Forest Ecology and Management 307 (2013) 101–111

Contents lists available at SciVerse ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Comparative study of biomass determinants of 12 poplar (*Populus*) genotypes in a high-density short-rotation culture ☆

M.S. Verlinden [a,*], L.S. Broeckx [a], J. Van den Bulcke [b], J. Van Acker [b], R. Ceulemans [a]

[a] Department of Biology, Research Group of Plant and Vegetation Ecology, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium
[b] Laboratory of Wood Technology, Department of Forest and Water Management, Faculty of Bioscience Engineering, Coupure Links 653, B-9000 Ghent, Belgium

ARTICLE INFO

Article history:
Received 15 April 2013
Received in revised form 24 June 2013
Accepted 30 June 2013

Keywords:
Poplar clones
Breeding and selection
POPFULL
Multivariate analysis
Bioenergy plantation

ABSTRACT

The success of the production of renewable bioenergy with short-rotation coppice (SRC) cultures primarily depends on their sustainability and biomass yield. The choice of the genotypic materials largely determines how much biomass can be produced; therefore there is a need to study the performance of genotypes in situ to select the best performing ones. Twelve poplar (*Populus*) genotypes, of which two only recently commercialized, were planted in a large-scale operational SRC culture for the production of biomass for bioenergy. The objectives of the study were: (i) to describe and compare the 12 genotypes based on their growth, structural and developmental characteristics, and (ii) to analyze causal relationships between determining traits and productivity characteristics assessed at leaf, tree and population level by performing a hierarchical cluster analysis. The clustering of the poplar genotypes was clearly determined by parentage and genetic origin. Distinct differences between clusters were expressed in the biomass related traits; genotypes of similar parentage and origin showed comparable characteristics. *Populus nigra* genotypes were the least performing among the studied genotypes. The recently commercialized *P. trichocarpa* × *P. maximowiczii* hybrids on the other hand, were among the most productive genotypes. The *P. deltoides* × *P. nigra* hybrids showed intermediary results, with genotype Hees showing the highest biomass production among the 12 genotypes. As higher heating value was rather uniform among the genotypes, biomass production appeared the primary trait with regard to bioenergy production. This has significant implications for SRC cultures aiming at maximization of biomass production for maximum bioenergy yield. Besides the direct measurements of woody biomass growth (i.e. stem diameter), leaf area index is one of the most important early selection criteria for poplar with bioenergy purposes. The negative correlation of biomass and leaf rust infection reconfirmed the importance of disease vulnerability in breeding and selection programs.

1. Introduction

Short-rotation coppice (SRC) with poplar or other fast-growing species for the production of bioenergy is currently gaining interest within the framework of global energy supply (Sadrul Islam and Ahiduzzaman, 2012). The success rate of renewable bioenergy from SRC cultures primarily depends on their sustainability and productivity or biomass yield. The choice of the genotypic materials used for the SRC cultures largely determines the amount of biomass that can be produced in a specific area or region (Kuiper, 2003). Therefore there is a need to study the performance of genotypes in situ to select the best performing genotypes. Nevertheless, on operational, large-scale plantations the use of a sufficiently broad genetic diversity among the planted genotypes is necessary to decrease cultivation risks such as diseases, insects or pests, rather than relying on the single highest performing genotype only. Moreover, mixing several genotypes with complementary strategies in a SRC plantation possibly results in a more efficient use of abiotic site resources (McCracken et al., 2001).

Continuous breeding and selection efforts are required to continuously improve productivity of the genotypic materials, in particular for short rotation biomass plantations, and to create a sufficiently large genetic variation in the commercially available genetic materials. In Belgium and in The Netherlands any new poplar genotype is submitted to a 20 yr screening and selection period before it is certified and put on the list of commercially available plant materials. Despite the historical popularity and preserved current importance of *Populus* tree species in both countries (De Cuyper, 2008; de Vries, 2008), the application in SRC cultures is limited. To our knowledge, the genotypes in the present study (cfr. 2.1) have rarely been studied (except for the oldest genotype

* Corresponding author. Tel.: +32 32652827; fax: +32 32652271.
E-mail address: Melanie.Verlinden@ua.ac.be (M.S. Verlinden).

0378-1127/$ - see front matter © 2013 The Authors. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.foreco.2013.06.062

'Robusta') and have never been planted in large-scale operational bio-energy plantations. Besides the fact that the other 11 genotypes were commercialized for a few decades, their use in SRC plantations is still new.

All 12 genotypes were planted in a large-scale SRC culture for the production of biomass for bioenergy. The establishment of such a large-scale multiclonal plantation allowed us to have ample replications per genotype (both areal replications to account for spatial variability, as well as replicated and harvestable plant material per tree/genotype). Growing several genotypes together while measuring their responses in a shared environment is commonly applied to understand how much genetic variation is available in particular traits (Dunlap and Stettler, 1998). The study of this variability is then valuable for determining the efficiency of selection for the trait in future breeding and selection processes (Rae et al., 2004). The objectives of the study are (i) to describe and compare 12 poplar genotypes, of which two recently commercialized, based on their growth, structural and developmental characteristics, and (ii) to analyze causal relationships between determining traits and productivity characteristics assessed at leaf, tree and population level. There are very few studies where genotypic variation among different poplar genotypes has been examined at different organisational levels for such a wide range of different parameters. Results of the present study can be of use for the management of future SRC plantations, particularly in Belgium, and can serve as a source of information for future poplar breeding programs and for the selection for biomass yield.

2. Material and methods

2.1. Plantation and site description

This study was performed on a large-scale operational SRC plantation as part of an ambitious multidisciplinary project POPFULL (http://webh01.ua.ac.be/popfull/). The overall aims of the project are to analyse the energy balance, the economic balance and the mitigation of greenhouse gases of bioenergy production. The POPFULL field site is located in Lochristi, province East-Flanders, Belgium (51°06′44″ N, 3°51′02″ E) at an elevation of 6.25 m above sea level. Subjected to an oceanic climate, the long-term mean annual temperature in the area of the site is 9.5 °C and the mean annual precipitation is 726 mm (Royal Meteorological Institute of Belgium). The area is situated in the sandy soil region of Flanders with poor natural drainage according to the Belgian soil classification (Van Ranst and Sys, 2000). Formerly, the 18.4 ha site was used as an agricultural area consisting of extensively grazed pasture and croplands, with corn as the most recent cultivated crop in rotation (Broeckx et al., 2012a). On 7–10 April 2010 an area of 14.5 ha (excluding the headlands that remained unplanted) was planted with 12 selected and commercially available poplar (*Populus*) and three willow (*Salix*) genotypes. The poplar genotypes represented different species and hybrids of *Populus deltoides*, *Populus maximowiczii*, *Populus nigra*, and *Populus trichocarpa*. The present study focuses on the poplar genotypes only; details on the origin and the parentage of the 12 genotypes are shown in Table 1 (after Broeckx et al., 2012a). Half of the genotypes were bred by and obtained from the Institute for Nature and Forestry Research in Geraardsbergen (Belgium). Genotype Robusta originates from an open-pollinated *P. deltoides* tree, first commercialized by the nursery Simon-Louis Frères (Metz, France). The other five genotypes were bred by "De Dorschkamp" Research Institute for Forestry and Landscape Planning in Wageningen (The Netherlands) and, as Robusta, obtained from the Propagation Nurseries in Zeewolde (The Netherlands).

The plantation was designed in two to four large monoclonal blocks of eight double rows wide per genotype with row lengths varying from 90 m to 340 m. Twenty-five centimeter long dormant and unrooted cuttings were planted in a double-row planting scheme with alternating inter-row spacings of 0.75 m and 1.50 m and a mean distance of 1.10 m between trees within a row, yielding a planting density of 8000 trees ha^{-1}. After two growing seasons (GS1 in 2010, and GS2 in 2011) the plantation was harvested on 2–3 February 2012 with commercially available SRC harvesters (Berhongaray et al., 2013). In the following two-year-rotations trees continue growing as a coppice culture with multiple stems per stool. More details on siteconditions and plantation lay-out are found in Broeckx et al. (2012a).

2.2. Determination of genotypic characteristics

All measurements – except those for the determination of wood characteristics, see below – were performed on the 12 planted poplar genotypes during the 2 yr of the first rotation, i.e. 2010 and 2011.

Table 1
Place of origin, botanical and parental characteristics of the twelve poplar (*Populus*) genotypes studied (adapted from Broeckx et al., 2012a).

