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

3
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20

21 **Abstract**

- 22 • Because of its vigorous growth, poplar can play an important role for sustainable
23 production of woody biomass to cover renewable energy needs. Hence, the selection
24 of suitable genotypes has to base on relevant traits among which intrinsic water-use
25 efficiency (W_i , estimated through leaf carbon isotope discrimination, Δ) may be a key
26 trait. Beside a large genetic variation in Δ among the frequently planted poplar
27 hybrids, the use of Δ in deployment or breeding programs requires insights in the
28 robustness of the genotype ranking for Δ across environments and years.
- 29 • Two F_1 full-sib families of poplar (*Populus deltoides* \times *P. nigra* and *Populus deltoides*
30 \times *P. trichocarpa*) were grown at two sites in Europe, i.e. northern Italy and central
31 France. For each family, leaf samples from 31 F_1 genotypes collected during different
32 field studies were used (i) to assess the effect of genotype, site and year on Δ in leaves,
33 as well as their mutual interactions; and (ii) to elucidate the relationships between Δ ,
34 leaf morphology and tree dimensions.
- 35 • Under the well-watered conditions of our study, a low to moderate genetic variability
36 was observed in the two poplar families. Within-family broad-sense heritability values
37 ranged from 0 to 0.49. The ranking of genotypes for Δ was more stable between years
38 than between sites.
- 39 • The study confirmed the occurrence of some degree of genetic variability of Δ in the
40 studied poplar families and the possibility to identify genotypes with low, stable Δ
41 values across years. However, the significant genotype by site interactions in our study
42 suggest that selection for larger water-use efficiency or lower Δ in these families has to
43 consider specific responses in different environments.

44
45 **Keywords:** genotype \times environment (G \times E) interaction, growth, heritability, hybrids, leaf
46 traits, *Populus*, water-use efficiency

47
48 **Running title:** Leaf carbon isotope discrimination in two full-sib poplar families

49

50 1. INTRODUCTION

51 According to the European Energy Directive, biomass will be the major renewable fuel
52 resource by 2020 (COM 2009/28/EC). Among the numerous biomass resources, woody
53 biomass sequesters carbon for longer periods of time and displays a more favorable
54 environmental balance than annual crops as it usually requires less fertilization inputs
55 (Updegraff et al. 2004, Gasol et al. 2009). The large genetic variability in the *Populus* genus
56 has been exploited to increase biomass yields under adequate water and nutrient supplies. In
57 particular, poplar hybrids appeared to be an interesting option for biomass production in short-
58 rotation coppice systems (SRC) due to their strong growth vigor that however is associated
59 with large water requirements (Tschaplinski et al. 1994, Souch and Stephens 1998). The
60 question arose (i) whether there is a significant variability in water-use efficiency (WUE, ratio
61 between biomass production and water use) or at an instant scale, in intrinsic water-use
62 efficiency (W_i , ratio between net CO₂ assimilation and stomatal conductance to water vapour)
63 among these highly productive hybrids, (ii) to which extent W_i of the parental species
64 combines in the hybrid progeny, and (iii) the ranking of the hybrid genotypes for W_i remains
65 stable among different environments and years. In drought-prone areas, poplar plantations are
66 often irrigated and the deployment of genotypes combining vigorous growth performance and
67 favorable WUE (or W_i) in these areas could save considerable amounts of water and, hence,
68 increase the economic viability of the plantations.

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

78 The genetic variability of leaf Δ as well as the relationships between Δ and growth
79 were described in poplar species or hybrids (see Table 1). These studies revealed a large
80 genetic variability of Δ in leaves and significant effects of soil conditions or water availability
81 on Δ . Generally, weak genotypic correlations between Δ and growth performance were
82 observed. Much less is known about the genotype by environment (G×E) and genotype by

83 year (G×YR) interactions for Δ in poplar, due to the limited availability of multiple-year
84 common garden studies of large poplar families or populations. For instance, a significant
85 change in genotype ranking between two environments may reduce the effectiveness of
86 deployment for a given site if genotypes with large water-use efficiency are selected on the
87 basis of Δ values at another site. The trial established under the framework of the European
88 POPYOMICs project (2003-2006) was an ideal opportunity to investigate the occurrence of
89 such interactions. The experiment comprised two F₁ full-sib poplar families from two hybrid
90 combinations (*Populus deltoides* × *P. trichocarpa* and *Populus deltoides* × *P. nigra*) grown
91 during three years.

