FOLIAR CONCENTRATIONS OF TEN MINERAL NUTRIENTS IN NINE *PINUS RADIATA* CLONES DURING A 15-MONTH PERIOD

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ABSTRACT

In a 15-month study, mature-length foliage samples were collected at 2-3 week intervals from the lower branch clusters of cuttings of nine non-select **Pinus radiata** clones. The cuttings which had been planted out four years previously were growing on a uniform pumice soil site at Rotorua.

Foliage samples representative of each clone on 25 sampling dates were analysed for 10 nutrient elements. Analysis of variance of the foliar data indicated that, despite the confounding for ramets within clones and dates, the effects of date and clone respectively were in almost all instances very highly significant ($p < .001$). In order of magnitude, the fractions of total variance accounted for by clone were: .48 (B), .37 (K, Zn), .23 (Ca, Mg), .12 (Mn), .07 (Na), .09 (N), .04 (P) and .02 (Cu); those accounted for by sampling date were: .77 (Cu), .68 (N), .60 (P), .54 (Na), .41 (Ca), .37 (Mg), .29 (Mn), .22 (K, B), and .11 (Zn).

Clonal repeatabilities as at any given date varied greatly with element, being highest for B and K (.60, .46), intermediate for Zn, Ca and Mg (.39, .37 and .35), low for N and Mn (.26, .17) and very low for Na, Cu and P (.11, .06 and .05). Clone-season interaction appeared to be minor.

Tree-to-tree coefficients of variation also varied widely with element and were (in order of magnitude): .32 (Na), .30 (Mn), .26 (B), .25 (Zn), .22 (Ca), .20 (Mg), .14 (K), .12 (P, Cu), .09 (N). To detect differences ($p < 0.05$) of at least 20% between the means of two sample populations for macronutrients would evidently require sampling from at least 10 trees per composite population; for micronutrients the number generally seems higher (**c.** 15-20).

INTRODUCTION

An active programme of selection and breeding has been in progress in New Zealand with radiata pine (*Pinus radiata* D.Don) now for about a quarter of a century. The main criteria for selection have been growth vigour, frequency of branch clusters on the stem, tree form and stem straightness. Tree-to-tree variations in other characteristics such as nutrient status and accumulation, which are not normally obvious to the observer in the forest, have received much less attention. In recent years however evidence of substantial variation in the ability of different *P. radiata* genotypes to utilise nutrients efficiently has been steadily accumulating (e.g. Fielding and Brown, 1961; Burdon, 1971; 1976; Forrest and Ovington, 1971; Humphreys *et al.*, 1975). Genotype-

nutritional interactions have also been reported for various other pine species (e.g. Squillace, 1970; Zobel and Roberds, 1970; Woessner, 1972; Woessner *et al.*, 1975; Goddard *et al.*, 1976; Jahromi *et al.*, 1976a; 1976b; Roberds *et al.*, 1976).

A small experimental plantation of *P. radiata* clones located at the FRI seemed to offer, in many ways, an ideal means of exploring genetic variation in foliar nutrient concentrations. The uniform nature of the site, latin square layout and both age and condition of the trees were all highly favourable. Also, the particular clones represented have been, and continue to be, used in a variety of experiments designed primarily to study environmental effects on growth (e.g. Jackson *et al., 1973;* Jackson *et al.*, 1976; Will and Hodgkiss, 1977); as a result of these and other as yet unpublished studies, a considerable body of data now exists for these clones.

The main objective of the present study was to determine the extent to which comparable foliage samples differ in concentrations of various elements over a full growing season, so that foliar analysis could be interpreted better in relation to sampling date. At the same time it was hoped that the data obtained would supplement the limited information currently available on between-tree variation in nutrient levels in *P. radiata* foliage (*see* Mead and Will, 1976), and help to improve estimates of the appropriate sampling intensity in terms of numbers of trees per sampling unit. In addition it was intended to obtain an insight into the nutritional peculiarities of the particular non-select experimental FRI clones represented.

METHODS

Description of the Study Area

The "Long Mile" plantation occupied about 0.47 ha of level ground in the "Long Mile" area at Forest Research Institute Headquarters grounds, Whakarewarewa. The site has a well drained, uniform, sandy-loam pumice soil with unrestricted rooting depth. The records for the official climatological station (Whakarewarewa B86124) located less than a kilometre from the study site must afford a good indication of climate in the study area.

History of the Clonal Plantation

The chronology is as follows (D. S. Jackson, pers. comm.):

October 1963 Seed sown in FRI nursery (open beds). No record of seedlot, thought to be a bulk collection from Kaingaroa Forest, exists.

April 1964 Of the tallest, most vigorous plants 16 were selected as clone ortets from a bed of the 6-month-old seedlings. Material from these seedlings was grafted on to other seedling rootstocks to propagate sufficient experimental material.

April 1965 Nine of the original 16 genotypes were retained for propagation, the 9 being chosen as those providing the greatest bulk of cutting material. As many uniform cuttings as practicable were taken from the 12-month-old grafts.

July 1966 Successfully set cuttings were lined out in the nursery.

July 1967 The 27-month-old cuttings of each clone were planted out.

July 1970 The stand was green pruned to a height of about 2 m.

Layout

In the plantation the trees were spaced at 2.1 × 2.1 m. Every fourth tree in a row was a ramet of one of the nine study clones. Trees between ramets were from control-

pollinated crosses. Ramets in alternate rows were staggered so that all ramets were equidistant at 8.5 × 8.5 m intervals. Most of the ramets sampled in the study were arranged in a 9 × 9 latin square configuration. A few which lay outside the latin square, but at the same spacing, were also included for sampling to keep defoliation to a minimum. The number of ramets per clone ranged from 8 to 12.