Genotype	Parentage	Section	Place of origin	Gender	Year of cross/ commercialization
Bakan[a]	T × M	Tacamahaca	(Washington US × Oregon US) × Japan	♂	1975/2005
Skado[a]	T × M	Tacamahaca	(Washington US × Oregon US) × Japan	♀	1975/2005
Muur[a]	D × N	Aigeiros	(Iowa US × Illinois US) × (Italy × Belgium)	♂	1978/1999
Oudenberg[a]	D × N	Aigeiros	(Iowa US × Illinois US) × (Italy × Belgium)	♀	1978/1999
Vesten[a]	D × N	Aigeiros	(Iowa US × Illinois US) × (Italy × Belgium)	♀	1978/1999
Ellert[b]	D × N	Aigeiros	Michigan US × France	♂	1969/1989
Hees[b]	D × N	Aigeiros	Michigan US × France	♀	1969/1989
Koster[b]	D × N	Aigeiros	Michigan US × The Netherlands	♂	1966/1988
Robusta[c]	D × N	Aigeiros	Eastern US × Europe	♂	1885–1890/1895
Grimminge[a]	D × (T × D)	Aigeiros × (Tacamahaca x Aigeiros)	(Michigan US × Connecticut US) × (Washington US × (Iowa US × Missouri US))	♂	1976/1999
Brandaris[b]	N	Aigeiros	The Netherlands x Italy	♂	1964/1976
Wolterson[b]	N	Aigeiros	The Netherlands	♀	1960/1976

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

[a] Genotypes bred by the Institute for Nature and Forestry Research (INBO, Geraardsbergen, Belgium). Main selection criteria: disease resistance, rooting capacity, adaptation to climate, soil and photoperiod, vigour, form and wood quality (de Cuyper, 2008).

[b] Genotypes bred by Research Institute for Forestry and Urban Ecology "De Dorschkamp" (Wageningen, The Netherlands). Main selection criteria: tolerance for leaf diseases (poplar rust and *Marssonina brunnea*), resistance to bacterial canker, growth, form, branch behaviour, wind tolerance (de Vries, 2008).

[c] Genotype originating from an open-pollinated *P. deltoides* tree, first commercialized by the nursery Simon-Louis Frères (Metz, France).

2.2.1. Woody biomass production

Stem diameter was assessed as the main tree characteristic for woody biomass production (Laureysens et al., 2004; Liberloo et al., 2006). Stem diameters were measured for all trees in one row (ranging from 71 to 328 trees) of each monoclonal block in the dormant season after GS1 and GS2 (February 2011 and December 2011). Diameters were measured with a digital caliper (Mitutoyo, CD-15DC, UK, 0.01 mm precision) at 22 cm above soil level (Ceulemans et al., 1993; Pontailler et al., 1997). For multiple-stem trees, every stem of the tree was measured, and the number of stems per tree was recorded as well. Tree height and woody biomass were calculated using allometric relationships with stem diameter. From a subset of trees comprised in the diameter inventories (i.e. every fourth tree in a row), tree height was measured with a telescopic rule (Nedo mEssfix, NL, 1 mm precision). From the resulting linear relationship of stem height versus diameter per genotype, the height of the remaining trees in the inventory was estimated. Secondly, for each genotype an allometric power relationship was established linking above-ground woody (dry) biomass to stem diameter. These allometric relationships were determined for each of the 12 genotypes in December 2011. Based on the stem diameter distribution after GS2, ten stems per genotype were selected for destructive harvest, covering the widest possible diameter range. Following a diameter measurement at 22 cm height (D), the stem was harvested at 15 cm above soil level, the mean harvesting height of the plantation. Dry biomass (DM) of each stem was determined by oven drying for 10 days at 70 °C. Biomass values were plotted against diameter and fitted as $DM = a \cdot D^b$ for each of the 12 genotypes (with a and b regression coefficients; cfr. Pontailler et al., 1997; Laureysens et al., 2004). Stem diameter inventory data were considered as spatially representative, resulting in genotypic means for the plantation. Genotypic means for tree height and woody biomass production were derived from the allometric equations combined with the inventory data. Biomass production values were converted to area based values (Mg ha^{-1}) using the planting distances. Means of annual genotypic diameter increment, height growth and annual biomass productivity over both GS1 and GS2 were calculated by halving the values at the end of GS2.

2.2.2. Foliage productivity

Leaf area index (LAI) (m^2 m^{-2}) was measured in four replicated measurement plots (of 5 × 6 trees) for each genotype in GS1 and in eight replicated measurement plots per genotype in GS2. The evolution of LAI was monitored throughout each of the two growing seasons from April to November using direct as well as indirect methods. The LAI-2200 Plant Canopy Analyzer (Li-COR Biosciences, Lincoln, NE, USA) was used to measure LAI indirectly by comparison of above- and below-canopy readings with a 45° view cap (see also Broeckx et al., 2012a). LAI_{max} was defined as the maximal LAI of the growing season and was averaged over all measurement plots per genotype. Direct LAI assessment consisted of leaf litter collection during the period of leaf fall, from September to December of GS1 and GS2. Three 0.57 × 0.39 m^2 litter traps were placed on the soil along a diagonal transect between the rows in four plots per genotype. The traps were emptied every two weeks and the cumulated dry mass of the collected leaf litter was converted to LAI_{max} using data of specific leaf area (SLA; cf. 2.2.3). Seasonal evolution of LAI in GS1 and GS2 was visualized as a curve of LAI versus day of the year. Leaf area duration (LAD) (m^2 day m^{-2}) was calculated as the area below the mean seasonal LAI curve per genotype by integrating over time.

The seasonal LAI curve was also used to estimate the radiation use efficiency (RUE) (g MJ^{-1}), representing the biomass produced per unit of intercepted short-wave radiation. The intercepted short-wave radiation was calculated from the Beer–Lambert extinction law (Eq. (1); Monsi and Saeki, 2005):

$$I = I_0 e^{-k\mathrm{LAI}} \quad (1)$$

where I_0 is the incident short-wave radiation, I is the radiation transmitted below the canopy and k is the extinction coefficient. The incoming short-wave radiation (0.3–3.0 μm) was continuously monitored at the site with a pyranometer (CNR1, Kipp & Zonen, Delft, The Netherlands) and logged automatically every 30 min (Zona et al., 2013). The value of k of Eq. (1) was derived from the LAI data using the converted Beer–Lambert law (Eq. (2)):

$$k = -\mathrm{LAI}^{-1} ln(I \cdot I_0^{-1}) \quad (2)$$

The LAI_{max} value determined through the direct leaf fall method was used as LAI value in Eq. (2). The ratio of $I \cdot I_0^{-1}$ was assessed during the LAI-2200 measurements at the time of LAI_{max}, taking into account the proportion of incoming radiation on the sensor angled between 7° and 53° zenith. The resulting k values for each genotype were then used for the calculation of the total cumulated intercepted radiation throughout GS1 and GS2. Following the quantification of the total above-ground biomass per genotype as explained above, RUE was calculated as the ratio of the annual above-ground biomass production and the annual intercepted short-wave radiation. The above-ground biomass production was taken as the sum of the woody biomass production (cf. 2.2.1) and the cumulated dry mass of the collected leaf fall (cf. supra).

2.2.3. Individual leaf properties

The ratio of fresh leaf area to leaf dry mass, defined as the specific leaf area (SLA) (m^2 kg^{-1}) (Larcher, 2003), was assessed for each genotype at the time of LAI_{max} in both GS1 and GS2. Three mature leaves of different individual leaf area and from different tree heights were randomly selected per measurement plot. Fresh leaf area was measured shortly after leaf collection with a Li-3000 Leaf Area Meter (Li-COR Biosciences, Lincoln, NE, USA). Leaves of plots of the same genotype were merged, oven dried at 70 °C and their combined dry mass determined by weighing. A measure for individual leaf area (cm^2) was obtained by averaging the aforementioned assessed fresh leaf areas per genotype (n = 12). Leaf nitrogen (N) concentrations were determined by dry combustion (with a NC-2100 element analyzer, Carlo Erba Instruments, Italy) of a subsample of the grounded dried leaves for each genotype and for both GS1 and GS2.

2.2.4. Phenology

The phenological onset and ending of GS2 was monitored by observing the apical buds of four selected trees per measurement plot during spring and autumn 2011. The timing of spring bud flush (day of the year; DOY) was defined at a stage according to the following: "Bud sprouting, with a tip of the small leaves emerging out of the bud scales, which could not be observed individually" (based on UPOV, 1981). The timing of bud set (DOY), accompanied by the end of leaf production and the end of height growth was set at the time when the "apical bud was present but not fully closed, bud scales were predominantly green and no more rolled-up leaves were present" (Rohde et al., 2010). The length of the growing season (days) was then defined as the period in between these well-defined phenological stages. A detailed description of phenological observations on poplar can be found in Pellis et al. (2004).

2.2.5. Wood characteristics

Wood characteristics were determined for six out of the 12 genotypes (i.e. Bakan, Grimminge, Koster, Oudenberg, Skado and Wolterson). After GS2, in January 2012, wood samples were taken from five trees in each of the eight measurement plots per genotype (n = 40). 2-cm-long specimens were cut from the main stem at a height of 5–7 cm from the base of the current-year shoot

and were stored at −20 °C. Thin (approx. 7 mm) disks were cut from these 2-cm-long samples for scanning with a flatbed scanner. The disk area was then determined semi-automatically in Matlab (7.12.0, 2011 Mathworks, Natick, MA, USA) on the scans. The exact thickness of the disks was measured with a Mitutoyo digital caliper and, by multiplication with the measured disk area, the fresh volume was derived. The disks were oven-dried for 48 h at 103 °C, from which wood density (kg m^{-3}) and moisture content (%) were derived. The remainder of the 2-cm-long specimens were oven-dried, milled and analysed using an oxygen bomb calorimeter (type 6200 Isoperibol Calorimeter, Parr Instrument Company, Moline, IL, USA) to determine their higher heating value (HHV) (MJ kg^{-1}), an indicator of the caloric heating potential of the wood.

