Genetic variation in carbon isotope discrimination and its relationship to growth under field conditions in full-sib families of Picea *mariana*

Lawrence B. Flanagan and Kurt H. Johnsen

Abstract: Measurements of the stable carbon isotope composition of leaf tissue were made on *Picea mariana* (Mill.) B.S.P. trees from four full-sib families grown on three different field sites at the Petawawa National Forestry Institute, Ontario, Canada. The four families chosen exhibited genetic variation for growth characteristics. Genetic variation was also observed for carbon isotopic discrimination (il) among the families of *P. mariana.* In addition, a strong correlation occurred between il values measured on trees in 1991 and 1992, two years that had very different precipitation and temperature conditions during the growing season, indicating that the ranking of individual trees remained almost constant between years. A strong, negative correlation was observed between average carbon isotope discrimination and average tree height for the four families on the driest, least productive site, as was expected based on leaf photosynthetic characteristics. There was no significant correlation, however, between il values and growth on the other two study sites, where productivity was higher.

Resume : Nous avons effectue des mesures de composition en isotopes stables de carbone sur des tissus foliaires d'epinettes noires (*Picea mariana* (Mill.) B.S.P.) de quatre families de descendance biparentale plantees sur trois sites differents a l'Institut national de recherche forestiere de Petawawa, Ontario, Canada. Les quatre families selectionnees montraient une variabilite genetique dans leurs caracteristiques de croissance. Nos mesures ont aussi revele une variabilite genetique dans la discrimination isotopique (il) parmi Jes families de *P. mariana.* De plus, Jes valeurs de il mesurees en 1991 et celles mesurees en 1992 sur Jes memes arbres etaient fortement correlees, et ce malgre des differences marquees de precipitation et de temperature au cours de la saison de croissance. Cette forte correlation indique que le rang d'arbres individuels etait quasi-constant d'une annee a l'autre. Sur le site le plus sec et le moins productif, Jes valeurs de discrimination isotopique moyennes des quatre familles etaient fortement et negativement correlees a la hauteur moyenne des arbres, ce qui concorde avec Jes proprietes photosynthetiques du feuillage. Sur Jes deux autres sites plus productifs, cette correlation entre il et la croissance etait cependant absente. [Traduit par la Redaction]

Introduction

Previous studies have documented genetic variation in growth characteristics in *Picea mariana* (Mill.) B.S.P. (Boyle 1987; Morgenstern and Mullin 1990). At present, however, there is very little information on the physiological basis for genetic variation in growth. In this paper we examine how differences in growth, observed among full-sib families of *P. mariana*, are related to leaf photosynthetic characteristics and carbon isotopic discrimination.

During ghotosynthetic gas exchange, the stable isotopic ratio (1^{3}C/ ^{2}C) of carbon dioxide assimilated differs from that of the source C0$_2$ available to plants (Farquhar et al. 1989). There are two primary processes that cause carbon isotope ratios to change during photosynthesis, diffusional fractionation and enzymatic fractionation. Carbon dioxide molecules containing ^{12}C are lighter and, therefore, diffuse into the leaf at a faster rate (by a factor of 1.0044, or 4.4%0) than CO? molecules containing ^{13}C (Craig 1954; Farquhar and Lioyd 1993). The primary carboxylating enzyme in C$_3$ plants, ribulose-1,5-bisphosphate carboxylase, preferentially uses ^{12}C0$_2$ (by a factor of 1.029, or *29%0)* and so discriminates against ^{13}C0$_2$ (Roeske and O'Leary

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1984; Guy et al. 1993). The carbon isotopic ratio of leaf organic material depends on the relative influence of diffusional and enzymatic fractionation, which in turn is determined by the ratio of leaf intercellular CO_2 *(pi)* and atmospheric CO_2 *(p.)* partial pressures (Farquhar et al. 1982, 1989). This ratio *(p/p.)* is important because it is a function of photosynthetic capacity and stomatal conductance. Changes in *p/p.* and leaf carbon isotopic ratio are, therefore, a function of changes in either, or both, photosynthetic capacity and stomatal conductance (Farquhar et al. 1989).

Several recent studies have documented how *p/p.* and carbon isotopic discrimination vary with changes in environmental conditions (Farquhar et al. 1989; Ehleringer et al. 1993). Measurement of the carbon isotopic ratio of leaf tissue provides an assimilation-weighted average of *p/p.*, a parameter that will vary as changes in environmental conditions induce variation in assimilation rate and stomatal conductance (Farquhar et al. 1989; Ehleringer et al. 1992). Since leaf carbon isotopic ratio provides information about processes integrated over the whole life of a leaf, it is particularly useful for examining subtle genetic differences in photosynthetic characteristics. Genetic variation in carbon isotopic discrimination has been demonstrated for a number of agricultural crops and tree species, and it has been suggested that it could be used as a selection criterion for improving water-use efficiency and yield of crop plants; e.g., wheat (Farquhar and Richards 1984; Condon and Richards 1993), barley (Hubick and Farquhar 1989), cowpea (Hall et al. 1990, 1993), peanut (Hubick et al. 1988), common bean (White et al. 1990), tomato (Martin and Thorstenson 1988), Douglas-fir (Zhang et al. 1993), and western larch (Zhang et al. 1994).

