

# Variation in nitrogen supply changes water-use efficiency of *Pseudotsuga menziesii* and *Populus × euroamericana*; a comparison of three approaches to determine water-use efficiency

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Received May 27, 2003; accepted October 26, 2003; published online April 1, 2004

**Summary** We studied the effects of three nitrogen (N) supply rates (low, intermediate and high) on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings and poplar clone “I-214” (*Populus × euroamericana* (Dole) Guinier) cuttings growing in mini-stands. Our specific objectives were to: (1) evaluate the effects of N supply on water-use efficiency (WUE) and biomass production; (2) determine if N affects WUE through control of carbon assimilation rates or through stomatal control of water loss; and (3) compare three methods of estimating WUE: one short-term method ( $WUE_i$ , based on gas exchange measurements) and two long-term methods ( $WUE_T$ , based on the ratio between biomass production and transpired water, and  $\Delta$ , based on leaf carbon isotope discrimination tested as a proxy of WUE). In both species, biomass production,  $WUE_i$  and  $WUE_T$  increased with increasing N supply, but there was no effect of N supply on either transpiration or stomatal conductance and  $\Delta$  was negatively related to leaf N concentration. Plots of  $\Delta$  versus both  $WUE_i$  and  $WUE_T$  revealed negative trends, but the regression between  $WUE_i$  and  $\Delta$  was significant only for Douglas-fir, and the regression between  $WUE_T$  and  $\Delta$  was significant only for poplar. Thus, the mechanisms underlying the response of WUE to N supply were mainly related to a positive effect of N supply on photosynthetic rates. The data confirm that carbon isotope discrimination may be a useful proxy of WUE. The finding that N availability enhances both biomass production and WUE may have practical implications in regions where these factors impose constraints on forest productivity.

**Keywords:** biomass production, carbon assimilation, carbon isotope discrimination, Douglas-fir, poplar, stomatal conductance.

## Introduction

Several studies have shown that nitrogen (N) supply enhances

plant productivity by improving water-use efficiency (WUE) (Field et al. 1983, Brix and Mitchell 1986, Lajtha and Whitford 1989, Livingston et al. 1999) by: (1) increasing water loss control by stomatal conductance, with no influence on assimilation rate (Toft et al. 1989, Guehl et al. 1995); or (2) increasing assimilation rate as a result of increased N investment in the photosynthetic apparatus (Ranjith et al. 1995) with no counterbalancing effect on stomatal conductance (Squire 1987, Reich et al. 1989, Liu and Dickmann 1996, Harvey and van den Driessche 1999, Welander and Ottosson 2000); or (3) causing a moderate increase in assimilation rate and a slight decrease in stomatal conductance (Wang et al. 1998, Livingston et al. 1999). In contrast, other studies have shown that N supply either has no effect on WUE (Sheriff and Nambiar 1991, Mitchell and Hinckley 1993, Guehl et al. 1995, Harvey and van den Driessche 1999, Korol et al. 1999) or decreases WUE (Lauteri et al. 1997, Walcroft et al. 1997, Meinzer and Zhu 1998).

These contrasting results are not easy to explain, partly because the mechanisms underlying the responses of stomatal conductance to altered concentrations of leaf N and intercellular  $CO_2$  are not fully understood (Mitchell and Hinckley 1993, Guehl et al. 1995, Grassi et al. 2002), and partly because it is unclear how changes in N concentrations affect the coupling between stomatal conductance and  $CO_2$  assimilation (sensu Wong et al. 1979). Moreover, there appear to be substantial differences among tree species in the response of WUE to nutritional status.

Differences in N effects on WUE may be also attributed to differences in experimental procedures such as: (a) the way WUE is estimated (instantaneous measurements versus measurements integrated over long periods; Hinckley et al. 1992, McMurtrie et al. 1992, Donovan and Ehleringer 1994); (b) the material studied (individual leaves versus the whole plant); (c) the experimental conditions (e.g., optimal or limiting water supply; Mitchell and Hinckley 1993); (d) plasticity in plant

physiological processes that could produce nonlinear responses (Lajtha and Getz 1993); and (e) nutrient imbalance, possibly leading to interspecific differences in the response of WUE to N (Smith and van den Driessche 1992).

We studied the effects of N supply on broad-leaved (poplar; *Populus × euroamericana* (Dole) Guinier) cuttings and conifer (Douglas-fir; *Pseudotsuga menziesii* (Mirb.) Franco) seedlings grown in large pots to allow free root development. In an earlier study (Ripullone et al. 2003), we observed interspecific differences in the photosynthesis–N relationship. At comparable N supply rates, poplar showed twofold higher photosynthetic nitrogen-use efficiency (PNUE) and a threefold steeper slope of the maximum assimilation rate ( $A_{\max}$ )–N relationship, primarily reflecting a higher investment of N in the photosynthetic apparatus compared with Douglas-fir. Our specific objectives in the present work were to: (1) evaluate the effect of N supply on WUE and biomass production; (2) assess if N influenced WUE through control of carbon assimilation rates or through stomatal control of water loss; (3) compare three estimates of water-use efficiency, namely short-term ( $WUE_i$ ), based on gas exchange measurements, and two long-term estimates ( $WUE_T$ ), one based on the ratio between biomass production and transpired water and one based on carbon isotope discrimination in leaves as a proxy of WUE.

