

Evaluation of leaf traits for indirect selection of high yielding poplar hybrids

Nicolas Marron^{*,1}, Sophie Y. Dillen¹, Reinhart Ceulemans

Research Group of Plant and Vegetation Ecology, Department of Biology, University of Antwerp (UA),
Campus Drie Eiken, Universiteitsplein 1, B-2610 Wilrijk, Belgium

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Abstract

Two *Populus* families growing at two sites in Europe (i.e., northern Italy versus central France) were used to investigate: (1) the relationships between various leaf structural and growth traits and biomass production, (2) the dependence of these relationships on environmental conditions and genetic background (i.e., *Populus deltoides* × *Populus nigra* family versus *P. deltoides* × *Populus trichocarpa* family), and (3) the subsequent relevance of the use of these morphophysiological traits as indirect indicators of productivity. Tree growth and leaf characteristics, as well as the links between them, were intensively studied for 3 weeks. The *P. deltoides* × *P. trichocarpa* family was more productive than the *P. deltoides* × *P. nigra* family at both sites. The two families inherited complementary leaf characteristics from their respective male parents, i.e., large leaves from *P. trichocarpa* and fast leaf production from *P. nigra*. The traits were clearly dependent on site conditions, trees being much bigger in Italy than in France. Moreover, the G × E interaction caused a significant change in the genotypic ranking between sites in terms of productivity for the *P. deltoides* × *P. trichocarpa* family, which might represent a limit for the selection of productive hybrids showing a large environmental spectrum. Three categories of leaf traits could be considered: (i) traits linked to whole tree growth irrespective of site and family (e.g., leaf area, petiole dimensions), (ii) traits for which the relationships with tree growth were dependent on site and/or family (e.g., specific leaf area, nitrogen content, leaf number increment), and (iii) traits showing no link with tree growth irrespective of site and family (e.g., chlorophyll and carbon contents).

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1. Introduction

Poplars are considered the fastest growing trees under temperate latitudes and are widely used in the wood industry for the production of paper, plywood, matches, and light packaging materials or for bioenergy (Hansen, 1991; Heilman et al., 1994; Zsuffa et al., 1996). The *Populus* genus is a very diverse genus in terms of the level of productivity and resistance to pathogens; many selection programmes have been developed in order to screen for the most promising genotypes. However, due to the extremely complex genetic basis for productivity, improving and maintaining plant performance under fluctuating environmental conditions remains a slow, laborious, and

mostly empirical process. Progress in increasing productivity and its stability through direct selection has been hampered by the relatively low heritability of biomass production and by its large genotype × environment (G × E) interaction (Ceccarelli and Grando, 1996). Moreover, from a technical point of view, the direct estimation of plant productivity is also a time-demanding and costly process, requiring a large amount of work by the breeders.

As an alternative to the direct selection for biomass production, morphophysiological traits genetically correlated with productivity have been targeted in selection programmes pursued in collaboration between physiologists and breeders. Even if the ideotype concept (i.e., a model plant of the desirable phenotype) has been more widely used by crop breeders than by forest tree breeders (with exception of the Finnish conifer ideotype and poplar ideotype; see review by Dickmann et al., 1994), Stettler et al. (1992) suggested the use of ideotypes as one possibility for breeding in short-rotation forestry (Rönnerberg-Wästljung and Gullberg, 1999). The successful application of this strategy

* Corresponding author. Present address: UMR 1137 INRA-UHP Écologie et écophysio-logie forestières, 54280 Champenoux, France.

Tel.: +33 3 83 68 42 48; fax: +33 3 83 68 42 40.

E-mail address: Nicolas.Marron@sbiol.uhp-nancy.fr (N. Marron).

¹ Both authors contributed equally to the work.

requires morphophysiological traits that are cheap and easy to score, characterized by a high genetic correlation with biomass production and/or growth performances, and by heritability higher than that of productivity (Tuberosa et al., 2002). In poplar, numerous traits, both at the whole plant and leaf levels, have been examined and evaluated as potential determinants of productivity. At crown level, tree architecture, canopy density, or sylleptic branchiness have been shown to be intimately related to stand productivity in some cases (Ceulemans, 1990; Dickmann et al., 1994; Wu and Stettler, 1998; Marron et al., 2006). At the leaf level, functional and structural components generally associated with high growth rates and productivity include total and individual leaf area, internal leaf structure, stomatal morphology and behaviour, leaf growth physiology, and functional traits such as photosynthetic performance (Isebrands and Nelson, 1982; Ceulemans et al., 1988; Pellis et al., 2004; Al Afas et al., 2006). Unfortunately, only a few success stories have so far been reported for enhancing productivity by applying indirect selection.

When identifying improved genotypes and potential cultivars, plant breeders routinely practice selection for genotypes that display stability for a given trait or set of traits across different environments. The need to develop well-buffered cultivars has led to a greater emphasis on phenotypic stability in breeding programs (Lin et al., 1986). Eberhart and Russell (1966) defined stability as the ability to show a minimum interaction with the environment. Hence, the stability of genotype performance is directly related to the effect of $G \times E$ interactions. $G \times E$ interactions are defined as the differential response of a genotype or cultivar for a given trait across environments, and they are important and essential components of plant breeding programmes dedicated to cultivar development.

To obtain information on $G \times E$ interactions and on the genetic background of the ecophysiological yield determinants in poplar, five inter- and intraspecific *Populus* hybrid families were planted at three sites in Europe (i.e., northern Italy, central France, and southern England) within the framework of an EC-funded research program (POPYOMICS; <http://www.soton.ac.uk/~popymic/>). Two of these families are full-sib families resulting from controlled crosses of the female parent *P. deltoides* 'S9-2' with *P. nigra* 'Ghoy' and *P. trichocarpa* 'V-24', respectively (Cervera et al., 2001). These two families were chosen because the genetic maps of the three parents are currently being established. Moreover, hybrids produced from these two interspecific crosses are of commercial importance. Indeed, most of the commercial clones planted throughout Europe and North America are derived from interspecific crosses between *P. deltoides* and *P. nigra*, or between *P. deltoides* and *P. trichocarpa* or their backcrosses, mainly because of the positive and high heterosis (superiority of the hybrids over the parents) often exhibited by the hybrids between these species (Dickmann and Stuart, 1983; Stettler et al., 1996; Cervera et al., 2001).

In a previous study, biomass production was shown to be tightly linked to the leaf number increment and to the area of the largest leaf along the stem in a subset of genotypes

belonging to the *P. deltoides* \times *P. nigra* family at the intermediate French site (Marron and Ceulemans, 2006). However, the afore-mentioned study was conducted at one site and with one of the two families only. In these conditions, it is difficult to know whether the observed relationships between productivity and its potential leaf determinant are robust and can be used across environments and for contrasted genetic background. In this context, the objective of the present study was to estimate the environmental, temporal, and genetic stability of the leaf determinants of productivity by relating differences in productivity among the hybrids to differences in leaf morphological and physiological traits for: (1) the two poplar families, (2) different environmental conditions (i.e., central France and northern Italy), and (3) different growing seasons (i.e., second growing season from Marron and Ceulemans (2006) and first growing season after coppicing in the present study). Furthermore, the replication of the experiment at the two sites allowed us to estimate the impact of the genotype \times site ($G \times S$) interactions on leaf growth, morphology, and physiology as well as on their links with tree productivity for the two families.

2. Materials and methods

2.1. Plant material

Two full-sib families resulting from controlled crosses and sharing the same female parent were used in this experiment. One family consisted of 180 genotypes (F_1) resulting from an interspecific cross between *P. deltoides* (Bartr. ex Marsh.) 'S9-2' and *P. nigra* (L.) 'Ghoy' ($D \times N$ family) (Cervera et al., 1996, 2001). The second family was composed of 182 genotypes and was generated from an interspecific cross involving *P. deltoides* 'S9-2' and *P. trichocarpa* (Torr. and Gray) 'V-24' ($D \times T$ family) (Marron et al., 2006). Both crosses were realized by the Institute of Forestry and Game Management (IBW, Geraardsbergen, Belgium) in 1987 and repeated in 1995 to enlarge the progeny. A sample of 31 genotypes of each family was selected to be representative of the genetic variation in the second-year biomass production. At least four replicates of each selected F_1 genotype were alive at each site.

2.2. Experimental design

The two experimental plantations were established in April 2003 from 25 cm uniform hardwood cuttings. The initial spacing was 0.75 m \times 2 m, accommodating an overall plant density of 6670 trees ha^{-1} . The experimental plantations were established according to a randomized block design (Marron et al., 2006). Six blocks were defined, and one replicate of each F_1 genotype and each parent was randomly allocated to each block. To reduce the border effects (Zavitkovski, 1981; Van Hecke et al., 1995), a double border row was planted around the plantations. The plantation management included irrigation, and the use of insecticides and fungicides as needed throughout the three growing seasons.

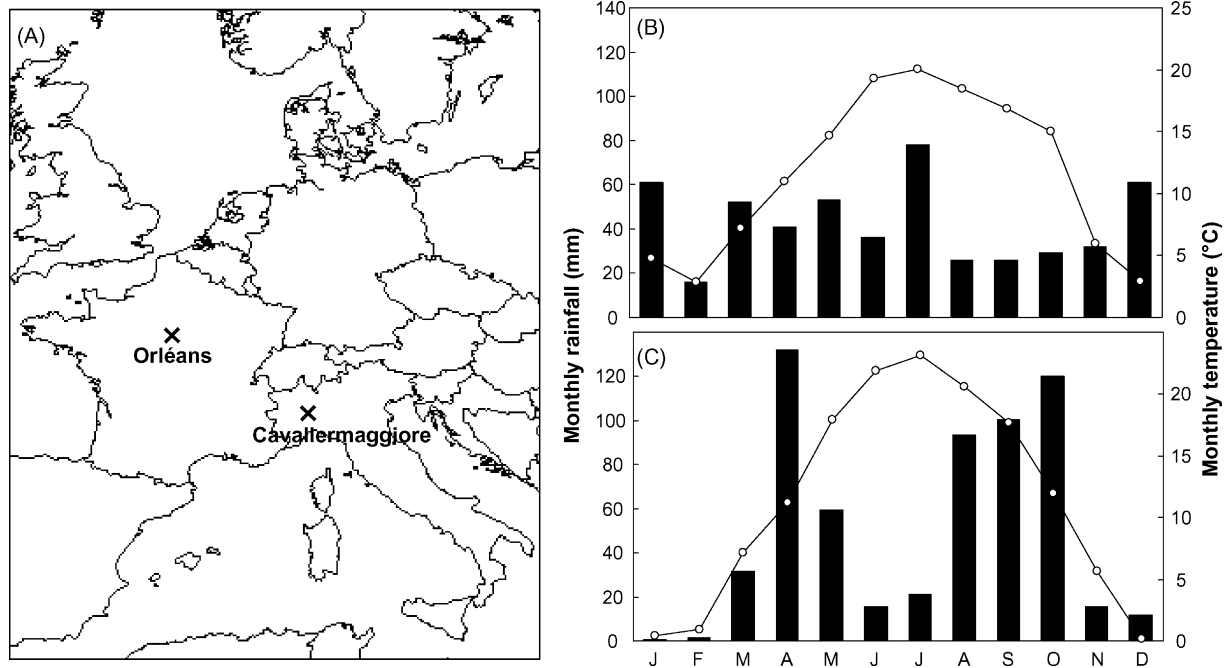


Fig. 1. Geographical location of the two experimental sites in Europe (A) and climatic conditions in 2005 (monthly rainfalls, black bars; monthly temperatures, curves) in Orléans, France (B) and Cavallermaggiore, Italy (C).