The application of carbon isotope techniques in breeding and selection programs requires an understanding of the relationship between variation in leaf carbon isotope discrimination values and plant growth under field conditions (Richards and Condon 1993). The growth of individual plants may be either positively or negatively correlated with leaf discrimination values depending on whether variation in discrimination is associated with changes in photosynthetic capacity or stomatal conductance (Farquhar et al. 1988, 1989). In some species genetic variation in discrimination is primarily a result of variation in photosynthetic capacity (e.g., cultivars of *Arachis*, Hubick et al. 1988), while in other species it is largely a result of variation in stomatal conductance (e.g., cultivars of *Phaseolus*, Ehleringer 1990). In addition, the relationship between discrimination values and growth can depend on field conditions. Condon and Richards (1993) have shown that in wheat genotypes, the relationship between crop biomass production and leaf carbon isotope discrimination values changed when crops were grown on different-quality sites (the amount of rainfall was the primary difference among sites). It was only on the driest site that the negative relationship between growth and leaf discrimination, predicted from leaf gas exchange characteristics, was supported for the wheat genotypes (Condon and Richards 1993).

In this study our objectives were (i) to determine whether genetic variation for leaf carbon isotopic discrimination occurs among full-sib families of *P. mariana* and *(ii)* to determine the relationship between leaf carbon isotopic discrimination and growth in *P. mariana* on three field sites that differ in productivity. Our results indicated that significant genetic variation for carbon isotopic discrimination occurred among families of *P. mariana* and that leaf carbon isotope discrimination values were strongly correlated with growth (tree height) only on the driest, least productive site.

Methods and materials

Plant material and experimental design

Four full-sib families of *P. mariana* were used from a complete 7 × 7 diallel cross experiment that was started at the Petawawa National Forestry Institute (PNFI), Ontario, Canada, in 1970 (Morgenstern 1974; Boyle 1987). The seven parental trees used for the diallel cross were from a plantation at PNFI, but the exact origin of the trees is unknown, except that they were grown from seed collected in the Lake Simcoe – Rideau region in Ontario (Morgenstern 1974). The four families (7122, 7125, 7143, 7146) that were used in the present experiment represent a 2 parent × 2 parent breeding structure, as shown below (we have dropped the prefix 71 from the family identifier throughout the rest of this paper):

	Female parent	
Male parent	59	63
52	22	25
62	43	46

The seeds resulting from the diallel cross were germinated, and seedlings were grown for 2 years in a greenhouse before being planted at three field sites at PNFI in 1973 (Morgenstern 1974). At each site trees from a full-sib family were planted in either 9-tree (sites 1 and 3) or 16-tree (site 2) square plots. The arrangement of family plots was randomized within a block at each site. At sites 1 and 3 there were 3 replicate blocks, and at site 2 there were 4 replicate blocks. Site 1 was established with 45 families from the original 49 families produced by the diallel cross, site 2 was planted with 37 of the families, and site 3 was initiated with 32 of the families.

For our experiment, 12 trees per family were chosen at each site. On sites 1 and 3, four trees were randomly chosen from each family plot in each of the 3 replicate blocks per site. On site 2, three trees were randomly chosen from each family plot in each of the 4 replicate blocks. This procedure resulted in a total of 144 tree samples collected in each year. One of the samples was lost, so data are presented for only 143 samples in the 1991 data set.

Carbon isotope discrimination

Carbon isotope analysis was done on foliage collected during October 1991 and November 1992. In 1991 small branches, from the four cardinal compass directions, were removed from a tree at each of two heights, the top one-third of the canopy and the bottom one-third of the canopy. In 1992 only four branches from the top one-third of the canopy were cut from a tree. In both years, foliage produced

during the previous summer was collected from the branches and combined for an individual tree. The tissue was dried at 65°C and ground to a fine powder with a mortar and pestle or with a tissue grinder.