2.2.6. Sensitivity to rust

The degree of rust infection (*Melampsora larici-populina*) was assessed in the field for each genotype at one single time during GS1 and GS2. Using a poplar-specific scoring system (Legionnet et al., 1999; Dowkiw and Bastien, 2004), each genotype was given a score for leaf rust infection by observing the grade of coverage by spore uredinia of 15–20 leaves (scores from 0 = 'no uredinium visible' to 8 = 'more than 75% of the leaf surface covered with uredinia'). In a similar approach trees were given an overall score (0–5) of rust infection based on the percentage of infected leaves and their location on the tree as well as the degree of leaf discoloration and/or leaf abscission (Steenackers, 2010).

2.3. Data analysis

Besides the determination of means and ranges, the coefficient of variance (COV) of every parameter was calculated as the ratio of its standard deviation to its mean value, reported as a percentage (%). To identify causal relationships between traits and productivity, bivariate correlations were made with biomass production and among all parameters mutually. The Pearson correlation coefficient – and its level of significance – was used to quantify the correlation. As an exception, the correlations with rust sensitivity scores and wood characteristics (Fig. 1) were assessed with the Kendall's tau rank coefficient since these data were not normally distributed. Furthermore, a hierarchical cluster analysis was performed to see whether genotypes of similar origin or parentage clustered together and to visualize the multivariate effects characterizing biomass production. Cluster analysis is an appropriate tool for evaluating and classifying genotypes according their productivity and related traits (Ares and Gutierrez, 1996; Tharakan et al., 2005; Guo and Zhang, 2010). Variables with correlation coefficients higher than 0.90 were eliminated for cluster analysis. The remaining variables were standardized on a range of −1 to 1; the Euclidean distance was used as measure for similarity; complete linkage (furthest neighbour) was applied as clustering algorithm. All data analyses were performed in SPSS (Version 20, SPSS Inc., Chicago, IL, USA).

3. Results

The minimum and maximum values observed for the 12 genotypes in terms of biomass production, growth traits, the different leaf and wood characteristics, phenological parameters and rust infections in GS1 and GS2 are shown in Table 2. The reported COV's indicated the variation among the genotypic averages; they are relative to the absolute values, though mutually comparable. Most prominently, the lowest COV of <1% was found for the HHV, ranging from 19.33 to 19.60 MJ kg^{-1}, showing that there was hardly any variation in the average HHV among the 12 genotypes. Other wood characteristics did neither vary much among genotypes (COV of only 5–7%). On the other hand, the individual leaf area differed tremendously among the different genotypes (COV of 51%), ranging from small leaves of 79.9 cm^2 for Brandaris to very large leaves of 306.3 cm^2 for Skado. LAI_{max} also showed a high variation among the genotypes, with a slightly higher variation in GS2 (COV of 32–35%) as compared to GS1 (COV of 23–32%). As a result of the very fast growth of all genotypes during the second year after establishment, values of LAI_{max} doubled or tripled from GS1 to GS2. Genotypic means (±standard deviation) of LAI_{max} of both growing seasons are shown in Table 3. LAD showed a somewhat higher variance (COV 31–41%). Minor genotypic and annual differences were expressed in SLA, with a mean value of 12.69 m^2 kg^{-1}. For both tree height and stem diameter, and hence also total biomass, variation among genotypes increased in GS2 as compared to GS1. Biomass production had a COV twice as large (38%) as stem diameter (15%) and tree height (19%). The mean biomass production over both growing seasons ranged from 1.52 Mg ha^{-1} yr^{-1} for Brandaris to 7.22 Mg ha^{-1} yr^{-1} for Hees (Tables 2 and 3). Differences in bud set and bud flush dates in GS2 were limited. Except for the T × M genotypes, which had both the earliest start and the latest end of GS2, all other genotypes had their bud flush as well as their bud set within maximal two weeks separated from each other. Sensitivity to rust also showed a rather high variation (COV's between 23 and 46) among the 12 genotypes (Tables 2 and 3), confirming the importance of this selection criterion in most breeding and selection programmes of poplar.

An overview of the results of the correlation analysis between biomass production and the different leaf characteristics, the growth traits, the phenological parameters and rust sensitivity is

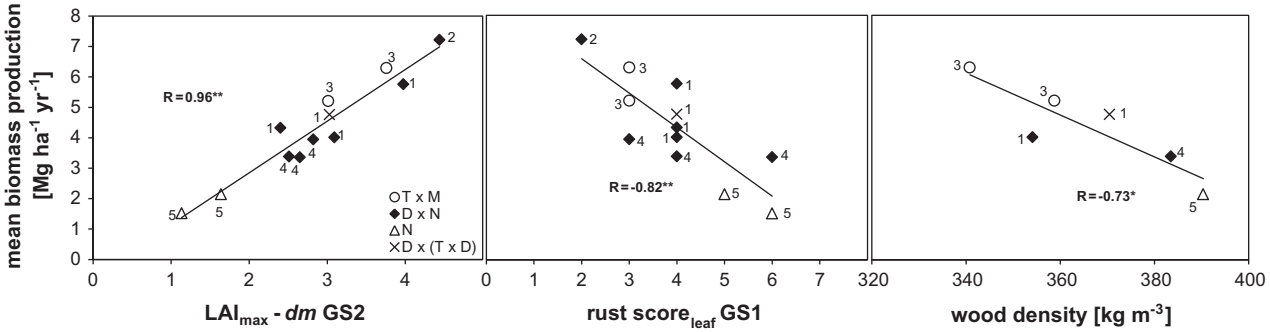

Fig. 1. Relationship of the maximum leaf area index - measured using the direct method (*dm*) - during the second growing season (left panel), the degree of poplar rust infection during the first growing season (middle panel) and wood density, determined after the second growing season (right panel) with the mean above-ground woody biomass production over the first two growing seasons. Each data point represents a genotypic mean. Symbol types indicate parentage (D = *Populus deltoides*, M = *Populus maximowiczii*, N = *Populus nigra*, T = *Populus trichocarpa*), symbol labels indicate cluster number (1–5; see Fig. 3). *R* represents the Pearson correlation coefficient (left and middle panel) or the Kendall's tau correlation coefficient (right panel); ** indicates a level of significance of $p \leqslant 0.01$, * $0.01 < p \leqslant 0.05$.

Table 2
Minimum and maximum values of growth traits, leaf and wood characteristics, phenological parameters and rust sensitivity of the 12 poplar genotypes in a short rotation coppice plantation during its first rotation.

		Minimum	Maximum	COV (%)
Height GS1	cm	199	263	10
Height GS2	cm	290	555	19
Mean height growth	m yr^{-1}	1.45	2.78	19
Diameter GS1	mm	19.1	26.2	10
Diameter GS2	mm	27.6	46.9	15
Mean diameter increment	mm yr^{-1}	13.8	23.5	15
Biomass GS1	Mg ha^{-1}	1.27	3.89	32
Biomass GS2	Mg ha^{-1}	3.04	14.43	38
Mean biomass production	Mg ha^{-1} yr^{-1}	1.52	7.22	38
Bud flush date GS2	DOY	70	108	–
Bud set date GS2	DOY	240	270	–
Length GS2	days	145	200	10
LAI_{max} *-dm* GS1	m^2 m^{-2}	0.67	1.40	23
LAI_{max} *-im* GS1	m^2 m^{-2}	0.58	1.87	32
LAI_{max} *-dm* GS2	m^2 m^{-2}	1.13	4.44	32
LAI_{max} *-im* GS2	m^2 m^{-2}	1.05	4.37	35
LAD GS1	m^2 m^{-2} days	53.5	182.9	31
LAD GS2	m^2 m^{-2} days	146.6	649.8	41
No. of stems per tree	#	1.03	1.55	14
SLA GS1	m^2 kg^{-1}	11.3	14.4	8
SLA GS2	m^2 kg^{-1}	10.8	14.3	9
Mean SLA	m^2 kg^{-1}	11.4	13.8	7
RUE GS1	g MJ^{-1}	0.332	0.532	15
RUE GS2	g MJ^{-1}	0.285	0.676	22
Wood density*	kg m^{-3}	340.8	390.3	5
Wood moisture content*	%	113	139	7
HHV*	MJ kg^{-1}	19.33	19.60	<1
Rust $score_{tree}$ GS1	–	0.5	3	46
Rust $score_{leaf}$ GS1	–	2	6	30
Rust $score_{tree}$ GS2	–	1	4	42
Rust $score_{leaf}$ GS2	–	3	6	23
Lamina nitrogen	mass%	2.78	4.06	12
	g m^{-2}	2.08	3.35	15
Individual leaf area	cm^2	79.7	306.3	51

