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Reference:

Gebauer Roman, Vanbeveren Stefan, Volařík Daniel, Plichta Roman, Ceulemans Reinhart.- Petiole and leaf traits of poplar in relation to parentage and biomass yield

Forest ecology and management - ISSN 0378-1127 - 362(2016), p. 1-9

Full text (Publishers DOI): http://dx.doi.org/doi:10.1016/j.foreco.2015.11.036

Petiole and leaf traits of poplar in relation to parentage and biomass yield

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13 Abstract

14 Poplars grown under a short-rotation coppice (SRC) regime for biomass production offer a promising
15 alternative source of renewable energy to fossil fuels. We examined the potential of leaf and petiole
16 traits of 12 different poplar genotypes as early selection criteria for breeding and selection
17 programmes. Petiole traits included theoretical hydraulic conductivity of the petiole, petiole xylem
18 area and the number of vessels in each petiole. The different genotypes clustered largely according
19 to their breeding programmes and to their parentage. Leaf and petiole traits showed strong
20 correlations, which enabled the prediction of difficult-to-measure petiole traits as xylem area, total
21 vessel lumen area and number of vessels based on the more common and easily measurable leaf dry
22 mass. We found significant correlations between above-ground woody biomass and nine leaf and
23 petiole traits. We developed three predictive correlative models based on the easy-to-measure
24 petiole and leaf traits (petiole cross-section area, petiole thickness and leaf dry mass). These simple
25 models can be used as early selection criteria for biomass yield in poplar breeding programmes. The
26 usefulness of the easy-to-measure petiole thickness for biomass prediction should be further tested
27 on other poplar genotypes.

28 Keywords: anatomy, leaf dry mass, petiole thickness, *Populus* spp., POPFULL, short-rotation coppice

29

1. Introduction

The culture of fast-growing trees under a short-rotation coppice (SRC) regime for biomass production offers one of the most promising alternatives to fossil fuels in the search for renewable energy sources (Foster, 1993). The concept of SRC is defined as carefully tended, high-density plantations of fast-growing perennial crops with rotations shorter than eight years (Herrick and Brown, 1967). Poplar (*Populus* spp.) and willow (*Salix* spp.) are the most commonly used species for SRC in Europe (Kauter et al., 2003; Aylott et al., 2008). Poplar is particularly suitable for SRC cultures in temperate regions because of its high growth rate and biomass yield, its easy vegetative propagation from cuttings and high coppice ability (Dillen et al., 2010). Since the early 1950's, intensive selection and breeding programmes for poplar have resulted in a wide range of highly productive genotypes. Several aspects of genotypic differences have already been examined and documented over the past decade: the importance of species and genotypes used in SRC (Willebrand et al., 1993; Dillen et al., 2011); the impact of coppicing (Herve and Ceulemans, 1996); the length of the coppice rotation cycle (Al Afas et al., 2008); and the interactions between soil type and genotype (Dillen et al., 2010).

Several studies have identified poplar traits that facilitate the poplar breeding process, as this remains a necessary and continuous requirement for SRC (Rae et al., 2004; Verlinden et al., 2013). A negative correlation between growth rate and wood density was shown in some studies (Pliura et al. 2007; Zhang et al. 2012), while others reported that there was no correlation (DeBell et al., 2002; Zhang et al., 2003). The reason for these conflicting observations could be that wood density changed with tree age in the study of DeBell et al. (2002); density increased after five years of growth in three poplar genotypes. So, the efficient selection of genotypes based on wood properties may require a standardized sampling at more than one height (DeBell et al., 2002, De Boever et al. 2007). On the other hand, individual leaf area and leaf area index were found to be very promising traits for early selection criteria, as they positively correlated with biomass (Barigah et al., 1994; Harrington et al., 1997; Verlinden et al., 2013). The petiole is an important part of the leaf. It plays a dual function

55 in leaves, i.e. providing mechanical support, and also serving as a pathway for water and nutrients, as
56 well as for retranslocation of photosynthates (Rost et al., 2006). There is evidence for allometric
57 relationships between leaf and petiole traits (Niinements et al., 2004; Al Afas et al., 2005). For
58 example, a positive correlation between individual leaf area and petiole diameter was observed for
59 12 different poplar genotypes (Al Afas et al., 2005). As there are allometric relationships between
60 leaf area characteristics and biomass, and as the petiole is a crucial part of the leaf, one might
61 assume that there are correlative relationships between the easy-to-measure petiole thickness and
62 biomass. The petiole thickness is therefore an interesting candidate for early selection criteria in
63 poplar breeding programmes.

64 This study was performed on a large-scale operational SRC plantation as part of an ambitious
65 multidisciplinary bio-energy project (POPFULL, 2015). Twelve different poplar genotypes were
66 planted at the POPFULL plantation which enabled us to measure their responses in a common
67 environment to quantify the degree of genotypic variation, in particular in leaf and petiole traits. Our
68 objective was to identify leaf and petiole traits that could be used as early selection criteria in future
69 breeding and selection programmes. We hypothesised that: (1) leaf and petiole traits are reliable
70 indicators of biomass yield; and (2) leaf and petiole traits are determined by parentage. If these
71 hypotheses were validated, it would allow us to construct a simple model to calculate difficult-to-
72 measure leaf and petiole traits from easier ones based on their correlation.

73

74 ## 2. Materials and Methods

75 ### 2.1. Site description

76 The POPFULL field site is located in Lochristi, province East-Flanders, Belgium (51°06´44´´ N, 3°51´02´´
77 E). The region has a temperate oceanic climate with a long-term average annual temperature and
78 precipitation rate of 9.5 °C and 726 mm, respectively (Royal Meteorological Institute of Belgium).

