# **Extension Note**

March 2001

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## Foliar Sampling Guidelines and Nutrient Interpretative Criteria for Lodgepole Pine

## Abstract

Lodgepole pine forests in British Columbia's interior are commonly nutrient deficient, and foliar analysis is widely used for evaluating their nutrient status. However, considerable uncertainty exists among users about the appropriate methodology to use when collecting and processing foliage samples and when interpreting results. This extension note presents updated conifer foliar sampling guidelines and revised foliar interpretative criteria for diagnosing lodgepole pine stand nutrient status.

## Introduction

Foliar analysis offers a useful and convenient method for evaluating the nutrient status of forested sites. It is based on the concept that the tree, not the soil, is the best indicator of soil nutrient availability. By measuring the concentration of essential nutrients in foliage, foliar analysis reflects both soil nutrient availability and the degree to which trees require, or are capable of using, soil nutrients.

Foliar analysis information can be used to:

- diagnose possible nutritional reasons for poor quality or rate of tree growth;
- 2. identify stands that will likely

respond well to nutrient additions;

- 3. prescribe fertilizer formulations to correct inferred nutrient deficiencies and stimulate growth; and
- 4. assess post-fertilization nutrient uptake.

Because of the apparent complexity of lodgepole pine (*Pinus contorta*) nutritional problems in portions of British Columbia's interior, foliar analysis has become an important component of the stand selection process for large-scale fertilizer operations. However, considerable uncertainty exists among users about the appropriate methodology to use when collecting and processing foliage samples and when interpreting results.

A manual prepared by Ballard and Carter (1986) offered guidelines for collecting, preparing, and analyzing soil and conifer foliage samples and for interpreting analytical results. Although no longer readily available, this publication is still a primary source of information for silviculturists assigned the task of collecting foliage samples and interpreting analytical data. Carter (1992) modified the interpretative criteria for macroand micronutrients.

In recent years, extensive research has been undertaken by the British Columbia Ministry of Forests to determine the nutritional status of



lodgepole pine and to document the effectiveness of fertilization in improving stand growth (Brockley 1996). In this extension note, these research results have been used to refine the interpretative criteria contained in the earlier publications and update guidelines for the collection and handling of foliage samples.

## **Foliar Sampling Guidelines**

Nutrient concentrations in conifer foliage can be strongly influenced by factors such as crown position, foliage age, time of year, and sample handling. It is essential, therefore, to use standardized procedures when sampling and processing foliage; otherwise, a reliable comparison of measured foliar values with published interpretive criteria may not be possible. Fortunately, standardized foliar sampling guidelines have gained general acceptance in British Columbia. These guidelines, applicable to most conifer species, are summarized below.

- Collect foliage during the dormant season, preferably between October 1 and December 31.
- 2. Confine sampling to current year's foliage.
- 3. Collect foliage from between the top one-quarter and the bottom one-half of the live crown.
- 4. Confine sampling to dominant and codominant trees.
- 5. Do not collect foliage from trees with heavy cone production, or with insect or disease problems.
- 6. Do not collect foliage from trees that are situated close to unpaved roads, where foliage may be contaminated by dust.

# Foliar sampling strategy for routine diagnostic use

Reliable interpretations of foliar analytical data depend on the assumption that the data represent the forest stand (or stratum) in question. Because substantial inter-tree variation exists in foliar nutrient levels, enough samples must be collected to ensure a suitable level of precision and confidence. An appropriate sampling strategy should account for this natural variation, and be reasonably simple and efficient.

Many studies have determined the number of samples necessary to provide particular levels of precision and confidence for individual foliar nutrients and species. To evaluate the nutrient status of candidate stands for operational fertilizations, foliage sampling of 20 trees per stand (or stratum) should provide adequate levels of precision and confidence for most macro- and micronutrients in lodgepole pine foliage.

Before sampling takes place, use available information (e.g., forest cover maps, history records, aerial photos, field observations) to divide the stand into uniform strata. For stands in which tree and site characteristics are uniform throughout (e.g., same site series), draw at least two wellseparated transect lines across the stand on a large-scale map of the block. These lines should also cross significant topographic features. After excluding portions of the lines adjacent to unpaved roads (guideline 6), establish equally spaced reference points on these lines corresponding to the trees that will be sampled. Using the appropriate compass bearing, pace these distances on the ground. At each sampling location, select the nearest tree that meets the criteria in guidelines 4 and 5.

Avoid collecting samples in small patches of minor strata (e.g., rocky knoll, gully) within an otherwise uniform stand. These small patches can either be identified before sampling or simply avoided when pacing the transect lines.

Where a stand can be divided into two or more large strata, sample each stratum as if it were an individual stand. If each stratum occurs as one contiguous entity, use the previously described transect approach to identify individual trees for sampling. If a stratum occurs as two or more "islands," use one transect line in each of two (or more) of the largest islands.

