INDIVIDUAL LEAF SELECTION TO BEST REPRESENT WHOLE-PLANT NUTRIENT STATUS IN MODERN CORN CROPPING SYSTEMS

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ABSTRACT

In modern corn cropping systems, fertilization is often required to maintain plant health. Tissue sampling is commonly utilized to evaluate plant nutrient status and determine fertilizer treatment needs. Recommendations exist on which partition/leaf to select for accurate representation of the whole-plant. Recommendations change with growth stage, suggesting to sample the whole-plant at early-vegetative stages, the top-collared leaf at late-vegetative stages, and the ear-leaf during reproductive stages. The primary goal of this study was to explore the ability of various individual-leaf sample selections to accurately represent the whole-plant concentrations of nitrogen (N), phosphorous (P), potassium (K), and sulfur (S) across multiple growth stages and N rates. Research was conducted at the Agronomy Center for Research and Education (ACRE) near West Lafayette, IN during the 2021 and 2022 growing seasons. The experiment included three N rates (0, 135, and 215 lbs. N ac^{-1}) sidedress applied as UAN (28-0-0) at V5. At V8, the 8th leaf and whole-plant were sampled. At V12, the 8th leaf, 12th leaf, and whole-plant were sampled. Grain yields responded positively to N application, increasing from 124 bu ac^{-1} without N to 234 bu ac^{-1} under 135 lbs. N ac^{-1} and 270 bu ac^{-1} under 215 lbs. N ac^{-1}. At both V8 and V12, leaf and whole-plant N concentrations showed a strong response to N application, increasing ($P<0.05$) by up to 60%. At V12, 8th and 12th leaf P, in addition to 8th leaf and whole-plant S were increased by N application. Plant K was not significantly influenced by N rate or year at any stage. Whole-plant nutrient concentrations averaged 3.41% N, 0.41% P, 2.54% K, and 0.24% S at V8. At V8, whole-plant N was 13% lower than 8th leaf N, whole-plant S was 14% lower than 8th leaf S, whole-plant P was 13% higher than 8th leaf P, and whole-plant K was 24% higher than 8th leaf K. At V12, whole plant nutrient concentrations averaged 2.04% N, 0.25% P, 2.01% K, and 0.13% S. Relative to the 8th leaf at V12, whole-plant N was 38% lower, S was 80% lower, P was 5% higher, and K was 14% higher. Relative to the 12th leaf at V12, whole-plant N was 31% lower, S was 30% lower, P was 10% lower, and K was 6% higher. Individual-leaf N and S were most similar to whole-plant N and S when the 8th leaf was sampled at V8. Leaf P was most similar to whole-plant P at V12 (both 8th and 12th leaves), while leaf K was most similar to whole plant K in the 12th leaf sampled at V12. Preliminary results indicated that (1) leaf P and K were similar to whole-plant P and K, (2) leaf N and S differed from whole-plant N and S, and (3) from V8 to V12, nutrient dilution led to decreased nutrient concentrations. Further analysis will incorporate leaf comparisons at R1, and stover versus grain comparisons at R6 to determine how the trends already observed continue into the reproductive period.

MATERIALS AND METHODS

This experiment was conducted in West Lafayette, IN at the Agronomy Center for Research and Education (ACRE) for both the 2021 and 2022 growing seasons. The study was conducted in separate field areas for each of the growing seasons to maintain that the previous crop was soybean in both site-years. The experimental design was a randomized complete block design (RCBD) consisting of 4 to 6 replications within a larger 8-replication N study. This analysis focused on 3 of 6 sidedress N rates (0, 135, and 215 lbs. N acre^{-1}) included in the study. Pioneer hybrid 1359AM was grown at a density of 31,000 plants acre^{-1} (2021) and 34,000 plants acre^{-1} (2022). N fertilizer treatments were sidedressed as urea ammonium nitrate (UAN; 28-0-0) using coulter injection at the V5 growth stage. Sulfur was pre-plant broadcast applied as ammonium thiosulfate (ATS) (12-0-0-26(S)) to supply 20 lbs. SO_4 ac^{-1} to the entire trial area. The N supply from the ATS was approximately 9.2 lbs. N ac^{-1}. Plots were planted on April 28th (2021) and May 2nd (2022) using a six-row John Deere 1780 planter. Each plot consisted of six rows with 30-inch spacing for a total width of 15 feet and a length of 90 feet. Grain yield was determined by combine harvesting the central 2 rows of the 6-row plots for a harvest area of 450 ft^2 in each plot. Grain yields were adjusted to 15.5% moisture based on moisture readings from the combines.