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

- 95 (i) there is a large genetic variability for Δ in each hybrid combination;
- 96 (ii) Δ differs between sites and years, but the genotype ranking remains stable
97 between contrasting environments and over different years;
- 98 (iii) there is no relationship between Δ and biomass production irrespective of
99 hybrid combination, site and year.

100

101 2. MATERIAL AND METHODS

102 2.1. Plant material

103 Two F₁ interspecific *Populus* full-sib families sharing the same female parent were examined:

104 – D×N: *Populus deltoides* (Bartr. ex Marsh) ‘S9-2’ × *Populus nigra* (L.) ‘Ghoy’ (180 F₁
105 genotypes; Cervera et al. 1996, 2001).

106 – D×T: *Populus deltoides* ‘S9-2’ × *P. trichocarpa* (Torr. & Gray) ‘V24’ (182 F₁
107 genotypes; Cervera et al. 1996, 2001).

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

112

113 2.2. Site description

114 The two sites were located (i) in northern Italy (Cavallermaggiore, Po valley, 44°42’ N, 7°40’
115 E) and (ii) in central France (Ardon, Loire valley, 47°46’ N, 1°52’ E). The elevation was 285
116 m for the Italian site and 110 m for the French site. During three growing seasons (2003 to
117 2005) the average annual temperature was only slightly lower at the French than at the Italian
118 site (11.5°C vs. 12.1°C). The climate at the Italian site had a more continental character than
119 the climate at the French site; the annual thermal excursion (i.e. difference between the
120 average daily temperatures of the warmest and the coldest month) was 35.8 and 28.1°C, in
121 Italy and France respectively. The sum of annual rainfall was 569 mm for the Italian and 645
122 mm for the French site (for a more detailed site description, see Marron et al. 2010). With
123 regard to soil conditions, soil texture was pure loam in Italy whereas the soil was composed of
124 75% sand in France.

125

126 2.3. Plantation layout

127 The field trials were established during April 2003 from 25-cm uniform hardwood cuttings at
128 a density of 6670 trees per ha at an initial spacing of 0.75 × 2 m. The two experimental
129 plantations were established according to a randomised block design. For each family, six
130 complete blocks were planted; each block contained one randomly planted replicate of each F₁
131 genotype and each of the parents. To limit border effects, a double border row was planted
132 around the plantations (*P. deltoides* × *P. nigra* ‘I-214’ and *P. deltoides* × *P. nigra* ‘Robusta’,
133 at the Italian and French site respectively). All stems were coppiced in December 2004 and

134 February 2006, after two and one growing seasons respectively. In early spring all shoots,
135 except the largest one, were removed from each stump. Throughout each growing season,
136 plantation management included weed removal, irrigation and the use of insecticides and
137 fungicides as needed. During the establishment year, there was a high mortality at the Italian
138 site probably due to rooting difficulties. Nevertheless, high growth rates at the Italian site
139 during the second growing season indicated that trees recovered from the rooting difficulties
140 (Dillen et al. 2009a). Throughout the whole experiment, at least three replicates of each
141 selected F₁ genotype and parent were alive at each site, except for *P. deltooides* 'S9-2' and *P.*
142 *nigra* 'Ghoy' in Italy.

