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# Is the ranking of poplar genotypes for leaf carbon isotope discrimination stable across sites and years in two different full-sib families?

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## Abstract

• **Introduction** Because of its vigorous growth, poplar can play an important role for sustainable production of woody biomass to cover renewable energy needs. Hence, the selection of suitable genotypes has to be based on relevant traits, among which intrinsic water use efficiency ( $W_i$ , estimated through leaf carbon isotope discrimination,  $\Delta$ ) may be a key trait. Besides a large genetic variation in  $\Delta$  among the frequently planted poplar hybrids, the use of  $\Delta$  in deployment or breeding programmes requires insights in

the robustness of the genotype ranking for  $\Delta$  across environments and years.

• **Methods** Two  $F_1$  full-sib families of poplar (*Populus deltoides* × *Populus nigra* and *Populus deltoides* × *Populus trichocarpa*) were grown at two sites in Europe, i.e. northern Italy and central France. For each family, leaf samples from 31  $F_1$  genotypes collected during different field studies were used (1) to assess the effect of genotype, site and year on  $\Delta$  in leaves, as well as their mutual interactions, and (2) to elucidate the relationships between  $\Delta$ , leaf morphology and tree dimensions.

• **Results** Under the well-watered conditions of our study, a low to moderate genetic variability was observed in the two poplar families. Within-family broad-sense heritability values ranged from 0 to 0.49. The ranking of genotypes for  $\Delta$  was more stable between years than between sites.

• **Conclusions** The study confirmed the occurrence of some degree of genetic variability of  $\Delta$  in the studied poplar families and the possibility to identify genotypes with low, stable  $\Delta$  values across years. However, the significant genotype-by-site interactions in our study suggest that selection for larger water use efficiency or lower  $\Delta$  in these families has to consider specific responses in different environments.

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Romain Monclus *in memoriam* (September 2006).

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Growth · Heritability · Hybrids · Leaf traits · *Populus* · Water  
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## 1 Introduction

According to the European Energy Directive, biomass will be the major renewable fuel resource by 2020 (COM 2009/28/EC). Among the numerous biomass resources, woody biomass sequesters carbon for longer periods of time and

displays a more favourable environmental balance than annual crops as it usually requires less fertilization inputs (Updegraff et al. 2004; Gasol et al. 2009). The large genetic variability in the *Populus* genus has been exploited to increase biomass yields under adequate water and nutrient supplies. In particular, poplar hybrids appeared to be an interesting option for biomass production in short-rotation coppice systems due to their strong growth vigour that, however, is associated with large water requirements (Tschaplinski et al. 1994; Souch and Stephens 1998). The question arose (1) whether there is a significant variability in water use efficiency (WUE, ratio between biomass production and water use), or at an instant scale, in intrinsic water use efficiency ( $W_i$ , ratio between net CO<sub>2</sub> assimilation and stomatal conductance to water vapour) among these highly productive hybrids; (2) to which extent  $W_i$  of the parental species combines in the hybrid progeny; and (3) whether the ranking of the hybrid genotypes for  $W_i$  remains stable among different environments and years. In drought-prone areas, poplar plantations are often irrigated, and the deployment of genotypes combining vigorous growth performance and favourable WUE (or  $W_i$ ) in these areas could save considerable amounts of water and, hence, increase the economic viability of the plantations.

Carbon isotope discrimination ( $\Delta$ ) is commonly used as a time-integrating surrogate of  $W_i$  (Farquhar and Richards 1984; Farquhar et al. 1989), high  $\Delta$  values being associated with low  $W_i$ . The occurrence of a negative and linear relationship between  $\Delta$  and  $W_i$  was confirmed among others in poplars (Monclus et al. 2006), in oaks (Roussel et al. 2009) and in a range of tropical trees (Cernusak et al. 2007), although it has been stated that the relation might differ among species (Warren and Adams 2006; Seibt et al. 2008). It is often assumed that a low  $\Delta$  (or large  $W_i$ ) comes at the expense of growth, i.e. that there is a trade-off between the two traits. In reality, the literature shows that within species, the relationship between growth performance and  $\Delta$  varies widely (Condon et al. 2004).

The genetic variability of leaf  $\Delta$  as well as the relationships between  $\Delta$  and growth were described in poplar species or hybrids (see Table 1). These studies revealed a large genetic variability of  $\Delta$  in leaves and significant effects of soil conditions or water availability on  $\Delta$ . Generally, weak genotypic correlations between  $\Delta$  and growth performance were observed. Much less is known about the genotype-by-environment ( $G \times E$ ) and genotype-by-year ( $G \times YR$ ) interactions for  $\Delta$  in poplar due to the limited availability of multiple-year common garden studies of large poplar families or populations. For instance, a significant change in genotype ranking between two environments may reduce the effectiveness of deployment for a given site if genotypes with large water use efficiency are selected on the basis of  $\Delta$  values at another site. The

trial established under the framework of the European POPYOMICs project (2003–2006) was an ideal opportunity to investigate the occurrence of such interactions. The experiment comprised two F<sub>1</sub> full-sib poplar families from two hybrid combinations (*Populus deltoides*  $\times$  *Populus trichocarpa* and *P. deltoides*  $\times$  *Populus nigra*) grown in 3 years.