Sampling Procedure

Foliage samples were generally collected at intervals ranging from 12-26 days and averaging 16 days. To avoid excessive defoliation a single ramet only was sampled for each clone on each collection date. The actual ramet to be sampled was randomly selected on each date. The record of individual ramets sampled is given in a more detailed account by Knight (1975).

Needles were usually collected during rainless periods, to minimise possible nutrient leaching. Occasionally, persistent wet weather made it necessary to sample foliage on some actual raindays. Sampling commenced in April 1971. During the first 11 months of the study, mature-length foliage (usually in the range 10-20 cm) which had developed during the preceding spring and summer, i.e. from the main 1970-71 flush, was collected. This was taken from first and second order branches of the lowest remaining branch clusters of each ramet. At the 20th collection (13 March 1972) a switch was made to the mature-length needles of the later season's main growth flush (1971-72). Foliage was collected from branches located on all sides of individual ramets rather than from a single directional aspect.

A conspicuous feature of clones 450 and 459 is that the older foliage of all their ramets exhibits pronounced visual symptoms of Mg deficiency each spring in contrast to other FRI clones in the stand.

To investigate this phenomenon, foliage samples were collected for chemical analysis from the two clones affected by needle chlorosis as well as from two apparently healthy clones (451 and 456) in the spring of 1975 (18 September 1975). For each clone separate samples were taken from the current season's flush on the uppermost branch cluster and from the previous season's flush on the lowermost branch cluster. Each foliage sample was a composite from two ramets.

Sample Preparation

After collection the needle samples were generally stored briefly in polythene bags in a deep freeze chest until needle measurements required for a related study had been recorded. The samples were subsequently placed in aluminium foil trays and were dried at *c.* 70° C in a forced draught oven for three days. The oven-dried samples were finely ground, to pass a 1-mm round-hole sieve, in a stainless steel Wiley mill.

Sample Analysis

Nitrogen was determined in the ammonium form after a semi-microKjeldahl digestion procedure using a selenium catalyst.

For other elements, the samples were dry-ashed at 480°C and the ash taken up in dilute hydrochloric acid. Phosphorus was determined colorimetrically as the yellow vanadomolybdate complex, and boron colorimetrically by the carmine method (Hatcher and Wilcox, 1950). Cations were determined by atomic absorption spectrophotometry.

All determinations of N, P, K, Ca, Mg, Na and Zn were made in duplicate within the same batch, and each batch of samples included a standard reference foliage sample. The samples were coded and later analysed in scrambled order. Determinations were repeated where duplicates differed from the duplicate mean by more than 6% for N, 4% for P, 8% for K and Mg, 10% for Ca, and 14% for Mn and Zn. After repeat analyses, original values were discarded. Not all of the B and Cu determinations were made in duplicate because of current workload.

Outline of Statistical Analysis

As a preliminary, polynomial regressions (i.e. quadratic models of the form $y = b_0 + b_1$ (days) $+ b_2$ (days)^2, were fitted to each of the nine clones' data for N, P, K and Ca. These four nutrients were selected as graphical examination (concentration on time) suggested the greatest chance of a satisfactory polynomial fit. The fit was restricted to data from the initial 10-month sampling period (May-February) because of the switch made in March 1972 from the 1970/71 to 1971/72 flush for foliage samples.

The purpose of this exercise was to test for parallelism of the fitted cubic equations and to see whether fitting curves with identical slope coefficients, i.e. differing only in intercept coefficients, would substantially reduce the explained sum of squares. If the individual clone regressions for a given nutrient were essentially parallel, i.e. the explained sum of squares was not substantially reduced, the inference would have been that clone-season interactions were negligible. In fact the regression fits proved so poor that this approach was abandoned.

The foliar data for each nutrient was then subjected to a two-way analysis of variance (clones and sampling dates) of the format shown in Table 1.

TABLE 1—Format of two-way analysis of variance model used to examine variation between clones (19) and sampling dates (25) for foliar concentrations of 10 mineral nutrients

Mean Squares	Format of Anova Degrees of freedom	Expectation
Clones	8	$V_E + 25\ V_C$
Dates	24	$V_E + 9\ V_D$
Error	192	V_E

V_C = component of variance due to differences between clones.

V_D = component of variance due to differences between sampling dates (includes variance due to different collectors and weather conditions).

V_E = component of residual variance which respresents, **inter alia**, a confounding of interaction of clone and sampling date, sampling variance (e.g. due to different collectors), or error within the tree, variance between ramets within clones, and analytical error.

Computations of the predicted sample size needed to detect significant differences between two means were carried out using the formula (Steyn, 1961):

$$N = \frac{2\ t^2 \times (CV)^2}{(D)^2}$$

where N = predicted number of individual tree samples needed
t = student's "t" relating to the confidence level desired and effective degrees of freedom of the original data. For 95% confidence level (two sided) a value of 1.96 was actually used
CV = coefficient of variation for individual tree samples from a stand
D = difference to be detected as a percent of the mean
2 = constant applied due to comparison of two means.

(A more precise value of t could be selected according to whether the predicted sample size (N) (see Table 5) is likely to be larger or smaller than the sample size used in the study (n).
Thus if $N > n$ the value of t should be based on N; conversely,
if $N \leqq n$ the value can be based on n.)

RESULTS AND DISCUSSION

Clonal Variation in Foliar Nutrient Concentrations

Clonal means over the whole study period (Table 2) differed significantly for all nutrients determined (Table 3). Results of the two-way analysis of variance (clones-dates) are summarised in the latter table. All F ratios for date effects exceed the 1% probability level except Zn ($p < 0.05$); similarly all F ratios for clone effects exceeded the 1% level except that for P ($p < 0.05$).

