# VARIATION IN PLANT TISSUE CONCENTRATION AMONG SUGARBEET VARIETIES

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**Justification**: Plant tissue analysis has increasingly been used for crops as a tool to fine tune nutrient management. Plant analysis was developed as a diagnostic tool and has generally not been used to determine nutrients to apply. For sulfur, analysis of sulfur in plant tissue is commonly determined using inductively coupled plasma emission spectroscopy (ICP) even though older data that is typically used to develop sufficiency ranges may have been determined by dry combustion. Recent work in Minnesota on corn and soybean has found differences in the assessment of sulfur concentration by ICP versus combustion. Comparison of methods of analysis for sulfur for additional crops such as sugarbeet would help to determine the accuracy of ICP and where additional research in correlation of plant tissue tests to crop yield should be conducted. If differences in the methods can be documented, it would indicate that sugarbeet growers should exercise extreme caution when interpreting plant tissue results for sulfur.

Plant tissue analysis has resulted in more recent questions on boron application than other micro-nutrients. Reports that list boron as being low typically suggest a foliar application of boron containing fertilizer sources. However, there is no documented evidence that tissue sufficiency ranges currently used are accurate and that when a low tissue boron concentration is reported that application will increase crop yield. Comparisons of yield response to tissue concentration are needed to provide evidence that a sufficiency range actually has meaning when deciding if fertilizer should be applied.

Recent surveys of corn, soybean, and hard red spring wheat plant tissue has shown significant variation in nutrient concentration when multiple hybrids/varieties are sampled in the same field at the same time. If taken at face value, tissue nutrient concentration should be reflective of soil nutrient status. Past research on corn, soybean, and wheat showed a significant portion of the variation in nutrient concentration was due to growth stage differences among hybrids/varieties at sampling. What needs to be addressed for sugarbeet is the degree of variation in tissue nutrient concentration in petioles and leaf blades for varieties grown at multiple locations and years and whether plant tissue analysis can be related to root or sugar yield. If there is significant variation in concentration that is reflective of genetics and not of yield potential, there should be a significant degree of caution when interpreting tissue results without further documentation of deficiencies with additional analysis such as soil tests.

**Objectives**:

1. Compare nutrient concentration in petioles and leaf blades among varieties at three sampling times.
2. Determine if tissue nutrient concentration is predictive of root and sugar yield when sampling adequately fertilized fields.

**Materials and Methods**: Six sugarbeet varieties (listed below) were planted at four locations [three locations were sampled in 2019 (Table 1)] and tissue analysis samples were collected at three sampling times over the growing season. Varieties were planted in four replications at each site. Sampling times were early to mid-June, early July, and late July to early August. The newest developed leaf was sampled. The petiole and leaf blade were sampled as one then separated for individual analysis. All samples were dried, ground, and analyzed for nitrate N and Cl via extraction with 5% acetic acid, total N by combustion, and P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn by ICP. A single composite soil sample consisting of six to eight cores was taken from the 0-6 and 6-24 inch depths from each site at each plant sampling date. Soil samples were analyzed using recommended procedures of N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn, and Cl and for pH (1:1 soil:water), soil organic matter (loss on ignition), and cation exchange capacity [CEC (ammonium saturation and displacement)]. Plant tissue nutrient concentration was correlated with yield and quality to determine what factors may be important for the prediction of root and sugar yield. All data was subject to an analysis of variance procedure assuming fixed effects of location, sampling time, and variety and random blocking effects.

Varieties used in the sampling trial:

1. Crystal RR018 – Check variety: Good disease tolerance, average yield but below average sugar.
2. Maribo 109 – Check variety: Good disease tolerance with average sugar content. Below average tons. Tends to have a smaller leaf canopy than other varieties.
3. Beta 92RR30 –Average tons and average sugar.
4. Beta 9475 –Good Cercospora leaf spot resistance, high yield and average sugar.
5. Crystal M579 –High sugar content.
6. Crystal M509 – Good cercospora resistance, low sugar content and high yield.

**Results**: Sample timings were targeted to occur within three week intervals near the 50-80 day suggested for sugarbeet sampling. Actual sampling dates averaged 45, 65, and 88 days after planting which was ideal for the trial to study early, suggested, and late sampling timings. Soil types, chemical properties, and cation exchange capacity was relatively similar among soils at the eight locations.

