GROWTH AND PHYSIOLOGICAL RESPONSES OF TWO DOUGLAS FIR PROVENANCES TO NITROGEN SUPPLY

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ABSTRACT

The genotypic variation of two commonly planted *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir) provenances (Ashley strain (Seedlot 93-273) referred to as 93 and Tramway strain (i.e., Beaumont strain) (Seedlot 98-514) referred to as 98) was investigated in growth and physiological responses of the seedlings to nitrogen (N) supply levels (40 and 100 mg N/litre) in sand culture in a glasshouse. The growth of Douglas fir seedlings was greater at 100 mg applied N/litre (high nitrogen) than 40 mg applied N/litre (low nitrogen). The high nitrogen supply treatment increased seedling growth rate and caused more biomass to be allocated to the shoots. Increased uptake rate of nitrogen and other nutrients (per unit mass of roots), increased percentage allocation of the nutrients to shoots, and enhanced net photosynthesis in needles were observed with high nitrogen supply.

There were very significant provenance differences in most measured parameters and significant nitrogen × provenance interactions in some parameters. Provenance 93 showed better growth than Provenance 98 at both nitrogen levels, with a larger difference in growth between the two provenances at the low nitrogen supply. Differences in response of the two provenances to nitrogen treatment levels were related to the ratio of needles to whole-plant dry weight, nitrogen productivity, uptake rate and allocation of nitrogen and other nutrients, but unrelated to the photosynthetic rate per unit leaf area. Provenance 93 had a greater ratio of needle to whole-plant dry weight, nitrogen productivity, uptake rate of nitrogen and other nutrients, and allocated greater proportions of absorbed nutrients to shoots for photosynthesis and new growth, which sustained a greater growth rate in Provenance 93, especially in the shoot. Among the growth parameters measured in this study, the relative growth rate of seedling height (RGR-Ht), the root/shoot ratio, and the ratio of needle to whole-plant dry weight seem to be the reliable and simple indicators to discriminate the provenance difference in response to nitrogen.

Keywords: nitrogen; net photosynthetic rate; seedlings; provenance differences; nutrient uptake and distribution; *Pseudotsuga menziesii*.

INTRODUCTION

Douglas fir is native to the Pacific Northwest of the United States and British Columbia, Canada. Now it is widely grown in plantations in many other counties. In New Zealand, Douglas fir is ranked as the second most important plantation tree (M.Belton unpubl. data).

As with other plantation species, nitrogen is the key nutrient most commonly limiting the growth of stands of Douglas fir in its natural range (Gessel *et al.* 1984). Several major studies have been carried out in the Pacific Northwest of the United States and British Columbia, Canada, on the effects of nitrogen fertiliser and thinning on Douglas fir ecosystems. Most stands in these areas had a good economic response to nitrogen fertiliser, especially on poor soils (Miller & Tarrant 1983; Barclay & Brix 1984; Stegemoeller & Chappell 1990; Brix 1993; Hopmans & Chappell 1994).

Although large-scale operational application of nitrogen fertilisers to a range of Douglas fir stands is routine in the Pacific Northwest (Turner 1977; Miller *et al.* 1988; Stegemoeller & Chappell 1990; Chappell *et al.* 1991) and British Columbia (Brix 1991; Marshall 1991), information on the nitrogen nutrition of Douglas fir, including nitrogen fertiliser responses in New Zealand, is very limited and inconsistent. Neither pines nor Douglas fir in the South Island high country responded to nitrogen, phosphorus (P), sulphur (S), potassium (K), or magnesium (Mg) fertilisers (Davis *et al.* 2001). Although there was no response by Douglas fir to urea-nitrogen applied in the field at Burnt Face, Douglas fir seedlings in a greenhouse trial responded to nitrogen applied as ammonium nitrate provided that phosphorus was also applied (Belton & Davis 1986). It is suggested that immobilisation of urea-nitrogen in soil organic matter may have contributed to the failure of trees in the field trial to respond to nitrogen (Belton & Davis 1986). Mineralisation of the immobilised nitrogen may have been limited by phosphorus deficiency, as is suggested by the fact that nitrogen uptake was increased significantly by superphosphate (Belton & Davis 1986). In various parts of New Zealand *Pinus radiata* D.Don is affected by nitrogen, phosphorus, potassium, magnesium, boron, or copper deficiencies but, except for nitrogen deficiency on areas denuded of topsoil, no nutrient deficiency is reported as restricting the growth of Douglas fir stands. This is almost certainly because Douglas fir has been planted on the better, more fertile sites (Will 1978).

The inconsistent growth responses of Douglas fir plantation stands to nitrogen fertiliser seem to be linked to many abiotic and biotic factors (Heilman *et al*. 1982; Edmonds & Hsiang 1987; Brix 1991), including provenance variation. The importance of foliar and soil N:P ratios and soil C:N ratios in predicting responses to nitrogen and phosphorus has been well documented (Peterson *et al.* 1984; Mohren *et al.* 1986; Edmonds & Hsiang 1987; Gessel *et al.* 1990; Radwan *et al.* 1991). However, provenance variation in nitrogen nutrition of Douglas fir has not been properly addressed (van den Driessche & EL-Kassaby 1990/91). A study of the response of Douglas fir provenances to nitrogen supply should provide an insight into the physiological basis of genotypic variation in nitrogen nutrition and also useful information for the management of Douglas fir nitrogen nutrition, especially in New Zealand.

