 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. These sufficiency ranges may be used to guide diagnosis of nutritional status of haskap plants. 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 some 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 tissue.

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Figure Captions

Figure 1. Box and whiskers plot of haskap cv. Indigo Gem leaf tissue nutrient concentrations. 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 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 ($p \leq 0.10$). Each data point represents an average value of 20 plants from a location.

Figure 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. Factor loading biplot of the principal component analysis performed on selected soil chemical properties, leaf tissue nutrients and plant parameters of haskap – cv. Indigo Gem. S.O.M - soil organic matter; S-pH - soil pH; S-P - available phosphorus; S-K - available potassium; S-Ca - soil calcium; S-Mg - soil magnesium; L-N - leaf nitrogen; L-P - leaf phosphorus; L-K - leaf potassium; L-Ca - leaf calcium; L-Mg - leaf magnesium, B.V - bush volume; L.S - leaf size; G.R - growth rate; SPAD - chlorophyll content.

Table 1. Methods of weed control and nutrient management history of the sampled farms from 2015 to 2016 in Nova Scotia.

Region-ID#	Soil	Plant age[a]	Method of weed control	Nutrient applied (method or placement)[c]
Annapolis-409	Fine loamy sand, well drained	2	Weeding (plant base)	Compost tea (foliar)
Annapolis-462	Friable sandy loam, well drained	4	Weeding (entire row)	Compost tea (foliar)
Annapolis-464	Friable sandy loam, well drained	4	Weeding (entire row)	Compost tea (foliar)
Annapolis-465	Sandy loam, well drained	4	Coconut fiber (plant base)	Organomex (foliar)
Annapolis-466	Sandy loam, well drained	4	Weeding (plant base)	Organomex (foliar)
Annapolis-469	Sandy loam, well drained	2	Weeding (plant base)	Organomex (foliar)
Annapolis-470	Sandy loam, well drained	5	Wood mulch (plant base)	N/A[b]
Bridgewater-419	Loam, moderately well drained	5	Oyster shell as mulch	Acer 17-7-10 (plant base)
Bridgewater-425	Loam, mod. well drained, recently cleared	4	Coconut fiber (plant base)	Acer 17-7-10 (plant base)
Bridgewater-429	Loam, mod. well drained, recently cleared	4	Coconut fiber (plant base)	Acer 17-7-10 (plant base)
Bridgewater-432	Loam, moderately well drained	2	Surface biochar application	Acer 17-7-10 (plant base)
Bridgewater-435	Loam, moderately well drained	3	Coconut fiber (plant base)	Acer 19-4-12 (plant base)
Bridgewater-440	Loam, well drained	3	Weeding (plant base)	Compost tea (foliar)
Bridgewater-442	Loam, well drained	4	Wood mulch (entire row)	Cow manure + Sea Agri-90 + compost tea
Bridgewater-444	Loam to sandy loam, well drained	2	Wood mulch (entire row)	N/A
Bridgewater-476	Loam, well drained	4	Weeding (plant base)	Compost tea (foliar)
Truro-402	Sandy loam, well drained	3	Weeding (plant base)	Cow manure
Truro-453	Loamy sand, rapidly drained	2	Black plastic + Wood mulch	MSW compost (trench)[d]
Truro-457	Gravelly loamy sand, rapidly drained	3	Wood mulch (entire row)	Compost tea (foliar) + Calphos (broadcast)

Note: Locations represent the major haskap production areas in Nova Scotia.

[a] Age of haskap plants on the field during sampling period.

[b] Not available.

[c] Nutrient application rates were not documented by the farmer.

[d] Municipal soil waste compost.

Table 2. Descriptive statistics of soil pH, soil organic matter and Mehlich III extractable nutrients observed from 19 selected locations growing haskap cv. Indigo Gem in Nova Scotia.

Parameters	Mean	Minimum	Maximum	CV (%)
pH	6.0	5.22	7.04	12.28
S.O.M[b] (%)	4.92	2.80	7.70	3.24
P_2O_5 (kg ha^{-1})	813.68	65.0	2320	1.16
K_2O (kg ha^{-1})	316.79	65.0	753.0	1.85
Ca (kg ha^{-1})	2920	1225	6497	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	745.0	1874	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

Note: CV represent coefficient of variation.

