Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides* × *Populus nigra*

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Summary

• We examined the relationships among productivity, water use efficiency (WUE) and drought tolerance in 29 genotypes of *Populus* × *euramericana* (*Populus deltoides* × *Populus nigra*), and investigated whether some leaf traits could be used as predictors for productivity, WUE and drought tolerance.

• At Orléans, France, drought was induced on one field plot by withholding water, while a second plot remained irrigated and was used as a control. Recorded variables included stem traits (e.g. biomass) and leaf structural (e.g. leaf area) and functional traits [e.g. intrinsic water use efficiency (W_i) and carbon isotope discrimination (Δ)].

• Productivity and Δ displayed large genotypic variability and were not correlated. Δ scaled negatively with W_i and positively with stomatal conductance under moderate drought, suggesting that the diversity for Δ was mainly driven by stomatal conductance.

• Most of the productive genotypes displayed a low level of drought tolerance (i.e. a large reduction of biomass), while the less productive genotypes presented a large range of drought tolerance. The ability to increase WUE in response to water deficit was necessary but not sufficient to explain the genotypic diversity of drought tolerance.

Key words: carbon isotope discrimination (Δ), drought tolerance, genotypic diversity, hybrid poplars, leaf traits, open field, productivity, water use efficiency (WUE).

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Introduction

Poplars are among the fastest growing trees in temperate latitudes. Their high productivity is associated with large water requirements. As a consequence, productivity depends closely on water availability (Ceulemans *et al.*, 1988; Tschaplinski & Blake, 1989; Tschaplinski *et al.*, 1994; Zsuffa *et al.*, 1996). In Europe, the most frequently used poplars are *Populus* × *euramericana* (*Populus deltoides* × *Populus nigra*) hybrids, and their cultivation is restricted to flood plains and bottomlands where water availability is not a limiting factor for growth. Consequently, commercial genotypes have been selected primarily on criteria such as high productivity, adequate wood properties and tolerance to pathogens such as foliar rusts. The global warming expected at the end of the 21st century will produce an increased probability of drought episodes, larger vapour pressure deficits and in general more frequent and more severe extreme climatic events (Saxe *et al.*, 2001). The counterbalancing effects of increasing water deficit and rising CO_2 concentration are the main factors expected to modify forest production (Loustau *et al.*, 2005). The effects of climate change are predicted to be the most severe in short rotation forestry such as poplar plantations, and the severity of the effects is expected to decrease for less intensively managed forests with longer revolutions and low productivities (Loustau *et al.*, 2005). Furthermore, to sustain the extension of poplar cultivation from flood plains and bottomlands to uplands, where soil water availability is subject

to seasonal shortages, would require hybrids with greater water use efficiency (WUE). Thus, it seems to be increasingly important to add traits of drought tolerance and WUE to the panels of criteria already used in poplar selection.

The concept of water deficit tolerance, when applied to cultivated tree species such as poplars, has been defined as the ability to limit the decrease of biomass production in response to a moderate water deficit (Passioura, 2002). Despite the fact that poplar species are among the most susceptible woody plants to drought, significant genotypic variability has been recorded in water deficit tolerance and patterns of response to water deficit (Ceulemans *et al.*, 1978; Pallardy & Kozlowski, 1981; Gebre & Kuhns, 1991; Liu & Dickmann, 1996; Chen *et al.*, 1997; Marron *et al.*, 2002, 2003). This variability encompasses several physiological and morphological traits, including stomatal sensitivity to water deficit (Liu & Dickmann, 1992; Blake *et al.*, 1996; Harvey & van den Driessche, 1997; Marron *et al.*, 2002), the potential for osmotic adjustment (Gebre *et al.*, 1994; Marron *et al.*, 2002), the sensitivity of leaf expansion, the extent of leaf abscission, and adjustment of the root : shoot ratio (Liu & Dickmann, 1992; Chen *et al.*, 1997; Ibrahim *et al.*, 1997; Tschaplinski *et al.*, 1998; Marron *et al.*, 2003). Water use efficiency (WUE) is defined at the whole-plant level as the ratio between biomass production and water consumption; it is difficult to measure as such. One functional trait that could be of interest as an index for improved or maintained productivity under reduced water availability is intrinsic water use efficiency (W_i), i.e. the ratio between net CO_2 assimilation and stomatal conductance. W_i can be indirectly estimated, via carbon isotope discrimination (Δ), assuming the occurrence of a linear and negative correlation between Δ and W_i as evidenced in cereals (Farquhar & Richards, 1984; Farquhar *et al.*, 1989) and trees (Ponton *et al.*, 2002). The identification of genotypes combining satisfactory productivity and high WUE would be a considerable advantage in moderately drought-constrained areas, as pointed out by Braatne *et al.* (1992) for poplar and by Condon *et al.* (2002) and Rebetzke *et al.* (2002) for cereals.

Δ shows great genotypic diversity among poplar species and is highly heritable (Rae *et al.*, 2004; Marron *et al.*, 2005; Monclus *et al.*, 2005). Nevertheless, relationships between productivity and Δ differ among studies: a positive relationship has been found for *Populus davidiana* (Dode) Schneider (Zhang *et al.*, 2004) while no link has been shown for *Populus* × *interamericana* Brockh. (Rae *et al.*, 2004). Thus, selection for high WUE may result in lower or higher productivity according to the predominant factor influencing variations of WUE for a considered species (Condon *et al.*, 2002). Previous studies with *P.* × *euramericana* hybrids did not show any relationship between productivity and Δ (Marron *et al.*, 2005; Monclus *et al.*, 2005). Productive genotypes were characterized by a large total leaf area and water use efficient genotypes were characterized by a large leaf nitrogen content (N_M), at least in the field (Marron *et al.*, 2005; Monclus *et al.*, 2005). The lack of correlation between productivity and Δ opens a way to select genotypes combining high productivity and high WUE. This lack of correlation also suggests that the intergenotype variability for WUE could be controlled by stomatal conductance (Farquhar *et al.*, 1989). However, the negative relationship between Δ and N_M could reflect the fact that the diversity for Δ may also be partly driven by photosynthetic capacity (Brugnoli & Farquhar, 2000).

The results described for *P.* × *euramericana* hybrids were obtained from experiments performed under well-watered conditions. In this context, the objectives of the present study were: (1) to test the impact of a moderate water deficit on productivity and on carbon isotope discrimination on a panel of *P.* × *euramericana* hybrids; (2) to highlight the relationships among Δ, net CO_2 assimilation, stomatal conductance, and W_i, and (3) to test the relationships among productivity, WUE and drought tolerance. These objectives have been addressed using a field trial comprising two plots with randomized blocks established during 2001 at 15-m spacing. Water deficit was induced during spring 2004 by withholding irrigation from one plot while the second plot, which was regularly irrigated, acted as a control. The second plot had already been analysed in detail in 2003 (Monclus *et al.*, 2005). Thus, a corollary of this study was the possibility to test the temporal stability (2004 vs 2003) of genotypic ranking for productivity, Δ, and leaf traits under well-watered conditions. Stem variables encompassed biomass, length and circumference and leaf traits consisted of maximal individual leaf area, carbon and nitrogen contents, specific leaf area, stomatal density, Δ, net CO_2 assimilation rate, photosynthetic capacity, stomatal conductance and W_i.