In New Zealand, there are three recommended seed sources of Douglas fir (Miller & Knowles 1994). These are the Californian provenance, the Ashley provenance, and the

Beaumont provenance. The Californian provenance is characterised by fast growth rate, especially at low elevations or on relatively sheltered sites. The Ashley provenance, whose ancestry is believed to be coastal Oregon, has proved vigorous and well adapted over a wide range of sites in New Zealand. The Beaumont provenance, primarily of Washington ancestry, is recommended as a suitable provenance for exposed, higher elevation sites in southern areas (Miller & Knowles 1994). The primary objective of this study was to determine if genotypic differences existed in growth and physiological responses of seedlings of two Douglas fir provenances (i.e., Ashley and Beaumont), commonly planted in New Zealand, to nitrogen supply in sand culture in a glasshouse.

MATERIALS AND METHODS

Treatments and Growth Conditions

This experiment was a factorial combination of two provenances × two nitrogen concentrations, with seven replicates per treatment. The concentrations of nitrogen applied as ammonium nitrate (NH_4NO_3) were 40 and 100 mg N/litre, representing a limiting (referred to as low nitrogen) and an optimum (referred to as high nitrogen) nitrogen supply, based on the work of Ingestad (1971). The ratio of NO_3^--N to NH_4^+-N in the treatment solutions was 40:60. Douglas fir provenances were Ashley strain (Seedlot 93-273) referred to as 93 and Tramway strain (i.e., Beaumont strain) (Seedlot 98-514) referred to as 98, which were commonly planted in the South Island of New Zealand.

Uniform 1-year-old bare-root seedlings (*ca* 11–14 cm in height) were selected from a commercial nursery, which was not inoculated with forest soil/duff or mycorrhizas. The selected seedlings were washed clean of soil and planted in PVC pots (12 cm in diameter and 30 cm in height), each of which was filled with 3.80 kg of well-washed fine river sand packed to 1.3 g/cm^3 dry bulk density. The bases of the pots were drilled with seven equally distributed holes (7 mm diameter) to facilitate free drainage. The pots were arranged randomly in a glasshouse where the temperature ranged from 15 to 25°C (daytime), and the photosynthetic photon flux density (PPFD) from 500 to 850 μmol/m^2·s. There was a 16-h/8-h day/night cycle and the pots were re-randomised every 2 weeks.

The seedlings were irrigated with deionised water for the first 2 weeks, with quarter strength nutrient solution in the third week, with half strength in the fourth week, and with full strength plus nitrogen treatments from the fifth week. The full strength nutrient solution was modified from that of Ingestad (1971) without nitrogen and contained 14 mg P/litre, 65 mg K/litre, 7 mg Ca/litre, 8.5 mg Mg/litre, 10 mg S/litre, 0.7 mg chelated Fe/litre, 0.4 mg Mn/litre, 0.03 mg Zn/litre, 0.03 mg Cu/litre, 0.007 mg Mo/litre, 0.2 mg B/litre, 0.03 mg Cl/litre, 0.003 mg Na/litre, and 0.006 mg Ni/litre. The pH of the nutrient solution was adjusted to pH 4.9–5.0, and 500 ml of solution were added on each irrigation occasion as this was sufficient to ensure that all prior nutrients in the soil solution were eluted. Irrigation with solutions was initially weekly but later twice per week. If necessary, just sufficient volumes of deionised water were added at intervals between the solution irrigations to bring the sand to field capacity (i.e., just at the point of drainage commencing).

After 6 months, the seedlings were harvested and separated into different parts, which were dried at 70°C for 72 h in an oven, weighed, and ground for nutrient analysis.

Measurement and Analytical Methods

Seedling height and basal diameter were measured periodically, and the time when each flush of new needles and shoot growth occurred was recorded. The relative growth rate (RGR) of seedling height or basal diameter was calculated as follows (van den Driessche 1992):

$$RGR = [\ \ln (h_f) - \ln (h_i)\ ] / (t_f - t_i)$$

where h_i is the initial height (or diameter) at time (t_i) and h_f is final height (or diameter) at time (t_f). The stem volume was calculated from the following equation (van den Driessche 1992):

$$\text{Stem volume} = (\pi D^2/4)h/3$$

where h is seedling height, D is basal diameter, and 1/3 is a calibration factor for stem volume of the seedlings.

After harvest, the number of first-order branches (emanating from the main stem) was counted on three positions on the stem. The lower position was the stem length before the first flush, the middle position the stem length between the first and second flushes, and the upper position the stem length between the second and third flushes. Second-order branches were present only on first-order branches at the lower position of the main stem.

During the experiment, the net photosynthetic rate of needles for each of the three flushes was measured at 20°C and a light saturated PPFD of 1000 μmol/m^2·s with a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, Neb., USA). The surface area of the needles was measured by using a portable LI-300 area meter (Li-Cor, Lincoln, Neb., USA). Two days before harvest, chlorophyll fluorescence was measured on needles that had a 20-min dark adaptation, using a portable chlorophyll fluorometer (PEA Plant Efficiency Analyser, Hansatech Instrument, Kings Lynn, UK). In all the treatments, four fully expanded needles were selected from each of first (6 months old), second (4 months old), and third (0.5 month old) flush needles located on the same (first-order) branch at a lower position on each seedling. Maximum and minimum chlorophyll *a* fluorescence emissions (F_m and F_0) of the needles were measured. Quantum yield of PSII (photosystem II), the amount of electron flow per unit energy absorbed by the photosynthetic apparatus, was estimated as directly proportional to $(F_m–F_0)/F_m$ (Genty *et al.* 1989).