[a] S.O.M - soil organic matter; P_2O_5 - phosphorus; K_2O - potassium; Ca - calcium; Mg - magnesium; Na - sodium; S - sulphur; Al - aluminum; B - boron; Cu - copper; Fe - iron; Mn - manganese; Zn – zinc.

Table 3. Descriptive statistics of haskap cv. Indigo Gem growth characteristics and leaf tissue nutrient composition from 19 selected haskap growing locations in Nova Scotia.

Parameters	Mean	Minimum	Maximum	CV (%)[a]
	Plant growth characteristics			
Bush volume (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 concentration			
N[c] (%)	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

Note: [a] CV - coefficient of variation.

[b] SPAD - chlorophyll content.

[c] N - nitrogen; P - phosphorus; K - potassium; Ca - calcium; Mg - magnesium; B - boron; Cu - copper; Fe - iron; Mn - manganese; Zn – zinc.

Table 4. Nutrient sufficiency ranges, and nutrient ratios for leaf tissue nutrient concentrations in haskap cv. Indigo Gem using boundary-line approach.

		Sufficiency ranges			Averaging approach
Nutrient/ratio	R^2	Optimum	Minimum	Maximum	Optimum[a]
N (%)[b]	0.84*	2.60	2.23	2.96	2.48
P (%)	0.30[ns]	0.25	0.22	0.28	0.27
K (%)	0.84*	1.08	0.84	1.32	1.09
Ca (%)	0.67[ns]	1.86	1.63	2.10	1.93
Mg (%)	0.91*	0.32	0.14	0.50	0.32
		Nutrient ratios			
N:P	0.81*	10.80	8.90	12.60	9.13
N:K	0.84[ns]	2.90	2.35	3.45	2.28
P:K	0.49[ns]	0.25	0.18	0.32	0.25
K:Ca	0.77*	0.57	0.43	0.72	0.56
K:Mg	0.57[ns]	2.90	2.18	3.60	3.34
Ca:Mg	0.80[ns]	6.40	5.10	7.70	5.94

Note: * represents regression coefficients are statistically significant ($p \leq 0.10$).

[a] Calculated from high-growth rate subpopulation (growth rate > 0.10 m^3 yr^{-1}).

[b] N - nitrogen; P - phosphorus; K - potassium; Ca - calcium; Mg – magnesium.

[ns] Not significant.

Table 5. Comparison of haskap cv. Indigo Gem estimated boundary-lines sufficiency ranges and optimum nutrient ratios to NSDA (2010b) nutrient recommendations for small fruit crops.

Nutrients	Haskap	Black currant[a]	Highbush blueberry[b]
N (%)[c]	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 ratios[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

Note: Haskap nutrient sufficiency ranges and ratios derived from boundary-line approach.

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

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

[c] N - nitrogen; P - phosphorus; K - potassium; Ca - calcium; Mg – magnesium.

[d] Nutrient ratios for black currant and highbush blueberry were calculated from recommended sufficiency ranges