Seasonal trends for individual clones (Fig. 1) suggest that clonal differences tended to be maintained over the entire study period. From this it seems that clone-season interaction was relatively unimportant.

Clonal variance (Table 3) represented much of the total variance ($V_C + V_D + V_E$) for Zn, K and B (37-48%), rather less for Mg and Ca (23% each) and relatively little for Cu, P, N, Na and Mn (2-12%).

Clonal coefficients of variation (given by $\sqrt{V_C}/x_{av}$) are shown in Table 4. They range from 0.03 to 0.20 with N, P and Cu at the lower end of the range ($\leqq 0.05$), Na, K. Mn, Mg and Ca intermediate (0.10-0.13), and Zn and B at the upper end of the range (0.16, 0.20). These coefficients, can, for macronutrients, be compared to the between-clone coefficients reported by Burdon (1976; Table 3), the values recorded in the present study, except for Ca, lie within the ranges reported in the earlier study.

Clonal repeatabilities calculated as $V_C/(V_C + V_E)$ (Burdon, 1971), are listed in Table 4. The test of significance of differences between clones (Table 3) is automatically a test of significance for clonal repeatability too. Assuming that clone-season interaction is negligible, the repeatabilities should be applicable to any given sampling date. These repeatabilities represent estimates of broadsense heritability provided that (1) the

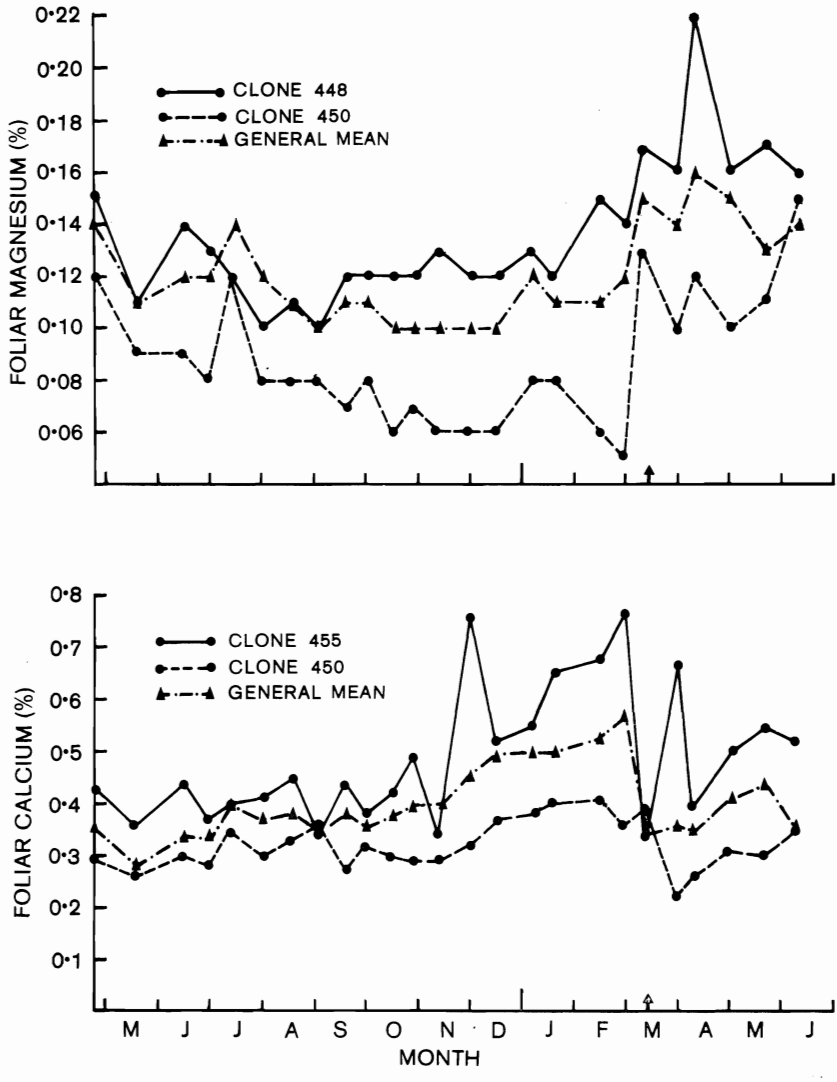
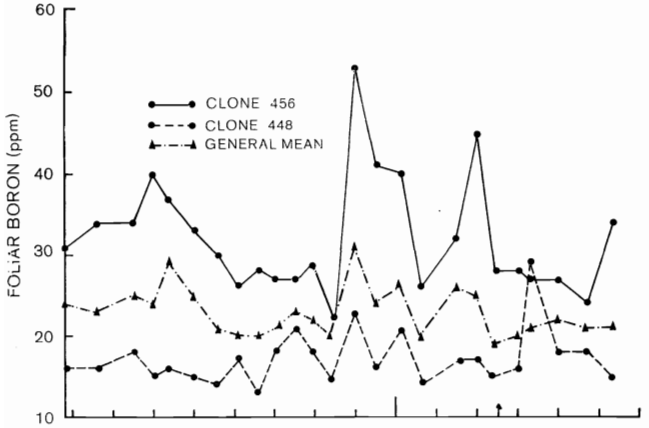
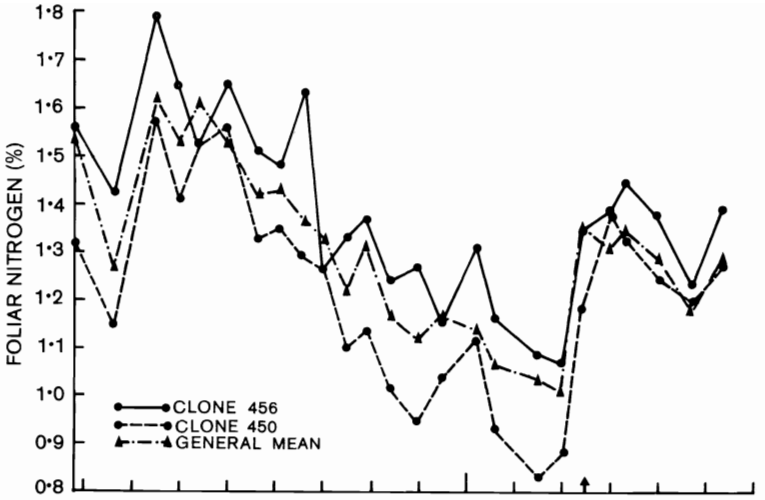
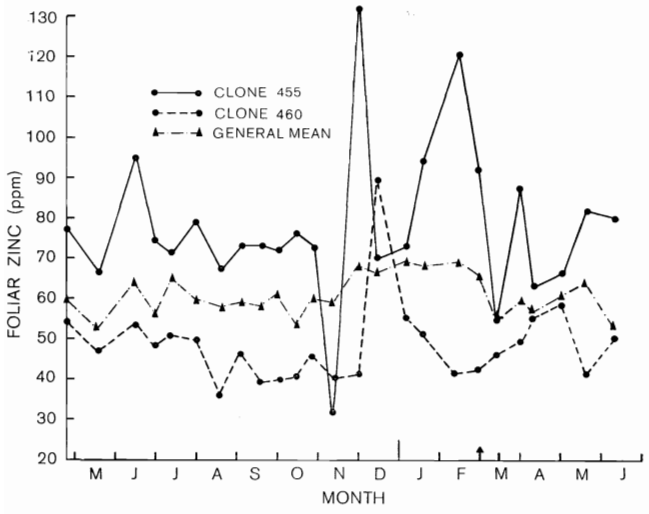