The plant samples after harvest were analysed for phosphorus, potassium, calcium, magnesium, boron, manganese, zinc, iron, copper, and aluminium by ICP spectrophotometry (Zarcinas 1980) and for nitrogen by LECO CNS-2000. Uptake rates of nutrients (U, μmol per gram of dry weight of root per day) were calculated as follows (Ingestad & Ågren 1988):

$$U = \frac{C_i}{M}\,(e^{RGR} - 1)\,\frac{DW_T}{DW_{Rt}}$$

where C_i is the concentration of the nutrient (μg/g) in the seedling, M the molecular weight of the nutrient, e the base of natural logarithm, DW_T and DW_{Rt} the dry weight (g) of the whole seedling and roots, and RGR the mean relative growth rate, which was calculated by:

$$RGR = \frac{\ln DW_T - \ln DW_0}{\Delta T}$$

where DW_0 is mean initial dry weight (g) of a random selection of 10 seedlings before treatment, and DW_T is mean seedling dry weight at the end of the experiment. Both were

estimated by destructively sampling the seedlings. ΔT is the duration of the experiment in days. Nitrogen productivity (*NP*, g/mol·day), a useful measure of the efficiency of nitrogen use for producing new biomass, was calculated as follows (Ingestad 1979):

$$NP = RGR/PNC$$

where *RGR* is the mean relative growth rate (g/g·day), and *PNC* the plant nitrogen concentration (mol/g).

Statistical Analysis

Analyses of variance were conducted to determine the effects of nitrogen, provenance, and their interactions on plant growth and physiology using the SAS software package. Duncan's multiple range test was used at the 95% probability limit ($p < 0.05$) to assess the differences when the interaction of provenance and nitrogen was significant.

RESULTS

Seedling Growth

Nitrogen and provenance main effects were significant ($p < 0.001$) for all the growth parameters (seedling height, height increment, basal diameter, relative growth rate of height, root/shoot ratio, ratio of needle to whole plant, branch number, and dry weight of shoot and root), which were greater (except the root/shoot ratio which was less) in Provenance 93 than 98 and at 100 mg N/litre than 40 (Table 1, Fig. 1 and 2). The nitrogen × provenance interactions were significant for relative growth rate of height (RGR-Ht) ($p < 0.05$), stem volume ($p < 0.05$), root/shoot ratio ($p < 0.01$), ratio of needle to whole plant ($p < 0.01$), and the number of first-order branches on the upper position of the stem ($p < 0.05$). Provenance 93 had a greater RGR-Ht than Provenance 98 at the low level of nitrogen (40 mg N/litre), but there was no difference between provenances at the higher level of 100 mg N/litre (Fig. 1). The stem volumes at 40 mg N/litre averaged 43% and 34% of the stem volumes at 100 mg N/litre in Provenances 93 and 98, respectively (Fig. 1). Although no significant differences were found between provenances at 100 mg N/litre, Provenance 98 had a significantly greater root/shoot ratio and a significantly lower ratio of needle to whole-plant (in dry weight) at 40 mg N/litre (Fig. 1). The numbers of first-order branches on the upper position of the stem were much fewer in Provenance 98 than 93 at 40 mg N/litre — they were equivalent to 30% and 63% of those at 100 mg N/litre (Fig. 2).

TABLE 1–Nitrogen and provenance effect on growth parameters of Douglas fir seedlings at the time of harvest. Values in the same column followed by different letters are significantly different.

Treatment	Seedling height (mm)	Basal diameter (mm)	Height increment (mm^3)	Dry weight (g)	
				Shoot	Root
Nitrogen main effect					
40	493 b	8.3 b	355 b	19.5 b	8.6 b
100	711 a	11.1 a	576 a	43.1 a	10.6 a
Provenance main effect					
93	656 a	10.2 a	528 a	35.0 a	10.2 a
98	554 b	9.3 b	411 b	27.7 b	9.1 b

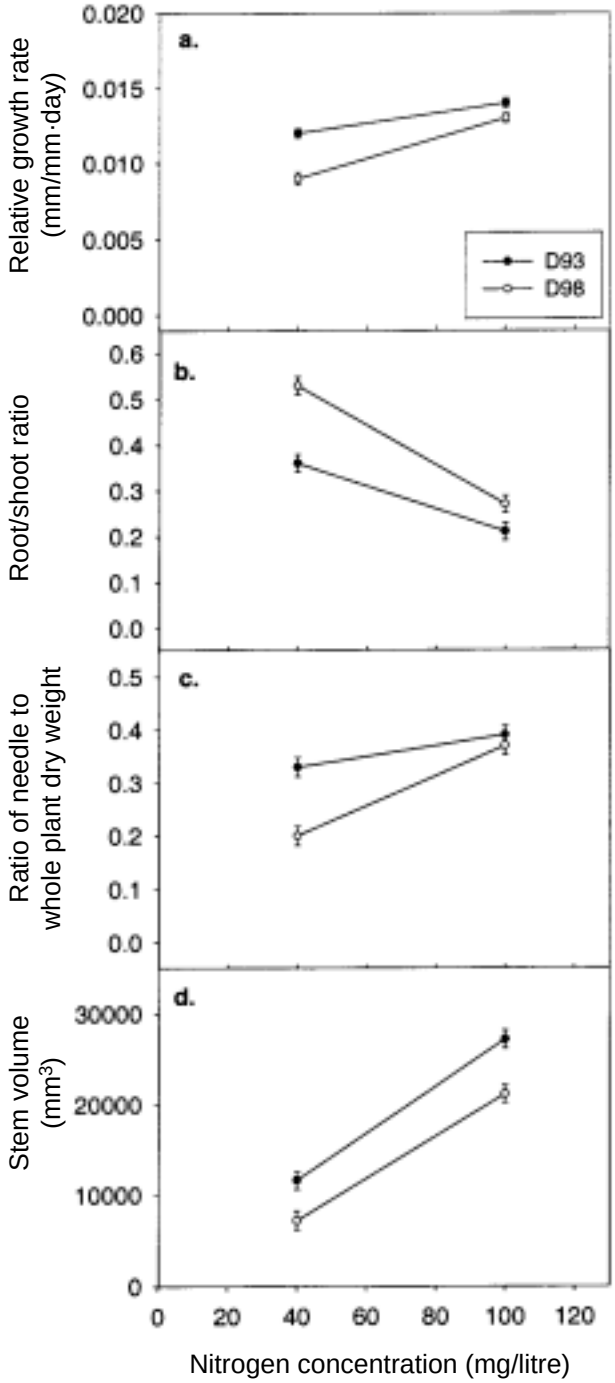

FIG. 1– Effect of nitrogen concentrations (40 and 100 mg N/litre) on (a) relative growth rate, (b) root/shoot ratio, (c) ratio of needle to whole-plant dry weight, and (d) stem volume of two Douglas fir provenances (D93 and D98) at the time of harvest. Error bars represent 1 S.E.

