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Terry M. Shaw

James A. Moore

Comparison of Foliar Nutrient Concentration Results and Protocol between Three Laboratories

INTERMOUNTAIN FOREST TREE NUTRITION COOPERATIVE (IFTNC)

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Summary

Foliar nutrient analysis results and laboratory analysis protocol were compared for three laboratories used by the IFTNC. Analysis of variance showed significant differences between laboratories for the same sample tissue. In addition, even though all three laboratories use ICP instrumentation, protocol techniques for preparing the foliage samples for analysis differ between laboratories. Various standards and internal checks are used routinely by each laboratory. It appears that the techniques used to digest and prepare the foliage samples are the main sources of variation between laboratories.

Introduction

Comparisons were performed between three independent laboratories that the IFTNC and a number of organizations frequently use in determining nutrient concentrations in conifer foliage. Our analysis was performed to assist the IFTNC and its members in selecting a laboratory for future foliar nutrient analysis. We compared results from the following laboratories: **Scotts Company**, Allentown, PA.; **Harris Laboratories**, Lincoln, NE.; and **University of Idaho Analytical Science Laboratory**, Moscow, ID. Specific objectives are to determine the variation between and within laboratories using the same conifer foliar samples sent to different laboratories and duplicates of the same samples analyzed twice by the same laboratory. The laboratories were not aware which samples were duplicates. Due to limitation in the data set not all comparisons could be explored. Therefore, determinations are based on the best statistical analysis permitted by the data and the interpretations of the IFTNC staff.

Results and Discussion

All three laboratories use a form of inductively coupled plasma emission spectrometry (ICP or ICAP) in determining macro and micro nutrient concentrations, excluding nitrogen. There are three main steps in the ICP analysis. Ground and dried samples are either ashed or chemically digested then digested with heat and chemicals' and finally analyzed using ICP spectrometry. Table 1 summarizes each laboratory's protocol for the ICP process is summarized in Table 1.

As part of each laboratory's methods and procedures, standards and duplicates are analyzed periodically to calibrate the analytical instruments and check accuracy and precision. All three laboratories use National Bureau of Standard (NBS) plant material samples to test and calibrate methods and analytical instruments. NBS plant materials are certified as to their elemental content. Types of standard materials used by the laboratories in our comparison vary. All three laboratories use a series of elemental and plant standards to ensure optimal operation, some which may be specific to various sample matrices. Our laboratories commonly use pine, peach and spinach tissue for plant material standards. In addition to NBS standards, internal duplicates are used. All three laboratories run standards and/or internal checks every 10 to 15 samples being analyzed (Table 1).

Table 1. ICP protocol procedures for the Harris, Scotts and U of I laboratories.

		Harris	Scotts	U of I
Method		Wet Ash Analysis	Dry Ash Analysis	Wet Ash Analysis
Step 1:	Temperature	60°C	500°C	Programmable heat block
	Chemical	5 ml. Concentrated nitric acid		Concentrated nitric acid
	Time	30 min.	4 hrs.	60 min.
Step 2:	Temperature	120°C	Ambient	125°C
	Chemical	30% hydrogen peroxide	5 ml. Concentrated nitric acid	
	Time	90-120 min.	60 min.	180 min.
		20% HCl for 15 min.	Dilution DI water	Dilution DI water
Step 3:	ICP	Perkin-Elmer	Thermo Jarrell	Perkin-Elmer
Sample Size		0.35 g.	0.40 g.	0.25-1.0 g.
Check Standards/Samples		Every 10-15 samples	Every 15 samples	Every 10-15 Samples

ICP is a common technique for examining foliar chemical levels and is used by all three laboratories in this comparison. In addition, all three laboratories calibrate their ICP apparatus through a series of check standards and duplication techniques. As part of the ICP preparation process, digestion of plant material is needed to achieve a medium that is suitable for spectrometry analysis. There are three main components in this process, amount and type of chemical, heating temperatures and digestion duration time. The preparation process varies between the three laboratories (Table 1). For example, Scotts protocol uses high temperatures to initially break down the plant material (otherwise known as dry ashing) followed by chemical digestion, while Harris and U of I use lower heating temperatures and a series of chemical digestions (wet ash). Furthermore, Scotts ICP instrument is a Thermo Jerell while Harris and U of I use a Perkin-Elmer ICP instrument. Even though Harris and U of I protocols are more similar

than Scotts, chemicals, temperatures and digestion duration times do differ between these two (Table 1). Moreover, Harris's laboratory protocol includes additional chemical digestion and buffers that are not present in U of I methods (Table 1).

