

Carbon isotope discrimination and oxygen isotope composition in clones of the F₁ hybrid between slash pine and Caribbean pine in relation to tree growth, water-use efficiency and foliar nutrient concentration

Z. H. XU,¹ P. G. SAFFIGNA,^{2,3} G. D. FARQUHAR,⁴ J. A. SIMPSON,¹ R. J. HAINES,¹ S. WALKER,¹ D. O. OSBORNE¹ and D. GUINTO^{2,5}

¹ Queensland Forestry Research Institute, MS 483, Fraser Road, Gympie, Queensland 4570, Australia

² Faculty of Environmental Sciences, Griffith University, Nathan, Queensland 4111, Australia

³ School of Agriculture and Horticulture, University of Queensland, Gatton, Queensland 4343, Australia

⁴ Research School of Biological Sciences, Institute of Advanced Studies, Australian National University, GPO Box 475, Canberra, ACT 2601, Australia

⁵ Present address: College of Agriculture, Forestry, Environment and Natural Resources, Cavite State University, Indang, Cavite, Philippines

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Summary The objectives of this study were: (1) to examine how foliar carbon isotope discrimination (Δ) and oxygen isotope composition ($\delta^{18}\text{O}$) are related to tree growth, ash mineral nutrient concentration and foliar nutrient concentration in 7-year-old clones of the F₁ hybrid between slash pine (*Pinus elliottii* Engelm.) and Caribbean pine (*P. caribaea* var. *hondurensis* Barr. et Golf.) in subtropical Australia; and (2) to evaluate the potential of using foliar Δ , ash mineral nutrient concentration and $\delta^{18}\text{O}$ measurements for selecting F₁ hybrid pine clones with high water-use efficiency (WUE) and growth potential. There were significant differences in tree growth, foliar Δ , $\delta^{18}\text{O}$ and ash mineral nutrient concentration among the eight clones tested. Significant negative linear relationships existed between tree growth and Δ , extrapolating to zero growth at $\Delta = 24\text{--}30\%$. There were strong genetic correlations ($r = -0.83$ to -0.96) between Δ and tree growth, particularly tree height. Significant non-genetic correlations ($r = -0.62$ to -0.80) existed between Δ and foliar K concentration. Foliar $\delta^{18}\text{O}$, ash mineral nutrient concentration and foliar nutrient concentration were unrelated to tree growth. In the F₁ hybrid pine clones, variation in tree WUE, as reflected by Δ , was largely attributed to a genetic effect on leaf photosynthetic capacity rather than on stomatal conductance, as reflected by foliar $\delta^{18}\text{O}$.

Keywords: ash mineral nutrient concentration, foliar K concentration, growth performance, photosynthetic capacity, *Pinus caribaea*, *Pinus elliottii*, selection, stomatal conductance.

Introduction

During photosynthesis, plants discriminate against the heavy

isotope of carbon (^{13}C) compared with the light isotope of carbon (^{12}C), resulting in a lower ratio of $^{13}\text{C}/^{12}\text{C}$ in plants relative to that in the atmosphere. Although discrimination against the stable isotope ^{13}C during photosynthesis has been related to plant growth and WUE only in the last two decades (Farquhar et al. 1982, 1989, Polley et al. 1993, Hall et al. 1994, Van de Water et al. 1994, Pennington et al. 1999), variation in carbon isotope composition of plants was first noted 50 years ago (Ehleringer and Vogel 1993). Carbon isotope discrimination (Δ) by plants has been defined by Farquhar and Richards (1984) as:

$$\Delta = (R_{\text{atmosphere}}/R_{\text{sample}} - 1), \quad (1)$$

where $R_{\text{atmosphere}}$ and R_{sample} ($\%$) are the $^{13}\text{C}/^{12}\text{C}$ molar ratios in atmospheric CO_2 and sample organic matter, respectively. Isotopic ratios, measured directly by isotope ratio mass spectrometry, can be more conveniently expressed relative to a reference standard (R_{standard}) as $\delta^{13}\text{C}$ ($\%$), defined as: $\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1)$. In practice, Δ is calculated as:

$$\Delta = (\delta^{13}\text{C}_A - \delta^{13}\text{C}_P)/(1 + \delta^{13}\text{C}_P), \quad (2)$$

where $\delta^{13}\text{C}_P$ and $\delta^{13}\text{C}_A$ are plant $\delta^{13}\text{C}$ and atmospheric $\delta^{13}\text{C}$, respectively.

Farquhar et al. (1982) developed a theory relating $\delta^{13}\text{C}$ to the intercellular CO_2 concentration in leaves of C_3 plants, which can be simplified to:

$$\Delta = a + (b - a)p_i/p_a - d, \quad (3)$$

where a (4.4%) is discrimination occurring during diffusion of CO_2 in air, b (between 27 and 30%) is discrimination attrib-

utable to carboxylation, p_i/p_a is the ratio of intercellular to atmospheric CO_2 partial pressures for C_3 plants, and d summarizes the effects of CO_2 dissolution, liquid phase diffusion, respiration and other processes that contribute to the discrimination. In turn, the ratio of carbon gain to water loss (WUE) is negatively related to p_i/p_a so that WUE and Δ are negatively related (Farquhar et al. 1982, Farquhar and Richards 1984, Farquhar et al. 1989, Hall et al. 1994). In drought-prone environments, there is potential to improve C_3 plant growth by selecting for high WUE on the basis of Δ . However, few studies have reported on the use of Δ in tree improvement programs. In one study with 13-month-old *Eucalyptus grandis* W. Hill ex Maiden clones, Bond and Stock (1990) found that tree height was significantly negatively related to foliar Δ . Subsequent studies have reported significant negative linear relationships between foliar Δ and WUE or tree growth, or both (Hogberg et al. 1993, Olbrich et al. 1993, Guehl et al. 1994, Medina and Francisco 1994, Osorio and Pereira 1994, Yoder et al. 1994, Zhang et al. 1994, Flanagan and Johnsen 1995, Zhang and Marshall 1995, Johnsen et al. 1999, Prasolova et al. 2000).