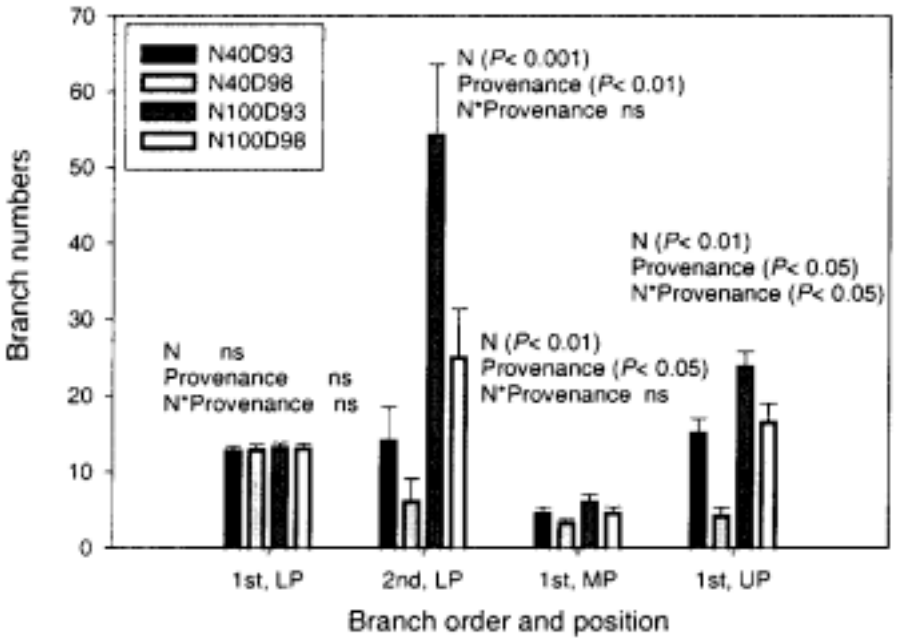

FIG. 2–Effect of nitrogen concentrations (40 and 100 mg N/litre) on branch numbers of first and second order at different positions of two Douglas fir provenances (D93 and D98) at the time of harvest. Error bars represent 1 S.E. (1st = first-order branch; 2nd = second-order branch; LP = lower position; MP = middle position; UP = upper position).

There were three periods of growth (i.e., flush) during the experiment, and the two provenances differed in flush time: Provenance 93 flushed 3 days earlier than 98. Nitrogen treatments had no effect on the time of flushing, but a very significant influence on branch numbers at lower, middle, and upper positions (Fig. 2). Except for the first-order lower position branches, the numbers of branches were greater in Provenance 93 than 98, and at 100 mg N/litre than 40 mg N/litre (Fig. 2).

Net Photosynthetic Rate and Quantum Yield of Needles

There were no significant differences in net photosynthetic rate between the provenances but there were significant differences ($p < 0.001$) between nitrogen treatments at all the measurement times. Net photosynthetic rate was greater for the higher nitrogen treatment in both the first- and second-flush needles (Fig. 3). The light response curves of both the second- and third-flush needles showed that the differences between the two nitrogen treatments in net photosynthetic rate commenced at about 250 $\mu mol/m^2 \cdot s$ PPFD and were greatest at the light-saturated value of 800–1000 $\mu mol/m^2 \cdot s$ PPFD (Fig. 4). The net photosynthetic rate also varied with different needle age when measured on the same day. The third-flush needles, about 10 days after emergence, had lower net photosynthetic rates than the second-flush needles (Table 2).

The measurement of the fluorescence parameters of needles did not show any difference between provenances or nitrogen treatments. Significant differences were found only

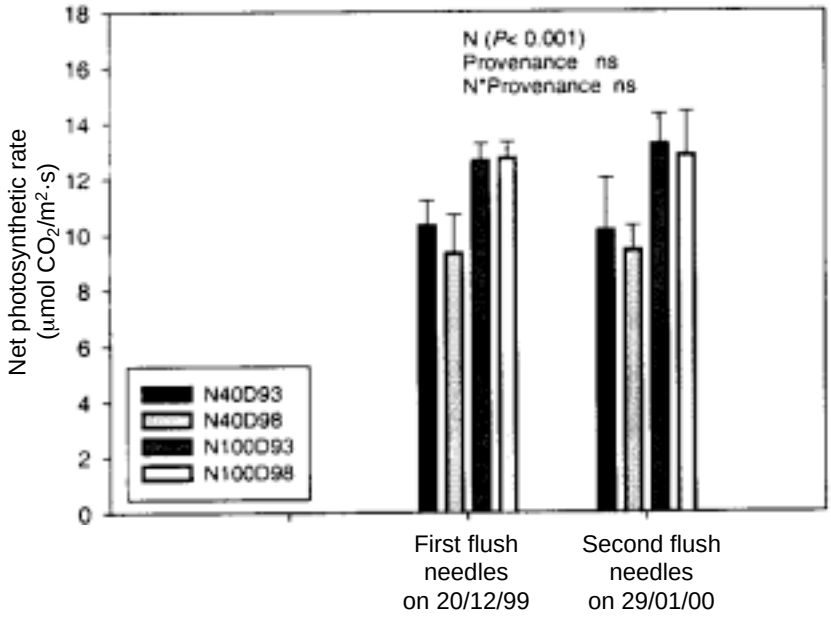

FIG. 3–Effect of nitrogen concentrations (40 and 100 mg N/litre) on net photosynthetic rate of first and second flush needles of two Douglas fir provenances (D93 and D98). Error bars represent 1 S.E.

among different-age needles. The third-flush needles, the youngest (about 2 weeks old), had a greater quantum yield than the first- (6 months old) and second-flush (4 months old) needles (Table 2).