Tissue samples were collected during the V8 and V12 growth stages. Individual-leaf and whole-plant samples came from the same 10 plant sample for each plot meaning leaves were removed from each of the 10 plants. At V8 this included separating the 8th leaf and whole-plant. At V12 the 8th leaf, 12th leaf, and whole-plant were separated. All tissue samples were dried at 60 C, weighed, and ground to a 1mm consistency before nutrient concentration could be determined. Samples were sent to Waypoint Analytical in Memphis, TN where the PT2 nutrient analysis was conducted. Upon receiving results, the weights and nutrient concentrations of individual leaves and their whole-plant counterparts were used to algebraically determine the true whole-plant nutrient concentrations, incorporating the leaves back into the whole-plant total. Prior to V8, aerosol spray paint was applied to the tip of the 7th leaf on each plant. This allowed researchers to distinguish specific leaf positions for accurate sampling as plants grew.

RESULTS AND DISCUSSION

It is important to consider nutrient sufficiency ranges and what partition these ranges are based upon. In Table 1, the V5 values are based upon a sample of the whole-plant. However, R1 samples are based upon just the ear-leaf. In many situations this may be a good representation of the whole-plant, but this study will investigate how this dynamic between a single leaf and the whole-plant can change with regard to growth stage, leaf position, soil N availability, and nutrient of interest. It is well documented in the literature that certain nutrients are mobile within the vasculature of a plant while others are not. Sulfur, for instance, is relatively immobile. Thus, once it is within the tissue of a

Table 1. Nutrient concentration sufficiency ranges in corn tissue during early vegetative (V5) and reproductive (R1) growth.

	Sufficiency Range (%)		
Nutrient	Whole Plant (~V5)	Ear Leaf (R1)	Mobility Within Plant
N	3.5-5.0	2.75-3.50	Mobile
P	0.3-0.5	0.25-0.45	Mobile
K	2.5-4.0	1.75-2.25	Mobile
S	0.2-0.5	0.15-0.50	Immobile

Adapted from "Plant analysis for testing nutrient levels in corn".

plant it is less likely to move to other areas if nutrient deficiency occurs. Alternatively, N, P, and K are relatively mobile.

In both years soil fertility samples were taken on an individual plot basis at planting to understand soil characteristics that could have implications on the plant tissue analysis to follow. Table 2 summarizes data from for both site-years to give averages of organic matter (OM), cation exchange capacity (CEC), P, K, and S levels. Results indicate a 10-ppm difference in P with lower phosphorous soil availability in 2021 compared to 2022. Inversely, soil levels of potassium were 10-ppm higher in the 2022 field site.

Table 2. Soil fertility characteristics measured at planting to a depth of 8" averaged across all plots for a given year

Year	OM (%)	CEC (meq/100g)	P (ppm)	K (ppm)	S (ppm)
2021	4.0	19.1	31	149	11
2022	3.7	18.9	41	139	11

Nitrogen

Nitrogen is often considered the "most" essential nutrient in corn production and is highly mobile within the plant (Table 1). During vegetative growth, N concentrations in the whole-plant decreased over time from 3.41% N to 2.04% N from V8 to V12 (Table 3). Similarly, the 8th leaf N concentration decreased from 3.85% N at V8 to 2.81% N at V12 (Table 3). At V8, the 8th leaf N concentration was 0.44% N higher than the whole-plant. Continuing with the idea of top-leaf versus whole-plant at the V12 growth stage, the 12th leaf averaged 2.68% N making it 0.64% N higher than the V12 whole-plant. At V12, the 8th leaf had a higher N concentration than the whole-plant with a concentration of 2.81% N in the 8th leaf compared to the 2.04% N in the whole-plant. Surprisingly, at V12 the N concentration of the 8th leaf was 0.13% N higher than the 12th leaf (Table 3). Overall, leaf N was higher than whole-plant N and grew as the season progressed.