Materials and methods

Plant material and growth conditions

Homogeneous 25-cm-long woody-stem cuttings of 29 *Populus deltoides* Bartr. ex Marsh. × *Populus nigra* L. genotypes were planted during January 2001 in a homogeneous open field at INRA Orléans (47°46′ N, 1°52′ E; 110 m above sea level) on a loamy sand soil (pH = 5.9) without fertilizer addition (see Appendix Table A1 for a detailed list of the genotypes and their origins). Two plots were established at 15-m spacing. Each plot was divided into five randomized complete blocks (one cutting of each genotype per block). Inside each plot, trees were planted at 0.5 × 1.2 m spacing. Two border rows of cuttings from the genotypes I214 and Branagesi surrounded each plot. Cuttings were coppiced at the end of 2001, 2002 and 2003. During 2004, bud flush occurred synchronously for all genotypes during the first week of April. Systematic pruning on 18 May 2004 left only one, 40-cm-high shoot on each stool. New shoots and branches of the main stem (sylleptics) were regularly removed during the experiment in order to produce stable continuous growth and

to minimize the variability potentially induced by different numbers of shoots per stool. The two plots were irrigated every second day (20 mm of water) with overhead sprinklers, from bud flush to 15 June 2004, the beginning of the experiment. From 15 June 2004 to the end of the growing season, a water deficit was induced by irrigation cessation on one of the plots while the second was kept irrigated and served as a control.

Environmental conditions and soil water potential

From 15 June 2004 to the end of the growing season, daily environmental conditions, in terms of irradiance ($J\ m^{-2}\ d^{-1}$), temperature (minimum, mean and maximum, $°C\ d^{-1}$), potential evapotranspiration (Penman; $mm\ d^{-1}$) and rainfall ($mm\ d^{-1}$), were measured with a meteorological recording station (Xaria; Degreane Horizon, Cuers, France) that was located near the experimental plot. Soil water availability was followed by measuring predawn leaf water potential (Ψ_{wp}, MPa) on a mature leaf with a Scholander-type pressure chamber. Homogeneity of soil water availability within each plot was tested by checking for the spatial stability of Ψ_{wp} in all cuttings from four genotypes: I45-51, Soligo, 2000_verde and Luisa_Avanzo (n = 20 plants per plot).

Stem and leaf traits recorded on all genotypes

From 15 June 2004 to 30 September 2004, stem length (*Lstem*, cm) and circumference 1 m above ground level (*Cir*, mm) were measured once a week from all cuttings of each genotype. On 7 January 2005, stems were coppiced and final stem length ($Lstem_F$, cm), circumference (Cir_F, mm) and fresh biomass ($Biom_F$, g FW) were recorded.

On 10 September 2004, one fully illuminated mature leaf, which presented the largest width (Foliar Index 17) (Monclus *et al.*, 2005), was collected on each cutting and photocopied in order to measure maximal individual leaf area (LA_{max}, cm^2). Photocopies were scanned and leaf area was estimated with an image analyser (UTHSCSA IMAGE TOOL program developed at the University of Texas Health Science Center at San Antonio, TX, USA, and available by anonymous FTP at http://ddsdx.uthscsa.edu/dig/itdesc.html). Six calibrated discs of lamina ($2\ cm^2$) were cut from this leaf; leaf discs were then dried and weighed, and specific leaf area (*SLA*, $cm^2\ g^{-1}$) was computed. Leaf carbon isotope composition ($\delta^{13}C$) and C and N concentrations were assessed for each cutting and genotype from 1-mg homogeneous dry powder obtained from calibrated discs. A continuous flux isotope ratio mass spectrometer (Delta S; Finnigan MAT, Bremen, Germany) was used for ^{13}C analyses. Carbon isotope composition was calculated relative to the Pee Dee Belemnite standard as in Craig (1957): $\delta^{13}C = [(R_{sa} - R_{sd})/(R_{sd}) \times 1000$ [‰], where R_{sa} and R_{sd} are the $^{13}C : {}^{12}C$ ratios of the sample and the standard, respectively. The discrimination between atmospheric CO_2 (δ_{air}, assumed to be close to −8‰) and plant material (δ_{plant}) was calculated as $\Delta = [\delta_{air} - \delta_{plant})/(1 + (\delta_{plant}/1000)]$ according to Farquhar & Richards (1984). Total carbon and nitrogen contents (C_M and N_M) were obtained with an elemental analyser on the same samples (NA 1500NC; Carlo Erba, Italy) and were expressed on a dry weight basis ($mg\ g^{-1}$ DW).

Leaf functional traits related to WUE

Photosynthetic capacity, net assimilation, stomatal conductance, W_i and stomatal density were measured in two groups of four genotypes selected for their contrasting carbon isotope discrimination ($\Delta^{13}C$, ‰) based on data collected during 2003 on the same plot in well-watered conditions (Monclus *et al.*, 2005): Eco_28, Robusta, Cima and Luisa_Avanzo showed high Δ values, while Pannonia, Agathe_F, Flevo and I45-51 showed low Δ values.

Photosynthetic capacity

In order to estimate the maximal carboxylation rate (V_{cmax}, $\mu mol\ CO_2\ m^{-2}\ s^{-1}$) and maximal light-driven electron flow (J_{max}, $\mu mole^-\ m^{-2}\ s^{-1}$), measurements of net CO_2 assimilation rate (A) vs internal CO_2 concentration (C_i) were taken on well-watered plants with a portable open infrared gas analyser (CIRAS-2; PP System, Hitchin, UK). Photon flux was maintained at $1500\ \mu mol\ m^{-2}\ s^{-1}$ and temperature at 25°C. Before measurements, photosynthesis was induced and the stomatal aperture was maximized by decreasing the CO_2 concentration in the leaf chamber ($200\ \mu mol\ mol^{-1}$) for about 30 min. A was measured at the following CO_2 mole fractions: 200, 400, 300, 250, 150, 100, 50, 500, 750, 1000, 1250, 1500 and 1800 $\mu mol\ mol^{-1}$. V_{cmax} and J_{max} were computed from the A/C_i relationships (Dreyer *et al.*, 2001).

Net CO_2 assimilation, stomatal conductance and W_i

These parameters were estimated both under well-watered and under water deficit conditions on a clear day (8 September 2004), near midday, on a fully illuminated mature leaf. Net CO_2 assimilation rates (A, $\mu mol\ CO_2\ m^{-2}\ s^{-1}$) and leaf conductance ($g_s$, $mmol\ H_2O\ m^{-2}\ s^{-1}$) were measured using a portable open infrared gas analyser (CIRAS-2) fitted to a leaf chamber operated at $200\ ml\ min^{-1}$. The leaf chamber covered an exposed area of $2\ cm^2$. The CO_2 concentration inside the chamber was kept at 400 ppm and stomatal distribution was fixed to be equal between the adaxial and abaxial sides. Ambient conditions in terms of radiation, temperature and evaporative demand were recorded. Readings were taken after steady-state conditions had developed (*c.* 1 min). Carbon dioxide and water vapour concentration differences between inlet and outlet gas circulating through the leaf chamber, as well as leaf temperatures obtained from energy balance equations, were used to calculate A,

transpiration (T) and g_s on a leaf area basis using von Caemmerer & Farquhar's equations (von Caemmerer & Farquhar, 1981). W_i was computed as the ratio between A and g_s.