143

144 **2.4. Carbon isotope discrimination and nitrogen analyses**

145 The largest sunlit mature leaves of a selection of 31 genotypes were collected in 2004 (D×N
146 in France), 2005 (D×N and D×T in Italy and France) and 2006 (D×N and D×T in France;
147 Table 2) during different field studies. Dry leaf material was ground to a fine powder. Leaf
148 carbon isotope composition ($\delta^{13}\text{C}$) and total nitrogen and carbon contents were assessed from
149 1-mg homogenous dry powder. A continuous flux isotope ratio mass spectrometer (IRMS1,
150 Delta S; Finnigan MAT, Bremen, Germany, Facility at UMR EEF, INRA-Nancy, with a long-
151 term (2001-2009) external precision of $\pm 0.16\text{‰}$) was used for ^{13}C , total carbon (C) and
152 nitrogen (N) analyses of 2004 and 2005 leaf samples. Carbon isotope composition was
153 calculated relative to the Vienna Pee Dee Belemnite (VPDB, standard) as in Craig (1957):
154 $\delta^{13}\text{C} = [(R_{\text{sa}} - R_{\text{sd}})/R_{\text{sd}}] \times 1000 \text{ [‰]}$, where R_{sa} and R_{sd} are the $^{13}\text{C}/^{12}\text{C}$ ratios of the sample
155 and the standard, respectively. The discrimination between atmospheric CO₂ (δ_{air} , assumed to
156 be close to -8‰ , with the hypothesis that δ_{air} was independent from site and year) and plant
157 material (δ_{plant}) was calculated as $\Delta = [\delta_{\text{air}} - \delta_{\text{plant}}]/(1 + (\delta_{\text{plant}}/1000))$ according to Farquhar and
158 Richards (1984).

159 A cross-calibration of two IRMS (IRMS2: 20-20 mass spectrometer, PDZEuropa,
160 Northwich, UK at UC Davis Stable Isotope Facility in California) has been performed from
161 leaf samples of 20 genotypes taken at random among D×N and D×T at the French site in 2006
162 (Table 2). Because no significant difference was observed between the two IRMS, the data
163 from the 31 common genotypes of the D×N and D×T families described in Dillen et al. (2008)
164 were compared to the data obtained in 2004 and 2005 in the present study. Total C and N
165 content of laminas were expressed on a dry mass basis (C_{M} , N_{M} , respectively; $\text{mg g}^{-1}_{\text{DW}}$).

166

167 2.5. Leaf traits and tree growth

168 In 2005 only, maximal leaf area (LA, cm²) was measured with a leaf area meter (CID, CI-203,
169 Inc., Camas, WA); leaves were then dried at 70°C, weighed and specific leaf area of the
170 largest leaf on the main stem was calculated (SLA, cm² g⁻¹_{DW}). Growth of genotypes was
171 assessed through stem circumference measurements at 1 m above ground level to the nearest
172 mm.

174 2.6. Data analyses

175 Statistical analyses were performed with the R software (version 2.10.1, A Language and
176 Environment Copyright, 2007). Assumptions on distributions of residuals of the linear models
177 were checked with Shapiro-Wilk statistics. In case of non-normality, the Box-Cox method
178 was used (Venables and Ripley 2002). Genotype means were calculated with their standard
179 error (\pm SE). The block effects were significant ($P \leq 0.05$) for all traits at both sites and were
180 therefore corrected by adding to each individual value the difference between the mean of its
181 respective block and μ . The following fixed or mixed models were used:

- 182 (a) To test family and site effects from data collected in 2005 (Table 2): $Y'_{ijkl} = \mu +$
183 $Fam_1 + G_j \langle Fam_1 \rangle + S_k + Fam_1 \times S_k + G_{j(i)} \times S_k + \epsilon_{ijkl}$, where Y'_{ijkl} are individual
184 values adjusted for the within-site block effects ($Y'_i = Y - B_i$ where B_i is the effect
185 of block i), μ is the general mean, Fam_1 is the family effect (fixed), $G_j \langle Fam \rangle_{(i)}$ is
186 the genotype effect nested in Fam_1 (random), S_k is the site effect (fixed), $Fam_1 \times S_k$
187 is the family by site interaction effect (fixed), $G_{j(i)} \times S_k$ is the genotype by site
188 interaction effect (random), and ϵ_{ijkl} is the residual error.
- 189 (b) To test year effect from 31 genotypes of D×N measured in France in 2004, 2005,
190 and 2006 and from 31 genotypes of D×T in 2005 and 2006 (Table 2): $Y'_{ijm} = \mu +$
191 $G_j + YR_m + G_j \times YR_m + \epsilon_{ijm}$, where G_j is the genotype effect (random), YR_m is the
192 year effect (fixed), and $G_j \times YR_m$ is the genotype by year interaction effect
193 (random).
- 194 (c) For comparison among parents of the two families: $Y'_{ij} = \mu + P_j + YR_m + P_j \times YR_m$
195 $+ \epsilon_{ij}$, where P_j is the effect of parent species j considered as fixed, YR_m is the year
196 effect (fixed), $P_j \times YR_m$ is the parent by year interaction effect (fixed). The Scheffé
197 method was chosen as post-hoc analysis (Maxwell and Delaney 2004).

