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Original article – Thematic Issue

# Leaf nutrients ranges and berry yield optimization in response to soil-applied nitrogen, phosphorus and potassium in wild blueberry (*Vaccinium angustifolium* Ait.)

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## Summary

**The study examined the main and interactive effects of soil-applied fertilizers (nitrogen, phosphorus, and potassium) and optimum fertilizer rate for the vegetative and crop phases leaf nutrients and berry yields of wild blueberry (*Vaccinium angustifolium* Ait.). Data were collected between 2004 and 2010 from previous and ongoing nutrition studies that were laid out in a central composite design (CCD). Experimental sites were located at Kemptown and Mount Thom, Nova Scotia, and Brantville, New Brunswick (Canada). Treatments consisted of five levels of soil-applied nitrogen (0, 12, 30, 48, and 60 kg N ha$^{-1}$), phosphorus (0, 18, 45, 78, and 90 kg P ha$^{-1}$), and potassium (0, 12, 30, 48, and 60 kg K ha$^{-1}$). Leaf nitrogen (N) and phosphorus (P) concentrations significantly increased with the addition of soil-applied N and P fertilizers, respectively. Leaf potassium (K) content in vegetative year had a significant quadratic response (convex shape) to soil-applied N and P. Leaf calcium (Ca) and magnesium (Mg) concentration exhibited significant concave quadratic (depression) response to soil added N, P and K. Nitrogen fertilizer significantly increased berry yields while soil-applied P and K had quadratic effects on berry yield. For commercial fields with similar soil nutrient levels, physical, biochemical and biological characteristics they recommend soil fertilizer rates of 30 kg ha$^{-1}$ N, 40 kg ha$^{-1}$ P, and 30 kg ha$^{-1}$ K applied at pre-emergence of shoots in the vegetative year of production to optimize foliar nutrient levels and berry yields. They suggest new optimal vegetative year leaf nutrient ranges N (1.80–2.03%), P (0.155–0.160%), K (0.53–0.55%), Ca (0.44–0.46%), Mg (0.115–0.13%), and B (24–26 ppm) for wild blueberry fields in Atlantic Canada.**

**Keywords**
lowbush blueberry, response surface, central composite design, berry yield, fertilizer

## Significance of this study

*What is already known on this subject?*

- Wild blueberry responds to balanced fertilization. Fertilizer programs in wild blueberry are often guided by leaf tissue analyses, but the optimization of leaf tissue standards have undergone limited study.

*What are the new findings?*

- Suggested are fertilizer rates of 30 kg ha$^{-1}$ N, 40 kg ha$^{-1}$ P, and 30 kg ha$^{-1}$ K and leaf nutrient ranges N (1.80–2.03%), P (0.155–0.160%), K (0.53–0.55%), Ca (0.44–0.46%), Mg (0.115–0.13%), and B (24–26 ppm) for commercial wild blueberry fields in Atlantic Canada. The fertilizer rates cost an extra $ 70 ha$^{-1}$ but increased net profits $ 500 ha$^{-1}$.

*What is the expected impact on horticulture?*

- Findings of this study could enable commercial growers to monitor their crop according to new leaf nutrient ranges and lead toward better farm profitability.

## Introduction

Wild blueberry plants have low nutrient requirements and grow slowly. Their natural habitats are characterized by nutritionally marginal soils (Korcak, 1988) and in commercial fields, fertilizers are routinely applied (20–40 kg NPK ha$^{-1}$) in early spring of the vegetative year of production with the aim to reestablish a functional canopy and sustain berry yields in the following crop year. Maqbool et al. (2016) showed 35 kg ha$^{-1}$ N, 40 kg ha$^{-1}$ P, and 30 kg ha$^{-1}$ K were optimum for fruit production and resulted in an average of 54% more floral buds, 25% more berries per stem, and 13% greater yield than previous recommend rates of 20 kg ha$^{-1}$ N, 10 kg ha$^{-1}$ P, and 15 kg ha$^{-1}$ K.

Fertilizer programs are often guided by leaf tissue analyses due to the inability of current soil analytical methods to determine accurately the wild blueberry nutritional demands (Ring, 2001). Leaf tissue nutrient standards have been developed for blueberry growing regions of Nova Scotia, Maine, New Brunswick and Quebec (Lockhart and Langille, 1962; Townsend and Hall, 1970; Trevett, 1972; Eaton et al., 2009; Lafond, 2009; Santiago, 2011). Recently, Eaton et al. (2009) proposed leaf tissue ranges from data collected in 1998–99 from 44 high yielding commercial fields across Nova Scotia. Since these sites received several different com-

binations of NPK fertilization and no statistical design was used therefore, this study may not account for potential main (N, P and K), quadratic (NN, PP and KK) and interactive (NP, NK, PK) effects. One criticism of this study was that these Nova Scotia fields may not have attained their full yield potentials and that the optimum ratio of leaf nutrient concentrations to yield needed to be determined with controlled experiments rather than by a survey.

Nutrient management of wild blueberries is dynamic with at least 12 nutrients involved and is complicated by the fact that this plant is naturally adapted to low soil nutrient environments (Korcak, 1988). The wild blueberry plant is a slow growing perennial calcifuge (i.e., acid-loving) that spreads by underground stems called rhizomes. The extensive root system occurs within the top 10 cm of soil and accounts for 85% of the total plant dry weight (Jeliazkova and Percival, 2003). The rhizomes serve as a reservoir for nitrogenous compounds as well as carbohydrates and some inorganic constituents, particularly P and Mg (Townsend et al., 1968). The numerous fine root hairs are heavily colonized by indigenous ericoid mycorrhizae that assist in nutrient uptake, notably N, P and organic forms of nutrients (Korcak, 1988).

Wild blueberry fields are pruned in the fall of the crop year or in the early spring of the vegetative year. Pruning the wild blueberry plant is necessary to encourage new vigorous shoot growth from the rhizomes and to maximize the production of floral buds and yields (Kinsman, 1993). Until the early 1980's, fields were pruned by burning but this has largely been replaced with flail mowing due to environmental risks (carbon footprint), increasing oil price, and organic matter loss caused by burning. The ash left after burning has been reported to supply nutrients (Smith and Hilton, 1971) but medium to long-term burning can gradually reduce soil fertility by depleting the organically bound nutrient reserves (Lafond, 2009). The amount of N lost from blueberry fields by burning was estimated at 35–50 kg N $ha^{-1}$ (Eaton, 1986). Conversely, pruning by mowing returns substantial amounts of plant material to the soil in each production cycle and gradually builds soil organic nutrient reserves (Lafond, 2009). In acidic conditions, soil P precipitates with soil aluminum (Al) and iron (Fe) to form relatively insoluble compounds. This slow mineralization, immobilization and formation of insoluble forms of P have been reported in short term incubation studies with wild blueberry (English, 2007). Therefore, continuous P supply may be required to meet crop demands in wild blueberry production systems.

Although wild blueberry nutrition has been studied extensively, earlier studies have focused mainly on the main effects of soil-applied fertilizers (NPK), leaf tissue nutrient standards, the effects of fertilizer formulations, timings for application and nutrient estimation (Lockhart and Langille, 1962; Townsend and Hall, 1970; Trevett, 1972; Jackson, 1976; Smagula and Ismail, 1981; Warman, 1987, 1988; Eaton, 1994; Smagula and Dunham, 1995; Penney and MacRae, 2000; Percival and Privé, 2002; Percival et al., 2003; Percival and Sanderson, 2004; Lafond, 2009; Eaton et al., 2009; Maqbool et al., 2012, 2016; Santiago and Smagula, 2012). However, quadratic (NN, PP and KK) and interactive effects (NP, NK and PK) of soil-applied NPK remain unknown. We also suspect that continuous mowing, repeated fertilizer applications, pest and weed management practices have changed the soil and plant nutrient dynamics and ultimately crop demands. These management changes may have left a gap in understanding the effect of soil-applied NPK on leaf nutrient concentrations. A nutrition management trial was started in early 2000 to better examine the long-term main and interactive effects of N, P, and K fertilizer applications, with one of the goals to determine the rates that provide the necessary leaf nutrient ranges to maximize yields under Nova Scotia (NS) growing conditions.

Response surface methodology (RSM) and canonical analysis in combination with central composite design (CCD) have been used by others to describe the effects of fertilization on foliar nutrients (Myers et al., 2009) and berry yield (Maqbool et al., 2016). For example, Chintala et al. (2012) used CCD and RSM to examine the effect of water potential and pH on N and P fertilizer additions to optimize Kentucky bluegrass (*Poa pratensis* L.) herbage yield and foliar nutrients.

The objectives of this research were: 1) to determine the main and interactive effects of soil-applied fertilizers (NPK) on wild blueberry leaf nutrients and berry yields, and 2) to provide new leaf nutrient ranges, pinpoint optimum leaf nutrient levels and corresponding fertilizer rates that maximize berry yield.