The analysis of variance comparisons between Harris versus U of I laboratories and Harris versus Scotts laboratories, respectively are shown in Tables 2 and 3. Foliar nutrient comparisons were made using common samples for each laboratory pairing. Comparisons used for this analysis were chosen solely based on availability of IFTNC sample materials. Overall, agreement was much better between Harris and U of I compared to Harris versus Scotts laboratories for foliar nutrient concentrations (Tables 2 and 3). Analysis of variance parameters and estimates in Tables 2 and 3 generally show higher R^2 values and lower coefficients of variation (CV) for the Harris versus U of I regression compared the Harris versus Scotts results. There is stronger agreement between Harris and U of I laboratory results than there is between Harris and Scotts. However, several nutrient comparisons differed significantly for both laboratory comparisons. Calcium, iron and copper showed low R^2 values and high CV's for both laboratory comparisons (Tables 2 and 3). Since both comparisons show high variation for these nutrients and Harris laboratory was included in both comparisons, one may conclude that Harris's estimates for these nutrients are inaccurate. However, we need additional analyses beyond the extent of our data, to fully explore this conclusion.

Table 2. Slope and intercept parameters estimates and goodness of fit statistics for foliar nutrient comparisons between Harris and U of I laboratories.

Nutrient	Intercept	Slope	R^2	CV
Nitrogen	0.1306	0.9977b	0.91	5.3
Phosphorus	0.0375	1.0652b	0.81	9.0
Potassium	0.0257a	1.2286	0.90	9.9
Sulfur	0.0433	0.8952b	0.57	10.1
Calcium	0.0185a	1.0844	0.92	15.6
Manganese	22.8819	1.0101b	0.99	7.4
Magnesium	0.031	0.9131	0.85	7.5
Iron	-13.4821	1.0013b	0.91	17.0
Zinc	1.8855a	1.2074	0.92	9.6
Copper	1.3440	0.4840	0.25	28.4
Boron	0.4505a	0.9464	0.96	10.0
Aluminum	2.2795a	1.0127b	0.98	9.5

a = not significantly different than zero (p = 0.10).
b = not significantly different than one (p = 0.10).
N = 36.

Table 3. Slope and intercept parameters estimates and goodness of fit statistics for foliar nutrient comparisons between Harris and Scotts laboratories.

Nutrient	Intercept	Slope	R^2	CV
Nitrogen	0.3349	0.8114	0.57	18.2
Phosphorus	0.4036	-0.7797	0.04	52.6
Potassium	0.2115	0.8999	0.77	13.0
Calcium	0.0560	0.8535	0.66	37.7
Manganese	65.6766	0.1599	0.40	35.1
Magnesium	0.0456	0.4964	0.40	16.8
Iron	27.4664	0.1232	0.21	27.0
Zinc	18.5294	0.8140b	0.04	79.1
Copper	-0.5886	0.9036	0.93	48.2
Boron	1.3509a	0.8741	0.53	37.8
Aluminum	23.3685	0.7544	0.71	43.0

a = not significantly different than zero (p = 0.10).
b = not significantly different than one (p = 0.10).
N = 316.

Analysis of variance results and parameter estimates from Harris's foliar chemical analysis of ten duplicate samples is given in Table 4. The analysis of variance shows that

Harris was able to duplicate results within acceptable limits (CV $\leq$ 15 %) on most nutrients. Copper and iron duplicate comparisons were outside acceptable limits and showed the highest variation with CV values of 45.6 % and 16%, respectively.

Table 4. Slope and intercept parameter estimates and goodness of fit statistics for Harris laboratory duplicate comparisons.

Nutrient	Intercept	Slope	R^2	C.V.
Nitrogen	-0.1123a	1.0610b	0.97	5.2
Phosphorus	0.0245a	0.8732b	0.83	4.5
Potassium	0.0839a	0.9185b	0.95	4.8
Sulfur	-0.0072a	1.0362b	0.90	6.6
Calcium	0.0091a	0.9534b	0.99	8.0
Manganese	10.6599	0.9307	1.00	4.5
Magnesium	-0.0096a	1.0764b	0.87	6.9
Iron	-6.1865a	0.9833b	0.75	16.1
Zinc	-3.7105a	1.0897b	0.96	5.4
Copper	0.6809a	0.6809	0.19	45.6
Boron	1.2944a	0.8409b	0.81	12.2
Aluminum	-8.4796a	0.9703b	0.98	11.5

a = not significantly different than zero (p = 0.10).
b = not significantly different than one (p = 0.10).
N = 10.

Conclusions and Recommendations

Analysis of variance regression has shown that variation between laboratory results can be significantly high even though all three laboratories are reporting their results after using ICP instrumentation. Moreover, NBS standards and internal checks are used extensively by all three laboratories to insure optimal operation and reduce variation, but still differences occur within and between laboratory results. It appears that much of the variation stems from methods used in preparing the samples before ICP analysis. Digestion chemicals, heating temperatures and heating duration vary by

laboratory. It would follow that different preparation techniques would result in different nutrient extraction levels and ICP results.

The IFTNC believes that all three laboratories are reputable and present results in as accurate and precise manner as possible for the protocol techniques used. However, our analysis shows that Harris and U of I results correlate more strongly than do Harris and Scotts. Therefore, based on statistical comparisons of the laboratories and Harris's ability to replicate within laboratory samples, we recommend that Harris be used as our primary contractor for foliar analysis, with a precautionary note that Harris's copper and iron results be accepted with some level of scrutiny and skepticism.