# FOLIAGE SAMPLING PROTOCOLS FOR DOUGLAS-FIR IN NEW ZEALAND

**J.D. Graham and M.O. Kimberley**

**Report No. 32 February 2003**

**DOUGLAS-FIR COOPERATIVE**

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# EXECUTIVE SUMMARY

## FOLIAGE SAMPLING PROTOCOLS FOR DOUGLAS-FIR IN NEW ZEALAND

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Sampling sites were set up in four Douglas-fir stands, one in North Island, three in South Island. Regular sampling over a period of two years enabled the following recommendations to be made.

- For routine monitoring of multi-element nutritional status of Douglas-fir stands, foliage should be collected from second order branches in the well lit part of the crown during the months of June or July

- A minimum sample of 25 trees is required to achieve a 10% accuracy, 35 trees is preferable.

## Introduction

For many years the sampling protocols for radiata pine have served as the default protocols for assessing the nutritional state of Douglas-fir, i.e. second order foliage is collected in the late summer from the well-lit part of the crown. With the exception of a preliminary report by the authors (Graham & Kimberley 2001), seasonal changes in elemental concentrations within Douglas-fir foliage have not been previously documented in New Zealand.

This whole study has three parts: (a) to monitor those seasonal changes, (b) to look at genetic variation and (c) formulate practical guidelines for collecting Douglas-fir foliage.
The first report on this study looked only at parts (a) and (c) by showing trends in Douglas-fir foliar nutrient levels on a monthly basis from November 1998 to November 1999.
This report looks at a second time sequence (March to October 2000) of samples to confirm or modify the earlier observations and recommendations. Also, the issue of sample size (part (b)) is addressed through the analysis of individual trees on one occasion.

## Methods

Four sites were chosen for a 2-year cycle of monthly foliage sampling beginning in November 1998. These were situated in Kaingaroa, Dalethorpe, Dusky and Flagstaff forests. Because of some logistical difficulties it was not possible to get a useful continuous cycle of samples from Flagstaff, but what data were available was used to check out trends shown by the other three forests. Site locations are shown in Figure 1 and more specific details are given in Table 1.

**Figure 1: General location of the four sample sites**

**Table 1: Details of the four sampling sites**

| FOREST | CPT | PL YR | LATITUDE | LONGITUDE | ALT. | ASPECT | SLOPE | RAINFALL |
|---|---|---|---|---|---|---|---|---|
| | | | | | m | | Degrees | mm |
| Kaingaroa | 1066 | 1989 | 38.3824 S | 176.4985 E | 500 | SW | 5 - 10 | 1200 |
| Fletcher Challenge Forests | | | | | | | | |
| Dalethorpe | 100 | 1986 | 43.3763 S | 171.8282 E | 495 | E | 5 - 10 | 1150 |
| Selwyn Plantation Board | | | | | | | | |
| Dusky | 106 | 1989 | 45.8213 S | 169.1734 E | 490 | NE | 0 - 5 | 950 |
| Ernslaw One | | | | | | | | |
| Flagstaff | 506 | 1986 | 45.8307 S | 170.4324 E | 380 | NW | 20 - 25 | 1000 |
| City Forests | | | | | | | | |

Sampling was carried out on twenty trees at each site by local operators. All trees (aged 9 or 12 at the start, depending on site) were climbed. Primary and secondary branches from the same twenty trees were sampled on each occasion. At each time an effort was made to collect from primaries pointing in the same direction. Sampling progressed around the upper well lit part of the crown, moving upwards when all branches of a primary whorl had been used. The primary branch was cut off just behind the most recent whorl of secondaries and pieces of the two branch types were sent to the Forest Research laboratory.

At the laboratory, equal amounts of foliage were removed from each branch piece and on most occasions the 20 samples of each type were bulked. For the March 2000 sampling (Flagstaff, May 2000) trees were treated individually. Foliage was dried at 70$^{o}$C, finely ground and analysed by standard methods. Moisture correction factors were applied to convert the element concentrations to those at "100$^{o}$C oven dried".