## Materials and methods

### Experimental design, plant material and growth conditions

The experiment comprised potted Douglas-fir seedlings in a greenhouse and potted poplar stem cuttings in an adjacent nursery at the University of Basilicata, Potenza, Italy. Seedlings and cuttings were planted in large pots to allow free root development and minimize the so-called pot effect (Heilman and Fu-Guang 1994, Lewis et al. 1999). Different spacing designs were applied to the species to account for the large differences in growth rate between poplar and Douglas-fir and to minimize plant competition.

In March 2000, 2-year-old Douglas-fir seedlings were selected for uniformity in size (height =  $41.7 \pm 0.5$  cm, mean  $\pm$  standard error,  $n = 144$ ), planted in nine cylindrical pots (diameter 130 cm, height 70 cm) according to a square spacing design (28  $\times$  28 cm; 16 seedlings per pot) and placed in a greenhouse. Throughout the experiment, the greenhouse maintained day/night temperatures and relative humidity at 30/19 °C and 60%, respectively. In June 2000, stem cuttings of poplar were selected for uniformity (height =  $20.4 \pm 0.5$  cm, mean  $\pm$  standard error,  $n = 30$ ), planted in nine large squared parallelepiped pots (side length = 220 cm, height 70 cm) according to a square spacing design (58  $\times$  58 cm; 16 plants per pot) and placed in a nursery. In the nursery, the cuttings were covered with a transparent plastic roof to prevent natural rainfall reaching the soil. Day/night temperatures and relative humidity were in the ranges 20–30/15–18 °C and 40–60%, respectively, over the entire experiment. All pots were filled with sieved (2 mm) siliceous sand; a 5-cm-thick layer of perlite was stratified on the top surface to minimize evapora-

tion from the soil. Clear days prevailed during the experiment with a photosynthetic photon flux (PPF) of about 900  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at the leaf level.

Three fertilization treatments were applied: low (LN), intermediate (MN) and high (HN) N supply; three pots were assigned randomly to each treatment. Nitrogen was supplied as ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) in solution (pH adjusted to 5.5 for Douglas-fir and 6.5 for poplar). To account for differences in growth rates between species, calculation of total amounts of N to be supplied in different treatments was based on initial tissue N content and expected biomass increase. Each week throughout the growing season, Douglas-fir plants were supplied with 0.006 g (LN), 0.02 g (MN) and 0.04 g (HN) of N; poplar plants were supplied with 0.285 g (LN), 0.95 g (MN) and 1.9 g (HN) of N. Macroelements in the solution were adjusted with respect to the amount of N supplied as suggested by Ingestad (1979).

All plants were well watered throughout the experiment by adding known amounts of water to each pot. At the beginning of the experiment, dimensions of Douglas-fir plants and poplar stem cuttings assigned to the different treatments were not statistically different.

### Biomass measurements

Dry mass production was estimated as the difference in total dry mass at the beginning (March 2000) and the end (November 2000) of the experiment. Initial biomass of each poplar stem cutting was assumed to be equivalent to the mean dry matter of the plant. Initial biomass of the Douglas-fir seedlings was estimated by randomly sampling 20 plants from the population of seedlings from which the experimental plants were selected. For determination of final biomass, 12 plants per species in each treatment (four plants per replicate) were sampled at the end of the experiment. Belowground and aboveground components were separated, and aboveground biomass was divided into stem and leaves. Biomass components were dried at 70 °C for 48 h and then weighed.

### Soil water and transpiration measurements

Soil water content ( $W_s$ ) was measured every 7–10 days by time domain reflectometry (TDR). The apparent dielectric constant of the soil was measured at three positions in each pot with a Tektronix reflectometer (Model 1502B, Tektronix, Corvallis, OR). The instrument was connected to the ends of three 11.4-cm-long stainless steel cylindrical probes, 0.6 mm in diameter, fully inserted into the soil 5 cm apart. Readings of soil dielectric constant were converted to a measure of soil water content as described by Topp and Davis (1985).

Transpiration from each pot was calculated as follows:

$$E_T = I - (E_S + \Delta W_S) \quad (1)$$

where  $E_T$  is transpiration,  $I$  is water added,  $E_S$  is evaporation from the soil and  $\Delta W_S$  is the change in soil water content over the time interval. Based on  $\Delta W_S$  values of three pots containing no plants,  $E_S$  was found to be negligible. Given the relative plant homogeneity, we assumed that, over the experimental

period, plants contributed equally to transpiration from each pot. Therefore, water consumption of the four plants sampled for biomass measurements at the end of the experiment was assumed to be one fourth of the total transpiration from the pot.