79 According to Belgian soil classification data, the area forms part of a sandy region with poor natural
80 drainage. The 18.4 ha site was formerly used for agricultural purposes consisting of croplands (62%;
81 with corn being the most recent cultivated crop) and extensively grazed pasture (38%). On 7-10 April
82 2010, an area of 14.5 ha (excluding the headlands) was planted with 12 selected poplar (*Populus*) and
83 three selected willow (*Salix*) genotypes, representing different pure native species and genotypes of
84 *Populus deltoides*, *P. maximowiczii*, *P. nigra*, *P. trichocarpa, Salix viminalis*, *S. dasyclados*, *S. alba* and
85 *S. schwerinii.* The present study focuses on the 12 poplar genotypes only (Table 1). Half of the
86 genotypes were sourced from and bred by the Institute for Nature and Forestry Research in
87 Geraardsbergen (Belgium) and half were bred by the ''De Dorschkamp'' Research Institute for
88 Forestry and Landscape Planning in Wageningen (The Netherlands; Table 1). Dormant and unrooted
89 cuttings were planted in a double-row system with alternating distances of 0.75 m and 1.50 m
90 between rows and an average 1.10 m between trees within the rows (i.e. 8000 plants ha^{-1}). After the
91 first two-year rotation, the plantation was harvested on 2-3 February 2012 (Berhongaray et al.,
92 2013). The second harvest took place after the second two-year rotation on 18-20 February 2014
93 (Vanbeveren et al., 2015). Plantation management was extensive, without fertilization or irrigation.
94 Only a minor influence of former land-use on the biomass production was observed during the first
95 growing season, and it disappeared during the second growing season (Broeckx et al., 2012). The
96 absence of an influence of former land-use was explained by the sufficient nutrient conditions and
97 optimal site conditions in terms of soil quality for both former cropland and pasture. Thus, former
98 land-use was not accounted for in the present study. More details on site conditions, on planting
99 material and on plantation layout have been previously reported (Broeckx et al., 2012).

100 2.2. Above-ground biomass

101 The above-ground biomass was inventoried for each of the 12 genotypes of the plantation in 3-7
102 February 2014, after the second year of the second rotation. The number of shoots per stump was
103 counted for each stump per row (one row per genotypic block, i.e. 80-310 stumps). Shoot diameters

were measured for every fifth stump in the same row. Shoot diameters were measured using a digital calliper (Mitutoyo, CD-15DC, UK, 0.01 mm precision) at 22 cm above the insertion height of the shoot on the stump. The biomass (dry mass in Mg ha^{-1}) was determined per genotype using allometric relationships established between above-ground woody dry mass (DM) and stem diameter (D) (eqn. 1 and 2)

If $D < 25$ mm $\rightarrow DM = a * D^b$ (equation 1)

If $D \geq 25$ mm $\rightarrow DM = c*D^2 + d*D + e$ (equation 2)

where a, b, c, d and e are regression coefficients specific to each genotype (Table 2).

2.3. Leaf and petiole traits

Leaf traits per genotype were determined by collecting mature leaves in August 2013, i.e. during the second year of the second rotation. Four trees per genotype were randomly selected and six mature leaves per tree were randomly collected from three different heights: two from the lower canopy, two from the middle canopy and two from the upper canopy. The fresh leaf area was measured immediately following leaf collection with a LI-3000 leaf area meter (Li-COR Bioscience, Lincoln, NE, USA). The average leaf area per leaf for each tree was then averaged per genotype to obtain the individual leaf area (A_{leaf}). Subsequently, the leaves were oven dried at 70 °C to allow for constant dry weight and individual dry mass (DM_{leaf}) to be weighed. The ratio of leaf dry mass to fresh leaf area – defined as the leaf mass per area (LMA) – was assessed per genotype.

Petiole traits were determined by collecting mature leaves of five trees per genotype from one randomly selected block, on 4-8 November 2013. Five leaves per tree were sampled, covering the complete range from the smallest to the largest leaf per tree. Immediately after sampling, petioles were fixed in a FAA solution (90 ml 70% ethanol, 5 ml acetic acid and 5 ml 40% formaldehyde). Ten randomly selected petioles per genotype were then used for anatomical analysis. In the laboratory,

cross-sections were manually obtained with a razor blade in the middle part of the petiole. The cross-sections were dyed using a saturated solution of phloroglucinol in 20% hydrochloric acid (HCl) to highlight the contrasting lignified cell walls in red. Stained cross-sections were examined under an Olympus BX51 light microscope (Olympus Corporation, Tokyo, Japan) and photographed using a digital Olympus E-330 camera (Olympus Corporation, Tokyo, Japan) and the QuickPHOTO Micro 3.0 software (Promicra, Prague, Czech Republic).

All vessel lumens in the micrographs were manually coloured using Adobe Photoshop 9.0.2 software (Adobe Systems Inc., San Jose, CA, USA). For each cross-section, the following traits were digitally measured using the ImageJ 1.45 software (Rasband, 2014): minimum and maximum vessel diameter (d_{min} and d_{max}, respectively); vessel lumen area (A_{lum}); number of vessels (N_{ves}); petiole cross-section area (A_{pet}); petiole thickness (D_1); petiole width (D_2); and petiole xylem area (A_x). The sum of lumen areas of individual vessels in the petiole was determined per petiole to obtain the total vessel lumen area per petiole (A_{lum_pet}). From the aforementioned measurements, the following parameters were calculated: vessel frequency per unit of petiole xylem area (V_f); petiole roundness (P_{round}) as the D_1 to D_2 ratio; vessel roundness (V_{round}) as the d_{max} to d_{min} ratio; the relative representation of total vessel lumen area in the petiole xylem area (A_{lum_x}); and the relative representation of petiole xylem area in the petiole cross-section area (A_{x_pet}). Only vessels with a d_{max} >10 µm were analysed as the manual colouring process did not allow the identification of vessels with smaller diameters.

