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THESIS

BIOMASS PRODUCTION AND SHOOT DYNAMICS OF SELECTED AND NATIVE POPLAR GENOTYPES GROWING UNDER SHORT ROTATION COPPICE

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Index

A mia Madre e mia Sorella

per la fiducia e il costante sostegno ricevuto

MAIN INTRODUCTION

Introduction

Bio-energy is probably one of the most well-known aspect of the use of renewable resources. It has received a lot of attention in the political arena and in the international media because of its relation with environmental problems (global warming, CO2 emission, green energy labels, etc.).

The production of energy from renewable sources has assumed a primary role in European Union (EU) policy that promotes their use and establishes their sustainability criteria (COM, 2009). Additionally, the current model for European development strategies aims to stimulate an efficient production of renewable energy (COM, 2014). In this framework, dedicated bioenergy crops are increasingly assuming a determinant role as renewable energy sources. In turn, bioenergy crops represent a potential cultivation system to produce biomass locally available and utilizable in different conversion process (Kauter et al. 2003). Moreover, bioenergy crops can have a positive effect on degraded land by adding organic matter and reducing soil erosion.

The development of sustainable, low-carbon, solid and liquid fuels from lignocellulosic biomass requires advances in many areas of science and engineering. From an agronomic perspective, the development and improvement in plant selection, breeding, cultivation and biomass modification techniques are important, because the provision of suitable and specialist bioenergy crops can greatly reduce costs associates with harvesting, pretreatment and bioconversion (Carroll and Somerville 2009; Rubin 2008).

Short Rotation Coppice (SRC) consist of fast growing tree species, such as *Populus* and *Salix* grown at high stem density and harvested at short time interval. In particular, SRWC system is characterized by two phases: i) single-stem (pre-coppice) and ii) multi-stem (post-coppice). The single stem phase consist of planted cuttings, which grow generally from one (Ceulemans and Deraedt 1999) to 2-3 years (Verlinden et al 2015). At the end of this phase, cuttings are coppiced at the base of the stem to promote sprouting of new shoots from the dormant buds arranged on stump (Ceulemans et al. 1996). The new shoots will be part of the multi-stem phase, coppiced regularly with a short rotation cycles, generally less than 4 years.

Throughout Europe hybrid clones are the most cultivated poplar genotypes (Sixto et al. 2013; Oliveira et al. 2015; Sixto et al. 2015) while, wild native European black poplar genotypes are rarely employed in SRC and also few considered in research studies. As reported by Benetka et al. (2007) *Populus nigra* is included in 60% of the interspecific hybrid poplar clones frequently cultivated in SRC systems. As an indigenous genotypes *P.nigra* is suitable to use in areas with particular environmental restrictions, such as areas in which is not legal the introduction of exotic genotypes.

In this context, the objectives of the present thesis were:

i) in Chapter 1 to compare field performance of hybrid clones and native black poplar genotypes both in terms of biomass production and biomass allocation, ii) assessing the influence of the length of the single-stem rotation on yield characteristics. In the Chapter 2, we analyzed and described two main diachronic processes of the SRC iii) the variation of shoots diameter frequency distribution and demography, iv) and the above-ground dry biomass partitioning according to shoots size class on stools. Finally, in the Chapter 3, we considered size inequality of population in the light of different pattern of resource partitioning and competition type. The differences between genotypes are discussed in the light of evolutionary trajectory, namely human breeding and natural selection.

Salient biological feature and systematics of the genus Populus

The genus *Populus*, commonly known as aspen, cottonwoods, and poplars, include morphologically diverse species of deciduous or, rarely, semi-evergreen trees that occur primarily in the boreal, temperate, and subtropical zones of the Northern hemisphere, characterized by a relative short-live and a very fast growth (Dickmann et al. 2002; Cronk 2005). Typically, trees belonging to this genus have tall and straight single stem, with bark that tend to remain tin and smooth until more advanced ages than in other tree species (Dickmann et al. 2002).

Most species of *Populus* have wide native range, spanning over than 20 degree of latitude and a great diversity of climates and soils (Stettler et al. 1996; Dickmann et al. 2002). Although *Populus* trees grow in a striking variety of habitats (Jansson et al. 2010), they occur mainly in two category of habitats: riparian areas and wetland or mountainous and upland areas. Riparian areas and wetlands are characterized by seasonal flooding and high water tables. One example in riparian area of Pacific North west of North America is represented by black cottonwood (*P. trichocarpa*), while in European countries black poplar (*P. nigra*) represent the most common tree species of this habitats (Stettler et al. 1996; Corenblit et al. 2014; Lefèvre et al. 2001). Some *Populus* riparian species are extremely phreatophytic (i.e. deep-rooting) especially during the establishment phase that occur after the major disturbances, such as fire or floods (Gurnell et al. 2001; Barsoum 2002). Aspen and some poplars grow in mountainous and upland habitats. It is the case of quaking aspen (*P. tremuloides*) in North America and of *P. tremula* in Europe uplands (Bernetti 1995).

One of the distinctive trait of the genus *Populus* is vegetative reproduction (Barsoum et al. 2004; Dickmann et al. 2002; Guilloy-Froget et al. 2002; Stettler et al. 1996) which strongly varies among species (Braatne et al. 1996; Rood et al. 2003; Rood et al. 2007). Vegetative reproduction occurs with different mechanisms that trees have evolved for asexual reproduction (Del Tredici 2001). One of these mechanism is the sprouting, that is the process whereby tree develops secondary replacement stems as response to injury or a dramatic change in surrounding environmental conditions (Del Tredici 2001). In *Populus* genus vegetative reproduction occurs commonly both through root sprouting and through rooting of shoots from broken branches or entire tree stem that have toppled during storm and floods and then buried in sediment (Braatne, Rood, and Heilman 1996; Rood et al. 2007; Bertoldi, Drake, and Gurnell 2011; Barsoum, Muller, and Skot 2004; Smulders et al. 2008).

As reported by Eckenwalder (1996) the number of species included in the *Populus* genus varies among classification from as few as 22 to as many as 85. One classification that is frequently used recognized 29 species subdivided into six section based on relative morphological and crossability (Eckenwalder 1996; Jansson, Bhalerao, and Groover 2010). Three of these sections, *Aigeiros*, *Tacamahaca* and *Populus* account for nearly the world's entire applied breeding work.

Study area

The study site is located in the Sele river valley (Latitude 40° 33' 33.21'' N; Longitude 14° 50' 15.60'' E, 19 m a.s.l., Eboli, Salerno, Italy) in a flat area previously used for agricultural crops in the Azienda Agricola Sperimentale Regionale "Improsta".

The soil is characterized by a deep of 80–100 cm, clay loam (sand 36.7%, clay 43.3%, loam 20%) with low N-levels (0.78 ± 0.27g/kg) in the upper layer (up to 40 cm). The soil organic matter decreases from the upper (1.42 %) to deeper (0.49 %) layers (100 cm).

During the experimental period (2007-2016), meteorological data obtained from a weather station 3 km distant from the study site (Battipaglia 72 m a.s.l.) indicate a Mediterranean humid-type climate characterized by a mean yearly temperature of 17.8 °C, mean annual cumulated precipitations of 1101.4 mm. The concomitance of a summer drought period (from June to August mean 6.25 % ±3% SD of cumulated annual precipitations) with the highest monthly mean temperature (August mean 26.5 ±0.8 °C SD).

Plant material and management of SRF

Five hybrid genotypes were bred by and obtained from research Institute for Nature and Forest in Geraardsbergen (INBO, Belgium). The hybrid clone I-214 was obtained by controlled crossing in 1929 by Poplar Research Institute in Casale Monferrato (CRF-PLF, Italy). Dormant cuttings of these six hybrid poplar clones were furnished by the nursery Allasia plant (Cavallermaggiore, Cuneo, Italy). Specifically, these hybrid clones were selected for peeling and sliced sheet production and frequently cultivated in traditional plantation systems characterized by a lower and longer densities and rotations, respectively, than SRC systems. They were crossed between 1969 to 1978 (excluding I-214) and commercialized in 1996-1999.

Dormant cuttings from three native *P. nigra* genotypes were collected in the wild from populations naturally growing in Campania region in Southern Italy (Table 1). These hardwood cuttings have been collected in February from adult and undetermined sex black poplar trees originated by seed, excluding individuals originated by roots and/or from stump re-sprouting.

Table 1 - *Populus* genotypes, commercial names, code, parental origins, gender and place of origin or site provenance of the six hybrid clone and three native black poplars genotypes studied in the short rotation coppice plantation. All genotypes are included (*P. nigra*) or are crossed from species belonging to *Aigeiros* botanical section, excepted for Grimminge and Hoogvorst hybrid clones which are crossed between species from *Aigeiros* and *Tacamahaca* sections.

Commercial name	Code	Parentage	Sex	Place of origin
I-214	I-214	*P. deltoides x P. nigra*	♀	Italy
Grimminge	GRI	*P. trichocarpa x (P. trichocarpa x P. deltoides)*	♂	Belgium
Hoogvorst	HOO	*P. trichocarpa x P. deltoides*	♀	
Oudenberg	OUD	*P. deltoides x P. nigra*	♀	
Muur	MUU	*P. deltoides x P. nigra*	♂	
Vesten	VES	*P. deltoides x P. nigra*	♀	
Common name	**Code**	**Species**	**Sex**	**Site provenance**
Limatola*	LIM	*Populus* spp.	nd	Valle Caudina, Italy
Isclero	ISC	*P. nigra*	nd	Isclero torrent -Valle Caudina, Italy
Ripiti	RIP	*P. nigra*	nd	Ripiti river-Cilento region, Italy
Badolato	BAD	*P. nigra*	nd	Badolato river - Cilento region, Italy

*Presumably spontaneous hybrid analyzed only in Chapter 1

All the 20-25 cm unrooted woody cuttings were first soaked into water for 48 hours and then carefully mechanically planted, leaving less than 3 cm of the cuttings with one or two buds above the soil surface. Unrooted cuttings were planted in a single-row layout with 3.0 m inter-row and 0.5 m within rows distances, obtaining the density of 6667 cuttings per hectare. A minimum of 3 rows was assigned to each clone or provenances.

In August 2006, 3.04 ha soil were ploughed at 0.8 m, thereafter leveled and harrowed, poplar plantation followed in April 2007. In the 1st year of the single stem cycle, ~320 m^3 ha^{-1} of water were supplied at six different times in 2007 (from April 17 to August 20) and at one time in 2008 (July 20) to counteract the extreme soil water shortage so as to assure the establishment of the poplar plantation. Mechanical weed control was done twice during the first growing season of single-stem cycle.

Experimental design and shoot measurements

A block design was used within 10 poplar genotypes stands x 10 replicate (stool with its shoots) plots (Figure 1). Each block consist of 3 rows with an assigned area of 1260 m^2 (140 m x 9 m). Within each block a core block area of 300 m^2 (100 m x 3 m) was spatially defined, containing the middle (central) row of 50 stools each. The core block area included the 10 replicate mono-stool plots of 30 m^2 each (Figure 1). Following a systematic sampling criteria, because of the fertility variation along the plot, were chosen 10 mapped stools (1 every 10 m) growing in the middle row of each poplar genotypes block (Figure 1). In order to assure homogeneity in space competition effects between stools, the constraint adopted in the systematic sampling is the full density within and between the rows.

The choice of such experimental design is mainly justified by the need to facilitate and carry out experimental tests on the harvest systems. This type of research study require large experimental blocks, with plants arranged in long rows to evaluate performance of harvest system. Additionally, as highlighted by Kauter et al. (2003) plot size strongly influences the estimation of biomass yield for poplar in SRC systems, reporting that the higher biomass yield was observed in small rather than large plots research trial as a results of the edge effect. For these reasons, we assigned a large area to each block overlooking the randomization.

Shoot collar diameter at 5 cm above soil level and total shoot height have been measured at the end of each growing season. The shoot collar diameter was performed with a digital Vernier calliper to the nearest 0.1 mm, in two perpendicular directions, while the total height of living shoots was determined by using a telescopic pole. Measurement were carried out in winter (December-February), so the dormant season, which coincide with the usual harvest time window.