For wood characteristics, indicated with *, only six genotypes were studied ($n = 6$). Height GS1 (GS2) = shoot height after the first (second) growing season; mean height growth = mean height growth over GS1 and GS2; diameter GS1 (GS2) = stem diameter at 22 cm height after the first (second) growing season; mean diameter increment = mean diameter increment over both seasons; biomass GS1 (GS2) = standing above-ground woody dry mass after the first (second) growing season; mean biomass production = mean biomass production over both seasons; LAI_{max} = maximum leaf area index of the growing season (measured with a direct method (*dm*) and with an indirect method (*im*)); LAD = leaf area duration; SLA = specific leaf area; RUE = radiation use efficiency; HHV = higher heating value; rust $score_{tree/leaf}$ = level of rust infection scored at tree/leaf level; lamina nitrogen = nitrogen concentration of leaves; DOY = day of year. The coefficient of variance (COV) is the ratio of the standard deviation among genotypic values to the mean value, reported as a percentage (%).

given in Table 4. The mean biomass production was strongly positively correlated with stem diameter and height growth, with LAI_{max} (see also Fig. 1) and LAD, and also with SLA. Negative correlations were found with the degree of rust infection (Fig. 1). Similar correlations as for biomass production were found for diameter growth. Height growth on the other hand, was neither correlated with LAI nor with LAD, and only weakly with rust infection. Tree height was significantly correlated with the individual leaf area as well as with the timing of bud set in GS2. Phenological dates were poorly related to other parameters. The few significant correlations with phenological dates showed that the later bud set, the higher the biomass production and the RUE; this was also explained by the lower rust infection. LAI_{max} and LAD of GS2 were negatively correlated with the rust infection during GS1. In GS1, the number of stems grown from a cutting was inversely proportional to the height reached after the first (establishment) year and also to the individual leaf area. The individual leaf area on its turn was negatively correlated with the nitrogen concentration in the leaf (Fig. 2). With regard to the wood characteristics, few correlations with other traits were observed. Overall, the wood density was negatively correlated to biomass production (Fig. 1). No correlation with wood moisture content was found, neither with the HHV of the wood which was very uniform among the six examined genotypes (Table 2). Only the moisture content was obviously, though not significantly, negatively correlated to wood density.

Following the elimination of highly correlated (Pearson correlation coefficient $\geqslant 0.90$) variables, a cluster analysis was performed on the remaining variables listed here: mean height growth, stem diameter in GS2, mean biomass production, bud flush and bud set dates in GS2, length of GS2, LAI_{max} in GS1 and GS2 (indirect method), LAD in GS1 and GS2, mean number of stems per tree, mean SLA, RUE in GS1 and GS2, individual leaf area and leaf N concentration. The resulting clustering dendrogram is shown in Fig. 3. Since there is no indisputable method for determining the number of clusters in a cluster analysis (Everitt, 1979), the number of clusters was determined by parsing this classification tree according to the rescaled cluster distance (Fig. 3). Starting from the right along the *x*-axis, two groups were distinctly differentiated from each other, namely on the one hand the group of all genotypes bred by and obtained from the Belgian Institute for Nature and Forestry Research (INBO) plus Hees, and on the other hand the group of the four other genotypes selected by "De Dorschkamp" Research Institute for Forestry and Landscape Planning in the Netherlands and Robusta (Table 1). Restricting the clustering to these two groups did not provide useful information apart from the origin of the poplar planting materials. When moving further to the left on the *x*-axis,

Table 3
Mean genotypic values (±standard deviation) of selected biomass production characteristics, of growing season length and of rust sensitivity.

Genotype	Biomass GS1		Biomass GS2		Length GS2		LAI_{max} *-im* GS1		LAI_{max} *-im* GS2		RUE GS2		Rust $score_{leaf}$ GS1	Rust $score_{leaf}$ GS2
	Mg ha^{-1}		Mg ha^{-1}		days		m^2 m^{-2}		m^2 m^{-2}		g MJ^{-1}			
Bakan	3.21	(1.81)	10.41	(5.28)	190	(3)	0.97	(0.37)	1.91	(0.56)	0.54	(0.05)	3	3
Skado	3.58	(1.78)	12.58	(5.63)	200	(4)	1.47	(0.13)	3.34	(0.79)	0.50	(0.06)	3	3
Muur	2.54	(1.30)	8.65	(4.24)	171	(3)	1.02	(0.15)	2.40	(0.50)	0.45	(0.10)	4	5
Oudenberg	2.56	(1.22)	8.03	(3.47)	163	(4)	0.98	(0.13)	3.09	(0.40)	0.50	(0.01)	4	5
Vesten	2.60	(1.31)	11.52	(5.34)	159	(2)	1.32	(0.18)	3.98	(0.72)	0.68	(0.05)	4	5
Ellert	2.03	(1.37)	7.89	(4.83)	154	(5)	1.30	(0.12)	2.82	(0.40)	0.40	(0.12)	3	5
Hees	3.89	(1.93)	14.43	(7.23)	167	(1)	1.87	(0.44)	4.44	(0.44)	0.58	(0.03)	2	3
Koster	1.79	(1.24)	6.76	(4.60)	146	(8)	1.10	(0.18)	2.51	(0.78)	0.39	(0.13)	4	4
Robusta	2.85	(1.56)	6.72	(4.24)	149	(8)	1.69	(0.59)	2.65	(0.79)	0.49	(0.19)	6	5
Grimminge	2.80	(1.51)	9.52	(4.70)	145	(5)	1.08	(0.55)	2.77	(0.71)	0.52	(0.06)	4	4
Brandaris	1.34	(0.86)	3.04	(1.73)	153	(7)	0.58	(0.12)	1.05	(0.14)	0.29	(0.10)	6	5
Wolterson	1.27	(0.78)	4.29	(2.31)	164	(2)	0.74	(0.27)	1.57	(0.55)	0.63	(0.08)	5	6

GS1 (GS2) = first (second) growing season; biomass = standing above-ground woody dry mass at the end of the growing season; LAI_{max} = maximum leaf area index of the growing season (measured with the indirect method (*im*)); RUE = radiation use efficiency; rust $score_{leaf}$ = level of rust infection scored at leaf level.

Table 4
Correlations between growth traits, leaf characteristics, phenological parameters and rust sensitivity of 12 poplar genotypes grown in a short rotation coppice plantation during its first rotation ($n = 12$). Significance levels of Pearson correlation: ns = not significant ($p > 0.05$); + = $0.01 < p \leqslant 0.05$; ++ = $p \leqslant 0.01$ for positive correlations, equally with – sign for negative correlations. For rust sensitivity parameters, symbols indicate significance of the non-parametric Kendall's tau correlation fit. Height GS1 (GS2) = stem height after the first (second) growing season; mean height growth = mean height growth over GS1 and GS2; diameter GS1 (GS2) = shoot diameter at 22 cm height after the first (second) growing season; mean diameter increment = mean diameter increment over both seasons; biomass GS1 (GS2) = standing above-ground woody dry mass after the first (second) growing season; mean biomass production = mean biomass production over both seasons; LAI_{max} = maximal leaf area index of the growing season (measured with a direct method (*dm*) and indirect method (*im*)); LAD = leaf area duration; SLA = specific leaf area; RUE = radiation use efficiency; rust $score_{tree/leaf}$ = level of rust infection scored at tree/leaf level; lamina nitrogen = leaf nitrogen concentration.

	Height GS2	Mean height growth	Diameter GS1	Diameter GS2	Mean diameter increment	Biomass GS1	Biomass GS2	Mean biomass production	Bud flush date GS2	Bud set date GS2	Length GS2	LAI_{max} -*dm* GS1	LAI_{max} -*im* GS1	LAI_{max} –*dm* GS2	LAI_{max} –*im* GS2	LAD GS1	LAD GS2	No. of stems per tree	SLA GS1	SLA GS2	Mean SLA	RUE GS1	RUE GS2	Rust $score_{tree}$ GS1	Rust $score_{leaf}$ GS1	Rust $score_{tree}$ GS2	Rust $score_{leaf}$ GS2	Lamina nitrogen	Individual leaf area
Height GS1	++	++	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	−−	++	ns	ns	++	ns	ns	ns	ns	ns	ns	++
Height GS2		++	+	++	++	+	++	++	ns	++	+	ns	ns	+	ns	ns	ns	ns	++	ns	++	ns	ns	–	–	ns	ns	ns	++
Mean height growth			+	++	++	+	++	++	ns	++	+	ns	ns	ns	ns	ns	ns	ns	++	ns	++	ns	ns	–	–	ns	ns	ns	++
Diameter GS1				++	++	++	++	++	ns	ns	ns	++	ns	++	+	+	+	ns	++	ns	++	ns	+	ns	ns	ns	ns	ns	ns
Diameter GS2					++	++	++	++	ns	ns	ns	ns	ns	++	++	+	++	ns	++	ns	++	ns	+	–	−−	ns	–	ns	ns
Mean diameter increment						++	++	++	ns	ns	ns	ns	ns	++	++	+	++	ns	++	ns	++	ns	+	–	−−	ns	–	ns	ns
Biomass GS1							++	++	ns	ns	ns	++	++	++	+	++	++	ns	+	ns	+	ns	ns	ns	–	ns	−−	–	+
Biomass GS2								++	ns	+	ns	++	+	++	++	++	++	ns	+	ns	++	ns	ns	−−	−−	ns	–	ns	ns
Mean biomass production									ns	+	ns	+	+	++	++	++	++	ns	+	ns	++	ns	ns	−−	−−	ns	–	ns	ns
Bud flush date GS2										ns	+	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Bud set date GS2											++	ns	ns	ns	ns	ns	ns	ns	ns	ns	+	+	+	–	ns	–	ns	ns	ns
Length GS2												ns	ns	ns	ns	ns	ns	ns	ns	+	+	ns	ns	ns	ns	–	ns	ns	ns
LAI_{max} -*dm* GS1													++	++	ns	++	ns	ns	ns	ns	ns	ns	+	ns	ns	ns	ns	ns	ns
LAI_{max} -*im* GS1														++	++	++	++	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
LAI_{max} -*dm* GS2															++	++	++	ns	+	ns	++	ns	ns	–	–	ns	ns	ns	ns
LAI_{max} -*im* GS2																++	++	ns	ns	ns	+	ns	ns	–	−−	ns	ns	ns	ns
LAD GS1																	++	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
LAD GS2																		ns	ns	ns	+	ns	ns	−−	−−	ns	–	ns	ns
No. of stems per tree																			–	ns	ns	ns	ns	ns	ns	ns	ns	ns	−−
SLA GS1																				ns	++	ns	ns	ns	ns	ns	ns	ns	++
SLA GS2																					++	ns	ns	−−	−−	–	ns	ns	ns
Mean SLA																						ns	ns	–	–	ns	ns	ns	ns
RUE GS1																							ns	ns	ns	ns	ns	ns	ns
RUE GS2																								ns	ns	ns	ns	ns	ns
Rust $score_{tree}$ GS1																									++	+	+	ns	ns
Rust $score_{leaf}$ GS1																										+	+	ns	ns
Rust $score_{tree}$ GS2																											ns	ns	ns
Rust $score_{leaf}$ GS2																												++	ns
Lamina nitrogen																													−−