In this study, we investigated whether genotypes combining low  $\Delta$  (or high  $W_i$ ) and stability between sites and years could be identified in the two full-sib poplar families. We tested the following hypotheses:

1. There is a large genetic variability for  $\Delta$  in each hybrid combination.
2.  $\Delta$  differs between sites and years, but the genotype ranking remains stable between contrasting environments and over different years.
3. There is no relationship between  $\Delta$  and biomass production irrespective of hybrid combination, site and year.

## 2 Material and methods

### 2.1 Plant material

Two F<sub>1</sub> interspecific *Populus* full-sib families sharing the same female parent were examined:

- D $\times$ N: *P. deltoides* (Bartr. ex Marsh) ‘S9-2’  $\times$  *P. nigra* (L.) ‘Ghoy’ (180 F<sub>1</sub> genotypes; Cervera et al. 1996, 2001).
- D $\times$ T: *P. deltoides* ‘S9-2’  $\times$  *P. trichocarpa* (Torr. & Gray) ‘V24’ (182 F<sub>1</sub> genotypes; Cervera et al. 1996, 2001).

The D $\times$ N and D $\times$ T crosses were made at the Research Institute for Nature and Forest (Geraardsbergen, Belgium, INBO). Thirty-one genotypes of D $\times$ N and D $\times$ T were selected to be representative of the genetic variation observed for the second-year biomass production at the Italian and French sites (Marron et al. 2007).

### 2.2 Site description

The two sites were located (1) in northern Italy (Cavallermaggiore, Po valley, 44°42′ N, 7°40′ E) and (2) in central France (Ardon, Loire valley, 47°46′ N, 1°52′ E). The elevation was 285 m for the Italian site and 110 m for the French site. During three growing seasons (2003 to 2005), the average annual temperature was only slightly lower at the French than at the Italian site (11.5°C vs. 12.1°C). The climate at the Italian site had a more continental character than the climate at the French site; the annual thermal excursion (i.e. difference between the average daily temper-

**Table 1** Literature review (1997–2010) of studies presenting leaf carbon isotope discrimination ( $\Delta$ , means $\pm$ standard error (SE) and ranges of variation (min and max)) in various poplar species

Reference	Year	Growth conditions	Plant material	Parentage	Age	No. of clones	$\Delta$ (‰)			Country	
							Mean $\pm$ SE	Min	Max		
Tanaka-Oda et al. (2010)		Plantation	<i>P. hopeinsis</i>		5–30 years			17.0	20.0	Japan	
Cao et al. (2009)	2008	Reserve	<i>P. euphratica</i>	Natural population	Unknown			20.3 $\pm$ 0.14	19.5	20.7	China
Monclus et al. (2009)	2004	Nursery	<i>P. deltoides</i> $\times$ <i>P. trichocarpa</i>	F <sub>1</sub>	1st year stem/4th year roots	33		21.6 $\pm$ 0.05	20.7	22.5	France
Soolanayakanahally et al. (2009)	2005–2006	Nursery/water deficit	<i>P. balsamifera</i>	21 provenances	15–30 years	210		22.0 $\pm$ 0.06	21.0	23.1	Canada
Yin et al. (2009)	2004	Outdoor common garden	<i>P. cathayana</i> , <i>P. przewalskii</i>	Cuttings	1st year stem/1st year roots			20.7 $\pm$ 0.34			China
Bonhomme et al. (2008)	2004	Greenhouse—control	<i>P. deltoides</i> $\times$ <i>P. nigra</i>	Unrelated	6–9 years	8		21.3 $\pm$ 0.26	20.1	22.3	France
	2006	Greenhouse—water deficit (25%)						20.2 $\pm$ 0.15			
Dillen et al. (2008)	2006	Commercial plantation	<i>P. deltoides</i> $\times$ <i>P. nigra</i>	Unrelated	6–9 years	8		21.3 $\pm$ 0.26	20.1	22.3	France
	2006	Nursery	<i>P. deltoides</i> $\times$ <i>P. nigra</i>	F <sub>1</sub>	1st year stem/4th year roots	50		22.2 $\pm$ 0.29	21.3	23.6	
	2006	Nursery	<i>P. deltoides</i> $\times$ <i>P. nigra</i>	F <sub>1</sub>	1st year stem/4th year roots	50		20.8 $\pm$ 0.04	19.9	21.6	
DesRochers et al. (2007)		Greenhouse	<i>P. deltoides</i> $\times$ <i>P. trichocarpa</i>	Cuttings	1st year stem/1st year roots	3		20.5 $\pm$ 0.04	19.8	21.7	Canada
		Greenhouse	<i>P. balsamifera</i> , <i>P. deltoides</i> $\times$ <i>P. petrowskyana</i>								
	2004	Greenhouse	<i>P. cathayana</i> , <i>P. kangdingensis</i>	Cuttings	1st year stem/1st year roots			18.0			China
DesRochers et al. (2006)	2002	Agricultural land	<i>P. balsamifera</i> $\times$ <i>P. simonii</i> , <i>P. deltoides</i> $\times$ <i>P. petrowskyana</i>	Cuttings	1st year stem/1st year roots	3		19.0 $\pm$ 0.07			Canada
Monclus et al. (2006)	2004	Nursery	<i>P. deltoides</i> $\times$ <i>P. nigra</i>	Unrelated	2nd year stem/2nd year roots	3		20.4 $\pm$ 0.04			France
	2004	Nursery	<i>P. deltoides</i> $\times$ <i>P. nigra</i>	Unrelated	1st year stem/4th year roots	29		21.6 $\pm$ 0.23	20.5	23.2	France
Voltas et al. (2006)	1999	Nursery/water deficit	<i>P. deltoides</i> $\times$ <i>P. nigra</i>	Rooted cuttings	1st year stem/2nd year roots	4		21.3 $\pm$ 0.19	19.8	22.3	Spain
	2000	Plantation			2nd year stem/3rd year roots	4		20.8	20.4	21.2	
Marron et al. (2005)	2001	Greenhouse	<i>P. deltoides</i> $\times$ <i>P. nigra</i>	Unrelated	2nd year stem/3rd year roots	4		21.1	20.3	21.6	France
Monclus et al. (2005)	2003	Nursery	<i>P. deltoides</i> $\times$ <i>P. nigra</i>	Unrelated	1st year stem/3rd year roots	29		21.8 $\pm$ 0.14	21.7	27.9	France
	2003	Nursery	<i>P. deltoides</i> $\times$ <i>P. nigra</i>	Unrelated	1st year stem/3rd year roots	29		20.0 $\pm$ 0.11	18.7	21.5	France