Nutrient Uptake and Distribution

There were significant differences ($p < 0.001$) between nitrogen treatments for nutrient concentrations in needles. Nutrient concentrations, except nitrogen, in needles (Table 3) were lower at 100 than 40 mg N/litre. There were also significant differences ($p < 0.01$) between provenances in the concentrations of some nutrients in needles (Table 3), but the concentrations of macronutrients (except for potassium being higher in Provenance 93) in needles were not significantly different between the two provenances. However, the concentrations of micronutrients and also sodium and aluminium were significantly greater in Provenance 98 than 93 (Table 3). There were significant interactions between nitrogen and provenance in the concentrations of sodium, boron, copper, iron, and aluminium, which was due mainly to the greater concentrations of these elements in Provenance 98 at the lower nitrogen supply. In both provenances, high nitrogen supply increased the nitrogen concentrations in needles, stems, and roots (Table 4). Concentration of nitrogen was significantly greater in Provenance 98 than in Provenance 93 for roots, but not for needles and stems (Table 4). Nitrogen productivity was significantly greater in Provenance 93 than in 98. There was significant interaction of nitrogen and provenance with nitrogen productivity (Table 4).

Significant differences were also found between nitrogen treatments ($p < 0.0001$) and between provenances ($p < 0.001$) for the uptake rate of nutrients (Table 5). The uptake rate

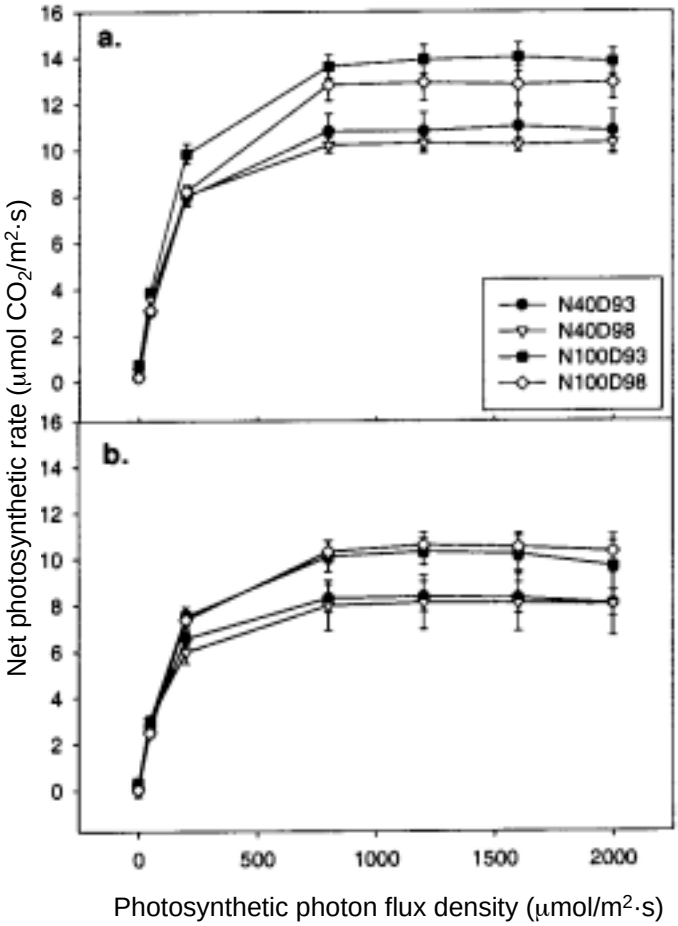

FIG. 4–Light response curves of second (a) and third (b) flush needles in two Douglas fir provenances (D93 and D98), as affected by nitrogen concentrations (40 and 100 mg N/litre). Error bars represent 1 S.E.

TABLE 2–Net photosynthetic rate and relative quantum yield in different flush needles of Douglas fir seedlings measured on 7 February 2000. Values in the same column followed by different letters are significantly different (Duncan's multiple range test, $p < 0.05$).

Needles	Net photosynthetic rate (μmol $CO_2/m^2 \cdot s$)	Relative quantum yield ($F_m–F_0/F_m$)
First flush (oldest)	n.d.	0.650 b
Second flush	12.0 a	0.673 b
Third flush (youngest)	9.3 b	0.766 a

was significantly greater at 100 than at 40 mg N/litre for all the nutrients analysed (some data not shown in Table 5) and in Provenance 93 than in 98 for most of the nutrients (Table 5).

TABLE 3–Nutrient concentrations in needles of Douglas fir seedlings at the time of harvest, as affected by nitrogen, provenance, and nitrogen × provenance interaction. Values in the same column followed by different letters are significantly different (Duncan's multiple range test, $p < 0.05$).