Root yield, sugar content per ton, and sugar content produced per acre varied among the six varieties across locations and years (Table 3). Overall, root yield, sugar content, and sugar production followed anticipated patterns based on past varietal response data, but variety

rankings did vary slightly by year (not shown). Some variation in varietal ranking may be due to differences in yield potential as a result of cercospora which had a greater incidence across locations in 2018 (not shown).

Differences in leaf blade nutrient concentration among varieties, when averaged across time and location, are summarized in Table 3. While significant, the relative differences in plant nutrient concentrations among the varieties were relatively small. The ranking among varieties (maximum to minimum concentrations) were not consistent indicating that varieties with greater nutrient concentration of a single nutrient were not greater for all nutrients. This indicates that plant nutrient uptake is not relatively greater for one variety versus another for all nutrients. Table 6 also lists the anticipated sufficiency range according to the Plant Analysis Handbook III for sugarbeet leaf blade tissue collected 50-80 days after planting. The average for boron tissue concentration was the only instance where a concentration average was close to the low end of the sufficiency range. However, the boron concentration in the leaf blade tissue did not necessarily indicate that boron was limiting yield. Results for leaf blade nitrate nitrogen and chloride are listed in Table 3, but there is no given sufficiency range for these nutrients.

Effects on all nutrient concentrations were similar for petioles (Table 4) as with leaf blades. However, the concentration of nutrients tended to be less in the petiole than in the leaf blade tissue. The major exceptions were potassium and chloride where the concentration was greater in the petiole than in the leaf blade. There is no identified sufficiency range for petiole tissue to compare results with established ranges.

The effect of time on macro and micronutrient concentrations is summarized in Figures 1 and 2, respectively. Most nutrients decreased in concentration in both the leaf blade and petiole samples over time starting at time one through time three. There were exceptions where some nutrients did not change over time or showed a temporary decrease from T1 to T2 but then increased from T2 to T3. Iron did exhibit a decrease over time, but this decrease was likely due to less soil contamination on leaves later in the growing season. As more leaves developed it was less likely that rain drops would reach the soil surface resulting in splashing of soil particles onto plant tissue. Due to contamination, tissue iron concentration should not be used as a predictor of yield and quality parameters. There was a large increase in copper from T2 to T3. The concentration of copper spiked in the leaf tissue at sampling time three as a result of copper being applied to treat cercospora. Tissue sulfur concentration generally increased in the leaf blade while it decreased in the petiole.

Plant tissue concentrations were correlated with root yield and sucrose content, but the data are not shown. Similar to root yield, there were no instances where sugar content or yield showed a consistent correlation with multiple nutrients. It would be expected that if a nutrient is limiting or if yield or quality is a function of nutrient concentration then there should be consistent correlation over time between these factors and the concentration of nutrients in the plant tissue. Nutrient concentration in plant tissue does not necessarily account for variations in plant growth

and differences in nutrient remobilization among varieties. The data overall indicates that some caution should be exercised when interpreting plant tissue results as a correlation between yield and quality and a concentration of a specific nutrient at a single point during the growing season does not prove that uptake of any nutrient is driving final yield or sugar production.

The correlations between yield and quality parameters did change as data were collected over years (not shown). The change in the best correlations between yield or quality parameters and plant tissue concentrations over time indicate that some caution should be exercised when using correlation data. Also, correlation does not prove that one factor drives the other factor rather it shows there is a relationship. In order to be certain that a tissue concentration impacts yield or quality separate research needs to be conducted using cause and effect to determine how application of nutrients change tissue nutrient concentrations and whether yield or quality factors are impacted.

Average nutrient concentrations by location were regressed with multiple soil and environmental factors to determine if variation in tissue concentrations could be explained by variations in factors which cannot be controlled. Multiple environmental factors were studied including average minimum and maximum temperature, total precipitation, and growing degree day. All the previous factors were summarized based on the time from planting to sampling, 1 day, 3 days, 1 week, 2 weeks, and 3 weeks prior to sampling. Significant factors were grouped into long term (greater than 2 weeks) or short term (2 weeks or less) factors for summary in Figures 3 and 4. All soil factors in Tables 2a and 2b were utilized and were grouped into soil test or other soil (soil) factors after the analysis. Time factor considers the time (days) between planting and sampling. The remaining variation which could not be explained by the model was marked as unknown. Two micronutrients, iron and copper, were not regressed with soil factors as contamination of iron and copper through soil adhering to the plant tissue or foliar application of the nutrient due to greater than expected concentrations of either nutrient not as a result of plant uptake.