## Materials and methods

### Field experimental sites

The field experiments were established on commercial fields located at Kemptown (NS), Mount Thom (NS) and Brantville, New Brunswick (NB), Canada. The commercial sites were developed by clear-cutting the competing vegetation from native stands in forests, with new plants growing from seedlings through growth of underground rhizomes, rather than from plants or seeds selected by man or set out in a controlled manner. The age of the crop system before the start of the trial was 25 years. The Kemptown experimental site (45°30'7.91"N, 63°7'27.72"W; elevation ~214 m) was established in spring 2001. The soil was a typically sandy loam, acidic, imperfectly drained, very stony and moderately rocky (Cobequid soil association). The soil pH was 4.3 ± 0.1 (0–15 cm depth). The soil organic matter was 10.23% ± 1.34 (0–15 cm depth). The Mount Thom experimental site (45°29'32.10"N, 62°59'30.65"W; elevation ~220 m) was established in May 2004. The soil was silt loam, strongly acidic, poorly drained, slightly to moderately stony and non-rocky (Thom soil association). The soil pH at 0–15 cm depth was 4.17 ± 0.13. The organic matter was 9.59% ± 1.21. The Brantville (47°22'22.74"N, 64°58'17.48"W; elevation ~12 m) experimental site was established in 2006. The soil was sandy, very acidic, poorly drained, non-stony and non-rocky (Acadie Siding). The soil pH at 0–15 cm depth was 4.31 ± 0.12. The organic matter was 8.85% ± 1.43.

### Experimental designs

A three factor central composite design (CCD) was used to study the response surfaces between soil-applied fertilizers and their impact on leaf nutritional levels (Myers et al., 2009). The three factors were: soil-applied N, P and K (kg $ha^{-1}$) at various rates. Treatments consisted of five levels of soil-applied N (0, 12, 30, 48, and 60 kg N $ha^{-1}$), P (0, 18, 45, 78, and 90 kg P $ha^{-1}$), and K (0, 12, 30, 48, and 60 kg K $ha^{-1}$) (Tables 1A and 1B). Treatment combinations were replicated four times. A plot size of 6 × 8 m was used. Experimental sites were fertilized in which urea/ammonium sulfate (source of N), triple superphosphate (source of P) and potassium chloride (source of K) in early May, every year prior to shoot emergence from the rhizomes in the vegetative stage

**Table 1A.** Experimental range and levels of the independent variables.

| | Range and levels | | | | |
|---|---|---|---|---|---|
| Original factors | -1.68 | -1 | 0 | 1 | 1.68 |
| N[z] (kg ha$^{-1}$): $X_1$ | 0 | 12 | 30 | 48 | 60 |
| P (kg ha$^{-1}$): $X_2$ | 0 | 18 | 45 | 72 | 90 |
| K (kg ha$^{-1}$): $X_3$ | 0 | 12 | 30 | 48 | 60 |

[z] The source of soil applied N was ammonium sulfate, P was triple superphosphate and K was potassium chloride.

**Table 1B.** Real and coded values of the central composite experimental design.

| | Coded values | | | Real values | | |
|---|---|---|---|---|---|---|
| | N | P | K | N | P | K |
| | | | | | (kg ha$^{-1}$) | |
| Factorial points | -1.00 | -1.00 | -1.00 | 12 | 18 | 12 |
| | 1.00 | -1.00 | -1.00 | 48 | 18 | 12 |
| | -1.00 | 1.00 | -1.00 | 12 | 72 | 12 |
| | 1.00 | 1.00 | -1.00 | 48 | 72 | 12 |
| | -1.00 | -1.00 | 1.00 | 12 | 18 | 48 |
| | 1.00 | -1.00 | 1.00 | 48 | 18 | 48 |
| | -1.00 | 1.00 | 1.00 | 12 | 72 | 48 |
| | 1.00 | 1.00 | 1.00 | 48 | 72 | 48 |
| Star points | -1.68 | 0.00 | 0.00 | 0 | 45 | 30 |
| | 1.68 | 0.00 | 0.00 | 60 | 45 | 30 |
| | 0.00 | -1.68 | 0.00 | 30 | 0 | 30 |
| | 0.00 | 1.68 | 0.00 | 30 | 90 | 30 |
| | 0.00 | 0.00 | -1.68 | 30 | 45 | 0 |
| | 0.00 | 0.00 | 1.68 | 30 | 45 | 60 |
| Center points | 0.00 | 0.00 | 0.00 | 30 | 45 | 30 |
| | 0.00 | 0.00 | 0.00 | 30 | 45 | 30 |
| | 0.00 | 0.00 | 0.00 | 30 | 45 | 30 |
| | 0.00 | 0.00 | 0.00 | 30 | 45 | 30 |

**Table 2.** Schedule of wild blueberry sampling dates and cropping cycles at three study sites.

| Sampling dates | Site | Cropping cycle | Foliar nutrient |
|---|---|---|---|
| 20th July 2004 | Mount Thom | Sprout | N |
| 27th June 2005 | Kemptown | Crop | N, P, K, Ca, Mg and B |
| 27th June 2005 | Mount Thom | Crop | N, P, K, Ca, Mg and B |
| 5th August 2005 | Kemptown | Crop | N, P, K, Ca, Mg and B |
| 8th August 2005 | Mount Thom | Crop | N, P, K, Ca, Mg and B |
| 30th June 2006 | Kemptown | Sprout | N, P, K, Ca, Mg and B |
| 3rd August 2006 | Brantville | Sprout | N, P, K, Ca, Mg and B |
| 14th August 2006 | Kemptown | Sprout | N, P, K, Ca, Mg and B |
| 15th September 2006 | Kemptown | Sprout | N, P, K, Ca, Mg and B |
| 25th June 2007 | Kemptown | Crop | N |
| 15th July 2009 | Kemptown | Crop | N |
| 25th June 2010 | Kemptown | Sprout | N |
| 16th August 2010 | Kemptown | Sprout | N |

of production. Fertilizers were applied using a Scotts SR2000 rotary fertilizer spreader (Marysville, Ohio). No fertilizers were applied in the crop year of production.

**Leaf sampling and foliar nutrient analysis**

Leaf tissue samples were obtained in vegetative and crop years of production in 2004, 2005, 2006, 2007, 2009 and 2010 (Table 2). A 7.5-m long rope was extended diagonally in each plot, marked at fifteen equally spaced points. One stem was randomly selected at each mark. The stem was grasped from its base and pulled gently to collect the leaves. Leaf samples were stored in paper bags, placed in a cooler and transported to the laboratory. The leaves were dried by placing the samples in an oven at 65°C for 8–10 hours. Samples were then ground using a Wiley Mill (Arthur H. Thomas Co, Philadelphia, PA, USA) with 2-mm mesh screen. Foliar N was measured by combustion using LECO CNS-1000 elemental auto analyzer (Leco Corporation, St. Joseph, MI) using the technique of Rutherford et al. (2008). One gram of the ground leaf sample was placed in crucibles for the P, K, Ca, Mg, and B analysis. The samples were burnt at 550°C for 4 hours, 5 mL of 30% (v.v.) of concentrated hydrochloric acid (HCl) was added and swirled gently to ensure the sample was completely wet. Resulting mixtures were transferred to 50-mL centrifuge tubes and filled with deionized water. Samples were centrifuged using an International Centrifuge (IEC), model K (International Equipment Co., Massachusetts) at 3,000 rpm for 15 minutes (Percival and Privé, 2002; Bourguignon, 2006). Supernatants were sent to the Soil and Feed Testing Laboratory, Agriculture Resource Division (Prince Edward Island Department of Agriculture and Forestry) for testing. Each sample was analyzed using a Thermo Jarrell Ash ICAP61-1100.

Berries were hand-raked (mid to end of August) from four randomly selected 1 $m^2$ quadrats in each plot and weighed using a digital balance (Mettler PE 6000, Burlington, ON) to calculate harvestable berry yield. Berry yield was measured from sites sampled for vegetative year and crop year leaf nutrients (Table 2). In the Results and discussion section, vegetative year berry yield means the yield obtained from sites that were sampled for the vegetative year leaf nutrients and, similarly, crop year berry yield means yield obtained from sites sampled for the crop year leaf nutrients.

**Statistical analysis**

The averages of the four replications (within each sampling date) were used to improve the precision of the parameter estimations, except the center point which the CCD calls for all replications to allow for the lack of fit test. The lack of fit test measures the adequacy of the quadratic response surface model. We tested models for lack of fit test to make sure there was no lack of second order model and proceeded to response surface analysis of the data (Myers et al., 2009). Foliar nutrient data for vegetative and crop year were analyzed separately. Within each production cycle, the data from all sampling dates were analyzed together using sampling date as a blocking factor to account for the date-to-date variability. Berry yield data from sites sampled for vegetative and crop year foliar nutrients were analyzed separately, using sites as a blocking factor to account for the site-to-site variability. Minitab 16 software (Minitab Inc., State College, PA) was used for regression and graphical analyses of the data. The second order model was used for the CCD response surface analysis:

$$Y = \beta_0 + \sum_{1=1}^{3} \beta_i X_i + \sum_{1=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + \varepsilon \qquad (1)$$

where Y is the response, $X_i$ are the coded levels of the factors, $\beta_0$, $\beta_i$, $\beta_{ii}$, and $\beta_{ij}$ are the regression coefficients, and ε is the error term assumed to be independent and have a normal distribution with constant variance (Myers et al., 2009). The regression coefficients of the response surface model were estimated using the least squares method. Three-dimensional (3D) surface plots were drawn to illustrate the interactive effects of factors on response variables. The overlaid contour plots were drawn for joint optimization of foliar nutrients and berry yield. Based on second order response surface models, the canonical analysis was performed to determine the location and the nature of the optimum setting of the factors of the response functions (Myers et al., 2009). Matlab 7.10 (The Mathworks, Inc., Natick, MA) was used to complete the canonical analysis of the data. The canonical analysis optimizes one response at a time, so canonical analysis results may be used if the interest is optimizing single response (e.g., berry yield). The overlaid surface plots simultaneously or jointly optimize (sweet spot-adequacy window) of number of responses, the overlaid contour plot results are more relevant for joint optimization.