Provenance	Nitrogen level (mg/litre)	Nutrient concentrations in needles											
		Macronutrients (mg/g)					Micronutrients (μg/g)						
		N	P	K	Ca	Mg	Na	B	Mn	Zn	Cu	Fe	Al
Nitrogen main effect													
	40	8.90 b	2.03 a	8.16 a	2.49 a	0.95 a	142 a	38.3 a	274 a	16.1 a	2.30 a	48.7 a	66.3 a
	100	11.9 a	1.21 b	6.72 b	1.79 b	0.71 b	100 b	22.3 b	195 b	11.9 b	1.40 b	38.7 b	58.8 a
Provenance main effect													
93		10.3 a	1.55 a	8.15 a	2.05 a	0.72 a	116 b	27.1 b	228 a	12.0 b	1.40 b	39.4 b	52.9 b
98		10.5 a	1.69 a	6.73 b	2.23 a	0.89 a	136 a	33.5 a	240 a	16.0 a	2.30 a	48.7 a	72.2 a
Provenance × nitrogen interaction													
93	40	8.70 a	1.94 a	9.17 a	2.29 a	0.85 a	128 b	31.8 b	258 a	12.8 a	1.40 b	39.4 b	52.2 c
93	100	11.8 a	1.16 a	7.12 a	1.82 a	0.71 a	104 bc	22.4 c	199 a	11.2 a	1.40 b	42.2 b	55.6 bc
98	40	9.10 a	2.12 a	7.15 a	2.67 a	1.04 a	156 a	44.8 a	289 a	19.4 a	3.20 a	56.0 a	80.4 a
98	100	11.9 a	1.26 a	6.31 a	1.76 a	0.73 a	96 c	22.2 c	192 a	12.6 a	1.40 b	35.4 b	62.0 b

TABLE 4–Tissue nitrogen concentration and plant nitrogen productivity. Values in the same column followed by different letters are significantly different (Duncan's multiple range test, $p < 0.05$).

Provenance	Nitrogen level (mg/litre)	Nitrogen concentration (mg/g)				Nitrogen productivity (g/mol·day)
		Needle	Stem	Root	Whole plant	
Nitrogen main effect						
	40	8.90 b	4.82 b	10.6 b	8.45 b	19.1 a
	100	11.9 a	6.02 a	13.0 a	10.1 a	18.7 a
Provenance main effect						
93		10.3 a	5.43 a	10.6 b	8.80 b	26.0 a
98		10.5 a	5.41 a	13.1 a	9.83 a	12.0 b
Provenance × nitrogen interaction						
93	40	8.70 a	4.85 a	9.21 a	7.76 a	27.1 a
93	100	11.8 a	6.00 a	11.9 a	9.83 a	24.5 a
98	40	9.10 a	4.78 a	11.9 a	9.15 a	11.2 b
98	100	11.9 a	6.04 a	14.2 a	10.5 a	12.9 b

TABLE 5–Nitrogen and provenance effects on the uptake rate of nutrients by Douglas fir seedlings during the period of experiment. Values in the same column followed by different letters are significantly different (Duncan's multiple range test, $p < 0.05$).

Provenance	Nitrogen level (mg/litre)	Nutrient uptake rate (μmol/g DW of root ·day)							
		N	P	K	Ca	Mg	Na	B	Mn
Nitrogen main effect									
	40	18.0 b	1.90 b	5.99 b	3.45 b	1.20 b	0.41 b	0.07 b	0.10b
	100	48.4 a	2.84 a	10.5 a	5.41 a	2.20 a	0.71 a	0.15 a	0.17a
Provenance main effect									
93		38.1 a	2.76 a	9.98 a	4.96 a	1.85 a	0.74 a	0.12 a	0.16 a
98		30.1 b	2.10 b	6.53 b	4.09 b	1.55 a	0.48 b	0.09 b	0.11 b

Significant nitrogen and provenance main effects ($p < 0.001$) and nitrogen × provenance interactions ($p < 0.01$) were found for nutrient distribution among different plant parts (Table 6). The relative amounts of nutrients in needles were larger in Provenance 93 than 98 while those in roots were smaller in Provenance 93 than 98 (Table 6). Compared to the high nitrogen supply, a greater proportion of the nutrients was retained in roots at the lower nitrogen supply and therefore a smaller proportion was allocated to needles at the lower nitrogen supply in both provenances. These effects were most pronounced for all nutrients in Provenance 98, which resulted in significant interactions between nitrogen and provenance (Table 6).

DISCUSSION

This study clearly demonstrated that, under glasshouse conditions, nitrogen treatments accounted for most of the variation in the measured parameters, with the provenance component being the next largest cause of variation. The interaction of nitrogen × provenance was relatively smaller, but significant for some parameters.

TABLE 6–Percentage distribution of nutrient contents in roots and needles of Douglas fir seedlings at the time of harvest, as affected by nitrogen, provenance, and nitrogen × provenance interaction. Values in the same column followed by different letters are significantly different (Duncan's multiple range test, $p < 0.05$).

Provenance	Nitrogen level (mg/litre)	N	P	K	Mg	Na	B	Zn	Mn
Percentage nutrient distribution in roots*									
Nitrogen main effect									
	40	55 a	54 a	39 a	52 a	74 a	40 a	54 a	37 a
	100	36 b	39 b	19 b	38 b	64 b	31 b	42 b	25 b
Provenance main effect									
93		37 b	38 b	20 b	40 b	63 b	29 b	40 b	23 b
98		53 a	54 a	38 a	50 a	75 a	42 a	56 a	38 a
Provenance × nitrogen interaction									
93	40	43 b	40 b	25 b	44 b	65 b	32 b	41 b	25 b
98	40	67 a	67 a	54 a	60 a	82 a	49 a	66 a	48 a
93	100	32 c	36 b	15 b	36 b	61 b	27 b	38 b	20 b
98	100	40 bc	41 b	22 b	31 b	68 b	35 b	46 b	29 b
***Percentage nutrient distribution in needles**†									
Nitrogen main effect									
	40	28 b	28 b	30 b	25 b	12 b	36 b	19 b	38 b
	100	43 a	35 a	41 a	34 a	16 a	39 a	24 a	50 a
Provenance main effect									
93		42 a	37 a	42 a	35 a	17 a	43 a	25 a	52 a
98		39 b	25 b	29 b	24 b	11 b	32 b	17 b	37 b
Provenance × nitrogen interaction									
93	40	39 a	39 a	41 a	34 a	17 a	45 a	27 a	50 a
98	40	18 b	17 b	18 b	11 b	8 b	27 b	11 b	26 b
93	100	46 a	36 a	42 a	36 a	18 a	41 a	24 a	53 a
98	100	41 a	34 a	40 a	32 a	15 a	38 a	24 a	48 a