Figure 3 summarizes the relationship between blade total N concentration and root yield, and blade total Ca concentration and recoverable sugar. Best fit models show a general relationship between the factors. However, in this case both graphs, clustering of values within sites result in the positive relationships and it is questioned how accurate a model developed to predict yield or quality can do so. The graphs presented use actual yield and recoverable sugar values and prediction models typically use values relative to a maximum value in order to reduce the impact of random factors not accounted for in the model from influencing the relationship between yield or quality factors and tissue concentrations. For example, crop yield is an interaction between the varieties genetic potential and optimal growth factors at an individual site. Soil nutrient availability is one factor impacting yield but not the sole factor thus adjusting yield data. For this report yield data was not adjusted on a relative basis as it is unclear how to make adjustments when differences in yield are based on genetic factors only. With nutrient availability trials the maximum yield produced by increasing rates of nutrient applied are used to compare the yield

produced by treatments to generate a relative yield as it relates to maximum yield potential by site for a specific cultivar.

The equations (a) through (f) below represent results from multiple regression analysis to determine if multiple factors combined can help predict root yield and recoverable sugar per ton. Equations a, b, and c identify significant prediction for root yield using plant tissue factors for sample times 1, 2, and 3, respectively. Equations e, f, and g identify prediction factors for recoverable sugar per ton for times 1, 2, and 3, respectively.

(a) root yield = -31.8 + 5.04(Blade N) + 1.28 (Blade B) – 0.000136 (Pet Cl)

(b) root yield = 57.0 – 27.7(Blade Mg) – 17.9 (Pet Ca) – 0.88 (Pet Cu)

(c) root yield = -20.7 + 0.82(Blade Zn) – 11.4 (Pet K) + 2.65 (Pet B)

(d) rec. sugar per ton = 80.6 -0.005(Blade NO3) + 20.9 (Blade P) -126.6 (Blade S) + 2.37 (Blade Zn) + 0.008 (Blade Cl) + 756.86 (Pet S)

(e) rec. sugar per ton = 446.6 – 213.9 (Blade Mg) – 332.7 (Blade S) + 1.09(Pet Mn)

(f) rec. sugar per ton = 351.7 – 183.3(Blade P) – 63.5(Blade Mg) – 0.17 (Blade Cu) + 1.41 (Blade Zn) – 80.4 (Pet Ca)

Time 1 prediction models could be used to predict 99% of the variability in yield and in recoverable sugar per ton with a combination of multiple factors. Combined $r^2$ values were poorer at time 2 compared to time 1 and for root yield at time 3 compared to time 1, but not for recoverable sugar at time 3 which had a total $r^2$ similar to time 1. This indicates that prediction is generally better for time 1 than the later sampling dates. What should be noted though is that all factors in the model do not necessarily have a positive impact on root yield or recoverable sugar. For example in equation a, root yield increased with increasing blade N and B concentration and decreasing petiole Cl content. One item to note is that there is some correlation between the different blade and petiole nutrient concentration as uptake of a single nutrient can impact the uptake of other nutrients. Also, prediction models are always better at backwards predicting values and seldom are good at forward predicting what may happen in future years. For example, many models exist to predict iron deficiency chlorosis in soybean but many fail to predict the severity and where IDC will occur when used in studies where the models did not generate data. Care should always be exercised when using multiple regression models as the data may be specific to the sites where the studies were conducted or cultivars used for the studies.