198 Differences between means were considered significant when the P -value of the ANOVA F -
199 test ≤ 0.05 . To characterize genetic variation present in each family separately, the following
200 random model was used:

201 (d) For variance decomposition within each family in 2005: $Y'_{ijk} = \mu + G_j + S_k$ (or
202 $YR_k) + G_j \times S_k$ (or $G_j \times YR_k) + \varepsilon_{ijk}$, where G_j is the genotype effect (random), S_k is
203 the site effect (random), YR_k is the year effect (random), $G_j \times S_k$ is the genotype by
204 site interaction effect (random) and $G_j \times YR_k$ is the genotype by year effect
205 (random). In order to quantify the relative importance of each effect, variance
206 components, σ^2_G , σ^2_S , σ^2_{YR} , $\sigma^2_{G \times S}$, $\sigma^2_{G \times YR}$ and σ^2_ε , were calculated by equating
207 observed mean squares to expected mean squares and solving the resulting
208 equations (Henderson 1953). We calculated the variance accounted for by the site
209 or year (σ^2_S or σ^2_{YR}) although the site and year effects were further on considered
210 as fixed as a result of their non-random sampling (Wu and Stettler 1997).

211 (e) For within-family broad-sense heritability estimation by site (H^2), across sites in
212 2005 (H^2_{Sites}) and across years, i.e. 2005 and 2006, in France (H^2_{Years}): $Y'_{ij} = \mu +$
213 $G_j + \varepsilon_{ij}$. When significant genotype effects were observed, within-family broad-
214 sense heritability was estimated as $\sigma^2_G / (\sigma^2_G + \sigma^2_\varepsilon)$ (Falconer 1989). The standard
215 errors were calculated using the method described by Singh et al. (1993).

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

221

222 3. RESULTS

223 3.1. Effect of genetic background

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

231 In France, *P. trichocarpa* ‘V24’ showed significantly lower Δ than *P. nigra* ‘Ghoy’ and
232 *P. deltooides* ‘S9-2’ ($P \leq 0.01$; Fig. 1). Mean values of Δ in D×N and D×T were comparable to
233 mid-parental values in 2004, 2005 and 2006. Only in France, D×T hybrids had a considerably
234 higher Δ (or lower W_i) compared to their father parent, *P. trichocarpa* ‘V24’ (average Δ values
235 and SE, 20.4 ± 0.0 and 19.5 ± 0.2 in 2005, and 20.8 ± 0.0 and 19.5 ± 0.2 in 2006 for hybrids and
236 father parent respectively; Fig. 1).

237

238 3.2. Site effect and G×S interactions

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

246

247 3.3. Year effect and G×YR interactions

248 Δ increased significantly in D×N and the parental species between 2004 and 2006 (Fig. 1). A
249 significant G×YR interaction was observed in D×T only (Table 4). Spearman rank
250 correlations calculated between years in D×T and D×N were always significant suggesting
251 that genotype ranking was relatively stable among the three years in these two families (Fig.
252 2). Nevertheless, moderate Spearman rank coefficients, ranging from 0.54 to 0.62, indicated
253 that some rank changes occur between years. Estimates of within-family broad-sense
254 heritability across years (H^2_{Years}) were higher than heritability estimates by site (H^2 ; Tables 3

255 and 4). Both Spearman rank coefficients and within-family broad-sense heritability estimates
256 by site, across sites and across years demonstrate that G×S are larger than G×YR interactions.