## Results and discussion

All results reported were for fertilization (NPK) that was applied in early spring of the vegetative year and hereafter is written as 'soil-applied fertilizers'. The analysis of variance (ANOVA) and *P*-values for all responses are provided in Tables 3 and 4 for the vegetative and crop year, respectively (Table 4). For all responses, the lack of fit test results is not significant, suggesting that the quadratic model adequately fits for all responses and the design settings are in the neighborhood of the optimum setting. All leaf elements were significant in both vegetative and crop year, except for leaf K in the crop year (Tables 3 and 4).

**Leaf nutrients**

Overall significant main and interactive effects were found between the soil-applied N, P, and K for leaf nutrient content (Tables 3 and 4). Leaf N content increased linearly in both years of production (vegetative and crop) with the soil-applied N but was found to be higher in the year of application (vegetative, Figure 1). The linear increase in leaf N agrees with previous findings (Trevett, 1972; Smagula and Ismail, 1981; Penney and McRae, 2000). Nitrogen has a beneficial effect on wild blueberry yield potential and berry yields (Maqbool et al., 2016). Berries per stem increased linearly with soil-applied N rate as it rose from 0 to 35 kg $ha^{-1}$ (Maqbool et al., 2016). The response foliar N (Figure 1) and berries per stem (Maqbool et al., 2016) are similar to soil-applied N and P fertilizers.

The vegetative and crop year leaf P content increased linearly with soil-applied P (Figure 2). A significant linear increase in leaf P with soil-applied P fertilizer rate (0–195 kg P $ha^{-1}$) was reported in Maine (Smagula and Ismail, 1981; Smagula and Dunham, 1995). Leaf K concentration was explained by convex quadratic effects only in the vegetative year (Figure 3a). The quadratic response of vegetative year leaf K is consistent with the findings of Smagula and Ismail (1981) who reported a significant quadratic response of vegetative-year leaf K. Initially leaf K increased with applied fertilizer 1:1:1 (0–135 kg $ha^{-1}$) and leaf K decreased at higher fertilizer rates (180 kg $ha^{-1}$). In our experiment, the fertilizers

**Table 3.** Analysis of variance (ANOVA) *P*-values of the central composite design in sprout year of production.

| Source of variation | N | P | K | Ca | Mg | B | Berry yield[z] |
|---|---|---|---|---|---|---|---|
| Blocks (samplings) | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Regression | 0.03 | 0.03 | <0.01 | 0.01 | <0.01 | 0.01 | <0.01 |
| Lack of fit | 0.88 | 0.98 | 0.96 | 0.84 | 0.99 | 0.99 | 0.53 |
| Adjusted $R^2$ | 0.95 | 0.96 | 0.70 | 0.95 | 0.91 | 0.75 | 0.85 |
| Significant terms | N | P | NN, PP | K, PP, KK, | KK, NP, PK | K, NP | N, K, PP, KK |

[z] Commercial blueberry fields follow two year production cycle and berries are harvested in alternate years (crop year). Berry yield results presented in this Table are from sites that are sampled sprout year leaf nutrients. Berry yields are measured from same sites in the crop year.

**Table 4.** Analysis of variance (ANOVA) *P*-values of the central composite design in crop year of production.

| Source of variation | N | P | K | Ca | Mg | B | Berry yield |
|---|---|---|---|---|---|---|---|
| Blocks (samplings) | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Regression | 0.03 | 0.04 | 0.57 | 0.04 | <0.01 | 0.02 | <0.01 |
| Lack of fit | 0.99 | 0.99 | 0.99 | 0.94 | 0.73 | 0.96 | 0.73 |
| Adjusted $R^2$ | 0.90 | 0.94 | 0.87 | 0.92 | 0.75 | 0.64 | 0.75 |
| Significant terms | N | P | | P, KK | K,NN, PP, KK | NN, PP | N, P, K, NN, PP, KK |

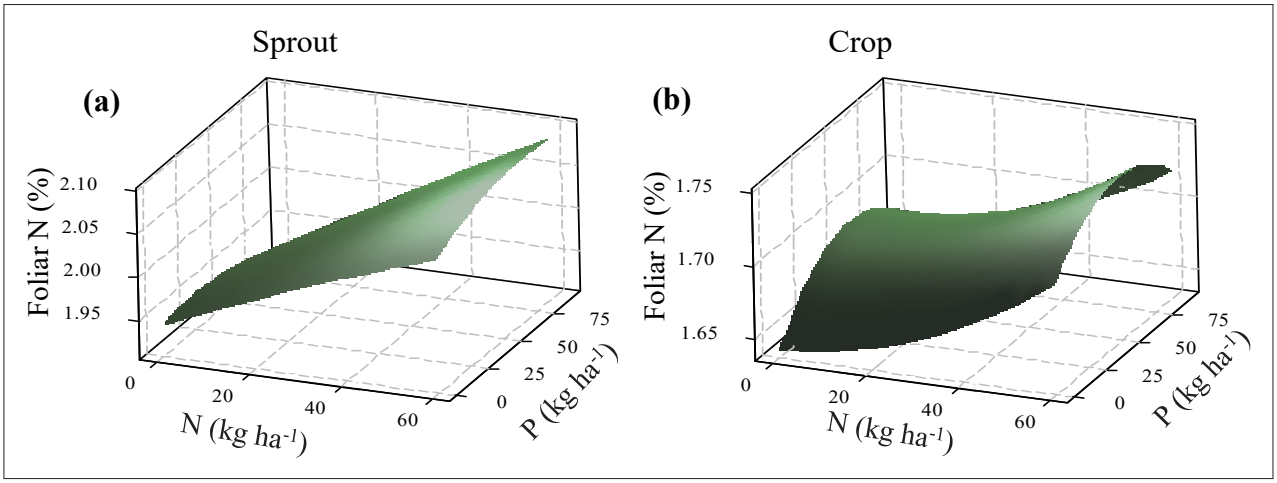



**Figure 1.** Response surface of foliar N (%), the effect of soil-applied nitrogen (kg N $ha^{-1}$) and phosphorus (kg P $ha^{-1}$) for potassium fixed at 30 kg K $ha^{-1}$, (a) in sprout year and (b) crop year.

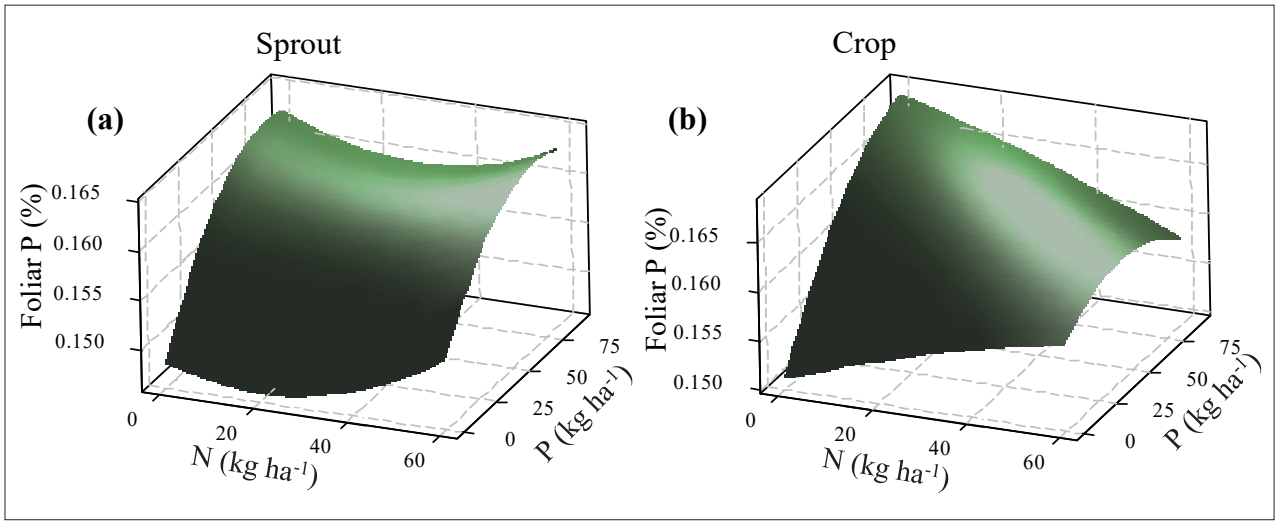



**Figure 2.** Response surface of foliar P (%) in sprout year, the effect of soil-applied nitrogen (kg N $ha^{-1}$) and phosphorus (kg P $ha^{-1}$) for potassium fixed at 30 kg K $ha^{-1}$, (a) in sprout year and (b) crop year.