Stomatal density

On 10 September 2004, two calibrated discs of lamina (2 cm^2) were cut from one fully illuminated mature leaf (Foliar Index 17) of each cutting, frozen in liquid nitrogen and stored at −80°C. Samples were washed in a mixed tetrahydrofurane/toluene [50/50 volume/volume (v/v)] solution for 30 s and were stuck to aluminium stubs on a Peltier stage (−50°C). They were then examined under a variable pressure scanning electron microscope (model 1450VP; Leo, Cambridge, UK). Backscattered secondary electron images were observed at an accelerating voltage of 20 kV, a probe intensity of 1 nA and a working distance of 10 mm, at ×180 magnification. Digital images of each sample (abaxial and adaxial sides) were captured from two areas on the leaf discs.

Analysis of stomatal density was carried out using the VISILOG 6.3 program (Noesis, Courtaboeuf, France) executing a macro command. The threshold to distinguish between stomata and leaf matrix in the image was obtained by an interactive image-segmentation based on selected image grey-level ranges that resulted in a binary image (stomata black and background white). Binary filter operations were performed on the recorded images with a single erosion step to remove pixel noise; finally, a dilatation operation was applied to compensate for the erosion steps before the stomatal number was calculated. The total number of stomata within an image was determined by counting, using the convention of excluding parts of stomata overlapping the margins of the image, and was converted to stomata per mm^2. Total stomatal density (*TSd*) was computed by summing adaxial and abaxial stomatal densities.

Statistical analyses

Results were evaluated by linear regression, linear correlation (Pearson's coefficients), rank correlation (Spearman's coefficient), and analysis of variance (ANOVA) using the SPSS statistical software package (SPSS, Chicago, IL, USA). For each variable, the normality of the distribution was tested by a Shapiro–Wilk test. All statistical tests were considered significant at $P \leq 0.05$. Genotypic means are expressed with their confidence interval ($\alpha = 0.05$) or their standard deviation.

In order to study the genotypic variability of traits on all genotypes in this experiment under well-watered conditions, we performed multivariate analyses using principal components analysis (PCA). The variables measured in well-watered conditions ($Biom_F$, $Lstem_F$, Cir_F, LA_{max}, N_M, C_M, *SLA* and Δ) were standardized and orthogonal factors (= F1 and F2 axes) were successively built as linear combinations of these variables to maximize the part of the variability explained by these factors. Variables were first represented on the plane defined by the two main factors of the PCA; their coordinates were their linear correlation coefficients (Pearson's coefficient) with these factors. Variables measured in water deficit conditions were projected as supplementary variables in the main plane F1 × F2 defined by variables measured in well-watered conditions. Coordinates of variables measured in water deficit conditions were obtained by computing linear correlations between these variables and scores F1 and F2 obtained from PCA measured in well-watered conditions.

Results

Genotypic diversity and correlations among variables under well-watered conditions

Substantial genotypic diversity was recorded in the well-watered plot for all tested variables (Table 1). $Biom_F$ differed between genotypes along a 3-fold range. N_M of mature leaves, LA_{max} and *SLA* ranged from 23 to 33 mg g^{-1}, 133.6 to 461.3 cm^2 and 107 to 145 cm^2 g^{-1}, respectively (Fig. 1). Δ differed by 2.7‰ among genotypes.

A general PCA was performed with the genotypic means of the variables recorded on all genotypes (Fig. 2a, Table 1). The main plane of the PCA (F1 × F2) explained 66% of the genotypic diversity, with 44% for F1 alone, while the F3 axis did not differentiate the genotypes (data not shown). Two independent groups of variables were identified from the F1 × F2 plane. The first group included $Biom_F$, $Lstem_F$, Cir_F and LA_{max} and the second group included *SLA* and Δ. In each group the variables were positively correlated (Table 2). The F1 axis of the PCA was only defined by the first group ($Biom_F$, $Lstem_F$, Cir_F and LA_{max}), while the F2 axis opposed N_M to *SLA* and Δ. There was no clear grouping among genotypes in the F1 × F2 plane (Fig. 2b). Genotypes NL-3972, Lambro and Mella displayed high productivity, Soligo and Mellone_Caro had high values of Δ, and I45-51 and Agathe_F showed both high productivity and low values of Δ. Interestingly, we were unable to detect any correlation between productivity (as estimated from $Biom_F$, $Lstem_F$ or Cir_F) and Δ under these conditions (Table 2).

Inter-annual stability of genotypic features

The data recorded during the present experiment (2004) were compared with those recorded on the same experimental plot under well-watered conditions during 2003 (Monclus *et al.*, 2005). Genotypic diversity was of the same magnitude during the two years for leaf Δ, leaf C_M and *SLA*, while it was lower during 2004 than during 2003 for N_M and LA_{max} (Fig. 1). Rank correlations (Spearman's coefficient) for leaf traits

Table 1 Abbreviations, descriptions and values of variables used in the study

		Genotypic diversity				Drought effect	
		WW		WD		WW vs WD	
Variable	Description	General mean ($IC_{\alpha=0.05}$)	P_{WW}	General mean ($IC_{\alpha=0.05}$)	P_{WD}	P	r_s
Stem Productivity							
$Biom_F$	Fresh biomass (g FW)	945 (106)	***	623 (80)	***	***	0.60**
$Lstem_F$	Final stem length (cm)	367 (12)	***	313 (12)	***	***	0.51**
Cir_F	Final stem circumference (mm)	74 (4.0)	***	63 (3.9)	***	***	0.67***
Leaf Structure							
LA_{max}	Maximal individual leaf area (cm^2)	264.5 (30.5)	***	223.2 (27.6)	***	***	0.78***
N_M	Leaf nitrogen content per dry mass (mg g^{-1} DW)	27 (1.2)	***	25 (1.0)	***	**	0.67***
C_M	Leaf carbon content per dry mass (mg g^{-1} DW)	431 (3.0)	***	430 (3.3)	***	ns	0.71***
SLA	Specific leaf area (cm^2 g^{-1} DW)	130 (3.0)	***	121 (3.4)	***	***	ns
Function							
A	Net assimilation rate (μmol CO_2 m^{-2} s^{-1})	20.3 (2.5)	***	20.4 (1.9)	**	ns	0.88**
V_{cmax}	Maximal carboxylation rate (μmol CO_2 m^{-2} s^{-1})	127.2 (27.2)	***	nd			
J_{max}	Maximal light driven electron flow (μmol e^- m^{-2} s^{-1})	195.3 (32)	***	nd			
g_s	Stomatal conductance (mmol H_2O m^{-2} s^{-1})	647.7 (41.7)	**	461.3 (49.5)	ns	***	0.88**
TSd	Total stomatal density (mm^{-2})	757 (117)	***	769 (119)	***	ns	0.93**
W_i	Intrinsic water-use efficiency (mmol mol^{-1})	0.031(0.003)	***	0.045(0.004)	ns	***	ns
Δ	Leaf carbon isotopic discrimination (‰)	21.63 (0.23)	***	21.33 (0.19)	***	*	0.41*

The variables *A*, V_{cmax}, J_{max}, g_s, *Tsd* and W_i were recorded on eight genotypes selected for their contrasting Δ measured during 2003. The other variables were recorded on the full set of 29 genotypes. All variables were recorded on trees in the two treatments [except for V_{cmax} and J_{max}, which were not determined (nd) in water deficit conditions]. Variables with the subscript F were recorded on 7 January 2005. For each variable and each treatment, well-watered (WW) vs water deficit (WD) conditions, general means, confidence intervals (CI; $\alpha = 0.05$), and genotypic diversity (ANOVA, P_{WW} and P_{WD}) are indicated. The effects of drought on each variable and on genotypic ranking were estimated by ANOVA (*P*) and rank correlations (Spearman's coefficient, r_s), respectively. Levels of significance are indicated by asterisks: $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$ and by 'ns' for nonsignificant values. DW, dry weight.