**Conclusions**: The data showed that there were clear differences in yield and quality among the sugarbeet varieties used in the study. Tissue (leaf blade and petiole) nutrient concentration will vary among sugarbeet varieties sampled in the same field at the same time. The concentration of most nutrients will decrease when sampling the same leaf relative to the top part of the canopy over time. The decrease or increase will occur for each nutrient similar for the leaf blade and

petiole sample.  Due to this variation, a large range in the recommended sampling time for leaf blade samples (50-80 days after planting) should not be used. The data indicates that earlier sampling around 40-50 days after planting may be more predictive of yield response compared to later samples. However, there was not strong evidence that root yield or recoverable sugar could be fully predicted by plant tissue concentration and that concentration of nutrients in leaf blade and petiole tissues could be explained by factors other than the soil test of a nutrient indicating much of the variation in plant tissue concentration is controlled by uncontrollable factors. The data indicates that significant caution should be exercised when collecting a single sample from a well fertilized field as there is no evidence that the concentration of a nutrient in the leaf or petiole has a direct impact on yield or quality.

Table 1. Location, planting and sampling information, dominant soil series, and cation exchange capacity (CEC) for each location (CC, Clara City; H, Hector; LL, Lake Lillian; M, Murdock; R, Renville).

| | Date of | | | | Soil | | CEC | | Particle Size | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Planting | Sample 1 | Sample 2 | Sample 3 | Series | Classification‡ | 0-6” | 6-24” | Sand | Silt | Clay |
| | | | | | | | meq/100g | | | % | |
| | | | | | 2017 | | | | | | |
| CC | 25-May | 12-Jul | 2-Aug | 22-Aug | Colvin-Quam | T Calciaquoll | 31.6 | 25.5 | 18 | 53 | 30 |
| LL | 8-May | 21-Jun | 12-Jul | 2-Aug | Nicollet | A Hapludoll | 33.7 | 28.7 | 25 | 40 | 35 |
| M | 29-Apr | 21-Jun | 12-Jul | 2-Aug | Bearden-Quam | Ae Calciaquoll | 28.0 | 22.2 | 14 | 48 | 38 |
| R | 6-May | 21-Jun | 11-Jul | 1-Aug | Chetomba | T Endoaquoll | 31.1 | 24.4 | 22 | 43 | 36 |
| | | | | | 2018 | | | | | | |
| CC | 17-May | 27-Jun | 18-Jul | 14-Aug | Bearden-Quam | Ae Calciaquoll | 30.9 | 20.9 | 16 | 48 | 37 |
| H | 10-May | 21-Jun | 9-Jul | 2-Aug | Crippin | A.P. Hapludoll | 35.8 | 28.5 | 10 | 49 | 41 |
| LL | 7-May | 21-Jun | 9-Jul | 2-Aug | Nicollet | A Hapludoll | 31.3 | 23.7 | 30 | 37 | 33 |
| M | 18-May | 27-Jun | 16-Jul | 14-Aug | Bearden-Quam | Ae Calciaquoll | 35.2 | 28.2 | 11 | 48 | 41 |
| | | | | | 2019 | | | | | | |
| H | 7-May | 17-Jun | 11-Jul | 31-Jul | Crippin | A.P. Hapludoll | 40.5 | 34.9 | 18 | 42 | 40 |
| LL | 6-May | 17-Jun | 11-Jul | 31-Jul | Okaboji-Canisteo | C.V. Endoaquoll | 36.0 | 30.9 | 13 | 50 | 37 |
| M | 31-May | 15-Jul | 31-Jul | 19-Aug | Byrne-Buse | C. Hapludoll | 27.7 | 23.9 | 21 | 50 | 29 |

‡A, aquic; Ae, aeric; A.P., aquic pachic; C, calcic; C.V., cuuulic vertic; T, typic.

Table 2. Summary of analysis of variance for the main effect of sugarbeet variety by and across 2017-2019 locations. Numbers within rows which are followed by the same letter are not significantly different at $P \leq 0.10$.

| Variety | | | | | | |
|---|---|---|---|---|---|---|
| Crystal RR018 | Maribo 109 | Beta 92RR30 | Beta 9475 | Crystal M579 | Crystal M509 | *P*>F |
| ----------Root Yield (tons/acre) ---------- | | | | | | |
| 28.3c | 26.2d | 26.7d | 30.5b | 29.5b | 33.0a | |
| ----------Recoverable Sugar (lbs/ton) ---------- | | | | | | |
| 265c | 269bc | 272b | 267c | 276a | 259d | |
| ----------Recoverable Sugar (lbs/acre) ---------- | | | | | | |
| 7633c | 7143d | 7313d | 8209b | 8223b | 8623a | |