#### Gas exchange measurements

In early October 2002, leaf gas exchange was measured with a portable infrared gas analyzer (CIRAS 1, PP Systems, Hitchin, U.K.) in 26 Douglas-fir plants (nine from each of the LN and HN treatments and eight from the MN treatment) and 14 poplar plants (six from each of the LN and HN treatments and two from the MN treatment). Only plants growing in the central portion of each pot were sampled, and measurements were made on one current-year fully illuminated shoot or leaf per plant, chosen in the upper crown portion. Gas exchange of Douglas-fir shoots were measured with a conifer-type chamber (PLC-conifer, PP Systems), and a broadleaf type chamber (PLC-broad, PP Systems) that enclosed 3.24 cm<sup>2</sup> of leaf surface was used for poplar leaves. Chambers were illuminated by halogen lamps, supplying a PPF of 1600 μmol m<sup>-2</sup> s<sup>-1</sup> at the shoot or leaf level. For Douglas-fir, lamps were positioned to minimize within-shoot shading. The following parameters were recorded after steady-state conditions had been attained for 5 min at ambient CO<sub>2</sub> concentration (370 μmol mol<sup>-1</sup>): net carbon assimilation (*A*), intercellular CO<sub>2</sub> concentration (*C<sub>i</sub>*), stomatal conductance (*g<sub>s</sub>*), transpiration (*E*) and leaf temperature (*T*). The temperature inside the cuvette was maintained at 26 ± 2 °C for Douglas-fir and 24 ± 2 °C for poplar. Light-saturated photosynthesis at 370 μmol mol<sup>-1</sup> (ambient CO<sub>2</sub>) was considered as maximum assimilation rate (*A<sub>max</sub>*). After the gas exchange measurements, needle area (projected) was determined with an LI-3000 (Li-Cor, Lincoln, NE) area meter. All photosynthetic parameters were expressed on a projected area basis.

#### Leaf N determinations

Immediately after the gas exchange measurements, 30 current-year Douglas-fir needles and four poplar leaves were collected from the upper part of the crown of 12 plants from each treatment (plants from gas exchange measurements were included), dried at 70 °C for 48 h and then finely ground for analysis of total N concentration according to the Kjeldahl method.

#### Carbon isotope discrimination analysis

About 1 mg of powder from the same leaf samples prepared for N analysis was used for determination of carbon isotope composition. Isotope ratios (<sup>13</sup>C/<sup>12</sup>C) were measured with a continuous flow triple collector isotope ratio mass spectrometer (CF-IRMS, ISOCHROM II VG Isotech, Middlewich, U.K.) connected to a Dumas-combustion elemental analyzer (NA-1500, Carlo Erba, Milan, Italy). All data were referenced to the standard PDB, a limestone fossil of *Belemnitella americana* from the Cretaceous Pee Dee formation in South Carolina, USA (Boutton 1991). The relative abundance of <sup>13</sup>C

in plant material was expressed in terms of carbon isotope composition ( $\delta_p$ ), according to the relationship:

$$\delta_p = \frac{R_s - R_b}{R_b} \quad (2)$$

where *R<sub>s</sub>* and *R<sub>b</sub>* refer to the <sup>13</sup>C/<sup>12</sup>C ratio in the sample and in PDB, respectively (Craig 1957). Carbon isotope discrimination ( $\Delta$ ), a factor related to isotope fractionation by the photosynthetic process relative to the source carbon, was estimated as:

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \quad (3)$$

where  $\delta_a$  is isotope composition of atmospheric CO<sub>2</sub>, which we assumed to be -8.0‰ (Farquhar et al. 1989a, Guehl et al. 1995) for both the greenhouse and the adjacent nursery. It is a reasonable assumption given the small differences in environmental conditions between the two sites, the effective air circulation and the small photosynthetic and respiratory biomass contained in the greenhouse.

#### Water-use efficiency

We estimated WUE by three methods. Instantaneous water-use efficiency (WUE<sub>i</sub>) was calculated from gas exchange measurements on individual plants, as the ratio of maximum carbon assimilation at saturating light (*A<sub>max</sub>*) to leaf transpiration rate (*E*) (Dang et al. 1991, Zhang and Marshall 1994). Long-term water-use efficiency (WUE<sub>T</sub>) was calculated, per pot, as the ratio between amount of total dry mass produced between the beginning and end of the growing season and water transpired during the same period, calculated by Equation 1. A long-term integrated estimate of WUE was also made based on  $\Delta$  analysis of leaf dry matter. This parameter provides an estimate of WUE integrated over the period of leaf structural carbon fixation (Farquhar et al. 1989b, Guehl et al. 1994, Osorio and Pereira 1994). The relationship between WUE and  $\Delta$  arises through their independent linkages to the ratio of internal to ambient CO<sub>2</sub> concentrations (*C<sub>i</sub>/C<sub>a</sub>*):

$$\Delta = a + \frac{(b - a)C_i}{C_a} \quad (4)$$

and

$$\text{WUE} = \frac{C_a(1 - C_i/C_a)}{1.6v} \quad (5)$$

where *C<sub>i</sub>* is intercellular CO<sub>2</sub> concentration, *C<sub>a</sub>* is ambient CO<sub>2</sub> concentration, *a* is the fractionation occurring as a result of diffusion of CO<sub>2</sub> in air (4.4‰), *b* is the net fractionation mainly caused by Rubisco in C<sub>3</sub> plants (27‰), and *v* is the water vapor pressure difference between the intercellular spaces and the atmosphere.