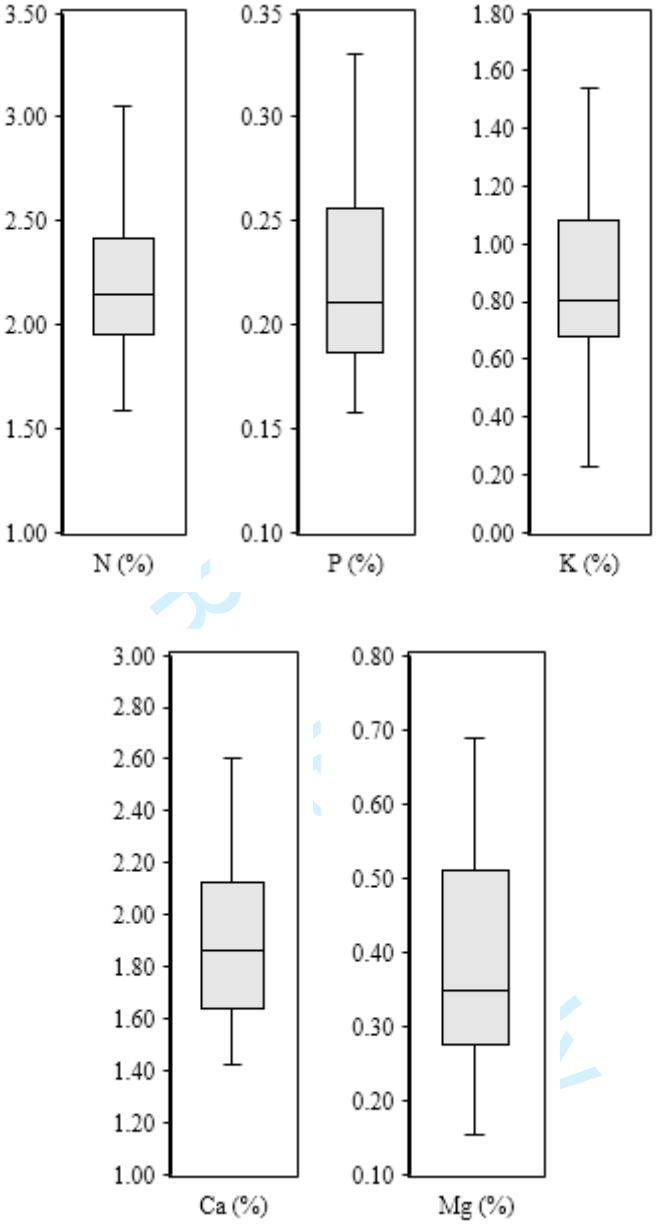
Table 6. Pearson correlation coefficients (r^2) and p-values of soil and Indigo Gem leaf tissue nutrient concentrations observed from 19 selected locations growing haskap cv. Indigo Gem in Nova Scotia.

	Soil parameter (kg/ha)					Leaf tissue nutrients (%)			
	pH	P_2O_5[a]	K_2O	Ca	Mg	N	P	K	Ca
Soil									
P_2O_5	0.51								
	0.02*								
K_2O	0.55	0.52							
	0.01**	0.02*							
Ca	0.86	0.70	0.60						
	0.00***	0.00***	0.01**						
Mg	0.65	0.28	0.19	0.47					
	0.00***	0.23[ns]	0.44[ns]	0.04*					
Leaf									
N	0.22	0.17	0.37	0.34	0.13				
	0.36[ns]	0.47[ns]	0.11[ns]	0.14[ns]	0.57[ns]				
P	-0.37	-0.20	-0.13	-0.33	-0.22	0.08			
	0.11[ns]	0.39[ns]	0.56[ns]	0.16[ns]	0.35[ns]	0.73[ns]			
K	-0.09	-0.18	0.07	0.12	-0.27	0.13	0.39		
	0.71[ns]	0.45[ns]	0.79[ns]	0.61[ns]	0.25[ns]	0.59[ns]	0.09[ns]		
Ca	0.12	0.23	0.20	0.17	0.45	0.15	0.11	-0.31	
	0.63[ns]	0.33[ns]	0.31[ns]	0.47[ns]	0.05*	0.52[ns]	0.63[ns]	0.18[ns]	
Mg	0.04	0.06	-0.18	-0.14	0.01	-0.28	0.04	-0.69	0.11
	0.87[ns]	0.81[ns]	0.46[ns]	0.56[ns]	0.98[ns]	0.24[ns]	0.86[ns]	0.00***	0.65[ns]

Note: *, **, *** represents significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively.

[a] P_2O_5 - available phosphorus; K_2O - available potassium; Ca - calcium; Mg - magnesium; N - nitrogen; P - phosphorus; K – potassium.