## How is foliage collected?

Foliage can be collected from the ground, or by climbing up the tree to retrieve it or shooting it down. In some cases (e.g., unthinned stands or stands with relatively high postthinning densities), it may be most efficient to fell individual trees at the time of sampling. A telescoping height pole (with five 6-foot sections) can be conveniently used to collect foliage samples from trees that are less than 11 m tall.

#### How much foliage is needed?

The amount of foliage collected per tree will depend on whether foliage from each tree will be analyzed separately or composited by amalgamating with foliage from other trees. Although composite analysis does not permit assessment of withinstand nutrient variability, it will, in most cases, provide reasonable estimates of mean foliar nutrient concentrations for the stand in question. For routine diagnostic purposes, composite sampling is desirable because it requires a smaller sample size per tree and greatly reduces the number, and hence the cost, of chemical analyses.

If foliage from each tree is to be analyzed separately, clip two branchends per tree following the instructions in guideline 3. Remove the current year's terminal shoot and one or two first-order lateral shoots from each branch and place the collected foliage in a labelled plastic bag (a separate bag for each tree). The cumulative length of collected lodgepole pine shoots should generally total at least 35 cm. When preparing composite samples, clip one branch-end per tree, remove the current year's terminal shoot and one or two first-order laterals, and put the foliage from each tree in a separate, labelled plastic bag.

Keep individual foliage samples cool  $(1-5^{\circ}C)$  until compositing or drying can be undertaken. If drying must be delayed for more than 5 days, freeze the foliage samples after sample collection.

# How are composite samples prepared?

When preparing composite samples, each tree must contribute the same mass of foliage to the composite. Take each individual sample and strip the fresh foliage from the twigs; the fascicle sheaths need not be removed from the needles. For each individual sample, mix the fresh foliage thoroughly and weigh out 4 g of foliage. If 20 trees are sampled, this procedure will yield 80 g (fresh weight) of foliage per composite sample. Sufficient accuracy can be obtained using inexpensive diet scales (250-g capacity), which are available from most kitchenware stores.

## How is foliage dried?

Foliage should be dried before shipping to the laboratory for nutrient analysis. However, if prompt shipping is possible, fresh foliage samples can be sent directly to the laboratory and then dried upon arrival. These samples should be packed in ice for shipment.

Fresh foliage is generally removed from twigs before composite samples are dried. Where foliage from individual trees is to be analyzed separately, it may be more convenient to dry the shoots without first stripping the needles from the twigs. The needles are easily removed from the twigs after drying.

In preparation for drying, transfer

each individual or composite sample to a short, fully opened paper bag (e.g., lunch bag with the top cut off), and ensure that these bags are clearly labelled. Fresh foliage should not be washed unless it is badly contaminated by road dust. Rinsing can leach mobile elements from foliage and detergent can contribute to contamination.

Large numbers of individual or composite foliage samples are most conveniently dried in a forced-air (i.e., convection) oven. Dry foliage at a temperature of 70°C for 16-20 hours, or until needles snap cleanly when bent. A microwave oven may be used to dry small numbers of composite samples; however, caution must be exercised so that foliage is not scorched. Avoid high power settings and lengthy drying cycles. The purpose of the microwave drying procedure is to dry foliage to the point where it can be safely shipped. Because the foliage may not be completely dry, instruct laboratory staff to dry samples in a forced-air oven at 70°C for 6-8 hours before analysis.

The following procedure for microwave drying is recommended:

- Divide the composite sample in half and place into two separate paper bags (~40 g of fresh needles per bag); dry each sub-sample separately.
- 2. Place one bag in the microwave oven and operate at medium power for 2 minutes.
- 3. Remove from oven briefly and then repeat step 2.
- 4. Repeat this process, using 1-minute drying times, until all needles snap cleanly when bent.
- 5. Recombine the two sub-samples into one composite sample and ship dried foliage to a laboratory for nutrient analysis.

Use shorter drying cycles when smaller quantities of foliage are dried in a microwave oven.

## How is dried foliage prepared for nutrient analysis?

Dried foliage samples can be shipped directly to the laboratory, where they will be ground up in preparation for nutrient analysis. Alternatively, foliage samples that have been dried in a forced-air oven can be ground up before shipment. Grinding of foliage can be conveniently done with a small stainless steel electric coffee mill. Grinding will be easier if the needles are crushed in the paper bag before loading the coffee mill with foliage. A number of short pulses (10–15 seconds each), rather than one lengthy grinding session, will reduce the risk of motor burnout. The largest particle dimension should be no more than about 1 mm after grinding. Place each ground foliage sample into a labelled, plastic snap-cap vial for shipment to the laboratory.