Initially Graham & Kimberley (2001) reported on an analytical sequence covering the months of November 1998 to November 1999. The apparent stability of concentration levels from late summer through to mid winter, and some disruptions to summer 1999-2000 sampling, led to a second sequence of March-October 2000 being chosen.

Mixed model ANOVAs (Analyses of Variance) incorporating terms for year, month nested within year, and foliage type (primary versus secondary branches) was performed for each element. The sites were treated as random, blocking effects in these analyses. Least significant differences were calculated for testing for differences between sampling times and foliage types. These analyses combined information from both sequences across all the sites to improve the calculation of significance levels.

Separate ANOVAs were applied to the individual tree samples from March 2000 (Kaingaroa, Dalethorpe & Dusky) and May 2000 (Flagstaff). By quantifying the variation between trees, these were used to determine appropriate sample sizes and also provided additional information on differences between foliage types. This analysis included individual minor secondary branches (developed later in the season from individual rather than clustered buds) from Kaingaroa as well as the primary and secondary branches included in the main analysis.

**Results:** *Unless stated otherwise, the level of statistical significance is taken to be 5%.*

For the six most generally important elements nitrogen(N), phosphorus(P), potassium(K), magnesium(Mg), boron(B) and copper(Cu) the average levels over the two sampling sequences are given in Table 2. Statistical analysis of overall mean concentrations indicated significant differences between sites. These differences should be regarded as similar to block effects in a field trial, and were not considered to be of great interest in themselves for this study which was concerned primarily with variation in foliage concentrations over time at rather than in specific site differences.

**Table 2: Average nutrient concentrations in the primary ($1^o$) and secondary ($2^o$) foliage**

| | | **N** | | **P** | | **K** | | **Mg** | | **B** | | **Cu** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Site** | | % | | % | | % | | % | | ppm | | ppm | |
| | | $1^o$ | $2^o$ | $1^o$ | $2^o$ | $1^o$ | $2^o$ | $1^o$ | $2^o$ | $1^o$ | $2^o$ | $1^o$ | $2^o$ |
| Kaingaroa | Dec 98 – Nov 99 | 1.40 | 1.45 | 0.141 | 0.136 | 0.634 | 0.638 | 0.110 | 0.111 | 17 | 16 | 2.9 | 2.9 |
| | Mar – Oct 2000 | 1.39 | 1.54 | 0.109 | 0.119 | 0.607 | 0.634 | 0.090 | 0.093 | 16 | 19 | 3.5 | 3.1 |
| | | | | | | | | | | | | | |
| Dalethorpe | Dec 98 – Nov 99 | 1.78 | 1.79 | 0.172 | 0.177 | 0.720 | 0.810 | 0.137 | 0.123 | 24 | 17 | 2.4 | 2.6 |
| | Mar – Oct 2000 | 1.82 | 1.93 | 0.113 | 0.132 | 0.659 | 0.703 | 0.100 | 0.107 | 21 | 23 | 2.3 | 1.7 |
| | | | | | | | | | | | | | |
| Dusky | Dec 98 – Nov 99 | 1.51 | 1.45 | 0.211 | 0.213 | 0.826 | 0.810 | 0.127 | 0.124 | 27 | 25 | 3.4 | 3.1 |
| | Mar – Oct 2000 | 1.39 | 1.41 | 0.162 | 0.173 | 0.800 | 0.825 | 0.117 | 0.118 | 23 | 27 | 2.9 | 2.8 |