[ns] Not significant.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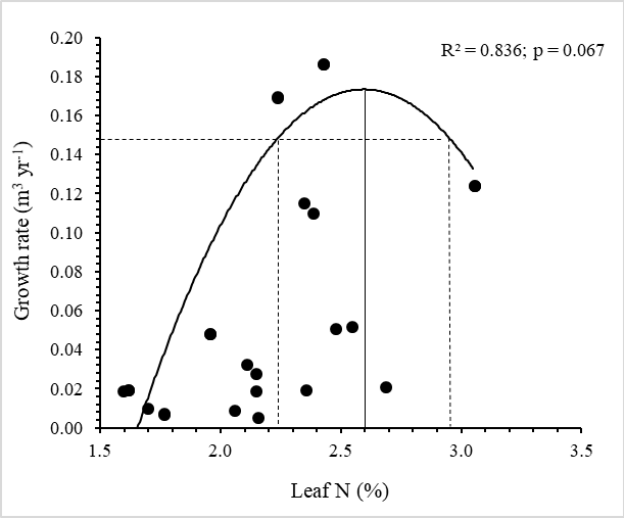
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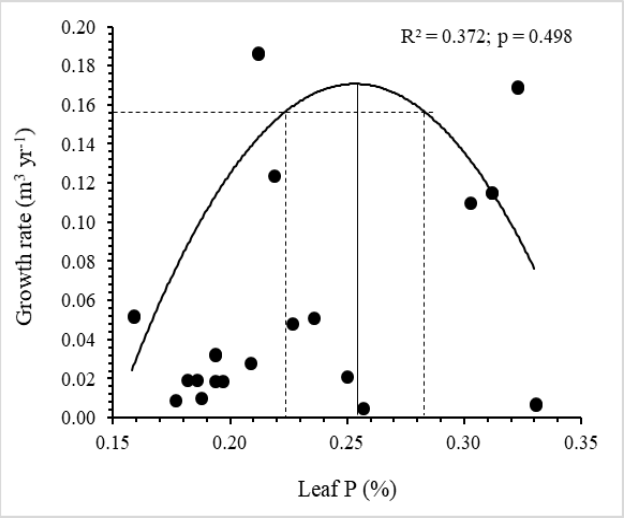
Leaf N (%)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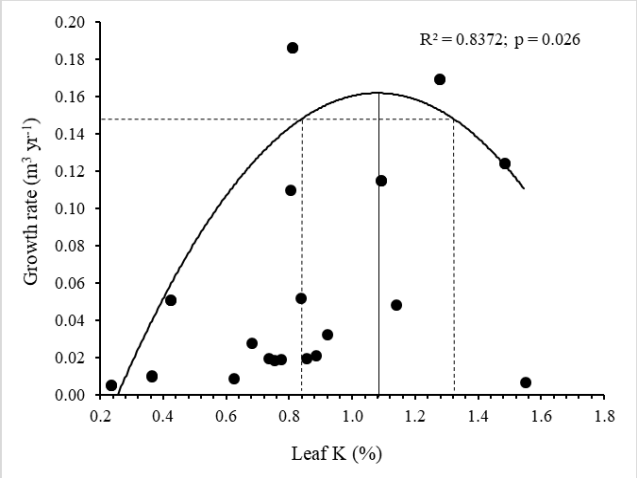
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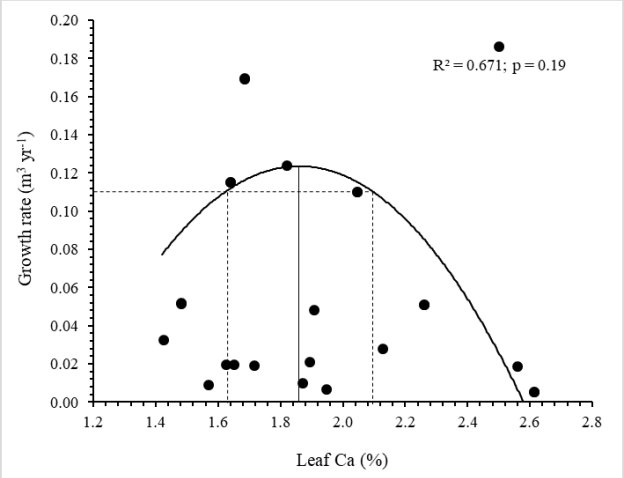