The theoretical hydraulic conductivity of each vessel (k_{ves}) was calculated according to the Hagen-Poiseuille law (eqn. 3). Because the cross-section of the vessel lumen was approximated as an ellipse, a modification to the formula was applied (Martre et al., 2000) (eqn. 4),

[kg m s^{-1} MPa^{-1}] (equation 3)

(equation 4)

where ρ is the density of water at 20 °C (998.205 kg m^{-3}), η is the viscosity of water at 20 °C (1.002 x 10^{-9} MPa s) and r_{lum} is the lumen radius.

The theoretical hydraulic conductivity of any petiole cross-section (K_{pet}) was calculated as the sum of all k_{ves} in the petiole. Xylem specific conductivity (K_{s_xylem}) and leaf specific conductivity (K_{s_leaf}) were then calculated as K_{pet} divided by A_x and A_{leaf}, respectively. All abbreviations used in this contribution are summarized and identified in Table 3.

2.4. Statistical analysis

Values averaged per genotype were used for all statistical analyses, which were performed in R (R Core Team, 2014). We calculated the coefficient of variance (CV) for each trait as the ratio of the standard deviation to the mean. The reported CVs indicate the variation among the genotypic averages; they are relative to the absolute values, while being mutually comparable. We constructed a correlation matrix for all traits using the Pearson's correlation coefficient (ranging from -1 to 1). Initial correlations to biomass revealed that genotype Hees differed from trends exhibited by all other genotypes. We therefore considered genotype Hees to be a special case and as such, constructed a second correlation matrix with all genotypes excluding genotype Hees. Additionally, allometric equations for biomass and leaf dry mass were calculated with selected traits (A_x, A_{pet}, D1, K_{pet}, N_{ves}) using linear regressions.

A hierarchical cluster analysis and a principal component analysis (PCA) were performed to assess similarities among genotypes and to differentiate different clusters. Some of the analysed traits were highly correlated ($R > 0.90$) and therefore only one of those traits per correlated group was chosen to be included in these analyses (i.e. A_{lum}, A_{pet}, A_{x_pet}, K_{s_xylem}, LMA, P_{round}, V_f and V_{round}). Biomass was considered as the dependent variable, and was therefore not directly used in the cluster analysis and in the PCA. The Euclidean distance was used to measure similarity and average linkage was used as a clustering algorithm for cluster analysis. Trait values were standardized to the range of -1 to 1 before

174 the analysis as they varied significantly across different scales. In the absence of a reliable method for
175 determining the number of clusters in a data set (Everitt, 1979; Verlinden et al., 2013), the number of
176 clusters was set to four to give each cluster at least two genotypes. The PCA was used in a similar
177 way as the cluster analysis: we used an ordination diagram with the first two PCA axes to visualise
178 similarities between genotypes. P-values and pseudo-R^2 values obtained from permutation tests
179 were then used to calculate the overall goodness of fit.

180 3. Results

181 For nine traits (A_l, A_{lum_pet}, A_{pet}, A_x, D_1, DM_{leaf}, K_{pet}, K_{s_leaf} and N_{ves}), we observed large variations among
182 the 12 genotypes (CV > 40%); the highest CVs were observed for A_{pet} (83%) and K_{pet} (79%) (Table 4).
183 The maximum values of these highly variable traits were observed for genotypes Bakan and Skado
184 (both TxM parentage); the only exception was K_{s_leaf} (Tables 4 and 5). On the other hand, these traits
185 had the lowest values for genotypes Ellert (DxN) and Brandaris (N). The smallest genotypic
186 differences (CV less than 10%) were found for d_{max}, d_{min} and V_{round}; the mean values of these traits
187 were 27.3, 19.0 and 1.5 µm, respectively. The CVs of other traits (including biomass) ranged from 12
188 to 34%.

189 All leaf and petiole traits (with the exception of V_f) were positively inter-correlated with each other.
190 The petiole hydraulic conductivity was also strongly correlated with other leaf and petiole traits that
191 were not involved in the conductivity calculation, as A_l, A_{pet}, A_x, DM_{leaf} or D_1. Only LMA, V_{round}, P_{round},
192 A_{x_pet} and A_{lum_x} were poorly correlated with other traits (Table 6). In general, heavier poplar leaves
193 were bigger and had larger A_{pet}, A_x, K_{pet} and N_{ves} (Table 6). The K_{pet} was more influenced by the N_{ves},
194 which was a more variable trait than the two vessel diameters. The relationship between the leaf and
195 petiole traits enabled us to construct allometric equations to calculate A_x, K_{pet} and N_{ves} based on the
196 commonly and easily measured leaf dry mass (Fig. 1). We also identified nine significant correlations
197 (out of 18) between the above-ground biomass and different leaf and petiole traits, after genotype

198 Hees was excluded from the analysis (Table 6). Exclusion of genotype Hees enabled us to construct
199 three models based on the easy-to-measure petiole and leaf traits (A_{pet}, D_1, DM_{leaf}) to calculate
200 biomass yield (Fig. 2). When the two T×M genotypes with the largest petioles and the heaviest leaves
201 were removed from the regression calculation, the regression was no longer significant. However, if
202 the weights of T×M genotypes were reduced to half, the regression was still significant with p-values
203 lower than 0.029.

204 The cluster analysis resulted in a dendrogram which clearly separated breeding programmes from
205 parentage groups (Fig. 3). A first distinction could be made between the genotypes bred at the
206 Research Institute for Forestry and Landscape Planning (the Netherlands) (cluster 1) and the
207 genotypes bred at the Flemish Institute for Nature and Forestry Research (Belgium) (Table 1 and Fig.
208 3). The only exceptions were genotypes Vesten and Robusta. The genotypes in cluster 1 contained a
209 mixture of DxN (Ellert, Koster, Hees and Vesten) and pure N genotypes (Brandaris and Wolterson).
210 Genotypes Hees and Vesten were more closely related to the pure N genotypes than to the other
211 DxN genotypes in the first group (Ellert and Koster). Within the second group, three clusters could be
212 distinguished: cluster 2 consisted of the TxM genotypes (Bakan and Skado), cluster 3 was composed
213 by the DxN genotypes (Muur and Oudenberg), and cluster 4 comprised the only Dx(TxD) genotype
214 (Grimminge) (cluster 4a) and the oldest DxN genotype (Robusta) (cluster 4b). The four previously
215 described clusters were also distinguished by the principal component analysis (PCA), with the
216 exception of genotype Grimminge (Fig. 4). Cluster 4 was therefore subdivided into clusters 4a and 4b.
217 Cluster 1 was separated due to higher values of V_f and lower values of almost all other traits (Fig. 4).
218 On the other hand, a high A_{pet} and a low V_f characterized cluster 2. The common characteristics of
219 cluster 3 were a high V_{round}, LMA, P_{round} and A_{x_pet}, while cluster 4 was characterized by high values for
220 A_{lum} and K_{s_xylem}. The first two principal components explained 74.4% of the variation among the
221 analysed traits. The traits used for the cluster analysis – described above – were correlated with