Masle et al. (1992) showed that ash mineral nutrient concentration and Δ were correlated across genotypes of several agricultural crop species. This result has been confirmed in other studies, but has never been examined in tree species. Although the mechanism underlying the correlation is not understood, it seems worthy of further study because of the potential to determine mineral nutrient concentration by means of an unsophisticated technique.

As shown in Equation 3, Δ is positively related to p_i/p_a , which may decrease as a result of either stomatal closure or increased photosynthetic capacity. Foliar organic oxygen isotope composition ($\delta^{18}\text{O}$; defined analogously to $\delta^{13}\text{C}$, but with ^{18}O and ^{16}O , and a standard that is the oxygen isotope ratio in sea water) is negatively related to leaf stomatal conductance (Farquhar et al. 1998, Barbour and Farquhar 2000, Barbour et al. 2000a, 2000b). Measurements of $\delta^{18}\text{O}$ can, therefore, help to ascertain if genetic effects on Δ are associated with effects on stomatal conductance to diffusion, or photosynthetic capacity, or both. The value of $\delta^{18}\text{O}$ in leaves of C_3 plants can be influenced by biological and environmental factors and sampling techniques (Dongmann et al. 1974, Epstein et al. 1977, Farquhar et al. 1993, Flanagan 1993, Yakir and Wang 1996, Saurer et al. 1997, Roden and Ehleringer 1999a, 1999b, Roden et al. 2000, N.V. Prasolova, Griffith University, Queensland, personal communication). However, there is little published information on the genetic and environmental effects on $\delta^{18}\text{O}$ in foliage of tree species in relation to tree growth, Δ , ash mineral nutrient concentration or foliar nutrient concentration.

Clonal forestry appears to offer an excellent opportunity for the early capture of the major benefits generated by tree improvement and biotechnology programs (Ahuja and Libby 1993, Libby and Ahuja 1993). Because ash mineral nutrient concentration, Δ and $\delta^{18}\text{O}$ could assist in the selection of clones for plantation forestry, we investigated: (1) how foliar Δ and $\delta^{18}\text{O}$ are related to tree growth, ash mineral nutrient concentration and foliar nutrient concentrations in 7-year-old

clones of the F_1 hybrid between slash pine and Caribbean pine in subtropical Australia; and (2) the potential of foliar Δ , ash mineral nutrient concentration and $\delta^{18}\text{O}$ measurements in selection of F_1 hybrid pine clones with improved WUE and growth.

Materials and methods

Site description

The experimental site is located at Toolara State Forest (26°00' S, 152°49' E), Queensland, Australia. The site has a humid subtropical climate with a mean annual rainfall of 1354 mm, about 60% of which falls in the summer (December to March). The July–September period is relatively dry, and occasionally this dry period may extend until November before summer storms occur. In January (midsummer), minimum, mean and maximum temperatures are 20.2, 24.9 and 29.6 °C, respectively. The corresponding temperatures in July (midwinter) are 6.3, 14.0 and 21.6 °C. Overall, the climate is characterized by hot moist summers with a mean relative humidity of 70% and mild winters with a mean relative humidity of 64%. In general, annual rainfall during the first 7 years after plantation establishment has been below the mean annual rainfall of 1354 mm for the last 25 years. Plantation tree species growing in the region often experience both well-watered and water-limited conditions in a year, even in the wet summer season. The soil is classified as a podzol according to the FAO/UNESCO system, with chemical properties and physical characteristics as shown in Table 1.

The site was cleared and burned in September 1985, ploughed in December 1985, and mounded in January 1986. It was planted in October 1986 at a density of 926 stems ha^{-1} (4.5 m between rows and 2.4 m between trees within rows)

Table 1. Physical and chemical properties of the soil at the experimental site.

Soil depth (cm)	0–10	10–20	20–70	70–100
<i>Physical properties</i>				
Electrical conductivity (dS m^{-1})	0.038	0.033	0.007	0.056
Clay (%)	10.0	10.0	16.0	28.0
Silt (%)	9.5	9.0	13.0	16.0
Sand (%)	80.5	81.0	71.0	56.0
<i>Chemical properties</i>				
pH (1:5 H_2O)	4.11	4.21	5.10	5.23
Organic C (%)	3.84	4.14	0.08	0.14
Total N (%)	0.054	0.055	0.001	0.005
Total P (mg kg^{-1})	20.9	18.1	4.6	6.8
Total K (mg kg^{-1})	47.0	39.7	17.1	180
Available P (mg kg^{-1})	1.6	0.8	0.5	1.6
Extractable Cu (mg kg^{-1})	0.7	0.8	0.9	0.8
Extractable Zn (mg kg^{-1})	0.5	0.6	0.4	0.3
Cation exchange capacity (cmol kg^{-1})	6.11	6.39	0.46	6.06

