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# Considerations for Successful Tissue Testing in Soybeans

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Tissue testing is an effective method for quantifying crop nutrient concentrations in-season and is becoming increasingly popular in soybeans as yield goals continue to rise. To ensure accurate nutrient analysis, it is critical to minimize errors associated with both tissue sample collection and the resulting interpretation. Obtaining accurate results involves collecting the correct plant part under optimal field conditions with adequate soil moisture, while also considering nutrient mobility when interpreting the results. With these considerations, tissue testing can be a valuable tool in managing plant nutrition in combination with routine soil sampling.

## Selecting the Correct Plant Part

Nutrient concentrations in soybeans vary in different plant parts (Bender et al. 2015). For example, the leaves contain about 2% potassium (K), while the petioles have as much as 5% K in a healthy soybean plant at full flower (R2) (Slaton et al. 2021). Therefore, it is crucial to sample the correct plant part, which may vary by growth stage (Dillon and Holshouser 2019) and the laboratory (table 1). Some laboratories and universities, including Virginia Tech, recommend sampling the uppermost fully expanded trifoliate leaf throughout all growth stages, while others may recommend sampling the entire plant during vegetative growth stages. Check with the lab processing the tissue sample to determine the plant part and the amount of material needed to run the analysis for the best results.

## Uppermost Fully Expanded Trifoliate Leaf

Selecting the correct leaf to sample — defined as the uppermost fully expanded trifoliate leaf (fig. 1) — is the sampler's responsibility, though interpretation of exactly which leaf that is can vary from person to person. To identify the correct leaf, the sampler should start at the top of the plant and work their way down two to four nodes from the top. Compare the leaves at nodes two, three, and four focusing on differences in size, color, and texture. The highest leaf with a dark green, full-size leaflet and coarse texture is the optimal choice (fig. 2). Avoid leaves with a velvety texture, as they are still developing and often have higher nutrient concentrations that can give misleading results. Depending on the plant's growth stage and the maturity of the emerging leaf on the top node, the correct leaf is typically found on the second, third, or fourth node from the top. Once identified, pinch the trifoliate leaf from the plant. A composite sample typically requires the uppermost fully expanded trifoliate leaf from 18-25 plants. These leaves should then be placed in a paper bag (not plastic) to facilitate proper drying and prevent mold growth. Complete the entire sample submission form and ship to the laboratory for analysis.

**Figure 1.** Soybean trifoliate leaf without the petiole. (Image by Carrie Ortel.)

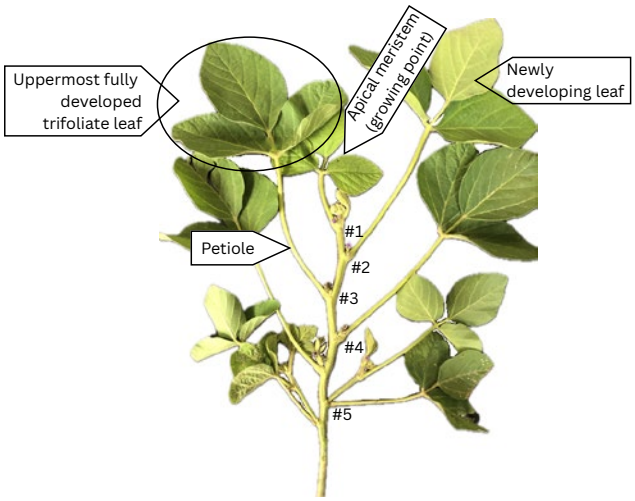


**Figure 2.** Soybean plant at the full flower (R2) growth stage. Purple flowers are visible at the top five nodes, with new developing leaves at the top of the plant. The uppermost fully expanded trifoliate leaf is at the third node from the top of the plant, identifiable by its full size and darker green color. The leaf developing on node 1 is the newest growth, while the leaf on node 2 is still developing, indicated by its smaller size, lighter green color, and velvety texture. (Image by Carrie Ortel.)