Table 3. Varietal differences in leaf blade nutrient concentration across eleven locations from 2017-2019 and three sampling times at each location. Within rows, numbers followed by the same letter are not significantly different at $P \leq 0.10$.

| | Variety | | | | | | |
|---|---|---|---|---|---|---|---|
| Nutrient | Crystal RR018 | Maribo 109 | Beta 92RR30 | Beta 9475 | Crystal M579 | Crystal M509 | Suffic.† |
| | ----------%---------- | | | | | | |
| Total-N | 5.25a | 4.87b | 4.84b | 4.88b | 4.79b | 4.87b | 4.3-5.0 |
| Phosphorus | 0.53a | 0.55a | 0.46c | 0.48bc | 0.45c | 0.51ab | 0.45-1.1 |
| Potassium | 3.95a | 3.74b | 3.63d | 3.62d | 3.71bc | 3.65cd | 2.0-6.0 |
| Calcium | 0.68b | 0.74a | 0.73a | 0.65c | 0.67bc | 0.69b | 0.5-1.5 |
| Magnesium | 0.48d | 0.52b | 0.56a | 0.50c | 0.50c | 0.52b | 0.25-1 |
| Sulfur | 0.38 | 0.36 | 0.35 | 0.37 | 0.36 | 0.38 | 0.21-0.5 |
| | ----------ppm---------- | | | | | | |
| Nitrate-N | 752a | 400e | 609bc | 634b | 478d | 580c | |
| Boron | 30 | 31 | 32 | 29 | 30 | 29 | 31-200 |
| Copper | 35c | 40a | 36bc | 33c | 39ab | 33c | 11-40 |
| Iron | 494a | 389c | 502a | 439b | 516a | 516a | 60-140 |
| Manganese | 65cd | 68b | 76a | 63d | 79a | 67bc | 26-360 |
| Zinc | 46ab | 39c | 44ab | 44b | 44ab | 47a | 10-80 |
| Chloride | 3059b | 3516a | 3076b | 3117b | 2996bc | 2895c | |

†Suffic, sufficiency range identified by Bryson et al., 2014.

Table 4. Varietal differences in petiole nutrient concentration across eleven locations from 2017-2019 and three sampling times at each location. Within rows, numbers followed by the same letter are not significantly different at $P \leq 0.10$.

| | Variety | | | | | |
|---|---|---|---|---|---|---|
| Nutrient | Crystal RR018 | Maribo 109 | Beta 92RR30 | Beta 9475 | Crystal M579 | Crystal M509 |
| | ----------%---------- | | | | | |
| Total-N | 2.54bc | 2.60ab | 2.65a | 2.52cd | 2.46d | 2.61ab |
| Phosphorus | 0.35bc | 0.43a | 0.35bc | 0.35bc | 0.33c | 0.37b |
| Potassium | 4.56 | 4.58 | 4.28 | 4.40 | 4.29 | 4.76 |
| Calcium | 0.44c | 0.56a | 0.49b | 0.45c | 0.49b | 0.57a |
| Magnesium | 0.26b | 0.28a | 0.28a | 0.24d | 0.24c | 0.24c |
| Sulfur | 0.14 | 0.15 | 0.13 | 0.14 | 0.14 | 0.14 |
| | ----------ppm---------- | | | | | |
| Nitrate-N | 4311c | | 5315a | 4281c | 3997c | 4777b |
| Boron | 23c | 25s | 24b | 24b | 23c | 26a |
| Copper | 9.6 | 9.5 | 8.6 | 9.9 | 9.0 | 9.5 |
| Iron | 307 | 300 | 267 | 257 | 289 | 285 |
| Manganese | 28b | 29b | 28b | 26b | 34a | 30b |
| Zinc | 20 | 21 | 18 | 18 | 19 | 20 |
| Chloride | 4980b | | 5880a | 5742a | 5665a | 6103a |