FIG. 1 (above and opposite)—Comparisons between foliar data recorded for extreme clone pairs and the nine-clone general mean. Changeover to a later flush is indicated by the vertical arrow on the time base.


FOLIAR BORON (ppm)
60
50
40
30
20
10
CLONE 456
CLONE 448
GENERAL MEAN


FOLIAR NITROGEN (%)
1·8
1·7
1·6
1·5
1·4
1·3
1·2
1·1
1·0
0·9
0·8
CLONE 456
CLONE 450
GENERAL MEAN


FOLIAR ZINC (ppm)
130
120
110
100
90
80
70
60
50
40
30
20
CLONE 455
CLONE 460
GENERAL MEAN
M J J A S O N D J F M A M J
MONTH

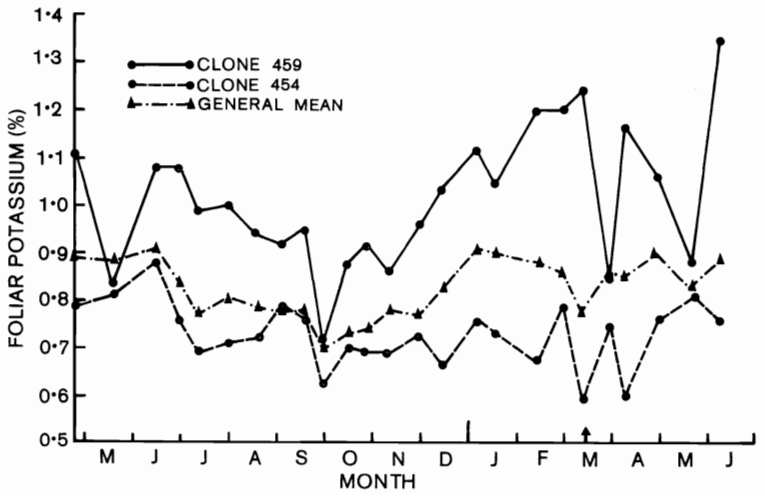

FOLIAR POTASSIUM (%)
1·4
1·3
1·2
1·1
1·0
0·9
0·8
0·7
0·6
0·5
CLONE 459
CLONE 454
GENERAL MEAN
M J J A S O N D J F M A M J
MONTH

TABLE 2—Clonal and overall means (x_{av}) of foliar concentrations recorded on all 25 collection dates

Clone	N	P	K	Ca	Mg	Na	B	Cu	Mn	Zn	Mean height	Mean d.b.h. o.b.	Mean CAI (ht)	Mean CAI (diam)
	%					ppm					cm	cm	cm	cm
448	1.25	.124	.77	.44	.14	.006	(17)	(3.7)	271	68	555 b	9.6 ab	179 ab	3.18 ab
450	(1.22)	.128	.84	(.32)	(.09)	.006	20	4.0	271	61	519 ab	8.3 a	179 ab	2.85 a
451	1.37	.126	.77	.37	.12	.006	28	3.7	319	63	623 c	10.2 b	221 bc	3.74 b
454	1.23	.121	(.73)	.45	.13	.007	21	3.8	322	59	547 b	9.3 ab	216 bc	3.21 ab
455	1.37	.132	.86	.49	.13	.006	25	4.1	361	78	630 c	9.8 ab	239 c	3.38 b
456	1.39	130	.84	.39	.12	(.006)	32	4.0	360	68	516 ab	8.2 a	215 bc	3.07 ab
457	1.26	(120)	.79	.38	.13	.008	21	3.8	(256)	51	508 ab	(7.7) a	202 abc	(2.75) a
459	1.35	131	1.02	.43	.11	.008	24	4.1	302	50	505 ab	8.7 ab	196 ab	3.13 ab
460	1.32	132	.82	.35	.12	.007	19	3.8	302	(48)	(459) a	8.2 a	(172) a	2.96 ab
x_{av}	1.31	.128	.83	.40	.12	.007	23	3.9	307	61	540	8.9	202	3.14

For growth parameters, values in vertical column with the same letters are not significantly different at the 5% probability level on the basis of the student-Newman-Keuls multiple range test (**see** Zar, 1974).