A 2- to 3-mg subsample of the ground tissue was sealed in an evacuated Vycor tube with cupric oxide wire and silver foil. The tubes were heated to 850°C for 6 h followed by an 8- to 9-h period of cooling to room temperature (Ehleringer and Osmond 1989). The carbon dioxide generated from the combustion was purified cryogenically within 2 days, and its ^{13}C/^{12}C ratio was measured on a gas isotope ratio mass spectrometer (Sira 12, VG Instruments Inc.) at the Ottawa-Carleton OCGC Stable Isotope Facility. Carbon isotope discrimination values (Ii) were calculated from carbon isotope composition values (o) as shown below (Farquhar et al. 1989):

$$\delta = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1$$

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p}$$

where

Rsample is the ^{13}C/^{12}C content of a sample

Rstandard is the ^{13}C/^{12}C content of the international PDB (Pee Dee Belemnite) standard (Ehleringer and Osmond 1989)

oa is the o^{13}C value for source atmospheric C02 (-0.0079, or -7.9%0)

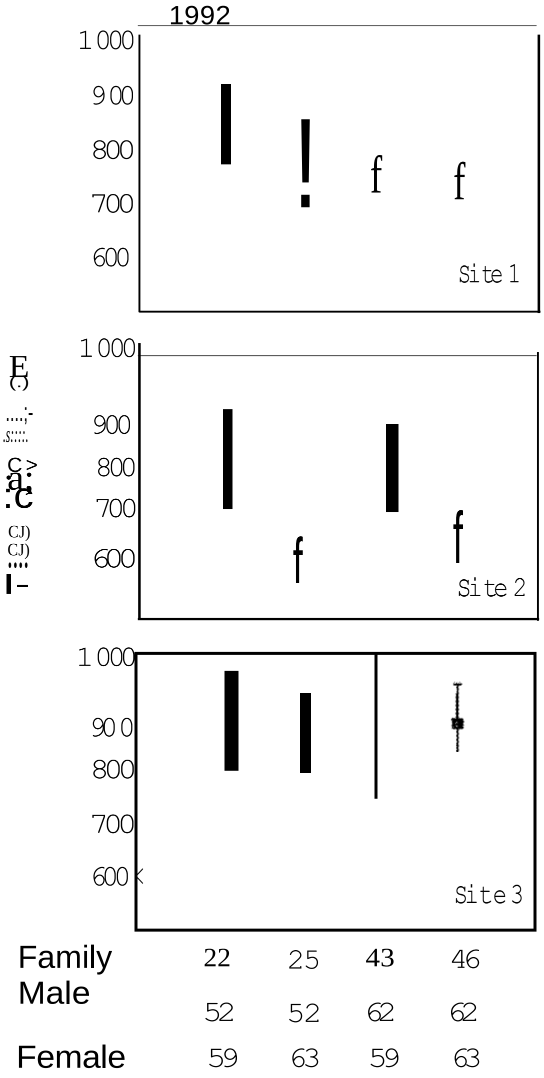
Op is the 0^{13}C value for plant foliage

The accuracy and precision of our analyses were checked by making repeated measurements of the carbon isotopic composition of a U.S. National Bureau of Standards sample (NBS-21, spectrographic graphite), an internal laboratory standard (Fisher graphite), and several tree samples. The mean value we obtained for 10 measurements of NBS-2 l was o^{13}Cp$_{08}$ = -28.15%0 (standard deviation = 0.040%0), which agrees well with the accepted o^{13}Cp$_{80}$ value of -28.10%0. Over a 2-year period, the standard deviation of 49 measurements of our internal graphite standard was 0.061*%0.* This graphite standard is the same laboratory standard used at the Stable Isotope Ratio Facility for Environmental Research at the University of Utah. Our mean o^{13}Cp$_{08}$ value (-26.11%o) for this laboratory standard is the same as the value measured at the University of Utah. Four replicate o^{13}C measurements on tissue samples from five different trees resulted in the following standard deviations *(%0);* 0.067, 0.109, 0.091, 0.115, and 0.020. The slightly higher standard deviations for the tree samples relative to the graphite standards result from foliage tissue inhomogeneity. We conclude that our carbon isotope measurements are repeatable and accurate to ±0.1*%0.*

Tree height measurements

Total tree height was determined by placing a IO-m pole, which was marked off in centimeter increments, next to a tree. While the pole was held in place by one person, a second person read the tree height and recorded the value. Tree height measurements were made for all trees in the three study plots in 1990 and 1992.

Fig. 1. Comparison of average tree height (±SD), measured in 1992, among four full-sib families of *Picea mariana* at Petawawa National Forestry Institute.



Statistical analysis

Carbon isotope discrimination values (6.) and tree height values were analyzed by analysis of variance (ANOVA) using a randomized complete block model, with blocks nested within a site as described above. All effects were considered fixed.

Results

Male parent, female parent, male x female, and female x site were all significant sources of variation for tree height measured in 1990 (data not shown) and 1992 (Fig. l ; Table 1), results similar to those observed for the same trees at earlier growth stages (Boyle 1987). Family 25 consistently had the shortest trees on all sites (Fig. 1). A different family had the largest average tree height on each of the three sites (Fig. 1). There was a strong positive correlation between tree height and stem diameter measured in 1992 (*r* = 0.734, *n* = 144, *P* < 0.001), indicating that tree height provides a good estimate of tree growth.