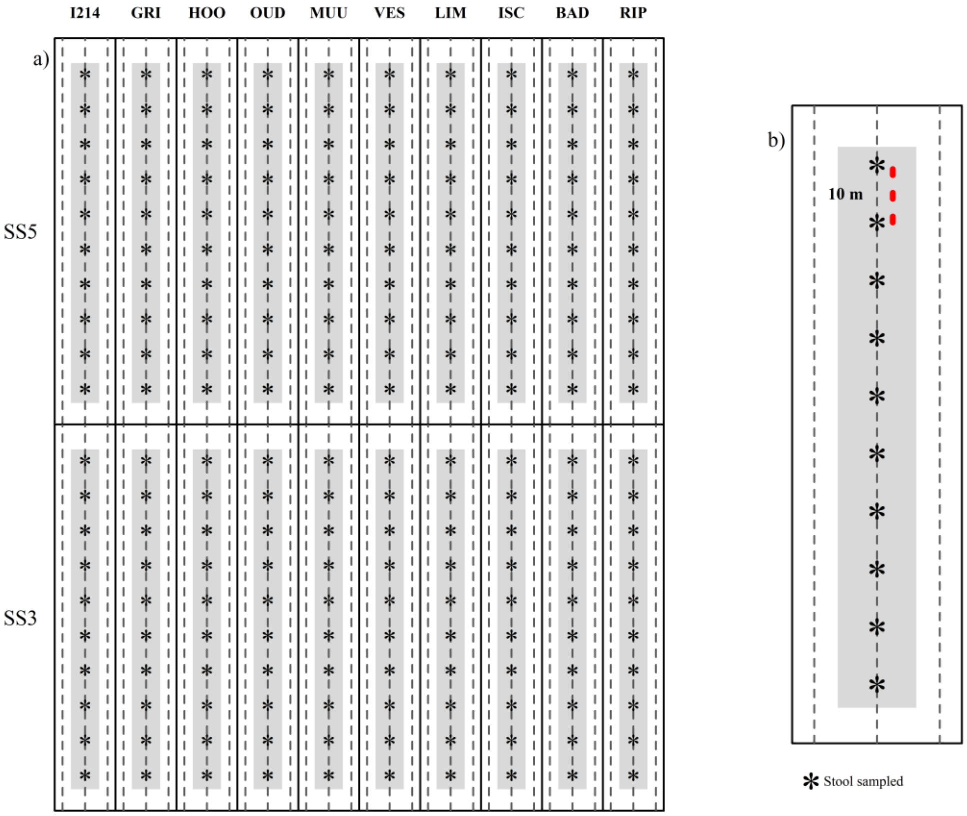

Figure 1 - Experimental design of poplar short rotation coppice plantation. a) The two treatments, characterized by different length of single stem rotation, are indicated as follow: SS3 for single-stem rotation length of 3 years and SS5 for single-stem rotation length of 5 years. b) Sampling design applied for each poplar genotypes. See table 1 for poplar genotype codes.

Shoot volume and specific density estimation

At the end of each growing season, in winter, three shoots for each poplar genotype (overall *n*= 81, 3 years x 3 shoots x 9 genotypes) were manually harvested to estimate shoot volume (stem and branches) and the corresponding specific density (limited to the first two years of rotation). After separating branches from the stem, stem volume over bark was calculate by section-wise measurements, using Huber section method (van Laar and Akça 2007), with length of butt logs of 0.50 or 0.20 m according to the total stem length. Live branches volume were estimated via branches dry biomass (see below), that were dried in a ventilated oven at 70 °C for 7-10 days, until the weight remains constant (West 2009).

Stem Specific density (Cornelissen et al. 2003) (SSD, dry weight of wood+bark for unit fresh volume in kg m^{-3}) of stem and branches was determined by water displacement. From each felled shoot six 8-10 cm length wood+bark segments were collected in the field: three on proximal, medial and distal point of stem, and three selected medium sized branches and hermetically sealed with parafilm®. In the laboratory, fresh volume was determined by promptly immerging each woody segment hanged from a metal needle into a beaker of 4 °C distilled water loaded on a top-loading electronic balance (Williamson and Wiemann 2010). Then segments were oven-dried at 70 °C for several days until its weight remain constant. Reciprocal values of live branches specific density was used in dry biomass-volume conversion and to estimate absolute form factor (*Fa*) (see below section). Mean specific density of stem and branches were also used for converting respectively standing stem and branches volumes to dry biomass, as detailed in the next section.

Specific density was determined on felled shoots collected according to three crown classes or strata (horizontal layers): suppressed, intermediate and dominant (Oliver and Larson 1996), that were related to the stool maximum height (<50%, 50-80%, >80% of maximum height for suppressed, intermediate and dominant shoots, respectively). Classification of crown classes reflect aboveground available growing space and light use efficiency which translate in a different biomass allocation pattern, more favourable in dominant related to the other subordinated crown category (Oliver and Larson 1996).

Stand volume estimation and dry biomass conversion

At the end of each growing season, the coppice standing volume was estimated for each poplar genotypes, according to felled sample tree method (van Laar and Akça 2007). Hence, the standing volume (V, m^3 ha^{-1}) was estimated as the product of total basal area (G, m^2 ha^{-1}), mean height (H_m, m) and average dimensionless absolute form factor F_a of the shoots. In this work, we use total basal area G as the actual density of plantation per hectare, excluding the unrooted cuttings and dead stool, H_m as the mean regressed height of quadratic mean diameter and F_a as the average value of absolute form factor (van Laar and Akça 2007):

F_a is the shoot volume (stem and branches) compared to a volume of a cylinder of the same height and as a reference point the over bark diameter measured at 5 cm above the base of the stem. Finally, volume was converted in aboveground dry biomass (AGDB, Mg ha^{-1}), applying an *ad hoc* stem and branches specific density as specified in the previous section.

CHAPTER 1

Aboveground dry biomass production in hybrid and native black poplar genotypes growing under two short rotation coppice treatments

Introduction

Fast growing trees planted as Short Rotation Coppice (SRC) represent an important source of biomass (Aylott et al. 2008; Rowe et al 2009), due to their high biomass yields, good combustion quality, ecological and social benefits (Groscurth et al. 2000; Hauk et al 2014) and relatively low biomass production costs (Kauter et al. 2003). Short rotation coppice has become very common, especially poplar cultivation, considered the most suitable species in European country (Dillen et al. 2007) and, more broadly, in temperate regions (Stettler et al., 1996). Poplar breeding programs in Europe allowed to obtain clones with high growth rates, across wide ranges of climates and sites (Liberloo et al. 2006; Deckmyn et al. 2004), and is still possible to achieve high biomass levels (Guo and Zhang 2010; Paris et al. 2011; Sixto et al. 2013).

Despite the high potential for SRC to produce biomass, no studies examined the effect of the length of single stem rotation on the biomass production of following multi-stem rotations. Currently, length of single stem or cuttings phase range between 1 and 3 years (Ceulemans and Deraedt 1999; Verlinden et al. 2015). We hypothesized that length of single stem rotation play an important role in poplar coppice plantation subjected to short cut time interval. Therefore, in this study it was evaluate the effect of the length of single stem rotation on the aboveground biomass production of subsequent first multi stem phase.

Material and methods

Plant material

See main introduction

Short rotation coppice treatments

Two different treatments were defined according to the length of the single-stem phase: 3 and 5 years respectively, namely TRTA and TRTB (from now onward TRTA and TRTB, Figure 2).

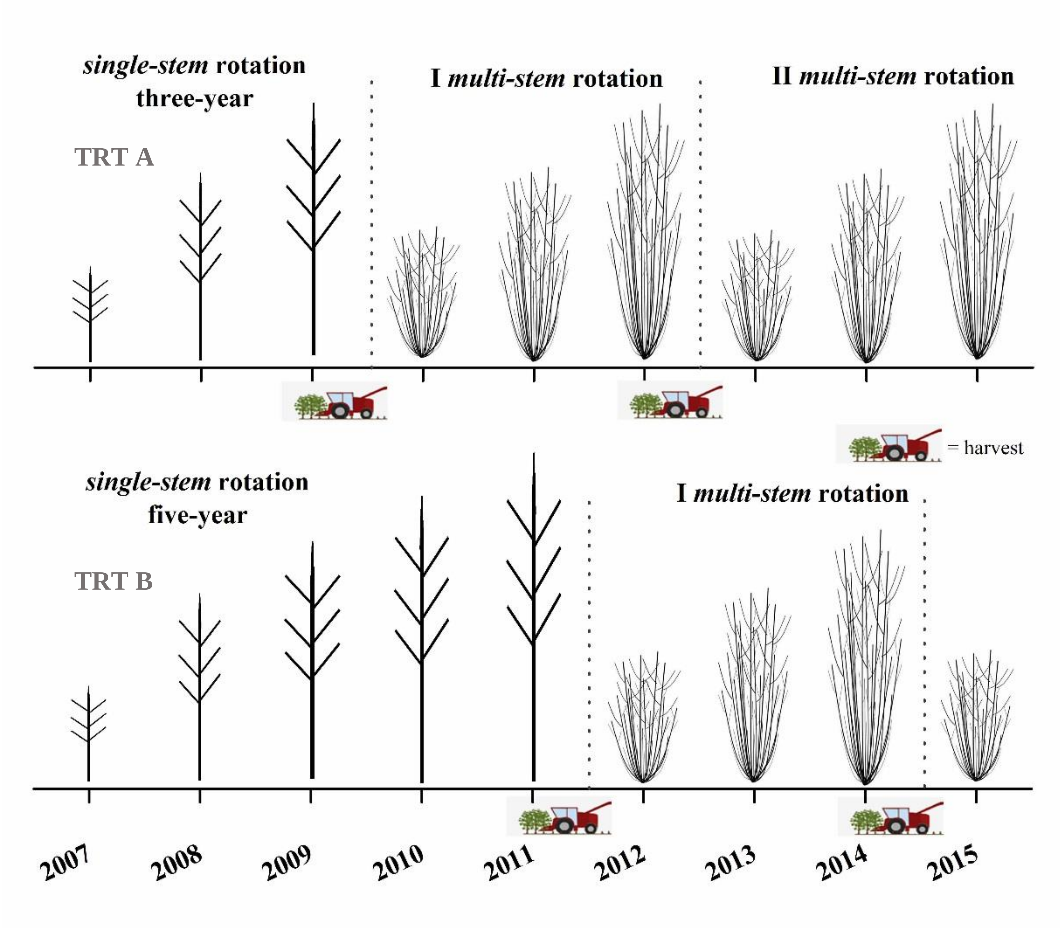

Figure 2 - Schematic representation of poplar short rotation coppice management regime. The coppice plantation was established in March 2007. In TRTA (TRTA) the plantation were harvested after 3 years from cuttings establishment in winter 2009, with an age of stem (S) and root (R) of 3 years (S3R3). In TRTB (TRTB) cuttings were harvested for the first time after 5 years from cuttings establishment in winter 2011 (S5R5). The length of multi-stem rotation cycle is the same for the two treatments A and B and equal to 3 years. Second coppicing occur at the end of the 2011 (R6S3) in the TRTA and at the end of 2014 (R8S3) in the TRTB.

To encourage the single stool to become multi-stemmed, i.e. composed by a stump and its shoots, in each half of the row length (~140 m), all trees were stumped back to 5 cm above ground level by the cut & chips harvesting system in winter 2009 and 2011 respectively. The length of multi-stem rotation cycle was the same for both treatments A and B and equal to 3 years (S3). Consequently, the second coppicing occurred at the end of the 2011 growing season when roots were 6 years old (R6) in the TRTA, and at the end of 2014 growing season when roots were 8 years old (R8) in the TRTB (Figure 2).

Experimental design and shoot measurements

See main introduction

Shoot volume and specific density estimation

See main introduction

Stand volume estimation and dry biomass conversion

See main introduction

Statistical analysis

A general model describing the relationship between aboveground stand dry biomass and year was developed for the single-stem and multi-stem rotation, while taking into account the differences in mean aboveground biomass and the different shape of the aboveground biomass to year relationships.

Selection model process via leave one-out cross validation approach was applied to entire dataset. The resulting test error rate was assessed by the mean square error (MSE) and the model was selected on the base of the minimum value of MSE.

In single stem, we introduce a second degree polynomial model without intercept, because starting value of aboveground biomass can be considered approximately equal to zero.

To test the difference in the parameters of regression equation, a dummy variable that identifies two levels of qualitative attribute was included in the model, as follow:

$$Y_i = a + bX_i + a_1D + b_1DX$$

Where Y_i and X_i are the dependent and independent variable, respectively. Categorical variable D (0,1) represent the dummy variable having two levels that can be the genotype group (hybrid and native poplar) and the SRC treatment (TRTA and TRTB). Dummy variable assume arbitrarily two value 0 and 1 so that, for example of a simple linear model we have:

We assign D=0, so that equation is:

$$Y_i = a + bX_i$$

When D=1, we obtain the following equation:

$$Y_i = (a + a_1) + (b + b_1)X_i$$

The parameters a_1 and b_1 represent the difference in intercept and slope for the two linear equation. The difference in parameters into the model was tested via *t*-test with a level of significance of 0.05.

Results

Aboveground dry biomass production in single stem rotation

First three years single-stem rotation of TRTA exhibited an average cumulative aboveground dry biomass production of 18.11 (± 2.25) and 21.06 (±0.99) Mg ha^{-1}, while at the end of first five years of TRTB dry biomass was 60.42 (± 2.25) and 80.97 (± 4.10) Mg ha^{-1}, respectively for hybrid clone and native black poplar genotype groups (Figure 3).