Highest values are underlined and lowest bracketed.

TABLE 3—Results of two-way analysis of variance for foliar concentrations of 10 mineral nutrients in 9 clones on 25 sampling dates

Variable (concentration in foliage)	Mean Squares			Fraction of total variance accounted for by:		Standard error of estimate[1]
	Date (df 24)	Clone (df 8)	Error (df 192)	Date	Clone	
N %	.271 61 ***	.111 83 ***	.011 13	.68	.09	.105
P %	.002 98 ***	.000 56 *	.000 23	.60	.04	.015
K %	.003 32 ***	.166 44 ***	.007 54	.22	.37	.087
Ca %	.044 93 ***	.075 43 ***	.004 79	.41	.23	.069
Mg %	.002 77 ***	.005 26 ***	.000 37	.37	.23	.019
Na %	.000 045 ***	.000 013 ***	.000 041	.54	.07	.002
B ppm	86.11 ***	551.8 ***	14.10	.22	.48	3.76
Cu ppm	6.20 ***	.556 **	.206	.77	.02	.454
Mn ppm	27.871 ***	35.612 ***	7.040	.29	.12	83.9
Zn ppm	240.0 *	2410.0 ***	140.6	.11	.37	11.85

*** P <.001 ** P <.010 * P <.050

[1] Square root of error mean square ($\sqrt{V_E}$)

TABLE 4—Mean foliar concentrations over all dates (x_{av}), clonal and non-clonal coefficients of variation, clonal repeatabilities and repeatabilities of clonal means

Foliar element	Mean overall dates (x_{av})	Coefficients of variation[1]		Clonal repeatability $V_C/(V_C + V_E)$	Repeatability of clonal means $V_C/(V_C + 0.04\ V_E)$
		Clonal	Non-clonal (within dates)		
N	1.306 %	.05	.08	.26	0.90
P	0.127 %	.03	.12	.05	0.59
K	0.826 %	.10	.10	.46	0.95
Ca	0.401 %	.13	.17	.37	0.94
Mg	0.120 %	.12	.16	.35	0.93
Na	0.0068%	.10	.30	.11	0.83
B	22.89 ppm	.20	.16	.60	0.97
Cu	3.90 ppm	.03	.12	.06	0.63
Mn	306.98 ppm	.12	.27	.17	0.83
Zn	60.62 ppm	.16	.20	.39	0.94

[1] for tree-to-tree coefficients see Table 5.

clonal differences recorded are in fact purely genetic, and (2) the clones are effectively a random sample from the population. For the study they range from 0.05 to 0.60 being relatively high for K and B (0.46, 0.60), intermediate for Zn, Ca and Mg (0.39, 0.37, 0.35), low for N and Mn (0.26, 0.17) and very low for Na, Cu and P (0.11, 0.06, 0.05). The macronutrient values accord reasonably well with those reported by Burdon (1976), especially in the strong repeatabilities for K at all four sites in the earlier study.

Repeatabilities of clonal means for foliar concentrations as at any particular date given by $V_C/(V_C + 0.04\ V_E)$ are also listed in Table 4. Apart from those for P (0.59) and Cu (0.63), repeatabilities are high and range from 0.83 to 0.97.

"Noise" Variation

Error ("noise") variance *(see* Table 3) was relatively low for Cu, N and B (20-30% of total variance), high for Zn and Mn (52-59%) and intermediate for the remainder (35-40%).

A measure of non-clonal variation is afforded by the non-clonal coefficient $\sqrt{V_E}/x_{av}$ (see Table 4). These range from 0.08 to 0.30 with values for N, P, K and Cu ($\leqq 0.12$) at the lower end of the range and values for Na and Mn at the upper ($\geqq 0.27$). The values recorded for macronutrients generally accord well with those reported by Burdon (1976; Table 3).

Interpretation of "noise" variance (V_E) is greatly helped by the apparent unimportance of clone-date interaction. Also, as coefficients of variation for chemical analysis technique for macronutrients range from about 2.0 to 2.8 (*see* Mead and Will, 1976; Table 3), analytical error, for macronutrients at least, can confidently be dismissed as only a minor component of V_E.

Consequently V_E seems to be very largely composed of sampling variance and between-ramet variation. Although no estimate of between-ramet variance is available for this study, the uniform nature of the site would make large ramet variation unlikely. Much of V_E therefore seems likely to stem from sampling error, and this would reflect variation within the tree (ramet) variance due to different collectors.

Tree-to-tree Variation in Foliar Concentrations and Implications for Sampling

The tree-to-tree (phenotypic) coefficient of variation for each element for any particular date can be estimated as $\sqrt{V_C + V_E}/x_{av}$. These coefficients are listed in Table 5 together with the average coefficients of between-tree variation for macronutrients reported by Mead and Will (1976).

The good agreement with the coefficients reported by Mead and Will (based on foliage samples collected from dominant or codominant trees on 127 New Zealand sites) indicates that the nine clones are indeed broadly representative of a normal New Zealand radiata pine population. For micronutrients, it is likely that the observed tree-to-tree coefficients of variation in foliar B, Cu, Mn and Zn are similarly representative. It is of interest that coefficients of variation in N, P, K, Cu and Mg reported by van Den Driessche (1974) for Douglas fir foliage also generally agree well with the coefficients shown in Table 5.