Fig. 2. Comparison of average family values for carbon isotope discrimination (±SD), measured on foliage collected in 1991, from four full-sib families of *Picea mariana* at Petawawa National Forestry Institute.

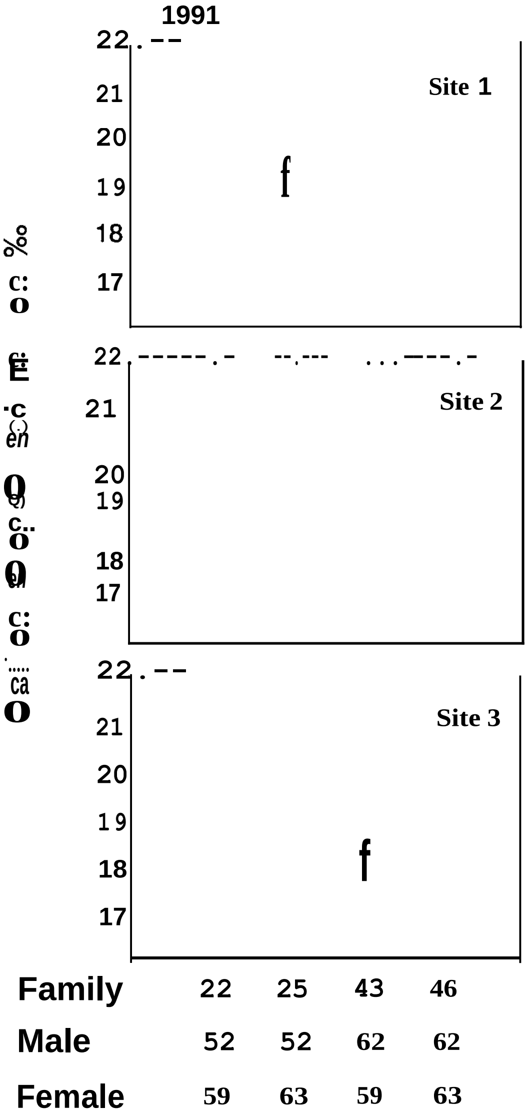


Fig. 3. Comparison of average family values for carbon isotope discrimination (±SD), measured on foliage collected in 1992, from four full-sib families of *Picea mariana* at Petawawa National Forestry Institute.

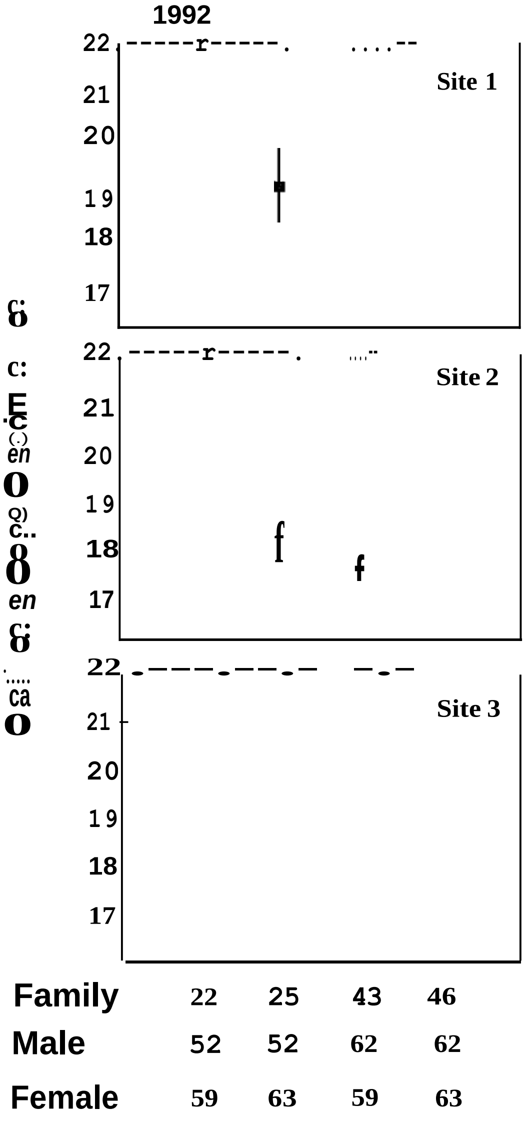


Table 1. Analysis of variance for tree height in *Picea mariana* measured in 1992.