(LA_{max}, N_M, C_M, *SLA* and Δ) showed that the genotypic ranking remained stable from 2003 to 2004 for all variables except *SLA* (Fig. 1). Values of *SLA* and Δ were significantly higher in 2004 than 2003, while values of LA_{max} were significantly lower in 2004 than 2003; these differences can be ascribed to differences in climate, 2003 having been warmer, with larger vapour pressure deficits, although the poplars were optimally irrigated and no drought stress occurred on the control plots in either year (Fig. 1a, Table 3). The differences could also have been related to a seasonal effect (earlier collection during 2003) or, most probably, to a combination of both seasonal and climatic effects. N_M was lower during 2004, possibly because of decreased nitrogen availability in the soil (Fig. 1). The genotypic ranking remained stable between 15 July 2003 and 7 January 2005 for biomass ($r_s = 0.58$, $P \leq 0.01$) and circumference ($r_s = 0.70$, $P \leq 0.001$), but not for stem length. During both years, biomass was positively correlated with stem length and circumference and maximal individual leaf area, but not with Δ ($r = -0.23$; $P = 0.23$) (Table 2). The negative correlation observed between Δ and N_M during 2003 was again detected during 2004, but was less significant ($P = 0.08$). Δ and *SLA* scaled positively during 2004 only (Table 2).

Impact of a moderate water deficit on genotypic diversity and correlations among variables

Withholding irrigation during the whole summer of 2004 induced significant decreases in Ψ_{wp} as compared with controls (Fig. 3a), with peak differences at the beginning of September. Ψ_{wp} remained high in controls (above −0.30 MPa) while it declined to −0.60 MPa in drought-stressed individuals. The spatial and genotypic stabilities of Ψ_{wp} were checked by comparing values from four different genotypes and individuals spread over the whole experimental area; no spatial or genotypic differences were detected. We may state from these observations that the drought was moderate but long-lasting (several weeks), with a peak during September, and that it was of similar intensity and duration in all genotypes over the whole experimental plot. The impact of water deficit on stem length and circumference became significant from 15 July 2004 and led to a significant decrease

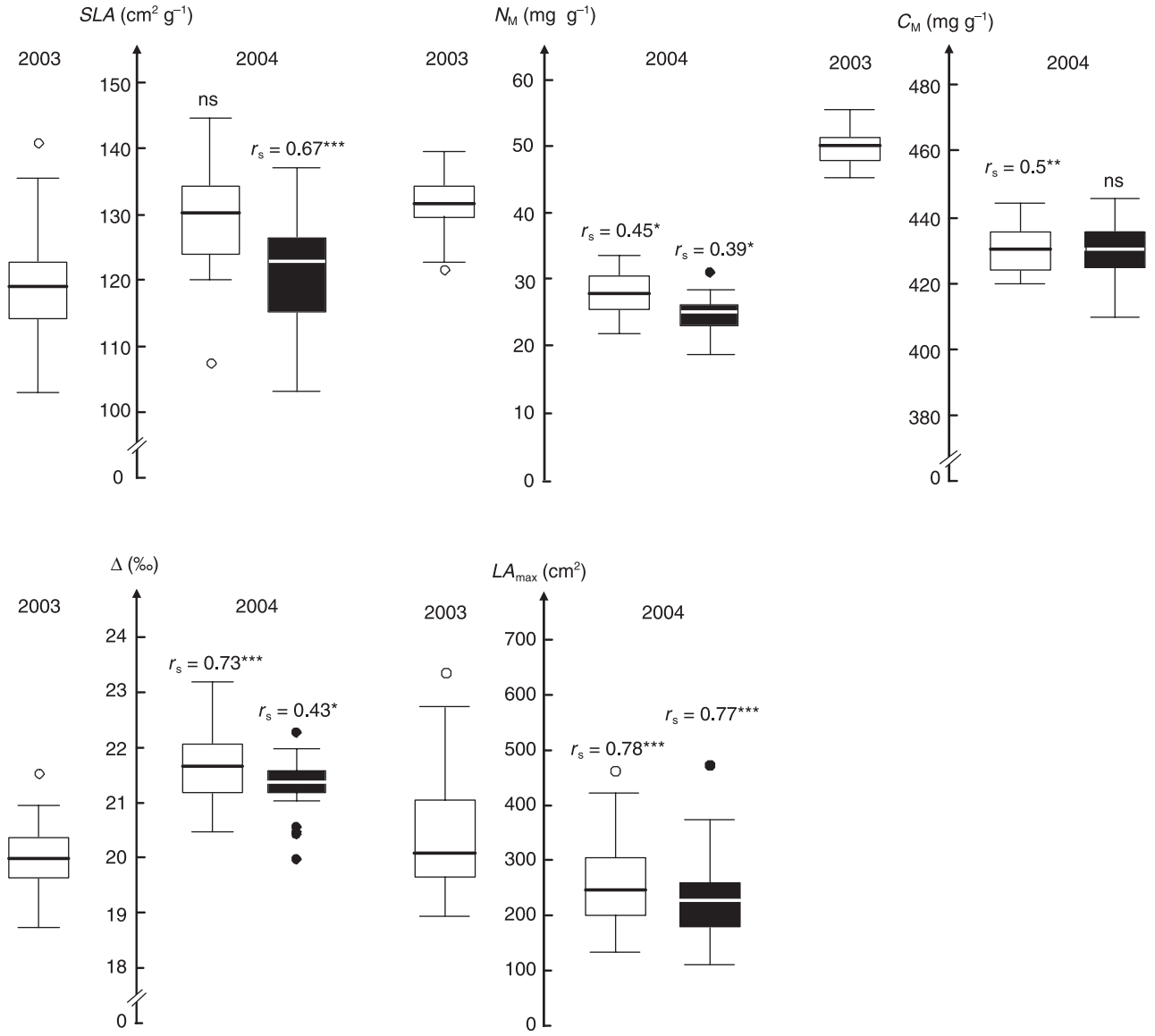


Fig. 1 Genotypic diversity and interannual stability of leaf traits in *Populus* × *euramericana* (*Populus deltoides* × *Populus nigra*). Box plots are shown of specific leaf area (*SLA*, $cm^2\ g^{-1}$), leaf nitrogen and carbon contents (N_M and C_M, $mg\ g^{-1}$), carbon isotope discrimination (Δ,‰) and maximal individual leaf area (LA_{max}, cm^2) recorded in 29 genotypes under well-watered conditions during 2003 (Monclus *et al*., 2005) and 2004 (white boxes) and under a moderate water deficit during 2004 (black boxes). Each box represents the quartile below (Q1) and above (Q3) the median value. Vertical bars represent minimum and maximum values except for genotypes that are away from 1.5 times from the top of the interquartile (Q3 – Q1) range. Values outside this range are represented as circles. Genotypic ranking was compared between 2003 and 2004 by rank correlations (Spearman's coefficient, r_s). Levels of significance are: $*P \le 0.05$; $**P \le 0.01$; $***P \le 0.001$; ns, nonsignificant.

in biomass production for most of the genotypes as recorded on 7 January 2005 (Figs 3b,c,4a, Table 1). While the reductions in Ψ_{wp} were comparable among cuttings, the impact on biomass differed widely among genotypes (Fig. 4a). For example, I45-51 and Soligo displayed the same level of productivity under well-watered conditions and were subjected to similar water deficit intensities, as attested by similar maximal decreases in Ψ_{wp} (−0.50 and −0.52 MPa, respectively). Nevertheless, they differed substantially in reduction in biomass in response to drought (Fig. 4a): I45-51 showed similar productivities in the two treatments, while a 50% decrease was observed for Soligo. It is noteworthy that most of the productive genotypes were very sensitive to drought (i.e. showed a large reduction in biomass), while the less productive genotypes showed a large range of biomass decreases, i.e. of drought tolerance.