2.3. Site description

The two field trials were located in central France (Orléans, Loire valley, 47°46'N, 1°52'E) and northern Italy (Cavallermaggiore, Po valley, 44°42'N, 7°40'E) (Fig. 1). The elevation was 110 m above sea level in Orléans and 285 m in Cavallermaggiore. The soil was mainly composed of loam in Italy and of sand in France. Annual temperatures were equal for the two sites in 2005 (11.6 °C). However, the climatic conditions were more variable in Italy than in France. Indeed, in 2005, monthly mean temperatures ranged from 0 to 23 °C in Cavallermaggiore, while they ranged from 3 to 20 °C in Orléans. In Orléans, monthly rainfalls were distributed almost equally during the year, while in Cavallermaggiore, they were concentrated mostly during spring and fall. The wettest month in Cavallermaggiore (i.e., April) received almost double the amount of rainfall as compared with the same period in Orléans.

2.4. Traits measured

On 25 June 2005, a label was attached to the main stem of each selected tree, 7 leaves below the youngest leaf just exceeding 20 mm in length (leaf *n*) (Larson and Isebrands, 1971; Marron and Ceulemans, 2006). From 27 June to 13 July 2005, (1) leaves between the label and leaf *n* were counted every second day, (2) stem height was measured to the nearest centimeter every second day using a graduated height pole, and (3) circumference was measured at ground level to the nearest millimeter using a taper at the beginning and at the end of the measuring period. Stem volume was calculated for each individual stem and measurement date from stem height and average circumference assuming a conical shape (Pontaiiller et al., 1997;

Marron et al., 2006). For each tree, a significant, linear, and positive relationship was obtained between time (day of year) and number of leaves (NL), total height (Ht, cm), and volume (Vol, cm³) of the stem. Leaf number increment (d NL/dt, day⁻¹), total height growth rate (d Ht/dt, cm day⁻¹), and volume growth rate (d Vol/dt, cm³ day⁻¹) were calculated as the regression coefficients of the relationships with time (Fig. 2).

Between 14 and 15 July 2005, the largest leaf on the main stem of each tree was harvested. The relative chlorophyll content (Chloro) was measured non-destructively with a SPAD-502 Minolta (Japan) chlorophyll meter and expressed in $\mu\text{mol g}_{\text{DW}}^{-1}$. The maximal leaf area (LA, cm²) was determined using a leaf area meter (CID, type CI-203, Inc., Camas, WA), dried at 70 °C in a drying oven for at least 48 h and weighed. The specific leaf area of the largest leaf on the main stem (SLA, cm² g_{DW}⁻¹) was calculated as the individual leaf area divided by the individual leaf dry weight. The petiole length (PetLg, mm) and dry weight (PetDW, g_{DW}) were also determined. Dry leaves and petioles were ground independently to a fine powder and analyzed for total nitrogen and carbon contents in 5 mg of powder by a dynamic Flush Combustion Method with the NC 2100 Soil Autoanalyzer (Carlo Erba, Italy). Total carbon and nitrogen contents of laminae and petioles were all expressed on a dry mass basis (C_M , N_M , $\text{Pet}C_M$, $\text{Pet}N_M$, respectively; mg g_{DW}⁻¹).

2.5. Data analyses

Statistical analyses were performed with R software (version 2.0.1, A Language and Environment Copyright, 2004). Data were tested for normality with the Shapiro–Wilk statistic. In case of non-normality, the Box–Cox test was performed. The lambda value of this test was used to transform the non-normal data.

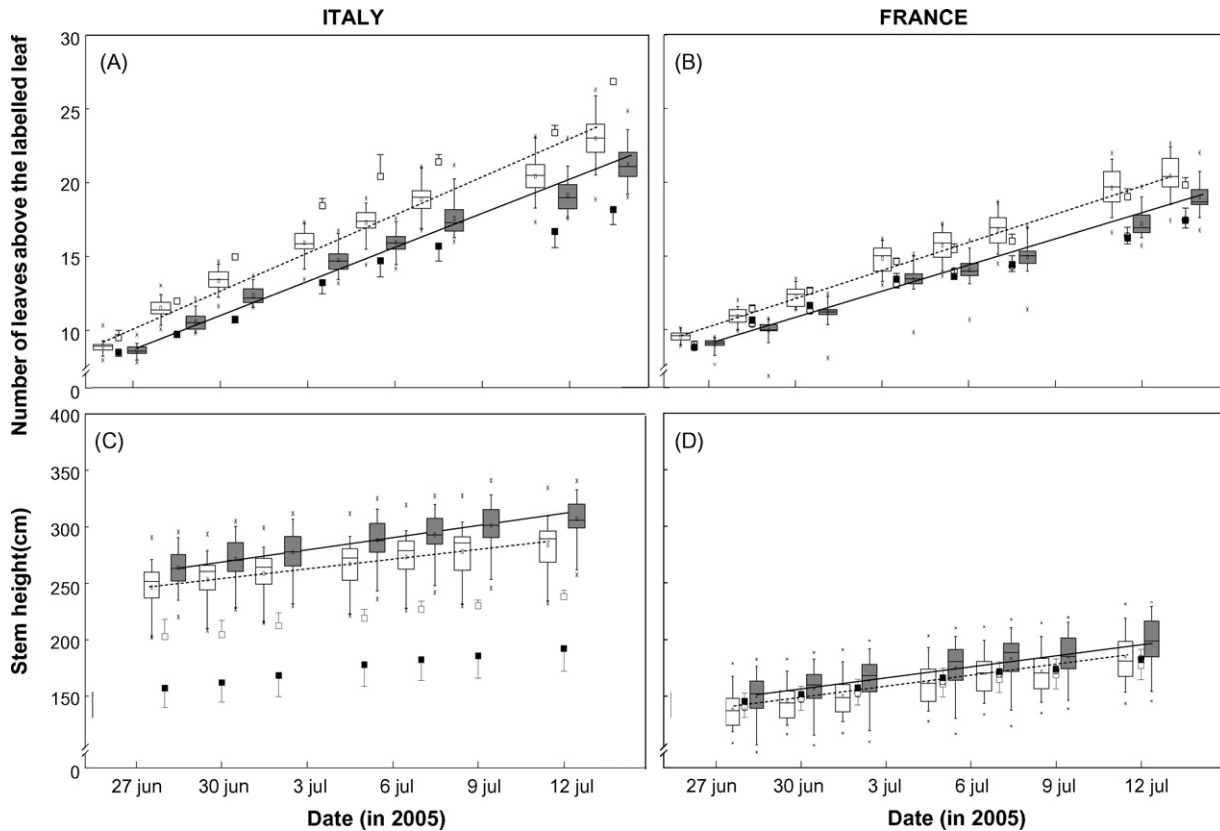


Fig. 2. Number of leaves (A and B) and stem height (C and D) during the 3-week experiment for the 31 D \times N (white boxes and dashed lines) and 31 D \times T (grey boxes and full lines) F₁ hybrids and their parents, *P. nigra* 'Ghoy' (black squares), *P. deltooides* 'S9-2' (white circles), and *P. trichocarpa* 'V-24' (white squares) in Cavallermaggiore, Italy (A and C) and in Orléans, France (B and D). For the parents, dots are means (\pm S.E.). The female parent *P. deltooides* 'S9-2' was absent in Italy, and only two replicates of *P. nigra* 'Ghoy' remained alive at this site.

Means were calculated with their standard error (\pm S.E.). For each family, data were evaluated by the following two models of analysis of variance (ANOVA):

- for within-site comparison: $Y_{ij} = \mu + B_i + G_j + \varepsilon_{ij}$, where μ is the general mean, B_i the effect of block i considered as fixed, and G_j is the effect of genotype j considered as random. Variance components of random effects (σ_G^2 and σ_ε^2), genetic parameters and their standard errors were calculated by equating observed mean squares to expected mean squares and solving the resulting equations (Henderson, 1953).
- for between-site comparison: $Y'_{jk} = \mu + G_j + S_k + G \times S_{jk} + \varepsilon_{ij}$, where Y'_{jk} are individual values adjusted for the within site block effects ($Y' = Y - B_i$), μ is the general mean, G the genotype effect (random), S the site effect (random), and $G \times S$ is the genotype by site interaction effect (random). In order to quantify the relative importance of each effect, variance components, σ_G^2 , σ_S^2 , $\sigma_{G \times S}^2$, and σ_ε^2 , were calculated by equating observed mean squares to expected mean squares and solving the resulting equations (Henderson, 1953).

Family means were compared by ANOVA. Parental performances were compared with the Scheffé method. Differences

between means were considered significant when the P value of the ANOVA F -test ≤ 0.05 .

Mean heterosis (or hybrid vigour) was expressed as the percentage of parental means (Li and Wu, 1997). The coefficients of genetic (CV_G) and residual (CV_ε) variation were estimated by σ_G/μ and σ_ε/μ , respectively. Broad-sense heritabilities (H^2) were estimated for each site on an individual basis as $H^2 = \sigma_G^2/(\sigma_G^2 + \sigma_\varepsilon^2)$ (Nyquist, 1991; Singh et al., 1993; Riemenschneider et al., 1996). The standard errors of broad-sense heritability were calculated using the method described by Singh et al. (1993). The change in genotype ranking across sites was described by the Spearman's rank coefficient (R_{Spearman}) on genotypic means for each site.