Source	df	Mean square	F-value	?-value
Female		201 754	26.17	0.0001
Male		51 384	6.67	0.0102
Female x male	1	36 347	4.71	0.0305
Site	2	360 959	46.82	0.0001
Site x male	2	8 666	1.12	0.3259
Site x female	2	95 549	12.39	0.0001
Site x male x female	2	2 152	0.28	0.7565
Block(site)	7	30 367	3.94	0.0004
Residual	401	7 709		

Average tree height differed significantly among the three sites (Table 1), with the shortest trees occurring on site 2, and the tallest trees occurring on site 3 (average tree height ± standard deviation: site 1, 809 ± 90 cm; site 2, 757 ± 103 cm; site 3, 866 ± 96 cm). The average height (± standard deviation) measured in 1992 for trees on all sites was 809 ± 93 cm, $n = 144$.

There was significant variation among families for foliage carbon isotope discrimination values in both 1991 and 1992 (Figs. 2, 3; Tables 2, 3). In addition, both male and female parent identity were significant sources of variation for a values in 1991 (Table 2). In 1992 female parent, **but** not male parent, was a significant source of variation for carbon isotope discrimination values (Table 3). The progeny of female parent 63 tended to have higher a values than the other families in both years (Figs. 2, 3). In 1991

Table 2. Analysis of variance for carbon isotopic discrimination in current-season foliage of *Picea mariana* collected during October 1991.

Source	df	Mean square	F-value	P-value
Female	I	14.256	39.687	0.0001
Male	I	5.422	15.094	0.0002
Female X male	I	3.515	9.786	0.0022
Site	2	4.449	12.387	0.0001
Site X male	2	0.140	0.389	0.6789
Site X female	2	0.375	1.044	0.3552
Site X male X female	2	0.308	0.856	0.4272
Block(site)	7	0.176	0.491	0.8396
Residual	124	0.359		

the largest difference in family mean Li value (1.17%0) occurred on site 1 between families 43 and 25 (Fig. 2). This difference indicates a 15.7% higher ratio of photosynthetic carbon assimilation to stomatal conductance in family 43 relative to family 25, based on calculations done using the Farquhar et al. (1982; 1989) model of leaf discrimination processes. In 1992 the largest difference in family mean Li value (1.10%0) occurred on site 1 between families 43 and 46 (Fig. 3). This difference corresponds to a 14.7% higher ratio of photosynthetic carbon assimilation to stomata] conductance in family 43 relative to family 46.

There was also significant variation in mean Li values among sites in 1991 (Fig. 4; Tables 2, 3). Trees on site 2 had lower discrimination, on average, than the other two sites. The magnitude of the difference between mean site Li values for site 2 and site 1 (approximately *0.56%0* difference in 1991) would translate into approximately a 6.9% increase in the ratio of carbon assimilation to stomata! conductance by the trees on site 2. The difference in Li values among sites followed a similar pattern in 1992, but was not as large as the variation observed among sites in 1991 (Fig. 4).

A strong significant correlation occurred between Li values measured on trees in 1991 and 1992 (Fig. 5), indicating that the ranking of individual trees remained almost constant between years.

There was a strong, negative correlation between average carbon isotope discrimination and average tree height for the four families on site 2 (Fig. 6). Sites 1 and 3 did not show any consistent relationship between average tree height and average Li values. The data plotted in Fig. 6 represent tree height measured in 1992 and Li values measured on current-season foliage collected in 1992. The patterns shown in Fig. 6 were consistent, however, if 1992 Li values and 1990 tree height values were plotted, or if 1991 Li values and 1990 tree height values were plotted (data not shown).

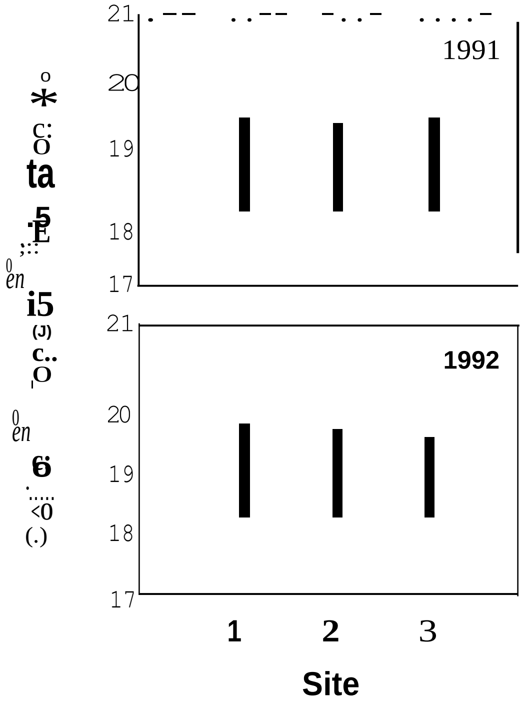
Discussion

Our data showed significant environmental influences on leaf discrimination values as demonstrated by the differences observed among sites and between years for the average

Table 3. Analysis of variance for carbon isotopic discrimination in current-season foliage of *Picea mariana* collected during November 1992.