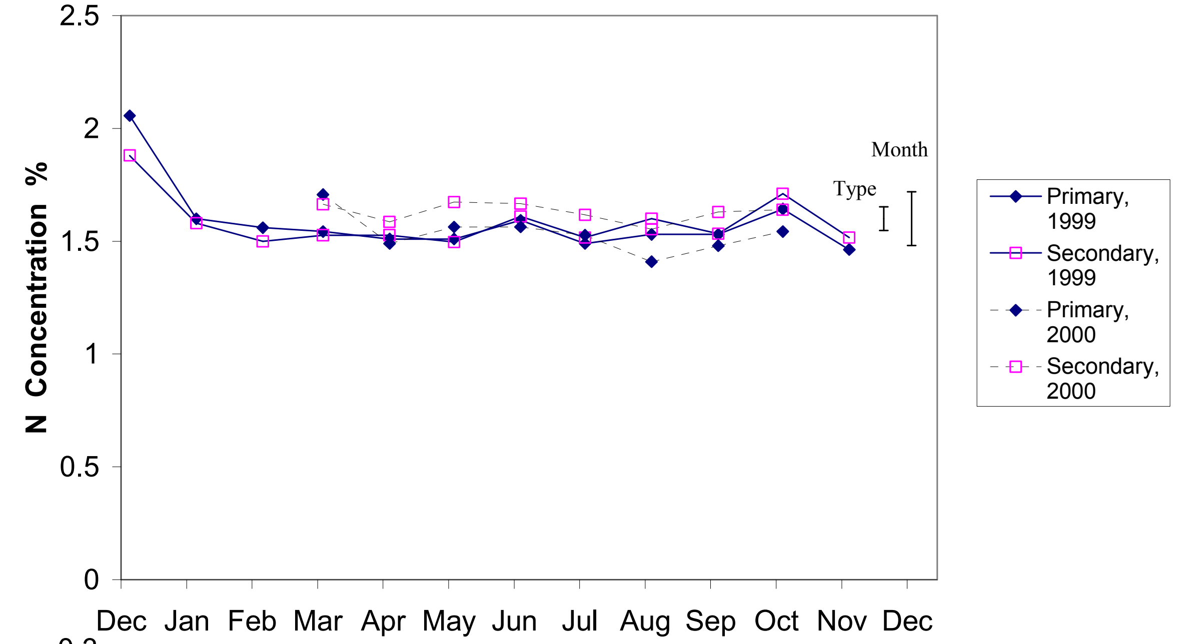
The monthly results for the two sequences are charted, by element, in Figures 2a – 2f. Primary and secondary foliage are shown separately. In general terms the month to month trends in 2000 are similar to those of 1999, the most obvious discrepancy being for magnesium in mid autumn to mid winter.

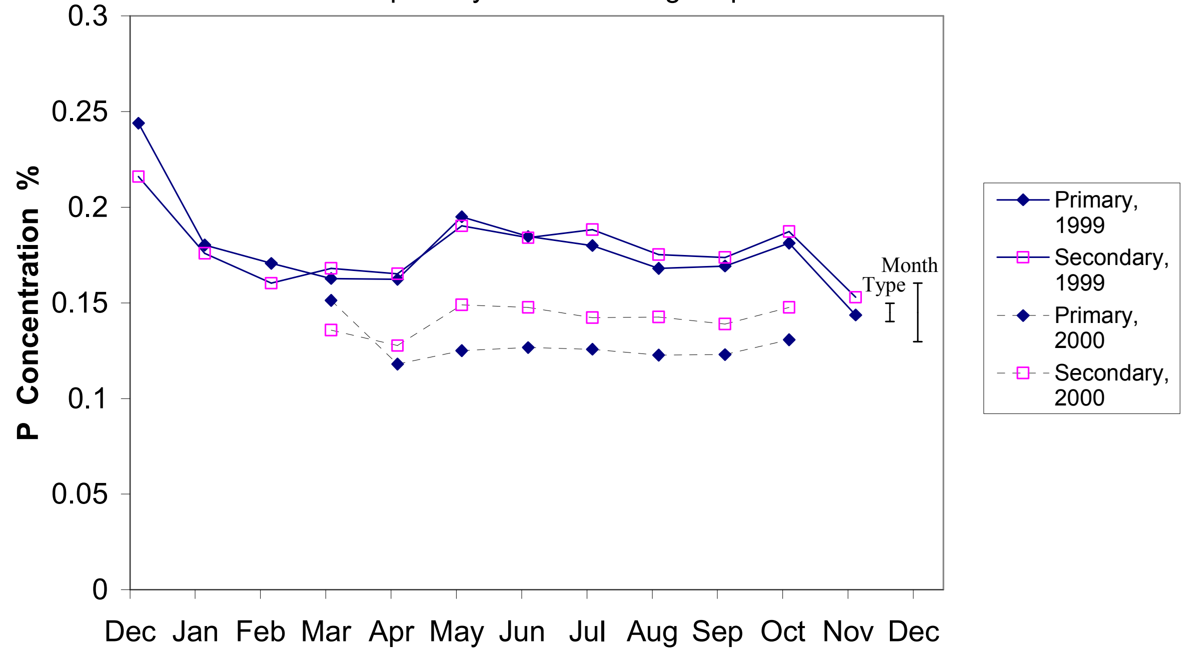
Least Significant Difference bars are shown for comparing foliage type (left hand bar) and month (right hand bar). If the difference between the two types exceeds the bar length at any one time, it is statistically significant. If the difference between two months for the same material exceeds the month bar, that difference is significant.