These tree-to-tree coefficients of variation obtained for this study allow estimates of sampling intensities needed to detect a significant difference of a given size between comparable samples of two similar-aged *P. radiata* stands, plots, or forests (Steyn's formula). For the particular sampling procedure adopted in this study, the number of trees needed per composite sample to detect differences variously $\geqq 5$, 10 or 20% with 95% confidence limits are listed by elements in Table 5. Where a difference of 20% or more will suffice, at least 20 trees per sample would apparently be needed if all 10 elements under investigation are to be determined, or 10 trees if the macronutrients (and Cu) only are of interest.

TABLE 5—Coefficients of variation (CV, %) within dates in foliar concentrations of 10 elements and estimated number of sample trees required to detect difference from the mean of 5, 10 and 20%; values in parentheses from Mead and Will (1976)

Element	CV[1]	Minimum sample size to detect[2] significant differences from mean value of:		
		≧ 5%	≧ 10%	≧ 20%
	%	number of trees[3]		
N	9.4 (8.5)	27	7 (4)	2 (1)
P	12.3 (13.8)	46	12 (11)	3 (3)
K	14.3 (15.6)	63	16 (14)	4 (4)
Ca	21.8 (24.0)	146	36 (35)	9 (9)
Mg	19.8 (20.4)	121	30 (24)	8 (6)
Na	31.5	306	76	19
B	26.1	209	52	13
Cu	12.0	44	11	3
Mn	30.0	276	69	17
Zn	25.1	194	48	12

[1] for the present study CV% = $\sqrt{V_C + V_E}/x_{av}$

[2] confidence level = 95% (two sided test)

[3] computed from the formula given by Steyn (1961) (see Methods).

Seasonal Fluctuations in Foliar Concentrations

Sampling date (Table 3) accounted for between 54 and 77% of total variance ($V_C + V_D + V_E$) for Na, P, N and Cu, between 22 and 41% for K, Mn, Mg and Ca, but only 11% for Zn. The large proportion of total variance associated with sampling date, as compared with clone for Na, P, N and Cu, underlines the particular importance of standardising time of sample collection (season) when employing diagnostic foliar techniques for these elements. By contrast season or date effect seems relatively unimportant for Zn.

Average seasonal trends for the 10 nutrients determined are shown in Fig. 2; in these graphs the general mean values for individual nutrients (i.e. at each sampling date) are expressed as percentage departures from the average overall dates for the particular nutrient concerned. The break shown in each trend in March 1972 indicates the changeover from the 1970-71 season's flush to the next season's flush. Peaks of varying magnitude were recorded for most nutrients during the winter months when growth is slowest and nutrient demand least. Concentrations of most foliar nutrients tended to be lowest when rapid spring growth was taking place. Exceptions to this generalisation were the two nutrients N and Ca. Nitrogen levels declined steadily from the winter maximum until March when the switch to the later flush was made. Calcium levels tended to increase linearly with time up to the changeover date, presumably as a consequence of the immobility of this nutrient in plant tissues. For the earlier flush samples the simple regression of foliar Ca concentration (y) on days from 1 January 1971 (x) is given by $y = .0007x + 0.209$ ($r = .889$; $p \leqq .01$). Summer maxima were recorded for K, Na, B, Mn and Zn; this may reflect the combined effects of decreased