Source	df	Mean square	F-value	P-value
Female	1	10.293	27.546	0.0001
Male	I	0.323	0.864	0.3543
Female X male	I	2.418	6.471	0.0122
Site	2	1.1ll	2.973	0.0548
Site X male	2	0.010	0.027	0.9733
Site X female	2	0.551	1.474	0.2329
Site X male X female	2	0.296	0.791	0.4555
Block(site)	7	0.298	0.798	0.5906
Residual	125	0.374		

Fig. 4. Comparison of average site values for carbon isotope discrimination (±SD), measured on foliage collected in 1991 and 1992, from four full-sib families of *Picea mariana* at Petawawa National Forestry Institute.



Li values (Figs. 2, 3, 4). A variety of environmental and physiological measurements indicate that site 2 is drier than the other study sites (Johnsen and Major 1995), which is consistent with the lower carbon isotope discrimination values observed for trees on site 2 (Fig. 4; Farquhar et al. 1989). In addition, the significant difference in average Li values among sites in 1991 was not observed in 1992 (Tables 2, 3; Fig. 4), which is consistent with the very different environmental conditions in the two years (Fig. 7). The summer of 1991 was substantially warmer and drier than the long-term average conditions at Petawawa National Forestry Institute. In contrast, the summer of 1992 was cooler, and had much higher precipitation, than the long-term average conditions (Fig. 7). The wetter, cooler growing

Fig. 5. Relationship between carbon isotope discrimination values measured in 1991 and 1992, on individual trees from four full-sib families of *Picea mariana* at Petawawa National Forestry Institute.

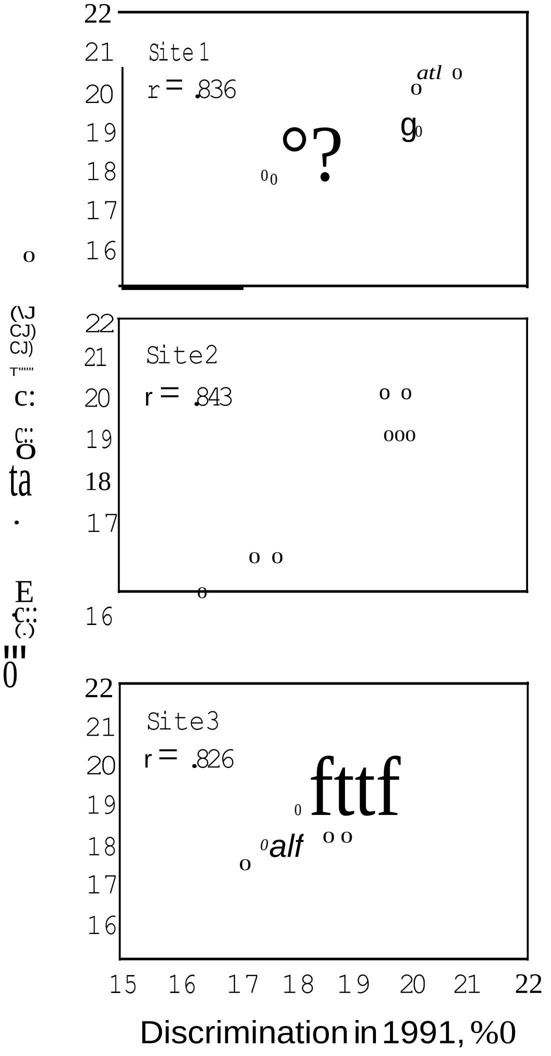
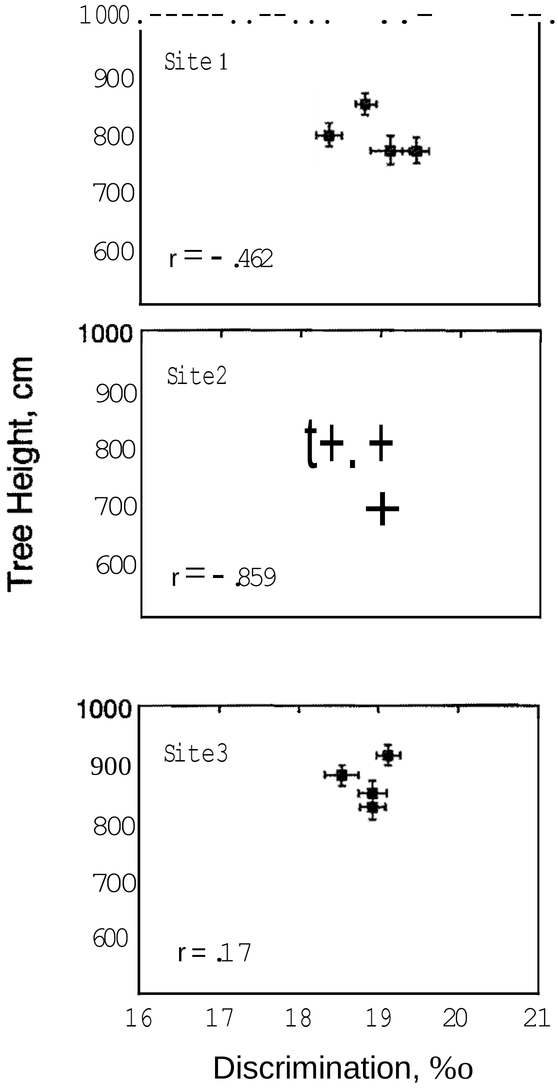