In general, leaf variables such as LA_{max}, *SLA*, N_M and Δ were affected by drought, and the expected changes occurred (i.e. decreases in LA_{max}, *SLA*, N_M and Δ) (Fig. 1, Table 1). Although no correlation has been previously reported

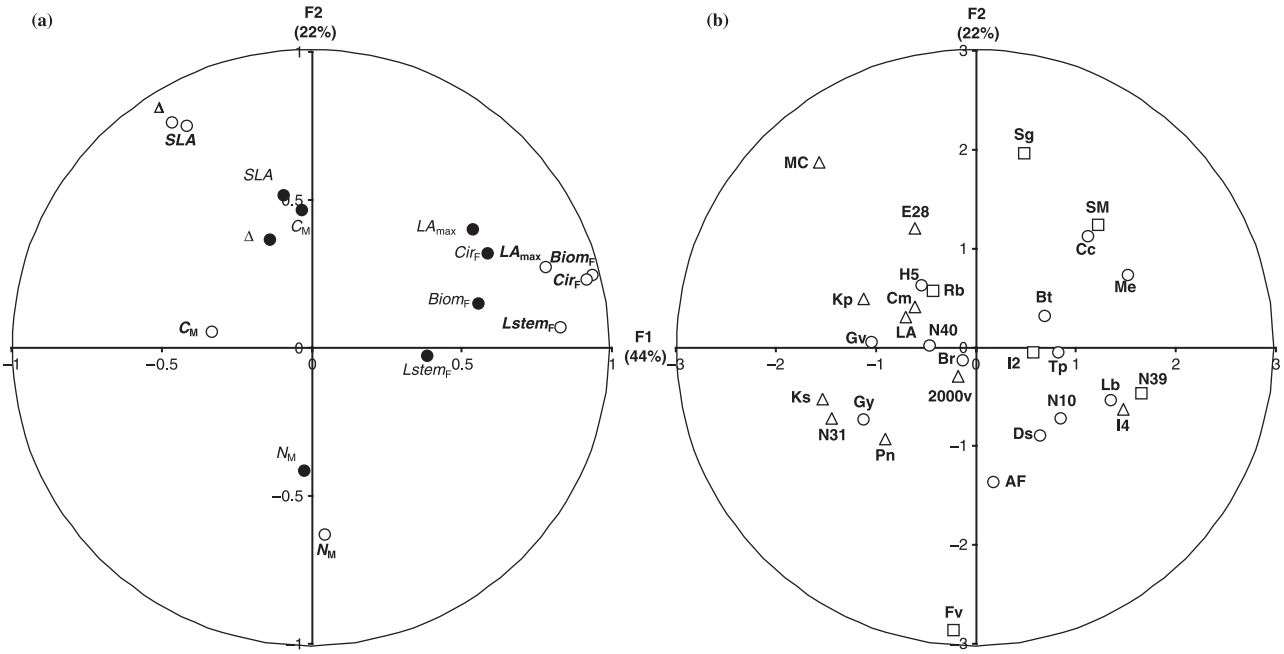


Fig. 2 Factorial analysis of genotypic diversity. The distribution of 16 variables (a) and the projection of 29 poplar [*Populus* × *euramericana* (*Populus deltoides* × *Populus nigra*)] genotypes (b) are shown in the main factorial plane F1 × F2 of a principal components analysis (PCA). F1 and F2 are linear combinations of the eight variables measured under well-watered conditions (open circles) and they were constructed to maximize the explained fraction of data variability. Variables recorded under water deficit conditions (closed circles) were projected as supplementary variables on the main F1 × F2 plane. See Table 1 for definitions of variable abbreviations. Genotype abbreviations: 2000v, 2000_verde; AF, Agathe_F; Br, Branagesi; Bt, Brenta; Cc, Carpaccio; Cm, Cima; Ds, Dorskamp; E28, Eco_28; Fv, Flevo; Gv, Gaver; Gy, Ghoy; H5, H523–9; I2, I214; I4, I45–51; Kp, Kopecky; Ks, Koster; Lb, Lambro; LA, Luisa_Avanzo; Me, Mella; MC, Mellone_Caro; N10, NL-1070; N31, NL-3149; N39, NL-3972; N40, NL-4040; Pn, Pannonia; Rb, Robusta; SM, San_Martino; Sg, Soligo; Tp, Triplo.

Table 2 Linear correlations (Pearson's coefficients) computed for well-watered (below the diagonal line) and for water deficit (above the line) conditions between variables from 29 (bold, $n = 29$) or eight (not bold, $n = 40$) genotypes

	$Biom_F$	$Lstem_F$	Cir_F	LA_{max}	SLA	N_M	C_M	Δ	A	V_{cmax}	J_{max}	g_s	TSd	W_i
$Biom_F$		**0.85*****	**0.95*****	**0.68****						nd	nd		–0.55**	
$Lstem_F$	**0.81*****		**0.76*****	**0.49***		**0.40***				nd	nd			
Cir_F	**0.94*****	**0.70*****		**0.66****						nd	nd		–0.59***	
LA_{max}	**0.78*****	**0.66****	**0.81*****							nd	nd			
SLA								**0.51****		nd	nd			
N_M										nd	nd		–0.50**	
C_M										nd	nd			
Δ					**0.72*****	**–0.33**$^{0.08}$				nd	nd	0.45**	0.54**	–0.49**
A										nd	nd	0.71***		
V_{cmax}	0.33$^{0.05}$	0.42*									nd			
J_{max}		0.39*			–0.35*	0.41*			0.45**	0.8***				
g_s									0.48**					–0.77***
TSd	–0.36*		–0.54**			–0.38*		0.36*						
W_i									0.83***		0.43**		–0.33*	

V_{cmax} and J_{max} were not determined in water deficit conditions (nd). The level of significance is indicated by asterisks: *$P \le 0.05$; **$P \le 0.01$; ***$P \le 0.001$. See Table 1 for definitions of variables.

between leaf traits measured in well-water conditions and the level of drought tolerance, genotypic variability in the decrease in LA_{max} was strongly correlated with genotypic variability in drought tolerance (Fig. 4b). However, a link has not been shown between the decrease of LA_{max} in response to drought and both LA_{max} and above-ground biomass measured under well-watered conditions (data not shown). Interestingly, 23 genotypes among the 29 studied decreased or tended to

	25 June to 15 July 2003	22 August to 10 September 2004	*P*
Daily maximum temperature (°C d^{-1})	27.7	24.8	***
Daily minimum temperature (°C d^{-1})	15.0	12.2	**
Daily mean temperature (°C d^{-1})	21.3	18.5	***
Evapotranspiration (mm d^{-1})	5.0	3.8	***
Daily irradiance (J cm^{-2} d^{-1})	2184	1715	**

Significant differences (ANOVA) are indicated by asterisks: $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.