222 other studied traits: A_{lum} with d_{max} and d_{min}; A_{pet} with K_{th}, N_{ves}, A_x, A_{lum_pet} and K_{s_xylem} with K_{s_leaf} (Table
223 6).

224 4. Discussion

225 4.1. Leaf and petiole morphology and anatomy

226 The measured hydraulic conductivity of different genotypes was found to be closely connected to
227 petiole anatomy. This corresponded with results reported for other genotypes and species (Sack et
228 al., 2003). The vessel diameter and the number of vessels were key traits determining petiole
229 conductivity (Sperry et al., 2006). Allometric equations constructed in the present study (Fig. 1) are
230 useful tools for better understanding tree hydraulic architecture, as leaves represent a
231 disproportionately large fraction (30% and more) of the whole-plant hydraulic resistance (Nardini
232 and Salleo, 2000; Sack and Holbrook, 2006).
233 The LMA in our study was within the range reported for other poplar species, from 70 g m^{-2} (D
234 genotype) (Turnbull et al., 2002) to 101 g m^{-2} (NxM genotype) (Green and Kruger, 2001). Although
235 LMA was closely correlated to the above-ground biomass of one-year old poplar cuttings in earlier
236 studies (Marron et al., 2005; Verlinden et al., 2013), where high-yielding DxN genotypes were
237 characterized by high LMA, no correlation was found in the present study. Excluding the high yielding
238 genotype Hees did not change this conclusion. Moreover, there were only minor genotypic
239 differences in LMA. The same pattern was observed for the same genotypes during the second
240 growing season of the first rotation (Verlinden et al., 2013) and for four five-year old *P. tremula* x *P.*
241 *tremuloides* genotypes (Yu, 2001). Thus, it seems that the variability in the LMA observed for
242 different poplar genotypes disappears when the trees get older.
243 Individual leaf area (A_l) differed significantly among the different genotypes and was positively
244 correlated with biomass for 11 out of the 12 genotypes. This relationship corresponds with previous
245 observations at the same plantation (Verlinden et al., 2013). As A_l was strongly correlated with

246 A_{lum_pet}, A_{pet}, A_x, D_1, D_2, DM_{leaf}, K_{pet} and N_{ves}, these traits were also closely connected to above-ground
247 biomass. This correlation enabled us to establish three models for calculating biomass based on
248 easily measured leaf and petiole traits. Nevertheless, the generality of these models should be
249 further evaluated for other poplar genotypes and parentage groups. Especially poplar genotypes
250 with thicker petioles are needed as only the two TxM genotypes had petioles thicker than 4.0 mm. A
251 correlation between K_{pet} and biomass yield was also observed in other studies (Brodribb et al., 2007;
252 Hajek et al., 2014). With regard to these correlations, genotype Hees appeared to be an exception as
253 it had a high above-ground biomass and a low A_l. Nevertheless, it was not possible to further
254 evaluate the unique behaviour of genotype Hees in the present study.
255
256 4.2. Cluster and principal component analysis

257 Clustering of the different genotypes depended largely on the selected breeding programmes. A
258 similar clustering pattern was found for 16 other traits studied on the same plantation during the
259 first rotation period (Verlinden et al., 2013). This pattern was explained by the different selection
260 criteria of the two breeding programmes (Verlinden et al., 2013). In general, the genotypes bred by
261 the Netherlands breeding institution had lower biomass yields compared to the genotypes bred by
262 the Belgium breeding institute. The Dutch genotypes were specifically screened for wind tolerance
263 (de Vries, 2014), a crucial characteristic for the lowlands in the Netherlands, while tolerance against
264 wind was not a primary selection criterion in the Belgian breeding programme (Steenackers et al.,
265 1990; De Cuyper, 2014). The same parent genotype was used for all Belgian-bred DxN genotypes
266 (Muur, Oudenberg and Vesten) and the same maternal genotype was used for all genotypes bred in
267 the Netherlands-bred DxN genotypes (Ellert, Hees and Koster) (Table 1); this might be another
268 explanation for the clustering observed in this study.

269 Clustering and PCA analysis also showed that genotypes of the same parentage were clustered
270 together. The strong parental effect has already been described for height, stem diameter, bud flush

and leaf area index by Verlinden et al. (2013) and for photosynthetic traits, the intrinsic water use efficiency and the leaf stable isotope composition by Broeckx et al. (2014). Nevertheless, DxN genotypes appeared to differ further from each other as they were grouped into two separate clusters. This finding also corresponds with a large variation of the stem xylem anatomy found for six DxN genotypes of different places of origin (Fichot et al., 2009). Because the number of TxM and Dx(TxD) genotypes was limited in this study, it was difficult to generalise about the impact of parentage.