with five ramets (cuttings) of each of 120 clones of the F_1 hybrid between slash pine and Caribbean pine. In October 1986, fertilizer comprising 60 kg P ha⁻¹, 25 kg N ha⁻¹ and 5 kg Cu ha⁻¹ was applied on an individual tree basis (ring application about 40 cm away from each tree). Other local routine management practices were carried out at the site as required. From the 120 clones, eight having the highest stem volume growth and the best stem form (i.e., straightness) at Age 7 years (Table 2) were selected for detailed growth measurements and analysis for foliar Δ , $\delta^{18}\text{O}$ and ash mineral nutrient concentration. The parents of the eight F_1 hybrid clones are: (1) E1-149 and CH4-37 (Family 10) for Clone 474; (2) E1-102 and CH4-98 (Family 9) for Clones 545, 887, 892 and 893; (3) E3-27 and CH4-98 (Family 18) for Clones 620 and 1264; and (4) E2-66 and CH4-73 (Family 15) for Clone 1073. One ramet of each of the eight clones was randomly located in each of the five blocks.

Measurement of tree growth and foliar sampling

Height and diameter at breast height (1.3 m above ground) of the eight clones in each of the five replications were measured at Age 7 years. The volume index of each tree was calculated as the volume of a cone. Merchantable stem volume (volume of wood under bark from the stem base, 0.2 m aboveground, to a height of 7.2 m) was measured at Age 7 years. The bottom third (V_B) of merchantable stem volume was measured first, followed by the middle and top thirds (V_M and V_T , respectively); each third was 2.4 m in length. The total merchantable stem volume was thus the sum of V_B , V_M and V_T .

Foliar samples were collected at Age 7 years following the standard procedure described by Xu et al. (1995b). Briefly, 200 of the most recent, fully expanded needles (about 1 year old) were collected from a branch on the northern (sunny) side of the basal spring whorl immediately below the tip of each sample tree. Foliar samples were oven-dried at 70 °C immediately after collection and ground to pass a 0.5-mm sieve for foliar nutrient analysis. The subsamples were ground again to pass a 0.14-mm sieve for foliar ^{13}C and ^{18}O analyses.

Both tree growth measurement and foliar sampling were performed around midwinter (July) when growth was minimal (cf. Xu et al. 1995a, 1995b) and soil water availability and evaporation were low.

Chemical, physical and statistical analyses

Available soil P, cation exchange capacity, electrical conductivity, extractable Cu and Zn, organic C, pH (1:5 H₂O) and total N, P and K were analyzed as described by Xu et al. (1995a). Soil particle size was analyzed by the method of Gee and Bauder (1986). Foliar Ca, Cu, K, Mg, Mn, N, P and Zn concentrations were determined as described by Xu et al. (1995b). Ash mineral nutrient concentration (mg kg⁻¹) was calculated as the sum of foliar P, K, Ca, Mg, Cu, Zn and Mn concentrations. Foliar ^{13}C was analyzed with a Roboprep CN (7001) coupled to a Tracermass mass spectrometer (9001) (PDZ Europa Ltd., Crewe, U.K.), with sucrose ($\delta^{13}\text{C}_{\text{PDB}} = -11.4\text{‰}$) and acetanilide ($\delta^{13}\text{C}_{\text{PDB}} = -31.8\text{‰}$) as reference standards. The spectrometer was calibrated with sucrose ($\delta^{13}\text{C}_{\text{PDB}} = -10.4\text{‰}$) supplied by the Australian National University. The mean standard deviation for $\delta^{13}\text{C}$ analysis of the sucrose reference standards was 0.14‰. The value of Δ was calculated by means of Equation 2, using a value of -8‰ for $\delta^{13}\text{C}_{\text{PDB}}$ of CO₂ in the atmosphere (Farquhar et al. 1989, O'Leary 1993). Foliar $\delta^{18}\text{O}$ (the ratio of $^{18}\text{O}/^{16}\text{O}$ in tree foliage (R) relative to that of Standard Mean Ocean Water (R_{SMOW}); $R/R_{\text{SMOW}} - 1$) was determined at the Australian National University based on a new technique reported by Farquhar et al. (1997), with a mean standard deviation of 0.2‰ for foliar ^{18}O analysis.

Statistical analysis including Duncan's Multiple Range Test and graphing were conducted with STATISTICA software (1999; Statsoft, Tulsa, OK). Because variations in foliar Δ and tree growth within clones would be mainly a result of environmental factors, correlations between Δ and tree growth of the corresponding clone means served as good indicators of genetic correlations between the measured traits. Therefore, both phenotypic correlations (based on individual tree data) and genetic correlations (based on only the corresponding clone means of the traits measured) are presented.