### Statistics

Effects of N treatments and species were assessed by factorial ANOVA and means were compared by Student-Newman-Keuls test. Slopes of regression equations were compared according to Gomez and Gomez (1984). All statistics were computed with the SPSS statistical package (SPSS, Chicago, IL).

### Results

Nitrogen supply increased leaf N concentration expressed on an area basis ( $N_a$ ) in both species (Table 1), although poplar had higher  $N_a$  than Douglas-fir. Similarly, biomass production was significantly increased by N supply in both species (Figure 1), with a much greater biomass response in poplar ( $r^2 = 0.75$ ) than in Douglas-fir ( $r^2 = 0.25$ ). The relative increase in total plant biomass in the HN treatment versus the LN treatment was also more pronounced in poplar (52%) than in Douglas-fir (41%). In contrast, there were no significant differences in biomass partitioning to root and shoot components between N treatments (Table 1).

Both  $WUE_i$  and  $WUE_T$  generally increased with increasing N supply in both Douglas-fir and poplar (Figure 2). In contrast, no effect of N supply on either transpiration or stomatal conductance was observed (Table 2). As a result of the increases in  $A_{max}$  with increasing N supply,  $WUE_i$  values of HN plants of Douglas-fir and poplar were about 13 and 11% higher, respectively, than the corresponding values for LN plants. The HN treatment increased  $WUE_T$  by 38% in Douglas-fir and 43% in poplar compared with values in the LN treatment (Table 2). The ratio between  $WUE_T$  and  $WUE_i$  was remarkably constant irrespective of the species and treatment (Table 2), with a mean value of 2.1 for Douglas-fir and 2.0 for poplar.

Carbon isotope discrimination was negatively related to  $N_a$  in Douglas-fir ( $r^2 = 0.22$ ,  $P < 0.01$ ) and poplar ( $r^2 = 0.23$ ,  $P < 0.01$ ; Figure 3). Values of  $\Delta$  were higher in poplar than in Douglas-fir in all treatments and were significantly lower in the HN treatment than in the LN treatment in both species (Table 2). There were no significant differences in  $\Delta$  between the HN and MN treatments in either Douglas-fir or poplar.

In both species, there was a negative relationship between  $WUE_i$  and  $\Delta$  ( $r^2 = 0.27$ ,  $P < 0.01$  in Douglas-fir, and  $r^2 = 0.22$ ,  $P > 0.1$  in poplar, Figure 4a), but this relationship was statistically significant in Douglas-fir only. In poplar,  $WUE_T$  was

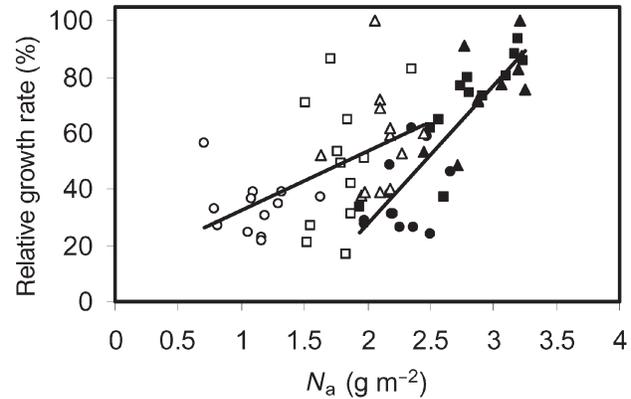


Figure 1. Relationship between relative growth rate (%) and leaf N concentrations ( $N_a$ ) in Douglas-fir (open symbols) and poplar (filled symbols). Symbols represent different N treatments: LN, circles; MN, squares; and HN, triangles. The regression relationships are: Douglas-fir,  $y = 21.20x + 11.32$  ( $r^2 = 0.25$ ,  $P < 0.01$ ); and poplar,  $y = 49.89x - 72.31$  ( $r^2 = 0.75$ ,  $P < 0.01$ ). Each value represents an individual plant.

strongly correlated with  $\Delta$  ( $r^2 = 0.91$ ,  $P < 0.01$ ), following an expected negative trend (Figure 4b). In Douglas-fir, the relationship between  $WUE_T$  and  $\Delta$  was negative but not significant ( $r^2 = 0.28$ ,  $P = 0.176$ ). There was a strong positive relationship between  $WUE_T$  and  $WUE_i$  ( $y = 0.74x - 1.10$ ,  $r^2 = 0.84$ ,  $P < 0.02$ ).