## Deciding Whether or Not to Include the Petiole

Virginia Tech recommends sampling only the leaf (without the petiole, fig. 1), while some laboratories recommend including the petiole in the sample. The petiole is the stalk that joins the leaflets to the stem (fig. 2) and acts as a storage organ for many nutrients, containing higher concentrations of most nutrients in a healthy soybean plant. Because nutrient concentrations differ significantly between the petiole and leaflets, it is important to match your sampling method with the critical concentration values for correct interpretation. Critical concentration is the threshold level that separates nutrient sufficiency from deficiency; values above this threshold indicate sufficient nutrient levels, while values below suggest deficiency and potential yield losses. Virginia Tech, along with several other universities and laboratories, determine critical concentrations based on research without the petiole, but other labs base their thresholds on testing that includes the petioles as part of the leaf sample (table 1).

**Table 1.** Commonly used laboratories in the Virginia area that analyze soybean tissue samples and their corresponding plant part.

| Laboratory or University | Growth Stage | Recommended Plant Part |
|---|---|---|
| AgroLab, Inc. | Seedling stage | All aboveground portion. |
| | Prior to or during initial flowering | First fully developed leaves from the top. |
| North Carolina Department of Agriculture | Seedlings V2-V3 | Cut the entire plant 1 inch above the soil line. Collect about 20 plants. |
| | Early growth (>V3) through bloom (R1-R2) | Collect the most recent mature trifoliate leaf (MRML) from ~20 plants. MRMLs are neither young and shiny nor old and dull and are about the third to fifth leaf down from the top. |
| Penn State | Seedling stage (less than 12”) | All aboveground portion. |
| | Prior to or during initial flowering | Two or three fully developed leaves at the top of the plant. |
| Virginia Tech* | Prior to or at initial bloom | Uppermost fully developed trifoliate leaf set (composed of three leaflets) per plant. Remove leaf stem (petiole). |
| | R1-R5 | Uppermost fully developed trifoliate leaf set (composed of three leaflets) per plant. Remove leaf stem (petiole). |
| Waters Agricultural Laboratories | Prior to flowering (V1-V6) | Fully developed leaves at the top of the plant. |
| | Flowering-Pod fill (R1-R5) | Fully developed leaves at the top of the plant. |
| Waypoint Analytical | Three to five open trifoliate leaves (V3-V5) | First mature trifoliate. |
| | First flower to early seed (R1-R5) | First mature trifoliate. |

*Virginia Tech does not accept commercial plant samples for analysis, but its recommendations for selecting soybean tissue samples, developed by Virginia Cooperative Extension specialists, are included here for comparison purposes with labs that do take outside samples for testing.

# Field Considerations

Tissue testing measures nutrient concentrations in the soybean plant but does not account for field conditions that affect nutrient uptake. For reliable results, collect samples during favorable conditions when the plant is fully transpiring and across management zones to capture in-field variability.

## Soil Moisture

Adequate soil moisture is essential to collecting a high-quality tissue sample. In extremely dry conditions, soybean plants may have plant-available nutrients available in the root zone but lack the sufficient water to facilitate nutrient uptake. When moisture returns, the soybean crop will take up these available nutrients. If tissue samples are collected during dry conditions, it may lead to a false deficiency diagnosis, as the crop may have adequate nutrients in the soil and will take them up once moisture returns. Similarly, saturated or compact soils can impact nutrient uptake and may skew the tissue test results. The use of both soil samples and tissue testing together will best describe the field situation and improve interpretation. Finally, if a foliar fertilizer application was recently made, allow ample time for the crop to utilize the treatment and for any residue to move from the leaf surface. This is typically at least one week following a rain event to facilitate uptake.

## In-Field Variability

Plant nutrient concentrations vary within fields due to differences in soil characteristics. Higher variability is expected in dryland soybean fields compared with irrigated fields. A composite tissue sample should be collected by the management zone and include at least 18 uppermost fully expanded trifoliate leaves (Ortel et al. 2023). Management zones can be defined by changes in soil texture, yield goals, or other relevant factors. To best represent a management zone, a good composite sample should be collected randomly throughout the area.

# Proactive vs. Reactive Sampling Methods

Tissue tests may be collected either proactively to monitor the crop status or reactively to address a problem. Depending on the condition of the field, different strategies may be used.