# Which nutrient analyses are required?

Most laboratories offer standard foliar analytical packages to which certain specialized analyses can be added. Standard packages generally analyze samples for the concentration of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), and boron (B). Total sulphur (S) may, or may not, be included in the standard package. Inorganic sulphate-S (SO<sub>4</sub>) is a non-standard analysis that must always be specifically requested. Because interior lodgepole pine forests are commonly S deficient, total S and SO<sub>4</sub> analyses should be requested to document their status and to develop appropriate fertilizer prescriptions.

## Interpretation of Foliar Nutrient Data

Interpretation of foliar analysis data involves an assessment of the foliar concentration of individual nutrients relative to published "critical levels," in combination with an evaluation of overall foliar nutrient balance. This combined approach is based on the premise that the relative proportions of nutrients in the foliage are as important (or more so) than absolute amounts.

The critical level for a particular nutrient is generally defined as the foliar nutrient concentration below which a significant decline in growth occurs, assuming all other nutrients are adequately supplied. Published critical levels for individual species and nutrients are based on a wide range of field or greenhouse experiments. However, specific critical levels are generally poorly defined because of the variability associated with sampling, climate, stand age, and other environmental factors. Differences in inter-laboratory analytical methodology are another important source of variation. Therefore, it is generally more useful to consider broader interpretative classes of foliar concentrations, which might indicate deficiency and sufficiency ranges. A similar approach may be appropriate when reporting foliar nutrient ratios.

Foliar nutrient interpretations are subject to serious shortcomings when foliar data are reviewed without knowledge, or consideration, of stand and site characteristics. The current growth performance of the stand, environmental characteristics of the site, and other factors (e.g., insect or disease problems) affecting foliar nutrient status should always be considered when interpreting foliar nutrient data.

Foliar analysis results can also be affected by differences in analytical methodology. Consequently, it is important to ask laboratory staff to

provide specific information about the methodologies used for nutrient extraction and determination. For most nutrients, methodological differences are likely too small to affect interpretation of nutrient sufficiency or deficiency. However, for SO<sub>4</sub> (and, to a lesser extent, total S), results obtained from different analytical methods can be large enough to seriously affect interpretative reliability. In addition, the comparison of year-toyear differences in foliar nutrient levels is facilitated when the same laboratory is used for subsequent analyses.

## Revised Interpretative Criteria for Lodgepole Pine

During the past 15 years, research experiments undertaken by the B.C. Ministry of Forests have yielded valuable information on the nutritional requirements and fertilization response potential of lodgepole pine (Brockley 1996, 2000). By using this new information, it is now possible to make some adjustments to the interpretative criteria for lodgepole pine suggested by Ballard and Carter (1986) and subsequently modified by Carter (1992). Revised interpretative criteria for individual nutrients are provided in Tables 1 and 2. Interpretative criteria for foliar nutrient ratios are shown in Table 3.

The revised deficiency thresholds for S and SO<sub>4</sub> are lower than those suggested by Ballard and Carter (1986), and are largely based on results from numerous research experiments with N and N+S fertilization conducted in the province's interior. Total S was determined by dry combustion in these experiments and the hydriodic acid (HI) reductionbismuth colorimetric method was used for SO<sub>4</sub> determination. However, inter-laboratory comparisons have shown that a wet digestion procedure followed by determination with an inductively coupled plasma

Element	Interpretation	Foliar concentration (% dry weight)
Nitrogen	Severely deficient Moderately to severely deficient Slightly to moderately deficient Adequate	< 1.00 1.00–1.15 1.15–1.35 > 1.35
Phosphorus	Severely deficient Moderately to severely deficient Slightly to moderately deficient Adequate	< 0.08 0.08-0.10 0.10-0.12 > 0.12
Potassium	Severely deficient Moderately to severely deficient Slightly to moderately deficient Adequate	< 0.30 0.30-0.35 0.35-0.40 > 0.40
Calcium	Severely deficient Moderately to severely deficient Slightly to moderately deficient Adequate	< 0.06 0.06-0.08 0.08-0.10 > 0.10
Magnesium	Severely deficient Moderately to severely deficient Slightly to moderately deficient Adequate	< 0.04 0.04-0.06 0.06-0.08 > 0.08
Sulphur	Severely deficient Moderately to severely deficient Slightly to moderately deficient Adequate	< 0.06 0.06-0.08 0.08-0.10 > 0.10

table 1 Interpretation of macronutrient concentrations in current year's lodgepole pine foliage

table 2 Interpretation of foliar sulphate-sulphur and micronutrient concentrations in current year's lodgepole pine foliage