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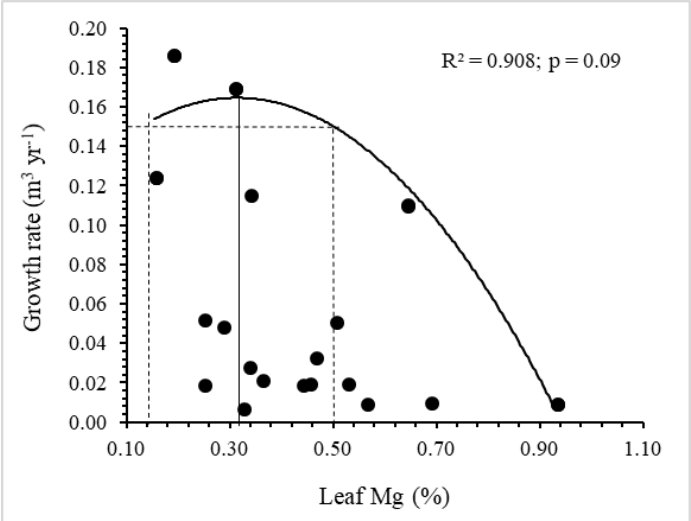
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Growth rate (m³ yr⁻¹)
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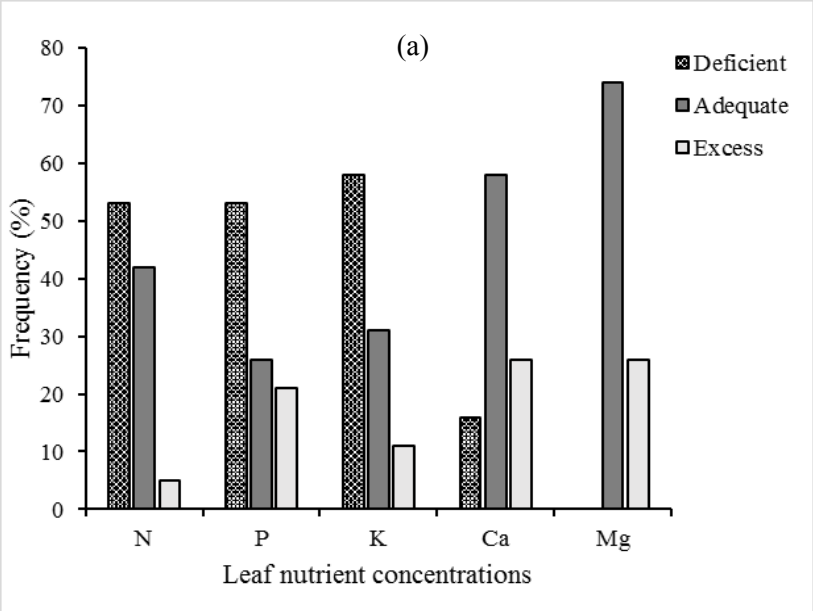
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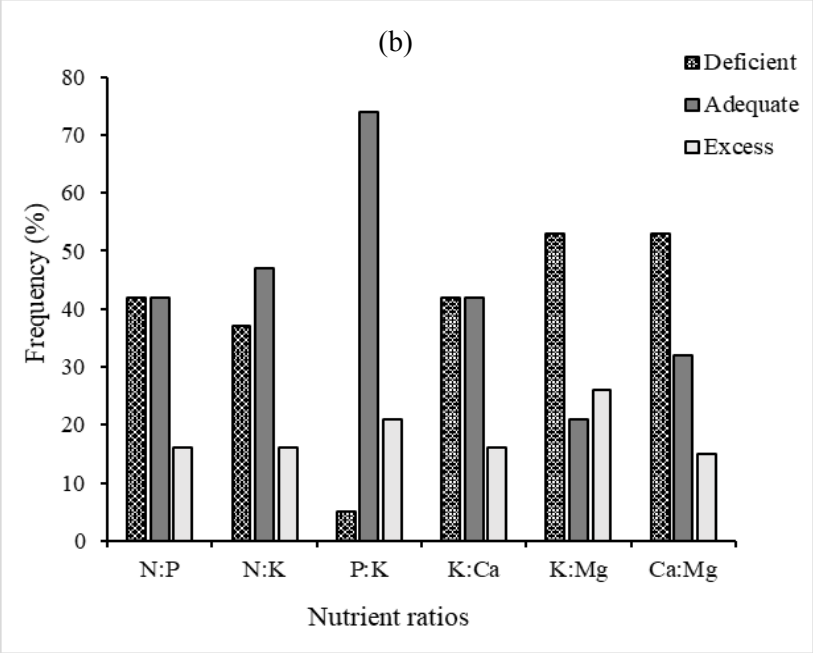
Leaf nutrient concentrations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Nutrient ratios