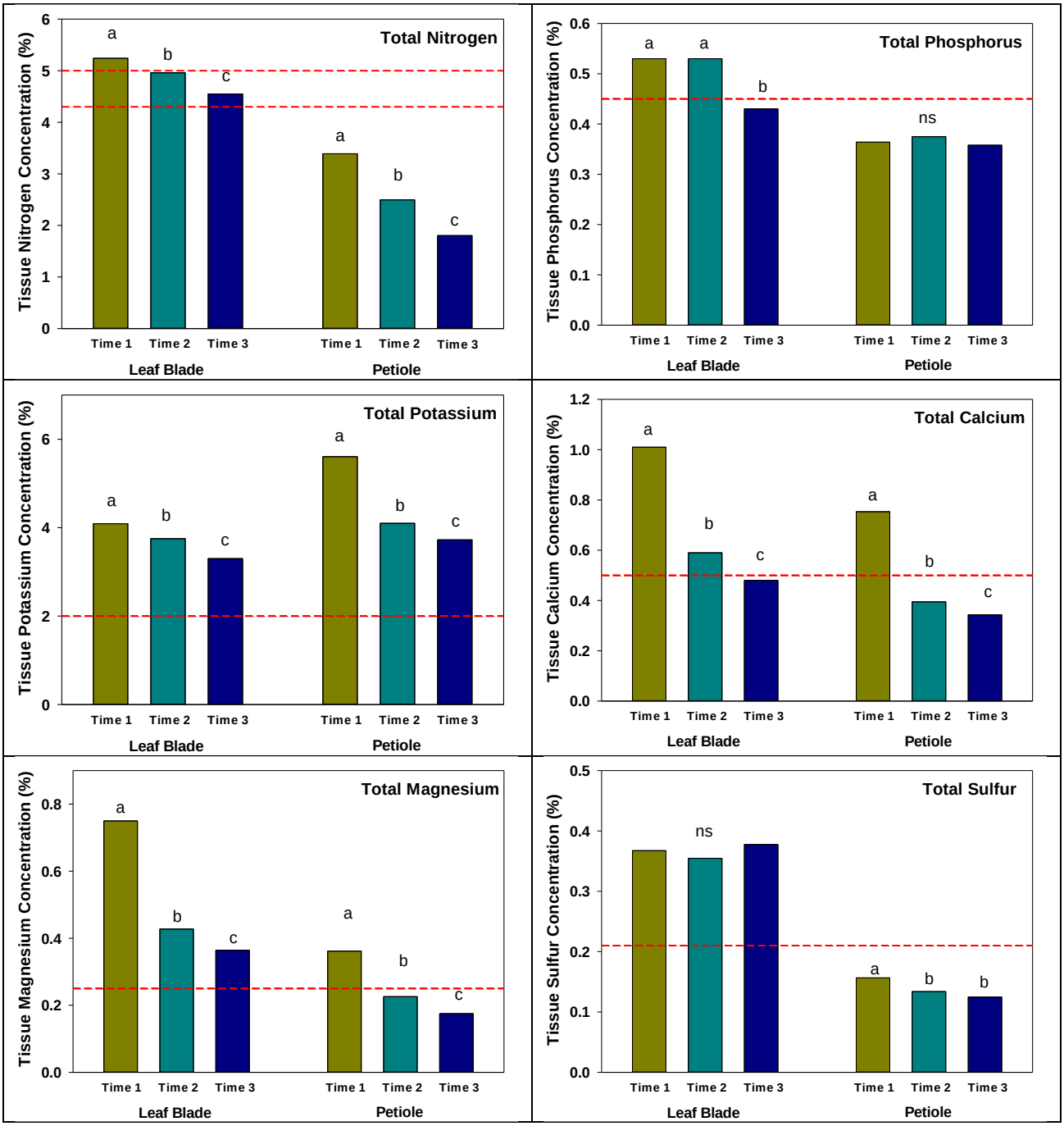


Figure 1. Summary of the impact of time on sugarbeet total macronutrient concentrations for leaf blade and petiole samples collected from six sugarbeet varieties. Letters denote significance among sampling times for leaf blade or petiole samples at $P \leq 0.10$. Horizontal dashed lines represent the upper and lower end of the sufficiency range for leaf blade samples according to Bryson et al., 2014. A single dashed line represents the low end of the sufficiency range.