In respect to the expected increase of drought occurrence due to climate change (Allen et al., 2010) breeders are actively looking for drought tolerant species. The lower vessel diameter of poplar genotypes in cluster 1 could be indicative of higher drought resistance as the risk for cavitation decreases with decreasing vessel diameter (Johnson et al., 2009; Hajek et al., 2014). Petioles of *P. balsamifera* were more resistant to cavitation than the branches which corresponded to the lower vessel diameter in the petioles (Hacke and Sauter, 1996). These results are contradictory as several authors failed to detect a relation between vessel diameter and cavitation resistance in closely related genotypes or between different poplar hybrids (Cochard et al., 2007; Fichot et al., 2010). The reason could be that several studies have linked tree drought resistance to vessel cell wall thickness (Hacke et al., 2006; Cochard et al., 2007), pit membrane structure (Choat et al., 2008; Jansen et al., 2009; Plavcová et al., 2013) or vessel grouping (Lens et al., 2011), rather than to vessel diameter. Thus, further studies should define anatomical and morphological traits involved in drought tolerance of poplar genotypes, which is important in future poplar breeding programs.

Conclusion

In our study leaf and petiole traits showed strong correlations between themselves. It enabled us to make three models to predict difficult-to-measure petiole traits as xylem area, total vessel lumen

295 area and number of vessels based on the more common and easily measurable leaf dry mass. The
296 first hypothesis was confirmed in our study, as significant correlations between above-ground
297 biomass and nine leaf and petiole traits were found. Three predictive correlative models for above-
298 ground biomass based on the leaf dry mass, petiole cross-section area and petiole thickness were
299 developed. The easy-to-measure petiole thickness can be used as early selection criteria in poplar
300 breeding programmes. The second hypothesis was also proved as different genotypes clustered
301 largely according to their breeding programmes and to their parentage.

302 Acknowledgments

303 This work was financially supported by Mendel University (Grant # IGA 73/2013), by the project
304 ''Indicators of Tree Vitality'' (Reg.# CZ.1.07/2.3.00/20.0265) co-financed by the European Social Fund
305 and the Czech Republic, and by ERC Advanced Grant agreement (# 233366, POPFULL) under the EC
306 7th Framework Programme (FP7/2007-2013). We also acknowledge the contribution of COST Action
307 FP1106, STReESS.

308 Conflict of interest

309 The authors declare that there are no conflicts of interest.

310

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467 Table 1. Breeding institution, place of origin, botanical and parental characteristics of the twelve poplar (*Populus*) genotypes studied. Adapted from Broeckx,
468 et al. (2012).

Genotype	Parentage	Section	Breeding Institution	Place of origin	Gender	Year of cross/ commercialization
Bakan	TxM	Tacamahaca	Institute for Nature and Forestry Research (Belgium)	(Washington USA x Oregon US) x Japan	male	1975/2005
Skado	TxM	Tacamahaca	Institute for Nature and Forestry Research (Belgium)	(Washington USA x Oregon US) x Japan	female	1975/2005
Muur	DxN	Aigeiros	Institute for Nature and Forestry Research (Belgium)	(Iowa USA x Illinois USA) x (Italy x Belgium)	male	1978/1999
Oudenberg	DxN	Aigeiros	Institute for Nature and Forestry Research (Belgium)	(Iowa USA x Illinois USA) x (Italy x Belgium)	female	1978/2000
Vesten	DxN	Aigeiros	Institute for Nature and Forestry Research (Belgium)	(Iowa USA x Illinois USA) x (Italy x Belgium)	female	1978/2001
Ellert	DxN	Aigeiros	Research Institute for Forestry and Landscape Planning (The Netherlands)	Michigan USA x France	male	1969/1989
Hees	DxN	Aigeiros	Research Institute for Forestry and Landscape Planning (The Netherlands)	Michigan USA x France	female	1969/1990
Koster	DxN	Aigeiros	Research Institute for Forestry and Landscape Planning (The Netherlands)	Michigan USA x The Netherlands	male	1966/1988
Robusta	DxN	Aigeiros	The nursery Simon-Louis Frères (France)	Eastern USA x Europe	male	1885-1890/1895
Grimminge	Dx(TxD)	Aigeiros x (Tacamahaca x Aigeiros)	Institute for Nature and Forestry Research (Belgium)	(Michigan USA x Connecticut USA) x (Washington USA x (Iowa USA x Missouri USA)	male	1976/1999
Brandaris	N	Aigeiros	Research Institute for Forestry and Landscape Planning (The Netherlands)	The Netherlands x Italy	male	1964/1976
Wolterson	N	Aigeiros	Research Institute for Forestry and Landscape Planning (The Netherlands)	The Netherlands	female	1960/1976

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470 D= *Populus deltoides*, M = *Populus maximowiczii*, N = *Populus nigra*, T = *Populus trichocarpa*

Table 2. Regression coefficients specific to each genotype used for above-ground woody dry mass (DM) calculation.

Genotype	Regression coefficients				
	a	b	c	d	e
Bakan	0.0681	2.6180	1.5225	-41.6450	312.4300
Skado	0.0419	2.7374	1.2550	-28.1980	196.8300
Muur	0.1045	2.5403	1.0779	-13.8550	79.9260
Oudenberg	0.0834	2.5684	0.9884	-14.1760	86.1700
Vesten	0.0825	2.5631	0.9137	-8.7014	19.4790
Ellert	0.1527	2.4120	0.6723	5.1370	-117.1300
Hees	0.1701	2.3590	0.9764	-16.6370	123.8400
Koster	0.0706	2.6145	1.0302	-15.4340	82.6730
Robusta	0.0655	2.6290	0.7842	-5.1988	0.5117
Grimminge	0.0571	2.7084	1.0258	-10.0300	33.8440
Brandaris	0.1531	2.3549	0.7147	-6.9515	38.5780
Wolterson	0.1097	2.5082	0.8833	-8.8460	38.3390

Table 3. List of the different traits with their respective units and abbreviations, as used throughout this contribution.