Table 1 (continued)

Reference	Year	Growth conditions	Plant material	Parentage	Age	No. of clones	$\Delta$ (%)			Country
							Mean $\pm$ SE	Min	Max	
Yin et al. (2005)	2003	Greenhouse—control	<i>P. cathayana</i> , <i>P. przewalskii</i>	Cuttings	1st year stem/1st year roots		24.7 $\pm$ 0.31			China
	2003	Greenhouse—water deficit (25%)					21.4 $\pm$ 0.32			
Zhang et al. (2005)		Greenhouse—control	<i>P. davidiana</i>	Seedlings	1st year stem/1st year roots		22.4 $\pm$ 0.41			Finland
		Greenhouse—water deficit (20%)					19.5 $\pm$ 0.34			
Li et al. (2004)	2002	Greenhouse—control	<i>P. davidiana</i>	Seedlings	1st year stem/1st year roots		22.4 $\pm$ 0.39			China
	2002	Greenhouse—water deficit (50%)					20.6 $\pm$ 0.30			
Rae et al. (2004)	2001	Nursery	<i>P. trichocarpa</i> x <i>P. deltooides</i>	F <sub>2</sub> , cuttings	1st year stem/2nd year roots	210		12.0	23.0	
Ripullone et al. (2004)	2000	Nursery	<i>P. deltooides</i> x <i>P. nigra</i>	Cuttings	1st year stem/1st year roots		22.6			Italy
Yin et al. (2004)	2003	Greenhouse—control	<i>P. cathayana</i> , <i>P. kangdingensis</i>	Cuttings	1st year stem/1st year roots		24.1 $\pm$ 0.38			China
Zhang et al. (2004)	2003	Greenhouse—water deficit (25%)					21.0 $\pm$ 0.30			
		Greenhouse—control	<i>P. davidiana</i>	Seedlings	1st year stem/1st year roots		22.4 $\pm$ 0.40			China
		Greenhouse—water deficit					19.4 $\pm$ 0.39			
Leffler and Evans (2001)	1997	Forest	<i>P. fremontii</i>	Natural population	Unknown			19.9	23.1	USA
Wang et al. (1997)		Desert	<i>P. euphratica</i>	Natural population	Mature tree			18.9	29.7	China
Brooks et al. (1997)	1993–1994	Forest	<i>P. tremulooides</i>	Natural population	Unknown			19.2	21.3	Canada
Buchmann et al. (1997)	1993	Forest	<i>P. tremulooides</i>	Natural population	Unknown			17.0	19.7	USA
Sparks and Ehleringer (1997)	1993	Forest	<i>P. fremontii</i> , <i>P. angustifolia</i>	Natural population	Unknown			19.8	22.6	USA
	1994							20.0	24.0	

For each study, the nature of the plant material (hybrid combination, parentage, age, number of studied clones) and the growth conditions are presented

atures of the warmest and the coldest months) was 35.8°C and 28.1°C in Italy and France, respectively. The sum of annual rainfall was 569 mm for the Italian and 645 mm for the French site (for a more detailed site description, see Marron et al. 2010). With regard to soil conditions, soil texture was pure loam in Italy, whereas the soil was composed of 75% sand in France.

### 2.3 Plantation layout

The field trials were established during April 2003 from 25-cm uniform hardwood cuttings at a density of 6,670 trees per hectare at an initial spacing of 0.75 × 2 m. The two experimental plantations were established according to a randomised block design. For each family, six complete blocks were planted; each block containing one randomly planted replicate of each F<sub>1</sub> genotype and each of the parents. To limit border effects, a double-border row was planted around the plantations (*P. deltoides* × *P. nigra* 'I-214' and *P. deltoides* × *P. nigra* 'Robusta', at the Italian and French sites, respectively). All stems were coppiced in December 2004 and February 2006, after two and one growing seasons, respectively. In early spring, all shoots, except the largest one, were removed from each stump. Throughout each growing season, plantation management included weed removal, irrigation and the use of insecticides and fungicides as needed. During the establishment year, there was a high mortality at the Italian site probably due to rooting difficulties. Nevertheless, high growth rates at the Italian site during the second growing season indicated that trees recovered from the rooting difficulties (Dillen et al. 2009a). Throughout the whole experiment, at least three replicates of each selected F<sub>1</sub> genotype and parent were alive at each site, except for *P. deltoides* 'S9-2' and *P. nigra* 'Ghoy' in Italy.