Table 3. Nitrogen Concentration of Various Individual-Leaf and Whole-Plant Tissue Samples from V8 and V12 Growth Stages across Multiple N Application Rates and Years

	Vegetative Nitrogen Concentration (%)				
	V8 Growth Stage		V12 Growth Stage		
Nitrogen Rate lbs N $acre^{-1}$	8th Leaf	Whole Plant	8th Leaf	12th Leaf	Whole Plant
2021	3.90	3.22	2.87	2.76	2.26
0	3.68	2.90	2.44	2.32	1.76
135	4.00	3.38	3.07	2.93	2.61
215	4.01	3.38	3.10	3.03	2.42
2022	3.83	3.54	2.77	2.62	1.89
0	3.49	3.12	2.14	1.95	1.41
135	3.90	3.71	2.96	2.92	2.08
215	4.10	3.80	3.21	3.00	2.17
Mean	3.85	3.41	2.81	2.68	2.04
Significance Test[1]					
N Rate	**	**	**	**	**
Year	ns	**	ns	*	**
N Rate*Year	ns	ns	*	*	ns

[1]A mixed model was run using JMP Pro 9.1 with N rate and Year as main effects, block as a random effect, and block*year as a random effect. P-Values greater than 0.05 considered non-significant (ns). P-Values less than 0.05 (*), 0.01 (**), and 0.001 (***).

Nitrogen application rate significantly influenced N concentrations of all plant partitions, particularly at the later growth stage. Plants that did not receive additional N fertilization had the lowest N concentrations. Differences between the higher N rates were often small due to both rates being sufficient for plant growth at V12 (plant N requirements increase as the season progresses).

Nitrogen concentrations differed significantly from year to year, varying by 10% in V8 whole-plants, 18% in V12 whole-plants, and 5% in V12 12th leaves. At V8, whole-plant N was higher in 2022 than in 2021 whereas the opposite was true at V12. Furthermore, a significant interaction between N rate and year was detected in V12 8th and 12th leaves due to much stronger N concentration responses to N application observed in 2022 compared to 2021.

Sulfur

Sulfur is considered immobile within the plant, meaning S usually remains in older tissues even after other nutrients have been remobilized (Table 4). From V8 to V12 the whole-plant S concentration decreased from 0.24 to 0.13%. Being far less mobile than N, Table 4 suggests that the 8th leaf retains a substantial amount of S from V8 to V12, decreasing to a lesser extent in the leaves, from 0.27 to 0.23% S, than the whole-plant. At V8, 8th leaf S was just 0.03% S higher than whole-plant S, however by V12, 8th leaf S was 0.10% S higher than whole-plant S (Table 4). At V12, the 12th leaf also had a higher S concentration than the whole-plant.

Table 4. Sulfur Concentration of Various Individual-Leaf and Whole-Plant Tissue Samples from V8 and V12 Growth Stages across Multiple N Application Rates and Years

	Vegetative Sulfur Concentration (%)				
	V8 Growth Stage		V12 Growth Stage		
Nitrogen Rate lbs N acre-1	8th Leaf	Whole Plant	8th Leaf	12th leaf	Whole Plant
2021	0.27	0.22	0.19	0.16	0.14
0	0.28	0.21	0.20	0.15	0.12
135	0.26	0.22	0.19	0.17	0.15
215	0.28	0.22	0.19	0.18	0.14
2022	0.27	0.25	0.26	0.17	0.12
0	0.26	0.24	0.23	0.14	0.11
135	0.27	0.26	0.26	0.18	0.13
215	0.29	0.27	0.28	0.19	0.13
Mean	0.27	0.24	0.23	0.17	0.13
Significance Test[1]					
N Rate	ns	ns	ns	**	**
Year	ns	**	**	ns	**
N Rate*Year	ns	ns	*	ns	ns

[1]A mixed model was run using JMP Pro 9.1 with N rate and Year as main effects, block as a random effect, and block*year as a random effect. P-Values greater than 0.05 considered non-significant (ns). P-Values less than 0.05 (*), 0.01 (**), and 0.001 (***).

The average difference in S concentration between the 8th and 12th leaf at V12 was 0.06%, with the 8th leaf having the higher S concentration than the 12th leaf (Table 4). This trend, however, is not consistent between years with the 2022 data showing a larger difference than 2021. Furthermore, the 12th leaf was more similar to the whole-plant S status than the 8th leaf at V12. This means that at both V8 and V12 the top collared leaf was approximately 0.03% higher than the whole-plant (Table 4).