257

258 **3.4. Phenotypic correlations**

259 In 2005, there were no clear relationships among Δ , leaf and growth traits across the two sites
260 and families. Leaves with higher nitrogen and carbon content appeared to have lower values
261 of Δ in D×N in Italy ($r = -0.73$ and $r = -0.63$ for leaf nitrogen and carbon content respectively;
262 $P \leq 0.001$). In France, the D×T hybrids showing higher W_i (or lower Δ) had denser leaves (or
263 lower SLA; $r = 0.74$, $P \leq 0.001$). In contrast, in Italy, denser D×N leaves tend to have lower
264 W_i (or higher Δ ; $r = -0.57$, $P \leq 0.01$). No significant relationships were detected between Δ
265 and stem circumference measured at the end of the growing season.

266

267 **4. DISCUSSION**

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

274

275 *Site effect*

276 In 2005 both families displayed significantly lower Δ or larger W_i at the Italian site. The
277 Italian site was characterized by higher temperatures, higher irradiance and a more fertile soil
278 than the French site. As a result, trees were significantly larger and leaves were richer in
279 nitrogen in Italy than in France (Marron et al. 2007). Moreover, leaf nitrogen content was
280 negatively correlated with Δ for the D \times N hybrids, as already observed by Monclus et al.
281 (2005). These findings suggest that the variation in Δ among genotypes and between sites was
282 partly caused by leaf nitrogen content and therefore possibly by photosynthetic assimilation
283 rates.

284 Significant G \times S interactions were detected depending on family and year. As a whole,
285 Spearman rank coefficients computed between sites indicated that genotype ranking for Δ
286 varied between sites for the two studied families. Although genotype ranking for Δ is usually
287 considered to be stable in contrasting environments, various results have been reported for
288 G \times S interactions. *Quercus* spp. seedlings displayed significant G \times S interactions in response to
289 varying irradiance levels, but not to drought (Ponton et al. 2002). In *Pinus taeda*, G \times S
290 interactions remained small (Baltunis et al. 2008). In *P. deltoides* \times *P. nigra* hybrids, Monclus
291 et al. (2006) found that drought treatment did not result in significant change in the genotype
292 ranking for Δ . On the other hand, 5-year-old commercial poplar hybrids ranked differently in
293 an alluvial and a non-alluvial site (Bonhomme et al. 2008). Accordingly, the contrasting
294 results for G \times S interactions for Δ could be explained by differences in (i) plant material or
295 relations among genotypes (genus, species, families, genotypes); (ii) number of genotypes
296 (Baltunis et al. 2008); and/or (iii) environments or treatments, used in the studies. In poplar,
297 the response of Δ to different environments or treatments appears to be more genotype
298 dependent than that of growth and leaf traits such as stem circumference and leaf area

299 (Marron et al. 2007; Dillen et al. 2009b), and less than that of leaf traits such as SLA
300 (Monclus et al. 2006).

301

302 *Year effect*

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

318

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

328

329 *Conclusions*

330 The present study showed that identification of genotypes with low Δ (or high W_i) across sites
331 and years was hampered by the occurrence of genotype by site interactions in the two studied

332 families. Each year, Δ values increased but the genotype ranking remained relatively stable. In
333 the case of a strong environmental influence affecting the relative ranking of genotypes, the
334 use of Δ in deployment populations would imply a genotype selection specific to site
335 conditions.
336

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350

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Table 1. Literature review (1997 to 2010) of studies presenting leaf carbon isotope discrimination (Δ , means \pm standard error (SE) and ranges of variation (Min and Max)) in various poplar species. For each study, the nature of the plant material (hybrid combination, parentage, age, number of studied clones) and the growth conditions are presented.