### 2.4 Carbon isotope discrimination and nitrogen analyses

The largest sunlit mature leaves of a selection of 31 genotypes were collected in 2004 (D×N in France), 2005 (D×N and D×T in Italy and France) and 2006 (D×N and D×T in France; Table 2) during different field studies. Dry leaf material was ground to a fine powder. Leaf carbon isotope composition ( $\delta^{13}\text{C}$ ) and total nitrogen and carbon contents were assessed from 1 mg homogenous dry powder. A continuous flux isotope ratio mass spectrometer (IRMS1, Delta S; Finnigan MAT, Bremen, Germany, Facility at UMR EEF, INRA-Nancy, with a long-term (2001–2009) external precision of  $\pm 0.16\text{‰}$ ) was used for  $^{13}\text{C}$ , total carbon (C) and nitrogen (N) analyses of 2004 and 2005 leaf samples. Carbon isotope composition was calculated relative to the Vienna Pee Dee Belemnite (standard) as in Craig (1957):  $\delta^{13}\text{C} = [(R_{\text{sa}} - R_{\text{sd}}) / (R_{\text{sd}}) \times 1,000 (\text{‰})]$ , where  $R_{\text{sa}}$  and  $R_{\text{sd}}$  are the  $^{13}\text{C}/^{12}\text{C}$  ratios

**Table 2** Schematic overview of the available leaf material for carbon isotope discrimination ( $\Delta$ ) analysis collected during different field studies from 2004 to 2006 in Italy and/or France

	D×N family		D×T family	
	Italy	France	Italy	France
2004		✓		
2005 <sup>a</sup>	✓	✓	✓	✓
2006 <sup>b</sup>		✓		✓

Each time, 31 common F<sub>1</sub> genotypes were selected from each of the two families. D×N=*P. deltoides* 'S9-2' × *P. nigra* 'Ghoy' and D×T=*P. deltoides* 'S9-2' × *P. trichocarpa* 'V24'

<sup>a</sup> In 2005, tree growth and additional leaf traits were measured

<sup>b</sup> Leaf carbon isotope discrimination ( $\Delta$ ) values have been described in Dillen et al. (2008)

of the sample and the standard, respectively. The discrimination between atmospheric CO<sub>2</sub> ( $\delta_{\text{air}}$ , assumed to be close to  $-8\text{‰}$ , with the hypothesis that  $\delta_{\text{air}}$  was independent from site and year) and plant material ( $\delta_{\text{plant}}$ ) was calculated as  $\Delta = [\delta_{\text{air}} - \delta_{\text{plant}}] / (1 + (\delta_{\text{plant}}/1,000))$  according to Farquhar and Richards (1984).

A cross-calibration of two IRMS (IRMS2: 20–20 mass spectrometer, PDZEuropa, Northwich, UK, at UC Davis Stable Isotope Facility in California) has been performed from leaf samples of 20 genotypes taken at random among D×N and D×T at the French site in 2006 (Table 2). Because no significant difference was observed between the two IRMS, the data from the 31 common genotypes of the D×N and D×T families described in Dillen et al. (2008) were compared with the data obtained in 2004 and 2005 in the present study. Total C and N contents of laminae were expressed on a dry mass basis ( $C_{\text{M}}$  and  $N_{\text{M}}$ , respectively, milligrams per gram dry weight).

### 2.5 Leaf traits and tree growth

In 2005 only, maximal leaf area (LA, square centimetres) was measured with a leaf area meter (CID, CI-203, Inc., Camas, WA); leaves were then dried at 70°C, weighed and specific leaf area of the largest leaf on the main stem was calculated (SLA, square centimetres per gram dry weight). Growth of genotypes was assessed through stem circumference measurements at 1 m above ground level to the nearest millimetre.

### 2.6 Data analyses

Statistical analyses were performed with the R software (version 2.10.1, A Language and Environment Copyright, 2007). Assumptions on distributions of residuals of the linear models were checked with Shapiro–Wilk statistics. In case of non-normality, the Box–Cox method was used



(Venables and Ripley 2002). Genotype means were calculated with their standard error ( $\pm$ SE). The block effects were significant ( $P \leq 0.05$ ) for all data and all sites, and were therefore corrected by adding to each individual value the difference between the mean of its respective block and  $\mu$ . The following fixed or mixed models were used:

- (a) To test family and site effects from data collected in 2005 (Table 2):  $Y'_{ijkl} = \mu + \text{Fam}_i + G_j < \text{Fam}_i > + S_k + \text{Fam}_i \times S_k + G_{j(i)} \times S_k + \varepsilon_{ijkl}$ , where  $Y'_{ijkl}$  are individual values adjusted for the within-site block effects ( $Y'_i = Y - B_i$ , where  $B_i$  is the effect of block  $i$ ),  $\mu$  is the general mean,  $\text{Fam}_i$  is the family effect (fixed),  $G_j < \text{Fam}_i >_{(i)}$  is the genotype effect nested in  $\text{Fam}_i$  (random),  $S_k$  is the site effect (fixed),  $\text{Fam}_i \times S_k$  is the family-by-site interaction effect (fixed),  $G_{j(i)} \times S_k$  is the genotype-by-site interaction effect (random) and  $\varepsilon_{ijkl}$  is the residual error.
- (b) To test year effect from 31 genotypes of D×N measured in France in 2004, 2005, and 2006 and from 31 genotypes of D×T in 2005 and 2006 (Table 2):  $Y'_{ijm} = \mu + G_j + \text{YR}_m + G_j \times \text{YR}_m + \varepsilon_{ijm}$ , where  $G_j$  is the genotype effect (random),  $\text{YR}_m$  is the year effect (fixed) and  $G_j \times \text{YR}_m$  is the genotype-by-year interaction effect (random).
- (c) For comparison among parents of the two families:  $Y'_{ij} = \mu + P_j + \text{YR}_m + P_j \times \text{YR}_m + \varepsilon_{ij}$ , where  $P_j$  is the effect of parent species  $j$  considered as fixed,  $\text{YR}_m$  is the year effect (fixed) and  $P_j \times \text{YR}_m$  is the parent-by-year interaction effect (fixed). The Scheffé method was chosen as post hoc analysis (Maxwell and Delaney 2004).  
Differences between means were considered significant when the  $P$  value of the ANOVA  $F$  test  $\leq 0.05$ . To characterize genetic variation present in each family separately, the following random model was used:
- (d) For variance decomposition within each family in 2005:  $Y'_{ijk} = \mu + G_j + S_k$  (or  $\text{YR}_k$ ) +  $G_j \times S_k$  (or  $G_j \times \text{YR}_k$ ) +  $\varepsilon_{ijk}$ , where  $G_j$  is the genotype effect (random),  $S_k$  is the site effect (random),  $\text{YR}_k$  is the year effect (random),  $G_j \times S_k$  is the genotype-by-site interaction effect (random) and  $G_j \times \text{YR}_k$  is the genotype-by-year effect (random). In order to quantify the relative importance of each effect, variance components,  $\sigma^2_G$ ,  $\sigma^2_S$ ,  $\sigma^2_{\text{YR}}$ ,  $\sigma^2_{G \times S}$ ,  $\sigma^2_{G \times \text{YR}}$  and  $\sigma^2_\varepsilon$ , were calculated by equating observed mean squares to expected mean squares and solving the resulting equations (Henderson 1953). We calculated the variance accounted for by the site or year ( $\sigma^2_S$  or  $\sigma^2_{\text{YR}}$ ), although the site and year effects were further on considered as fixed as a result of their non-random sampling (Wu and Stettler 1997).
- (e) For within-family broad-sense heritability estimation by site ( $H^2$ ), across sites in 2005 ( $H^2_{\text{Sites}}$ ) and across years, i.e. 2005 and 2006, in France ( $H^2_{\text{Years}}$ ):  $Y'_{ij} = \mu +$

$G_j + \varepsilon_{ij}$ . When significant genotype effects were observed, within-family broad-sense heritability was estimated as  $\sigma^2_G / (\sigma^2_G + \sigma^2_\varepsilon)$  (Falconer 1989). The standard errors were calculated using the method described by Singh et al. (1993).

For all traits, values of the best linear unbiased predictions (BLUP) for each genotype were obtained using the *lmer* function in R on model *e*. Changes in genotype ranking across sites were analysed with Spearman rank coefficients ( $\rho$ ). Linear correlations between traits in 2005 were estimated with Pearson correlation coefficient. Both correlations were based on BLUP values.

### 3 Results

#### 3.1 Effect of genetic background

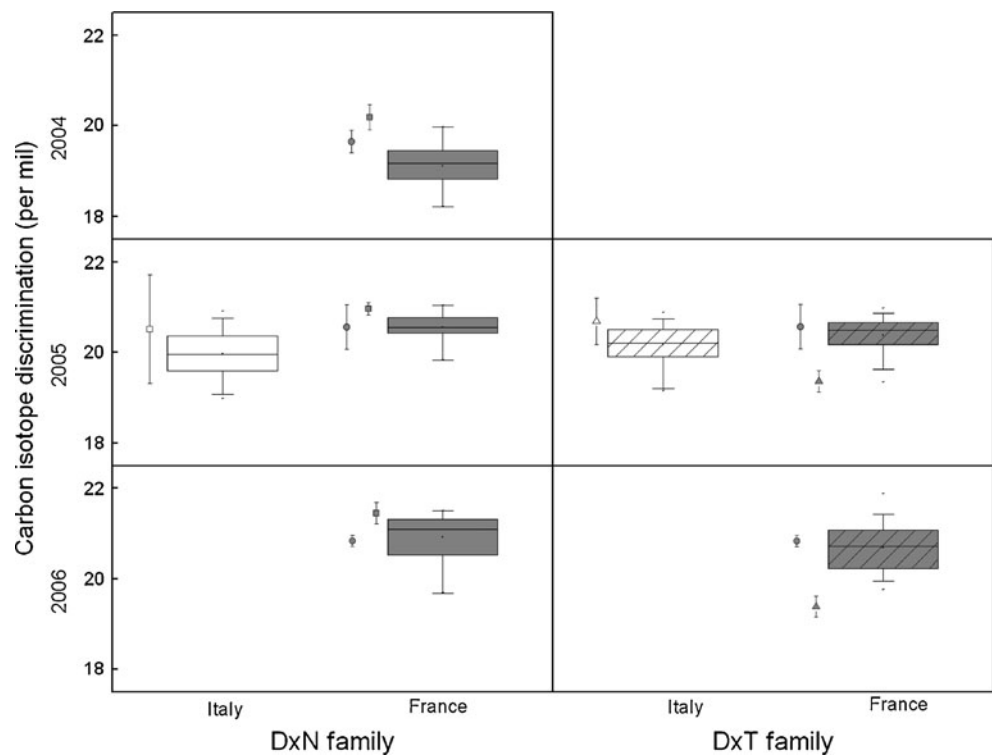
Irrespective of site, no significant difference was observed between the average  $\Delta$  values of D×N and D×T in 2005 (Fig. 1). One year later, in 2006, D×N showed significantly higher  $\Delta$  values than D×T in France (Fig. 1; Dillen et al. 2008). Each year, highly significant differences among genotypes were observed, except for D×T in Italy in 2005 (Table 3). By and large, moderate within-family broad-sense heritability ( $H^2$ ) values were obtained for  $\Delta$  in the studied families and sites, ranging from 0 to 0.49 (Table 3). Lower  $H^2$  values were obtained at the Italian compared with the French site in 2005.