Element	Interpretation	Foliar concentration (ppm dry weight)
Sulphate-S <sup>a</sup>	Severely deficient <sup>b</sup> Moderately to severely deficient <sup>b</sup> Slightly to moderately deficient Adequate	< 40 40-60 60-80 > 80
Copper	Probable deficiency Possible deficiency No deficiency	< 1 1-3 > 3
Zinc	Probable deficiency Possible deficiency No deficiency	< 10 10–15 > 15
Iron	Probable deficiency Possible deficiency No deficiency	< 20 20–30 > 30
Manganese	Probable deficiency Possible deficiency No deficiency	< 15 15–25 > 25
Boron	Severely deficient <sup>c</sup> Probable deficiency <sup>d</sup> Possible deficiency <sup>e</sup> Likely not deficient <sup>e</sup> No deficiency	< 3 3-6 6-12 12-15 > 15

- a Interpretations for sulphate-S apply only to unfertilized foliage and not to sulphate-S analytical procedures that use an inductively coupled plasma spectrophotometer (icp).
- b Growth response following N fertilization is unlikely unless S is added in combination with N.
- c Visual symptoms of B deficiency (i.e., top dieback) likely present.
- d Sub-acute B deficiency, causing reduced height increment, likely exists in the absence of visual deficiency symptoms (i.e., top dieback).
- e A B deficiency, causing reduced height increment or top dieback, may be induced by N fertilization.

Ratio	Interpretation	Threshold value
N:P	Moderate to severe P deficiency	> 13
	Slight to moderate P deficiency	11–13
	Possible slight P deficiency <sup>a</sup>	9–11
	No P deficiency	< 9
N:K	Moderate to severe K deficiency	> 4.5
	Slight to moderate K deficiency	3.5 - 4.5
	Possible slight K deficiency <sup>a</sup>	2.5 - 3.5
	No K deficiency	< 2.5
N:Mg	Moderate to severe Mg deficiency	> 30
0	Slight to moderate Mg deficiency	20-30
	Possible slight Mg deficiency <sup>a</sup>	15-20
	No Mg deficiency	< 15
N:S <sup>b</sup>	Severe S deficiency	> 25
	Moderate to severe S deficiency	20-25
	Slight to moderate S deficiency	14-20
	No S deficiency <sup>c</sup>	< 14

table 3 Interpretation of foliar nutrient ratios in current year's lodgepole pine foliage

a Deficiency may be induced by N fertilization.

b Interpretive reliability may be affected by analytical methodology.

c S deficiency likely to be induced by N fertilization if pre-fertilization N:S > 12.

> (icp) spectrophotometer gives slightly lower (~ 5 to 10%) total S results than with dry combustion. This should be considered when using the interpretative criteria for total S in Table 1. For SO4, results with ion chromatography compare favourably with the colorimetric method. However, results obtained with ICP are generally much higher and less precise than those obtained with the other two methods. Therefore, confirm the analytical methodology with the laboratory before using the SO4 interpretative criteria in Table 2.

> The revised deficiency thresholds for P, K, and Mg are also lower than those suggested in earlier publications. Although localized deficiencies of P, K, and Mg may exist in interior lodgepole pine forests, significant growth responses following additions of these nutrients have not been documented. For these nutrients, revised deficiency thresholds are based largely on favourable responses of lodgepole pine to N (and N+S) fertilization when foliar P, K, and Mg levels are at, or near. these thresholds. In "maximum productivity" research experiments, foliar responses following repeated additions of these nutrients were also useful in setting the revised

deficiency thresholds.

The revised thresholds for B and Fe are also slightly lower than previously indicated. The adjustment for B is based on documentation of acute and sub-acute symptoms of B deficiencies in the interior and on growth and foliar responses following B fertilization. The revised deficiency threshold for Fe is based on favourable responses of lodgepole pine to N (and N+S) additions in stands with foliar Fe levels that are at, or slightly below, the revised threshold.

Foliar nutrient concentrations of Ca, Mn, and Zn in lodgepole pine foliage are almost always much higher than published threshold levels. In the absence of any new information for these nutrients, the thresholds suggested by Ballard and Carter (1986) have not been revised.

Foliar analytical data from fertilization research experiments established by the B.C. Ministry of Forests were used to develop the interpretative criteria for foliar nutrient ratios shown in Table 3. Changes in foliar nutrient balance following repeated nutrient additions in maximum productivity research experiments were particularly useful, as were published interpretative criteria from European forests. Unfortunately, the interpretative reliability of ratios may be strongly affected by differences in analytical methodology. For example, the threshold values for N:S ratios (Table 3) are based on wet digestion for N and dry combustion for total S. However, determination of N by dry combustion and total S by wet digestion followed by ICP gives higher and lower values, respectively, than the wet digestion and dry combustion methods for N and S analysis. The resulting differences in N:S ratios can be quite large.

#### References

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