	Unit	Abbreviation
biomass	$Mg\ ha^{-1}$	
individual leaf area	cm^2	A_l
leaf dry mass	g	DM_{leaf}
leaf mass per area	$g\ m^{-2}$	LMA
leaf specific hydraulic conductivity (K_{pet}/A_l)	$mg\ m^{-1}\ s^{-1}\ Mpa^{-1}$	K_{s_leaf}
maximum vessel diameter	µm	d_{max}
minimum vessel diameter	µm	d_{min}
number of vessels in a petiole	-	N_{ves}
petiole cross-section area	mm^2	A_{pet}
petiole roundness (D_1/D_2)	-	P_{round}
petiole thickness	mm	D_1
petiole width	mm	D_2
petiole xylem area	mm^2	A_x
relative representation of lumen area in xylem area ($100\ A_{lum\_pet}/A_x$)	%	A_{lum_x}
relative representation of xylem area in petiole cross-section area ($100\ A_x/A_{pet}$)	%	A_{x_pet}
theoretical hydraulic conductivity of petiole	$mg\ m\ s^{-1}\ Mpa^{-1}$	K_{pet}
total lumen area in a petiole	mm^2	A_{lum_pet}
vessel roundness (d_{max}/d_{min})	-	V_{round}
vessel lumen area	μm^2	A_{lum}
vessel frequency (N_{ves}/A_x)	μm^{-2}	V_f
xylem specific hydraulic conductivity (K_{pet}/A_x)	$kg\ m^{-1}\ s^{-1}\ Mpa^{-1}$	K_{s_xylem}

Table 4. Minimum and maximum values of petiole traits of the 12 poplar genotypes together with their coefficient of variance (CV; %). The genotype for which the minimum or the maximum value has been observed, is shown in brackets. For a definition of all abbreviations and acronyms, and their units, see Table 2.

	Min		Max		CV
A_l	322	(Ellert)	1724	(Bakan)	58
A_{lum}	333	(Ellert)	578	(Grimminge)	19
A_{lum_pet}	0.13	(Ellert)	1.13	(Skado)	74
A_{lum_x}	25	(Ellert)	42	(Muur)	12
A_{pet}	2.41	(Brandaris)	21.25	(Bakan)	83
A_x	0.42	(Brandaris)	3.45	(Bakan)	73
A_{x_pet}	12	(Ellert)	22	(Koster)	21
Biomass	14.8	(Brandaris)	32.4	(Hees)	24
D_1	1.82	(Brandaris)	5.44	(Skado)	42
D_2	1.86	(Brandaris)	5.34	(Bakan)	34
d_{max}	23.23	(Ellert)	30.81	(Grimminge)	9
d_{min}	16.91	(Ellert)	21.57	(Grimminge)	8
DM_{leaf}	2.49	(Ellert)	12.30	(Bakan)	56
K_{pet}	2.3	(Ellert)	35.5	(Skado)	79
K_{s_leaf}	73	(Ellert)	381	(Muur)	51
K_{s_xylem}	4.23	(Ellert)	15.29	(Muur)	33
LMA	77	(Hees)	101	(Robusta)	8
N_{ves}	365	(Ellert)	2232	(Bakan)	65
P_{round}	1.08	(Robusta)	1.33	(Oudenberg)	7
V_f	545	(Grimminge)	943	(Brandaris)	15
V_{round}	1.40	(Ellert)	1.52	(Muur)	2

Table 5. Mean values (±SD) for petiole traits (n=10; except Biomass and K_{s_leaf} where n=1) with a coefficient of variance > 40%, per genotype. For explanation and definition of the acronyms of the different petiole traits, see Table 2.

Parentage	Genotype	Biomass (Mg ha^{-1})	A_{lum_pet} (mm^2)	N_{ves}	A_x (mm^2)	A_{pet} (mm^2)	K_{pet} (mg $m\ s^{-1}\ MPa^{-1}$)	K_{s_leaf} (mg $m^{-1}\ s^{-1}\ MPa^{-1}$)
TxM	Bakan	25.80	1.10 (0.39)	2232 (792)	3.45 (1.18)	21.25 (3.62)	30.1 (10,9)	175
TxM	Skado	31.95	1.13 (0.53)	1996 (862)	3.31 (1.47)	21.90 (8.46)	35.5 (18,8)	265
DxN	Muur	23.10	0.56 (0.06)	1008 (203)	1.37 (0.21)	7.12 (1.21)	20.4 (3,0)	381
DxN	Oudenberg	25.46	0.44 (0.15)	926 (267)	1.29 (0.40)	5.76 (1.54)	13.7 (6.4)	226
DxN	Vesten	24.87	0.35 (0.15)	792 (331)	1.08 (0.40)	5.73 (0.68)	10.0 (5,2)	120
DxN	Ellert	21.69	0.13 (0.07)	365 (150)	0.50 (0.23)	3.94 (1.36)	2.3 (1,6)	73
DxN	Hees	32.36	0.24 (0.14)	525 (229)	0.74 (0.39)	4.42 (1.77)	6.8 (4,7)	132
DxN	Koster	19.20	0.34 (0.14)	870 (269)	1.11 (0.36)	4.85 (1.43)	7.6 (4,5)	126
DxN	Robusta	15.01	0.35 (0.20)	655 (327)	1.03 (0.49)	4.77 (1.42)	11.6 (8,0)	198
Dx(TxD)	Grimminge	22,09	0.50 (0.20)	851 (300)	1.57 (0.58)	9.72 (2.85)	15.7 (7.0)	184
N	Brandaris	14.84	0.15 (0.09)	385 (190)	0.42 (0.22)	2.41 (0.86)	3.0 (2,5)	78
N	Wolterson	21.62	0.18 (0.09)	451 (194)	0.63 (0.27)	3.16 (1.15)	4.0 (2.3)	110

Table 6.Correlation matrix of leaf and petiole traits of twelve poplar genotypes. Significance levels of Pearson correlation: ns = not significant ($p > 0.05$); + = $0.01 < p \leq 0.05$; ++ = $p \leq 0.01$ for positive correlations. Negative correlations are marked equally with a – sign. Signs in brackets show correlations without genotype Hees. For explanation of abbreviations and definition of acronyms, see Table 2.