Detailed comparisons indicate a significant difference between primary and secondary foliar P for most of the year 2000 sequence. Differences in foliar N were almost significant during August-September 2000.

There were distinct differences between years for P, K and Mg concentrations.

**Figure 2a–c: Foliar nitrogen, phosphorus & potassium concentrations (3 sites combined)**



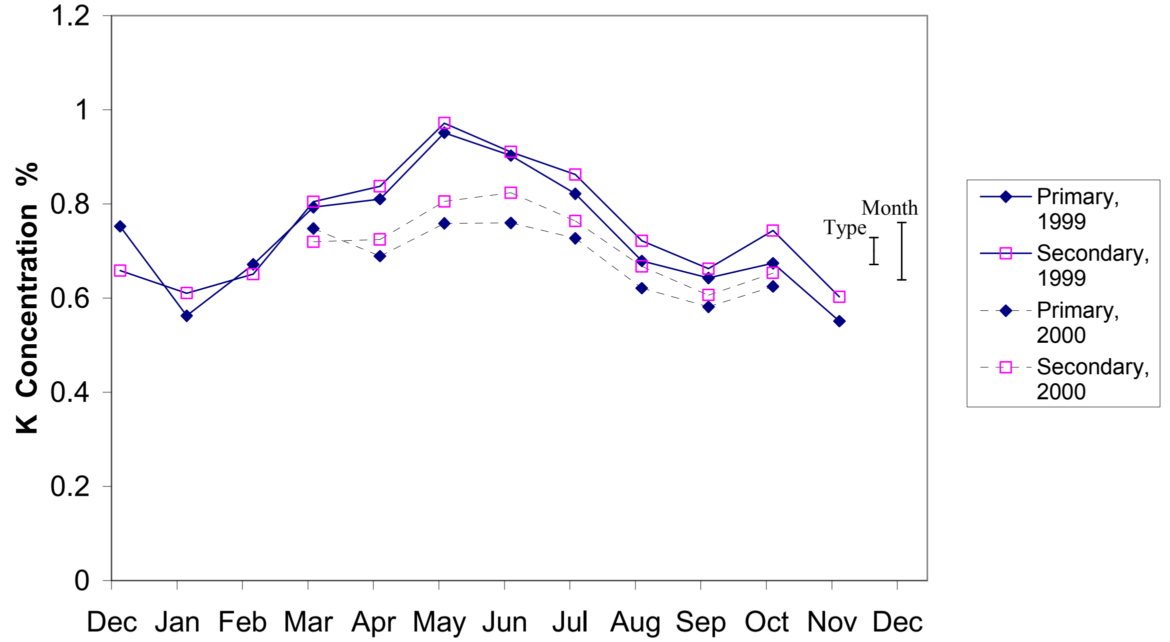
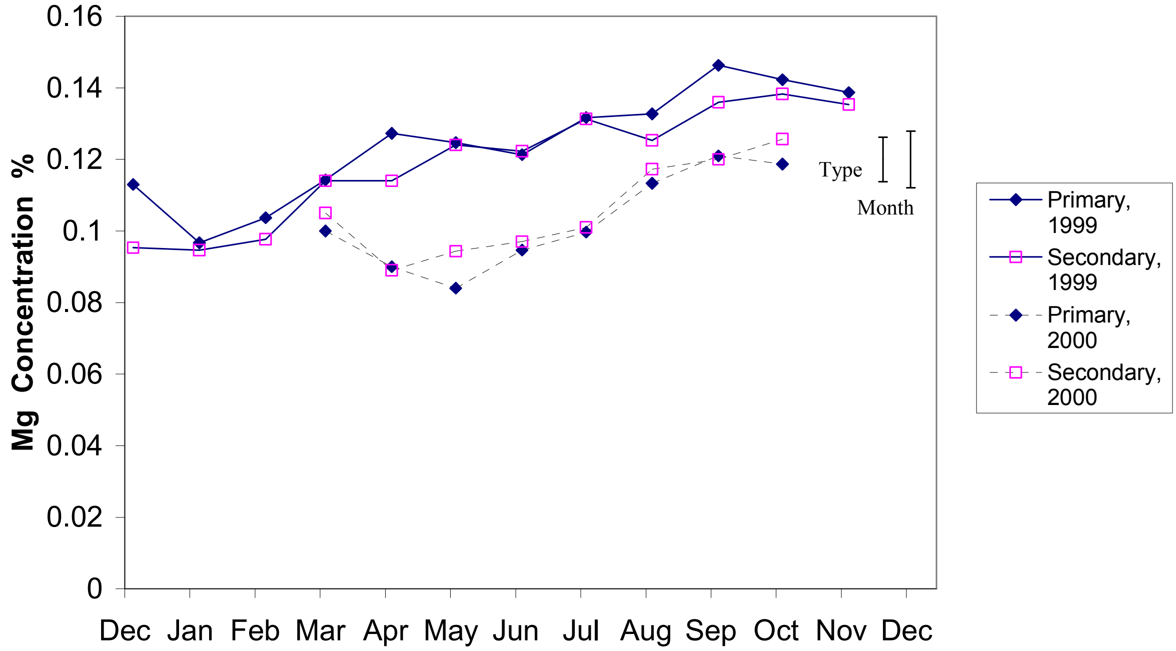
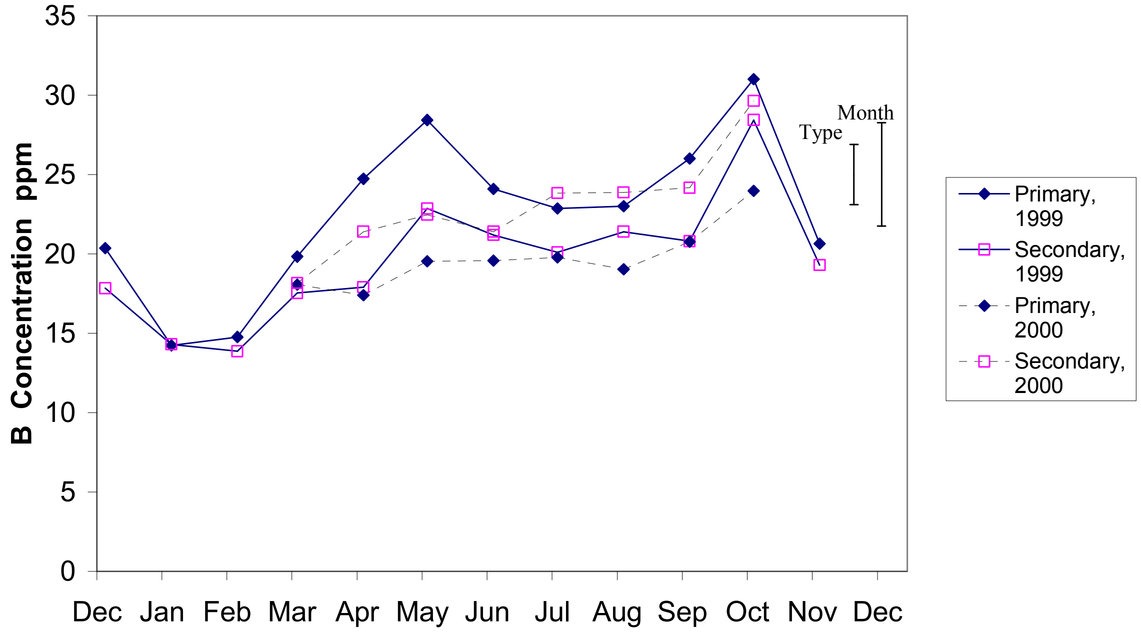
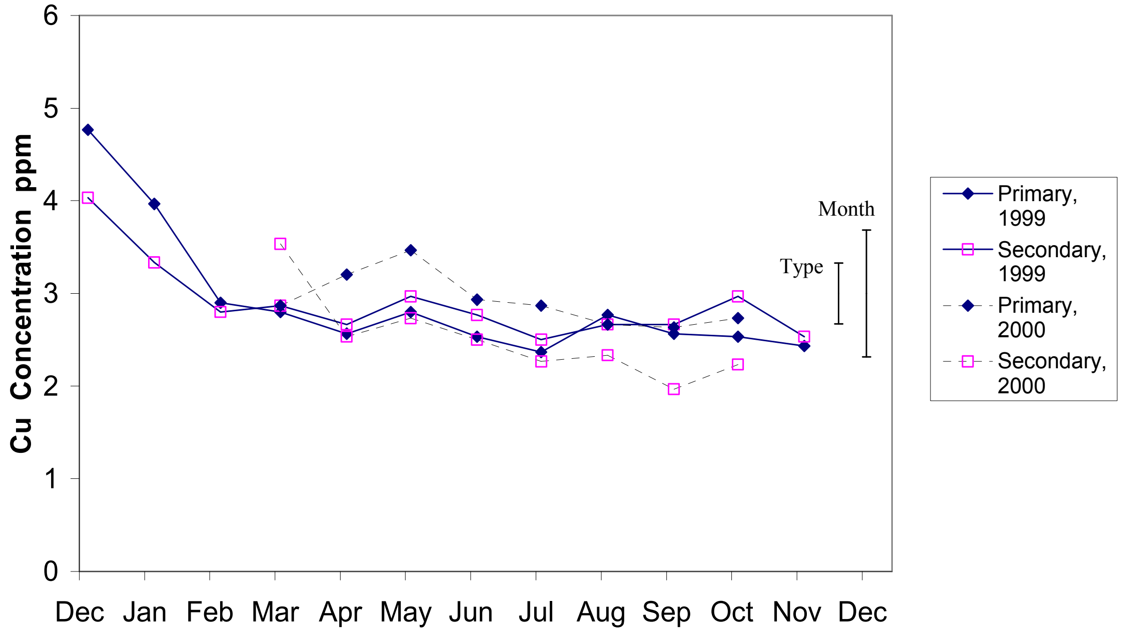