For each site and family, linear regressions were used to relate stem volume growth rate ($d\text{Vol}/dt$) to the foliar traits. The mathematical expression of the fitted model was $d\text{Vol}/dt = a + bV_1 + cV_2 + dV_3 + \dots + xV_n$, where letters a – d represent the fitted regression constants and V_n the measured experimental variables. To avoid collinearity between traits, measured variables included in the regression analysis were $d\text{NL}/dt$, LA, SLA, Chloro, N_M , C_M , PetDW, PetLg, Pet N_M , and Pet C_M .

Linear correlations between traits were estimated with Pearson's correlation coefficient on a genotypic mean basis. To summarize the variability of both families at the two sites as well as the relationships between traits, multivariate analyses

using principal components analysis (PCA) were performed. The same traits as for the linear regressions, plus stem volume (Vol), were used. The traits were standardized, and orthogonal factors (i.e., PC1 and PC2 axes) were successively established as linear combinations of these traits to maximize 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 coefficients) with these factors. PCA's were realized for each family and each site independently.

3. Results

3.1. Within-site variability

3.1.1. Family comparison

At the two sites, significant differences between mean values of the two families were observed for most of the studied traits ($P \leq 0.001$) (Table 1). The D × T family showed a significantly faster tree growth in height, circumference, and volume than the

D × N family (Fig. 2). However, the D × N family exhibited a faster leaf number increment than the D × T family. The produced leaves were larger (Fig. 3), denser (i.e., with lower SLA), richer in carbon, and, on the contrary, poorer in nitrogen for the D × T than for the D × N family. Furthermore, leaves of the D × T family showed longer and heavier petioles than the ones of the D × N family.

3.1.2. Hybrids–parents comparison

Parents of both families showed only few differences in most leaf traits (Table 2). In France, leaves were produced faster and they reached a larger mature size for the *P. nigra* 'Ghoy' male parent compared with both of the other parents ($P \leq 0.05$). On the other hand, the *P. deltooides* 'S9-2' female parent showed significantly heavier and longer petioles than both of the other parents. Leaves of *P. trichocarpa* 'V-24' were significantly richer in carbon than the ones of *P. deltooides* 'S9-2', *P. nigra* 'Ghoy' showing intermediate values. Both families showed hybrid vigour in growth traits compared with the mean of their two parents (Table 3). The highest values of heterosis

Table 1

General means (\pm S.E.), range of variation, and level of significance of differences between the families (D × N and D × T hybrids) for each site, and between sites (Orléans, F and Cavallermaggiore, I) for each family, for plant growth, lamina and petiole traits

	D × N family (31 F ₁)				Pedigree variation	D × T family (31 F ₁)		
	Site	Mean \pm S.E.	Genotypic range	Site variation		Mean \pm S.E.	Genotypic range	Site variation
Plant growth traits								
Height (cm)	F	145.2 \pm 2.8	116.3–182.8	***	ns	152.9 \pm 3.8	108.5–186.2	***
	I	246.5 \pm 4.0	202.6–290.5		<***	264.0 \pm 3.1	220.8–295.4	
Circumference (mm)	F	65.4 \pm 1.8	47.2–85.2	***	<***	77.4 \pm 2.0	49.7–95.4	***
	I	89.3 \pm 2.1	64.5–111.25		<***	98.9 \pm 1.7	74.8–116.5	
Volume (cm ³)	F	219.7 \pm 16.2	81.3–418.8	***	<***	334.3 \pm 20.1	124.0–543.3	***
	I	558.6 \pm 30.3	230.9–934.9		<***	717.3 \pm 28.7	354.7–1040.2	
dH/dt (cm day ⁻¹)	F	2.85 \pm 0.07	1.87–3.60	ns	<***	3.11 \pm 0.06	2.27–3.88	ns
	I	2.78 \pm 0.06	2.01–3.47		<***	3.22 \pm 0.07	1.56–3.22	
dVol/dt (cm ³ day ⁻¹)	F	4.28 \pm 0.31	1.30–7.65	***	<***	6.56 \pm 0.32	2.40–9.69	***
	I	7.37 \pm 0.46	3.41–12.82		<***	9.96 \pm 0.41	3.67–13.68	
dNL/dt (day ⁻¹)	F	0.72 \pm 0.01	0.55–0.83	***	>***	0.60 \pm 0.01	0.48–0.73	***
	I	0.85 \pm 0.01	0.69–1.01		>***	0.73 \pm 0.01	0.63–0.92	
Lamina traits								
LA (cm ²)	F	151.3 \pm 5.7	97.2–212.9	***	<***	337.6 \pm 13.6	157.0–475.8	ns
	I	196.8 \pm 3.8	123.1–262.1		<***	354.9 \pm 6.4	275.0–465.2	
SLA (cm ² g _{DW} ⁻¹)	F	182.8 \pm 2.4	156.9–215.7	***	>***	169.7 \pm 3.1	109.4–194.6	***
	I	162.7 \pm 1.9	130.5–200.0		>***	145.2 \pm 1.9	127.0–170.2	
Chloro (μmol g _{DW} ⁻¹)	F	8.38 \pm 0.19	6.87–11.35	ns	ns	8.06 \pm 0.15	6.53–9.80	*
	I	8.29 \pm 0.18	6.69–10.38		ns	8.47 \pm 0.12	7.60–10.27	
N _M (mg g _{DW} ⁻¹)	F	34.3 \pm 0.3	28.2–37.1	***	>***	29.2 \pm 0.4	20.8–36.2	***
	I	40.5 \pm 0.4	34.0–45.9		>***	34.4 \pm 0.3	31.3–37.8	
C _M (mg g _{DW} ⁻¹)	F	474.5 \pm 0.7	458.1–492.5	***	<***	485.9 \pm 1.5	474.5–499.1	***
	I	453.4 \pm 1.3	429.5–472.1		<***	461.3 \pm 1.1	440.8–474.0	
Petiole traits								
PetLg (mm)	F	82.1 \pm 1.8	62.3–107.3	*	<***	102.9 \pm 2.7	68.2–127.8	ns
	I	89.2 \pm 0.9	64.7–104.3		<***	106.8 \pm 1.1	89.6–126.2	
PetDW (g)	F	0.112 \pm 0.008	0.062–0.310	***	<***	0.233 \pm 0.010	0.097–0.307	***
	I	0.159 \pm 0.005	0.076–0.234		<***	0.299 \pm 0.007	0.213–0.406	
PetN _M (mg g _{DW} ⁻¹)	F	10.6 \pm 0.1	8.9–13.8	***	>***	8.6 \pm 0.1	7.3–10.4	***
	I	11.8 \pm 0.2	9.4–13.9		ns	12.1 \pm 0.2	10.6–14.7	
PetC _M (mg g _{DW} ⁻¹)	F	458.7 \pm 0.5	449.9–464.3	***	>***	444.4 \pm 0.7	430.8–458./	***
	I	438.4 \pm 2.2	419.7–455.5		ns	435.0 \pm 0.2	416.7–452.9	

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$; and ns = non-significant. See Section 2 for abbreviations.

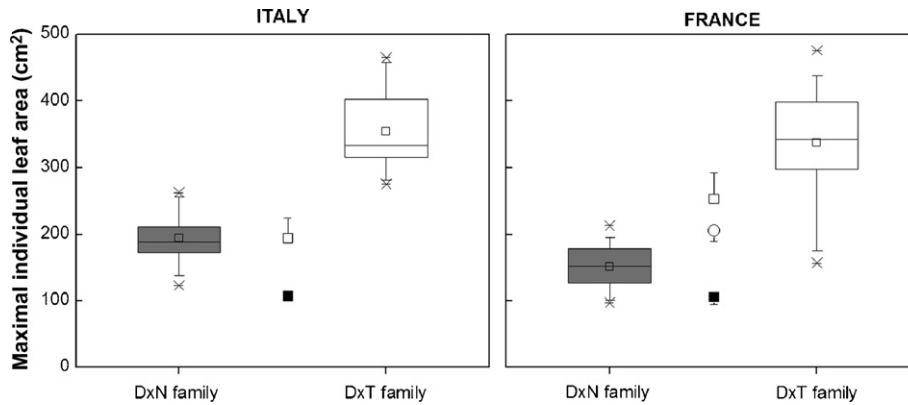


Fig. 3. Boxplots of the maximal individual leaf area along the stem (LA) measured at the end of the experiment for the 31 D × N (white boxes) and 31 D × T (grey boxes) F₁ hybrids and their parents, *P. nigra* ‘Ghoy’ (black squares), *P. deltooides* ‘S9-2’ (white circles), and *P. trichocarpa* ‘V-24’ (white squares) in Cavallermaggiore, Italy and in Orléans, France. For the parents, dots are means (±S.E.). The female parent *P. deltooides* ‘S9-2’ was absent in Italy, and only two replicates of *P. nigra* ‘Ghoy’ remained alive at this site.

Table 2
General means (±S.E.) and significance of differences between parents (*P. nigra* ‘Ghoy’, *P. deltooides* ‘S9-2’, and *P. trichocarpa* ‘V-24’), indicated by different capital letters for $P \leq 0.05$, and between sites (Orléans, F and Cavallermaggiore, I), indicated by asterisks: * $P \leq 0.05$, ** $P \leq 0.01$ or *** $P \leq 0.001$, for plant growth, lamina and petiole traits