### Discussion

The greater increase in total plant biomass in poplar than in Douglas-fir in response to increasing N supply paralleled the pattern observed at the foliar level, where the slope of the  $A_{max}$ -N relationship was threefold steeper for poplar than for Douglas-fir, reflecting mainly a higher N investment in the photosynthetic apparatus (Ripullone et al. 2003). Douglas-fir reduced the proportion of foliar N allocated to photosynthetic components (Rubisco and light harvesting complex) in response to increased N availability, whereas poplar maintained this proportion constant. Compared with deciduous trees, conifers generally show a smaller increase in amounts of photosynthetic enzymes (especially Rubisco) in response to enhanced N supply (Wullschlegel 1993).

Biomass partitioning was unaffected by N supply in either

Table 1. Leaf N concentration on a leaf area basis ( $N_a$ ), total dry biomass produced and root to shoot dry biomass ratio in Douglas-fir and poplar seedlings grown in three N treatments (LN = low N supply; MN = intermediate N supply; HN = high N supply). Mean values not sharing common letters are significantly different, according to Student-Newman-Keuls test ( $P < 0.05$ ). Values of  $N_a$  were derived from previous experimental work (Ripullone et al. 2003).

	Douglas-fir			Poplar		
	LN	MN	HN	LN	MN	HN
$N_a$ ( $g\ m^{-2}$ )	1.11 a	1.80 b	2.10 c	2.28 a	2.80 b	3.05 c
Plant dry biomass (g)	23.3 a	34.6 b	39.6 b	199.6 a	377.2 b	417.2 b
Root/shoot dry biomass	0.66 a	0.71 a	0.62 a	0.28 a	0.20 a	0.25 a

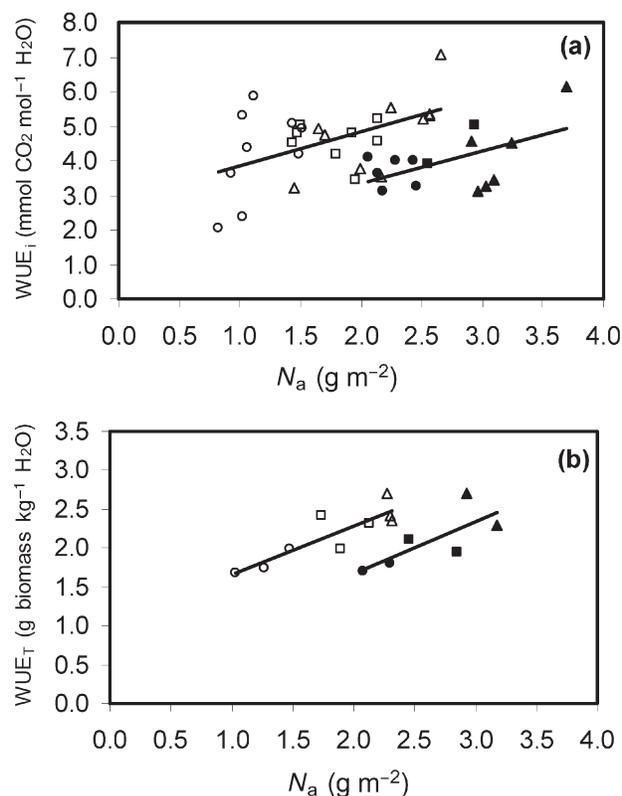


Figure 2. Relationship between (a) instantaneous water-use efficiency ( $WUE_i = \text{mmol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ) or (b) long-term water-use efficiency ( $WUE_T$ , dry biomass plant produced (g) per kg water used) and leaf N concentrations ( $N_a$ ) in Douglas-fir (open symbols) and poplar (filled symbols). Symbols represent different N treatments: LN, circles; MN, squares; and HN, triangles. The regression relationships are: (a) Douglas-fir,  $y = 0.97x + 2.89$  ( $r^2 = 0.22$ ,  $P < 0.02$ ), poplar,  $y = 0.93x + 1.48$  ( $r^2 = 0.28$ ,  $P < 0.05$ ); and (b) Douglas-fir,  $y = 0.62x + 1.04$  ( $r^2 = 0.76$ ,  $P < 0.01$ ), poplar,  $y = 0.65x + 0.37$  ( $r^2 = 0.56$ , not significant, line not shown). Each value represents an individual plant in panel (a), and the mean of a replicate in panel (b).

species. This contrasts with the findings of Walters and Reich (1989) on *Ulmus americana* L. and Pregitzer et al. (1990) on two hybrid poplars (*Populus*  $\times$  *euramericana* cv. Eugenei and *P. tristis*  $\times$  *P. balsamifera*) in which shoot/root ratio increased

in response to N fertilization under both well-watered and water-limited conditions. Similarly, Shaw et al. (1998) reported that Douglas-fir seedlings grown at high N supply allocate a significantly larger proportion of their carbon to the shoot compared with seedlings grown at lower N supply. In contrast, Grassi and Minotta (2000) observed that well-watered *Picea abies* plants growing on sandy soil substrate, as in our study, were not stimulated to produce more roots in response to a decrease in N supply.