Nitrogen application rate affected V12 12th leaf and whole-plant S concentrations. When no N was applied, S concentrations were decreased by up to 36% relative to treatments receiving an N application (Table 4). Sulfur concentrations differed significantly from year to year, varying by 15% in V8 whole-plants, by 30% in V12 8th leaves, and by 13% in V12 whole-plant samples (Table 4). At V8, whole-plant S concentrations were higher in 2022, a trend also seen in the 8th leaf at V12. However, the V12 whole-plant had a lower S concentration in 2022 than in 2021. An interaction between N rate and year effects was detected from the V12 8th leaf due to S concentrations responding positively to N rates in 2022, yet remaining stable across N rates in 2021 (Table 4).

Table 5. Phosphorous Concentration of Various Individual-Leaf and Whole-Plant Tissue Samples from V8 and V12 Growth Stages across Multiple N Application Rates and Years

	Vegetative Phosphorous Concentration (%)				
	V8 Growth Stage		V12 Growth Stage		
Nitrogen Rate lbs N acre-1	8th Leaf	Whole Plant	8th Leaf	12th Leaf	Whole Plant
2021	0.38	0.41	0.23	0.29	0.25
0	0.39	0.41	0.23	0.27	0.26
135	0.37	0.42	0.22	0.28	0.25
215	0.38	0.41	0.23	0.31	0.25
2022	0.33	0.40	0.25	0.27	0.25
0	0.31	0.38	0.21	0.25	0.25
135	0.34	0.40	0.26	0.28	0.25
215	0.36	0.42	0.27	0.28	0.25
Mean	0.35	0.41	0.24	0.28	0.25
Significance Test[1]					
N Rate	ns	ns	*	**	ns
Year	**	ns	*	ns	ns
N Rate*Year	ns	ns	**	ns	ns

[1]A mixed model was run using JMP Pro 9.1 with N rate and Year as main effects, block as a random effect, and block*year as a random effect. P-Values greater than 0.05 considered non-significant (ns). P-Values less than 0.05 (*), 0.01 (**), and 0.001 (***).

Phosphorous

Phosphorus is mobile within the plant despite being considered the most immobile nutrient in the soil. Table 5 illustrates that P concentration decreased from V8 to V12 in both the 8th leaf and whole-plant. The 8th leaf decreased from 0.35 to 0.24% P, but the decrease was more dramatic in 2021 (Table 5). The whole-plant P concentration also decreased from 0.41 to 0.25% P (Table 5). Whole-plant P trends over time were consistent across years with no major differences between N rate treatments.

At V8, 8th leaf P was 0.35% P which was lower than the 0.41% P measured in the whole-plant (Table 5). At V12,12th leaf P was slightly higher than both the 8th leaf and whole-plant status. The 8th leaf and whole-plant P concentrations were similar at V12.

Nitrogen application rate had a significant effect on both the 8th and 12th leaf at V12. In general, leaf P concentrations increased by up to 25% with N application. Phosphorus concentrations differed significantly from year to year, varying by 12% in the V8 8th leaf and 7% in the V12 8th leaf (Table 5). At V8, 8th leaf P concentrations were higher in 2021. However, at V12, 8th leaf P concentrations were higher in 2022. There was a significant interaction between year and N rate in the 8th leaf at V12 due to a positive P concentration response to N rate in 2022 but no response to N rate in 2021.

Potassium

Potassium demand peaks during the vegetative period and is mobile within the plant. From V8 to V12, K concentrations decreased in the whole-plant by about 0.6% K (Table 6). At V8, 8th leaf K decreased dramatically in 2021 from 2.16% to 1.72% but remained relatively stable during the same time period in 2022, only decreasing from 1.77 to 1.73% K (Table 6). Whole-plant K concentrations were consistently higher than individual-leaf concentrations.