	Site	<i>P. nigra</i> ‘Ghoy’		<i>P. deltooides</i> ‘S9-2’		<i>P. trichocarpa</i> ‘V-24’	
		Mean ± S.E.	Variation	Mean ± S.E.	Variation	Mean ± S.E.	Variation
Plant growth traits							
Height (cm)	F	142.8 ± 11.7	*	125.7 ± 9.9		133.6 ± 12.9	
	I	203.5 ± 15.5		–		157.8 ± 17.0	
Circumference (mm)	F	63.6 ± 9.4		58.3 ± 4.1		71.8 ± 4.8	*
	I	65.0 ± 9.0		–		50.8 ± 5.9	
Volume (cm ³)	F	201.0 ± 59.7		147.7 ± 24.2		224.7 ± 45.0	
	I	237.2 ± 80.9		–		119.8 ± 41.8	
dHt/dt (cm day ⁻¹)	F	2.36 ± 0.10		2.97 ± 0.27		2.46 ± 0.24	
	I	2.67 ± 0.76		–		2.57 ± 0.63	
dVol/dt (cm ³ day ⁻¹)	F	3.18 ± 0.86		3.66 ± 0.47		4.14 ± 0.88	
	I	3.16 ± 0.11		–		1.90 ± 0.51	
dNL/dt (day ⁻¹)	F	0.66 ± 0.03	A***	0.53 ± 0.04	B	0.50 ± 0.04	B
	I	1.05 ± 0.02		–		0.60 ± 0.07	
Lamina traits							
LA (cm ²)	F	106.4 ± 10.8	A	206.3 ± 16.4	B	252.8 ± 40.1	B
	I	108.0 ± 2.2		–		194.7 ± 31.4	
SLA (cm ² g _{DW} ⁻¹)	F	162.1 ± 6.1		174.7 ± 11.0		163.3 ± 9.3	
	I	154.0 ± 16.9		–		199.1 ± 18.6	
Chloro (μmol g _{DW} ⁻¹)	F	8.63 ± 1.16		7.36 ± 0.55		7.74 ± 0.46	
	I	7.42 ± 0.85		–		6.57 ± 1.14	
N _M (mg g _{DW} ⁻¹)	F	30.7 ± 0.8		32.2 ± 2.6		27.1 ± 1.8	
	I	31.3 ± 2.3		–		33.1 ± 3.9	
C _M (mg g _{DW} ⁻¹)	F	483.4 ± 2.7	AB**	469.4 ± 4.2	A	494.2 ± 5.0	B*
	I	446.1 ± 7.9		–		463.6 ± 9.6	
Petiole traits							
PetLg (mm)	F	65.4 ± 3.5	A	113.1 ± 5.8	B	55.0 ± 5.4	A
	I	63.5 ± 5.5		–		40.5 ± 6.8	
PetDW (g)	F	0.081 ± 0.009	A	0.164 ± 0.020	B	0.098 ± 0.020	A
	I	0.093 ± 0.010		–		0.062 ± 0.017	
PetN _M (mg g _{DW} ⁻¹)	F	8.5 ± 0.3	**	9.0 ± 1.3		11.4 ± 0.8	
	I	10.8 ± 0.4		–		17.3 ± 3.0	
PetC _M (mg g _{DW} ⁻¹)	F	451.1 ± 2.6	*	449.3 ± 5.4		440.3 ± 4.3	
	I	418.7 ± 20.8		–		426.5 ± 4.7	

The female parent *P. deltooides* ‘S9-2’ was absent in Italy, and only two replicates of *P. nigra* ‘Ghoy’ remained alive at this site. See Section 2 for abbreviations.

Table 3

Mean heterosis, broad-sense heritability ($H^2 \pm \text{S.E.}$), and coefficients of genetic (CV_G) and residual (CV_ε) variation for plant growth, lamina and petiole traits of $D \times N$ and $D \times T$ families in Orléans (F) and in Cavalleremaggiore (I)

	D × N family (31 F ₁)					D × T family (31 F ₁)			
	Site	Mean heterosis (%)	$H^2 \pm \text{S.E.}$	CV_G (%)	CV_ε (%)	Mean heterosis (%)	$H^2 \pm \text{S.E.}$	CV_G (%)	CV_ε (%)
Plant growth traits									
Height (cm)	F	7.55	0.30 ± 0.08	8.99	13.78	15.2	0.25 ± 0.08	10.93	18.94
	I	17.4	0.35 ± 0.09	7.03	9.53	40.3	0.16 ± 0.07	4.40	10.20
Circumference (mm)	F	6.78	0.28 ± 0.08	12.50	19.87	15.9	0.20 ± 0.08	10.90	22.05
	I	27.2	0.26 ± 0.08	7.01	13.83	48.7	0.20 ± 0.08	9.69	16.46
Volume (cm ³)	F	20.6	0.42 ± 0.09	36.24	43.00	44.3	0.28 ± 0.08	27.62	44.45
	I	57.5	0.27 ± 0.08	23.38	38.30	83.3	0.18 ± 0.07	15.90	33.76
dHt/dt (cm day ⁻¹)	F	6.49	0.44 ± 0.09	11.84	13.44	12.7	0.28 ± 0.08	8.71	13.82
	I	4.03	0.25 ± 0.08	12.50	22.77	20.0	0.18 ± 0.07	8.75	22.04
dVol/dt (cm ³ day ⁻¹)	F	20.1	0.44 ± 0.09	35.99	40.87	40.6	0.27 ± 0.08	22.14	36.84
	I	57.1	0.35 ± 0.09	28.29	38.62	63.2	0.24 ± 0.08	17.53	31.15
dNL/dt (day ⁻¹)	F	17.4	0.38 ± 0.09	8.33	10.71	14.2	0.20 ± 0.08	5.84	11.62
	I	-24.0	0.26 ± 0.08	7.15	11.75	18.5	0.41 ± 0.06	7.76	9.76
Lamina traits									
LA (cm ²)	F	-3.34	0.62 ± 0.07	19.26	15.08	32.0	0.52 ± 0.08	20.88	19.94
	I	45.1	0.54 ± 0.08	14.73	18.00	45.1	0.33 ± 0.09	12.63	18.08
SLA (cm ² g _{DW} ⁻¹)	F	7.88	0.32 ± 0.08	6.03	8.80	0.41	0.32 ± 0.08	8.82	12.81
	I	5.30	0.23 ± 0.08	6.79	12.40	-37.2	0.03 ± 0.05	3.02	15.97
Chloro (μmol g _{DW} ⁻¹)	F	4.59	0.07 ± 0.06	6.90	24.43	6.33	0.42 ± 0.09	9.00	10.59
	I	10.5	0.47 ± 0.09	10.4	11.2	22.4	0.28 ± 0.08	6.55	10.45
N _M (mg g _{DW} ⁻¹)	F	8.26	0.34 ± 0.09	6.03	8.30	-1.56	0.63 ± 0.07	14.03	10.66
	I	22.8	0.25 ± 0.08	6.00	10.34	3.88	0.06 ± 0.06	2.28	9.83
C _M (mg g _{DW} ⁻¹)	F	-0.40	0.60 ± 0.08	1.44	1.21	0.84	0.00 ± 0.00	0.00	3.99
	I	1.62	0.37 ± 0.09	2.06	2.90	-0.48	0.22 ± 0.08	1.27	2.58
Petiole traits									
PetLg (mm)	F	-8.71	0.53 ± 0.08	11.22	10.57	18.3	0.54 ± 0.08	13.68	12.60
	I	28.1	0.60 ± 0.08	10.00	8.12	61.7	0.62 ± 0.08	10.04	8.18
PetDW (g)	F	-9.38	0.23 ± 0.08	27.13	48.96	43.8	0.35 ± 0.09	20.30	27.62
	I	41.5	0.53 ± 0.08	22.92	26.53	79.3	0.33 ± 0.09	15.82	22.32
PetN _M (mg g _{DW} ⁻¹)	F	17.3	0.35 ± 0.09	9.16	11.78	-18.8	0.07 ± 0.06	3.57	18.17
	I	8.97	0.18 ± 0.07	7.15	15.47	-42.9	0.03 ± 0.05	3.39	15.76
PetC _M (mg g _{DW} ⁻¹)	F	1.85	0.23 ± 0.08	0.70	1.30	-0.08	0.26 ± 0.08	1.05	1.85
	I	4.51	0.00 ± 0.00	0.00	6.23	1.96	0.00 ± 0.00	0.00	5.71

See Section 2 for abbreviations.

were observed for stem volume (Vol) and the corresponding growth rate (dVol/dt) (up to 83.3% for stem volume of the $D \times T$ family in Italy). On average, heterosis was larger for the $D \times T$ than for the $D \times N$ family at both sites. Concerning leaf traits, important heterosis was observed in terms of leaf number increment (dNL/dt) (except for the $D \times N$ family in Italy), leaf area (LA), and petiole length (PetLg) and petiole dry weight (PetDW) (except for the $D \times N$ family in France).

3.1.3. Within-family variability

For most of the traits, the genetic variability was greater for the $D \times N$ than for the $D \times T$ family (Table 3). Overall, the expression of the variability was more important in France than in Italy for both families. The highest values of coefficients of genetic (CV_G) and residual (CV_ε) variations were observed for Vol and dVol/dt of the $D \times N$ family at both sites. In all cases, broad-sense heritability (H^2) was low to moderate. The highest values of H^2 were observed for LA (0.62 for the $D \times N$ family in France), PetLg (0.62 for the $D \times T$ family in Italy), lamina

nitrogen ($N_M = 0.63$ for the $D \times T$ family in France), and carbon ($C_M = 0.60$ for the $D \times N$ family in France) contents.

3.2. Between-site variability

3.2.1. Comparison between sites

All traits showed significant differences between sites, except concerning the stem height growth rate (dHt/dt) of both families, and LA and PetLg of the $D \times T$ family (Table 1). Tree dimensions and growth as well as dNL/dt were higher in Italy than in France for both families. Stem height growth rates (dHt/dt) did not differ between sites for both families. Italian leaves of the two families were larger, denser (i.e., with lower SLA), with longer and heavier petioles, richer in nitrogen (both laminas and petioles), and, on the contrary, poorer in carbon (both laminas and petioles) compared with the French leaves.

Only a few differences between sites were found for the male parents (Table 2). Trees of *P. nigra* 'Ghoy' were significantly taller ($P \leq 0.05$), and they produced leaves at a faster rate ($P \leq 0.001$) in Italy than in France. On the contrary, trees of

the male parent *P. trichocarpa* ‘V-24’ exhibited a significantly larger stem circumference in France than in Italy ($P \leq 0.05$). As observed for the two families, leaves of both male parents were richer in carbon in France than in Italy. Because of the absence of the female parent in Italy, due to important mortality during the establishment of the plantation at that site, a comparison of the performances of *P. deltoides* ‘S9-2’ between sites was not possible.

3.2.2. Genotype \times site interactions

In the multi-site ANOVA, the highest values of genetic variance (σ_G) were found for the D \times N family, and more precisely for d Ht/dt (22.3%), for d Vol/dt (25.1%), for LA (32.4%), Chloro (33.5%), and for PetLg (48.7%) (Table 4). For the D \times T family, the highest values of σ_G were observed for LA (23.1%), Chloro (28.6%), and for PetLg (50.7%). For the D \times N family, most of the G \times S interactions of growth traits were not significant, except for d Ht/dt and d NL/dt. In contrast, for the D \times T family, G \times S interactions were significant for all growth traits. However, for the growth traits of this family, the Spearman’s rank coefficients were not significant, suggesting that the ranking of the genotypes in terms of tree dimensions and growth between sites was affected by the G \times S interaction. The highest values of G \times S interaction were observed for d Ht/dt of both families ($\sigma_{G \times S} = 14.5\%$ of the phenotypic variance for the D \times T family), for LA (20.6%), SLA (12.5%), and N_M (19.8%) of the D \times T family, and for PetDW (22.2%) of the D \times N family. However, these values were lower than those of the site effect (σ_S), and thus, site effects were not hidden by G \times S interactions and could be interpreted.