Table 2. Stem growth of clones of the F_1 hybrid between slash pine and Caribbean pine at Age 7 years.¹

Clone	Height (m)	DBH (m)	VI (m ³)	Merchantable volume (m ³ × 10 ³)			
				Bottom	Middle	Top	Total
474	11.6 bc ²	0.209 ab	0.134 abc	59.1 abc	40.8 ab	23.8 a	124 ab
545	12.4 ab	0.221 a	0.159 a	66.1 a	44.2 a	26.6 a	137 a
620	11.5 bc	0.220 a	0.148 ab	62.6 ab	29.5 c	22.0 ab	114 abc
887	12.7 a	0.204 ab	0.138 ab	57.0 abc	41.1 ab	26.7 a	125 ab
892	12.0 ab	0.194 bc	0.119 bcd	53.5 abcd	36.8 abc	22.0 ab	112 abc
893	10.2 d	0.178 c	0.087 e	41.7 d	27.0 c	14.0 c	83 d
1073	10.5 d	0.188 bc	0.100 de	49.1 cd	30.1 c	15.8 bc	95 cd
1264	10.8 cd	0.192 bc	0.105 cde	52.7 bcd	30.6 bc	15.8 bc	99 bcd

¹ Tree height, diameter at breast height (1.3 m above ground), volume index (VI) and merchantable volume are means of five replications for each clone.

² Means within a column followed by the same letter are not different from each other at the 5% level of significance according to Duncan's Multiple Range Test.

Results

Tree growth

Tree growth of the F₁ hybrid pine clones at Age 7 is shown in Table 2. There were significant differences in tree height, diameter at breast height (DBH) and volume index among the clones. Clone 887, with a height of 12.7 m, was 24% taller than Clone 893. The DBH of Clone 545 (0.221 m) was 24% greater than that of Clone 893. Volume index of Clone 545 (0.159 m³) was 83% higher than that of Clone 893. There were also significant differences in merchantable stem volume among the eight clones. The bottom third of merchantable stem volume (which has a relatively smaller percentage of recent growth compared with the top third) for Clone 545 was 0.0661 m³, which was 59% higher than that of Clone 893. The middle and top thirds of merchantable stem volume for Clone 545 were 0.0442 and 0.0266 m³, respectively, which were 64 and 90% higher than the corresponding values for Clone 893. Overall, total merchantable stem volume for Clone 545 (0.137 m³) was 65% higher than that of Clone 893. Most of the other clones had growth intermediate between that of Clones 545 and 893. Total merchantable stem volume accounted for 86% of the variation in volume index of whole trees.

Foliar Δ , $\delta^{18}\text{O}$ and nutrient concentration

Foliar Δ , $\delta^{18}\text{O}$, nutrient concentration and ash mineral nutrient concentration of the eight clones at Age 7 are presented in Table 3. Mean Δ differed significantly among the eight clones, ranging from 19.6 to 20.7‰. Foliar $\delta^{18}\text{O}$ also differed significantly among the clones, although it ranged only from 27.5 to 28.1‰. There were significant differences in foliar N (0.83–1.04%) and P concentrations (0.052–0.073%) among the clones. Significant differences among clones also existed for foliar Ca, Mg, Mn and ash mineral nutrient concentrations. However, foliar Cu, K and Zn concentrations did not vary significantly among the clones.

Foliar Δ and $\delta^{18}\text{O}$ in relation to tree growth, ash mineral nutrient concentration and foliar nutrient concentration

Both tree height ($r = -0.67$) and total merchantable stem volume ($r = -0.51$) were negatively related to Δ at Age 7 (Figure 1). Carbon isotope discrimination was better related to tree height or total merchantable stem volume than to tree DBH ($r = -0.36$) (Table 4). Furthermore, middle and top merchantable stem volume appeared to be better related to Δ ($r = -0.55$) than bottom merchantable stem volume ($r = -0.34$). Foliar $\delta^{18}\text{O}$ was not significantly related to tree growth for any of the 40 trees of the eight clones examined.

Although foliar K concentration sampled at Age 7 was unrelated to either clone (Table 3) or tree growth (data analysis not shown), there was a significant, negative linear relationship between foliar K concentration and Δ ($r = -0.62$) (Table 4 and Figure 2a). In addition, there was a significant, positive linear relationship between Δ and foliar Ca concentration ($r = 0.42$). However, Δ was unrelated to foliar N, P, Mg, Cu, Zn, Mn and ash mineral nutrient concentrations. The mean foliar K concentration of seven trees in one experimental block with $\Delta < 19.6\text{‰}$ was significantly higher than that of the remaining 33 trees with $\Delta > 19.6\text{‰}$ (0.394 versus 0.256%; $P < 0.001$). When data for the seven trees associated with $\Delta < 19.6\text{‰}$ and high foliar K concentration were excluded, there was no significant relationship between Δ and foliar K concentration, and the negative linear relationship between tree growth and Δ ($n = 33$) was improved (Table 4). Foliar $\delta^{18}\text{O}$ was only weakly related to foliar K concentration and Δ (Figures 2b and 2c).

In terms of genetic correlations, based on clone means, Δ was well related to tree growth with a correlation coefficient of -0.96 for tree height at Age 7, compared with -0.88 , -0.90 and -0.83 for middle, top and total merchantable stem volume, respectively (Figure 3 and Table 4). These results indicate that there were strong genetic correlations between Δ and tree growth, particularly tree height, among the eight clones examined. In addition, Δ was related to foliar K concentration ($r = -0.80$).