**Fig 2d–f: Foliar magnesium, boron & copper concentrations (3 sites combined)**



From chemical analysis of the individual trees, the relevant means and coefficients of variation for all four sites were calculated (Table 3). Put together they yield information on the minimum number of trees that should be sampled (in a reasonably homogenous stand) to achieve a certain accuracy expressed as a 95% confidence interval (Table 4).

**Table 3: Means and (Coefficients of Variation) from individual sample trees**

| **Forest** | **Material** | **N** | **P** | **K** | **Mg** | **B** | **Cu** |
|---|---|---|---|---|---|---|---|
| Kaingaroa | minor secondary | 1.58(9) | 0.122(18) | 0.777(19) | . | . | . |
| | Primary | 1.54(10) | 0.123(13) | 0.742(23) | 0.086(15) | 15(18) | 3.8(18) |
| | Secondary | 1.54(10) | 0.114(14) | 0.660(19) | 0.086(19) | 17(21) | 4.6(11) |
| Dalethorpe | Primary | 2.11(16) | 0.142(14) | 0.721(17) | 0.096(21) | 17(34) | 2.0(32) |
| | Secondary | 2.06(14) | 0.129(18) | 0.742(17) | 0.108(16) | 17(22) | 2.7(32) |
| Dusky | Primary | 1.47(12) | 0.190(29) | 0.781(18) | 0.117(21) | 21(27) | 2.8(19) |
| | Secondary | 1.43(11) | 0.164(28) | 0.759(20) | 0.121(19) | 21(20) | 3.3(22) |
| Flagstaff | Primary | 1.75(7) | 0.179(18) | 1.047(17) | 0.120(26) | 36(24) | 3.3(20) |
| | Secondary | 1.76(9) | 0.158(15) | 0.978(16) | 0.122(23) | 28(17) | 3.6(19) |
| Overall | | 1.69(11) | 0.147(19) | 0.801(19) | 0.107(20) | 22(23) | 3.3(22) |