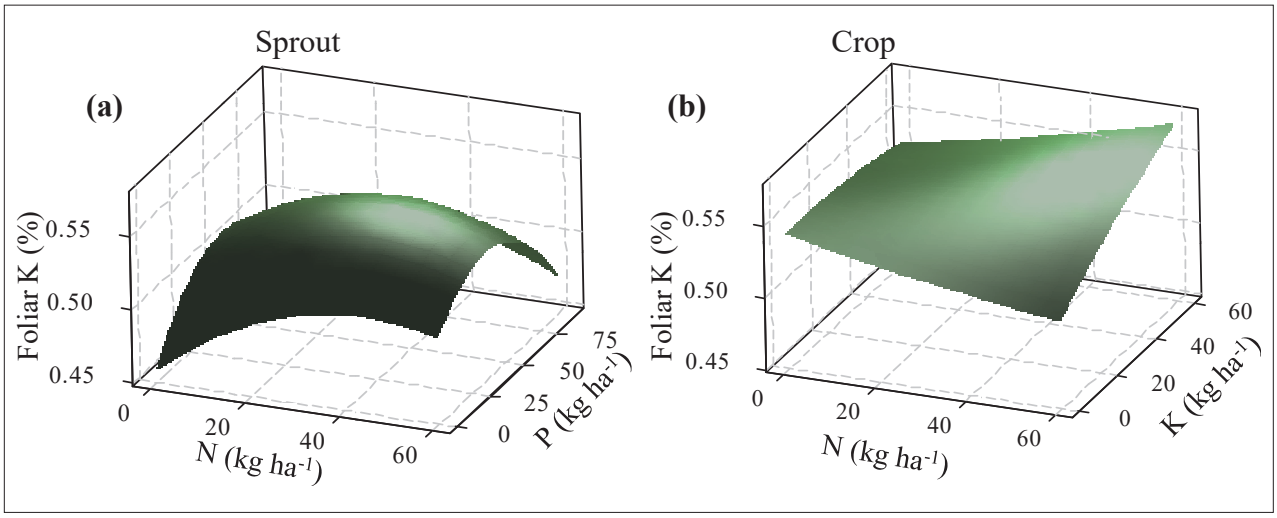



**Figure 3.** Response surface of foliar K (%) in sprout year (a), the effect of soil-applied nitrogen (kg N ha$^{-1}$) and phosphorus (kg P ha$^{-1}$) for potassium fixed at 30 kg K ha$^{-1}$. Response surface of foliar K (%) in crop year (b), the effect of soil-applied nitrogen (kg N ha$^{-1}$) and potassium (kg K ha$^{-1}$) for phosphorus fixed at 45 kg P ha$^{-1}$.

were applied in the vegetative-year of production. It appears that P remained available to the plant through mineralization in the longer term (crop year). Alternatively, wild blueberry plants might have taken up all the P required during whole production cycle (two-year), when fertilizers (vegetative year) were applied and stored in their rhizomes to be utilized when required (vegetative and crop year) in order to maintain foliar P and K levels. Repeated applications of fertilizer increased soil P and K in wild blueberry soils (Eaton and Patriquin, 1988). Phosphorus concentrations in rhizome tissues increased in fertilized plots (NPK) but K content did not (Eaton, 1994). This may explain the lack of K effect on leaf K levels (Table 4). Therefore, the residual effect of P fertilizer on leaf P can be attributed to efficient nutrient cycling, retention of K (soil colloids and hums) and special ability of wild blueberry plant to store nutrients in rhizome systems (Eaton, 1994). The leaf K in vegetative year benefited from soil-applied N and P when applied at optimum levels (Figure 3).

Leaf tissue Ca response is illustrated with a concave quadratic effect of soil-applied P and K, a response similar in both vegetative and crop year of production (Figures 4a and 4b). Smagula and Ismail (1981) reported quadratic trend of leaf Ca levels 0.387, 0.369, 0.344, 0.361 and 0.35% at 0, 45, 90, 135, and 180 kg ha$^{-1}$ fertilizer (1:1:1). Calcium concentrations are low in the acidic soil. Wild blueberries (*Ericaceae* plants) grow in acidic environments and have efficient Ca uptake system (Korcak, 1989) and foliar leaf Ca is not affected by pH over the range of 4.1–6.8 (Korcak, 1989).

Leaf Mg content varies significantly with a concave quadratic (K) and interactive (N × P and P × K) effects in the vegetative year of production (Figures 5a and 5b). Significant concave quadratic effects of N, P and K were also found for leaf Mg concentrations in crop year (Figures 5c and 5d). Soil pH shifts may partially explain. These complex interactions found in the vegetative year, may be partially explained by soil pH shifts, cation exchange capacity, cation competition and soil $K^+$ concentrations (Brady and Weil, 2008). The uptake of cations such as $K^+$ and $NH_4^+$, and the naturally low soil pH conditions found in blueberry fields may depress the uptake of $Mg^{+2}$ from the soil. Ammonium built up in plant tissue can suppress uptake of cationic nutrients such as $Ca^{+2}$ and $Mg^{+2}$ (Britto and Kronzucker, 2013) and the suppression ef-

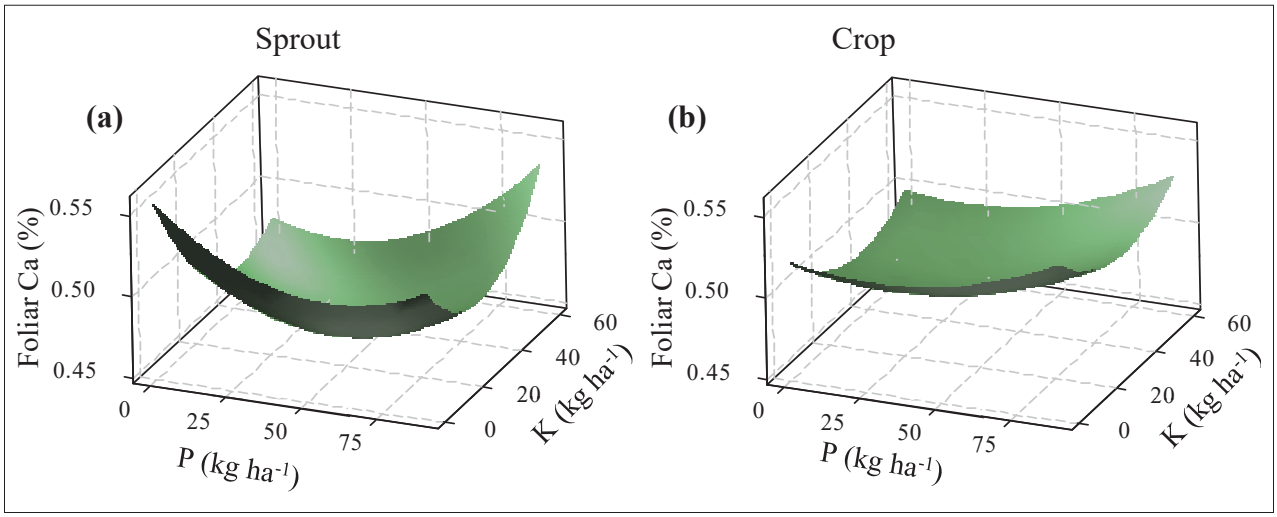



**Figure 4.** Response surface of foliar Ca (%), the effect of soil-applied phosphorus (kg P ha$^{-1}$) and potassium (kg K ha$^{-1}$) for nitrogen fixed at 30 kg N ha$^{-1}$, (a) in sprout year and (b) crop year.

fect can be viewed by combining leaf N, Ca and Mg results (Figures 1, 4, and 5). The significant liner decrease in leaf Mg levels was also found by Smagula and Ismail (1981) when they applied N fertilizer (0–180 kg ha$^{-1}$). However, in their study the fertilizer treatments were laid out in a randomized complete block design and ignored any possible interactions between the nutrients. Ammonium-based fertilizers are reported to reduce the soil pH, which in turn reduces leaf Mg levels (Trevett et al., 1968; Warman, 1988).

We found significant NP interaction for vegetative year leaf B (Figure 6a). Soil-applied K linearly (0–60 kg ha$^{-1}$) reduced foliar B (Figure 6b). NP effect on soil organic matter decomposition may explain the complex NP interaction (shape of Figure 6a). Among the soil B pools (soluble, weathering of minerals, adsorbed on clay, iron hydroxides or both, soil organic matter), the soil organic matter pool tends to contain the largest amount of B which is released into soil solution on decomposition (Marschner, 1995).

A quadratic effect (concave) of the soil-applied N and P rates explained crop year foliar B (Figure 6c). This concave shape of NP may be explained by the allocation of B towards beneficial reproductive growth during the crop year. Maximum reproductive growth (floral nodes) occurred at mid to high soil-applied fertilizers (N 30–40 kg ha$^{-1}$, P 40–60 kg ha$^{-1}$, K 30 kg ha$^{-1}$) (Maqbool et al., 2016), thus utilizing plant available B to maximum extent and creating concave shape of foliar B in the crop year of production. The low leaf B concentration at middle values of NPK in crop year may indicate the allocation of leaf B to the developing inflorescence (Lahav and Whiley, 2002). The B is an essential micronutrient for plants and plays a vital role in reproductive growth (Marschner, 1995).

**Berry yields**

The soil-applied N linearly increased berry yields on sites sampled for foliar nutrients in the vegetative year. These results are in agreement with previous studies (Percival and Sanderson, 2004; Maqbool, 2013). Maqbool et al. (2016) recommended 35 kg N ha$^{-1}$, 40 kg P ha$^{-1}$ and 30 kg K ha$^{-1}$ to optimize yield potentials and berry yields by analyzing 6 production cycles of data. In this study, we found 20 kg N ha$^{-1}$ for vegetative sites, which is 10 kg N ha$^{-1}$ lower than that of Maqbool et al. (2016) to optimize yields. The P and K fertilizer rates to optimize berry yields were similar in both studies. This difference in N rate to optimize berry yields may be due to year-to-year variation in N supplying capacity of soils (mineralization) that is affected by climatic factors, such as variations in temperature and soil moisture. Crop sites had N rates (28 kg N ha$^{-1}$) compared to Maqbool et al. (2016) (35 kg N ha$^{-1}$).