Table 3. Carbon isotope discrimination (Δ), oxygen isotope composition ($\delta^{18}\text{O}$), foliar nutrient concentrations and ash mineral nutrient (AMN) concentration in clones of the F₁ hybrid between slash pine and Caribbean pine at Age 7 years.¹

Clone	Δ (‰)	$\delta^{18}\text{O}$ (‰)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Cu (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Mn (mg kg ⁻¹)	AMN (mg kg ⁻¹)
474	20.1 abc ²	28.0 ab	0.95 ab	0.060 b	0.252 a	0.097 bc	0.110 bcd	2.0 a	17 a	50 b	5205 bc
545	19.7 c	27.8 abc	1.04 a	0.061 b	0.294 a	0.153 a	0.125 ab	2.2 a	16 a	80 a	6346 a
620	20.4 ab	27.6 bc	0.83 b	0.052 b	0.245 a	0.091 bc	0.094 cd	1.8 a	17 a	60 ab	4849 c
887	19.6 c	27.6 bc	1.02 a	0.058 b	0.329 a	0.057 c	0.083 d	2.5 a	14 a	40 b	5287 bc
892	19.7 c	28.1 a	0.92 ab	0.054 b	0.365 a	0.100 bc	0.092 cd	1.9 a	16 a	50 b	6128 ab
893	20.7 a	27.8 abc	0.99 a	0.073 a	0.264 a	0.115 ab	0.111 bc	3.0 a	16 a	50 b	5664 abc
1073	20.7 a	27.5 c	1.03 a	0.062 ab	0.256 a	0.123 ab	0.144 a	2.3 a	16 a	50 b	5867 abc
1264	20.6 a	27.5 c	0.91 ab	0.052 b	0.235 a	0.083 bc	0.098 cd	2.2 a	17 a	60 ab	4708 c

¹ Ash mineral nutrient concentration (mg kg⁻¹) is the sum of the mineral nutrients including P, K, Ca, Mg, Cu, Zn and Mn.

² Means within a column followed by the same letter are not different from each other at the 5% level of significance according to Duncan's Multiple Range Test.

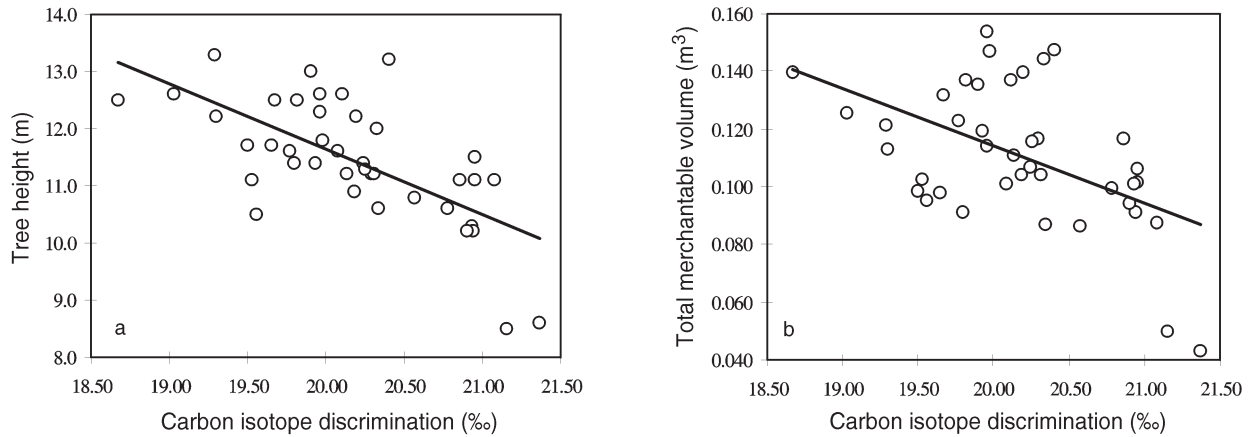


Figure 1. Relationship between carbon isotope discrimination (x) of 7-year-old clones of the F₁ hybrid pine and (a) tree height (y) with the fitted curve: $y = 34.5 - 1.14x$ ($r^2 = 0.43$, $n = 40$, $P < 0.001$, $rsd = 0.8$); and (b) total merchantable stem volume (y) with the fitted curve: $y = 0.512 - 0.0199x$ ($r^2 = 0.24$, $n = 40$, $P < 0.001$, $rsd = 0.021$).

Table 4. Simple linear correlation coefficients between carbon isotope discrimination (Δ), oxygen isotope composition ($\delta^{18}O$) and tree growth, foliar nutrient concentration and ash mineral nutrient (AMN) concentration in clones of the F₁ hybrid between slash pine and Caribbean pine at Age 7 years.

Variable ¹	Δ (‰)			$\delta^{18}O$ (‰)
	A ² (n = 40)	B (n = 33)	C (n = 8)	
Height (m)	-0.665** ³	-0.721**	-0.960**	ns
DBH (m)	-0.363*	-0.470**	ns	ns
Volume index (m ³)	-0.449**	-0.534**	ns	ns
Bottom merchantable stem volume (m ³)	-0.342*	-0.439**	ns	ns
Middle merchantable stem volume (m ³)	-0.547**	-0.654**	-0.884**	ns
Top merchantable stem volume (m ³)	-0.550**	-0.649**	-0.901**	ns
Total merchantable stem volume (m ³)	-0.510**	-0.622**	-0.834**	ns
K (%)	-0.615**	ns	-0.798*	0.414**
Ca (%)	0.417**	ns	ns	ns
AMN (mg kg ⁻¹) ⁴	ns	ns	ns	ns

¹ DBH: diameter at breast height (1.3 m above ground); and volume index calculated as: $\pi DBH^2 H/12$, where H is tree height (m).