Table 6. Potassium Concentration of Various Individual-Leaf and Whole-Plant Tissue Samples from V8 and V12 Growth Stages across Multiple N Application Rates and Years

	Vegetative Potassium Concentration (%)				
	V8 Growth Stage		V12 Growth Stage		
Nitrogen Rate lbs N acre-1	8th Leaf	Whole Plant	8th Leaf	12th Leaf	Whole Plant
2021	2.16	2.81	1.72	2.05	2.21
0	2.39	3.09	2.15	2.22	2.52
135	1.74	2.30	1.36	1.85	1.95
215	2.34	3.05	1.65	2.07	2.17
2022	1.77	2.35	1.73	1.79	1.87
0	1.82	2.33	1.94	2.01	2.16
135	1.66	2.15	1.56	1.66	1.72
215	1.85	2.58	1.70	1.70	1.74
Mean	1.93	2.54	1.73	1.89	2.01
Significance Test[1]					
N Rate	ns	ns	ns	ns	ns
Year	ns	ns	ns	ns	ns
N Rate*Year	ns	ns	ns	ns	ns

[1]A mixed model was run using JMP Pro 9.1 with N rate and Year as main effects, block as a random effect, and block*year as a random effect. P-Values greater than 0.05 considered non-significant (ns). P-Values less than 0.05 (*), 0.01 (**), and 0.001 (***).

Despite large year and N rate differences in treatment means shown in Table 6, significant N rate and year effects were not detected due to variable K concentration results. Still notable however, the 0 lbs. N ac^{-1} treatment had a K concentration in the 8th leaf, 12th leaf, and whole-plant. At both V8 and V12 the median N rate of 135 lbs. N ac^{-1} consistently had low mean K concentration values for all partitions across both years.

Grain yields in both 2021 and 2022 were above the Indiana state average. Grain yields responded positively to N application, increasing from 124 bu ac^{-1} without N to 234 bu ac^{-1} under 135 lbs. N ac^{-1} and 270 bu ac^{-1} under 215 lbs. N ac^{-1} (Table 7).

Table 7. Mean Grain Yield for N Rate Treatments

Grain Yield (bu. ac.-1)	N Rate (lbs. N ac-1)		
	0	135	215
2021	120	240	283
2022	127	227	257

CONCLUSIONS

The results of this study show that N, P, K, and S concentrations can vary depending upon growth stage and individual-leaf sample selection. In all nutrients measured, whole-plant and 8th leaf concentrations declined from V8 to V12. Leaf N and S concentrations exceeded whole-plant N and S by 13% and 11% at V8, respectively.

Leaf P concentrations were lower than whole-plant concentrations at V8, but were similar to or higher than whole-plant P at V12. Leaf K was always lower than whole-plant K. At V12, whole-plant N concentration was lower than both 8^{th} and 12^{th} leaf N concentration. The 8^{th} and 12^{th} leaf N concentrations were similar at V12. K and S concentrations in the V12 12^{th} leaf showed strong similarity to their respective V12 whole-plant samples, while the V12 8^{th} leaf was less similar to the V12 whole plant for K and S concentrations. For K, the 8^{th} leaf concentration was lower than the whole-plant concentration at V12, but for S the 8^{th} leaf had a higher concentration than the whole-plant at V12. P demonstrated that the 8^{th} leaf, 12^{th} leaf, and whole-plant were all similar at V12, but the 8^{th} leaf may have been slightly more similar to the whole-plant. In both years of this study grain yield was increased with higher N rates. However, plant nutrient concentration response to N rate was variable depending upon the nutrient of interest and the growth stage. At V8, the only nutrient concentration affected by N rate was nitrogen. However, by V12 the S and P concentration of some partitions were affected by N rate. Plant nutrient concentrations varied by year for N, S, and P depending on sample selection and growth stage. Significant interactions between year and N rate, likely due to varying soil nutrient availability and plant growth rates caused by annual differences in temperature and precipitation trends. When tissue sampling it is important to acknowledge that last year's nutrient concentrations may not be a perfect benchmark. The unique mobility of N, P, K, and S within the plant influenced the relationships between individual leaves and whole-plant units. Special consideration must be given to immobile nutrients such as S, which may be overrepresented if older leaves are sampled. On the other hand, sampling newer or still-developing leaves may lead to higher-than-expected concentrations of mobile nutrients such as P. Interestingly, this study found that new leaves at V12 did not have higher N concentrations than older leaves. Preliminary results indicate that individual leaf sampling may be most effective at earlier vegetative growth stages, such as V8, due to increasing disparities between leaf and whole-plant nutrient concentrations as the season progresses.

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