3.3. Relationships between traits

The models derived from the linear regression analysis for d Vol/dt were as follows:

- for the D \times N family in Italy: d Vol/dt = $-14.1 + 0.13(\text{PetLg}) + 2.41(N_M)$,
- for the D \times N family in France: d Vol/dt = $-9.6 + 0.03(\text{LA}) + 13.2(d \text{NL}/dt)$,
- for the D \times T family in Italy: d Vol/dt = $0.36(\text{Chloro}) + 0.013(\text{LA})$,
- and for the D \times T family in France: d Vol/dt = $0.019(\text{LA})$.

No significant collinearity was observed between the traits included in the analysis.

For the D \times N family, the main planes of the PCA (PC1 \times PC2) established for Italy and for France independently explained 56.9% and 57.1% of the variability, respectively, with PC1 explaining 35.2% in Italy and 37.1% in France (Fig. 4). For the D \times T family, the main planes of the PCA explained 45.5% of the variability in Italy and 56.5% in France, with PC1 explaining 31.0% and 40.1%, respectively. In all cases, PC1 axes were defined by a group of traits composed of Vol, d Vol/dt, LA, PetLg, and PetDW. This group of traits was opposed, along PC1, to d NL/dt, except for the D \times N family in France. PC2 axes of the PCA were defined depending on families and sites. The PC2 axis was defined by the carbon and nitrogen contents of laminae and petioles (C_M , N_M , $\text{Pet}C_M$, and $\text{Pet}N_M$) for the D \times N family in Italy (Fig. 4A), by the opposition between the carbon contents (C_M and $\text{Pet}C_M$) and SLA for the D \times N family in France (Fig. 4B), by N_M for the D \times T family in Italy (Fig. 4C), and by

Table 4
Relative importance of genetic (σ_G^2), site (σ_S^2), genotype by site ($\sigma_{G \times S}^2$), and residual (σ_ε^2) effects in the phenotypic variation (σ_P^2), significance of the G \times S effect, and Spearman’s rank coefficient (R_{Spearman}) between France and Italy, based on genotypic means, for traits related to plant growth, lamina and petiole for D \times N and D \times T families at the two experimental sites: Orléans, France and Cavallermaggiore, Italy

	D \times N family (31 F ₁)						D \times T family (31 F ₁)					
	σ_G^2/σ_P^2	σ_S^2/σ_P^2	$\sigma_{G \times S}^2/\sigma_P^2$	$\sigma_\varepsilon^2/\sigma_P^2$	G \times S	R_{Spearman}	σ_G^2/σ_P^2	σ_S^2/σ_P^2	$\sigma_{G \times S}^2/\sigma_P^2$	$\sigma_\varepsilon^2/\sigma_P^2$	G \times S	R_{Spearman}
Plant growth traits												
Height (cm)	3.9	87.8	0.2	8.1	ns	0.65***	0.3	86.2	2.6	11.0	***	ns
Circumference (mm)	14.0	50.4	0.2	35.3	ns	0.68***	0.0	43.3	10.9	45.8	***	ns
Volume (cm ³)	11.5	59.2	1.7	27.6	ns	0.53**	0.5	59.3	8.3	31.9	***	ns
d Ht/dt (cm day ⁻¹)	22.3	2.8	9.3	65.6	***	ns	6.6	0.3	14.5	78.6	***	ns
d Vol/dt (cm ³ day ⁻¹)	25.1	31.2	1.7	42.0	ns	0.80***	5.3	35.9	11.3	47.5	***	ns
d NL/dt (day ⁻¹)	18.9	37.4	1.9	41.9	***	ns	11.4	56.9	3.3	28.5	*	ns
Lamina traits												
LA (cm ²)	32.4	34.7	2.8	30.1	ns	0.84***	23.1	0.6	20.6	55.7	***	0.40*
SLA (cm ² g _{DW} ⁻¹)	8.7	33.0	9.3	49.0	***	ns	2.2	34.4	12.5	51.0	***	ns
Chloro ($\mu\text{mol g}_{\text{DW}}^{-1}$)	33.5	0.0	0.5	66.0	ns	ns	28.6	6.3	4.7	60.4	ns	0.53**
N_M (mg g _{DW} ⁻¹)	9.3	53.5	4.0	33.2	*	ns	3.9	41.0	19.8	35.3	***	ns
C_M (mg g _{DW} ⁻¹)	13.4	59.4	6.9	20.3	**	ns	1.0	14.1	0.0	84.9	ns	ns
Petiole traits												
PetLg (mm)	48.7	11.1	1.0	39.2	ns	0.85***	50.7	0.6	8.1	40.6	***	0.67***
PetDW (g)	4.7	18.1	22.2	55.0	***	0.54**	15.0	23.8	10.4	50.8	***	0.44*
$\text{Pet}N_M$ (mg g _{DW} ⁻¹)	0.0	98.0	0.6	1.4	**	0.39*	0.1	69.7	1.5	28.7	ns	ns
$\text{Pet}C_M$ (mg g _{DW} ⁻¹)	2.8	34.3	0.0	62.9	ns	0.52**	1.1	2.6	3.5	92.9	ns	ns

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$; and ns = non-significant. See Section 2 for abbreviations.

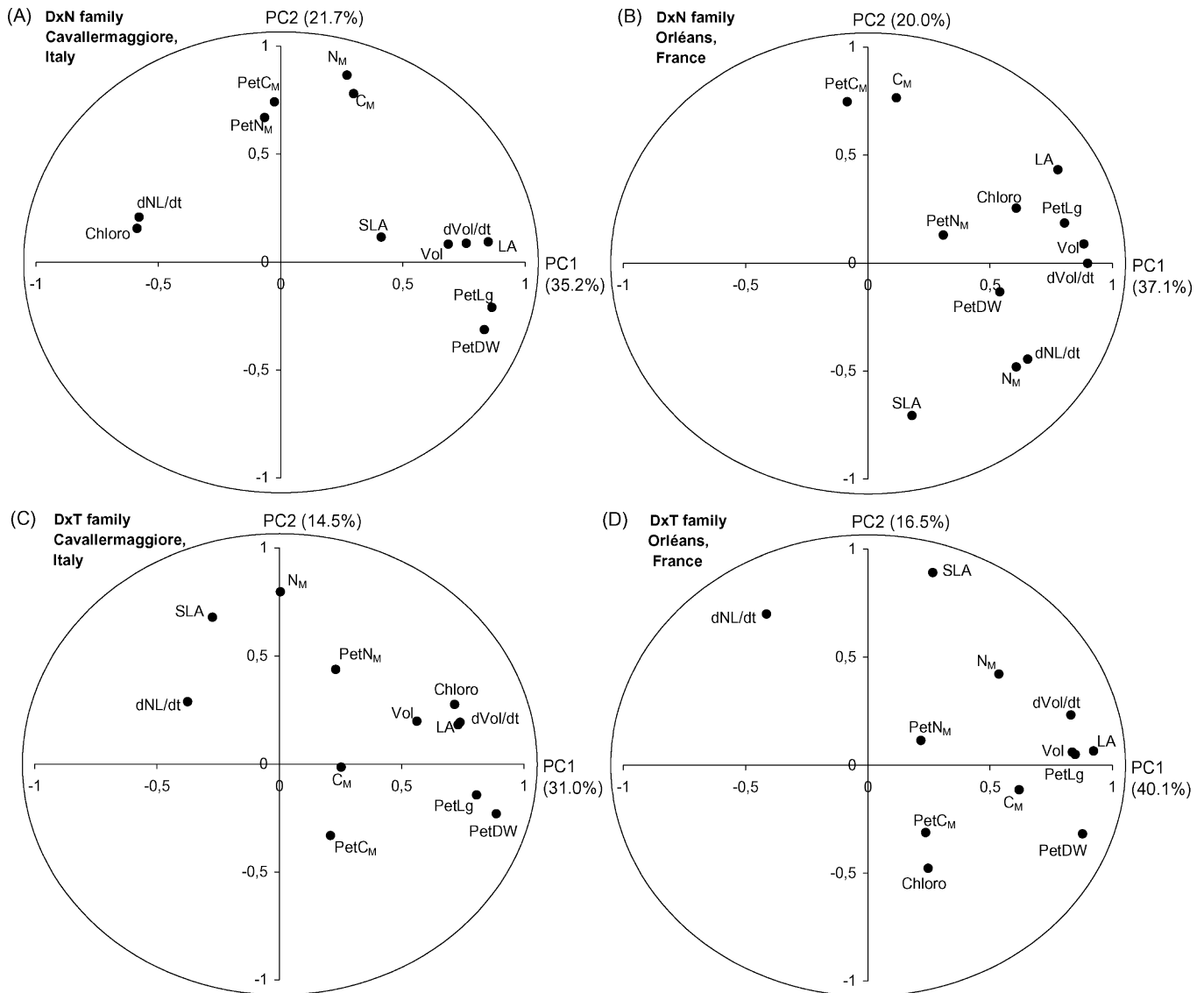


Fig. 4. Distribution in the factorial planes PC1 \times PC2 of the principal components analysis (PCA) of growth, lamina and petiole traits of the D \times N (A and B) and D \times T (C and D) families in Cavallermaggiore, Italy (A and C) and in Orléans, France (B and D). PC1 and PC2 are linear combinations of the variables and were constructed to maximize the part of the data variability that they explained. The numbers (in %) above or next to each panel represent the variation explained by the respective principal components. See Section 2 for abbreviations.

the opposition between SLA and Chloro for the D \times T family in France (Fig. 4D).

All growth traits were positively inter-correlated, except dHt/dt and dNL/dt for the D \times N family in Italy and for the D \times T family at both sites (Table 5). The strongest correlations were observed for PetLg, PetDW, and LA with all growth traits, except dHt/dt (Fig. 5). Chloro was also positively correlated with tree growth, with the exception of the D \times T family in Italy. In contrast, for SLA, C_M , N_M , Pet C_M , and Pet N_M , only few significant inter-correlations were found.