Reference	Year	Growth conditions	Plant material				Δ (‰)			Country	
			Hybrid formula	Parentage	Age	Number of clones	Mean ± SE	Min	Max		
Tanaka-Oda et al. (2010)		plantation	<i>P. hopeinsis</i>		5 to 30 years			17.0	20.0	Japan	
Cao et al. (2009)	2008	reserve	<i>P. euphratica</i>	natural population	unknown			20.3 ± 0.14	19.5	20.7	China
Monclus et al. (2009)	2004	nursery	<i>P. deltooides</i> x <i>P. trichocarpa</i>	F ₁	1 st year stem / 4 th year roots	33		21.6 ± 0.05	20.7	22.5	France
		nursery / water deficit						22.0 ± 0.06	21.0	23.1	
Soolanayakanahally et al. (2009)	2005 - 2006	outdoor common garden	<i>P. balsamifera</i>	21 provenances	15 to 30 years	210			22.7	23.7	Canada
Yin et al. (2009)	2004	greenhouse - control	<i>P. cathayana</i> , <i>P. przewalskii</i>	cuttings	1 st year stem / 1 st year roots			20.7 ± 0.34			China
	2004	greenhouse - water deficit (25%)						20.2 ± 0.15			
Bonhomme et al. (2008)	2005	commercial plantation	<i>P. deltooides</i> x <i>P. nigra</i>	unrelated	6 to 9 years	8		21.3 ± 0.26	20.1	22.3	France
	2006							22.2 ± 0.29	21.3	23.6	
Dillen et al. (2008)	2006	nursery	<i>P. deltooides</i> x <i>P. nigra</i>	F ₁	1 st year stem / 4 th year roots	50		20.8 ± 0.04	19.9	21.6	
		nursery	<i>P. deltooides</i> x <i>P. trichocarpa</i>	F ₁	1 st year stem / 4 th year roots	50		20.5 ± 0.04	19.8	21.7	
DesRochers et al. (2007)		greenhouse	<i>P. balsamifera</i> , <i>P. deltooides</i> x <i>P. petrowskyana</i>	cuttings	1 st year stem / 1 st year roots	3			21.5	23.5	Canada
Ren et al. (2007)	2004	greenhouse	<i>P. cathayana</i> , <i>P. kangdingensis</i>	cuttings	1 st year stem / 1 st year roots			18.0			China
DesRochers et al. (2006)	2002	agricultural land	<i>P. balsamifera</i> x <i>P. simonii</i> , <i>P. deltooides</i> x <i>P. petrowskyana</i>	cuttings	1 st year stem / 1 st year roots	3		19.0 ± 0.07			Canada
	2003							20.4 ± 0.04			
Monclus et al. (2006)	2004	nursery	<i>P. deltooides</i> x <i>P. nigra</i>	unrelated	1 st year stem / 4 th year roots	29		21.6 ± 0.23	20.5	23.2	France
		nursery / water deficit						21.3 ± 0.19	19.8	22.3	
Voltas et al. (2006)	1999	plantation	<i>P. deltooides</i> x <i>P. nigra</i>	rooted cuttings	1 st year stem / 2 nd year roots	4		20.8	20.4	21.2	Spain
	2000							21.1	20.3	21.6	
Marron et al. (2005)	2001	greenhouse	<i>P. deltooides</i> x <i>P. nigra</i>	unrelated	3 months	31		21.8 ± 0.14	21.7	27.9	France
Monclus et al. (2005)	2003	nursery	<i>P. deltooides</i> x <i>P. nigra</i>	unrelated	1 st year stem / 3 rd year roots	29		20.0 ± 0.11	18.7	21.5	France
Yin et al. (2005)	2003	greenhouse - control	<i>P. cathayana</i> , <i>P. przewalskii</i>	cuttings	1 st year stem / 1 st year roots			24.7 ± 0.31			China
	2003	greenhouse - water deficit (25%)					21.4 ± 0.32				
Zhang et al. (2005)		greenhouse - control	<i>P. davidiana</i>	seedlings	1 st year stem / 1 st year roots			22.4 ± 0.41			Finland