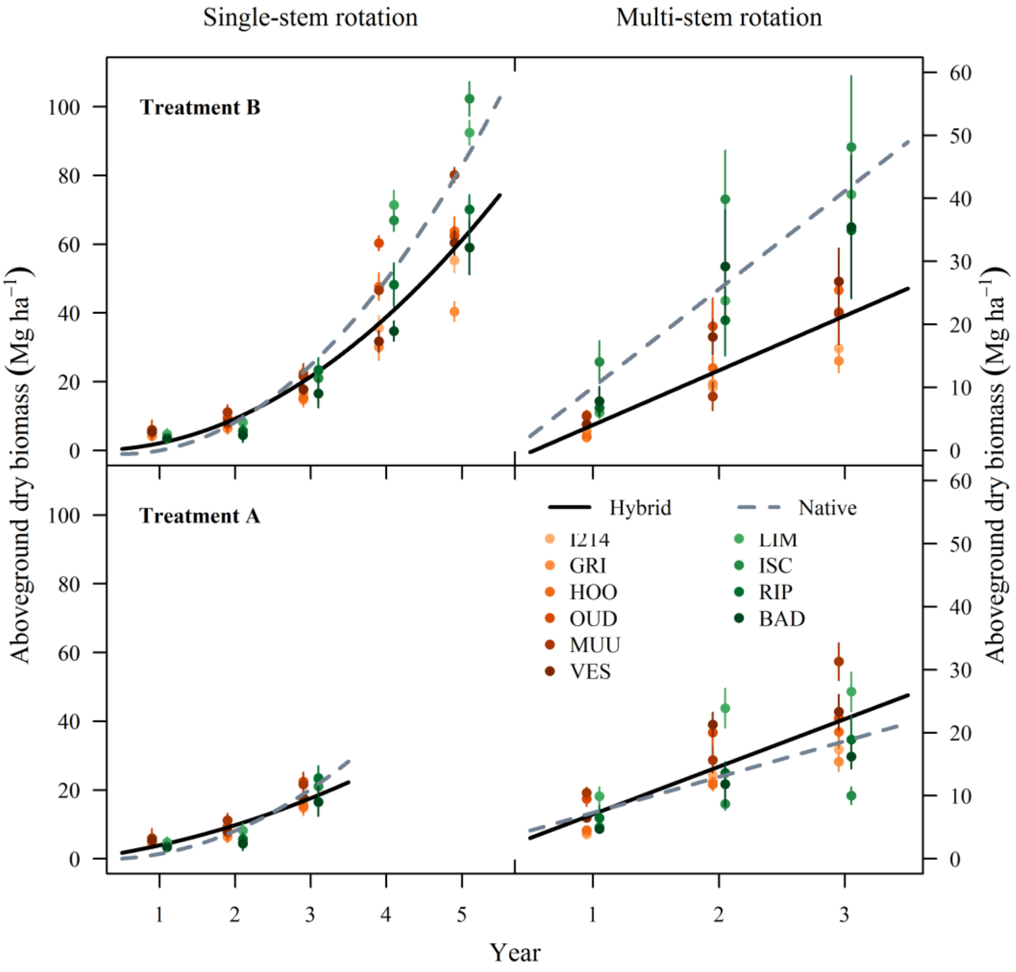

Figure 3 - Aboveground dry biomass production in SRC culture for hybrid (oranges) and native black poplar (greens) genotypes. TRTA consist of a 3-year single stem and 3-year multi-stem rotation; TRTB consist of a 5-year single stem and 3-year single stem rotation. See Table 1 (Main Introduction) for genotype codes.

In single stem of TRTA, the average mean annual increment was 6.04 and 7.02 Mg ha^{-1} yr^{-1} for hybrid clone and native black poplar genotypes, respectively. In TRTB mean increments were 12.08 and 16.19 Mg ha^{-1} yr^{-1}, respectively.

A significant regression on cumulative aboveground dry biomass was found for each treatment with an adjR^2 that ranged between 0.57 and 0.90 (Table 2).

Table 2 - Fit statistics for regression model describing the relationship between aboveground dry biomass (Mg ha^{-1}) and years of rotation cycle, for poplar genotypes grown under two short rotation coppice treatment: TRT A (3 years single-stem rotation) and TRT B (5 years single-stem rotation). Multi-stem rotation length is 3 years in both treatments. In each regression model group of genotypes are encoded as dummy variable where the two levels are Hybrid and Native. Where MSE is mean square error obtained from leave one out cross validation; *adj*R^2 is adjusted coefficient of regression; F and p-value are Fisher's statistic and significance level.

		MSE	***adj*R^2**	**F-value***	***p*-value**
TRT A	Single-stem $^+$	5.66	0.90	55.30	<0.001
	Multi-stem $^{++}$	16.54	0.57	14.10	<0.001
TRT B	Single-stem $^+$	11.32	0.87	68.32	<0.001
	Multi-stem $^{++}$	18.81	0.84	53.29	<0.001

*F$_{(5,24)}$ for single-stem of TRT A and F$_{(5,44)}$ for single stem of TRT B; F$_{(3,26)}$ for multi stem for both treatments

$^+$In single stem was applied a second-degree polynomial regression.

$^{++}$In multi stem rotation was applied a linear regression.

In single stem rotation of TRT A and TRT B a second-degree polynomial regression model was selected because it showed a low MSE than linear regression model while, in multi-stem rotation the MSE obtained by leave one-out cross validation was lower for linear regression model in both TRT A and B.

For hybrid poplar genotype group in the single-stem rotation of TRTA, aboveground dry biomass predicted is equal to: -7.088*(year) + 3.367*(year^2) (Table 3), where the linear coefficient (b, t = -1.651, p=0.112) is not statistically different from zero, and quadratic coefficient (c_1: t = 3.168, p=0.004) of second-degree polynomial regression is significantly different from zero. For black poplar genotype group in the single-stem rotation of TRTA, aboveground dry biomass predicted from regression is equal to (-7.88-10.964)*(year) + (3.367+3.303)*(year^2). The latest equation not differ significantly from hybrid in quadratic coefficient of regression equation (b_1, t = -1.615, p=0.119) and linear term is not significantly different (c_1, t=-1.966, p=0.061), indicating that cumulative aboveground dry biomass in black poplar genotype not differ from hybrid poplar genotypes (Table 3).

Table 3 - Last squares coefficients estimates of second-degree polynomial regression describing relationship between aboveground dry biomass (Mg ha^{-1}) and year of single-stem rotation, for poplar genotypes grown under two short rotation coppice treatment: TRT A (3 years single-stem rotation) and TRT B (5 years single-stem rotation). Group of Genotypes are encoded as dummy variable where the two levels are Hybrid and Native. SE represent standard error of estimated parameter.

	Parameter	**Estimate**	**SE**	***t*-value**	***p*-value**
TRT B	b (year) [hybrid]	-7.088	4.295	-1.651	0.112
	c (year^2) [hybrid]	3.367	1.063	3.168	0.004
	b_1 (year x genotype) [native]	-10.964	6.790	-1.615	0.119
	c_1 (year^2 x genotype) [native]	3.303	1.680	1.966	0.061
TRT A	b (year) [hybrid]	-4.872	6.424	-0.758	0.452
	c (year^2) [hybrid]	3.208	1.050	3.054	0.004
	b_1 (year x genotype) [native]	-3.180	10.157	-0.313	0.756
	c_1 (year^2 x genotype) [native]	1.531	1.661	0.922	0.362

In single stem of TRTB (Table 3), regression equation that predict aboveground dry biomass is equal to -4.872*(year) + 3.208*(year^2) for genotype hybrid poplar group, where linear coefficient is not statistically different from zero (b, t=-0.758, p=0.452), while quadratic coefficient is statistically significant different from zero (c, t=3.054, p = 0.004). For native black poplar genotypes the regression equation is equal to (-4.872 -3.180)*(year) + (3.208+1.531) *(year^2), where linear (b_1, t=-0.313, p=0.756) and quadratic (c_1, t=0.922, p=0.362) coefficients are not different from zero (Table 3).

Aboveground dry biomass production in multi-stem rotation

Under TRTA, the cumulated tree-years multi-stem rotation aboveground woody dry biomass averaged between 21.61 (± 1.16) and 18.18 (± 1.66) Mg ha^{-1} for hybrid clones and native black poplars respectively

(Figure 3). In TRTB, cumulative dry biomass yielded by hybrid clone genotypes is 21.07 (± 1.72) and 39.96 (± 4.38) Mg ha^{-1}, for native black poplar genotypes.

The regression equation that predicted aboveground dry biomass for the first multi-stem rotation of TRTA (Table 4) is equal to -0.491+7.561*(year) for hybrid poplar genotype group and is equal to (-0.491+2.106) + (7.561-1.871)*(year) for black poplars. Both two intercepts (a, a_1) are not statistically different from zero (a: t=-0.165, p=0.870, a_1: t=0.447, p=0.658). Unlike the intercepts, slopes b but not b_1 differs significantly from zero and the slope of regression equation for hybrid genotype group is higher than black poplar genotype group of about 33%.

Table 4 - Last squares coefficients estimates of linear regression describing relationship between aboveground dry biomass (Mg ha^{-1}) and year of multi-stem rotation, for poplar genotypes grown under two short rotation coppice treatment: TRT A (3 years single-stem rotation) and TRT B (5 years single-stem rotation). Multi-stem rotation length is 3 years in both treatments. Group of genotypes are encoded as dummy variable where the two levels are hybrid and native. SE represent standard error of estimated parameter.

	Parameter	**Estimate**	**SE**	***t*-value**	***p*-value**
TRT A	a [hybrid]	-0.491	2.977	-0.165	0.870
	b (year) [hybrid]	7.561	1.378	5.487	0.000
	a_1 (genotype) [native]	2.106	4.707	0.447	0.658
	b_1 (year x genotype) [native]	-1.871	2.179	-0.859	0.398
TRT B	a [hybrid]	-4.621	3.134	-1.474	0.152
	b (year) [hybrid]	8.667	1.451	5.974	0.000
	a_1 (genotype) [native]	-0.926	4.956	-0.187	0.853
	b_1 (year x genotype) [native]	6.909	2.294	3.012	0.006

In multi-stem rotation of TRTB, regression equation for aboveground dry biomass predicted is equal to -4.621 + 8.667 *(year) for hybrid poplar genotypes and (-4.261-0.926) + (8.667+6.909)* (year) in native black poplar group (Table 4). The intercepts (a, a_1) are not statistically different from zero with t=-1.474, p=0.152, and t=-0.187, p=0.853, respectively. Unlike first multi stem-rotation of TRTA, in

TRTB the slopes (b, b_1) of linear regression are significant (b: t= 5.974, p<0.000; b_1: t= 3.012, p=0.006) and markedly higher for black poplar than hybrid poplar genotypes group of about 44% (Table 4).

A significant regression equation on cumulative aboveground dry biomass was found for each multi-stem rotation of hybrid and native poplar genotypes with an adjR^2 that ranged between 0.73 and 0.78 (Table 5).

Table 5 - Fit statistics for linear regression model describing the relationship between aboveground dry biomass (Mg ha^{-1}) and year or rotation cycle, for poplar genotypes grown under two short rotation coppice treatment: TRT A (3 years single-stem rotation) and TRT B (5 years single-stem rotation). Multi-stem rotation length is 3 years in both treatments. In each regression, SRC treatments are encoded as dummy variable where the two levels are TRT A and TRT B. Where MSE is mean square error obtained from leave one out cross validation; *adj*R^2 is adjusted coefficient of regression; F and p-value are Fisher's statistic and significance level.

		MSE	***adj*R^2**	**F-value***	***p*-value**
Multi-stem	Hybrid	8.78	0.73	32.34	<0.001
	Native	11.02	0.78	29.42	<0.001

$^*F_{(3,32)}$ for hybrid clone and $F_{(3,20)}$ for native black poplar genotypes, respectively

In first multi-stem rotation of hybrid genotypes, the equation for aboveground dry biomass is equal to -0.491 + 7.561*(year) for TRTA and (-0.491 - 4.130) + (7.561 + 1.106) *(year) in TRTB, respectively (Table 6). Aboveground dry biomass predicted in TRTA not differ statistically from TRTB in hybrid genotypes, as observed from the *p*-values associated to a_1 and b_1 (Table 6). In the regression, only the slope is statistically different from zero (b, t=6.408, p<0.001)

In first multi-stem rotation of native black poplar genotypes, regression equation for aboveground dry biomass predicted is equal to 1.614 + 5.689*(year) for TRTA and (1.614 -7.161) + (5.689 + 9.886)*(year) in TRTB (Table 6). Unlike hybrid genotypes, in native black poplar genotypes aboveground dry biomass predicted in TRTA differ statistically from TRTB, as we can see from the *p*-values associated to a_1 and

b_1 (Table 6). In regression model the slopes of two linear regression are statistically different from zero (b, t=2.692, p<0.014 and b_1, t=3.307, p=0.004), that is 63% higher in TRTB than TRTA.

Table 6 - Last squares coefficients estimates of linear regression describing relationship between aboveground dry biomass (Mg ha^{-1}) and year of multi-stem rotation, for poplar genotypes grown under two short rotation coppice treatment: TRT A (3 years single-stem rotation) and TRT B (5 years single-stem rotation). Multi-stem rotation length is 3 years in both treatments. SRC treatments are encoded as dummy variable where the two levels are TRT A and TRT B. SE represent standard error of estimated parameter.