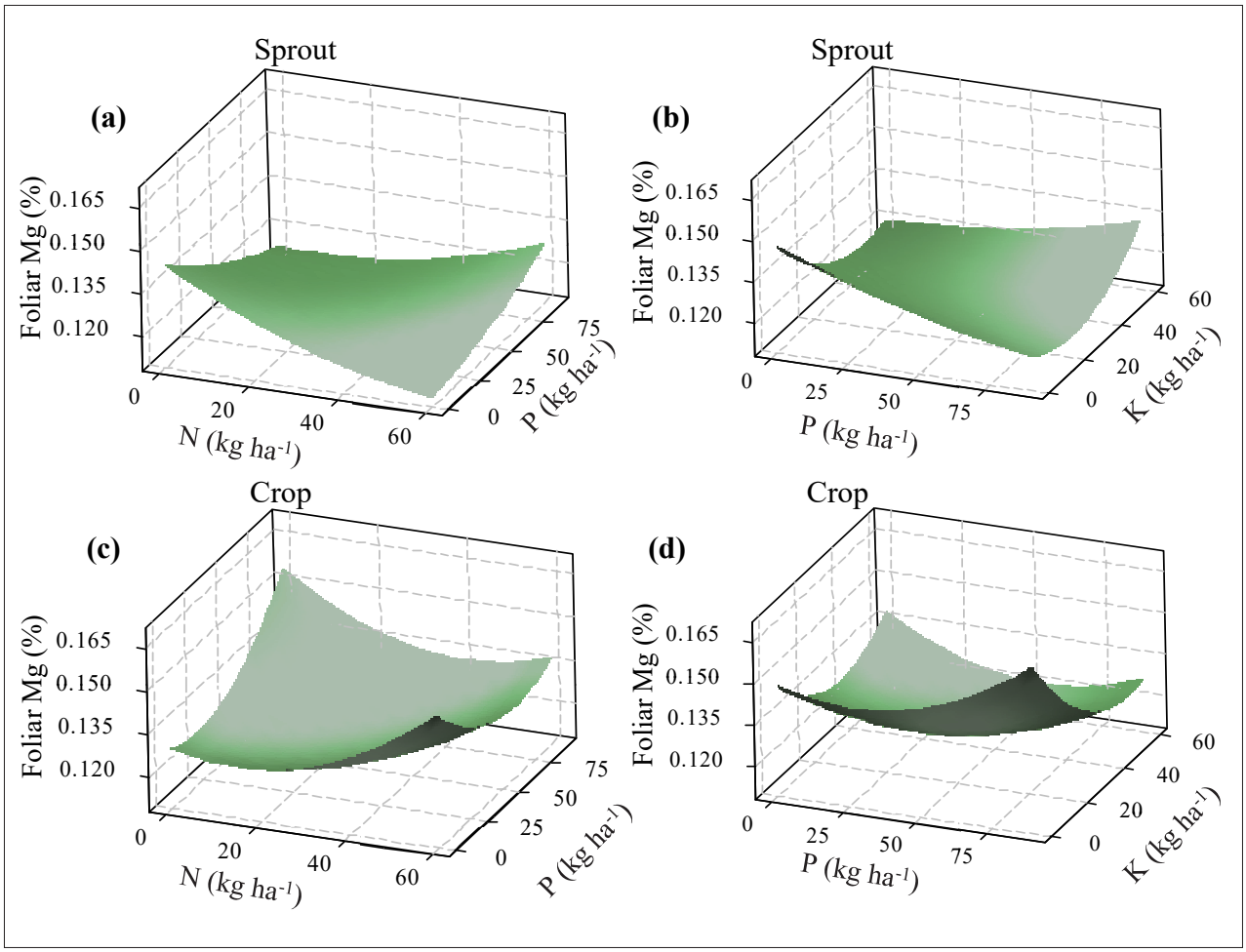



**Figure 5.** Response surface of foliar Mg (%) in sprout year: (a) the effect of soil-applied nitrogen (kg N ha$^{-1}$) and phosphorus (kg P ha$^{-1}$) for potassium fixed at 30 kg K ha$^{-1}$ and (b), the effect of soil-applied phosphorus (kg P ha$^{-1}$) and potassium (kg K ha$^{-1}$) for nitrogen fixed at 30 kg ha$^{-1}$. Response surface of foliar Mg (%) in crop year: (c) the effect of soil applied N (kg N ha$^{-1}$) and phosphorus (kg P ha$^{-1}$) for potassium fixed at 30 kg K ha$^{-1}$ and (d), the effect of soil applied phosphorus (kg P ha$^{-1}$) and potassium (kg K ha$^{-1}$) for nitrogen fixed at 30 kg ha$^{-1}$.

We found a convex quadratic effect of soil-applied P and K for berry yield on sites sampled for vegetative year foliar nutrients (Tables 3 and 4; Figures 7b and 7d). Studies have reported significant linear increases in berry yield with soil-applied P (0–195 kg P ha$^{-1}$) in Maine (Smagula and Dunham, 1995). The reason for the higher rates of P necessary in Maine is because the soils are sandy in nature but with a lower organic matter than in NS soils. Wild blueberry plants in NS are better able to acquire P from the high soil organic matter decomposition, therefore, comparatively low P fertilizer (40–60 kg ha$^{-1}$) may be adequate in NS soils. The quadratic effect of soil-applied N, P and was also significant for berry yield sites sampled for crop year foliar nutrients (Table 4). The same trend holds valid for foliar nodes (yield potential) (Maqbool et al., 2016). Berry yields were different between sites sampled for vegetative (3,620 kg ha$^{-1}$) and crop (3,170 kg ha$^{-1}$) year foliar nutrients. These site-to-site optimal berry yield differences may be attributed to uncontrollable factors such as inherited yield potentials due to clone variably among blueberry fields, weed and disease pressures and bee pollinator densities.

**Simultaneous optimization of leaf nutrients and berry yield**

All the response surface plots were overlaid to simultaneously optimize all the foliar nutrients and berry yield. We used stationary points for each response from canonical analysis to guide ourselves to get a common optimal point (sweet spot-adequacy window) for all the responses. By doing so, we get fertilizer rates (25–30 kg N ha$^{-1}$, 30–55 kg P ha$^{-1}$ and 25–45 kg K ha$^{-1}$) that simultaneously optimized vegetative year leaf nutrients and berry yields (sites sampled for vegetative leaf nutrients). For crop year leaf nutrients, fertilizer rates were 15–35 kg N ha$^{-1}$, 15–50 kg P ha$^{-1}$ and 20–40 kg K ha$^{-1}$. From overlaid plots, we obtained optimal leaf nutrient ranges for vegetative and crop year of production (Table 7). The optimum settings to optimize leaf N, P, K, Ca, Mg, B and berry yield were obtained by overlaying the plots and looking at the sweet spots-adequacy window in Figures 8 and 9 (Myers et al., 2009). The primary objective of this study was to determine the optimal leaf nutrient ranges that resulted in producing maximum berry yields. The canonical analysis was used to identify the optimum levels of the leaf nutrients and berry yields whereas window of adequacy analyses provided optimum ranges (Tables 5 and 6).