In France, *P. trichocarpa* 'V24' showed significantly lower  $\Delta$  than *P. nigra* 'Ghoy' and *P. deltoides* 'S9-2' ( $P \leq 0.01$ ; Fig. 1). Mean values of  $\Delta$  in D×N and D×T were comparable to mid-parental values in 2004, 2005 and 2006. Only in France did D×T hybrids have a considerably higher  $\Delta$  (or lower  $W_i$ ) compared with their father parent, *P. trichocarpa* 'V24' (average  $\Delta$  values and SE,  $20.4 \pm 0.0$  and  $19.5 \pm 0.2$  in 2005, and  $20.8 \pm 0.0$  and  $19.5 \pm 0.2$  in 2006 for hybrids and father parent, respectively; Fig. 1).

#### 3.2 Site effect and G × S interactions

The average  $\Delta$  values differed between sites, with significantly lower values ( $P \leq 0.01$ ) at the Italian than at the French site for each family in 2005. On the other hand, *P. trichocarpa* 'V24' had the smallest  $W_i$  or highest  $\Delta$  at the French site (Fig. 1). Significant G × S interactions were detected in both families in 2005 (Table 4). In addition, non-significant Spearman rank correlation coefficients calculated between sites indicated that the genotype ranking was site-dependent for each family in 2005. Estimates of within-family broad-sense heritability across sites ( $H^2_{\text{Sites}}$ ) were lower than heritability estimates by site ( $H^2$ ; Tables 3 and 4).

**Fig. 1** Box plots of genotypic means of leaf carbon isotope discrimination ( $\Delta$ , ‰) estimated in 2004, 2005 and/or 2006 in Italy (white) and/or in France (grey) for the two poplar families: D×N (non-hatched, 31 F<sub>1</sub>)=*P. deltoides* ‘S9-2’ × *P. nigra* ‘Ghoy’, D×T (hatched, 31 F<sub>1</sub>)=*P. deltoides* ‘S9-2’ × *P. trichocarpa* ‘V24’. Means ( $\pm$ SE) are indicated for the parents: *P. deltoides* ‘S9-2’ (circle), *P. nigra* ‘Ghoy’ (square), *P. trichocarpa* ‘V24’ (triangle)



3.3 Year effect and G × YR interactions

$\Delta$  increased significantly in D×N and the parental species between 2004 and 2006 (Fig. 1). A significant G × YR interaction was observed in D×T only (Table 4). Spearman rank correlations calculated between years in D×T and D×N were always significant, suggesting that genotype ranking was relatively stable among the 3 years in these two families (Fig. 2). Nevertheless, moderate Spearman rank coefficients, ranging from 0.54 to 0.62,

indicated that some rank changes occur between years. Estimates of within-family broad-sense heritability across years ( $H^2_{Years}$ ) were higher than heritability estimates by site ( $H^2$ ; Tables 3 and 4). Both Spearman rank coefficients and within-family broad-sense heritability estimates by site, across sites and across years demonstrate that G×S are larger than G×YR interactions.

**Table 3** Within-family broad-sense heritability ( $H^2$ ) and standard error (SE) for leaf carbon isotope discrimination ( $\Delta$ ) in Italy and/or in France during 2004, 2005 and/or 2006

	D×N family		D×T family	
	$H^2_{Italy}$	$H^2_{France}$	$H^2_{Italy}$	$H^2_{France}$
2004		0.49±0.02***		
2005	0.21±0.08**	0.41±0.09***	0 ns	0.49±0.08***
2006 <sup>a</sup>		0.40±0.10***		0.40±0.09***

D×N=*P. deltoides* ‘S9-2’ × *P. nigra* ‘Ghoy’, D×T=*P. deltoides* ‘S9-2’ × *P. trichocarpa* ‘V24’

ns non-significant

\*\*\* $P \leq 0.01$ , \*\* $P \leq 0.001$

<sup>a</sup>  $H^2$  values were recalculated on 31 F<sub>1</sub> genotypes from the study of Dillen et al. (2008)