	Parameter	**Estimate**	**SE**	***t*-value**	***p*-value**
Hybrid	a [TRT A]	-0.491	2.549	-0.193	0.848
	b (year) [TRT A]	7.561	1.180	6.408	0.000
	a_1 (treatment) [TRT B]	-4.130	3.604	-1.146	0.260
	b_1 (year x treatment) [TRT B]	1.106	1.668	0.663	0.512
Native	a [TRT A]	1.614	4.566	0.354	0.727
	b (year) [TRT A]	5.689	2.114	2.692	0.014
	a_1 (treatment) [TRT B]	-7.161	6.457	-1.109	0.281
	b_1 (year x treatment) [TRT B]	9.886	2.989	3.307	0.004

Discussion

According to literature data on intensive SRC systems, hybrid poplar biomass production in multi-stem phases ranges from 1.3 to 24 Mg ha^{-1} yr $^{-1}$ with a mean biomass yield of 9.3 (±4.2) Mg ha^{-1} yr $^{-1}$ (Njakou Djomo et al. 2014). The values of biomass production of our crops are included in this large range, falling around the literature mean. It is remarkable the value of 13.3 Mg ha^{-1} yr^{-1} produced by the native black poplar genotypes, which is higher than the literature mean value, despite our coppice plantation were managed as semi-extensive crops, with low energy input, mainly irrigation, limited to the first two years to assure the establishment under dry Mediterranean climate.

It was difficult to compare aboveground biomass production observed in our SRC system with values reported in literature, because environmental constraints and cultivation practices are largely different.

In northern Italy, biomass production of hybrid poplar genotypes ranged from 3.0 to 15.0 Mg ha^{-1} yr^{-1} (Facciott et al. 2006; Paris et al. 2011) with a mean value of 7-12 Mg ha^{-1} yr^{-1} (Bergante et al. 2010; Sixto et al. 2013). It is important to considerer that hybrid poplar genotypes, most largely cultivated, are produced by few breeding centers based in 7 countries (Italy, Belgium, France, Holland, Germany, Scandinavia and United Kingdom) of the central or northern Europe (Isebrands and Richardson 2015), under climatic condition different from southern Mediterranean areas.

The aboveground biomass production better modelised by a second-degree polynomial regression during single stem phase, suggests a different pattern of biomass allocation with respect to the multi-stem phases significantly described by a linear regression model (Figure 3). Indeed, single stem phases are constituted by unrooted cuttings, which grow allocating part of photosynthates mainly to root system, which in turn determine a deviation from linearity in aboveground biomass production. In contrast, under multi-stem phase the linearity can be ascribed to preexistence of root system, which are able to support the growth of aboveground biomass.

Concerning the response of the two group of genotypes to the different management regimes, the biomass production in the multi-stem phase consistently differed according the length of the previous mono-stem phases. Indeed, the biomass production of hybrid genotypes do not vary according the length of the mono stem phase, whereas notably, black poplar native genotype showed a consistently higher amount of biomass, with almost double values, produced when the mono-stem phase was longer.

From an eco-physiological perspective, we wonder why the root age significantly affect aboveground biomass of native black poplars whereas do not influence hybrid genotypes production. Since resprouting shoots profit of an already established root system and that the resprouting ability depend on the size of their belowground carbohydrates reserves (Shibata et al. 2016) we should consider the possibly of different root system between hybrid clone and black poplar genotypes.

Wild population of European black poplars are characterized by a deep root system as a response of riparian environmental constraints. In the establishment phase, European black poplar as obligate phreatophytic species allocated more resources in belowground biomass to cope with water table level fluctuations, intensity and frequency of flooding (Barsoum and Hughes 1998). Indeed, in riparian environments wood fragments (functionally comparable to cuttings) of European black poplar develops both below and aboveground biomass rapidly (Barsoum and Hughes 1998). In other words, to increase anchorage under flooding events and permit to explore more deep soil levels they need to allocate most resources in root system.

Although parent plants material of hybrid clones comes at the same from wild riparian poplar (Braatne et al. 1996), we should hypothesize that the phreatophytic behavior of the parent wild population became overshadowed, may be because the human selection was more interested in having high above ground biomass production (Riemenschneider et al. 2001). Indeed, human selection emphasized aboveground traits rather than below ground traits (Riemenschneider et al. 2001; Stettler et al. 1996), site adaptability and diseases tolerance (Jansson et al. 2010).

Our results emphasizes the role of root system for aboveground biomass production and stimulate do not forget to take in account this trait in poplar breeding programs for SRC purposes.

An alternative explanation for the better performances of the native black poplars should be a greater resprouting bud bank on the stump. This hypothesis could be supported by the evidence (as reported in the chapter 2) of a greater number of shoots produced by the native black poplar than the hybrid clone genotypes. The lacking of previous literature studies on native genotypes for SRC do not help in discussing these data and our hypothesis remain at the moment merely speculative.

In terms of research extension, we can say that native black poplar are a valid alternative to hybrid poplar genotypes in Mediterranean environment. A longer single stem phase in native black poplars increases aboveground biomass production in the multi-stem phase. However, from an agronomic perspective, five-years single-stem phase influences harvesting system. Indeed, the large basal diameter and wood hardness of five-years old hardwood cuttings (as few as 15 cm) constrains the use of single pass cut and chips system which is the most useful and economic advantageous (Fiala and Bacenetti 2012). Since harvesting of SRC represents a significant cost in the overall supply chain of biomass production (Mitchell et al. 1999) still remains to assess the existence of a real economic benefit of five year single stem.

Conclusion

For the first time our study demonstrate the importance of the length of single stem rotation for biomass production in multi stem phases. Native black poplar from Campania Region revealed itself suitable for SRC, supporting the cultivation and conservation of autochthonous plant materials from native forest genetic resources as an alternative, ecologically viable and economically profitable plantation.

The better biomass yield observed for *P. nigra* under longer single stem rotation suggest that it can be utilized in a medium rotation coppice obtaining feedstocks for the production of Oriented Strand Board panel and pulp wood in addition to energy.

The use of native poplars genotypes for SRC should be strongly considered as possible biomass source mainly in areas subjected to particular conservation regime, where the introduction of exotic genotypes is forbidden.

To sum up, considering the large lacking of knowledge about the use of native tree genotypes for SRC in Italy, testing their filed performance under different management types and environmental condition should become one of the main stream of SRC research.

CHAPTER 2

Comparative study of hybrids and native black poplar genotypes under short rotation coppice system: insight from the first rotation cycle

Introduction

Tree species of the genus *Populus* are commonly utilized in short-rotation coppice (SRC) cultivation of temperate and Mediterranean regions of Europe. Suitability of poplars to biomass production are related to their (i) high productivity stand level, (ii) fast shoot growth and (iii) high resprouting ability. Generally, the traits targeted for *Populus* improvement are classified as either agronomic and wood quality (Stanton et al. 2015). Additionally, most of these hybrid clones that are currently being cultivated in Europe, grow vigorously as single-stem plants (Ceulemans et al. 1996; Stettler et al. 1996). Indeed, they are selected mainly for high quality straight stem production (i.e. production of long single stems with little branching, due to a strong apical dominance), rather than for multi-stem coppice biomass production which, instead, is more associated with sprouting ability (Dickmann and Keathley 1996; Laureysens et al. 2003; Stanton et al. 2010; Sixto et al. 2013). Native species and related local populations of *Populus* spp. exhibit high resprouting ability after disturbance, but they are receiving less consideration for SRC plantation (Benetka et al. 2014). Among these species, *Populus nigra* L., widespread throughout Europe, northern Africa and central and west Asia (Lefèvre et al. 2001), is a light-demanding opportunistic pioneer species growing in riparian forest and wetlands from medium to low elevation in areas with episodic fluvial, geomorphic and fire physical disturbances. Under Mediterranean climate these disturbances usually occurs asynchronous over time and space like for instance along rivers (Guilloy-Froget et al. 2002). Physical disturbances in black poplar promote vegetative propagation from stumps, broken shoots and root suckers sprouting from shallow horizontal roots (soboliferous), while seedlings regeneration is a rare opportunity in spring-summer, mainly on bare soils (Barsoum 2002; Rood et al. 2003; Lytle and Poff 2004; Slavov and Zhelev 2010).

According to Koyama and Kira (1956) frequency size distribution may be used to scaling from individual to stand level. Many authors have studied development dynamic of even-aged forest stands by a shifting of diameter distribution along the time (Pretzsch 2009). Nevertheless, few data are available in literature on the time-course of diameter evolution for SRC, particularly when shoots density decreases over time because of competition processes. Classically, modeling of diameter distribution are based on the assumption that at any point in time, the underlying diameter distribution of the stand can be adequately characterized by a pdf, say $f(D, \vartheta)$, where D is diameter and ϑ is a vector of distribution parameters (Knoebel and Burkhart 1991). So far, diameter distribution has been characterized by continuous, unimodal pdf's functions and several equation are used for fitting it. Among them, the Weibull diameter distribution as the best fit for coppice stands with different stocking levels (Sandoval et al. 2012).

This study provides an important opportunity to advance the selection criteria underling poplar biomass production. The side-by-side cultivation of native black poplar selections (cultivars) of regional provenances and selected hybrid poplar clones managed in a multi-stem sprout SRC system, allows gaining insight into its growth and demographic patterns in a shared environment. With this aim, our work, carried out in a SRC plantation in the first multi-stemmed cycle, studies the over time: i) variation of shoots diameter frequency distribution and demography, ii) above-ground dry biomass partitioning according to shoots size class on stools. As ultimate goal is to shed light on the contrasting evolutionary and man-made (humankind) time frame (trajectory) which characterize the life history of different studied poplars genotypes.

Materials and methods

Study area and climate

See main introduction

Plant material and management of SRF

See main introduction

Data used in the following study came from multi-stem rotation of treatment A.

Experimental design and shoot measurements

See main introduction

Shoots mortality

Cumulative percentage shoot mortality was determined on annual basis from the second year of rotation onwards as ratio between the number of dead shoots and the total number of shoots at the end of each growing season. Besides total number of shoots include starting cohort and the new shoots sprouted in the second and third year after coppicing.

Probability density function (PDF) model

Diameter distributions of living shoots was modelled by two parameters Weibull probability density function (PDF) that have the following form (Bailey and Dell 1973):

$$\boldsymbol{f(d) = \left(\frac{\beta}{\alpha}\right) \cdot \left(\frac{d}{\alpha}\right)^{\beta-1} \cdot e^{\left[-\left(\frac{d}{\alpha}\right)^{\beta}\right]}}$$

where α is the scale, β is the shape parameter and d the basal diameter of shoot. The parameter β is the key of flexibility of the distribution because it permits covers most of the shapes of diameter distribution (Merganic and Sterba 2006). If $\beta < 1$ the Weibull PDF show an inverse J-shape distribution that asymptotically approaches the x and y axis. For $\beta = 1$ the density function becomes an exponential PDF. It approximates the normal distribution when $\beta = 3.602$, so the skewness of the PDF approaches zero and the distribution becomes symmetric. When the shape parameter is $1<\beta< 3.602$ or $\beta > 3.602$ the Weibull density function is positively or negatively skewed, respectively.

Model estimation and evaluation

The Weibull's parameters were estimated for all poplars stands using the maximum likelihood method, widely used in adult, young and coppice plantations (Zarnoch and Dell 1985; Sandoval et al. 2012; Calzado Carretero et al. 2013). Goodness-of-fit was evaluate by one-sample Kolmogorov-Smirnov statistic (Zar 2010) at 0.05 α level. For each value of the continue variable shoot diameter (d), the test compare the proportion of value less than d with the expected proportion predicted by 2-parameters Weibull distribution. The statistic D of test, which compute the maximum differences overall D values, can be written as:

$$\boldsymbol{D = max[F(x_i) - G(x_i)]}$$

where $F(x)$ is the cumulative observed frequency and $G(x)$ is the cumulative expected frequency by Weibull distribution. Critical value for this test are referred to cut-off value *CV* used for determining if statistic *D* is significant at level specified. The accuracy of selected 2-parameters Weibull function was assessed according to the mean square error *RMSE*:

$$\mathrm{RMSE}=\sqrt{\frac{\sum_{\mathrm{i=1}}^{\mathrm{n}}(\mathrm{F(x_i)\ \text{-}G(x_i)})^2}{\mathrm{n\text{-}m}}}$$

where F_i and G_i are respectively the observed and the predicted cumulative relative frequency for all values of shoot diameter, while n and m are the number of alive shoots and the number of the parameters of model, respectively.

The error index (*EI*) was calculated by multiplying the sum of absolute differences between observed and estimated cumulative relative frequency to the basal area weighted factor $w(x_i)$ (Reynolds, Burk, and Huang 1988; Mehtätalo 2004):

$$EI = \sum_{i=1}^{n} w(x_i)\,|F(x_i) - G(x_i)|$$

where:

$$w(x_i) = \frac{g(x_i)}{\sum_{i=}^{n} g(x_i)}$$

is the ratio of basal area of each single shoot $g(x_i)$ to the stand basal area $\sum g(x_i)$. The error index (EI) is a goodness-of-fit indicator, similar to RMSE and used to compare predicted PDFs with different number of diametric classes (Sandoval et al. 2012).