The three methods used to estimate WUE yielded similar results. Nitrogen availability increased WUE in both species. Similar findings have been reported for *U. americana* (Reich et al. 1989), *Eucalyptus* spp. (Sheriff 1992), maritime pine (Guehl et al. 1995), *Pinus radiata* D. Don (Sheriff and Mattay 1995), poplar clones (Liu and Dickmann 1996), white spruce (Livingston et al. 1999) and *Quercus robur* L. (Welander and Ottosson 2000). In these studies, the increase in WUE was related to the effects of enhanced leaf N concentrations on both  $g_s$  and  $A_{\text{max}}$ , with  $g_s$  increasing less than  $A_{\text{max}}$ . In contrast, other studies (e.g., *Eucalyptus* spp. (Stark 1992), pedunculate oak (Guehl et al. 1995), *P. radiata* (Korol et al. 1999), Douglas-fir (Chapman 1983, Hinckley et al. 1992) and poplar (Siegwolf et al. 2001)) have shown no change in WUE with increased N supply. In these studies, the relationship between the carbon supply through the stomata and carbon demand by the mesophyll was unaffected by changes in foliar N concentration. This is likely to be a non-causal link because of the parallel development of mesophyll and epidermis or because of partial control of stomata by mesophyll functions (Wong et al. 1979, 1985).

Although species-specific mechanisms may modulate the effect of N availability on WUE, such contrasting results suggest that our understanding of the mechanisms involved in the response of stomatal conductance to N availability is incomplete (Blackman and Davies 1985, Grassi et al. 2002). There is evidence that soil N availability may have a direct effect on stomatal conductance, independently of its effect on leaf N concentration, mediated by hormonal signals from roots to leaves (Radin et al. 1982, Chapin 1991, Bowmann and Conant 1994).

The strong positive relationship between  $WUE_T$  and  $WUE_i$  indicates that, at least under the relatively stable environmen-

Table 2. Maximum assimilation rate at saturating light ( $A_{\text{max}}$ ), leaf evapotranspiration rate ( $E$ ), stomatal conductance ( $g_s$ ), instantaneous water-use efficiency ( $WUE_i = A_{\text{max}}/E$ ), long-term water-use efficiency ( $WUE_T$ ) and leaf carbon isotope discrimination ( $\Delta$ ) in Douglas-fir seedlings and poplar cuttings grown in three N treatments (LN = low N supply; MN = intermediate N supply; and HN = high N supply). Mean values not sharing common letters are significantly different, according to Student-Newman-Keuls test ( $P < 0.05$ ).

	Douglas-fir			Poplar		
	LN	MN	HN	LN	MN	HN
$A_{\text{max}}$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	4.76 b	5.17 ab	5.26 a	12.13 b	14.10 ab	14.73 a
$E$ ( $\text{mmol m}^{-2} \text{ s}^{-1}$ )	1.28 a	1.15 a	1.15 a	3.33 a	3.28 a	3.68 a
$g_s$ ( $\text{mmol m}^{-2} \text{ s}^{-1}$ )	245.0 a	276.7 a	202.7 a	349.2 a	328.5 a	394.7 a
$WUE_i$ ( $\text{mmol CO}_2 \text{ mol H}_2\text{O}^{-1}$ )	4.20 b	4.57 a	4.81 a	3.69 b	4.46 a	4.16 a
$WUE_T$ ( $\text{g DM kg}^{-1} \text{ H}_2\text{O}$ )	1.81 c	2.24 b	2.49 a	1.75 c	2.02 b	2.50 a
$\Delta$ (%)	19.37 b	18.73 a	18.76 a	22.55 b	22.25 a	22.10 a

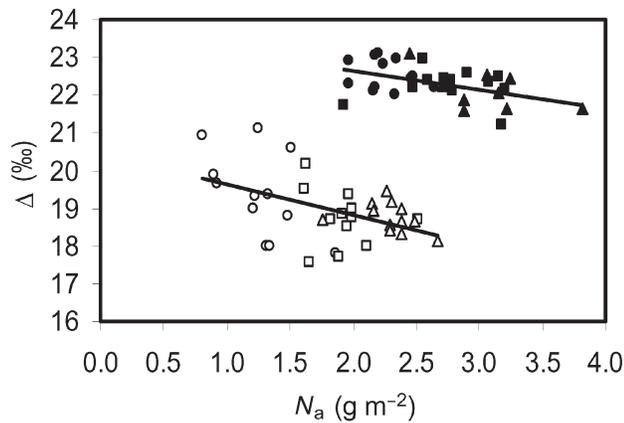


Figure 3. Relationship between leaf carbon isotope discrimination ( $\Delta$ ) and leaf N concentrations ( $N_a$ ) in Douglas-fir (open symbols) and poplar (filled symbols). Symbols represent different N treatments: LN, circles; MN, squares; and HN, triangles. The regression relationships are: Douglas-fir,  $y = -0.79x + 20.41$  ( $r^2 = 0.22$ ,  $P < 0.01$ ); and poplar,  $y = -0.49x + 23.63$  ( $r^2 = 0.23$ ,  $P < 0.01$ ). Each value represents an individual plant.