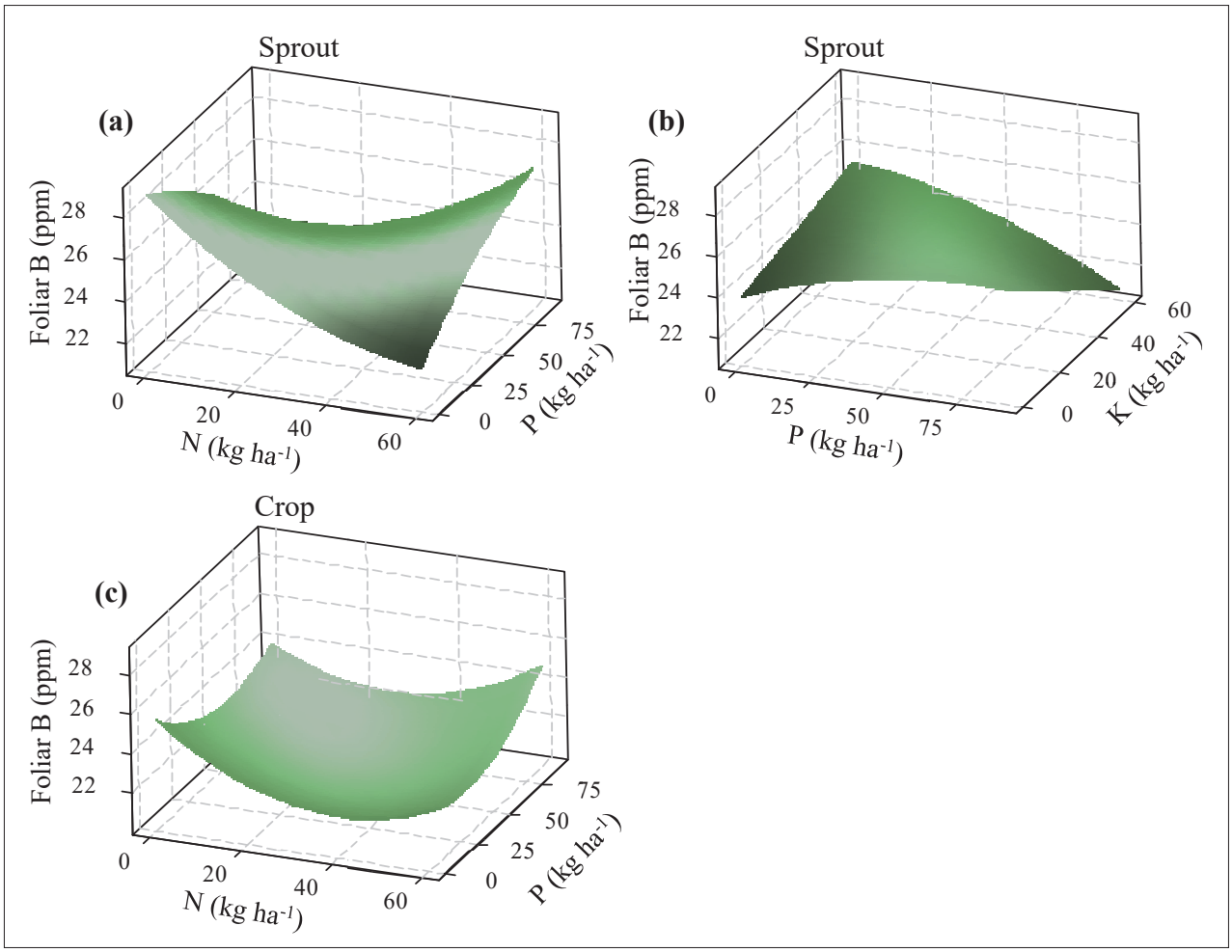



**Figure 6.** Response surface of foliar B (ppm) in sprout year: (a) the effect of soil-applied nitrogen (kg N ha$^{-1}$) and phosphorus (kg P ha$^{-1}$) for potassium fixed at 30 kg K ha$^{-1}$ and (b), the effect of soil-applied phosphorus (kg P ha$^{-1}$) and potassium (kg K ha$^{-1}$) for nitrogen fixed at 30 kg ha$^{-1}$. Response surface of foliar B (ppm) in crop year, (c) the effect of soil-applied (kg N ha$^{-1}$) and phosphorus (kg P ha$^{-1}$) for potassium fixed at 30 kg K ha$^{-1}$.

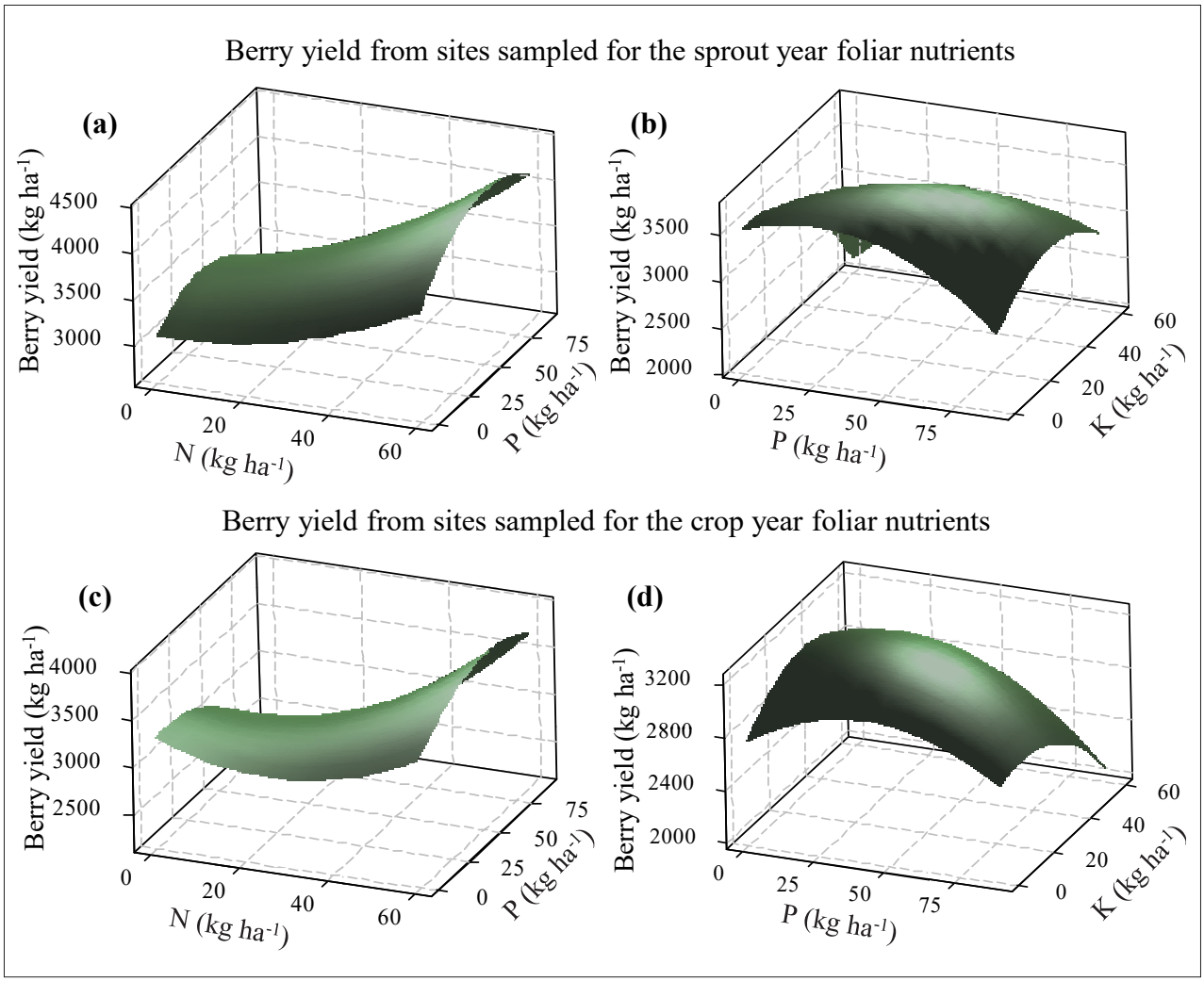



**Figure 7.** Response surface of berry yield (a) the effect of nitrogen (kg N ha$^{-1}$) and phosphorus (kg P ha$^{-1}$) for potassium fixed at 30 kg K ha$^{-1}$ and, (b) the effect of phosphorus (kg P ha$^{-1}$) and potassium (kg K ha$^{-1}$) for nitrogen fixed at 30 kg N ha$^{-1}$ for sites sampled for foliar nutrients in sprout year of production. Response surface of berry yield (c) the effect of nitrogen (kg N ha$^{-1}$) and phosphorus (kg P ha$^{-1}$) for potassium fixed at 30 kg K ha$^{-1}$ and, (d) the effect of phosphorus (kg P ha$^{-1}$) and potassium (kg K ha$^{-1}$) for nitrogen fixed at 30 kg N ha$^{-1}$ for sites sampled for foliar nutrients in crop year of production.

**Table 7.** Mineral concentration of wild blueberry leaves in sprout year and crop year of production.

| Nutrient | Sprout year | | | | Crop year | | | |
|---|---|---|---|---|---|---|---|---|
| | Optimal ranges obtained through overlaying surface plots | | Optimal concentrations reported by Trevett (1972) | | Optimal ranges obtained through overlaying surface plots | | Optimal concentration reported by Trevett (1972) | |
| | Min. | Max. | Min. | Max. | Min. | Max. | Min. | Max. |
| N (%) | 1.8 | 2.03 | 1.60 | 2.00 | 1.5 | 1.7 | 1.50 | 1.75 |
| P (%) | 0.155 | 0.160 | 0.125 | 0.222 | 0.158 | 0.164 | 0.119 | 0.158 |
| K (%) | 0.53 | 0.55 | 0.400 | 0.900 | 0.535 | 0.545 | 0.300 | 0.700 |
| Ca (%) | 0.44 | 0.46 | 0.270 | 0.517 | 0.465 | 0.495 | 0.440 | 0.644 |
| Mg (%) | 0.115 | 0.130 | 0.130 | 0.249 | 0.115 | 0.125 | 0.150 | 0.330 |
| B (ppm) | 24 | 26 | 24 | 60 | 18 | 22 | 30 | 70 |