Statistical analysis

A multiway factorial analysis of variance (Zar 2010) was applied to detect the effect of three following factors: year of rotation, genotypes and crown class on the aboveground woody dry biomass partitioning. Consequently, data were calculated for each of the nine poplar genotypes, in each of three-year multi-stemmed rotation, and for the tree crown classes. For each combination of the three factors, there were ten replicate values: therefore, (3 x 9 x 3 x 10) = 810 values were used in the analysis. Before the above mentioned analysis, the dataset was transformed into its logarithms ln (xi + 1) that is advisable when some of the data are small numbers (particularly zero) (Zar 2010).

Shoot diameter distributions

Based on the results of one sample goodness of fit Kolmogorov-Smirnov (Table 7), shoot diameter distributions can be predicted by a two-parameters Weibull PDF (Figure 4), even if about 7% of cases (Vesten and Ripiti, respectively at the end of first and third year of rotation) did not show a Weibull PDF distribution (Table 7). The indicator RMSE revealed that the Weibull PDF have an optimum result in terms of precision, even when evaluated the corresponding basal area weighted EI (Table 7).

The trends of the scale α and shape β parameters (Figure 5) indicate that the α values are always higher in hybrids clones compared to black poplars ($\alpha \leq 2.0$ and $\alpha \geq 2.0$, respectively) in all three years. The α value also increase in all poplars genotypes over time: in black poplars does not exceed never the threshold of 2.0 in all provenances, while in Grimminge and Vesten hybrid genotypes α values ranged from 2.362 and 2.862 in all three years.

Table 7 - Summary of fit statistics of two-parameters Weibull shoot diameter distribution for six hybrid clone (white lines) and three native black poplar provenances (grey lines) for the first coppice cycle (2010-2012). RMSE = root mean square error, EI = error index, D_{ks} and CV are Kolmogorov–Smirnov statistic and cut-off values, respectively.

Poplar genotype	**Year**	***RMSE***	***EI***	**D_{KS}**	**CV**
I-214	1st	0.027	0.015	0.076	0.153
	2nd	0.063	0.040	0.146	0.172
	3th	0.072	0.056	0.180	0.224
Grimminge	1st	0.045	0.030	0.103	0.246
	2nd	0.041	0.027	0.075	0.192
	3th	0.043	0.021	0.108	0.192
Hoogvorst	1st	0.034	0.024	0.076	0.185
	2nd	0.057	0.045	0.152	0.218
	3th	0.057	0.042	0.182	0.294
Oudenberg	1st	0.056	0.037	0.143	0.167
	2nd	0.051	0.041	0.116	0.177
	3th	0.051	0.040	0.101	0.175
Muur	1st	0.046	0.042	0.112	0.170
	2nd	0.036	0.037	0.122	0.178
	3th	0.056	0.037	0.116	0.196
Vesten	1st	0.078	0.054	0.177*	0.170
	2nd	0.065	0.048	0.145	0.174
	3th	0.085	0.041	0.161	0.185
Isclero	1st	0.053	0.032	0.132	0.116
	2nd	0.052	0.037	0.099	0.6956
	3th	0.070	0.057	0.137	0.147
Ripiti	1st	0.048	0.040	0.075	0.135
	2nd	0.044	0.030	0.117	0.150
	3th	0.041	0.040	0.094*	0.093
Badolato	1st	0.049	0.023	0.094	0.103
	2nd	0.047	0.050	0.092	0.133
	3th	0.023	0.035	0.057	0.089

*Values of *Kolmogorov-Smirnov* statistic significantly different from two-parameter Weibull at α=0.05.

In other hybrids, the threshold of α >3.0 is overcome in the third year (I-214 and Oudenberg), from the second years (Hoogvorst), and in the entire rotation (Muur) (Figure 5).

On the other hand, the β values decreases over time in hybrid genotypes, with the exception of Hoogvorst, and increase in black poplar genotypes with a narrow range of β values: from 1.42 to 2.38 and from 1.47 and 2.03 for hybrid and black poplar genotypes, respectively. The standard error values of two parameters was influenced by size of the dataset, in our case by the reduction of the absolute number of shoots over time, due to mortality and, specularly by recruitment of new shoots.

At the end of first growth seasons (2010), all PDFs are right skewed (β<3.68) because of rapid establishment of social hierarchy among shoots, with exception for Grimminge that approximate normal diameter distribution (β=3.68).

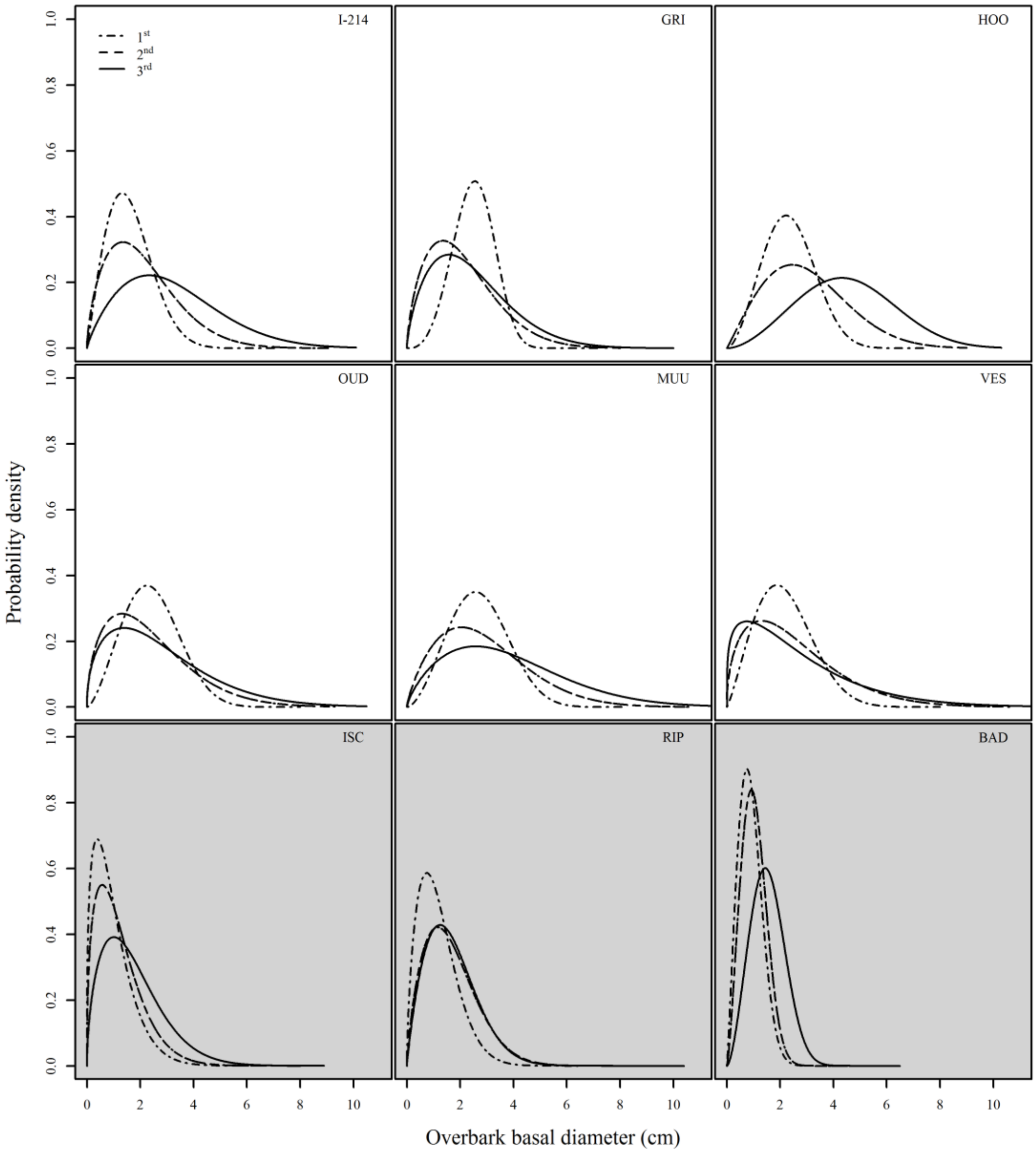

Figure 4 - Weibull function for living shoot diameter distributions of six hybrid clones (white background) and three native black poplar provenances (grey background) of Table 1. Shoot basal overbark diameter data, measured at 0.05 m from the base, come from the first coppice cycle 2010-2012 (1st is 2010, 2nd is 2011 and 3rd is 2012). See Table 1 (Main Introduction) for poplar genotype codes.

During the subsequent year (2011) the shape parameter decreases ($1.47 < \beta < 2.03$) in hybrids genotypes and distribution become markedly skewed and right tailed as a results of growth, mortality and in any cases recruitment population processes.

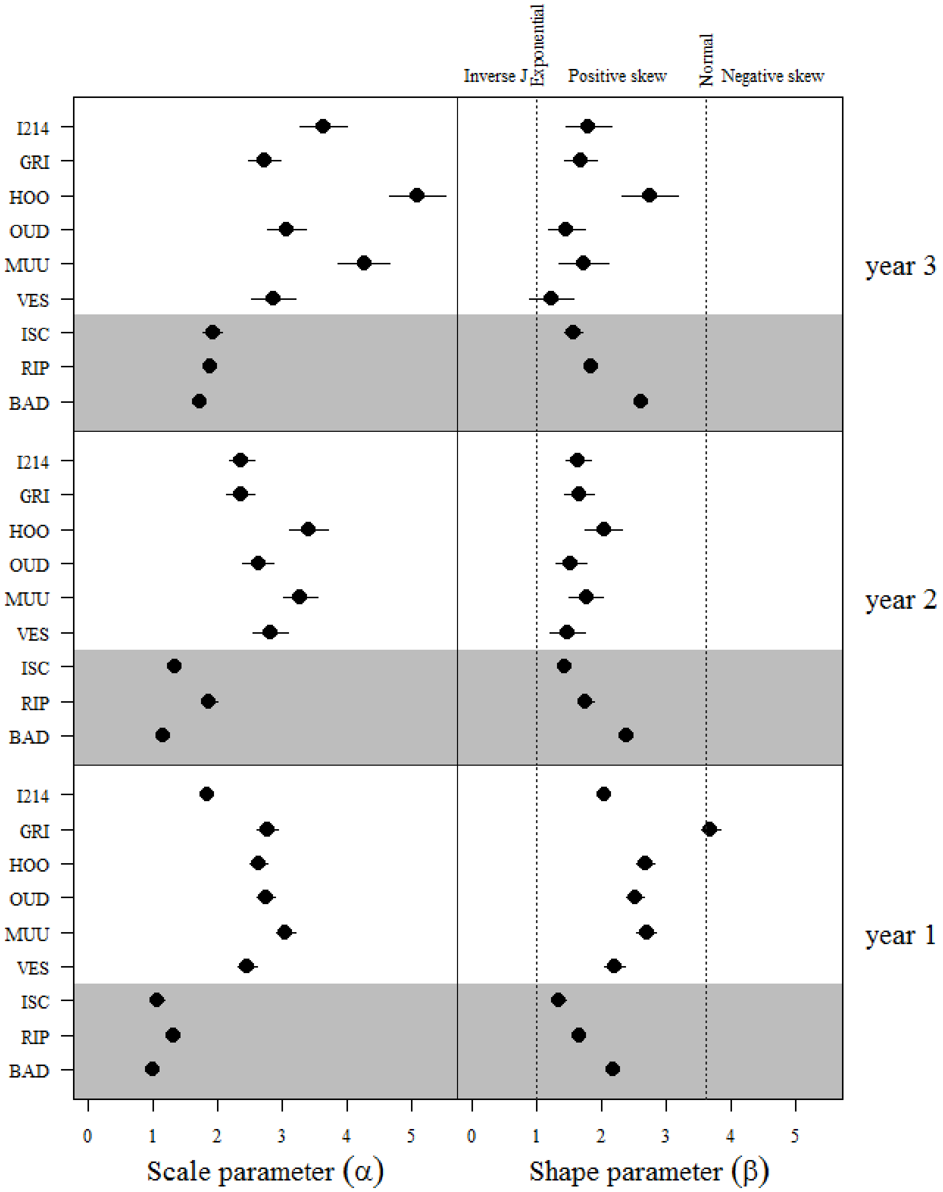

Figure 5 - Scatterplot of the estimate scale (α) and shape (β) parameters of Weibull pdf for six hybrid clones and three black poplar provenances at the end of each growing season of first coppice cycle 2010-2012. Horizontal bar is standard error of estimated parameter values. See Table 1 (main introduction) for poplar genotype codes. Positive and negative skew represent right and left asymmetry, respectively.

Grimminge, Oudenberg, Muur and Vesten hybrid genotypes exhibit a clear recruitment of suppressed shoots with shift of distributions to the origin of x-axis. This process was absent in black poplar genotypes.