² A: correlation of Δ with tree growth and foliar nutrient concentration in all the data sets; B: correlation of Δ with tree growth and foliar nutrient concentration in the 33 data sets—the data sets for seven trees associated with $\Delta < 19.6\%$ and high foliar K concentration (mean [K] of 0.394% compared with 0.256% for the remaining trees) are omitted; and C: correlation of mean clone Δ with the corresponding tree growth and foliar nutrient concentration.

³ Asterisks ** and * indicate $P < 0.01$ and 0.05 , respectively, and ns indicates not significant.

⁴ Ash mineral nutrient concentration (mg kg⁻¹) is the sum of the mineral nutrients including P, K, Ca, Mg, Cu, Zn and Mn; foliar N, P, Mg, Cu, Zn and Mn concentrations are not significantly related to either Δ or $\delta^{18}O$.

Discussion

Tree growth, Δ , $\delta^{18}O$ and foliar nutrient concentration

There were significant differences in tree growth at Age 7 among the eight clones of the F₁ hybrid between slash pine and Caribbean pine. This indicates that further genetic gains in tree growth can be achieved through selection of clones with high growth performance, such as Clones 545 and 887 from the initially assessed top eight clones. Several clones exhibited moderate growth but other desirable traits such as stem straightness that are likely to be of value to the forest industry (Kleinschmit et al. 1993, Libby and Ahuja 1993).

Foliar Δ values of 19.6–20.7‰ for the eight clones of the F₁ hybrid pine at Age 7 are comparable with those of 16.7–21.4‰ for other tree species (Gutierrez and Meinzer 1994, Marshall and Zhang 1994, Zhang et al. 1994). Although the largest difference in foliar Δ between clones was only about 1‰ in this study, the differences were statistically significant. Similarly, Olbrich et al. (1993) found significant differences in foliar Δ between four *Eucalyptus grandis* clones, although the largest difference was only 0.8‰. Significant genetic effects on foliar Δ have also been observed in other tree species (Aitken et al. 1995, Flanagan and Johnsen 1995, Johnsen et al. 1999, Pennington et al. 1999, Prasolova et al. 2000).

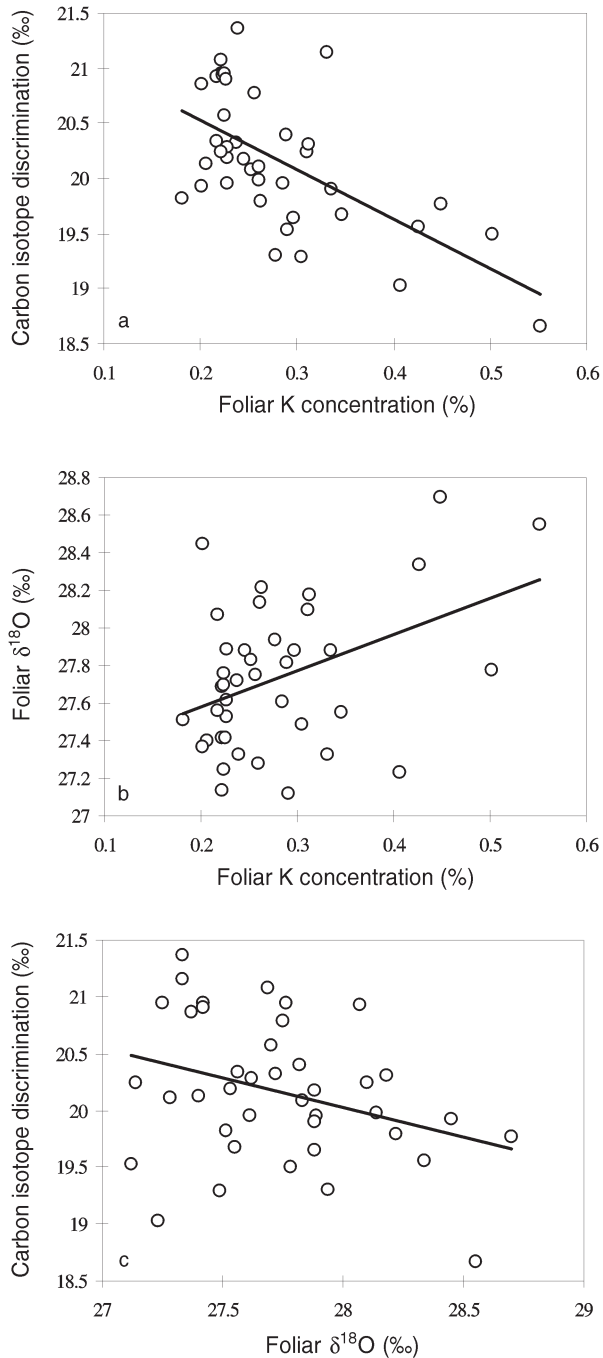


Figure 2. Relationships between (a) foliar K concentration (x) and carbon isotope discrimination (y) with the fitted curve: $y = 21.4 - 4.51x$ ($r^2 = 0.36$, $n = 40$, $P < 0.001$, $rsd = 0.5$); (b) foliar K concentration (x) and foliar $\delta^{18}\text{O}$ (y) with the fitted curve: $y = 27.2 + 1.92x$ ($r^2 = 0.15$, $n = 40$, $P < 0.001$, $rsd = 0.4$); and (c) foliar $\delta^{18}\text{O}$ (x) and carbon isotope discrimination (y) with the fitted curve: $y = 34.7 - 0.523x$ ($r^2 = 0.11$, $n = 40$, $P < 0.05$, $rsd = 0.6$) in 7-year-old clones of the F_1 hybrid pine.