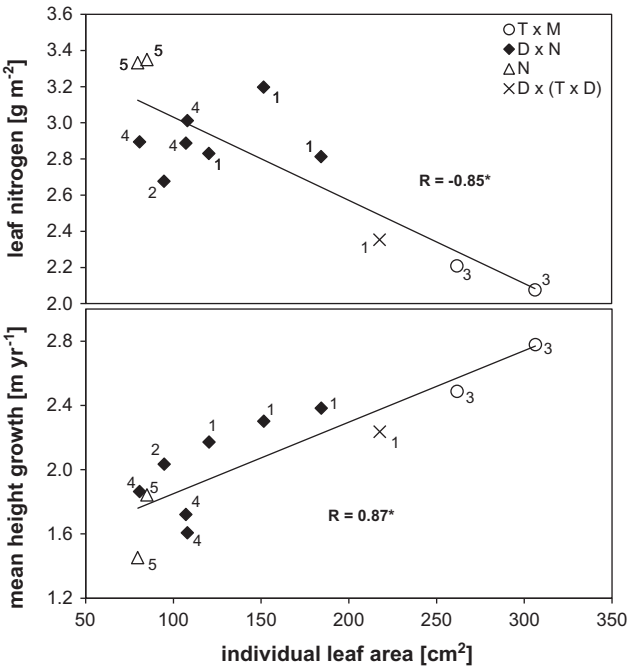

Fig. 2. Relationship of individual leaf area with leaf nitrogen content on a leaf area basis (upper panel), and with mean tree height growth (lower panel). Each data point represents a genotypic mean. Symbol types indicate parentage (D = *Populus deltoides*, M = *Populus maximowiczii*, N = *Populus nigra*, T = *Populus trichocarpa*), symbol labels indicate cluster number (1–5; see Fig. 3). *R* represents the Pearson correlation coefficient; ** indicates a level of significance of $p \leqslant 0.01$.

the T × M genotypes first dissociated from the first group followed by Hees. At the following larger gap in the distance coefficients, five clusters were identified (Fig. 3). The mean values of the different parameters used in the analysis and the variance within clusters are reported in Table 5.

The five clusters were clearly related to the genetic background of the genotypic plant materials, as well as to their biomass production. Cluster 1 consisted of three D × N genotypes and the only D × (T × D) genotype, all four genotypes bred by INBO. The four genotypes of this cluster were characterized by intermediate, but similar production characteristics within the cluster, indicated by the low COV for e.g. LAI (11%) and height growth (4%) (Table 5). There was high variation in the individual leaf area (COV of 25%) in this cluster (see also Fig. 2). Cluster 4 contained three D × N genotypes, i.e. Ellert and Koster, both selected by "De Dorschkamp" and Robusta. Genotypes of this cluster all showed a biomass production performance in the lower range and had a higher tendency to grow in multiple stems from the planted cuttings in comparison with genotypes from cluster 1, 3 (and 5). Genotype Hees (D × N) was separated from all other genotypes in a singleton cluster. Hees had strong similarities in external morphology (e.g. crown structure, pyramidal tree shape; Broeckx et al. (2012b) and personal observations) with genotypes of cluster 4, but Hees was characterized by a deviating high biomass production. Hees had a mean above-ground woody biomass productivity of 7.22 Mg ha^{-1} yr^{-1} and in GS2 its LAI_{max} reached a value of 4.37. Genotypes Bakan and Skado, forming cluster 3, were distinguished by their single and high stems, long growing period, high woody biomass production and typically large leaves. In contrast, Brandaris and Wolterson (cluster 5) were the least productive of all studied genotypes. So, the different traits and characteristics studied allowed to discriminate genotypes based on their genetic background and on their biomass production.

4. Discussion

Obviously, genotypes of the same parentage clustered together based on the traits and parameters examined, except for the

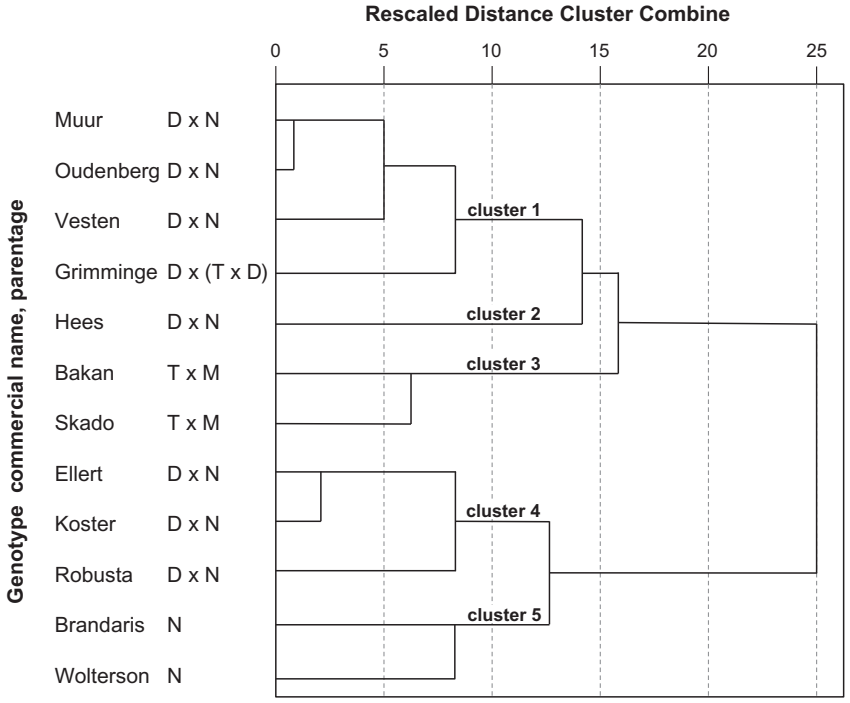

Fig. 3. Dendrogram of the hierarchical cluster analysis conducted on 16 tree characteristics measured on 12 *Populus* genotypes (shown on the *y*-axis). The five restrained clusters are indicated on the dendrogram branches. The following characteristics were included in the analysis: mean height growth, stem diameter in GS2, mean biomass production, bud flush and bud set dates in GS2, length of GS2, LAI_{max} in GS1 and GS2 (indirect method), LAD in GS1 and GS2, mean number of stems per tree, mean SLA, RUE in GS1 and GS2, individual leaf area and leaf N concentration. GS = growing season.

Table 5
Mean cluster values of growth traits, leaf characteristics, phenological parameters and rust sensitivity. Mean height growth = mean height growth over the first (GS1) and second growing season (GS2); diameter GS2 = stem diameter at 22 cm height after the two growing seasons; mean biomass production = mean above-ground woody biomass production over both seasons; LAI_{max} = maximal leaf area index of the growing season measured with the indirect method (*im*); LAD = leaf area duration; SLA = specific leaf area; RUE = radiation use efficiency; lamina nitrogen = nitrogen concentration of leaves. The coefficient of variance (COV) is the ratio of the standard deviation among genotypic values in the cluster to the mean cluster value, reported as a percentage (%).