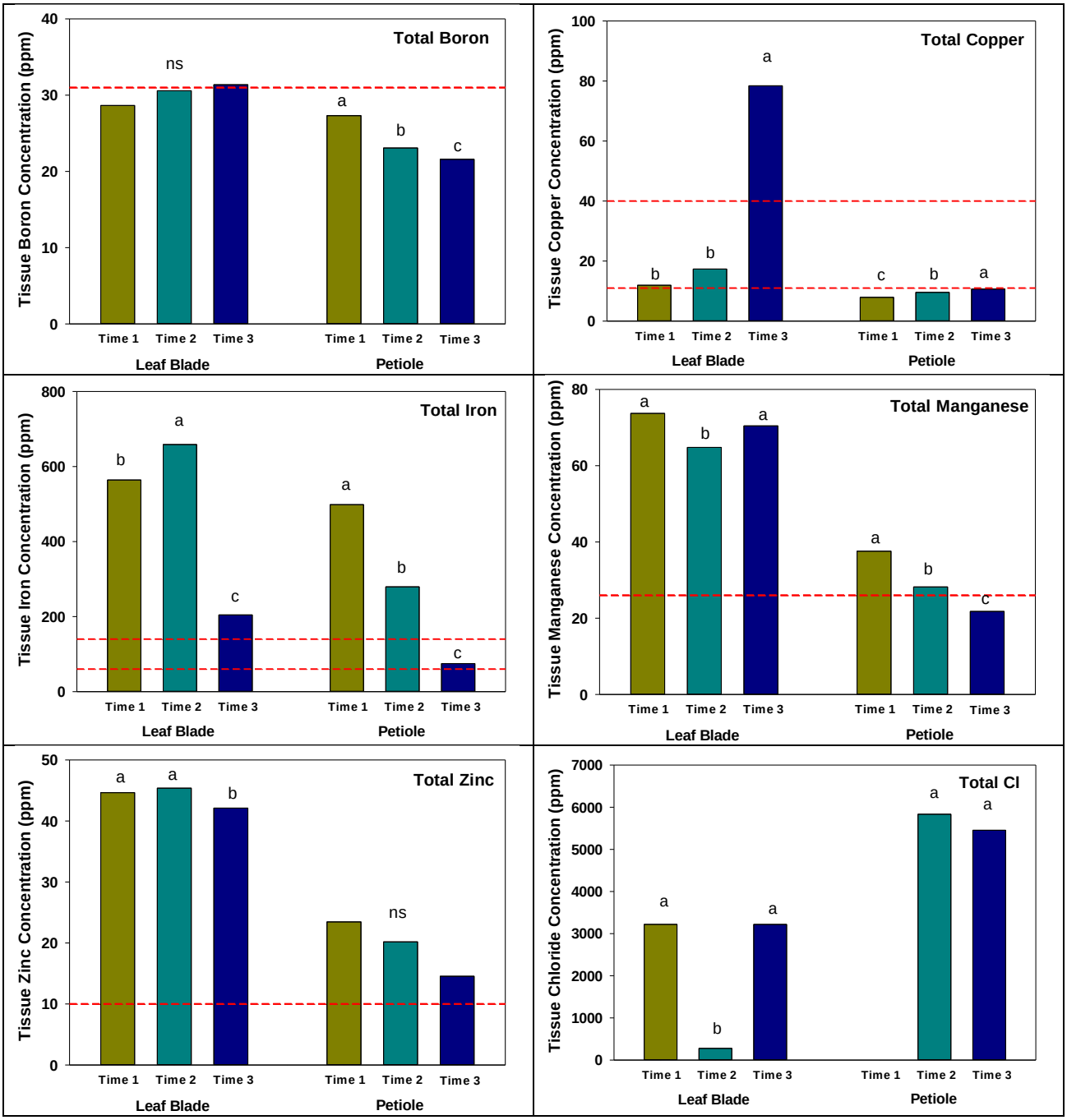


Figure 2. Summary of the impact of time on sugarbeet total micronutrient concentrations for leaf blade and petiole samples collected from six sugarbeet varieties. Letters denote significance among sampling times for leaf blade or petiole samples at $P \leq 0.10$. Horizontal dashed lines represent the upper and lower end of the sufficiency range for leaf blade samples according to Bryson et al., 2014. A single dashed line represents the low end of the sufficiency range.