* Calculated as (nutrient contents in roots / total nutrient contents in whole seedlings) × 100
† Calculated as (nutrient contents in needles / total nutrient contents in whole seedlings) × 100

Effect of Nitrogen on Growth and Physiology of Douglas Fir Seedlings

Nitrogen treatments had a large effect on growth, nutrient uptake, and distribution, and the physiological parameters measured in this study. More biomass was produced from the seedlings at high nitrogen supply due to improved nutrition and enhanced net photosynthesis. Increasing nitrogen supply enhanced total plant growth. Shoot growth, however, was stimulated more than root growth, leading to a lower root/shoot ratio than that produced with a lower nitrogen supply. Nutrient status of the growing medium affects partitioning of biomass (Clarkson & Hanson 1980) and nitrogen influences the growth primarily of leaf area (Ingestad & Lund 1979). In this study, the root to shoot ratio in both provenances was related inversely to the level of nitrogen supply, which agrees with the previous observations made on other tree species (Ingestad 1979; Walters & Reich 1989; Sun *et al.* 2001). Greater growth of the seedlings at high nitrogen supply increased nutrient uptake, but decreased the concentration of nutrients (except nitrogen) as a result of dilution. There was no significant

difference between nitrogen treatments for nitrogen productivity, which suggests the low nitrogen treatment was not low enough to cause severe nitrogen deficiency in the seedlings. In this study, the concentrations of nutrients (except potassium and boron) in the needles at high nitrogen treatment were a little bit lower than the critical levels of nutrients (12.5 g N/kg, 1.6 g P/kg, 6.0 g K/kg, 2.5 g Ca/kg, 1.7 g Mg/kg, and 20 mg B/kg) derived from pot-grown Douglas fir seedlings (Walker & Gessel 1991). This is because in our study river sand was used as pot material and the nutrient solution was irrigated and free draining. Walker & Gessel's (1991) critical levels of nutrients, however, may be too high for New Zealand conditions though originally a reasonable starting point (Tim Payn pers. comm.).

The increased net photosynthetic rate with increasing nitrogen supply reflects the structural and functional roles of nitrogen in the photosynthetic apparatus. In this study, the lack of difference between the two nitrogen treatments in quantum yield of PSII, i.e., the electrons transferred per quantum of light absorbed by chlorophyll as estimated from fluorescence parameters (Genty *et al.* 1989), implies that the lower nitrogen supply did not have an adverse effect on photochemical processes of chlorophyll. Therefore, the reduced net photosynthetic rate in the needles at the lower nitrogen supply was probably due to the limitation of enzymatic processes in photosynthesis. Rubisco is a rate-limiting factor for potential photosynthesis in plants under the present atmospheric air conditions. Needle content of Rubisco-nitrogen is positively related to nitrogen content per unit area. However, Rubisco functions increasingly as a storage protein in addition to its catalytic functions with increasing nitrogen content per unit area (Warren *et al*. 2003). Ripullone *et al*. (2003) reported that nitrogen supply significantly affected nitrogen content per unit area in Douglas fir and *Populus* × *euroamericana* (Dole) Guinier, and in both species there were positive correlations between nitrogen content per unit area and chlorophyll concentration, and between nitrogen content per unit area and light-saturated photosynthesis and maximum carboxylation. The net photosynthetic rate in the third-flush needles (the youngest) did not achieve the same level as in the second-flush needles, which may be due to the physiological immaturity of those needles. In contrast, the quantum yield of PSII was greater in the third-flush needles. This indicates that those photons that were absorbed by the photosynthetic apparatus in the younger needles were used more efficiently.

Provenance Difference in Response to Nitrogen

In this study, Provenance 93 consistently had greater growth than Provenance 98 at both nitrogen levels, which may reflect genetic adaptation of the two provenances to the different environments prevailing in the natural habitats. Provenance 93 or the Ashley strain is believed to originate from coastal Oregon and has proved vigorous and well adapted over a wide range of sites in New Zealand (Miller & Knowles 1994). Provenance 98 or the Tramway strain (i.e., Beaumont strain), is primarily of Washington ancestry and is recommended as a suitable strain for exposed, higher elevation sites in southern areas (Miller & Knowles 1994). Provenances originating from the coastal fog-area of California and Oregon are generally more vigorous and faster-growing (Miller & Knowles 1994). Plant species characteristic of favourable (e.g., nutrient-rich) environments often have greater maximum relative growth rates (RGR) than do species from less favourable (e.g., nutrient-poor) environments (Lambers *et al*. 1998). The physiological basis and exact mechanisms for genetic variation in RGR are not fully understood. However, it is well

documented that a high RGR is associated with a high leaf area ratio, or net assimilate rate, or high nutrient concentration, or nutrient productivity (Lambers *et al*. 1998). When comparing more-productive cultivars of tree species with less-productive ones, leaf area ratio, rather than photosynthesis (per unit leaf area), is the main factor that accounts for variation in RGR (Ceulemans 1989). Analysis of the correlation RGR with plant traits suggests that leaf area ratio is the key trait because it enables the plant to expose a large leaf area to light and carbon dioxide per given biomass invested in leaves (Lambers *et al*. 1998). Although the leaf area ratio was not measured in this study, the greater ratio of needle to whole-plant dry weight in Provenance 93 than 98 (Fig. 1) to some extent accounted for the difference between the two provenances in growth. In this study, the photosynthesis (per unit leaf area) was not significantly different between the two provenances that showed considerable difference in growth, which implies that photosynthetic rate (per unit leaf area) was not the major cause for the difference between the two provenances in growth. This agrees with the previous study that rate of photosynthesis per unit leaf area shows no correlation with RGR among closely related taxa or among morphologically similar taxa (Lambers & Poorter 1992). In this study, it was not clear if earlier flush time and more branches in Provenance 93 contributed to the greater growth in this provenance. Further study is needed to investigate the relationship between flush time or branch number and the rate of leaf elongation and leaf appearance. It has been reported that the greater RGR and leaf area ratio of fast-growing grass species is associated with a more rapid leaf appearance and elongation (Groeneveld & Bergkotte 1996).

