STANDARD METHODS FOR ANALYZING TOTAL NITROGEN OF WHITE SPRUCE SEEDLING TISSUES YIELD SIGNIFICANTLY DIFFERENT RESULTS, SHOWING NEED FOR CAUTION IN USING THIS DATA FOR NURSERY SOIL MANAGEMENT

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Tissue chemical analysis is gaining interest and use as an effective diagnostic technique in forest nursery soil management practices. It frequently is used with nursery stock to help evaluate seedling quality and fertilization schedules. However it does have limitations.

For instance, the analytical techniques used, though their reproducibility is good, may not give the accurate nutrient element levels of the tissues. Different analytical techniques may give different values for the same sample. These differences are serious in that they are not consistent and are therefore not subject to simple correction; rather they are due to the complex nature of the chemical matrix encountered in plant tissue.

This article evaluates total N analyses of seedling tissues as determined by the Kjeldahl and Dumas procedures.

Materials and Methods

In 1963 a randomized complete block-designed study with four replications was established in nursery beds with white spruce *(Picea glauca* (Moench.) Voss.) seed of known origin. Each replicate contained 27 fertility treatments in the form of a 3 x 3 x3 factorial arrangement. The factors were N, P, and K fertilizers, and the levels were 0, 100, and 200 elemental pounds per acre. The fertilizers were incorporated into the 6 surface inches of seedbed soil just prior to methyl bromide fumigation and seeding. The analysis of the sandy loam nursery soil at time of treatment ranged from 4.5 to 6.0 percent organic matter, 5.6 to 6.2 pH values, 0.135 to 0.165 percent total N, 22 to 26.5 ppm. available P, and 49 to 71, 1390 to 1830, and 80 to 96 ppm. exchangeable K, Ca, and Mg. respectively[2]. Each treatment plot consisted of a 24-square-foot portion of a nursery bed. Seedling density was kept uniform among plots throughout the rotation of the white spruce crop.

In October, at the end of the third growing season in the seedbed, representative samples of seedlings from the interior of the seedbed plots were lifted out and immediately separated into "new needles" (third or current year) , "old needles" (first and second year) , "roots", "new stem" (third or current year stem and branches with bark), and "old stem" (other stem and branches with bark). Weights were determined on each tissue component following drying to constant weight at 70° C. The tissue was then ground in a Wiley mill to pass a 20-mesh sieve (Wilde *et. al.,* 1964) and subsamples were taken for Kjeldahl and Dumas total N analyses.

The macro-Kjeldahl procedure with 0.5 g. needle tissue or 1.0 g. root, and stem tissue was used with a copper sulfate, selenium metal, and sodium sulfate digestion mixture (Wilde *et. al.,* 1964). The micro-Dumas procedure with 30 mg. of tissue was used with the Coleman Model 29 analyzer. Prior to the comparative analyses, each procedure was conducted numerous times on a variety of the seedling tissues to satisfy the question of reproducibility of results. Each tissue sample was analyzed at least in

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2 Talli, A. R. 1966. Growth and nutrition of *Picea glauca* (Moench.) Voss.I seedlings. Ph.D. thesis, State University Coll. of For. at Syracuse Univ., Syracuse, New York.

duplicate by each chemical procedure.

The N analytical results were statistically evaluated: (1) By analysis of variance involving analytical procedure and tissue as factors in a 2 x 5 factorial arrangement, followed by Scheffe multiple comparison tests to identify the tissues in which there were significant differences between analytical procedures; and (2) by analyses of variance involving the N, P, and K fertilizer treatments as factors within a separate 3 x 3 x 3 factorial arrangement for each of the five tissues as analyzed by the two analytical procedures. All statistical tests were evaluated at the 95 percent probability level.

Results and Discussion

The results of the two methods of total N analyses are summarized in table 1. Statistically significant differences in the data were found by the two methods for all tissues. The Kjeldahl N data was consistently lower than that for comparable tissues analyzed for N by the Dumas procedure. Using the results of the Dumas procedure as a basis for comparison, the Kjeldahl N data ranged from 50 to 94 percent of the Dumas N data, depending on tissues involved:

New Needles		Old Needles		Roots		New Stems		Old Stems
93%	≅	94%	>	84%	>	66%	>	50%

This range in values between the two procedures resulted in altering the order of N levels in the various tissues. The Kjeldahl results indicated the N levels in the various tissues occur as:

New Needles > Old Needles > New Stems > Old Roots > Stems

The Dumas results, however, indicated a different order of N levels for the various tissues:

New Needles > New Stems > Old Needles > Old Stems > Roots

When the N, P, K fertilizer treatments were introduced to the evaluation, significant differences due to the P and K fertilizer additions were indicated for the old-stem tissue N data as obtained by the Dumas method, while no significant differences due to fertilization were indicated with the same tissue for N concentrations obtained by the Kjeldahl procedure (table 2). Thus, the results of a fertilization study may lead to conclusions about the effects of the treatments on the N levels in plant tissues that could not be corroborated by a different technique of N analysis.