Table 3 Climate (air temperature, Penman evapotranspiration and daily irradiance) during the expansion of the leaves collected during 2003 and 2004

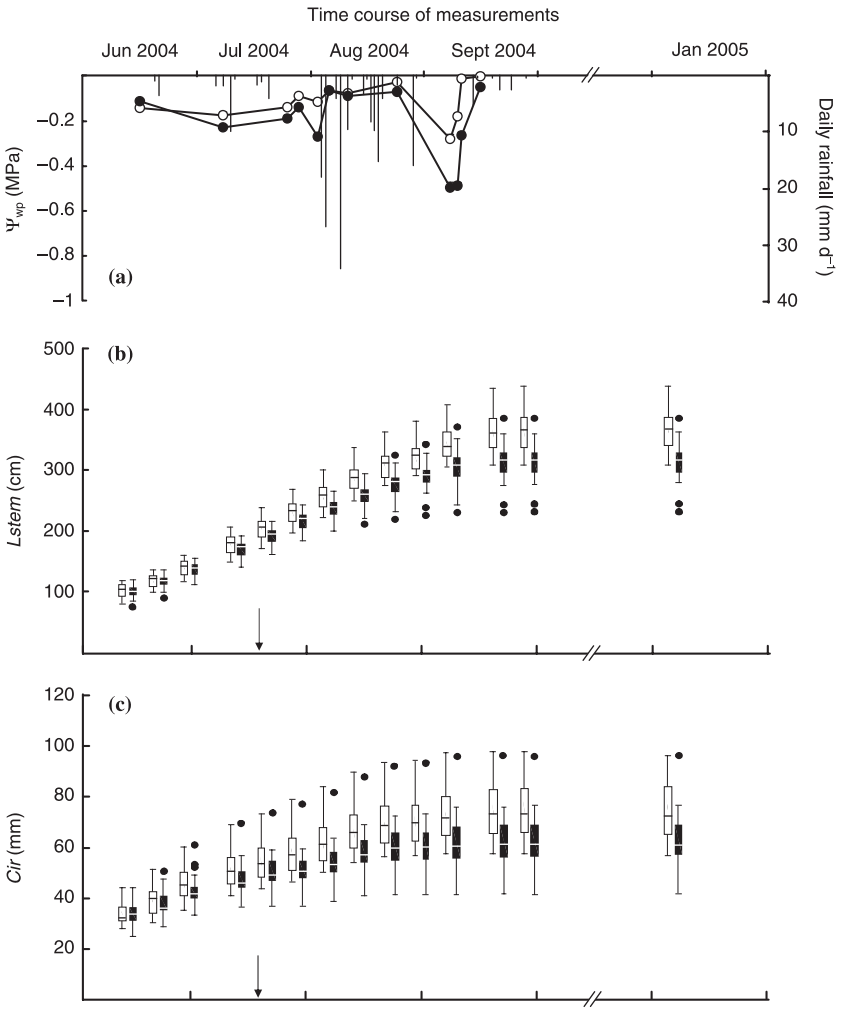
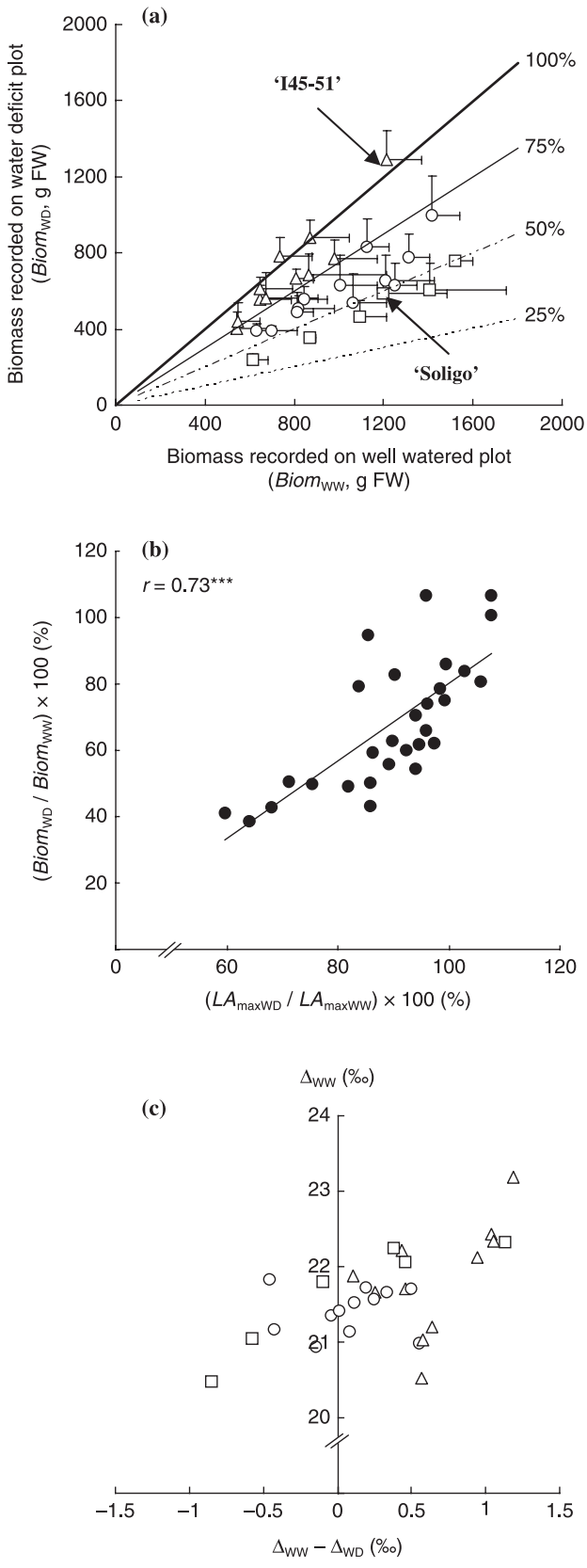


Fig. 3 Seasonal dynamics of drought stress. (a) Time course of predawn leaf water potential (Ψ_{wp}, MPa; circles) and daily rainfall (mm d^{-1}; vertical bars) during the experiment. Predawn leaf water potential was measured on 20 well-watered (open circles) and drought-stressed (close circles) cuttings (means with standard deviations). (b and c) Time course of stem height (*Lstem*, cm) and circumference (*Cir*, mm) in 29 genotypes in well-watered (white boxes) and watered deficit (black boxes) conditions. Each box represents the quartile below (Q1) and above (Q3) the median value. Vertical bars represent minimum and maximum values except for genotypes that are away from 1.5 times from the top of the interquartile (Q3 – Q1) range. Values outside this range are represented as circles. Arrows indicate the date from which the stem height and circumference of drought-stressed genotypes differed significantly from those of well-watered genotypes.

decrease their Δ in response to drought (Fig. 4b). Of the six genotypes that tended to increase Δ, none was drought tolerant (triangles, Figs 4a,c). Conversely, the whole range of drought tolerance levels was observed among those for which Δ decreased or tended to decrease (Fig. 4c). Although most of the variables were affected by drought, the genotypic diversity remained comparable to that observed under well-watered conditions (Fig. 1, Table 1). Furthermore, the moderate drought constraint did not induce any significant change in genotypic ranking for variables related to productivity and for leaf variables such as LA_{max}, N_M and Δ. *SLA* was the only variable for which genotypic ranking was significantly modified by drought (Table 1). It is noteworthy that genotypic ranking for *SLA* did not differ between well-watered conditions during 2003 and moderate water deficit conditions during 2004 (Fig. 1).