	K_{s_leaf}	K_{s_xylem}	K_{pet}	A_{lum_x}	A_{x_pet}	V_f	P_{round}	D_2	D_1	A_{pet}	A_x	N_{ves}	A_{lum_pet}	A_{lum}	V_{round}	d_{min}	d_{max}	A_l	LMA	DM_{leaf}
Biomass	ns	ns	ns (+)	ns	ns	ns	ns	ns (+)	ns (+)	ns (+)	ns (+)	ns (+)	ns (+)	ns	ns	ns	ns	ns (+)	ns	ns (+)
DM_{leaf}	ns	ns	++	ns	ns	-	ns	++	++	++	++	++	++	+ (ns)	ns	+	+	++	ns	
LMA	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
A_l	ns	ns	++	ns	ns	- (ns)	ns	++	++	++	++	++	++	ns	ns	ns	ns			
d_{max}	++	++	++	+	ns	--	ns	+ (ns)	+	+ (ns)	+	ns	+	++	+	++				
d_{min}	++	++	++	ns	ns	--	ns	+	+	+	+	+ (ns)	+	++	ns					
V_{round}	++	++	ns	++	+	ns	ns	ns	ns	ns	ns	ns	ns	+						
A_{lum}	++	++	++	+	ns	--	ns	ns	+	ns	+	ns	+							
A_{lum_pet}	ns	ns	++	ns	ns	- (ns)	ns	++	++	++	++	++								
N_{ves}	ns	ns	++	ns	ns	ns	ns	++	++	++	++									
A_x	ns	ns	++	ns	ns	-	ns	++	++	++										
A_{pet}	ns	ns	++	ns	ns	- (ns)	ns	++	++											
D_1	ns	ns	++	ns	ns	-	ns	++												
D_2	ns	ns	++	ns	ns	- (ns)	ns													
P_{round}	ns	ns	ns	ns	ns	ns														
V_f	ns	ns	-	ns	ns															
A_{x_pet}	ns	ns	ns	ns																
A_{lum_x}	++	++	ns																	
K_{pet}	+	+																		
K_{s_xylem}	++																			

Fig. 1. Linear regressions of petiole xylem area (A_x), theoretical hydraulic conductivity of the petiole (K_{pet}) and number of vessels (N_{ves}) with leaf dry mass (DM_{leaf}). Each data point represents a genotypic mean. The types of symbols indicate the parentages (D = *Populus deltoides*,M = *P. maximowiczii*, N = *P. nigra*, T = *P. trichocarpa*). The p-value was lower than 0.001 for each regression line.

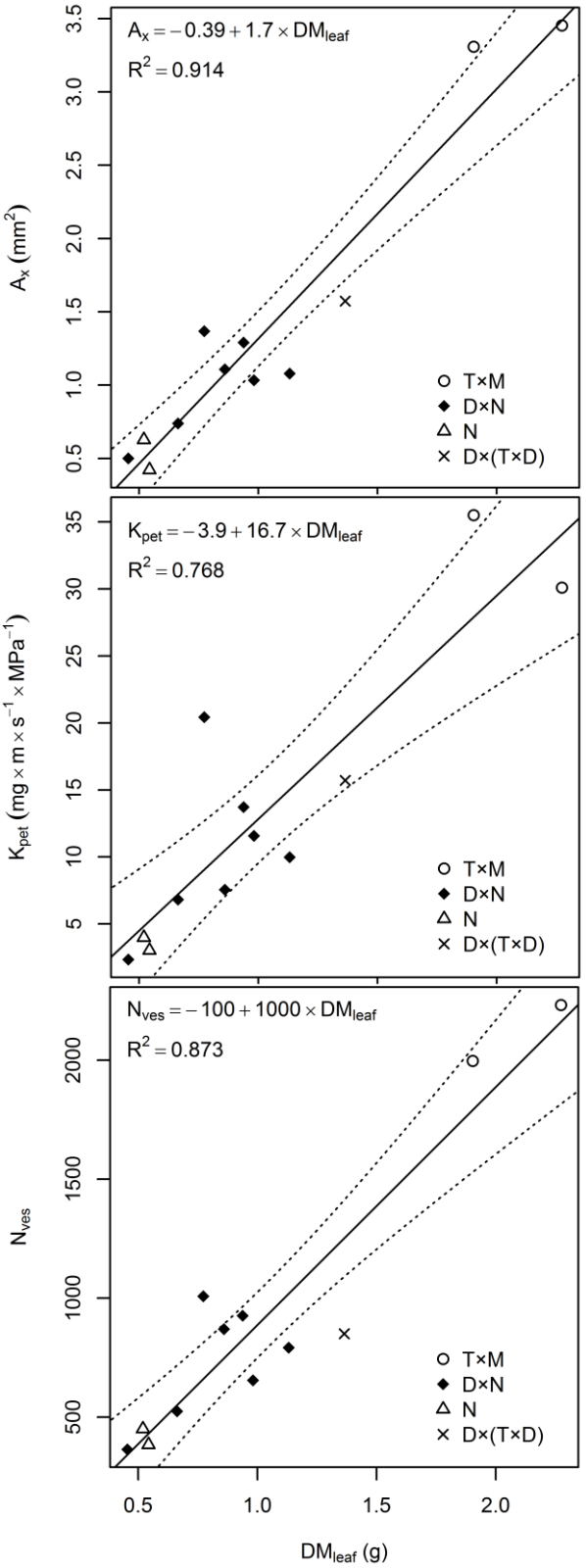


Fig. 2. Linear regressions of petiole cross-sectional area (A_{pet}), petiole thickness (D_1) and leaf dry mass (DM_{leaf}) with biomass (excluding genotype Hees). Each data point represents a genotypic mean. D = *Populus deltoides*, M = *P. maximowiczii*, N = *P. nigra*, T = *P. trichocarpa*. The p-value was lower than 0.001 for each regression line.