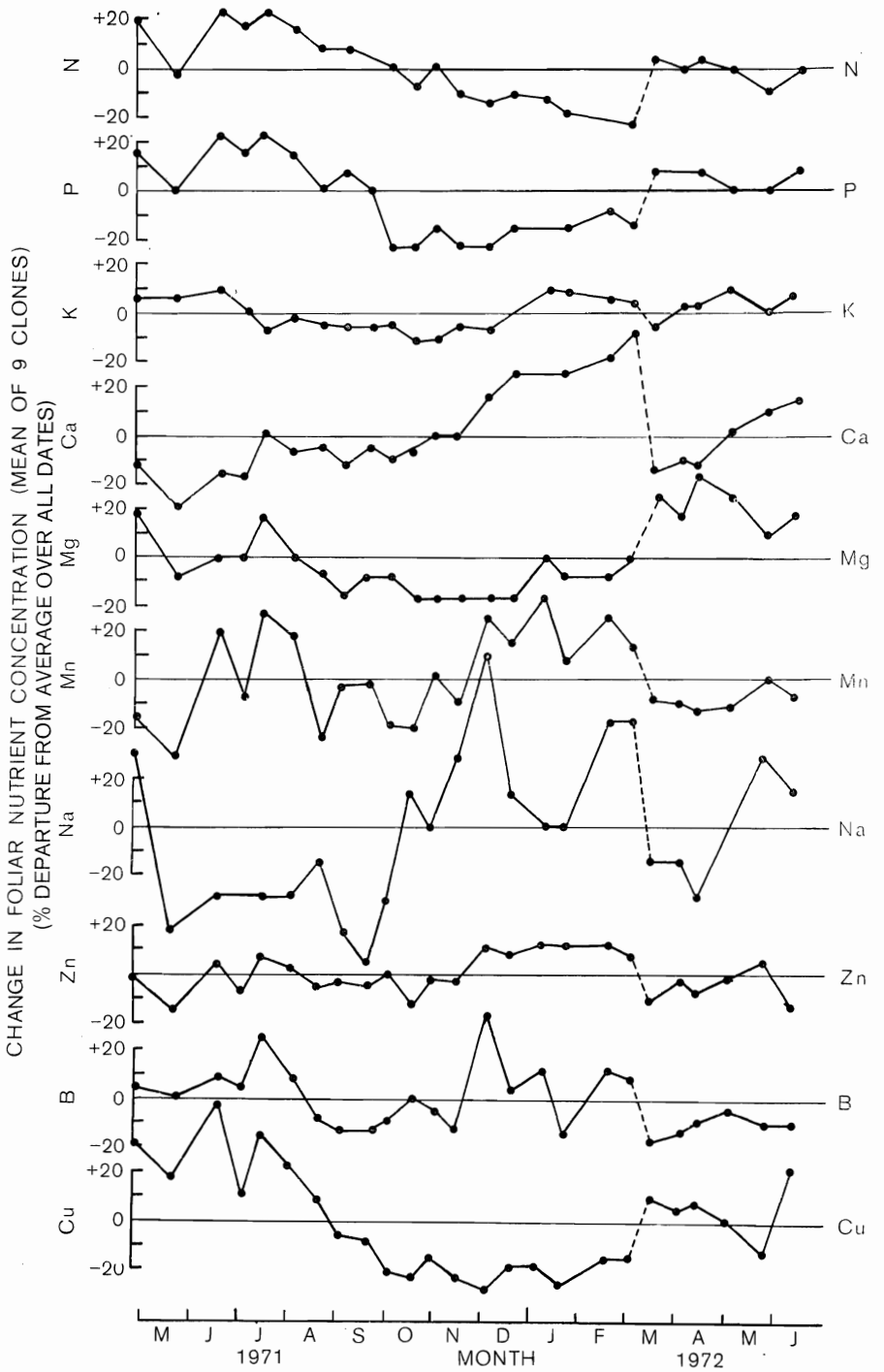


FIG. 2—Comparison of seasonal fluctuations of 10 nutrient elements in the foliage of **P. radiata**. The switch to needles of a later main flush at the 20th collection is indicated by the broken line.

demand as the growth rate slackens off, and increased supply resulting from heightened microbial and root activity during the warmer summer months. The closely parallel trends recorded for Cu and P suggest a possible synergism between these two nutrients.

Although several accounts of studies dealing with seasonal fluctuations in concentrations of nutrients in the foliage of *P. radiata* have been published (Raupach *et al.,* 1972; Duranti, 1974; Mead and Will, 1976), only that of Mead and Will relates directly to New Zeaand conditions and sites. These workers followed seasonal variations in N, P, K, Ca and Mg at 4 widely separated sites over a single 12-month period. At one site, viz. Compartment 69 in Kaingaroa Forest, one of the categories of foliage sampled corresponded fairly closely to that sampled in the present study as regards needle age and position in crown. Tree age, site and year of sampling, however, were different. Despite this, most of the trends that they reported for this category of foliage accord with those which have been described above. The pattern which they described for K, however, showed a summer minimum in contrast to the summer maximum recorded in the present study.

General Nutritional Status

The seasonal minima (Fig. 2) for N and P approached the accepted threshold of deficiency (Will, 1978), but it is not known whether this reflected seasonally limiting supplies. Average clonal concentration of N declined fairly steadily from July until the switch to foliage of a later flush in March, falling from 1.6 to 1.0%. P levels fell to the lowest level between October and February (0.10-0.11%).

Although the general clonal mean foliar Mg concentration did not fall below 0.10% during the study period, certain of the study clones (450 and 459) did in fact regularly display spring needletip chlorosis. The over-all-dates means for these two clones were lower than for all other clones (*see* Table 2). The values for foliar Mg (%) in current and previous season's flush were as follows:

Clone	451	450	459	456
current season's flush uppermost branch cluster	0.08	0.11	0.11	0.07
previous season's flush lowermost branch cluster	0.10	0.06	0.06	0.08

It was the needles with values at 0.06%, critically low according to Will (1966), that displayed needletip chlorosis. This suggests that the deficiency symptoms may arise as a result of substantial remobilisation of Mg from older foliage on lower branch clusters and movement to newer foliage, e.g. on upper branch clusters.

Year-to-Year Variation in Foliar Levels

From Fig. 1, it is evident that considerable variation in foliar levels of most nutrients can occur between the same months in different years. This accords with other findings, both in *P. radiata* (Humphries *et al.,* 1971) and other conifers (*see* Tamm, 1968; Everard, 1973 and Van den Driessche, 1974). Humphreys *et al.* examined foliage samples, collected in the same manner each winter for nine years, for N, P, K, S, Ca, Mg, Al, Na, Mn, Fe and Cl and found that except for P there was a significant difference between the values recorded in different years; the differences were mostly related to rainfall

or the number of wet days. It is evident therefore that annual variation can reduce the precision of foliar analysis as a diagnostic method (Van den Driessche, *loc. cit.*). The seasonal trends recorded in the present study and those reported by Mead and Will (*loc. cit.*) for different year and site (Kaingaroa) agree tolerably well for N and Ca but less so for other macronutrients, in particular K.

Relevance of Seasonal Trends to Recommended Sampling Period

The importance of time of year in relation to sampling has been discussed by Van den Driessche (1974). Mead and Will (1976) make specific recommendations as regards the most appropriate sampling period for individual macronutrients in young *P. radiata* foliage growing under New Zealand conditions, suggesting as a compromise late January to March for all-purpose sampling. Their recommendations are based on a single season's sampling and therefore take no account of year-to-year variations in the pattern of seasonal trends. Also, their study has shown that seasonal changes in foliar patterns do not necessarily follow the same pattern on different sites.

Because of the non-standard crown position sampled in the present study, and the strong likelihood of appreciable variation in seasonal patterns from year to year, no attempt is made to revise recommended sampling procedures from this study.

The present study confirms the observation made by Mead and Will that N concentrations were never very stable during the year. For other macronutrient the most stable periods of the year differ between the studies.