**Table 4** Relative importance of (A) genetic ( $\sigma_G^2$ ), site ( $\sigma_S^2$ ), genotype by site ( $\sigma_{G \times S}^2$ ), and residual ( $\sigma_\epsilon^2$ ) variances in the phenotypic variation ( $\sigma_P^2$ ) across the two sites, (B) genetic ( $\sigma_G^2$ ), year ( $\sigma_Y^2$ ), genotype by year ( $\sigma_{G \times Y}^2$ ), and residual ( $\sigma_\epsilon^2$ ) variances in the phenotypic variation ( $\sigma_P^2$ ) across three (D×N) or two (D×T) years, and (C) the ratio of genotypic to phenotypic variance across sites in 2005 ( $H_{Sites}^2$ ) and across years in France ( $H_{Years}^2$ ), for carbon isotope discrimination of the two poplar families: D×N=*P. deltoides* ‘S9-2’ × *P. nigra* ‘Ghoy’ and D×T=*P. deltoides* ‘S9-2’ × *P. trichocarpa* ‘V24’

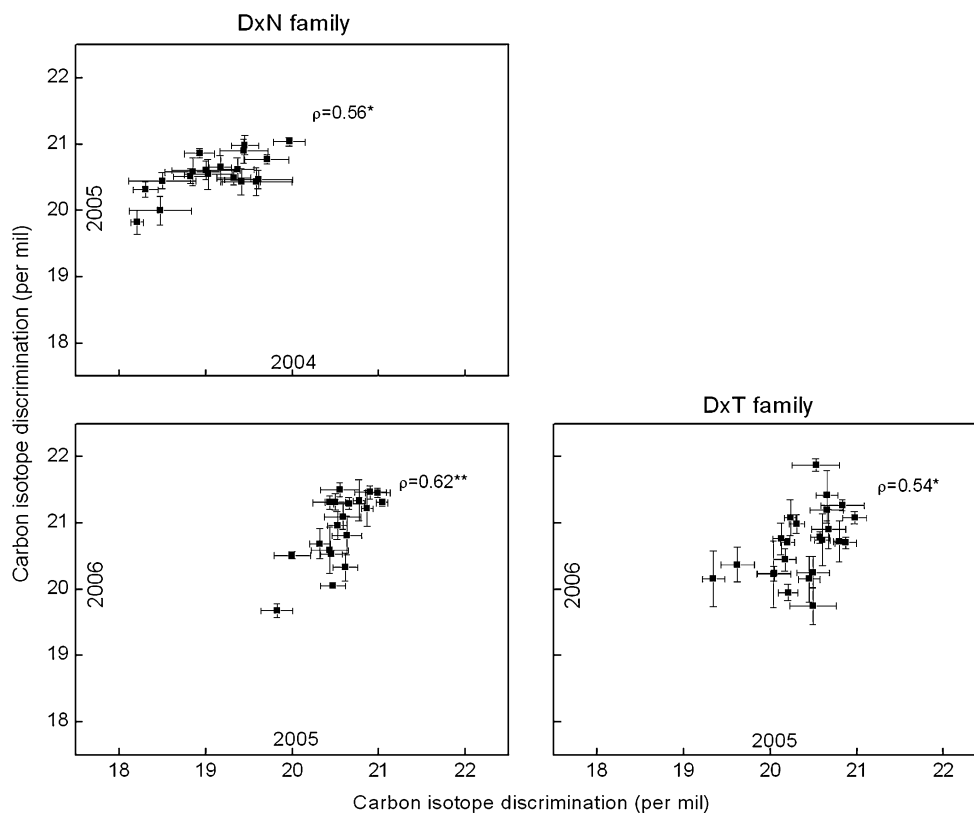
A	$\sigma_G^2/\sigma_P^2$	$\sigma_S^2/\sigma_P^2$	$\sigma_{G \times S}^2/\sigma_P^2$	$\sigma_\epsilon^2/\sigma_P^2$
D×N	12.4*	20.3***	9.2*	58.1
D×T	3.4 ns	6.3*	13.9**	76.4
B	$\sigma_G^2/\sigma_P^2$	$\sigma_Y^2/\sigma_P^2$	$\sigma_{G \times Y}^2/\sigma_P^2$	$\sigma_\epsilon^2/\sigma_P^2$
D×N	10.6***	67.8***	2.9 ns	18.7
D×T	34.9***	1.9*	16.9**	46.3
C	$H_{Sites}^2$		$H_{Years}^2$	
D×N	0.32±0.07		0.70±0.06	
D×T	0.18±0.08		0.73±0.06	

ns non-significant

\* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$



**Fig. 2** Between-year relationships for leaf carbon isotope discrimination ( $\Delta$ ) in France are presented on a genotypic mean basis ( $\pm$ SE). For the D $\times$ N family,  $\Delta$  in 2004 vs. 2005 and in 2005 vs. 2006 were plotted, and for D $\times$ T family,  $\Delta$  was measured in 2005 and 2006 only. Spearman rank coefficients ( $\rho$ ) are indicated: \*\* $P \leq 0.01$  and \* $P \leq 0.05$



### 3.4 Phenotypic correlations

In 2005, there were no clear relationships among  $\Delta$ , leaf and growth traits across the two sites and families. Leaves with higher nitrogen and carbon content appeared to have lower values of  $\Delta$  in D $\times$ N in Italy ( $r = -0.73$  and  $r = -0.63$  for leaf nitrogen and carbon content, respectively;  $P \leq 0.001$ ). In France, the D $\times$ T hybrids showing higher  $W_i$  (or lower  $\Delta$ ) had denser leaves (or lower SLA;  $r = 0.74$ ,  $P \leq 0.001$ ). In contrast, in Italy, denser D $\times$ N leaves tend to have lower  $W_i$  (or higher  $\Delta$ ;  $r = -0.57$ ,  $P \leq 0.01$ ). No significant relationships were detected between  $\Delta$  and stem circumference measured at the end of the growing season.