4. Discussion

4.1. Variability in growth and leaf traits

As already observed for the two families used in the present study as well as for various crosses between *P. deltooides* and

P. nigra species (Euramerican hybrids) and between *P. deltooides* and *P. trichocarpa* species (Interamerican hybrids), the F_1 hybrids exhibited a fast growth and a high hybrid vigour for the growth traits as compared with the mean values of their two parents (Ranney et al., 1987; Marron et al., 2006). The *P. deltooides* \times *P. trichocarpa* hybrids were significantly more productive than the *P. deltooides* \times *P. nigra* hybrids irrespective of site, showing once again the high potential of the Interamerican hybrids when growth and sanitary conditions are optimal. Italian conditions were the most favourable for the growth of both types of hybrids, i.e., higher temperature, longer day length, and better soil (loam versus sand in France). Although impressive differences in tree dimensions were recorded between sites for both families, differences in stem height and stem volume increase rates were weaker. This phenomenon could be partly explained by the fact that the experimental period was quite short with

Table 5

Pearson's correlation coefficients, calculated from genotypic means, between plant growth, lamina, and petiole traits, of D × N (above) and D × T (below) families in Orléans (normal font) and in Cavallermaggiore (bold italic font)

		Plant growth traits					Lamina traits					Petiole traits				
		Height	Circum.	Volume	dHt/dt	dVol/dt	dNL/dt	LA	SLA	Chloro	N _M	C _M	PetLg	PetDW	PetN _M	PetC _M
Plant growth traits																
Height	F		0.85 ***	0.91 ***	0.68 ***	0.85 ***	0.61 ***	0.64 ***		0.45 *			0.57 ***	0.41 *		
	I		0.86 ***	0.89 ***		0.72 ***		0.55 **					0.60 ***	0.50 **		
Circum.	F	0.89 ***		0.97 ***	0.70 ***	0.94 ***	0.48 **	0.69 ***		0.51 **			0.62 ***	0.38 *		
	I	0.69 ***		0.86 ***		0.86 ***		0.57 **	0.42 *				0.72 ***	0.63 ***		
Volume	F	0.93 ***	0.95 ***		0.71 ***	0.97 ***	0.52 **	0.66 ***		0.49 **	0.37 *		0.64 ***	0.38 *		
	I	0.82 ***	0.69 ***			0.72 ***		0.54 **					0.54 **	0.47 **		
dHt/dt	F					0.84 ***	0.70 ***	0.52 **			0.42 *		0.55 **	0.42 *		
	I						0.98 ***									
dVol/dt	F	0.76 ***	0.83 ***	0.89 ***	0.46 **		0.62 ***	0.62 ***		0.47 **	0.42 *		0.66 ***	0.37 *		
	I	0.58 ***	0.80 ***	0.58 ***				0.47 **					0.54 **	0.49 **		
dNL/dt	F				0.45 *				0.41 *		0.56 **					
	I				0.92 ***									-0.39 *		
Lamina traits																
LA	F	0.77 ***	0.83 ***	0.84 ***		0.83 ***	-0.36 *			0.58 ***			0.80 ***	0.39 **		
	I		0.46 *			0.45 *							0.80 ***	0.83 ***		
SLA	F				0.47 **	0.37 *	0.38 *				0.48 **	-0.43 *				
	I															
Chloro	F								-0.42 *		0.37 *		0.40 *			
	I	0.42 *	0.49 **	0.42 *		0.49 **		0.40 *					-0.40 *	-0.38 *		
N _M	F					0.37 *			0.49 **							-0.39 *
	I													0.51 **	0.49 **	
C _M	F	0.45 *	0.37 *					0.39 *			0.53 **		0.75 ***			0.58 ***
	I															0.55 **
Petiole traits																
PetLg	F	0.55 **	0.59 ***	0.56 **		0.59 ***		0.77 ***			0.39 *	0.50 **		0.40 *		
	I		0.38 *			0.38 *		0.58 **		0.53 **				0.88 ***		
PetDW	F	0.61 ***	0.73 ***	0.67 ***		0.64 ***	-0.55 **	0.84 ***			0.39 *		0.47 **	0.84 ***		
	I		0.42 *			0.48 **		0.73 ***	-0.39 *	0.54 **				0.82 ***		
PetN _M	F										0.53 **					
	I										0.39 *					
PetC _M	F											0.62 ***				
	I											0.41 *				

See Section 2 for abbreviations.

regard to the duration of the entire growing season and might not be the most representative, within the whole growing season, of the growth potential of the two families. However, it has already been shown, for various *P. deltooides* × *P. nigra* genotypes after coppicing, that, when conditions are non-limiting, tree growth is linear in June and July, before slowing down during August and stopping in late August (circumference growth) or in mid-September (height growth) (Monclus et al., 2006). Therefore, the length of the growing period is probably a factor having a larger influence on differences in tree dimensions between the two families. Indeed, the onset of the growing seasons is known to greatly differ between the two families (4 days and 10 days earlier for the D × T than for the D × N family at the French and Italian sites, respectively, in 2003; unpublished data). So, differences in productivity between the two families could be related to a larger extent to differences in the length of the growing season rather than to inherent differences in growth rates. This might also explain why stem height at the beginning of the experiment was not necessarily correlated with the stem height increase rate.

Both families have inherited complementary leaf characteristics from their respective male parents: the D × T family expanded larger leaves with longer and heavier petioles, but were less rapidly produced than the leaves of the D × N family. These differences in foliage deployment strategies of the parental species and of their respective progenies have already been well-documented. *P. nigra* is known to promote a high numbers of leaves, while *P. deltooides* and *P. trichocarpa* promote large individual leaf size (Ridge et al., 1986; Ceulemans et al., 1988; Ferris et al., 2001; Gielen et al., 2001; Rae et al.,

2004; Monclus et al., 2005; Marron and Ceulemans, 2006). Leaf structure also differed between both families. The leaves of the D × T family were denser and/or thicker (i.e., with lower SLA), and exhibited higher values of lamina carbon content and, on the contrary, lower lamina as well as petiole nitrogen contents than the leaves of the D × N family. Leaves of *P. trichocarpa* are adapted to a diffuse light regime and they usually showed thicker spongy and palisade mesophyll layers than the leaves of both *P. nigra* and *P. deltooides* species (Figliola, 1986; Scarascia-Mugnozza et al., 1986). A tight link between high SLA and high nitrogen content is characteristic of leaves with short life spans because these traits increase vulnerability to herbivory and to physical hazards (Wright et al., 2004). Thus, the D × N family seems to be more adapted to the easier and faster renewal (higher leaf number increment) of smaller (lower individual leaf area) and more sensitive (higher SLA and nitrogen content) leaves than the D × T family. This latter hybrid, on the contrary, promotes the duration of a more limited number of much larger leaves (leaves twice as large for the D × T than for the D × N family at both sites). Between both families, the larger leaves and lower nitrogen contents for the D × T than for the D × N family could also be explained by the fact that large leaves need a strong support, and the support tissues are known to contain a lower nitrogen content than the lamina (Niinemets et al., 2006). However, lower leaf nitrogen content could also potentially reduce the integrated photosynthetic capacity of larger leaves, which is not in line with the positive links that are generally observed between leaf size and whole plant productivity. This is probably due to the fact that, in a large leaf, a reduction of the photosynthesis per unit of leaf area or per unit of leaf

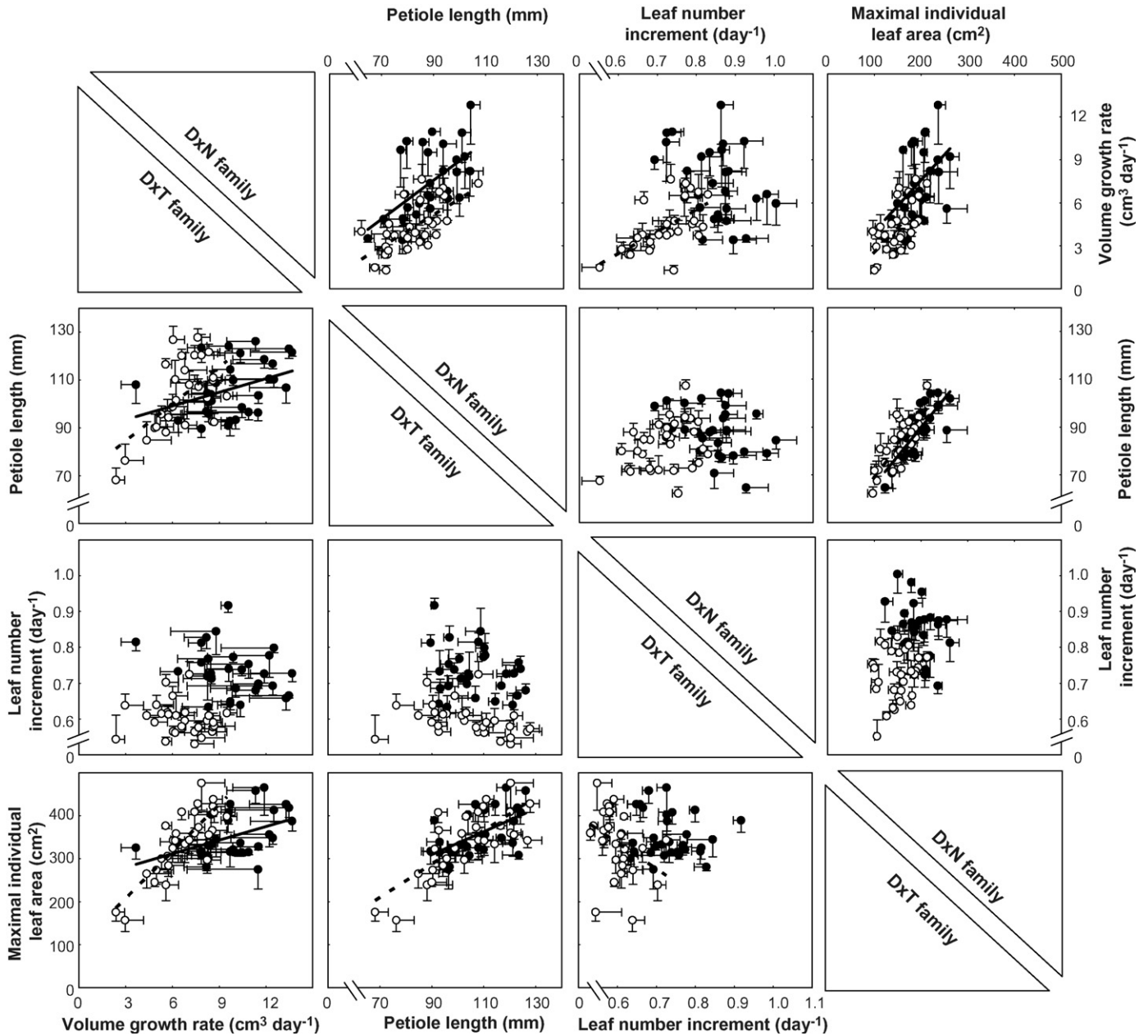


Fig. 5. Correlations between volume growth rate ($d\text{Vol}/dt$), petiole length (PetLg), maximal individual leaf area (LA), and leaf number increment ($d\text{NL}/dt$) of the $\text{D} \times \text{N}$ (above) and $\text{D} \times \text{T}$ (below) families in Orléans, France (empty dots and dashed lines) and in Cavallermaggiore, Italy (solid dots and full lines). Each dot is the genotypic mean ($\pm\text{S.E.}$). See Table 5 for the corresponding Pearson's correlation coefficients.

weight is compensated by the photosynthesis of a larger leaf area.