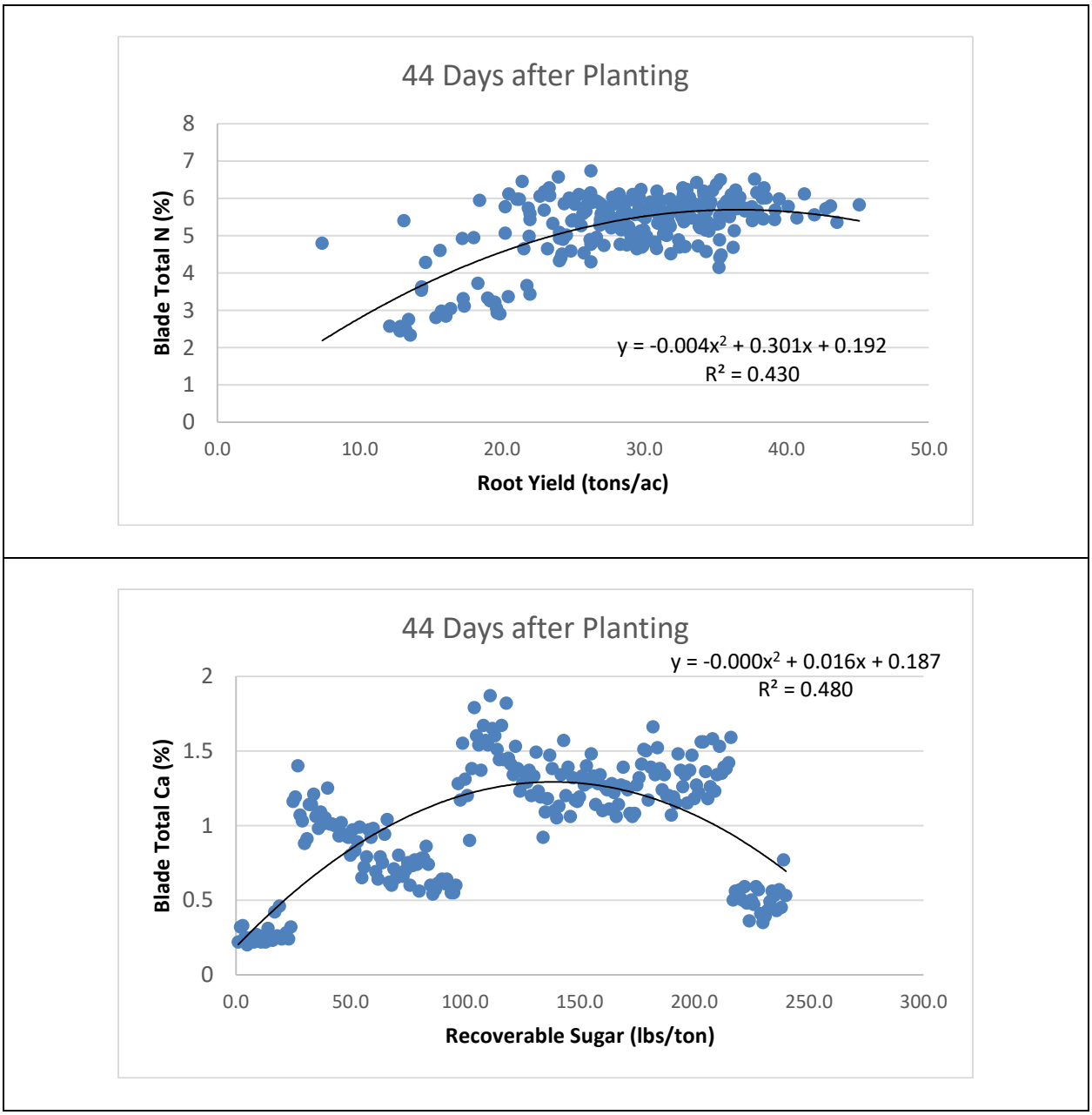


Figure 3. Relationship between blade total N concentration and root yield and blade total Ca concentration on recoverable sugar for tissue samples collected 44 days after planting.