## 4 Discussion

Among the studies using leaf carbon isotope discrimination ( $\Delta$ ) as a surrogate of intrinsic water use efficiency ( $W_i$ ) in poplar (summarized in Table 1), few were focused on the effects of the genotype  $\times$  site and genotype  $\times$  year interactions on  $\Delta$  in full-sib poplar families, as well as their implications for deployment. In line with earlier studies, a moderate genetic variability occurred for  $\Delta$  within the two studied poplar families (Table 1). Average values of  $\Delta$  did not always differ among families: the variability was much larger within than between families.

### 4.1 Site effect

In 2005, both families displayed significantly lower  $\Delta$  or larger  $W_i$  at the Italian site. The Italian site was characterized by higher temperatures, higher irradiance and a more fertile soil than the French site. As a result, trees were significantly larger and leaves were richer in nitrogen in Italy than in France (Marron et al. 2007). Moreover, leaf nitrogen content was negatively correlated with  $\Delta$  for the D $\times$ N hybrids, as already observed by Monclus et al. (2005). These findings suggest that the variation in  $\Delta$  among genotypes and between sites was partly caused by leaf nitrogen content and therefore possibly by photosynthetic assimilation rates.

Significant G  $\times$  S interactions were detected depending on family and year. As a whole, Spearman rank coefficients computed between sites indicated that genotype ranking for  $\Delta$  varied between sites for the two studied families. Although genotype ranking for  $\Delta$  is usually considered to be stable in contrasting environments, various results have been reported for G  $\times$  S interactions. *Quercus* spp. seedlings displayed significant G  $\times$  S interactions in response to varying irradiance levels, but not to drought (Ponton et al. 2002). In *Pinus taeda*, G  $\times$  S interactions remained small (Baltunis et al. 2008). In *P. deltoides*  $\times$  *P. nigra* hybrids, Monclus et al. (2006) found that drought treatment did not result in a significant change in the

genotype ranking for  $\Delta$ . On the other hand, 5-year-old commercial poplar hybrids ranked differently in an alluvial and a non-alluvial site (Bonhomme et al. 2008). Accordingly, the contrasting results for  $G \times S$  interactions for  $\Delta$  could be explained by differences in (1) plant material or relations among genotypes (genus, species, families, genotypes); (2) number of genotypes (Baltunis et al. 2008); and/or (3) environments or treatments used in the studies. In poplar, the response of  $\Delta$  to different environments or treatments appears to be more genotype-dependent than that of growth and leaf traits such as stem circumference and leaf area (Marron et al. 2007; Dillen et al. 2009b) and less than that of leaf traits such as SLA (Monclus et al. 2006).

#### 4.2 Year effect

For the  $D \times N$  hybrids in France,  $\Delta$  was measured in 3 years (2004–2006) and values of  $\Delta$  increased each year, indicating a gradual decrease in  $W_i$ . Interannual differences in  $\Delta$  can be due to climatic differences between years. However, the plantations were irrigated and the temperatures during the three growing seasons were not significantly different (16.5°C on average between April and September). Thus, the observed time course of  $\Delta$  is unlikely to be due to interannual differences in water availability or temperature. This interannual pattern may result from the yearly increase of the stool and root system with respect to the leaf area index. This is primarily due to coppicing and may result in a smaller hydraulic constraint to water flow from soil to leaves and, hence, a larger stomatal conductance or lower  $W_i$ . This hypothesis is supported by observations in coppiced trees by Kruger and Reich (1993) and Wildy et al. (2004). The genotype ranking was more stable among years in a given plantation than among sites in a given year, as suggested by the Spearman rank coefficients and ratios of genotypic to phenotypic variance across sites and years. This can be partly explained by the fact that for a given genotype, the same trees were measured among years at a given site, whilst different trees were measured at the different sites.

As commonly observed, we were unable to detect any correlation between  $\Delta$  and growth (represented by stem circumference at the end of the growing season; Marron et al. 2005; Monclus et al. 2005, 2006, 2009; Bonhomme et al. 2008; Dillen et al. 2008). In principle, negative correlation between  $\Delta$  and growth may be expected given that leaf nitrogen contributes to the variation in  $\Delta$ , suggesting that photosynthetic capacity is at least for a minor part driving the variability in  $\Delta$ . Tree growth, however, is a complex interaction of process-related and structural traits. The lack of relationship excludes a trade-off between growth and  $\Delta$  within the studied poplar families and confirms the possibility of breeding genotypes

combining vigorous growth performance and large efficiency for water use.

## 5 Conclusions

The present study showed that identification of genotypes with low  $\Delta$  (or high  $W_i$ ) across sites and years was hampered by the occurrence of genotype-by-site interactions in the two studied families. Each year,  $\Delta$  values increased, but the genotype ranking remained relatively stable. In the case of a strong environmental influence affecting the relative ranking of genotypes, the use of  $\Delta$  in deployment populations would imply a genotype selection specific to site conditions.

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