Table 8 - Coppice stand attributes of six hybrid clones (withe lines) and three native black poplars provenances (grey lines) for the first coppice rotation (2010-2012). The stand was established in March 2007, and coppiced in December 2009. Values are mean number of shoot per stump (N), quadratic mean diameter (cm), mean regressed height of quadratic mean diameter of shoots (m), SSD is the stem specific density (kg m-3), AGDB is the cumulated stand aboveground dry biomass of stem and branches (Mg ha-1), and AGBI is dry biomass percentage annual increment (%). In brackets standard deviation. Lower case letters indicate differences at α=0.05 according to Newman-Keuls multiple range test. See Table 1 in main introduction for poplar genotype codes.

Year of coppice cycle	**Poplar genotype**	**Mean shoots per stump** (N)	**Quadratic mean shoot diameter** (cm)	**Shoot mean height** (m)	**SSD** (kg m^{-3})	**AGDB** (Mg ha^{-1})	**AGBI** (%)
1st	I-214	7.6(4.6) ***cd***	1.83	2.61	333.10	3.88 (1.8) ***c***	
	GRI	2.9(1.3) ***d***	2.61	3.01	381.08	4.21 (2.3) ***c***	
	HOO	5.2(2.2) ***cd***	2.53	2.89	411.32	4.53 (1.2) ***c***	
	OUD	6.4 (2.0) ***cd***	2.64	3.47	397.37	9.50 (3.6) ***b***	
	MUU	6.2 (3.3) ***cd***	2.92	3.51	363.80	10.47 (2.7) ***a***	
	VES	6.2 (3.8) ***cd***	2.42	2.84	359.30	6.50 (2.0) ***bc***	
	ISC	17.4 (6.3) ***b***	1.37	2.08	570.90	5.05 (1.7) ***c***	
	RIP	21.7 (9.9) ***a***	1.23	2.15	496.80	6.45 (5.3) ***bc***	
	BAD	28.1 (9.8) ***a***	0.96	1.82	587.01	4.73 (1.4) ***c***	
2nd	I-214	6.0 (2.2) ***c***	2.54	3.64	407.5 (57.0)	13.30 (7.8) ***b***	243.01
	GRI	4.8 (2.1) ***c***	2.49	3.22	457.7 (35.0)	12.27 (2.6) ***b***	191.26
	HOO	3.7 (1.8) ***c***	3.41	4.00	414.7 (27.5)	11.82 (3.0) ***bc***	160.64
	OUD	5.7 (3.5) ***c***	2.89	4.14	417.8 (41.6)	20.04 (9.1) ***ab***	110.93
	MUU	5.6 (2.4) ***c***	3.40	4.34	395.2 (51.2)	23.65 (6.5) ***a***	125.82
	VES	5.9 (3.0) ***c***	3.14	3.98	404.3 (33.3)	21.32 (6.1) ***a***	228.13
	ISC	13.4 (6.6) ***b***	1.96	2.63	431.4 (16.4)	8.72 (3.1) ***c***	72.58
	RIP	19.8 (5.3) ***b***	1.53	3.14	454.0 (26.8)	13.34 (±3.7) ***b***	105.91
	BAD	26.0 (7.0) ***a***	1.12	2.40	463.6 (23.8)	11.83 (6.1) ***bc***	150.41
3rd	I-214	3.5(1.1) ***de***	3.73	4.96		17.34 (7.0) ***bc***	30.34
	GRI	4.8(1.9) ***d***	2.87	3.23		15.39 (4.8) ***bc***	25.46
	HOO	2.0(1.1) ***e***	4.91	5.56		20.12 (8.1) ***b***	70.21
	OUD	5.8(3.0) ***cd***	3.41	4.85		22.33 (6.9) ***b***	11.40
	MUU	4.6(1.3) ***d***	4.45	5.49		31.30 (9.4) ***a***	32.34
	VES	5.8(3.3) ***cd***	3.56	4.45		23.24 (8.2) ***b***	9.48
	ISC	8.3(2.7) ***c***	2.09	3.04		10.03 (4.4) ***c***	14.86
	RIP	16.8(4.9) ***b***	2.10	3.25		18.92 (3.5) ***bc***	42.31
	BAD	22.7(6.9) ***a***	1.67	3.18		16.23 (6.1) ***bc***	36.97

During the third growing season (2012) the shape of the diameter distribution change for Hoogvorst (β from 2.03 to 2.74) and Vesten (β from 1.45 to 1.23) because of the high mortality rate and the additional shoot recruitment, respectively. The others poplar clones as I-214, Grimminge, Oudenberg and Muur during the latest year of rotation, and black poplar provenances during all three years did not show a clear change in the shape diameter distribution, but only a shift towards higher x-axis values. This pattern is due to the persistence of starting shoot cohort. Native black poplars show lower values of scale parameter than hybrid clones in all three years of rotation because the relative smaller size of shoots. However, their diameter distribution appears more leptokurtic ($1.00<\alpha<1.93$) than hybrid clones ($1.84<\alpha<5.09$). A marked enhancement of the scale parameters was showed by the hybrid genotypes I-214, Hoogvorst and Muur that are clones with a high growth rates (see AGBI in Table 8)

Shoots mortality

Sprouting ability documented after the first growing season in hybrid clones is about four times lower than black poplar provenances (5.8 *vs*. 22.5 shoots stool^{-1}). The same proportionality is roughly maintained over the rotation (Table 9) because of mortality and recruitment processes. Shoot mortality onset in the second growing season and reached an average of 48.8% and 31.4% at the last of third year for hybrid and native genotypes, respectively (Table 9). Splitting mortality in density-dependent self-thinning and by insect predation, the cumulated frequencies indicate higher values in hybrids clones than in black poplar genotypes for both processes (41.1 % *vs*. 29.5% and 7.4% *vs*. 1.9%, respectively). Additionally, self-thinning pertain exclusively among smallest shoots, while insect predation operate mainly on intermediate dimensional classes of shoots (Table 9). Sprouting of new shoots in the second and third year after coppicing characterize all hybrids poplar genotypes but not black poplar provenances (Table 9). It was marked in Grimminge (110%) and Oudenberg (48.5%) hybrid clones in the second and third year of rotation, respectively. In other hybrid genotypes, the recruitment not increased the number of living shoots (Table 9).

Table 9 - New sprouting and mortality of shoots (%) during the first coppice cycle (2010-2012) in hybrid clones (withe lines) and native black poplars provenances (grey lines). Mortality was classified in intraspecific competition (Self-thinning) and predation (Insect) processes at the end of the second (GS2) and third (GS3) growing season. Mean basal diameter (cm) of new sprouted shoots (d_{NS}) dead from self-thinning (d_{ST}) and by insect predation (d_I), are reported. In brackets standard deviation. See Table 1 in main introduction for poplar genotype codes.

Poplar genotype (code)	**New sprouting (%)**		**d_{NS} (cm)**		**Self-thinning (%)**		**d_{ST} (cm)**		**Insect (%)**		**d_I (cm)**	
	GS2	GS3	GS2	GS3	GS2	GS3	GS2	GS3	GS2	GS3	GS2	GS3
I-214	5.3	13.8	0.36 (0.27)	0.28 (0.56)	22.5	53.8	0.56 (0.27)	0.98 (0.56)	2.5	7.7	1.55 (0.49)	2.34 (1.23)
GRI	110.3	16.4	0.50 (0.32)	0.43 (0.21)	21.3	29.6	0.40 (0.12)	0.67 (0.26)	0.0	2.8	-	1.45 (0.49)
HOO	21.2	14.3	0.33 (0.21)	0.28 (0.53)	41.3	65.3	1.33 (1.11)	1.03 (0.60)	0.0	6.9	-	1.92 (1.02)
OUD	19.3	48.5	0.43 (0.27)	0.33 (0.36)	10.3	35.6	0.76 (0.57)	0.83 (0.36)	5.9	6.9	4.15 (0.84)	3.13 (1.16)
MUU	3.6	25.8	0.38 (0.27)	0.25 (0.90)	8.1	26.9	0.48 (0.20)	1.25 (0.90)	1.6	14.1	3.30 (0.41)	2.81 (0.62)
VES	4.8	35.4	0.22 (0.17)	0.26 (0.32)	7.7	35.2	0.52 (0.27)	0.70 (0.35)	1.5	5.7	1.90 (0.33)	2.72 (1.13)
ISC	-	-	-	-	23.0	51.7	-	0.68 (0.29)	0.0	0.6	-	1.36 (0.41)
RIP	-	-	-	-	6.9	18.9	0.43 (0.19)	0.47 (0.14)	1.8	3.7	0.90 (0.42)	1.92 (0.48)
BAD	-	-	-	-	6.8	17.8	0.34 (0.14)	0.45 (0.12)	0.7	1.4	0.82 (0.54)	1.74 (0.37)

Biomass partitioning

Factorial ANOVA of partitioned dry biomass, detected significantly differences between poplar genotypes (*df* =8, *F* =18.31, *p* <0.001), strata (*df* =2, *F* =952.98, *p* <0.001) and years (*df* =2, *F* =142.11, *p* <0.001) (Figure 5). Additionally, differences in mean dry biomass among genotypes was dependent on shoots age and crown class (*df* =32, *F* =1.77, *p* <0.01). Dominant shoots account an average of ~80% of aboveground biomass (range:76-83% during the rotation) in hybrid clones, while black poplar genotypes showed an average value of 65% (range:64-68% during the same period). The intermediate shoots of black poplar genotypes showed a mean value of 31% than 16% displayed by hybrids genotypes, while in suppressed shoots values ranged from 3% to 4%. This partitioning biomass pattern was steady during the entire course of first rotation.

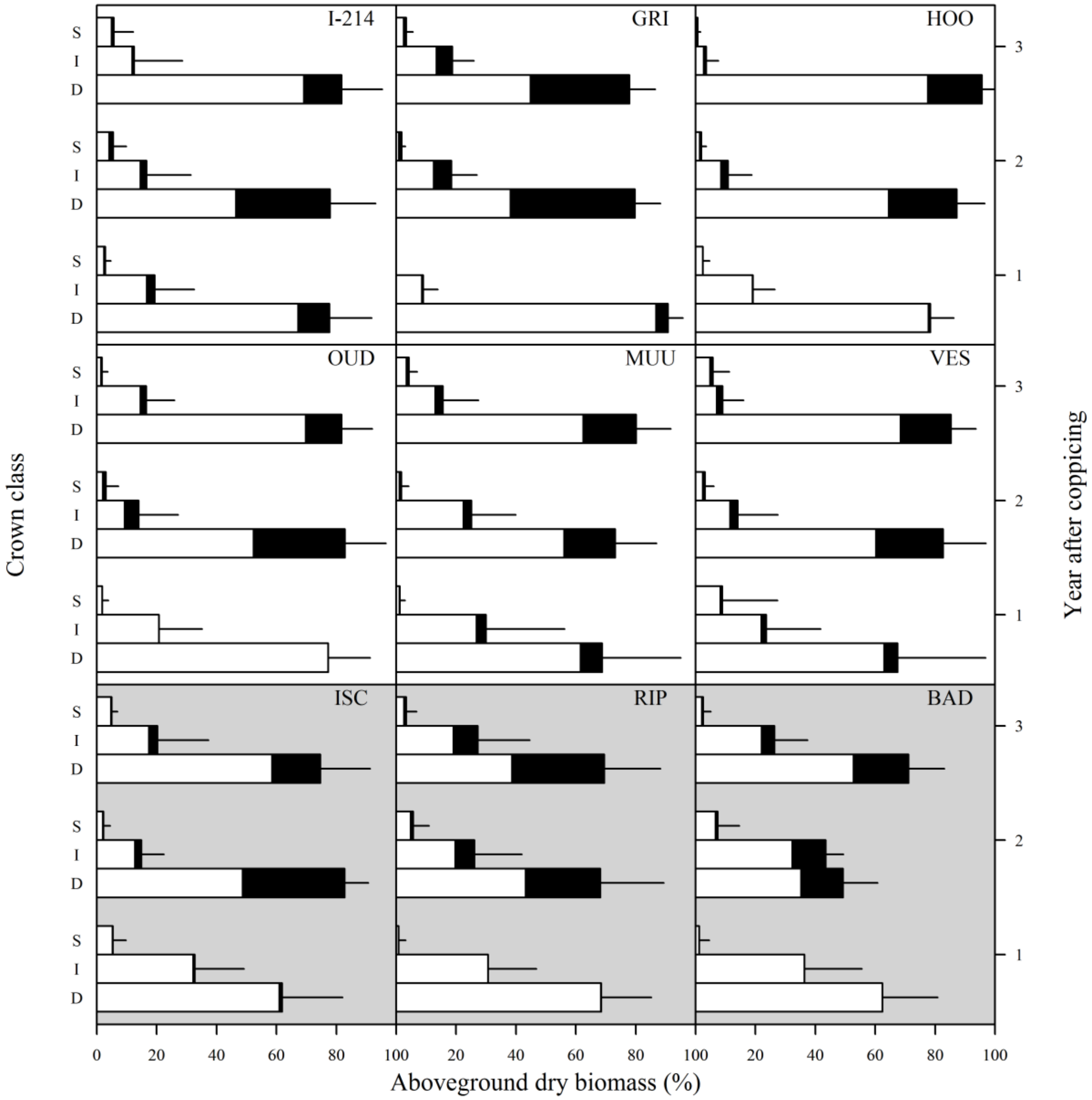

Figure 6 - Aboveground dry biomass allocated in stem and branches of shoots classified according to three crown classes (D=dominant, I=intermediate and S=suppressed) after 2010 (1st), 2011 (2nd) and 2012 (3rd) growing seasons, respectively. In the upper two panels are presented the six hybrid poplar genotypes, in the lower panels the three native black poplar genotypes. The horizontal columns shows mean value of dry biomass with associated bars of standard deviation of whole shoot crown classes. See Table 1 (main introduction) for poplar genotype codes.