Under both control and drought conditions, Δ did not correlate with biomass, while it was positively correlated with



SLA (Table 2). The projection of variables measured after water deficit on the main plane of the previous PCA resulted in the same grouping of variables as in controls, and, within each group, variables remained positively correlated (Fig. 2a, Table 2). Taking into account the finding that drought led to decreases in productivity and in Δ, the relative positions of variables of drought plants vs control plants allow us to identify an axis related to genotypic productivity and another related to Δ. In this context, the F1 axis can be regarded as the productivity axis, but F2 differs slightly from a Δ axis, for which an inclined axis would be more appropriate.

Relationship between Δ and other leaf functional variables

Under well-watered conditions, great genotypic diversity was recorded for all functional variables (Table 1): W_i (= A/g_s), A and g_s ranged from 0.028 to 0.045 (mmol mol^{-1}), from 14.4 to 27.0 (μmol m^{-2} s^{-1}) and from 515 to 712 (mmol m^{-2} s^{-1}), respectively. V_{cmax}, J_{max} and *TSd* differed among genotypes by more than a 2-fold range. Under drought, significant genotypic diversity was observed for A and *TSd* only.

Under moderate drought conditions only, a linear and negative relationship was found between Δ and W_i (Table 2). Δ scaled positively with g_s under moderate drought conditions. A and g_s were positively correlated, but W_i scaled positively with A in controls, and negatively with g_s under drought (Table 2). *TSd* scaled positively with Δ whatever the water conditions and negatively with W_i in controls. Under well-watered conditions only, J_{max} scaled positively with A, V_{cmax} and N_M and negatively with *SLA*. No correlation was observed between W_i and variables of productivity. This observation corroborates the lack of relationship described, at the genotypic level, between productivity and Δ. J_{max} and V_{cmax} scaled positively with $Lstem_F$, and V_{cmax} tended to be positively linked with biomass in well-watered conditions (P = 0.053). *TSd* scaled negatively with $Biom_F$, Cir_F and N_M whatever the water availability.

Fig. 4 Diversity of drought tolerance and relationship with water use efficiency. (a) Relationship for each genotype between fresh biomass (*Biom*, g FW) measured on 7 January 2005 on well-watered ($Biom_{WW}$) plots and that measured on water deficit ($Biom_{WD}$) plots. Means are presented with their standard deviations. Triangles, circles and squares distinguish genotypes for which the decrease in mean biomass in response to water deficit was 0–25%, 25–50% and 50–75%, respectively. (b) Linear correlation between the ratio of above-ground biomass measured in water deficit conditions ($Biom_{WD}$) to above-ground biomass measured in well-watered conditions ($Biom_{WW}$) and the ratio of leaf area measured in water deficit conditions (LA_{maxWD}) to leaf area measured in well-watered conditions (LA_{maxWW}). (c) Relationship between carbon isotope discrimination (Δ) measured on each genotype in well-watered conditions (Δ_{WW}) and the difference in Δ computed between well-watered and water deficit conditions (Δ_{WD}). Symbols (triangles, circles and squares) distinguish genotypes as for Fig. 4(a).

Discussion

Genotypic diversity for productivity, Δ and leaf traits under well-watered conditions

Great genotypic diversity was found among *Populus* × *euramericana* genotypes for all tested variables. This confirms similar observations in a glasshouse and in the field for the same panel of genotypes under optimal water supply (Marron *et al.*, 2005; Monclus *et al.*, 2005). Although genotypic ranking differed between glasshouse and field experiments (Monclus *et al.*, 2005), it did not differ for most of the variables when measurements were performed in the field during two successive years (2003 and 2004). Genotypic ranking did not differ for stem biomass and circumference, while it varied for the length of the stem, confirming that stem circumference is the more reliable and early index of genotypic productivity. Inter-annual differences in leaf Δ, LA_{max}, leaf N_M and C_M and *SLA* may be imputed, at least in part, to differences in environmental growth conditions (Niinemets *et al.*, 1998; Barbour *et al.*, 2002; Monclus *et al.*, 2005). Nevertheless, genotypic ranking was modified for *SLA* only, suggesting that the plasticity of leaf density/thickness to environmental changes is genotype dependent (Monclus *et al.*, 2005).

As previously described for *P.* × *interamericana* (Rae *et al.*, 2004) and for *P.* × *euramericana* growing in a glasshouse and in the field (Marron *et al.*, 2005; Monclus *et al.*, 2005), biomass did not correlate with Δ. This result, combined with the finding that Δ and productivity showed broad-sense heritabilities above 0.5 in *P.* × *interamericana* (Rae *et al.*, 2004) and *P.* × *euramericana* (Monclus *et al.*, 2005), suggests that there is potential for improving W_i in poplar without reducing overall productivity. Stomatal density, which was negatively correlated with biomass and circumference and positively correlated with Δ, could be used as one index for the selection of genotypes combining high productivity and WUE.

The lack of correlation between productivity and Δ suggests that the genotypic variability for WUE is controlled by stomatal conductance (Farquhar *et al.*, 1989). Nevertheless, the negative correlation observed between Δ and N_M during 2003 and 2004 suggests that genotypic diversity of photosynthetic capacity could also contribute to the intergenotype variability for WUE (Monclus *et al.*, 2005). This negative correlation is usually explained in terms of increases in concentration of chlorophyll and Rubisco in leaves resulting in a higher photosynthetic capacity per unit leaf area, a smaller concentration of carbon dioxide at sites of carboxylation for the same stomatal conductance and lower discrimination against ^{13}C (Anderson *et al.*, 2000; Macfarlane *et al.*, 2004). Δ and *SLA* scaled positively during 2004, suggesting that the variability of internal conductance to CO_2 transfer into the leaves could also contribute to intergenotype variability for WUE. This link, already demonstrated by Vitousek *et al.* (1990), has been cited as evidence of the effect on Δ of the internal conductance for CO_2 transfer. However, genotypic ranking for *SLA* differed significantly between 2003 and 2004, while it was conserved for Δ, indicating a limited contribution of *SLA* to intergenotype variability for WUE in *P.* × *euramericana*.

Impact of a moderate water deficit on productivity, Δ and leaf traits

Withholding of irrigation induced a moderate but long-lasting drought, as shown by the significant reduction in predawn leaf water potential. Moreover, the intensity of drought was homogeneous over the whole experimental plot and no genotypic effect was visible on leaf predawn water potential. Although the pruning process has produced differences between genotypes in terms of rootstock over the years, we therefore assume that all genotypes were subjected to similar stress during a large fraction of the growth season. As shown by the early deviations of both stem length and circumference increments between controls and drought-stressed plants (15 July), the water deficit persisted over a large fraction of the growth season. Because of this homogeneity in drought within and between blocks, we may state that the impact of drought on biomass production was to a large extent genotype dependent. Except for I45-51, the most productive genotypes displayed large drought-induced decreases in biomass, i.e. a low water deficit tolerance. The less productive genotypes displayed a large range of tolerance. Productive genotypes probably had higher rootstocks than less productive ones, but, because most of the productive genotypes were very sensitive to drought, we can hypothesize that the effect of the rootstock did not reduce the impact of withholding irrigation in our study.