The structure and growth of the leaves of the two families were also affected by site conditions. Leaves were produced more rapidly and they reached a larger final size and higher density and/or thickness (i.e., with lower SLA) in Italy than in France. The better climatic as well as edaphic conditions in Italy than in France allowed the trees from both families to support both a high leaf production (number) and a fast leaf expansion (size). This result is in contradiction with studies demonstrating that smaller leaves are advantageous in hot and dry environments and at high intensities of solar radiation, while large leaves are

advantageous in lower irradiance, and cooler and moister environments (Ackerly et al., 2002). Leaves and petioles of both families were significantly richer in nitrogen in Italy than in France, possibly due to the presence of a pig breeding farm close to the Italian plantation. This additional indirect source of fertilization, inducing higher leaf nitrogen contents, is also probably responsible for higher photosynthetic capacities and thus for a higher assimilation rate and biomass accumulation in Italy than in France (Wright et al., 2004). Petioles are photosynthetically active to a certain degree, but their photosynthetic rates are likely to be very small compared with laminas (Niinemets, 1999).

4.2. Time stability of leaf determinants of productivity

During the summer of 2004, a similar experiment was conducted on the D × N family at the French site only (Marron and Ceulemans, 2006). The main perspectives of this previous experiment were to test the robustness of the observed relationships between leaf traits and tree growth for varying environmental conditions, growing seasons, and genetic backgrounds. In the previous study, the dimensions and growth of the trees were higher (e.g., $d\text{Vol}/dt = 13.7\text{ cm}^3\text{ day}^{-1}$ versus $4.3\text{ cm}^3\text{ day}^{-1}$ in the present study) due to the fact that the trees were in their second growing season while they were in their first growing season after coppicing in the present study. However, the leaf number increment was much faster in this study ($d\text{NL}/dt = 0.72\text{ day}^{-1}$ versus 0.45 day^{-1} in Marron and Ceulemans, 2006), illustrating the higher growth potential of the trees after coppicing. Moreover, the leaves produced after coppicing were significantly smaller and less dense and/or thick (i.e., with higher SLA) than the leaves produced during the second growing season. The behaviour of the trees with respect to their foliage development evolved according to the type of growth they need to support: primary growth or secondary growth. After coppicing, the production of a large number of small leaves was preferred, possibly in order to promote growth in height (primary growth) as well as the production of sylleptic branches (developing from leaf basal buds) which are known to carry most of the total leaf area of the plant (Ceulemans, 1990; Wu and Stettler, 1998). Moreover, leaves were much richer in nitrogen after coppicing, probably highlighting a more efficient photosynthesis compared with the second growing season. In contrast, during the second growing season (Marron and Ceulemans, 2006), the slower production of larger leaves seemed to be preferred in order to promote growth in circumference (secondary growth) when the foliage was already well-developed and established.

In spite of these differences, the two major conclusions drawn during the second growing season (Marron and Ceulemans, 2006) were still valid during the first growing season after coppicing of the present study: (1) hybrids generated from the cross between *P. deltoides* 'S9-2' and *P. nigra* 'Ghoy' inherited the faster growth increment of their male parent *P. nigra* and the larger leaf size of their female parent *P. deltoides* and, (2) for this family and at the French site, the stem volume increase rate ($d\text{Vol}/dt$) can be simply decomposed into leaf number increment ($d\text{NL}/dt$) and area of the largest leaf along the main stem (LA). The relevance of the use of these two traits as selection criteria, for comparable crosses and environmental conditions, is supported by their tight links with tree growth and their broad-sense heritabilities that are higher than the ones of most of the growth traits. Furthermore, these relationships are stable in time.

4.3. Plasticity of leaf determinants of productivity

As shown by the very diverse equations linking stem volume growth rate to leaf traits, specific for each site and family, leaf determinants of growth are strongly affected by the environment as well as by the genetic background. However, some of the relationships between traits are valid irrespective of conditions: stem

volume as well as stem volume growth rate were closely linked to the leaf area of the largest leaf along the stem, and to the length and dry weight of the petiole of this leaf, for both families and both sites. Leaf area has of course a prime effect on light interception, on carbon assimilation, and consequently on biomass accumulation (Wu, 1994a,b; Harrington et al., 1997). Evidently, large leaves need stronger support which will be associated with longer and heavier petioles. Moreover, large petioles can allow the leaf to more efficiently adapt its orientation and the subsequent light interception, decrease leaf aggregation, and represent wider and more efficient ways for transport of water and carbon compounds (Niinemets and Fleck, 2002; Niinemets et al., 2004). The leaf area of the largest leaf and the petiole dimensions exhibited higher broad-sense heritabilities and coefficients of genetic variation than most of the growth traits. The latter are easy and cheap to score and they appear to be robust and useful early indicators of tree vigour in poplar.

In contrast with the afore-mentioned environmentally and genetically stable relationships between traits, some of the correlations were strongly dependent on site conditions and/or on family. For instance, stem growth was linked to the nitrogen content of the leaf lamina and, on the contrary, independent of SLA for both families in France only. In Italy, the links between stem growth and SLA as well as with nitrogen content were less obvious, even if SLA was nearby (for the D × N family) or opposed (for the D × T family) to the growth traits in the main planes of the PCA. The dependence of the links between the relative growth rate (RGR) and its main factors, i.e., SLA and net assimilation rate (NAR), on environmental conditions is a well-documented phenomenon. It has been shown that under high irradiance, RGR is mainly controlled by NAR while SLA becomes dominant under low irradiance (Poorter and Van der Werf, 1998; Shipley, 2002). Due to longer day length and less rainy days in Italy than in France, the light conditions in Italy were more favourable than the French ones. However, in France, stem growth was linked to lamina nitrogen content and thus probably to NAR, and independent of SLA. Most probably, the natural light environment of both sites was non-limiting, and this factor was not responsible for the differences observed between both sites. On the other hand, the components of RGR are also strongly influenced by growth temperature (Loveys et al., 2002). For a wide range of species, these authors have observed that NAR becomes more important than SLA for explaining variations in RGR when the temperature was inferior to 20 °C. On the contrary, when the temperature was superior to 20 °C, inter-specific variations in SLA were more important in determining variations in RGR amongst the species. The average temperatures in June and July 2005 were 21.9 °C at the Italian site and 19.2 °C at the French one. These differences in temperatures between sites might be partly responsible for the different patterns concerning the links between stem growth, SLA, and lamina nitrogen content.

4.4. Conclusions and breeding implications

In general, the environment can act on pedigree performances in three different ways: (1) all the genotypes respond similarly

to the different environments, i.e., effects of genotype and environment are statistically additive and so, the $G \times E$ interaction is not significant; (2) the $G \times E$ interaction is significant, but due to changes in the differences among genotypes without change in genotype ranking, and (3) the $G \times E$ interaction is significant with a change in genotype rank from one environment to another. Only the last case will cause problems for the breeder because a genotype selected for its growth vigour will not necessarily be vigorous if grown in a different environment. In our study, the $D \times N$ family belongs to the first case (i.e., no significant genotype \times site ($G \times S$) interaction), while the $D \times T$ family belongs to the last case (i.e., significant $G \times S$ interaction with change in ranking among environments). So this could be challenging for the selection of productive hybrids showing a large environmental spectrum. However, these results are not in line with those obtained in previous experiments conducted with the two families, which show significant $G \times S$ interactions without a complete trade-off in performance between sites (Marron et al., 2006). This inconsistency could be due to the more limited number of genotypes studied here (31 versus 180), influencing the degree of significance of the effects and coefficients. Moreover, the age of the plantations could also have had an impact on the temporal evolution of the $G \times S$ interaction, with the differences between both sites getting more pronounced with time.

In terms of leaf determinants of productivity, three different categories of traits could be considered: (i) traits linked to tree growth in all cases, (ii) traits linked to tree growth depending on environment and/or family, or (iii) traits never correlated with tree vigour. Individual maximal leaf area and petiole dimensions remain the most reliable indirect indicators of tree performances irrespective of environmental conditions and pedigree. On the other hand, the links between tree growth, SLA, and lamina nitrogen content were strongly dependent on site conditions, while leaf number increment had a more pronounced influence on tree growth for the $D \times N$ than for the $D \times T$ family, notably in France. Finally, traits such as chlorophyll, petiole nitrogen, and lamina and petiole carbon contents showed only very weak or unreliable correlations with tree growth.