After the first growing season, biomass branches proportion of dominant shoots was close to zero in all black poplar genotypes, Hoogvorst and Oudenberg, while in others hybrid clones values ranged from 4.5% to 10.5% (Figure 5). These proportions increased during second grow season for all genotypes reaching an average of 26%, 6% and <1% for dominant, intermediate and suppressed crown classes (maximum value of 43% was showed by dominant class in Grimminge genotype). It decrease proportionally after the third growing season with mean values of 19.1% and 3.6% for dominant and intermediate strata, but was unchanged for suppressed strata.

Discussion

In the present study, we document a short-time differential response in reiteration of aboveground biomass induced by coppice disturbance in hybrid clone and wild black poplars genotypes growing under homogeneous conditions in terms of climate, assigned stool-space and ontogenetic stage of roots. The ensemble of metrics analyzed over the first multi-stemmed rotation documents a different pattern of space occupancy by shoots for both genotype groups. In hybrid clones the aboveground growing space was shared by a significantly lower number of larger and taller shoots compared to black poplars. Furthermore, a more pronounced onset of size inequality because of intraspecific competition and recruitment in shoot cohorts of hybrid genotypes, is inductive of a different pattern in biomass allocation between shoots crown classes compared to black poplar genotypes. As a result, dominant shoots of hybrid clones grubbed up in average ~ 80% of aboveground biomass, while in black poplars it was ~ 65% and another one third allocated in intermediate shoots.

Inventory of resprouting after coppicing provided useful insight into the dynamic processes of our multi-stemmed rotation that occur between shoots (i.e. intraspecific competition, recruitment, mortality and growth) which can be examined through the development of two-parms Weibull PDF.

This finding was in agreement with other studies that noticed Weibull PDF as an appropriate analytical tool to describe the dynamic of tree stand, due to its flexibility and ability to fit various diameter distribution shapes. In our study, variation of diameter distribution over short time (3 years), from near symmetric to skewed asymmetric unimodal curves, depend by the frequency of intermediate and suppressed shoots, the degree of competition and, by the recruitment processes and growth rate.

Moreover, the presence of dominant shoots, belonging to starting cohort in all poplar genotypes, at the end of the third year diameter distributions are long tailed and they appear skewed with high frequency of intermediate and suppressed shoots in all poplar genotypes.

Overall, mortality processes mainly operate on the intermediate and suppressed crown classes with different magnitude among studied poplars (Table 9). Mortality by self-thinning involve the suppressed and intermediate shoots that die in any given time interval with different magnitude among studied poplars, while predation processes act mostly toward intermediate shoots of poplar hybrid clones. These two mortality processes starting from second year after harvesting with higher magnitude in hybrid than native black poplar genotypes (Table 9). In our extensively managed SRC plantation, nor chemical or biological control was used against pests and pathogens. Moreover, mortality from predation processes was mainly induced by stem borer larvae attacks (*Cryptorhyncus lepathi)* that weakened and liable stem to wind breakage.

Additionally, no wood killing fungus were observed. Recruitment processes characterized hybrids genotypes alone, contributing during the second year to the shift of PDFs to axis origin. In some hybrid genotypes, many of the sprouted new shoots death during the season of the formation without change the shoot population numbers, whereas in others genotypes these shoots were recruited with a consequent increasing of shoot number population. Many of this shoot sprouted at the beginning of spring (personal observation) and die before the end of growing season because they will be shaded when closed canopy stand will be reached. Compared to the results from the literature, we found only one studies that reported recruitment of new shoots many years after coppicing (Giudici and Zingg 2005).

Although the studies on diameter distribution in SRC plantation are few, a positive correlation of α with stand attribute such as mean or quadratic mean diameter was identified (Sandoval et al. 2012). In our case, the scale parameter (α) of PDF is a useful attribute to discriminate population size of hybrids clones from native black poplar. Additionally, the scale parameter increase over time consistent with those reported by Bullock and Burkhart (Bullock and Burkhart 2005). Indeed, shape parameter (β) was used to characterize size inequality in tree population (Knox and Peet 1989). Over the analysed rotation time, our PDFs had shape parameters that clearly indicate diameter distribution right tailed, which in turn characterize a plant population with a marked size hierarchy (Weiner and Solbrig 1984). Most of diameter distributions observed for hybrid genotypes has exhibited a decreasing value of β over time. By contrast, the native black poplar genotypes in all case showed a clearly temporal increase of such parameter. Since the change of shape parameter values depend of the high frequency of lower diameter classes and related mortality rate, it can be indicative of different shade-tolerant behaviour of shoots under coppice growing conditions.

During three years of rotation, as shown by shoot diameter distribution, the largest number of shoots is in the suppressed intermediate layers, but the greater contribution to stool dry biomass are made by dominant shoots in hybrid poplars, dominant and intermediate in black poplar genotypes. So, this different biomass-partitioning mode can be related to divergent life history among hybrids and native poplar genotypes. Selection criteria of poplar hybrids were directed to achievement of trees with long and straight stem and low branches growing under even-aged single stem plantation (Laureysens et al. 2003; Laureysens et al. 2005). Therefore, hybrids poplar accumulate proportionally the greater portion of dry biomass in few dominant shoots. In contrast, native black poplar genotypes, which growing along river channel and subjected to high physical disturbances tend to deployed aboveground biomass near-equally between dominant and intermediate crown classes.

Therefore, the relative high number of the shoots in the suppressed and intermediate classes, able to survive in shaded condition, may be related to supply of carbon to the lower portion of the stem and roots of the dominant shoots, as demonstrate by Tschaplinski and Blake (1995). Since, the latter authors evidenced that the role of suppressed shoots is clone specific, we found that in hybrid genotypes characterized by their relative high shoot mortality rate, the role of these shoots are limited to the first year, in agreement with the results of Laureysens et al. (2005), while for wild native genotypes these shoots carry out this function for more years.

The ability of poplar species to resprout after coppicing is an important criteria for their selection. In our work black poplars exhibited a higher number of shoots than hybrids clones. This finding was in agreement with Benetka et al. (2002) reported for *P. nigra* provenances after first coppicing. Indeed the majority of the hybrid genotypes included in this work were not selected for their sprouting ability (Laureysens et al. 2005). On the other hand, black poplar is one of the most relevant species of the floodplain forests (Corenblit et al. 2014; Karrenberg et al. 2002; Braatne et al. 1996) subjected to frequent injury of the aboveground biomass. Moreover, species with high sprouting ability showed a relevant carbohydrates allocation to below-ground specialized organs (Del Tredici 2001; Sakai et al. 1995; Sakai and Sakai 1998). Therefore, resprouting is clearly a crucial trait for the persistence of the species (Bond and Midgley 2001) in highly dynamic river ecosystem with episodic major disturbance, such as flooding and other short-term erosion events.

Conclusion

The cultivation of black poplar and hybrids poplar genotypes under the same site conditions has allowed us highlighting their different behaviour under the high disturbance regime inflicted by the short rotation. Unlike hybrid poplars, the black poplar genotypes have a higher number of shoots per stool, and a biomass mainly distributed among shoots belonging to intermediate and dominant classes. Instead, hybrid poplars, with a lower number of shoots, tend to amass aboveground biomass mainly into the dominant class. Therefore, life history of selected and wild poplar genotypes contributed markedly to different behaviours under short rotation coppice regime.

The allocation of biomass to harvestable organs, such as stem and branches (Ceulemans 1992) and relative proportion of wood and bark tissues on plant parts are also matter of interest because influences directly the energetic properties of lignocellulosic biomass (Adler et al. 2005; Guidi et al. 2008; Quilhó and Pereira 2001).

CHAPTER 3

Size inequality in poplar short rotation coppice system

Introduction

Management regime in SRC foresee coppicing iterated under short time interval, generally 2-3 years. Coppicing affects resprouting ability, recruitment and mortality of shoots and their longitudinal and radial growth. Lastly, these ecological processes affect aboveground biomass yield of tree plantations. In fact, stem population is allometrically related to canopy stand structure, which in turn affects solar radiation interception and consequently, the potential growth rate of the plants (Newton 2007). Moreover, stem number affects water and nutrient availability, primarily through belowground competition between individual trees. In high density tree plantations, intraspecific competition for resources and space increases with growth, leading to stem number decline through mortality, while individual biomass of the surviving stems increase, following the negative density-dependent self-thinning rule (Oliver and Larson 1996).

Due to ecological, evolutionary and economic implication, plant population dynamics has received much attention, in particular for even-aged and monospecific tree stands (Damgaard 2005). In plant monoculture, each individual plant competes with conspecifics for the limiting resources. In this context, studies on size inequality were used to detect competition among individual plants (Weiner and Thomas 1986). The basic concept of competitive interaction, described by size inequality, is the way of sharing limiting resources between individuals which can lead to size symmetric or asymmetric competition (Weiner and Thomas 1986; Schwinning and Weiner 1998). When resources are proportionally shared by size, the competition type is defined symmetric, while it is defined asymmetric if a disproportional resource sharing does exist. Light represent a resource that generates size asymmetric competition. In fact, in crowded plant population, large individuals intercept most of the light resource, causing a steep vertical gradient of resource availability and inducing the suppression of small individuals (DeMalach et al. 2016). For these reasons, competitive asymmetry has been considered a driving force of mortality and size inequality in plant population. In contrast, below ground competition for water and soil nutrients appears to be symmetric where contested resources are shared in proportion to competitor size (Schwinning and Weiner 1998)

Many studies evidence the existence of an asymmetric competition in plant population. These studies focused their attention mainly on the effect of competition on population structure or on its time variation (Thomas and Weiner 1989; Bourdier et al. 2016).

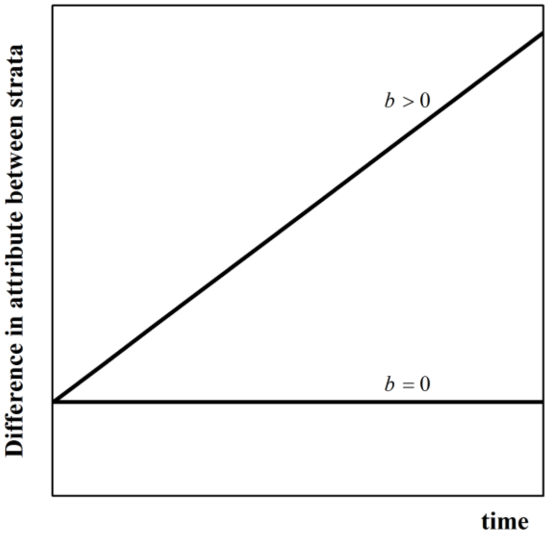

Figure 7 – Schematic representation of competition types as proposed in this study for coppice plantations. The values of slope (b) of linear relationship between differences in size attribute among strata as a function of time can be used to define competition types. Slope is b=0 as consequence of proportional sharing of resources by size, the differences between strata is absent and the competition can be defined as symmetric. Under asymmetric competition slope b>0, implying that resources are disproportional shared by size, with a greater differences in attributes between strata.

Based on this background we hypothesized that the size asymmetry or inequality can be quantified by calculating the coefficient in size attribute differences (delta) among crown classes (as defined in chapter

2) of shoots as a function of the time (Figure 6). It was choose to conduct the study using only two crown classes (dominant and intermediate) because, as emerged in the chapter 2, more than 95% of the aboveground biomass is allocated to these two crown classes.

The coefficient can be identified by the slope of a linear function relating the differences in size attributes among individuals. The lack of differences in size attributes among classified individual over time is the result of a symmetric partitioning of the resources and can be described by a slope value equal to zero (b=0). When the differences in size attributes among individuals increase over time, the slope of linear relationship is positive and greater than zero (b>0). This implies that resources are disproportionally shared by size and the crown classes differ strongly in size attributes.

Therefore, the aim of the study is to compare the traditional indices of variability used into the analysis of competition and resources partitioning to the proposed method in high-density coppice stand.

Size inequality in tree plantation

Each individual in a community competes both with conspecific and with plant belonging to others species. More generally, Begon et al. (2006) define competition among organisms in the following terms: “Competition is an interaction between individuals, brought about by a shared requirement for a resource, and leading to a reduction in the survivorship, growth and/or reproduction of at least some of the competing individuals concerned.” When a population is composed by competing individuals belonging to the same species the competition is called intraspecific.