tal conditions of our experiment, short-term measurement ( $A_{\max}/E$ ; transpiration efficiency) is a relevant component in integrating long-term WUE. When considering WUE at the plant level, however, additional carbon and water loss have to be considered in addition to those occurring during the photosynthetic carbon gain and the transpiration process. Because respiration of non-photosynthetic organs throughout the day and respiration of autotrophic tissues in the dark affect plant carbon balance and non-transpirative (cuticular and lenticellular) water losses influence plant water economy (Farquhar et al. 1989b),  $WUE_T$  is expected to be lower than  $WUE_i$ . The  $WUE_i/WUE_T$  ratio was close to 2 in both Douglas fir and poplar, indicating a reduction of about 50%, irrespective of species and treatment, from instantaneous transpiration efficiency to long-term whole-plant water-use efficiency. This reduction is likely attributable to the requirements for maintenance respiration, which appeared to be similar in the two species, at least under the tested conditions. According to McCree (1986) and Guehl et al. (1995) about 30 to 50% of assimilated carbon is respired.

Theoretically, the imposition of differential N treatments accompanied by any gradual effects on the ratio of exposed mesophyll cell surface to leaf area, and likely also on the surface area of chloroplasts exposed to intercellular airspace, would have important effects on mesophyll conductance to  $CO_2$  diffusion (Evans and Loreto 2000) that would affect both instantaneous and long-term WUE. In our study species, however, we previously demonstrated that N supply has no effect on leaf mass per area (Ripullone et al. 2003).

In both species, we observed a decrease in  $\Delta$  with increasing leaf N concentrations, confirming previous reports (Bender and Berge 1979, Gebauer et al. 1987, Warren et al. 2001). This response may be associated with the higher carboxylation ca-

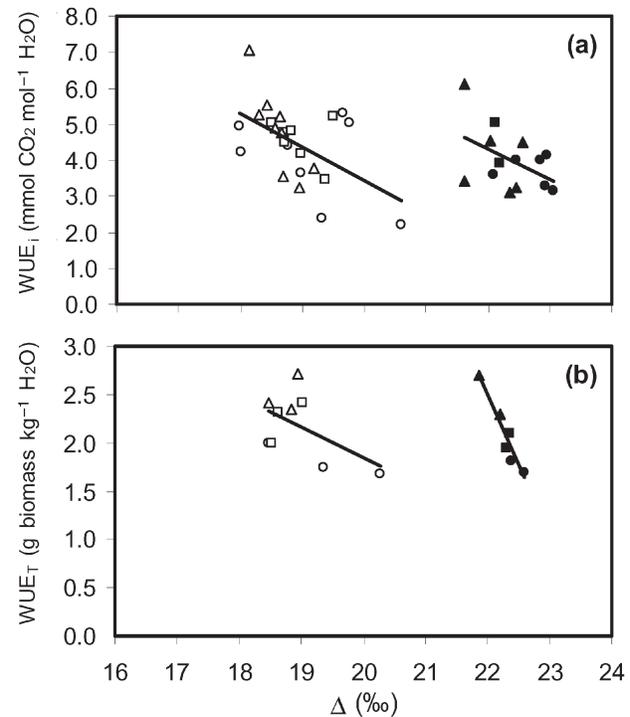


Figure 4. Relationship between (a) instantaneous water-use efficiency ( $WUE_i$ ) or (b) long-term water-use efficiency ( $WUE_T$ ) and leaf carbon isotope discrimination ( $\Delta$ ) in Douglas-fir (open symbols) and poplar (filled symbols). Symbols represent different N treatments: LN, circles; MN, squares; and HN, triangles. The regression relationships are: (a) Douglas-fir,  $y = -0.92x + 21.80$  ( $r^2 = 0.27$ ,  $P < 0.01$ ); poplar,  $y = -0.85x + 22.98$  ( $r^2 = 0.22$ , not significant); and (b) Douglas-fir,  $y = -0.31x + 8.09$  ( $r^2 = 0.28$ , not significant); poplar,  $y = -1.42x + 33.68$  ( $r^2 = 0.91$ ,  $P < 0.01$ ). Each plotted value represents an individual plant in panel (a) and the mean of a replicate in panel (b).

capacity and the lower  $C_i/C_a$  of the HN-treated plants (Ripullone et al. 2003). The response may also be related to a modification of the resistance to  $CO_2$  diffusion in the water phase (Stitt and Schulze 1994).