		CLUSTER 1 {Muur, Oudenberg, Vesten, Grimminge}		CLUSTER 2 {Hees}		CLUSTER 3 {Bakan, Skado}		CLUSTER 4 {Ellert, Koster, Robusta}		CLUSTER 5 {Brandaris, Wolterson}	
		Mean	COV (%)	Mean	COV (%)	Mean	COV (%)	Mean	COV (%)	Mean	COV (%)
Mean height growth	$m\ yr^{-1}$	2.27	4	2.03	–	2.63	8	1.73	7	1.65	17
Diameter GS2	mm	41.9	8	46.9	–	42.6	8	36.3	8	30.8	15
Mean biomass production	$Mg\ ha^{-1}\ yr^{-1}$	4.72	16	7.22	–	5.75	13	3.56	9	1.83	24
Bud flush date GS2	DOY	99	–	96	–	75	–	97	–	97	–
Bud set date GS2	DOY	258	–	263	–	270	–	247	–	255	–
Length GS2	days	159	7	167	–	195	4	150	3	158	5
LAI_{max} -*im* GS1	$m^2\ m^{-2}$	1.105	14	1.87	–	1.22	29	1.36	22	0.66	16
LAI_{max} -*im* GS2	$m^2\ m^{-2}$	2.62	11	4.37	–	2.63	38	2.43	22	1.31	28
LAD GS1	$m^2\ m^{-2}$ days	105.6	11	182.9	–	125.2	29	119.7	16	63.3	22
LAD GS2	$m^2\ m^{-2}$ days	356.4	13	649.8	–	429.9	43	336.1	28	164.9	16
No. of stems per tree	#	1.14	9	1.50	–	1.04	1	1.35	13	1.32	9
Mean SLA	$m^2\ kg^{-1}$	13.0	5	13.4	–	13.5	1	12.1	6	11.8	4
RUE GS1	$g\ MJ^{-1}$	0.487	6	0.469	–	0.502	8	0.366	15	0.501	9
RUE GS2	$g\ MJ^{-1}$	0.538	18	0.584	–	0.520	5	0.426	13	0.457	53
Individual leaf area	cm^2	168.5	25	94.8	–	284.1	11	98.7	16	82.4	5
Lamina nitrogen	mass%	3.65	14	3.57	–	2.89	5	3.54	4	3.93	5

D × (T × D) genotype Grimminge which was grouped with three other D × N genotypes. Both the N and the T × M genotypes formed a cluster each, whereas the D × N genotypes were distributed over three clusters. Apparently the D × N genotypes were clustered largely according their origin of selection and production. Cluster 1 contained the D × N genotypes bred by INBO, whereas the D × N genotypes bred by "De Dorschkamp" Research Institute for Forestry and Landscape Planning (The Netherlands) were grouped in cluster (2 and) 4, also including Robusta. This clustering pattern can be explained by the fact that hybrid genotypes bred by a particular producer/breeder were selected according to specific criteria and, by the fact that they frequently have the same or genetically highly related parents. For example, Muur, Oudenberg and Vesten have the same *P. deltoides* genotype as their maternal parent (Table 1); the paternal parents of Oudenberg and Vesten are full-sibs and the parents of the paternal parent of Muur have the same origin as the parents of Vesten's and Oudenberg's paternal parent (Van Slycken et al., 2005). The parental trees of Ellert, Hees, Koster have the same origin, obviously different from Robusta which is a much older genotype from French origin with an unknown paternal tree (Centrum voor Genetische Bronnen). The most prominent results were shown by Hees, which was expected to cluster with Koster and Ellert; however, due to its particularly high productivity Hees formed a cluster on its own. On the contrary, Brandaris and Wolterson jointly forming cluster 5 were the least productive genotypes. These are both *P. nigra* genotypes and also the only pure native European species of the study. The biomass production of all hydrids involving *P. nigra* as a parent was higher than this of the parent species itself, suggesting a positive heterosis (i.e. hybrid vigour; Stettler and Ceulemans, 1993). Although no pure *P. deltoides* genotype was incorporated in our study, D × N hybrids outperforming their parents was frequently shown before (Stettler et al., 1996; Marron and Ceulemans, 2006). Leaves of *P. deltoides* are larger in size and smaller in quantity compared to *P. nigra,* typically having small leaves (Fig. 2; Ridge et al., 1986; Ceulemans, 1990; Marron and Ceulemans, 2006). Hybrids of D × N combine both strategies, resulting in a larger total leaf area and associated biomass production than both parental species (Orlović et al., 1998; Marron and Ceulemans, 2006). Both individual leaf size and LAI_{max} were lowest for the *P. nigra* species in comparison with the D × N hybrids in the present study (Table 5; Figs. 1 and 2). On the other hand the T × M genotypes Bakan and in particular Skado were among the highest productive genotypes (Table 5). Their early bud flush was the most distinctive trait of these two T × M genotypes, which could be attributable to the southern (Japanese) origin of their parents (Table 1; Michiels et al., 2008). Together with their late bud set date, the long growing period was one of the factors contributing to their high growth performance. However the positive correlation of mean biomass vs. growing season length was not significant ($p = 0.099$; Table 4), genotypes Skado and Bakan (cluster 3) had the longest growing season and showed the highest biomass production after Hees (Table 5). The strong correlation of LAD with biomass furthermore confirms these results. Whereas LAI_{max} of Bakan and Skado had the same magnitude compared to clusters 1 and 4, their LAD was much higher, indicating the higher importance of the growing season length.

In GS2 frequent events of windsnap of the upper and poorly lignified part of the main stem were observed for both T × M genotypes (personal observations). Due to their tall height and large, heavy leaves (Fig. 2), they experienced a higher wind pressure. Moreover, the higher in the canopy, the larger their individual leaf area (unpublished results). Since the T × M genotypes had the highest slenderness (ratio of stem height to diameter) among the studied genotypes, in combination with their high above-ground biomass (Table 5), they were more susceptible to windsnap (Harrington and DeBell, 1996). In contrast, the N and D × N genotypes, and in particular the hybrids of clusters 2, 4 and 5 were generally shorter and had smaller leaves (Fig. 2). Moreover, due the higher branchiness of these D × N genotypes (Broeckx et al., 2012b), they experienced higher mutual support, decreasing the risk to sway in the wind (Harrington and DeBell, 1996). During the breeding and selection procedure, the Dutch genotypes (from "De Dorschkamp" Research Institute for Forestry and Landscape Planning in Wageningen) were specifically screened for wind tolerance (de Vries, 2008), a crucial characteristic for the low lands of The Netherlands. In the Flemish poplar breeding programme wind tolerance as such was not taken up as one of the primary selection criteria (Steenackers et al., 1990; De Cuyper, 2008).

One of the main criteria in both breeding and selection programmes in Flanders and The Netherlands, is disease (including leaf rust) resistance. Resistance to leaf rust in *Populus* has been shown to be under strong genetic control (Rajora et al., 1994; Dunlap and Stettler, 1998). Since leaf rust resistance is often strongly correlated at different tree ages, early selection for this trait appears feasible (Rajora et al., 1994). The results of this study confirmed that biomass production decreased with increasing rust infection (Fig. 1) in line with previous reports (Royle and Ostry, 1996; Steenackers et al., 1996; Dunlap and Stettler, 1998). The infection of rust was more severe and started earlier in the year in GS1 than in GS2, and had therefore a larger impact on biomass growth. In GS1 the rust infection on Robusta caused a sudden decrease in LAI, a black coloration of leaves and leaf fall after week 35 (Broeckx et al., 2012a). As expected, genotype Robusta was most susceptible to rust among all the genotypes. Robusta is the oldest of the genotypes (Table 1), and is known for poor rust resistance (Centrum voor Genetische Bronnen; Steenackers et al., 1990) and slow growth (Barigah et al., 1994; Ceulemans et al., 1996; Meiresonne, 2006). *P. deltoides* species are frequently used for (back)crossing to breed rust-resistant genotypes (Steenackers et al., 1990; Steenackers, 2010). Genotype Grimminge, which is a back-cross of (*P. trichocarpa* × *P. deltoides*) with a *P. deltoides* maternal parent, showed an intermediate rust infection of all genotypes of this study. Besides Robusta, also the *P. nigra* genotypes showed a rust score in the higher range (Fig. 1; Table 3); this probably also explained their low productivity. Nowadays, breeding and selection strategies in Flanders aim at partial resistance and tolerance to rust rather than complete resistance due to newly arising, more virulent pathotypes that caused the breakdown of rust resistance between 1980 and 2000 (De Cuyper, 2008).

Concerning the wood characteristics, only poor genotypic variation was observed (Table 2). Similar to previous findings (Benetka et al., 2002), very small differences (COV <1%) in the HHV between the six genotypes were found, with a mean value of 19.45 MJ kg^{-1}. This is well within the range reported in a review study on biomass quality of poplar which also concluded the small variation in HHV that exists among poplar species (Kenney et al., 1990). Therefore, selection for this trait presumably only brings about little genetic improvement. Wood quality has the lowest priority among the selection criteria for breeding and selection programs for poplar cultivars in Flanders, in particular with regard to SRC cultivation (Steenackers et al., 1990). Despite poor variation in wood density as well, a significant negative correlation with biomass production was found (Fig. 1). The lower yielding genotypes (e.g. Brandaris, Wolterson, Koster) had a shorter growing season, grew slower and less vigorously, and produced a denser structured wood. Nevertheless, due to their lower growth the analysed wood disks had a smaller diameter and an associated larger proportion of bark as compared to the more productive genotypes, which could have influenced the relationship of wood density and biomass production. A negative correlation between growth rate and wood density in *Populus* spp. was shown in a number of studies (Beaudoin et al., 1992; Pliura et al., 2007; Zhang et al., 2012), although no relationship was reported in other studies (Farmer, 1970; DeBell et al., 2002; Zhang et al., 2003) since growth rate usually has little or no influence on wood density in diffuse-porous hardwoods (Barnett and Jeronimidis, 2003). Despite a high genetic control of wood density in poplar (Kenney et al., 1990), minor importance was attributed to this trait due to the low variation and its poor effect on biomass yield. A similar reasoning held true for wood moisture content, which also showed little variation among genotypes (COV of only 7%). Also little variation of these wood characteristics within the studied genotypes was observed (slightly higher than the variation among the genotypic averages; data not shown), which is likewise important regarding the conversion efficiency to bioenergy. Nevertheless, despite the uniformity of wood characteristics observed in this study and hence their assumed minor importance in breeding and selection for bioenergy purposes the selection for high calorific values, high wood densities and low moisture contents remains overall important.