In relation to micronutrients (not reported by Mead and Will), the foliar data indicate that sampling date is least important for Zn, most important for Cu, and of intermediate importance for B and Mn. For Cu, values fluctuated least between October and February (a period of maximum stress); for Zn they fluctuated least in the December-February period (the period of least stress). Concentrations of B were most stable from late August to early October (a period of maximum stress). Concentrations of Mn never seemed to be particularly stable.

For soundly based recommendations on the most appropriate period of foliar sampling, a comprehensive study lasting several years seems necessary. This would enable the normal seasonal pattern to be established for each nutrient, and would also make it feasible to recognise the particular climatic factors which cause anomalies in a given year. In the interim, however, the compromise standard sampling procedure recommended by Mead and Will must stand.

Clonal Relationships between Growth Rates and Nutrient Concentrations

Highly significant ($p < 0.01$) differences exist between clones for height and d.b.h.o.b. as measured on 19 July 1971, as well as for current annual increment (CAI) in height (25 June 1970 to 19 July 1971) and d.b.h.o.b. (26 August 1970 to 20 July 1971) (*see* Table 2).

Clonal repeatabilities for these growth parameters range from *c.* 0.2 to 0.6, while repeatabilities of the clonal means ranged from *c.* 0.7 to 0.9. One particular clone (457) suffered high mortality; by the measurement date (19 July 1971) only 5 of the original 13 ramets had survived, and of these 2 more appeared moribund. The high mortality in this clone could well stem from an inherently poor control of water loss (Jackson *et al.*, 1973).

Sample correlations of clonal means between the four growth parameters (height, diameter, CAI in height and diameter) and foliar concentration over-all-dates are shown in Table 6. The correlations were for the most part weak and inconsistent. Exceptions

TABLE 6—Correlation matrix for selected clone growth parameters on mean[1] foliar concentrations for each of the nine clones

Dependent variable	Independent variable													
	N	P	K	Ca	Mg	Na	B	Cu	Mn	Zn	Dia	CAI (ht)	CAI (diam)
	Correlation Coefficient ("r")													
Ht	.28	—.06	—.21	.49	.30	—.14	.30	.02	.42	.75*	.87**	.73*	.81**
Dia	.25	<.01	—.20	.54	.37	.04	.10	—.19	.36	.59		.47	.91**
CAI (ht)	.50	—.01	—.04	.55	.39	—.43	.66[2]	.40	.73*	.50			.58
CAI (diam)	.54	.12	—.12	.38	.30	—.21	.41	—.13	.53	.48			

* significant at 5% level

** significant at 1% level

1 i.e. overall mean for study period based on 25 fortnightly samples collected per clone

2 significant at 10% level.

were mean height on foliar Zn ($r = 0.75$ $p < 0.05$). The repeatabilities of clonal means for foliar nutrient concentration given in Table 4 (last column) help to evaluate these correlations. The relatively low repeatabilities of means recorded for P and Cu (0.59 and 0.63 respectively) suggest that the very weak correlations for these elements would tend to underestimate the true clonal correlations.

The correlation between height and foliar Zn is interesting since (1) there is known to be an important relationship between Zn and auxin (Skoog, 1940), and (2) auxins are know to be important in the apical control of growth (Kozlowski, 1971). Clone 460, which in this study was appreciably below average as regards foliar Zn concentration (*see* Table 2) has notoriously weak leader dominance (Will, 1971; Will and Hodgkiss, 1974). However, Zn levels were consistently well above reported thresholds of deficiency (1-5 ppm; Leaf, 1973). Marcos de Lanuza (1970) has however reported that the transitional or critical range for *P. radiata* seedlings is quite wide (10-40 ppm). It is of interest therefore that three clones ranked lowest in mean height (Table 2) were well below average for Zn and on several sampling dates had foliar concentrations of 40 ppm (Knight, 1975; Table 11).

Because of the very restricted sampling in this study (which takes no account of possible clonal differences in within-crown distribution of nutrients) and the lack of biomass data, the foliar data gives no clear indication of efficiency of nutrient utilisation, except possibly for Mg, where visual symptoms of stress were seasonally apparent. Thus the question remains unanswered as to whether a clone with consistently low foliar concentrations (1) has an inherently lower requirement than other clones but is not under nutritional stress, (2) is actually less efficient and is therefore at a disadvantage relative to other clones on the same site, or (3) has the same net content of that nutrient as other clones but a different distribution pattern within the crown.

Despite the selection of larger (more vigorous) seedlings in the nursery, there is little reason to believe that the nine study clones have given an unrepresentative picture of genetic variability in regards to foliar nutrient concentrations and nutritional characteristics.

CONCLUSIONS

1. The large consistent clonal differences in foliar nutrient concentrations found to exist among nine non-select *P. radiata* clones over the study period, indicate that there is considerable genetic variation in foliar levels of specific nutrients within populations of this species.
2. The seasonal appearance of visual symptoms of magnesium stress in two clones and the pattern of Mg distribution within the crown, provide some evidence of genetic variation in the efficiency of nutrient utilisation. Thus further indications have been provided that tolerance of low soil nutrient status could be a criterion for selection in breeding programmes with *P. radiata.*
3. The limited data from three crown positions support other published reports that different within-crown distribution patterns exist among clones; consequently very restricted sampling within the crown is liable to give misleading results as regards nutrient status. Additional data and sampling details are available in an unpublished appendix available from the editor on request.

4. Realistic evaluation of nutrient status at any one site can only be made if an adequate number of trees is sampled.
5. The results of the study underscore the need for taking into account seasonal and year-to-year fluctuations for individual nutrients in any attempt to refine sampling procedure and interpretation of foliar data.

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