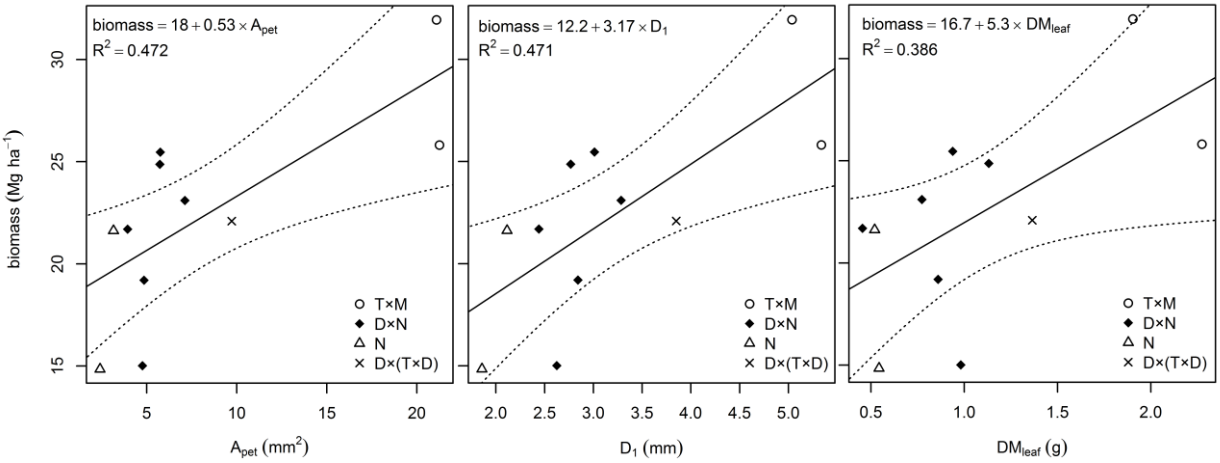
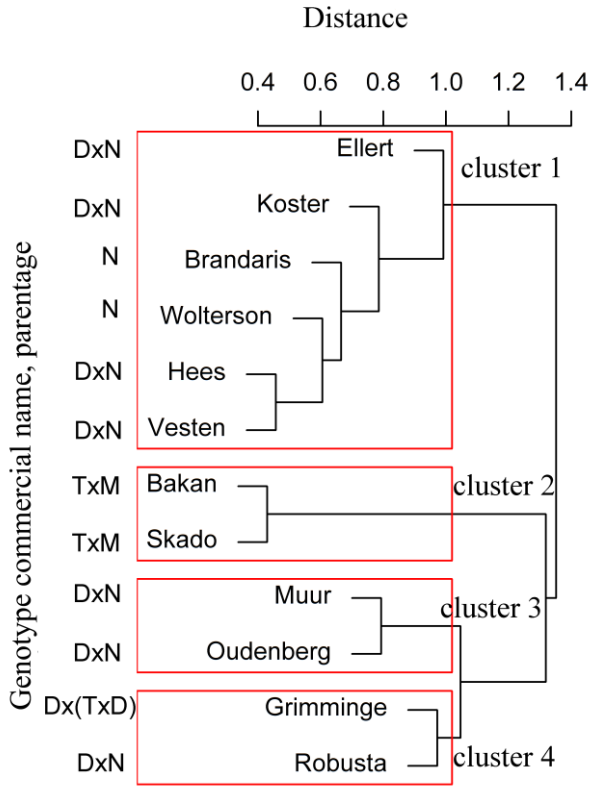
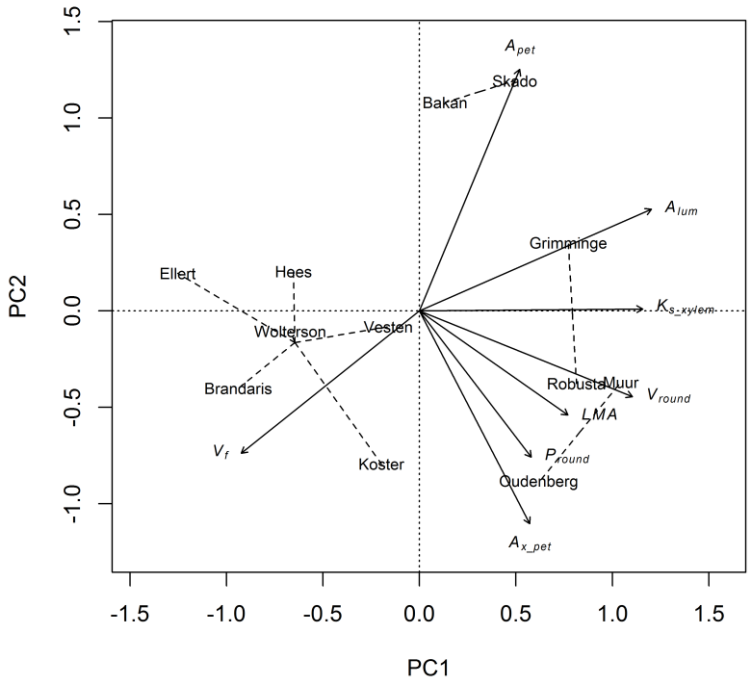


Fig. 3. Dendrogram of the hierarchical cluster analysis conducted on eight leaf and petiole traits measured on 12 *Populus* genotypes (shown on the y-axis). The four restrained clusters are indicated on the dendrogram branches. The following traits were included in the analysis: vessel lumen area, petiole cross-section area, vessel frequency, vessel roundness, petiole roundness, relative representation of xylem area in petiole cross-section area, xylem specific hydraulic conductivity and leaf mass per area.



571 Fig. 4. Ordination plot from the principal component analysis (PCA) showing the different genotype
572 clusters. The PCA has been based on the same traits as the dendrogram in Figure 3. The first two
573 principal components explained 48.7% and 25.7% of the total variance. Dashed lines connect the
574 genotypes to the same cluster as obtained in the hierarchical cluster analysis.



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