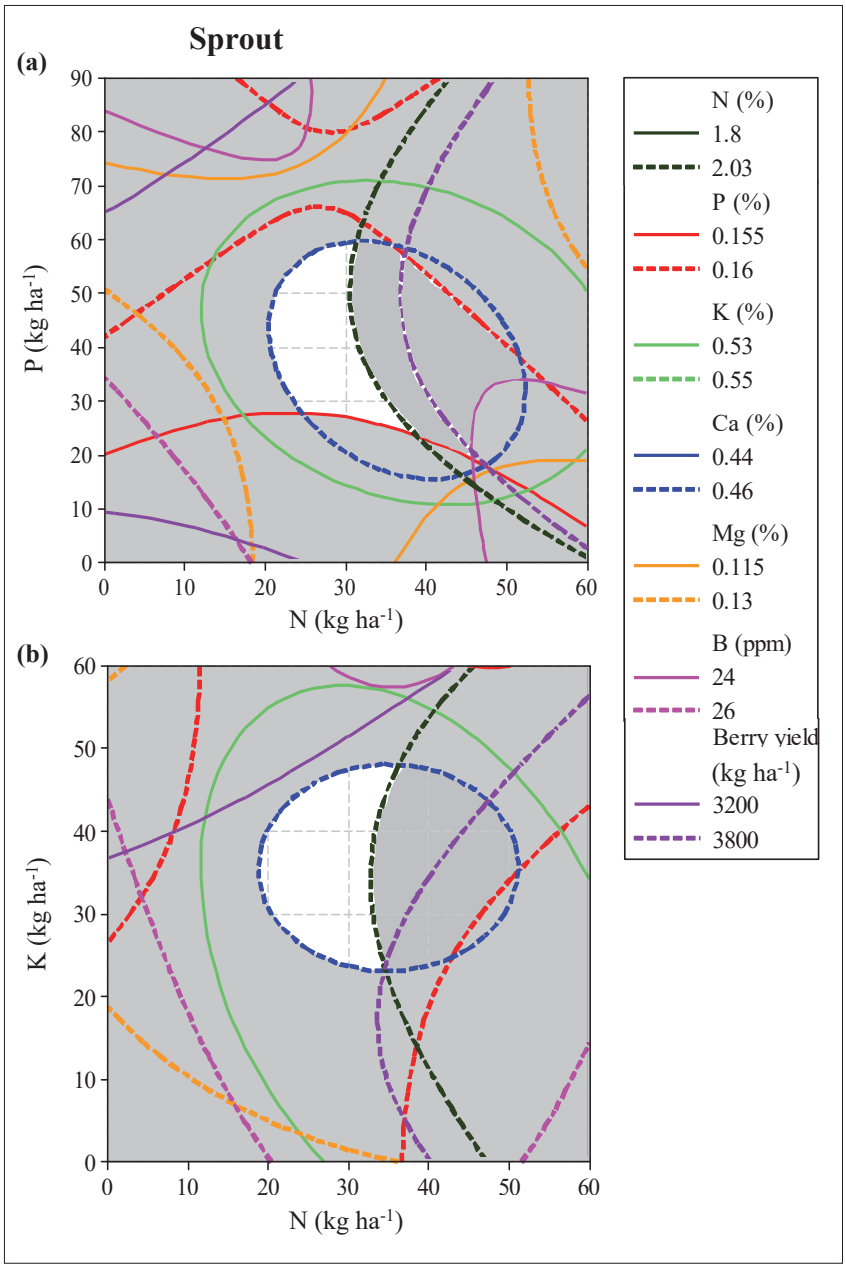



**Figure 8.** Overlaid contour plots of response surface models for sprout year: (a) the effect of soil applied nitrogen (kg N $ha^{-1}$) and phosphorus (kg P $ha^{-1}$) for potassium fixed at 30 kg K $ha^{-1}$ on foliar N (%), P (%), K (%), Ca (%), Mg (%), B (ppm), and berry yield (kg $ha^{-1}$), and (b) the effect of soil applied nitrogen (kg N $ha^{-1}$) and potassium (kg K $ha^{-1}$) for phosphorus fixed at 45 kg $ha^{-1}$ on foliar N (%), P (%), K (%), Ca (%), Mg (%), B (ppm), and berry yield (kg $ha^{-1}$). The white area is the Sweet Spot where the criteria of all responses are fulfilled.

**Table 5.** Canonical analysis performed for each response surface model in sprout year.

| Responses | Stationary points in real units (kg $ha^{-1}$) | | | $Y_S$ | Nature of stationary point |
|---|---|---|---|---|---|
| | N | P | K | | |
| N (%) | 14 | 41 | 36 | 2.011 | Saddle |
| P (%) | 27 | 59 | 27 | 0.159 | Saddle |
| K (%) | 35 | 43 | 27 | 0.548 | Maximum |
| Ca (%) | 34 | 38 | 34 | 0.454 | Minimum |
| Mg (%) | 32 | 50 | 34 | 0.116 | Saddle |
| B (ppm) | 32 | 50 | 31 | 24.7 | Saddle |
| Berry yield (kg $ha^{-1}$) | 20 | 37 | 24 | 3620 | Saddle |
| SD | 7.9 | 7.9 | 4.5 | | |

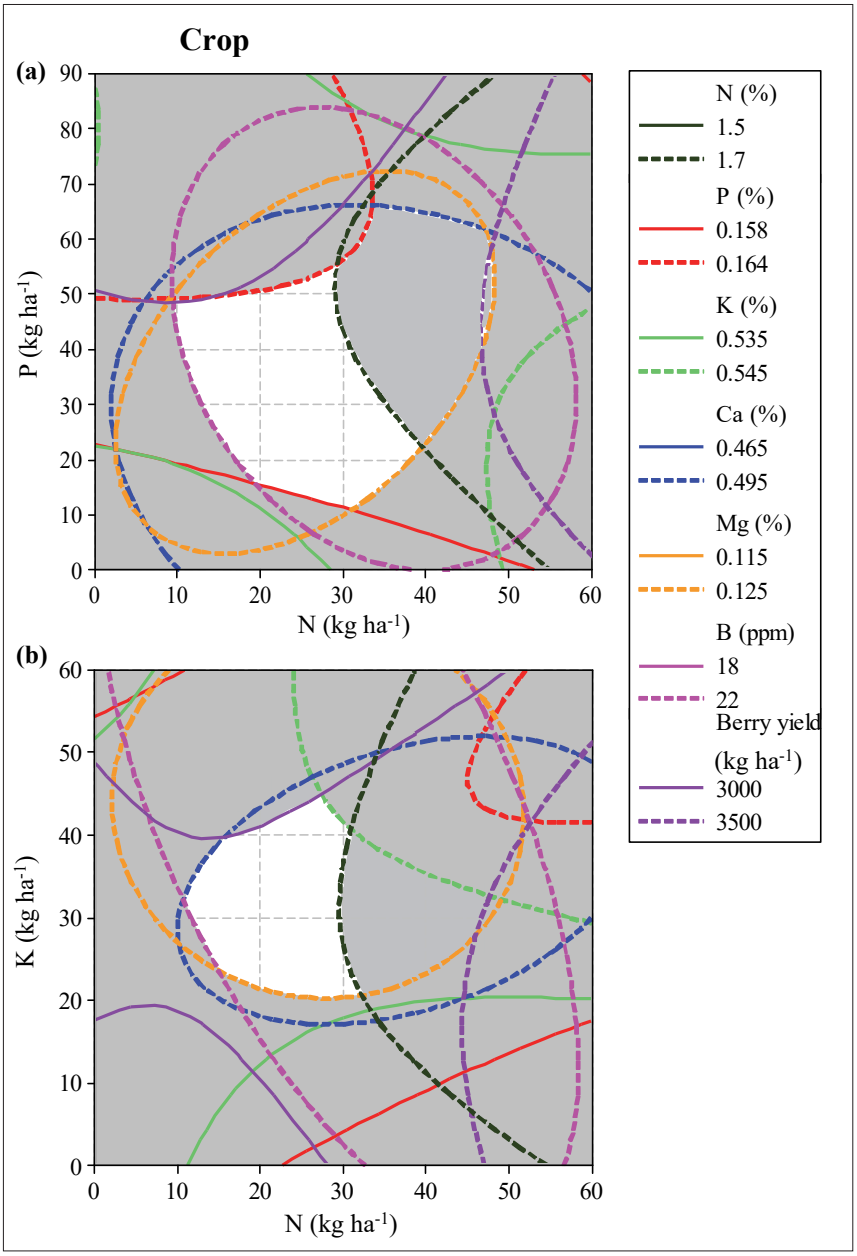



**Figure 9.** Overlaid contour plot of response surface models for crop year: (a) the effect of soil applied nitrogen (kg N ha$^{-1}$) and phosphorus (kg P ha$^{-1}$) for potassium fixed at 30 kg K ha$^{-1}$ on foliar N (%), P (%), K (%), Ca (%), Mg (%), B (ppm), and berry yield (kg ha$^{-1}$), and (b), the effect of soil applied nitrogen (kg N ha$^{-1}$) and potassium (kg K ha$^{-1}$) for phosphorus fixed at 45 kg ha$^{-1}$ on foliar N (%), P (%), K (%), Ca (%), Mg (%), B (ppm), and berry yield (kg ha$^{-1}$). The white area is the Sweet Spot where the criteria of all responses are fulfilled.