		greenhouse - water deficit (20%)					19.5 ± 0.34		
Li et al. (2004)	2002	greenhouse - control					22.4 ± 0.39		China
	2002	greenhouse - water deficit (50%)	<i>P. davidiana</i>	seedlings	1 st year stem / 1 st year roots		20.6 ± 0.30		
Rae et al. (2004)	2001	nursery	<i>P. trichocarpa</i> x <i>P. deltooides</i>	F ₂ cuttings	1 st year stem / 2 nd year roots	210	12.0	23.0	
Rippulone et al. (2004)	2000	nursery	<i>P. deltooides</i> x <i>P. nigra</i>	cuttings	1 st year stem / 1 st year roots		22.6		Italy
Yin et al. (2004)	2003	greenhouse - control	<i>P. cathayana</i> , <i>P. kangdingensis</i>	cuttings	1 st year stem / 1 st year roots		24.1 ± 0.38		China
	2003	greenhouse - water deficit (25%)					21.0 ± 0.30		
Zhang et al. (2004)		greenhouse - control					22.4 ± 0.40		China
		greenhouse - water deficit	<i>P. davidiana</i>	seedlings	1 st year stem / 1 st year roots		19.4 ± 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	forest					20.0	24.0	

Table 2. Schematic overview of the available leaf material for carbon isotope discrimination (Δ) analysis collected during different field studies from 2004 to 2006 in Italy and/or France. Each time 31 common F₁ genotypes were selected from each of the two families. D×N = *P. deltooides* ‘S9-2’ × *P. nigra* ‘Ghoy’ and D×T = *P. deltooides* ‘S9-2’ × *P. trichocarpa* ‘V24’.

	D×N family		D×T family	
	Italy	France	Italy	France
2004		✓		
2005*	✓	✓	✓	✓
2006†		✓		✓

*In 2005, tree growth and additional leaf traits were measured.

†Leaf carbon isotope discrimination (Δ) values have been described in Dillen et al. (2008)

Table 3. Within-family broad-sense heritability (H^2) and standard error (SE) for leaf carbon isotope discrimination (Δ) in Italy and/or in France during 2004, 2005 and/or 2006; D×N = *P. deltooides* ‘S9-2’ × *P. nigra* ‘Ghoy’, D×T = *P. deltooides* ‘S9-2’ × *P. trichocarpa* ‘V24’, and level of significance of the genotype effect is indicated: *** = $P \leq 0.001$, ** = $P \leq 0.01$ and ns = non-significant.

	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 [†]			0.40 ± 0.10	***			0.40 ± 0.09	***

[†] H^2 values were recalculated on 31 F_1 genotypes from the study of Dillen et al. (2008)

Table 4. Relative importance of (A) genetic (σ^2_G), site (σ^2_S), genotype by site ($\sigma^2_{G \times S}$), and residual (σ^2_ϵ) variances in the phenotypic variation (σ^2_P) across the two sites, (B) genetic (σ^2_G), year (σ^2_Y), genotype by year ($\sigma^2_{G \times Y}$), and residual (σ^2_ϵ) variances in the phenotypic variation (σ^2_P) across three (D×N) or two (D×T) years, and (C) the ratio of genotypic to phenotypic variance across sites in 2005 (H^2_{Sites}) and across years in France (H^2_{Years}), 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’ ; level of significance of the effects: *** = $P \leq 0.001$, ** = $P \leq 0.01$, * = $P \leq 0.05$ and ns = non-significant.

A	σ^2_G/σ^2_P	σ^2_S/σ^2_P	$\sigma^2_{G \times S}/\sigma^2_P$	$\sigma^2_\epsilon/\sigma^2_P$
D×N	12.4 *	20.3 ***	9.2 *	58.1
D×T	3.4 ns	6.3 *	13.9 **	76.4
B	σ^2_G/σ^2_P	σ^2_Y/σ^2_P	$\sigma^2_{G \times Y}/\sigma^2_P$	$\sigma^2_\epsilon/\sigma^2_P$
D×N	10.6 ***	67.8 ***	2.9 ns	18.7
D×T	34.9 ***	1.9 *	16.9 **	46.3
C	H^2_{Sites}		H^2_{Years}	
D×N	0.32 ± 0.07		0.70 ± 0.06	
D×T	0.18 ± 0.08		0.73 ± 0.06	

Figure 1. Box plots of genotypic means of leaf carbon isotope discrimination (Δ , ‰) 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 F₁) = *P. deltoides* ‘S9-2’ × *P. nigra* ‘Ghoy’, D×T (hatched, 31 F₁) = *P. deltoides* ‘S9-2’ × *P. trichocarpa* ‘V24’. Means (\pm standard errors) are indicated for the parents: circle = *P. deltoides* ‘S9-2’, square = *P. nigra* ‘Ghoy’, triangle = *P. trichocarpa* ‘V24’.