An increase in mesophyll conductance could be associated with elevated foliar N concentrations, which might result in increased photosynthetic rates, without affecting stomatal conductance and transpiration (Grassi et al. 2002). No link between stomatal and mesophyll conductance was found in maritime pine (Guehl et al. 1995) or in shrubs and grasses in a cold desert environment (Toft et al. 1989). However, in these studies, the increase in WUE was the result of a negative effect on stomatal conductance and there was no effect of increasing leaf N concentration on photosynthesis.

A comparison of the methodologies used to assess WUE highlighted some differences. By combining Equations 4 and 5, we obtained a simplified version of the relationships linking  $WUE_i$  to  $\Delta$  (for review see Farquhar et al. 1989b); however,  $\Delta$  of leaf dry matter represents a long-term assimilation weighted integration of  $C_i/C_a$ , and so it cannot be simply related to  $WUE_i$ . To relate  $\Delta$  to  $WUE_T$ , the proportions of respiratory carbon losses and non-transpirative water losses should

be considered. In this experiment, the proportion of non-transpirative water losses is limited to cuticular and lenticellular losses because evaporation from the soil was negligible. Therefore, we assumed that most of the observed reduction in  $WUE_T$  with respect to  $WUE_i$  was related to the amount of carbon lost through whole-plant respiration, which amounted to about 50% for both species in the three N treatments. In this case, by combining Equations 4 and 5 and solving for  $v$  based on data from Table 2, we estimated that Douglas-fir had a 65% higher long-term vapor pressure ( $v$ ) difference than poplar. This implies that any long-term difference in intrinsic WUE suggested by substantial difference in  $\Delta$  between the study species would be accounted for by the environmental control on WUE exerted by  $v$ . That is, the higher intrinsic WUE of Douglas-fir compared with poplar would be reduced when expressed as  $WUE_T$  because of a higher long-term leaf-to-air vapor pressure difference caused by the differences in plant structure and leaf morphology between the species.

Poplar had higher  $\Delta$  values than Douglas-fir in all N treatments, presumably because of higher mesophyll conductance in broad-leaved species than in conifer species (Warren et al. 2001). Although the difference may also be associated with differences in leaf chemistry between the species, we conclude that this is unlikely for the following reasons. If it is assumed that cellulose is the predominant compound in foliage, then a difference as large as 3‰ in  $\Delta$  could be attributed only partially to specific variations in leaf chemistry. Furthermore, possible effects of metabolic compounds such as resins and terpenoids (which are particularly abundant in conifer needles and more depleted in  $^{13}C$  than carbohydrates because of fractionation during the fatty acid biosynthetic pathway) would reduce the isotopic composition of conifer species, resulting in higher  $\Delta$  values. This implies that the observed differences in  $\Delta$  may be somewhat underestimated. Differences between broad-leaved trees and conifers in the carbohydrates stored in foliage might also affect the isotopic signals retained in whole-leaf dry matter (Oliver Brendel, personal communication). On the other hand, because our experimental conditions were relatively stable, it seems unlikely that the large differences in  $\Delta$  observed between the two species could be mostly caused by differences in the proportion and isotopic composition of nonstructural carbohydrates, which should retain an isotopic signature relatively similar to that of the structural carbon of earlier assimilation.

Instantaneous water-use efficiency and  $\Delta$  were negatively correlated in Douglas-fir and in poplar, although the correlation was not significant in poplar. This linear negative relationship is in agreement with the results of many studies under both controlled laboratory and field conditions (Farquhar et al. 1982, 1989b, Zhang et al. 1993, Guehl et al. 1994, Aitken et al. 1995, Warren et al. 2001), confirming that  $\Delta$  is a useful complement to the short-term evaluation of WUE by gas exchange measurements.

In conclusion, elevated N supply increased both biomass production and WUE. Gas exchange measurements revealed that the mechanisms underlying the response of WUE to N

supply were mainly related to the positive effect of N on photosynthetic rates (Ripullone et al. 2003), because no influence of N supply on stomatal conductance or transpiration was observed in either species. Short-term and long-term estimates of WUE showed convergent patterns and similar relationships with N supply. Furthermore, we confirmed that carbon isotope discrimination is a useful proxy of WUE, thus providing a long-term measure of physiological activity.

#### Acknowledgments

Research was supported by the Italian National Project COFIN-1999 'Produttività e uso delle risorse idriche in ecosistemi forestali: potenzialità applicative delle tecniche di frazionamento isotopico di carbonio, ossigeno e idrogeno' and by funds from 'Dottorato di ricerca in arboricoltura da legno' (University of Basilicata). We gratefully thank Lioudmila Alexeeva, Santina Dapoto, Antonio Lapolla, Mario Pompeo and Mimma Zuardi for technical help and laboratory assistance. Thanks also to Federico Magnani, Giuseppe Montanaro and Giuseppe Celano for stimulating discussions.

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