The negative correlation of individual leaf area with leaf nitrogen content (Fig. 2) indicated that in the larger-leaved trees leaf nitrogen was diluted over the larger leaf area as compared to the high nitrogen concentration in the leaves of the smaller-leaved genotypes. This dilution hence meant an optimization of nitrogen use since the larger leaves allow more light interception. In large leaves, a lower photosynthesis per unit of leaf area is often compensated by photosynthesis of a larger leaf area (Tharakan et al., 2005; Marron et al., 2007). Preliminary, unpublished results indeed showed a positive correlation of photosynthetic capacity with leaf nitrogen content (Beernaert, 2012). Nevertheless, the relative differences in individual leaf area among genotypes (Fig. 2) were much larger than the relative variation in photosynthesis, suggesting that leaf area is the most influencing factor in total photosynthesis. This was partly evidenced by the positive correlation between individual leaf area and biomass production in GS1 (Table 4), which was previously demonstrated for several poplar genotypes (Ridge et al., 1986; Barigah et al., 1994; Harrington et al., 1997). This correlation between individual leaf area and biomass production was also valid in GS2, and for the pooled data of GS1 and GS2, although less significant ($p = 0.060$). When ignoring Hees – i.e. the isolated genotype in cluster 2 – the aforementioned correlation was perfectly true ($p = 0.001$). Furthermore, individual leaf area showed a strong positive correlation with tree height growth (Fig. 2), which on its turn was positively correlated with biomass production. As is also evidenced from the present study, LAI rather than individual leaf area is a logic and reliable indicator of biomass production (Ceulemans, 1990; Barigah et al., 1994; Orlović et al., 1998; Tharakan et al., 2005). LAI_{max} showed a strong positive correlation with biomass productivity, both in GS1 and GS2 (Fig. 1, Table 4). Furthermore LAD, incorporating the evolution of LAI over the growing season, was also strongly correlated to biomass production (Table 4). Leaf area development was reported earlier as a reliable trait for the early selection of high productivity in poplars (Ceulemans et al. 1994; Bunn et al. 2004). In contrast to the expectations, RUE was not correlated to the biomass production related traits (except for the positive correlation between RUE and height growth in GS1). The genotypic mean of RUE in GS2 was 0.50 g MJ^{-1}, which is low compared to the range of 1-2 g MJ^{-1} frequently reported for poplar (Cannell et al., 1988; Landsberg and Wright, 1989; Green et al., 2001), but much higher than the 0.002–0.041 g MJ^{-1} reported for a comparable poplar SRC culture in Belgium (Deraedt and Ceulemans 1998).

5. Conclusions

The clustering of the poplar genotypes studied here was clearly determined by parentage and genetic origin. Distinct differences between clusters were expressed in the biomass related characteristics; genotypes of similar parentage and origin showed comparable characteristics. *P. nigra* genotypes were the least performing among the studied genotypes. The most recently commercialized *P. trichocarpa* × *P. maximowiczii* hybrids on the other hand, were among the most productive genotypes. As HHV values were rather similar among genotypes, biomass production appeared the more important trait with regard to bioenergy production; this has important implications with regard to SRC cultures aimed to maximize biomass production for maximum bioenergy yield. Besides the direct (diameter) measurements of woody biomass growth, LAI is one of the most important early selection criteria for poplar

with bioenergy purposes. The negative correlation of biomass and leaf rust infection reconfirmed the importance of disease vulnerability in breeding and selection programs.

Acknowledgements

This research has received funding from the European Research Council under the European Commission's Seventh Framework Programme (FP7/2007-2013) as ERC Grant Agreement No. 233366 (POPFULL), as well as from the Flemish Hercules Foundation as Infrastructure contract ZW09-06. Further funding was provided by the Flemish Methusalem Programme and by the Research Council of the University of Antwerp. We gratefully acknowledge the excellent technical assistance of Joris Cools, the logistic support of Kristof Mouton at the field site as well as the laboratory analyses of Gerrit Switsers and Nadine Calluy.