There have been few studies of genetic effects on foliar $\delta^{18}\text{O}$ in tree species. The foliar $\delta^{18}\text{O}$ value of 27.5–28.1‰ in the 7-year-old F_1 hybrid pine clones was higher than the value of 17.6–20.0‰ in whole wood of tree rings in *Picea glauca* Voss (Gray and Thompson 1977), but lower than the value of

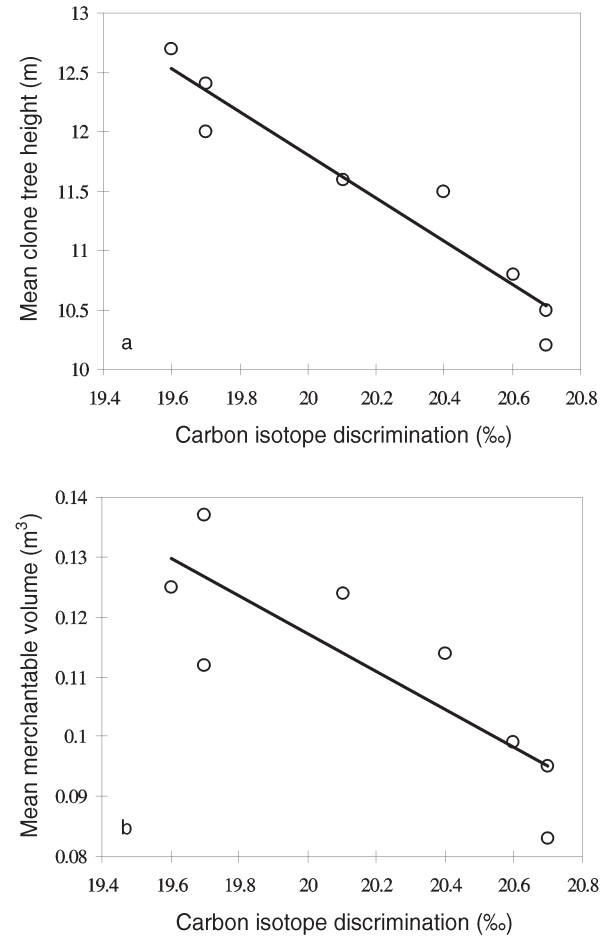


Figure 3. Relationships between (a) carbon isotope discrimination (x) of 7-year-old clones of the F_1 hybrid pine and mean clone tree height (y) with the fitted curve: $y = 48.3 - 1.83x$ ($r^2 = 0.91$, $n = 8$, $P < 0.001$, $rsd = 0.3$); and (b) mean total merchantable stem volume (y) with the fitted curve: $y = 0.747 - 0.0315x$ ($r^2 = 0.65$, $n = 8$, $P < 0.01$, $rsd = 0.011$).

30.2–32.6‰ in foliage of 9-year-old hoop pine (*Araucaria cunninghamii* D. Don) families (N.V. Prasolova, personal communication). There were significant differences in foliar $\delta^{18}\text{O}$ among the clones, which is similar to the findings in hoop pine families.

Foliar P concentrations of 0.052–0.062% in seven of the eight clones at Age 7 were generally below the critical foliar P concentration of 0.065–0.080% for growth of exotic pines in subtropical Australia (Bevege and Richards 1972, Simpson and Osborne 1993, Xu et al. 1995b, 1995c). Both foliar N and K concentrations were in the range of acceptable values reported by Simpson and Osborne (1993). All of the other foliar nutrient concentrations were generally above the acceptable values for growth of pine trees in subtropical Australia.

Correlation of Δ with tree growth

When data for all the eight clones were examined, Δ was negatively related to both tree height and total merchantable stem volume at Age 7. When the seven data sets with $\Delta < 19.6‰$

and a foliar K concentration of 0.395% (compared with a mean [K] of 0.256% for the remaining 33 data sets) were excluded from the analysis, the negative linear relationship between Δ and tree growth improved. Our approach of relating Δ measured for 1-year-old needles of trees at Age 7 to cumulative tree growth for a period of 7 years differs from most other studies where Δ is related to plant growth over a period of less than 2 years. This may, in part, explain why the middle and top parts of merchantable stem volume are generally better related to Δ than the bottom part, because Δ appears to correlate best with volume growth in the period closest to the Δ measurement.