## Proactive

Proactive tissue testing in fields with no visual problem areas may be beneficial in avoiding nutrient deficiencies that often begin as “hidden hunger.” Hidden hunger refers to an existing yield-limiting nutrient deficiency with no visual symptoms that would indicate a problem. In these situations, composite samples from each management zone are compared to critical concentrations (see “Interpreting the Results,” below) rather than to other areas of the field.

## Reactive

After a problem occurs, such as marginal chlorosis which may indicate a potassium deficiency, reactive tissue tests may be used to quantify the crop nutrient status. Additionally, collecting soil and nematode samples may also be necessary to help identify the issue. The field should be divided into zones, defined as “good” and “bad” areas, with a composite sample collected from each zone. The results from these samples can be compared with one another to help aid in diagnosing the problem. It is also a good idea to include soil sample analysis and nematode assays in this strategy to consider all possible causes of the problem.

# Interpreting the Results

In addition to carefully collecting the sample to minimize errors, it is important to consider key factors when interpreting the laboratory results. Whenever available, critical concentrations should be used to interpret the results for the exact growth stage, with sufficiency ranges as a secondary option (tables 2a and 2b). Critical concentration levels are based on replicated research but are not available for all nutrients. Sufficiency ranges are based on survey data rather than replicated research and should be interpreted with careful consideration of the nutrient mobility and crop growth stage.

**Table 2a** Soybean nutrient sufficiency ranges and critical concentration levels for macronutrients.

| Macronutrients | % |
|---|---|
| Nitrogen (N) | 4.25 – 5.00 |
| Phosphorus (P) | 0.30 – 0.50 |
| Potassium (K)* | 1.97 at R1 |
| | 1.89 at 15 days after R1 |
| | 1.72 at 30 days after R1 |
| | 1.47 at 45 days after R1 |
| Calcium (Ca) | 0.50 – 1.50 |
| Magnesium (Mg) | 0.25 – 0.80 |
| Sulfur (S) | 0.25 – 0.60 |

*Critical concentration established in Arkansas (Slaton et al. 2021)

**Table 2b** Soybean nutrient sufficiency ranges and critical concentration levels in micronutrients.

| Micronutrients | PPM |
|---|---|
| Manganese (Mn) | 20 – 200 |
| Iron (Fe) | 50 – 300 |
| Boron (B) | 25 – 60 |
| Copper (Cu) | 6 – 30 |
| Zinc (Zn) | 20 – 50 |
| Molybdenum (Mo) | 0.5 |

Table 2 provides nutrient sufficiency ranges when testing the uppermost fully expanded trifoliate leaf (no petiole) for nitrogen, phosphorus, calcium, and magnesium, as well as for six micronutrients (Donohue 2023; Sabbe et al. 2000). The potassium critical concentrations vary depending on the number of days after the first flower growth stage; the levels are based on Arkansas research (Slaton et al. 2021). The sufficiency range for sulfur is based on Sabbe et al. (2000).

## Nutrient Mobility

Not all nutrients are equally mobile in a soybean plant. Nitrogen (N), phosphorus (P), magnesium (Mg), and K are mobile and can be remobilized from one plant part to another, being deposited into the developing pod and seed during the reproductive growth stages (Bender et al. 2015). This is important to remember when collecting tissue samples over multiple growth stages, particularly in the reproductive phase. A decline in leaf nutrient concentrations may not indicate deficiency, but rather the remobilization of nutrients from the leaves into the developing pods and seeds (Slaton et al. 2021). Meanwhile, calcium (Ca), sulfur (S), and many micronutrients are immobile and generally remain in the original plant part, not moving from one part to another.

## Growth Stage

Vegetative soybeans begin accumulating nutrients in the stems, leaves, and petioles (Bender et al. 2015). As flowers, pods, and seeds develop, mobile nutrients are redistributed into these organs, leaving leaves and petioles depleted (Slaton et al. 2021). However, the redistribution of mobile nutrients within the plant only signifies a yield-limiting deficiency when the crop measures below the critical concentration or sufficiency range for a nutrient.