References

Ares, A., Gutierrez, L., 1996. Selection of poplar clones for the Lower Valley of the Colorado River, Argentina. Forestry 69, 75–82.
Barigah, T.S., Saugier, B., Mousseau, M., Guittet, J., Ceulemans, R., 1994. Photosynthesis, leaf area and productivity of 5 poplar clones during their establishment year. Ann. For. Sci. 51, 613–625.
Barnett, J.R., Jeronimidis, G., 2003. Wood quality and its biological basis. Blackwell Publishing, Oxford, UK.
Beaudoin, M., Hernandez, R.E., Koubaa, A., Poliquin, J., 1992. Interclonal, intraclonal, and within-tree variation in wood density of poplar hybrid clones. Wood Fiber Sci. 24, 147–153.
Beernaert, E., 2012. Inter-specific and seasonal variation in photosynthetic rate of different poplar clones. Master Thesis Biology, University of Antwerp (in Dutch).
Benetka, V., Bartáková, I., Mottl, J., 2002. Productivity of *Populus nigra* L. ssp. *nigra* under short-rotation culture in marginal areas. Biomass Bioenerg. 23, 327–336.
Berhongaray, G., El Kasmioui, O., Ceulemans, R., 2013. Comparative analysis of harvesting machines on an operational high-density short rotation woody crop (SRWC) culture: one-process versus two-process harvest operation. Biomass Bioenerg. (http://dx.doi.org/10.1016/j.biombioe.2013.07.003).
Broeckx, L.S., Verlinden, M.S., Ceulemans, R., 2012a. Establishment and two-year growth of a bio-energy plantation with fast-growing *Populus* trees in Flanders (Belgium): effects of genotype and former land use. Biomass Bioenerg. 42, 151–163.
Broeckx, L.S., Verlinden, M.S., Vangronsveld, J., Ceulemans, R., 2012b. Importance of crown architecture for leaf area index of different *Populus* genotypes in a high-density plantation. Tree Physiol. 32, 1214–1226.
Bunn, S.M., Rae, A.M., Herbert, C.S., Taylor, G., 2004. Leaf-level productivity traits in *Populus* grown in short rotation coppice for biomass energy. Forestry 77, 307–323.
Cannell, M.G.R., Sheppard, L.J., Milne, R., 1988. Light use efficiency and woody biomass production of poplar and willow. Forestry 61, 125–136.
Centrum voor Genetische Bronnen, Nederland. Website Rassenlijst bomen. URL: <http://www.wageningenur.nl/rassenlijstbomen.htm>. (accessed 12.02.13) (in Dutch).
Ceulemans, R., 1990. Genetic variation in functional and structural productivity determinants in poplar. Thesis Publishers, Amsterdam.
Ceulemans, R., Pontailler, J.Y., Mau, F., Guittet, J., 1993. Leaf allometry in young poplar stands: reliability of leaf area index estimation, site and clone effects. Biomass Bioenerg. 4, 315–321.
Ceulemans, R., Perez-Leroux, A., Shao, B.Y., 1994. Physiology growth and development of young poplar plants under elevated atmospheric CO_2 levels. In: Veroustraete, F., Ceulemans, R. (Eds.), Vegetation Modelling and Climate Change Effects. SPB Academic Publishing, The Hague, The Netherlands, pp. 81–98.
Ceulemans, R., Shao, B.Y., Jiang, X.N., Kalina, J., 1996. First- and second-year aboveground growth and productivity of two *Populus* hybrids grown at ambient and elevated CO_2. Tree Physiol. 16, 61–68.
De Cuyper, B., 2008. Poplar breeding programme in Flanders, Belgium. TREEBREEDEX, INRA-Orléans, 18–19 September, 2008 (abstract URL: <http://treebreedex.eu/IMG/pdf/Summaries-2.pdf>).
de Vries, S.M.G., 2008. Breeding and selection of poplar in Netherlands. TREEBREEDEX, INRA-Orléans, 18–19 September, 2008 (abstract URL: <http://treebreedex.eu/IMG/pdf/Summaries-2.pdf>).
DeBell, D.S., Singleton, R., Harrington, C.A., Gartner, B.L., 2002. Wood density and fiber length in young *Populus* stems: relation to clone, age, growth rate, and pruning. Wood Fiber Sci. 34, 529–539.
Deraedt, W., Ceulemans, R., 1998. Clonal variability in biomass production and conversion efficiency of poplar during the establishment year of a short rotation coppice plantation. Biomass Bioenerg. 15, 391–398.
Dowkiw, A., Bastien, C., 2004. Characterization of two major genetic factors controlling quantitative resistance to *Melampsora larici-populina* leaf rust in hybrid poplars: strain specificity, field expression, combined effects, and relationship with a defeated qualitative resistance gene. Phytopathology 94, 1358–1367.
Dunlap, J.M., Stettler, R.F., 1998. Genetic variation and productivity of *Populus trichocarpa* and its hybrids. X. Trait correlations in young black cottonwood from four river valleys in Washington. Trees 13, 28–39.
Everitt, B.S., 1979. Unresolved problems in cluster-analysis. Biometrics 35, 169–181.
Farmer Jr., R.E., 1970. Variation and inheritance of Eastern Cottonwood growth and wood properties under two soil-moisture regimes. Silvae Genet. 19, 5–8.
Green, D.S., Kruger, E.L., Stanosz, G.R., Isebrands, J.G., 2001. Light-use efficiency of native and hybrid poplar genotypes at high levels of intracanopy competition. Can. J. Forest Res. 31, 1030–1037.
Guo, X.Y., Zhang, X.S., 2010. Performance of 14 hybrid poplar clones grown in Beijing, China. Biomass Bioenerg. 34, 906–911.
Harrington, C.A., DeBell, D.S., 1996. Above- and below-ground characteristics associated with wind toppling in a young *Populus* plantation. Trees 11, 109–118.
Harrington, C.A., Radwan, M.A., DeBell, D.S., 1997. Leaf characteristics reflect growth rates in 2-year-old *Populus* trees. In: DeBell, D.S., Harrington, C.A., Clendenen, G.W., Radwan, M.A., Zasada, J.C. (Eds.), Increasing the productivity of short-rotation *Populus* plantations. USDA Forest Service, Washington, pp. 64–68.
Kenney, W.A., Sennerby-Forsse, L., Layton, P., 1990. A review of biomass quality research relevant to the use of poplar and willow for energy conversion. Biomass 21, 163–188.
Kuiper, L., 2003. Summary of the results of six years of research on energy crops. Centrum voor biomassa innovatie, Wageningen (in Dutch).
Landsberg, J.J., Wright, L.L., 1989. Comparisons among *Populus* clones and intensive culture conditions, using an energy-conservation model. Forest Ecol. Manage. 27, 129–147.
Larcher, W., 2003. Physiological plant ecology: ecophysiology and stress physiology of functional groups. Springer-Verlag, Berlin.
Laureysens, I., Bogaert, J., Blust, R., Ceulemans, R., 2004. Biomass production of 17 poplar clones in a short-rotation coppice culture on a waste disposal site and its relation to soil characteristics. Forest Ecol. Manage. 187, 295–309.
Legionnet, A., Muranty, H., Lefèvre, F., 1999. Genetic variation of the riparian pioneer tree species *Populus nigra*. II. Variation in susceptibility to the foliar rust *Melampsora larici-populina*. Heredity 82, 318–327.
Liberloo, M., Calfapietra, C., Lukac, M., Godbold, D., Luo, Z.B., Polle, A., Hoosbeek, M.R., Kull, O., Marek, M., Raines, C., Rubino, M., Scarascia-Mugnozza, G., Ceulemans, R., 2006. Woody biomass production during the second rotation of a bio-energy *Populus* plantation increases in a future high CO_2 world. Glob. Change Biol. 12, 1094–1106.
Marron, N., Ceulemans, R., 2006. Genetic variation of leaf traits related to productivity in a *Populus deltoides* × *Populus nigra* family. Can. J. Forest Res. 36, 390–400.
Marron, N., Dillen, S.Y., Ceulemans, R., 2007. Evaluation of leaf traits for indirect selection of high yielding poplar hybrids. Environ. Exp. Bot. 61, 103–116.
McCracken, A.R., Dawson, W.M., Bowden, G., 2001. Yield responses of willow (*Salix*) grown in mixtures in short rotation coppice (SRC). Biomass Bioenerg. 21, 311–319.
Meiresonne, L., 2006. Opportunities, possibilities and future for poplar cultivation in Flanders: short-rotation coppice for energy: position in the Flemish forest policy, INBO.R.2006.11. Research Institute for Nature and Forest, Brussels (in Dutch).
Michiels, B., Van Slycken, J., Steenackers, M., De Boever, L., 2008. Bakan and Skado: Two new INBO – poplar cultivars. Research Institute for Nature and Forest, Brussels (in Dutch).
Monsi, M., Saeki, T., 2005. On the factor light in plant communities and its importance for matter production, 1953. Ann. Bot. 95, 549–567.
Orlović, S., Guzina, V., Krstić, B., Merkulov, L., 1998. Genetic variability in anatomical, physiological and growth characteristics of hybrid poplar (*Populus x euramericana* DODE (GUINIER)) and eastern cottonwood (*Populus deltoides* BARTR.) clones. Silvae Genet. 47, 183–190.
Pellis, A., Laureysens, I., Ceulemans, R., 2004. Genetic variation of the bud and leaf phenology of seventeen poplar clones in a short rotation coppice culture. Plant Biol. 6, 38–46.
Pliura, A., Zhang, S.Y., MacKay, J., Bousquet, J., 2007. Genotypic variation in wood density and growth traits of poplar hybrids at four clonal trials. Forest Ecol. Manage. 238, 92–106.
Pontailler, 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. For. Sci. 54, 335–345.
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.
Rajora, O.P., Zsuffa, L., Yeh, F.C., 1994. Variation, inheritance and correlations of growth characters and *Melampsora* leaf rust resistance in full-sib families of *Populus*. Silvae Genet. 43, 219–225.
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.
Rohde, A., Storme, V., Jorge, V., Gaudet, M., Vitacolonna, N., Fabbrini, F., Ruttink, T., Zaina, G., Marron, N., Dillen, S., Steenackers, M., Sabatti, M., Morgante, M., Boerjan, W., Bastien, C., 2010. Bud set in poplar – genetic dissection of a complex trait in natural and hybrid populations. New Phytol. 189, 106–121.

Royal Meteorological Institute of Belgium. Klimaat in de wereld – klimatogram Gent-Melle. URL: <http://www.meteo.be/meteo/view/nl/6042865-Klimaat+in+de+wereld.html>. (Accessed 15.01.2013) (in Dutch).

Royle, D.J., Ostry, M.E., 1996. Disease and pest control in the bioenergy crops poplar and willow. Biomass Bioenerg. 9, 69–79.

Sadrul Islam, A.K.M., Ahiduzzaman, M., 2012. Biomass energy: sustainable solution for greenhouse gas emission. AIP Conf. Proc. 1440, 23–32.

Steenackers, J., Steenackers, M., Steenackers, V., Stevens, M., 1996. Poplar diseases, consequences on growth and wood quality. Biomass Bioenerg. 10, 267–274.

Steenackers, M., 2010. Old versus new poplar clones: what is the difference? Presentation at 'info- en debatdag: Populier in het vlaamse bos-en landschap', Brussels, 16 November, 2010 (in Dutch).

Steenackers, V., Strobl, S., Steenackers, M., 1990. Collection and distribution of poplar species, hybrids and clones. Biomass 22, 1–20.

Stettler, R.F., Ceulemans, R., 1993. Clonal material as a focus for genetic and physiological research in forest trees. In: Ahuja, M.R., Libby, W.J. (Eds.), Clonal forestry I, genetics and biotechnology. Springer-Verlag, Berlin, pp. 68–86.

Stettler, R.F., Bradshaw Jr., H.D., Heilman, P.E., Hinckley, T.M., 1996. Biology of *Populus* and its implications for management and conservation. NRC Research Press, Ottawa.

Tharakan, P.J., Volk, T.A., Nowak, C.A., Abrahamson, L.P., 2005. Morphological traits of 30 willow clones and their relationship to biomass production. Can. J. Forest Res. 35, 421–431.

UPOV (1981) Guidelines for the conduct of tests for distinctness, homogeneity and stability: Poplar (Populus L), UPOV, TG/21/7.

Van Ranst, E., Sys, C., 2000. Eenduidige legende voor de digitale bodemkaart van Vlaanderen (Schaal 1:20,000). Laboratorium voor Bodemkunde, University of Ghent, Ghent (in Dutch).

Van Slycken, J., Michiels, B., Steenackers, M., 2005. The new IBW-clones 'Muur', 'Vesten', 'Oudenberg' and 'Grimminge'. Research Institute for Nature and Forest, Brussels (in Dutch).

Zhang, S.Y., Yu, Q., Chauret, G., Koubaa, A., 2003. Selection for both growth and wood properties in hybrid poplar clones. Forest Sci. 49, 901–908.

Zhang, P., Wu, F., Kang, X., 2012. Genotypic variation in wood properties and growth traits of triploid hybrid clones of *Populus tomentosa* at three clonal trials. Tree Gen. Genomes 8, 1041–1050.

Zona, D., Janssens, I.A., Aubinet, M., Vicca, S., Gioli, B., Fichot, R., Ceulemans, R., 2013. Fluxes of the greenhouse gases (CO_2, CH_4 and N_2O) above a short-rotation poplar plantation after conversion from agricultural land. Agr. Forest Met. 169, 100–110.