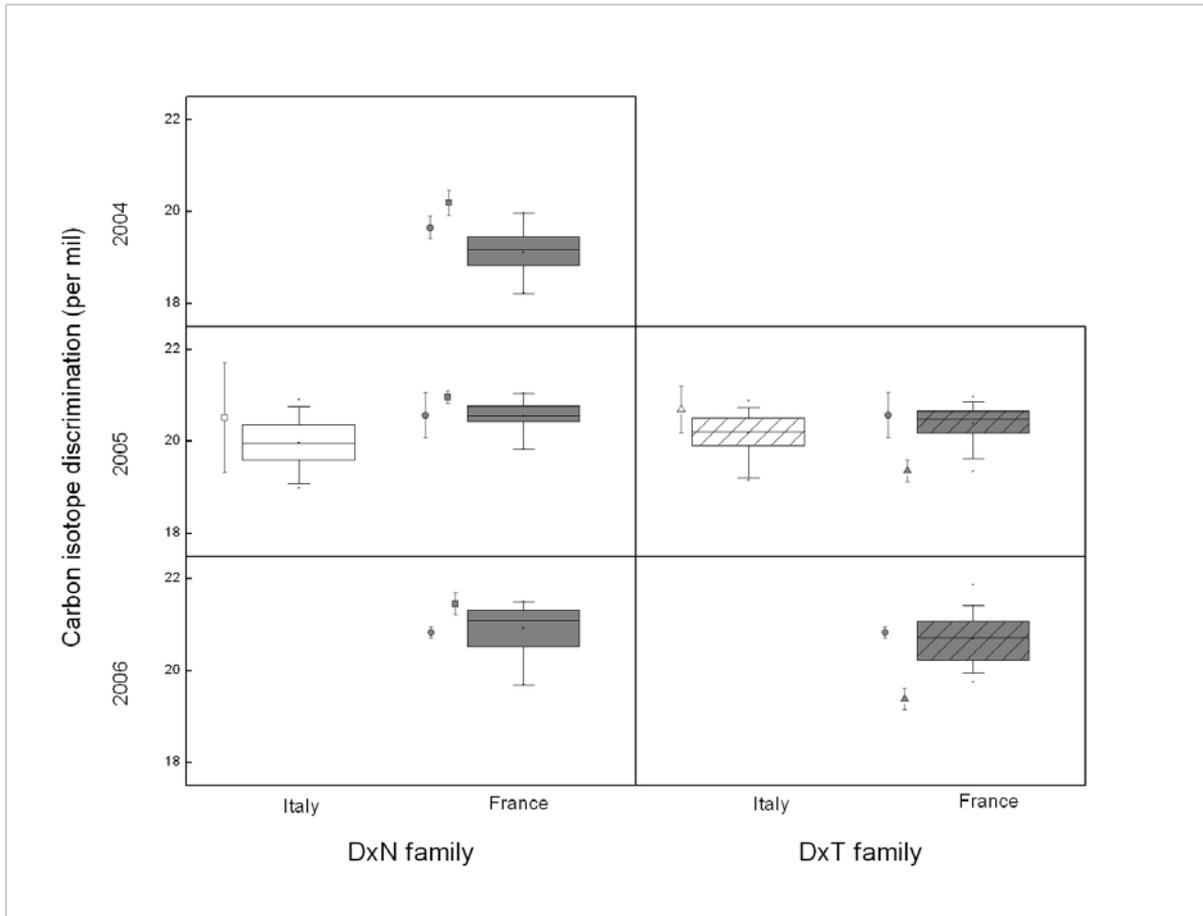


Figure 2. Between-year relationships for leaf carbon isotope discrimination (Δ) in France are presented on a genotypic mean basis (\pm standard error). For the D×N family, Δ in 2004 vs. 2005 and in 2005 vs. 2006 were plotted, and for D×T family, Δ was measured in 2005 and 2006 only. Spearman rank coefficients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