The genotypic variation between Provenances 93 and 98 in growth was also related to their nitrogen productivity (Table 4), uptake rate of nutrients per unit root mass (Table 5), and nutrient distribution within plants (Table 6). Provenance 93 had greater nitrogen productivity and uptake rate of nitrogen and other nutrients. It also allocated greater proportions of absorbed nutrients to shoots for photosynthesis and new growth. All those sustained a greater growth rate in Provenance 93, especially in the shoot. Nutrient productivity is a useful measure of the efficiency of nutrient use in producing new biomass (Ingestad 1979). It has been reported that greater nitrogen productivity is associated with rapid growth, a relatively large investment of nitrogen in photosynthetic tissue, efficient use of the nitrogen invested in the leaves for the process of photosynthesis, and relatively low carbon use in respiration (Garnier *et al*. 1995; Poorter *et al*. 1990). Variation in nitrogen requirement and nutrient productivity depends much more on the balance between requirements for protein synthesis for new growth and nitrogen storage (Lambers *et al*. 1998). In this study, Provenance 98 had greater concentration of nitrogen in whole seedlings, especially in roots (Table 4), but slower growth and less dry weight than Provenance 93. This implies that Provenance 98 might have higher nitrogen requirement. However, further study is needed to investigate the difference between the two provenances in critical concentration of nitrogen for shoot and root growth. A study of physiological and biochemical mechanisms at cellular and molecular levels, which could be under genetic control, should provide insight for the difference between the two provenances in nitrogen requirement. Greater uptake rate of nutrients per unit root mass is another plant trait associated with rapid growth (Lambers & Poorter 1992), which was confirmed by this study (Table 5). In addition, fast-growing species allocate relatively more to their leaves, in terms of both biomass and nitrogen (Lambers *et al*. 1998; Hawkins *et al*. 1999). Our results were in agreement with their report (Fig. 1 and Table 6). In this study, the seedlings of two

provenances were not inoculated with forest soil/duff or mycorrhizal spores and no significant colonisation of mycorrhizas was found in roots of either provenance at harvest. Therefore, the provenance difference in growth and nitrogen nutrition should not be due to mycorrhizal development.

Provenance interaction with nitrogen treatments was observed in this study for some growth and physiological parameters (Fig. 1 and 2, Tables 3, 4, and 6), suggesting that one provenance may have performed relatively better at one nitrogen level than another. The interactions were due mainly to greater differences in growth (especially of shoot) between provenances at the low nitrogen supply. Provenance 98 retained more nutrients in the roots than Provenance 93 did, and allocated smaller amounts of the nutrients to needles, especially at the low nitrogen supply. This may be responsible for more severe inhibition of shoot growth than root growth in Provenance 98 at the low nitrogen treatment, which resulted in a much greater root/shoot ratio and a significant increase in the concentration of some micronutrients in the needles of this provenance (Table 3). The different responses of the two provenances to nitrogen levels were related to nitrogen productivity, and the distribution of nitrogen and other nutrients within plants, as well as the ratio of needle to whole-plant dry weight. It has been reported that differences in response of Douglas fir families to nitrogen and phosphorus treatment levels were related to nutrient productivity (van den Driessche & El-Kassaby 1990/1991).

Among the growth parameters measured in this study, the relative growth rates of seedling height (RGR-Ht), the root/shoot ratio, and the ratio of needles to whole-plant dry weight seem to be the reliable and simple indicators for discriminating the provenance difference in response to nitrogen. The percentage nitrogen distribution in the plant parts was a good indicator too, although it may be costly to use it in practice due to the analysis of this nutrient for all plant parts.

CONCLUSIONS

Nitrogen had a large effect on the growth and nutrition of Douglas fir seedlings. Provenance 93 grew better at both nitrogen levels, and this was associated with the greater ratio of needles to whole-plant dry weight, nitrogen productivity and uptake rate of nutrients, and the ability to allocate a greater proportion of the dry matter and nutrients to the shoots.

Implications for Management

It was concluded from this study that Provenance 93 was a relatively efficient nitrogen responder while Provenance 98 was a relatively inefficient nitrogen responder. Although a field trial is needed to validate their responses because of the lack of mycorrhizas in this experiment, it would be expected that nitrogen fertiliser could improve the seedling growth of these two Douglas fir provenances at sites with low nitrogen availability. The provenance difference in response to nitrogen supply levels has implications for sustainable Douglas fir production in selection of provenances most suited to sites of particular nitrogen status. However, further work is needed with a larger genetic pool of Douglas fir clones or provenances to select a better provenance than Provenance 93 for a site with more severe nitrogen limitation than that tested in this study. The identification of new provenances or

clones with greater nitrogen use efficiency, coupled with best management practices, will enhance the efficiency of applied nitrogen fertiliser, reduce the cost of input, and prevent losses of nitrogen to the ecosystem, all of which could contribute to sustainable Douglas fir ecosystems that protect and promote soil, water, and air quality.

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