Drought also induced significant decreases in Δ, LA_{max}, N_M and *SLA* but did not modify the genotypic ranking for productivity, Δ, LA_{max} and N_M. The genotypic ranking obtained for *SLA* under water deficit differed significantly from that obtained under irrigation in the same year but was comparable to that measured during 2003 under well-watered conditions. Taking into account the fact that *SLA* shows high plasticity to soil water content and climatic variations (Abrams *et al.*, 1990; Niinemets *et al.*, 1998; Marron *et al.*, 2002; Wright *et al.*, 2004), and that temperatures and cumulated irradiance were significantly higher during July 2003 than September 2004, we conclude that each genotype is characterized by a different range of plastic responses of *SLA*. Many other studies have found for woody species a similar impact of indices of water availability such as soil water potential and vapour pressure deficit on *SLA* and Δ (Abrams *et al.*, 1990; Damesin *et al.*, 1997; Niinemets *et al.*, 1998; Korol *et al.*, 1999; Miller *et al.*, 2001; Barbour *et al.*, 2002; Marron *et al.*, 2002; Wright *et al.*, 2004). However, similar variations of environmental conditions modified genotypic ranking for *SLA* only, suggesting that the response of Δ to water availability is less genotype dependent than that of *SLA*.

Drought did not modify the relationships between variables and no correlation was found between productivity and Δ under these conditions. Productive genotypes were very sensitive to drought (as seen from the large reduction in shoot biomass) and showed, simultaneously, a large range of Δ values. It is noteworthy that, of the genotypes for which Δ tended to increase in response to drought, none was a drought-tolerant genotype. This result suggested that the genotypic ability to increase WUE is necessary to produce a high level of drought tolerance. However, the whole range of drought tolerance levels was observed among those genotypes for which Δ decreased or tended to decrease, suggesting that the ability to increase WUE is necessary but not sufficient to explain genotypic diversity of drought tolerance among *P.* × *euramericana* hybrids. It is clear that drought tolerance it is not only governed by WUE but probably includes a lot of other traits that also contribute to the overall drought tolerance capacity of the genotype (Liu & Dickmann, 1992; Gebre *et al.*, 1994; Chen *et al.*, 1997; Ibrahim *et al.*, 1997; Tschaplinski *et al.*, 1998; Marron *et al.*, 2002, 2003). Although no direct link was detected between leaf traits measured in well-watered conditions and the level of drought tolerance of the genotypes, it is noteworthy that the magnitude of leaf area reduction is a good indicator of the magnitude of above-ground biomass reduction and so of the drought sensitivity of the genotype. The lack of correlation observed between the magnitude of leaf area reduction and leaf area or above-ground biomass measured in well-watered conditions supported the idea that there is potential for improving concomitantly productivity and drought tolerance. One of the genotypes (I45-51) displayed not only a high productivity and a low Δ, but also a rather high level of tolerance to a moderate drought constraint. This genotype clearly warrants additional attention from this point of view.

Carbon isotope discrimination (Δ) scaled negatively with W_i during drought only. This negative correlation is in agreement with the model of Farquhar (Farquhar & Richards, 1984; Farquhar *et al.*, 1989) and with previous experiments with woody species such as *Quercus robur* (Ponton *et al.*, 2002) and *Populus davidiana* (Zhang *et al.*, 2004). The lack of correlation in well-watered genotypes may be a result of the fact that we measured gas exchange at peak values of g_s during diurnal cycles. Taking into account that Δ scaled positively with stomatal conductance (g_s), these results show that the diversity for Δ is mainly driven by stomatal conductance and support our previous conclusion regarding the lack of correlation between Δ and productivity. While W_i scaled positively with A and J_{max} in well-watered conditions, no direct correlation was found between Δ and photosynthetic capacity; this again supports the important contribution of stomatal conductance rather than net CO_2 assimilation to the diversity of Δ measured in leaf tissues. However, in controls, J_{max} scaled positively with A, V_{cmax} and N_M, and N_M was negatively correlated with Δ, suggesting at least a minor contribution of photosynthetic capacity to the diversity of Δ in *P.* × *euramericana*.

In this context, additional studies are needed to determine whether these results can be extended to other poplar genetic backgrounds such as *P.* × *interamericana* and to evaluate the genetic determinism of traits related to productivity, WUE and drought tolerance in *P.* × *euramericana* and *P.* × *interamericana*. The results obtained in this study were derived from coppiced shoots that behave as plants in their juvenile growth phase. Thus genotypic diversity for productivity, WUE and drought tolerance must be validated in more mature plants with a physiology typical of older trees.

Conclusion

This study demonstrated great diversity in the levels of drought tolerance, assessed from the reduction of biomass accumulation under moderate drought, in *P.* × *euramericana* genotypes. We observed that, of the most productive genotypes, many were drought susceptible, and that the less productive genotypes displayed a large range of drought tolerance. We have shown that the magnitude of leaf area reduction under water deficit is a good indicator of the drought susceptibility of the genotype. We have also shown that the decrease of Δ in response to moderate drought is necessary but not sufficient to explain the diversity of drought tolerance in *P.* × *euramericana*. Because Δ scaled negatively with W_i and positively with stomatal conductance in moderate drought conditions, these results support the idea that diversity for Δ is mainly driven by stomatal conductance. This study confirms that productivity and leaf Δ display large genotypic variability among *P.* × *euramericana* hybrids and that genotypic differences remain stable over time in field experiments. It also confirmed the lack of correlation between productivity and Δ that was previously detected in the glasshouse and in the field under different growth conditions (Marron *et al.*, 2005; Monclus *et al.*, 2005) and thus supports the conclusion that there is potential for improving WUE in poplar without necessarily reducing overall productivity. The operational deployment of productive, water-use-efficient genotypes will reduce the cost of plantation management by reducing the need for irrigation. Interestingly, stomatal density could be used to identify genotypes combining productivity and WUE. We have identified among *P.* × *euramericana* hybrids at least one genotype combining high productivity, high WUE and drought tolerance, the cultivar I45-51; this cultivar warrants additional attention to determine its potential for afforestation in stations with moderate soil water deficits.

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Appendix

Table A1 List of abbreviations and origins of the 29 *Populus* x *euramericana* genotypes studied

Genotype	Abbreviation	Origin
Gaver	Gv	Belgium
Ghoy	Gy	Belgium
Robusta	Rb	France
H 523-9	H5	Hungary
Kopecky	Kp	Hungary
Pannonia	Pn	Hungary
Branagesi	Br	Italy
Brenta	Bt	Italy
Carpaccio	Cc	Italy
Cima	Cm	Italy
2000_verde	2000v	Italy
Eco_28	E28	Italy
I214	I2	Italy
I45-51	I4	Italy
Lambro	Lb	Italy
Luisa_Avanzo	LA	Italy
Mella	Me	Italy
Mellone_Caro	MC	Italy
San_Martino	SM	Italy
Soligo	Sg	Italy
Triplo	Tp	Italy
Dorskamp	Ds	The Netherlands
Flevo	Fv	The Netherlands
Koster	Ks	The Netherlands
NL-1070	N10	The Netherlands
NL-3149	N31	The Netherlands
NL-3972	N39	The Netherlands
NL-4040	N40	The Netherlands
Agathe_F	AF	USA