The strong genetic correlation between Δ and tree height ($r = -0.96$) among the 7-year-old F_1 hybrid pine clones growing in a drought-prone environment is similar to that observed ($r = -0.97$) for families of 22-year-old *Picea mariana* Britton (Johnsen et al. 1999). Prasolova et al. (2000) reported that foliar Δ for 9-year-old hoop pine families can be either positively or negatively related to tree height under well-watered conditions, depending on the canopy positions sampled. However, foliar Δ is consistently and negatively related to tree height of the same hoop pine families grown in a more water- and N-limiting growth environment, irrespective of the canopy positions sampled (N.V. Prasolova, personal communication). The Δ intercept values of 25.6–30.2‰ (calculated from the relationships between tree growth and Δ for all 40 individual tree data sets in Figure 1) and 23.7–26.4‰ (determined from the relationships between tree growth and Δ for the eight clone means in Figure 3) are within the range of Δ intercept values (22–32‰) reported for the relationship between plant WUE and Δ in plant leaves by Hall et al. (1994). Based on theoretical considerations (Farquhar et al. 1982, Hall et al. 1994), the Δ intercept for the simple linear relationship between WUE and Δ in C_3 plants should equal $b - d$, where b is the carboxylation parameter (27–30‰) and d is the parameter (0–3‰) describing the discrimination due to respiration and other processes. The Δ intercepts of 24–30‰ for tree growth and Δ found in this study, which are close to the theoretical value of about 27‰ for WUE and Δ , suggest not only that Δ is well related to tree growth, but that either water is completely limiting or that photosynthetic capacity is the underlying source of variation. Regardless of the underlying cause, Δ may be an important physiological criterion for selection of elite clones with improved tree growth.

Relationships between Δ and $\delta^{18}\text{O}$, ash mineral nutrient concentration and foliar nutrient concentration

There was a significant negative linear relationship between foliar K concentration and Δ of the eight clones sampled at Age 7 years. This relationship was not attributable to growth dilution because foliar K concentration was unrelated to either tree growth or clone. This suggests that higher foliar K concentration is associated with lower Δ , but not tree growth. The reason for the high foliar K concentrations of the seven trees with $\Delta < 19.6\%$ in one block is unknown. One possible explanation is that the trees were growing in an area of ash spots resulting from site preparation burning. Alternatively, the area

might have inherently higher soil K fertility. Carbon isotope discrimination was positively related to foliar Ca concentration when data for all 40 individual trees were included, but unrelated to foliar Ca concentration when the means of the eight clones were analyzed. White (1993) reported that, in *Phaseolus vulgaris* L., Δ was negatively related to foliar K concentration. Masle et al. (1992) found that foliar K and Ca concentrations were negatively related to WUE for both C_3 crop species and C_4 crop species, and positively related to Δ for C_3 species, but negatively related to Δ for C_4 species. Our finding that foliar Δ and tree growth were unrelated to foliar N concentration corroborates the results of a study on *Picea mariana* families (Johnsen et al. 1999). We found no significant correlation between Δ and ash mineral nutrient concentration, whereas Masle et al. (1992) reported that the ratio of ash mineral concentration to ash concentration in several agricultural crop leaves was significantly related to Δ . This discrepancy may be explained by species differences or growth conditions.

Foliar $\delta^{18}\text{O}$ in the F_1 hybrid pine clones was unrelated to tree growth and was only weakly related to Δ . This suggests that tree WUE as reflected by Δ might be largely a result of a genetic effect on photosynthetic capacity rather on stomatal conductance, because $\delta^{18}\text{O}$ in tree foliage is a good index of stomatal conductance (Farquhar et al. 1998). Foliar $\delta^{18}\text{O}$ data for 9-year-old hoop pine families (N.V. Prasolova, personal communication) have revealed that fast-growing trees in a well-watered environment with close to an adequate N supply have low WUE (high Δ) and high stomatal conductance (low $\delta^{18}\text{O}$), indicating that tree WUE is primarily associated with genetic effects on stomatal conductance; however, fast-growing trees in a drought-prone, N-limited environment have high WUE (low Δ) and high photosynthetic capacity (high N concentration), suggesting that tree WUE, in this case, is mainly associated with genetic effects on photosynthetic capacity. Johnsen et al. (1999) reported that significant genetic variation in foliar Δ of *Picea mariana* families can largely be attributed to genetic control of photosynthetic capacity.

Mechanisms of tree growth and foliar K concentration relating to Δ

We found a significant, negative linear relationship between Δ and foliar K concentration in clones of F_1 hybrid pine. Foliar K concentration was unrelated to either tree growth or clone, suggesting that it is not under genetic control in this species. We speculate that foliar K concentration was not associated with regulation of p_i/p_a (see Equation 3), for two reasons. First, foliar K concentration was generally in the optimum range for tree growth and was only weakly related to stomatal conductance as reflected by foliar $\delta^{18}\text{O}$. Second, foliar K concentration was weakly related to tree WUE as reflected by Δ . Our study provided no information about the mechanism by which foliar K regulates foliar Δ (Kupka 1992, Hirsch et al. 1998).

In conclusion, foliar Δ in the eight clones of the F_1 hybrid pine at Age 7 was significantly correlated with tree growth, foliar $\delta^{18}\text{O}$ and nutrient concentrations. Tree growth was nega-

tively related to Δ but unrelated to foliar $\delta^{18}\text{O}$, ash mineral nutrient concentration and foliar nutrient concentration. There were strong genetic correlations between Δ and tree growth. Among clones, variation in tree WUE, as reflected in Δ , was largely attributable to the genetic effect on leaf photosynthetic capacity rather than on stomatal conductance, as reflected in foliar $\delta^{18}\text{O}$. Overall, foliar Δ appears promising as a physiological parameter for selecting clones of F_1 hybrid pine for improved WUE and increased growth in drought-prone environments in subtropical Australia.

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