# Conclusions

Tissue testing soybeans may be an effective approach for monitoring crop nutrient status throughout the season when done correctly. To minimize errors associated with sample collection, be sure to: 1) collect tissue samples during optimal field conditions when the plant is fully transpiring; 2) select the uppermost fully expanded trifoliate leaf of 18 or more plants, following the laboratory's recommendations for which plant parts to include in the sample (table 1); 3) walk the entire management zone to account for inherent field variability; 4) continue to use the same selected laboratory for analysis consistency; and 5) interpret the results considering the context, including soil characteristics, nutrient mobility, and growth stage.

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# Glossary

**Critical concentration** – The threshold for sufficiency of a nutrient for the specific crop at a given growth stage, based on replicated research. Concentrations at or above this threshold are sufficient; any below this threshold are deficient and could limit yields.

**Hidden hunger** – A yield-limiting nutrient deficiency that expresses no visual symptoms, complicating diagnosis.

**Petiole** – The stalk that joins a leaf to a stem.

**Sufficiency range** – The range for sufficiency of a nutrient for a specific crop at a given growth stage, based on survey data.

**Trifoliate** – A leaf with three leaflets.

**Uppermost fully expanded trifoliate** – The trifoliate near the top of the soybean plant, typically two to four nodes from the top, that is fully expanded. This can be identified by its darker color and coarser texture than immature, developing leaves.

# References


AgroLab, Inc. 2012. Tissue Sampling Instructions. https://www.agrolab.us/pdfs/AgroLab_Plant_Tissue_Sampling.pdf.

Bender, R. R., J. W. Haegele, and F. E. Below. 2015. "Nutrient Uptake, Partitioning, and Remobilization in Modern Soybean Varieties." *Agronomy Journal*, 107(2), 563–573. https://doi.org/10.2134/agronj14.0435.

Dillon, K., and D. Holshouser. 2019. Soybean Reproductive Development Stages. Virginia Cooperative Extension. SPES-156NP. https://vtechworks.lib.vt.edu/server/api/core/bitstreams/63ce5cc6-2563-4e50-b1e1-e431867d1193/content.

Donohue, S. J. 2023. Agronomy Handbook Part VIII. Soil Testing and Plant Analysis. Virginia Cooperative Extension. SPES-299P. https://www.pubs.ext.vt.edu/content/dam/pubs_ext_vt_edu/424/424-100/spes-299-H.pdf.

North Carolina Department of Agriculture and Consumer Services, Agronomic Division. n.d. Plant Tissue Sampling for Soybean. North Carolina Department of Agriculture. https://www.ncagr.gov/media/3366/download?attachment.

Ortel, C. C., T. L. Roberts, K. A. Hoegenauer, A. M. Poncet, N. A. Slaton, and W. J. Ross. 2023. "Mapping Variability of Soybean Leaf Potassium Concentrations to Develop a Sampling Protocol." *Agrosystems, Geosciences and Environment*, 6(4). https://doi.org/10.1002/agg2.20439.

Penn State College of Agriculture and Life Sciences. 2025. Instructions for Taking Samples for Plant Analysis. https://agsci.psu.edu/aasl/plant-analysis/plant-tissue-total-analysis/instructions-for-taking-samples-for-plant-analysis.

Sabbe, W. E., G. M. Lessman, and P. F. Bell. 2000. Reference Sufficiency Ranges for Plant Analysis in the Southern Region of the United States. Southern Cooperative Series Bulletin #394, 33-34.

Slaton, N. A., G. L. Drescher, R. Parvej, and T. L. Roberts. 2021. "Dynamic Critical Potassium Concentrations in Soybean Leaves and Petioles for Monitoring Potassium Nutrition." *Agronomy Journal*, 113(6), 5472–5482. https://doi.org/10.1002/agj2.20819.

Waters Agricultural Laboratories, Inc. 2025. Plant Sampling for Field Crops. https://watersag.com/service/plant-analysis/.

Waypoint Analytical. 2025. Soybean Tissue Sampling. https://waypointanalytical.com/docs/SoybeanTissueSampling.pdf.



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