**Table 4: Number of sample trees required for specified accuracy**

| | **N** | **P** | **K** | **Mg** | **B** | **Cu** |
|---|---|---|---|---|---|---|
| | | | | | | |
| Overall mean ( 80 individuals, 2 fol types ) | 1.69 | 0.147 | 0.80 | 0.107 | 21.6 | 3.3 |
| | | | | | | |
| Accuracy required, "10% of true mean" | 0.17 | 0.015 | 0.08 | 0.01 | 2 | 0.3 |
| Number of sample trees required | 5 | 13 | 14 | 18 | 25 | 22 |
| | | | | | | |
| Accuracy required, "5% of true mean" | 0.085 | 0.0075 | 0.04 | 0.005 | 1 | 0.16 |
| Number of sample trees required | 19 | 54 | 55 | 73 | 98 | 78 |

## Discussion

Our sample sites seem to meet at least the “upper-marginal” nutrient levels as presented by Reuter & Robinson (1997). Their data, summarised from a series of American field trials, are presented in Table 5 for comparison with our annual means of Table 2 and the monthly averages of Figures 2(a) – 2(c). It is important not to focus too much on differences between sites (Table 2) as these sites can just be considered random blocks within an experiment. A report by Prince (1990), looking at a small number of New Zealand field trials where N and/or P were applied, supports Reuter & Robinson’s figures. It is therefore reasonable to accept our sites as meeting recognised levels of nutrition.

**Table 5: Douglas-fir foliar nutrient concentrations (Reuter & Robinson 1997)**

| | N % | P % | K % | Mg % | B ppm | Cu ppm |
|---|---|---|---|---|---|---|
| Marginal | 1.3 | 0.10 | 0.45-0.80 | 0.08-0.10 | n.a. | 1-2.6 |
| Adequate | 1.45 | 0.15 | n.a. | 0.12 | 15-20 | 4 |

The rise and decline of different elements follows a generally similar pattern in both sampling years with the partial exception of magnesium and the high variability of boron. However, the additional information provided by the second time series indicates some changes in the periods of greatest element stability derived from the first sequence (Graham & Kimberley 2001).

Based on these results, sampling during the following months is now recommended:

Nitrogen, January-July
Phosphorus, May-August
Potassium, May-July
Magnesium, May-July
Boron, June-August
Copper, June-August

To obtain consistent results across all the elements, it is recommended that sampling should be performed during June and July.

On the whole there is less year-to-year variation between samples of secondary foliage than there is between samples of primary foliage. Therefore secondary foliage should collected.

To obtain analytical results that are within 10% of the true stand mean when expressed as a 95% confidence interval, for the six elements under study a minimum of 25 trees must be sampled. A larger sample, of 35 trees, yields an accuracy of 3.5%, 6%, 6%, 6.5%, 8%, and 7.5% for N, P, K, Mg, B and Cu respectively.

## Recommendations

For routine monitoring of multi-element nutritional status of Douglas-fir stands, foliage should be collected from second order branches in the well-lit part of the crown during the months of June or July.

A minimum sample of 25 trees is required, 35 trees is preferable.

## Acknowledgements

A study of this type would not be possible without the help of local people from Selwyn Plantation Board, Ernslaw One Ltd. and City Forests Ltd. and the co-operation of Fletcher Challenge Forests Ltd.
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Appraisal and comments on the report content were provided by Dr M Skinner (Forest Research)

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