Fig. 6. Relationship between foliage carbon isotope discrimination and tree height in four full-sib families of *Picea mariana* at Petawawa National Forestry Institute. Values are the average ± SE for each family. Both carbon isotope discrimination and tree height measurements were made in 1992.



season of 1992 should have ameliorated the poor conditions on site 2, which was apparent in the carbon isotope data.

Since conditions were much warmer and drier in 1991, one might expect that average discrimination values should be lower in 1991 relative to 1992 (Farquhar et al. 1989). This pattern was not observed (Figs. 2, 3, 4) because of the different methods of leaf sample collection used in the two study years. Foliage samples were collected only from the top one-third of the canopy in 1992, while foliage samples were collected from both the top and bottom one-third of the canopy in 1991. Leaves from the lower layers of tree canopies incorporate less ^{13}C into organic material than foliage at the top of the canopy because of two processes. First, the low light intensities apparent within lower layers of a canopy result in a decrease in the ratio of photosynthesis to stomatal conductance and, consequently, increase discrimination against ^{13}C during gas exchange (Farquhar et al. 1989; Pearcy and Pfitsch 1991). Second, foliage in the lower layers of a canopy may assimilate some CO$_2$ released from soil respiration, which is relatively depleted in ^{13}C, in addition to carbon dioxide assimilated from the bulk atmosphere (Farquhar et al. 1989; Sternberg et al. 1989; Broadmeadow et al. 1992; Broadmeadow and Griffiths 1993). These two processes would act to increase the average leaf discrimination values observed in 1991, despite the fact that the 1991 growing season was much warmer and drier than 1992.

Our data also indicated that significant genetic variation exists among full-sib families of *P. mariana* for carbon isotope discrimination (Tables 2, 3). Genetic variation for carbon isotope discrimination has also been demonstrated for a number of agricultural crop and forage species (see Ehleringer et al. 1993 for a review). Variation in photosynthetic capacity among the different families of *P. mariana* was the cause of the genetic differences in carbon isotope discrimination (Johnsen and Major 1995). Stomatal conductance rates do not vary significantly among the different *P. mariana* families studied. Differences in photosynthetic capacity have also been observed to be the primary cause of genetic variation in carbon isotope discrimination in peanut (Rubick et al. 1988).

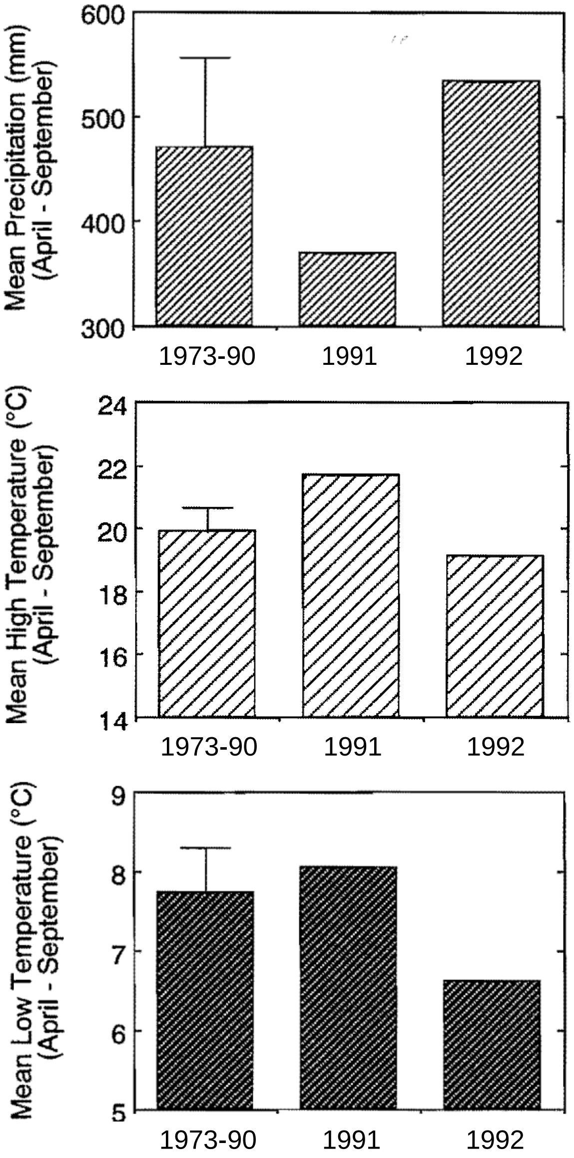
Based on the Farquhar et al. (1982, 1989) model of carbon isotopic discrimination during photosynthetic gas exchange, discrimination will be reduced in a plant when photosynthetic capacity is increased, if stomatal conductance remains constant. The higher photosynthetic capacity would also translate into higher growth, if all other things remained constant. Therefore, carbon isotope discrimination values should be negatively correlated with plant growth when variation in discrimination is a result of changes in photosynthetic capacity (Farquhar et al. 1989). In contrast, if variation in discrimination is caused predominantly by a change in stomatal conductance, then carbon isotope discrimination values should be positively correlated with growth. This is because an increase in stomatal conductance will result in higher assimilation of carbon, thereby increasing growth, and will also enhance discrimination against ^{13}C during gas exchange (Farquhar et al. 1989). Since differences in photosynthetic capacity were the source of variation in carbon isotope discrimination in the *P. mariana* families we studied (Johnsen and Major 1994), growth was expected to be negatively correlated with discrimination values.