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References

- Ackerly, D.D., Knight, C.A., Weiss, S.B., Barton, K., Starmer, K.P., 2002. Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level analyses. *Oecologia* 130, 449–457.
- Al Afas, N., Marron, N., Ceulemans, R., 2006. Clonal variation in stomatal characteristics related to biomass production under short rotation coppice culture. *Environ. Exp. Bot.* 58, 279–286.
- Ceccarelli, S., Grando, S., 1996. Drought as a challenge for the plant breeder. *Plant Growth Regul.* 20, 149–155.
- Cervera, M.-T., Gusm ao, J., Steenackers, M., Peleman, J., Storme, V., Vanden Broeck, A., Van Montagu, M., Boerjan, W., 1996. Identification of AFLP molecular markers for resistance against *Melampsora larici-populina* in *Populus*. *Theor. Appl. Genet.* 93, 733–737.
- Cervera, M.-T., Storme, V., Ivens, B., Gusm ao, J., Liu, B.H., Hostyn, V., Van Slycken, J., Van Montagu, M., Boerjan, W., 2001. Dense genetic linkage maps of three *Populus* species (*Populus deltoids*, *P. nigra* and *P. trichocarpa*) based on AFLP and microsatellite markers. *Genetics* 158, 787–809.
- Ceulemans, R., 1990. Genetic Variation in Functional and Structural Productivity Determinants in Poplar. Thesis Publishers, Amsterdam, The Netherlands.
- Ceulemans, R., Impens, I., Steenackers, V., 1988. Genetic variation in aspects of leaf growth of *Populus* clones, using the leaf plastochron index. *Can. J. Forest Res.* 18, 1069–1077.
- Dickmann, D.I., Stuart, K.W., 1983. *The Culture of Poplars*. MSU Press, East Lansing, MI.
- Dickmann, D.I., Gold, M.A., Flore, J.A., 1994. The ideotype concept and the genetic improvement of tree crops. *Plant Breeding Rev.* 12, 163–193.
- Eberhart, S.A., Russell, W.A., 1966. Stability parameters for comparing varieties. *Crop Sci.* 6, 36–40.
- Figliola, A.L., 1986. Studies in the physiology, morphology, and anatomy of *Populus trichocarpa*, *Populus deltoides*, and their hybrids. M.Sc. Thesis, University of Washington, Seattle, WA.
- Ferris, R., Sabatti, M., Miglietta, F., Mills, R.F., Taylor, G., 2001. Leaf area is stimulated in *Populus* by free air CO₂ enrichment (POPFACE), through increased cell expansion and production. *Plant Cell Environ.* 24, 305–315.
- Gielen, B., Calfapietra, C., Sabatti, M., Ceulemans, R., 2001. Leaf area dynamics in a closed poplar plantation under free-air carbon dioxide enrichment. *Tree Physiol.* 21, 1245–1255.
- Hansen, E.A., 1991. Poplar woody biomass yields: a look to the future. *Biomass Bioenerg.* 1, 1–7.
- Harrington, C.A., Radwan, M.A., Dean, D.S., 1997. Leaf characteristics reflect growth rates of 2-year-old *Populus* trees. *Can. J. Forest Res.* 27, 1321–1325.
- Henderson, C.R., 1953. Estimation of variance and co-variance components. *Biometrics* 9, 226–252.
- Heilman, P.E., Ekuon, G., Fogle, D., 1994. Above- and below-ground biomass and fine roots of 4-year-old hybrids of *Populus trichocarpa* \times *Populus deltoides* and parental species in short-rotation culture. *Can. J. Forest Res.* 24, 1186–1192.
- Isebrands, J.G., Nelson, N.D., 1982. Crown architecture of short-rotation, intensively cultured *Populus*. II. Branch morphology and distribution of leaves within the crown of *Populus* ‘Tristis’ as related to biomass production. *Can. J. Forest Res.* 12, 853–864.
- Larson, P.R., Isebrands, J.G., 1971. The plastochron index as applied to developmental studies of cottonwood. *Can. J. Forest Res.* 1, 1–11.
- Li, B., Wu, R., 1997. Heterosis and genotype \times environment interactions of juvenile aspens in two contrasting sites. *Can. J. Forest Res.* 27, 1525–1537.
- Lin, C.S., Binns, M.R., Lefkovich, L.P., 1986. Stability analysis: where do we stand? *Crop Sci.* 26, 894–900.
- Loveys, B.R., Scheurwater, I., Pons, T.L., Fitter, A.H., Atkin, O.K., 2002. Growth temperature influences the underlying components of relative growth rate: an investigation using inherently fast- and slow-growing plant species. *Plant Cell Environ.* 25, 975–987.
- Marron, N., Ceulemans, R., 2006. Genetic variation of leaf traits related to productivity in a *Populus deltoides* \times *Populus nigra* family. *Can. J. Forest Res.* 36, 390–400.

- Marron, N., Bastien, C., Sabatti, M., Taylor, G., Ceulemans, R., 2006. Plasticity of growth and sylleptic branchiness in two poplar families grown at three sites across Europe. *Tree Physiol.* 26, 935–956.
- Monclus, R., Dreyer, E., Delmotte, F.M., Villar, M., Delay, D., Boudouresque, E., Petit, J.-M., Marron, N., Bréchet, C., Brignolas, F., 2005. Productivity, leaf traits and carbon isotope discrimination in 29 *Populus deltoides* × *P. nigra* clones. *New Phytol.* 167, 53–62.
- Monclus, R., Dreyer, E., Villar, M., Delmotte, F.M., Delay, D., Petit, J.-M., Barbaroux, C., Le Thiec, D., Bréchet, C., Brignolas, F., 2006. Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides* × *Populus nigra*. *New Phytol.* 169, 765–777.
- Niinemets, Ü., 1999. Differences in chemical composition relative to functional differentiation between petioles and laminae of *Fraxinus excelsior*. *Tree Physiol.* 19, 39–45.
- Niinemets, Ü., Fleck, S., 2002. Petiole mechanics, leaf inclination, morphology, and investment in support in relation to light availability in the canopy of *Liriodendron tulipifera*. *Oecologia* 132, 21–33.
- Niinemets, Ü., Al Afas, N., Cescatti, A., Pellis, A., Ceulemans, R., 2004. Petiole length and biomass investment in support modify light-interception efficiency in dense poplar plantations. *Tree Physiol.* 24, 141–154.
- Niinemets, Ü., Portsmouth, A., Tobias, M., 2006. Leaf size modifies support biomass distribution among stems, petioles and mid-ribs in temperate plants. *New Phytol.* 171, 91–104.
- Nyquist, W.E., 1991. Estimation of heritability and prediction of selection response in plant populations. *Crit. Rev. Plant Sci.* 10, 235–322.
- Pellis, A., Laureysens, I., Ceulemans, R., 2004. Growth and production of a short rotation coppice culture of poplar. I. Clonal differences in leaf characteristics in relation to biomass production. *Biomass Bioenerg.* 27, 9–19.
- Pontaiiller, J.Y., Ceulemans, R., Guittet, J., Mau, F., 1997. Linear and non-linear functions of volume index to estimate woody biomass in high density young poplar stands. *Ann. Forest Sci.* 54, 335–345.
- Poorter, H., Van der Werf, A., 1998. Is inherent variation in RGR determined by LAR at low irradiance and by NAR at high irradiance? A review of herbaceous species. In: Lambers, H., Poorter, H., Van Vuuren, M.M.I. (Eds.), *Inherent Variation in Plant Growth. Physiological Mechanisms and Ecological Consequences*. Backhuys Publishers, Leiden, The Netherlands, pp. 309–336.
- Rae, A.M., Robinson, K.M., Street, N.R., Taylor, G., 2004. Morphological and physiological traits influencing biomass productivity in short-rotation coppice poplar. *Can. J. Forest Res.* 34, 1488–1498.
- Ranney, J.W., Wright, L.L., Layton, P.A., 1987. Hardwood energy crops: the technology of intensive culture. *J. Forest.* 85, 17–28.
- Ridge, C.R., Hinckley, T.M., Stettler, R.F., Van Volkenburgh, E., 1986. Leaf growth characteristics of fast growing poplar hybrids *Populus trichocarpa* × *P. deltoides*. *Tree Physiol.* 1, 209–216.
- Riemenschneider, D.E., Stelzer, H.E., Foster, G.S., 1996. Quantitative genetics of poplars and poplar hybrids. In: Stettler, R.F., Bradshaw Jr., H.D., Heilman, P.E., Hinckley, T.M. (Eds.), *Biology of Populus and its Implications for Management and Conservation, Part I*. NRC Research Press, National Research Council of Canada, Ottawa, ON, Canada, pp. 159–181 (Chapter 7).
- Rönnerberg-Wästljung, A.C., Gullberg, U., 1999. Genetics of breeding characters with possible effects on biomass production in *Salix viminalis* (L.). *Theor. Appl. Genet.* 98, 531–540.
- Scarascia-Mugnozza, G.E., Hinckley, T.M., Stettler, R.F., 1986. Evidence for nonstomatal inhibition of net photosynthesis in rapidly dehydrated shoots of *Populus*. *Can. J. Forest Res.* 16, 1371–1375.
- Shipley, B., 2002. Trade-offs between net assimilation rate and specific leaf area in determining relative growth rate: relationship with daily irradiance. *Funct. Ecol.* 16, 682–689.
- Singh, M., Ceccarelli, S., Hamblin, J., 1993. Estimation of heritability from varietal trials data. *Theor. Appl. Genet.* 86, 437–441.
- Stettler, R.F., Bradshaw Jr., H.D., Zsuffa, L., 1992. The role of genetic improvement in short rotation forestry. In: Mitchell, J.B., Ford-Robertson, J.B., Hinckley, T., Sennerby-Forsse, D. (Eds.), *Ecophysiology of Short Rotation Forest Crops*. Elsevier Applied Sciences, London, UK, pp. 285–308.
- Stettler, R.F., Zsuffa, L., Wu, R., 1996. The role of hybridization in the genetic manipulation of *Populus*. In: Stettler, R.F., Bradshaw Jr., H.D., Heilman, P.E., Hinckley, T.M. (Eds.), *Biology of Populus and its Implications for Management and Conservation, Part I*. NRC Research Press, National Research Council of Canada, Ottawa, ON, Canada, pp. 87–112 (Chapter 4).
- Tuberosa, R., Salvi, S., Sanguineti, M.C., Landi, P., MacCaferri, M., Conti, S., 2002. Mapping QTLs regulating morpho-physiological traits and yield: case studies, shortcomings and perspectives in drought-stressed maize. *Ann. Bot.* 89, 941–963.
- Van Hecke, P., Moermans, R., Mau, F., Guittet, J., 1995. Border effects and size inequality in experimental even-aged stands of poplar clones (*Populus*). *Ann. Sci. Forest.* 52, 193–200.
- Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J., Villar, R., 2004. The worldwide leaf economics spectrum. *Nature* 428, 821–827.
- Wu, R.-L., 1994a. Quantitative genetics of yield breeding for *Populus* short rotation culture. II. Genetic determination and expected selection response of tree geometry. *Can. J. Forest Res.* 24, 155–165.
- Wu, R.-L., 1994b. Quantitative genetics of yield breeding for *Populus* short rotation culture. III. Efficiency of indirect selection on tree geometry. *Theor. Appl. Genet.* 88, 803–811.
- Wu, R., Stettler, R.F., 1998. Quantitative genetics of growth and development in *Populus*. III. Phenotypic plasticity of crown structure and function. *Heredity* 81, 299–310.
- Zavitkovski, J., 1981. Small plots with unplanted plot border can distort data in biomass production studies. *Can. J. Forest Res.* 11, 9–12.
- Zsuffa, L., Giordano, E., Pryor, L.D., Stettler, R.F., 1996. Trends in poplar culture: some global and regional perspectives. In: Stettler, R.F., Bradshaw Jr., H.D., Heilman, P.E., Hinckley, T.M. (Eds.), *Biology of Populus and its Implications for Management and Conservation, Part II*. NRC Research Press, National Research Council of Canada, Ottawa, ON, Canada, pp. 515–539 (Chapter 19).