.Statistically significant differences in N uptake, which would normally be attributed to fertilization, may be more dependent on the peculiarities the chemical analysis procedures than biologically important effects. The implications of these differences are particularly important to stud-

TABLE 1.—*Results of Kjeldahl and Dumas N analytical procedures on various tissues of 3–0 white spruce seedlings. Mean values are based on analytical results of 108 samples of each tissue*

Tissues[1]	Kjeldahl		Dumas	
	Mean	Range	Mean	Range
	Nitrogen Concentration			
	Percent	*Percent*	*Percent*	*Percent*
New Needles	1.57	1.43–1.74	1.69	1.54–1.83
Old Needles	1.00	0.92–1.06	1.05	0.98–1.14
Roots	0.62	0.56–0.73	0.73	0.62–0.83
New Stems	0.80	0.66–0.93	1.21	1.05–1.42
Old Stems	0.46	0.30–0.56	0.91	0.71–1.16

[1] See text for explanation of tissues.

TABLE 2.—*A comparison of the analyses of variance of N concentrations determined by the Kjeldahl and Dumas analytical procedures as affected by fertilization treatments for old stem tissue* [1]

Source	df	Kjeldahl		Dumas	
		F	Significance[2]	F	Significance[2]
N	2	0.017	N.S.	0.723	N.S.
P	2	0.331	N.S.	3.370	*
K	2	2.414	N.S.	3.887	*
NP	4	0.637	N.S.	0.708	N.S.
NK	4	0.507	N.S.	0.690	N.S.
KP	4	0.386	N.S.	1.869	N.S.
NPK	8	1.932	N.S.	1.978	N.S.

[1] See text for explanation of tissue.

[2] N.S. indicates no significant difference.

* indicates a significant difference at the 95 percent probability level.

ies of N uptake in seedlings.

Both the Kjeldahl and Dumas total N procedures are subject to sources of error (Rennie, 1965) and no attempt is made to state that one method gives a truer measure of total N than the other. The Kjeldahl procedure, a wet-combustion method, is known to possibly underestimate total N in organic matter because of the inability of this procedure to oxidize certain complex heterocyclic N compounds. Also, the procedure may result in losses of nitrate-N unless special precautions, for example, modified digestion mix, are employed (Bremner 1960, 1965).

On the other hand, the Dumas procedure, a dry-combustion method, is known to possibly overestimate total N in organic matter because incomplete combustion may result in methane gas being produced, which is not removed prior to measurement of the gas volume (Bremner, 1965). The measurement of the gas volume determines the N concentration of the dry-combusted organic matter.

These differences in N results of the two procedures appear to be greater for the more woody tissue, for example, stemwood and bark, than the more succulent tissues such as new and old needles, with root tissue being intermediate.

If forest nurserymen are utilizing tissue analysis results, which they should be doing as an aid in determining nursery soil management practices, the differences expressed above may have considerable practical implications. *Nurserymen who compare the results of N analyses of their stock with published guidelines for "adequate" or "deficient" N levels, must be certain that the analytical procedure used with their stock is comparable to the procedure used in developing the guideline levels.*

If the fertilization practices used in the nursery are based, at least in part, on the replenishment of nutrient elements removed from the soil by a crop of seedlings, it can be readily demonstrated that the analytical procedure markedly affects this estimate and would in turn possibly influence soil management practices. Table 3, based on a seedling density of 30 per square foot, shows the difference in N uptake on an area basis by the 3-0 crop of white spruce seedlings, depending upon the N analytical procedure employed. Compared to the data based on the Dumas procedure, that from the Kjeldahl analyses underestimated the total N uptake of the 3-0 seedling by approximately 20 percent.

Summary

Evaluation of data from two analytical procedures for total N of seedling tissues demonstrated:

(1) There were significant differences between the two, with the Kjeldahl procedure resulting in consistently lower values than the Dumas; (2) these differences depended on the type of tissue being analyzed, the larger differences occurring with the more woody tissue; and (3) significant differences in N uptake following fertilizer treatments de-

TABLE 3.—*Uptake of N by 3–0 white spruce seedlings as calculated from mean values of N concentration determined by the Kjeldahl and Dumas analytical procedures*

Tissue[1]	Mean Dry Weight per Seedling	Kjeldahl	Dumas
		Nitrogen Concentration	
	grams	*lbs./acre*	*lbs./acre*
New Needles	0.93	42.2	45.1
Old Needles	0.33	9.6	9.7
Roots	0.63	11.5	13.4
New Stems	0.49	11.5	17.3
Old Stems	0.57	7.7	15.4
Total	2.95	82.5	100.9

[1] See text for explanation of tissues.

pended partly on the analytical procedure used. The importance of these differences in the interpretation of the analytical data is stressed.

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