**Table 6.** Canonical analysis performed for each response surface model in crop year.

| Responses | Stationary points in real units (kg ha$^{-1}$) | | | $Y_S$ | Nature of stationary point |
|---|---|---|---|---|---|
| | N | P | K | | |
| N (%) | 24 | 47 | 27 | 1.698 | Saddle |
| P (%) | 36 | 50 | 37 | 0.163 | Maximum |
| K (%) | 22 | 51 | 33 | 0.541 | Maximum |
| Ca (%) | 36 | 32 | 34 | 0.484 | Minimum |
| Mg (%) | 27 | 45 | 37 | 0.119 | Minimum |
| B (ppm) | 31 | 45 | 34 | 20.77 | Minimum |
| Berry yield (kg ha$^{-1}$) | 27 | 38 | 30 | 3170 | Saddle |
| SD | 5.6 | 6.8 | 3.6 | | |

The vegetative year leaf N (dotted dark green) and berry yield (dotted purple color) followed the upward trend, suggesting leaf N (vegetative) and berry yield are closely related and nitrogen fertilization increased leaf N maximized berry yields when supplied with P (40 kg P $ha^{-1}$) and K (30 kg K $ha^{-1}$) fertilizers (Figure 8a). The relationship of crop year leaf N with berry yields followed the similar pattern as vegetative year leaf N and berry yields (Figures 9a and 9b), but optimum levels in crop year leaf N (1.5–1.7%) were lower than that of vegetative year leaf N (1.8–2.03%). The lower leaf N in crop year may be explained by the fact that wild blueberry plant acquires N (mainly $NH_4^+$ and organic N fractions) during the vegetative year of production (Maqbool et al., 2016), store N in rhizomes and use during the whole production cycle (2-year commercial cycle). These lower leaf N levels in the crop year may be due to allocation of recourses towards reproductive organs (flowering, berry production). Fertilizers were applied in the vegetative year of production and earlier studies have suggested an increase in berry yields by splitting fertilizer doses between the vegetative and crop phases of production. In our sites, the fertilizers and soil N supply seem to fulfill the crop demand during both the vegetative and crop year of production. Soil N data from the Kemptown site suggested that the wild blueberry crop uptake soil N mainly in the form of ammonium and organic fractions in the vegetative year of production and store it in their rhizomes for later use in the crop year of production. Eaton (1994) found that N concentration in leaf and rhizome tissues were elevated in fertilized (NPK) plots for 5 production cycles. However, N-based fertilizer applications may be required in crop years where wild blueberry plants do not acquire enough N in the vegetative year of production due to the small N supplying capacity of the soil (e.g., sandy soils and low organic matter). The decision to make split fertilizer applications (vegetative and crop) could be guided through local soil knowledge and comparing leaf N status in the vegetative and the crop year with the suggested leaf N nutrition ranges reported in this study. The soil-applied N rates (14 kg N $ha^{-1}$) required to optimize foliar N in the vegetative year was lower than that required to optimize other foliar nutrients, yield potentials (Maqbool et al., 2016) and berry yields (Tables 5 and 6). These lower N rates (14 kg N $ha^{-1}$) may be enough to optimize foliar N, because some of the leaf N nutrient demands are coming from the stored reserves in the rhizomes. However, higher N applications (30 kg N $ha^{-1}$) may be required to replenish this rhizome pool of N, and/or perform other metabolic processes in the plant to achieve optimal yield potential. The relatively high N requirement in the crop year may indicate that higher rates of N fertilizer in the vegetative year are required to keep crop year leaf N within the recommended range due to competition for nutrients by the berries (Penney and MacRae, 2000).

The amount of N fertilizer required to optimize other vegetative year foliar nutrients (P, K, Ca, Mg and B) ranged from 27–30 kg N $ha^{-1}$ (Tables 5 and 6), which is very similar to the requirement to optimize yield potentials and berry yields (Maqbool et al., 2016). The optimal dose of N ranged from 22–36 kg N $ha^{-1}$ with an average of 30 kg N $ha^{-1}$ required to optimize foliar nutrients (P, K, Ca, Mg, and B). Again, this N dose agreed with the one required to optimize yield components and berry yields (Maqbool et al., 2016).

Contrary to N, where a lower level of N fertilizer was required to optimize foliar concentration than that of yield and harvestable yields, higher P (59 and 50 kg P $ha^{-1}$) was required to optimize vegetative and crop year leaf P levels than the amount needed to optimize berry yields (Maqbool et al., 2016). The amount of K fertilization needed to optimize vegetative (27 kg K $ha^{-1}$) and crop year (33 kg K $ha^{-1}$) berry yield were similar (Tables 5 and 6). The amount of K fertilizer required to optimize foliar nutrients and berry had the smallest SD (4.5 and 3.6 for vegetative and crop year, respectively) compared to soil-applied N and P (Tables 5 and 6).

Our vegetative year leaf N, P, and K nutrient concentration ranges (N = 1.8 – 2.03%, P = 0.155 – 0.160% and K = 0.53 – 0.55%) were higher than existing nutrient ranges (Eaton et al., 2009) in NS (N = 1.6 – 2.0%, P = 0.110 – 0.144% and K = 0.41 – 0.52%). Our vegetative year leaf Ca and B nutrient ranges (Ca = 0.44 – 0.46% and B = 24 – 26 ppm) fell within similar ranges (Eaton et al., 2009) but were narrower in scope (Ca = 0.32 – 0.47% and B = 19 – 31 ppm). The optimal vegetative year leaf nutrient levels obtained in this study may be due to the enhanced nutrient uptake through a more balanced fertilization (NPK). This relatively high N, P and K leaf nutrient levels may also be required for better growth, development and to maximize yield potentials. For example, availability of ammonium was increased after fertilization and enhanced uptake of soluble organic nitrogen was also observed in the vegetative year of production (Maqbool et al., 2016). These optimum nutrient ranges also reflect the effect of fertilization on soil N pools and N uptake.

Our vegetative year leaf Mg nutrient ranges (0.115 – 0.130%) were lower than existing nutrient ranges for NS (0.15 – 0.19%). Optimum leaf Mg content (0.11 – 0.13%) in the vegetative year was also lower compared to ranges obtained in earlier studies (Lockhart and Langille, 1962; Townsend and Hall, 1970; Trevett, 1972; Lafond, 2009). Lower leaf Mg content could be due to the suppression effect of ammonium build-up (Britto and Kronzucker, 2013) from our increased N applications and/or due to application of K fertilizer (antagonistic effect) (Mengel et al., 2001; Milošević et al., 2013), but these lower foliar Mg levels were not correlated with reduced berry yields. However, plants require a specific ratio of nutrients depending on the life cycle, genetic characteristics and environment to realize full genetic yield potentials. Therefore, the ratio of nutrients may be more critical than the actual nutrient levels (Marschner, 1995).

Optimum leaf B concentration in vegetative (24–26 ppm) and crop year (18–22 ppm) were below the Maine critical values for vegetative (24 ppm) and crop (30 ppm) proposed by Trevett (1972), but within NS optimal ranges of 19–31 ppm (Eaton et al., 2009). B deficiency occurs mostly on coarse-textured soils with low organic matter content and high rainfall conditions like those found in Maine, USA. The optimal leaf B content (18–22 ppm) in the crop year supplied enough B to the inflorescences for good berries per stem and optimum yield. Wild blueberry plants may not require the high B levels (30–70 ppm) proposed by Trevett (1972). The additional B supply from external sources may not be necessary in low pH soils with high soil organic matter until foliar B content falls below a critical level (< 18 ppm) that affects berry yields.

Our crop year optimal leaf N (1.5–1.7%), K (0.535–0.545%), Ca (0.465–0.495%) and B (18–22 ppm) ranges agrees with those previously reported by Trevett (1972) (Table 7). However, our crop year leaf P (0.158–0.164%) ranges were higher while leaf Mg (0.115–0.125%) ranges were lower (antagonistic effect of K application or ammonium built up) than previously suggested by Trevett (1972) (Table 7). Interestingly, our results differ from the literature and propose a narrower range of leaf nutrient concentrations for

foliar N, P, K, Ca, Mg and B be used as standards during the crop year. Our crop year leaf nutrient ranges were narrower (Figures 7 and 8) than suggested by Trevett (1972) (Table 7).

Maqbool et al. (2016) recommended 35 kg N $ha^{-1}$, 40 kg P $ha^{-1}$ and 30 kg K $ha^{-1}$ to maximize yield potentials and optimize berry yields while this study, based on our canonical analysis and overlaid surface plots recommends a slight reduction in N (30 kg N $ha^{-1}$, 45 kg P $ha^{-1}$, and 30 kg K $ha^{-1}$) fertilizer application in the vegetative year of production). This reduction may be due to the supplying capacity of the soil through mineralization and can be affected by climatic factors such as variations in temperature and soil moisture. The new proposed leaf nutrient (N, P, K, Ca, Mg and B) ranges for vegetative and crop year are presented in Table 7.

## Conclusion

Results from this study have provided insight into the main and interactive effects of soil-applied N, P and K, their optimum rates of application, and the corresponding predicted foliar nutrient contents for optimum berry yields of wild blueberries grown in areas of NS. The results of this study emphasize the importance of applying balanced fertilization. Based on the canonical analysis and overlaid surface plots, they recommend 30 kg N $ha^{-1}$, 45 kg P $ha^{-1}$ and 30 kg K $ha^{-1}$ soil-applied fertilizer at shoot pre-emergence in the spring of the vegetative year. We also present the optimal vegetative year leaf nutrient ranges N (1.8–2.03%), P (0.155–0.160%), K (0.53–0.55%), Ca (0.44–0.46%), Mg (0.115–0.13%) and B (24–26 ppm) for wild blueberry fields in Atlantic Canada. We suggest optimum leaf N (1.5–1.7%), P (0.158–0.164%), K (0.535–0.545%), Ca (0.465–0.495%), Mg (0.115–0.125%) and B (18–22 ppm) for crop year leaf nutrient levels. The leaf nutrient ranges in response to optimum fertilizer doses may be used as guidelines for wild blueberry production in all of the Atlantic Canadian provinces, Quebec and the State of Maine.

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