A strong, negative correlation was observed between average carbon isotope discrimination and average tree height (growth) for the four families on site 2 (Fig. 6), as was expected based on leaf photosynthetic characteristics. The higher carbon assimilation and more conservative water use by the families with lower discrimination values would be particularly beneficial on site 2, where water availability limits growth. There was no significant correlation, however, between A values and growth on the other two study sites, where productivity was higher. Condon and Richards (1993) have also observed that the relationship between growth and leaf carbon isotope discrimination values changed when plants were grown in the field on different-quality sites. In addition, the negative relationship between A values and growth, predicted from leaf gas exchange characteristics in wheat genotypes, was also only observed on the lowest quality (driest) site (Condon and Richards 1993).

When making links between leaf carbon isotope discrimination and growth, it is necessary to consider genetic differences in carbon allocation pattern in addition to leaf photosynthetic gas exchange properties (Cowan 1986; Masle and Farquhar 1988; Virgona et al. 1990). Differences in allocation of carbon between photosynthetic tissue and roots can alter the relationship between A values and growth when water is not limiting. For example, the *P. mariana* genotypes that have high discrimination, and a low ratio of photosynthesis to stomatal conductance, may also have a high ratio of photosynthetic tissue to root tissue. Such an allocation pattern would magnify the problem of high stomatal conductance and low assimilation on a site, like site 2, where water availability limits growth (Cowan 1986). A higher allocation to photosynthetic tissue on a site that is not limited by water availability, however, may overcome any restriction on growth imposed by low assimilation rates. Therefore, there may be genetic differences in carbon allocation patterns among the families of *P. mariana* considered in this study, that explain the changed relationship between A values and tree height when plants were grown on different-quality sites. Virgona et al. (1990) showed that there is genetic variation in carbon allocation patterns in sunflower. Sunflower genotypes exhibited a positive correlation between A values and higher ratio of leaf area to total plant carbon, which is consistent with our predictions for differences among the families of *P. mariana*.

The results of this study suggest that trees with low A values and high photosynthetic capacity relative to water loss have an advantage on water stressed sites, while trees with high A values perform well on sites where water is not limiting growth. Since the exact origin of the parent trees used for the diallel cross is unknown, our results shed no

Fig. 7. Comparison of temperature and precipitation amounts during the growing season (April-September) in 1991 and 1992 with the long-term average conditions (average during 1973-1990 ± SD) at Petawawa National Forestry Institute.



light on the adaptive nature of genetic variation in Li in *P. mariana.* Studies with plants in arid land systems have suggested that different genotypes within a population have distinct suites of characteristics that are advantageous in certain conditions and unfavorable in other conditions (Ehleringer 1993a, 1993b). Large temporal and spatial variability in environmental conditions in the arid land systems maintain a diversity of physiological types within a population (Ehleringer 1993a). This may also be true for *P. mariana,* which is long-lived and, in its natural habitat, grows under extremely diverse environmental conditions.

A better understanding of the relationships among carbon gain, water loss, carbon allocation and growth in *P. mariana* would greatly facilitate efforts to breed trees that grow well in a specific range of environmental conditions. The results of this study indicated that carbon isotope discrimination measurements provide information about genetic variation in photosynthetic processes, which was highly repeatable in two different study years, and which greatly contributed to understanding genotype x environment interactions influencing the growth of *P. mariana.* Because of the well-developed mechanistic understanding of physiological processes influencing carbon isotope discrimination (Farquhar et al. 1989; Ehleringer et al. 1993), the repeatability of the measurements, and the ease of measuring large numbers of mature trees, carbon isotope discrimination shows promise for incorporating physiological assessment into currently established tree breeding programs.

Acknowledgments

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