In a plant-plant interaction, neighbouring plants limit the growth of each other because they compete for the access to a specific limiting resource. Therefore, the competition types that can be observed in a plant population depends exclusively by the resources and the size of competing plants. In fact, different individuals, may respond to intraspecific competition in different way. When a generic plants population is characterized by a weak intraspecific competition, the individual size attributes such as weight, diameter or height, are symmetrically distributed around the mean (Figure *7*). When intraspecific competition is at its most intense level, the distribution of the attributes become positively skewed, with many small individuals and few large ones (Figure 7).

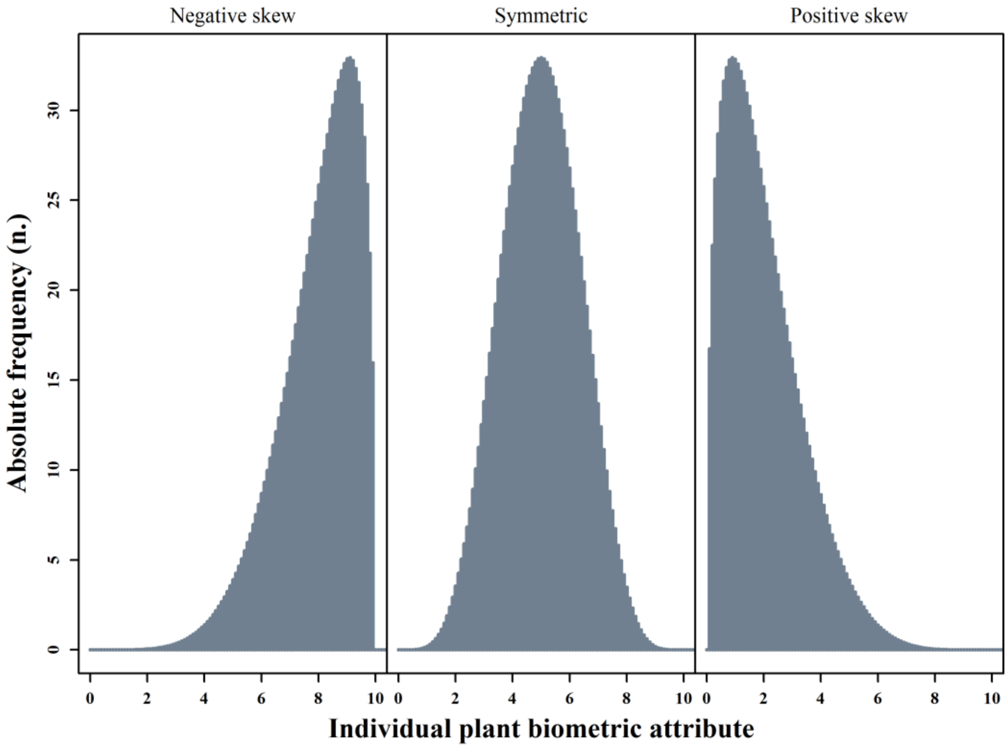

Figure 8 – Types of unimodal frequency distribution for individual size attributes in a generic plant populations.

Plant size frequency distribution have been described in several different ways by ecologists (Newton 2007). In some cases, size distributions are not characterized mathematically, in several other cases histograms have been presented to depict graphically the inequality embedded in frequency distribution, restricting statistical analysis to normality (Benjamin and Hardwick 1986).

Generally, variation in plant size has been described and analysed by using statistical moment or statistics derived from the moment of the size distribution (Weiner and Thomas 1986; Bendel et al. 1989). Two statistics derived from moment of distribution are used for many years in describing of size inequality of plant populations: Skewness and Coefficient of Variation (Ford 1975; Higgins et al. 1984; Bendel et al. 1989).

Typically, plant population showed positively skewed size distributions as a results of an intense competition among conspecific individuals (Higgins et al. 1984; Knox and Peet 1989). This characteristic of size distribution suggests that plant populations have an high size inequality.

Weiner and Solbrig (1984) have argued that inequality more directly reflect competitive interaction and that size inequality or size hierarchy, as the term used by ecologists, are often synonymous with the concept of size inequality. They highlighted that the concept of size inequality or concentration correspond to the notion of size hierarchy and not asymmetry, and proposed as approach the use of Lorenz curve to describe inequality in plant populations (Weiner and Solbrig 1984).

In the Lorenz curve, individuals are ranked by size (*x*-axis), and the cumulative proportion of plants is plotted against the corresponding cumulative proportion of their total size (*y*-axis) (Figure 9). Lorenz curve is a straight diagonal line, called line of equality if all individuals of population are the same size. If population has any inequality in size, then the Lorenz curve fall below the line of equality. From the Lorenz curve, the size-inequality may be summarized by the Gini coefficient (see Material and Methods section) representing the ratio between the area enclosed by the line of equality and the Lorenz curve, and the total triangular area under the line of equality (Figure 9). Although, Gini coefficient has been used as measure of inequality in size in plant population in numerous studies (Geber 1989; Laureysens et al. 2005; Nord-Larsen et al. 2006) it does not include all the information of the Lorenz curve. Otherwise, the same Author that proposed the use of Gini coefficient to describe inequality in plant population highlighted that different Lorenz curves can exhibit the same Gini coefficient (Weiner and Solbrig 1984).

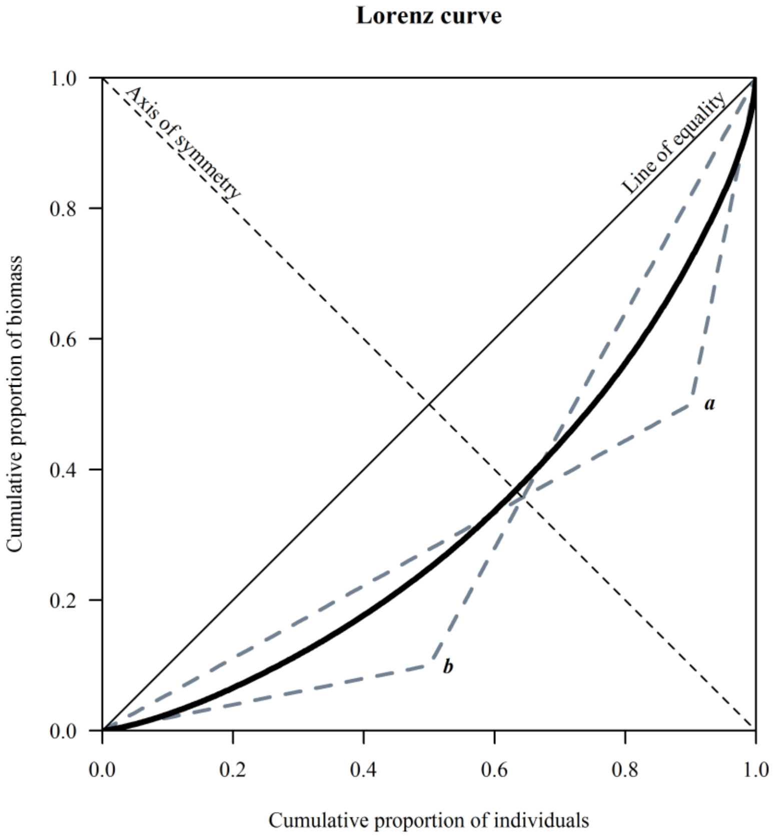

Figure 9 – The Lorenz curve. A generic symmetric case (dark and bold line) and two size-asymmetric cases (grey and dotted line). In case ***a*** size inequality of plant population is due to the very few individuals that contain a very large proportion of population's biomass. In contrast, case ***b*** represent plant population with the same degree of inequality related to the relative large number of very small individuals which in turn contribute very little to population's biomass. Data of the cases ***a*** and ***b*** came from Damgaard and Weiner (2000).

The curve ***a*** and ***b*** (Figure 9) represent two cases of size inequality in plant population (Damgaard and Weiner 2000) related to biomass distribution. In curve ***a*** the size inequality is from the occurrence of very few and large individuals, that have a large proportion of total population's biomass, while in the case ***b*** the same degree of inequality is due to the relatively large number of small individuals that are contributing very little to the total biomass of the plant population. This two cases can be analysed by the asymmetry statistic derived from Lorenz curve through the use of the Lorenz asymmetry coefficient (Damgaard and Weiner 2000).

A Lorenz curve is defined symmetric if the estimated curve is parallel to the line of equality at the axis of the asymmetry, and since the value of Lorenz asymmetry coefficient is equal to 1 (Figure 8). When the point in which Lorenz curve is parallel to line of inequality is above to the axis of asymmetry, the value of Lorenz asymmetry coefficient is greater to 1 (Figure 9, case ***a***). Correspondingly, if Lorenz asymmetry coefficient is lower than 1, the point where Lorenz curve is parallel to the line of equality is below the axis of asymmetry (Figure 9, case ***b***).

Material and methods

Study site

See main introduction

Plant materials

See main introduction

Data used in the following study came from multi-stem rotation of treatment A.

Shoot classification and size attributes differences

Shoot population were classified according to crown classes or strata (horizontal layers): suppressed, intermediate and dominant (Oliver and Larson 1996), that were related to the stool maximum height <50%, 50-80%, >80% of maximum height for suppressed, intermediate and dominant shoots, respectively. Classification of crown classes reflect aboveground available growing space and light use efficiency which translate in a different biomass allocation pattern, more favourable in dominant related to the other subordinated crown category (Oliver and Larson 1996). In order to determine the type of competition we calculated absolute differences in mean size attributes (basal diameter, quadratic diameter, mean quadratic diameter, total height, dry weight) between dominant and intermediate shoots, where dry biomass is mainly allocated (Figure 5, Chapter 2).

Size inequality indices

Size inequality of poplar shoot populations over time is described by the coefficient of variation (CV), skewness (g_1), Gini coefficient (Ĝ) and Lorenz's asymmetry (S) (Damgaard 2005). Size variability indexes were determined on stand attributes namely the basal diameter and the total height of the shoots, which are indicated in the following equations with the letter *x*. Therefore, we calculate CV as ratio between standard deviation values of underlying stand variable to their arithmetic mean. CV ranges between 0 and 1, when variability of sampled population is low or high respectively. According to Bendel *et al.* (1989) CV is invariant to frequency distribution scale changes but not for location changes.

$$CV = \frac{\sqrt{\dfrac{\sum_{i=1}^{n} x_i^2 - \dfrac{\left(\sum_{i=1}^{n} x_i\right)^2}{n}}{n-1}}}{\dfrac{\sum_{i=1}^{n} x_i}{n}}$$

The skewness (g_1) was estimated as the ratio between third and second central moments of frequency distribution of stand variable considered. According to Bendel *et al.* (1989), g_1 is invariant to frequency distribution scale and location changes. Values $g_1>0$, indicate a right-tailed frequency distribution, on the contrary values $g_1<0$ depicted distribution left-tailed.

$$g_1 = \frac{\dfrac{n\sum_{i=1}^{n}(x-\bar{x})^3}{n-1}}{\left(\dfrac{\sum_{i=1}^{n}(x-\bar{x})^2}{n-1}\right)^{3/2}}$$

Gini coefficient (Ĝ) was estimated as proposed by Weiner and Solbrig (1984) for measurement of size inequality among plant population. Its values ranged between Ĝ=0, if all observation are the same, to Ĝ=1, if only one observation differs from the others. As CV, coefficient Ĝ is a measure of relative precision invariant to scale but not invariant to location changes (Bendel et al. 1989). Ĝ was calculated as a function of the sum of the absolute differences between all pairs of observation scaled by mean and sample size to obtain dimensionless coefficient (Allison 1978):

$$\hat{G} = \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} \left| x_i - x_j \right|}{2n^2 \bar{x}}$$

Weiner and Solbrig (Weiner and Solbrig 1984) argued that different frequency distribution might exhibit the same values of coefficient Ĝ. In these cases, was proposed the measure of Lorenz asymmetry coefficient (S) to detect differences in frequency distribution and describe the population inequality (Damgaard and Weiner 2000). Coefficient S conveys symmetry (equality) when its value is equal to S=1, and asymmetry (inequality) when S≠1. S and g_1 shared the same properties of invariant. We calculate the coefficient S for an ordered sample (x_1, x_2,..x_m,x_{m+1}, .. x_n), such that x_i is the size (basal diameter or total height) of shoot *i*:

$$S = \frac{m + \delta}{n} + \frac{L_m + \delta x_{m+1}}{L_n}$$

$$\delta = \frac{\mu - x_{m+1}}{x_{m+1} - x_m}$$

where *n* is the sample size, *m* is the number of plants with a size less than average μ, x_{m+1} is the plant with a size above than μ, x_m is the plant with a size equal to μ, $L_m = \sum_{i=1}^{m} x_i$ is the sum of the plants with a size less average value than μ and $L_n = \sum_{i=}^{n} x_i$ is the sum of the plant sampled.

Statistical analysis

A general linear model was applied to analyze the relationship between absolute differences in size attribute of classified shoots as a function of time. To test the difference in the parameters of regression equation, a dummy variable that identifies two level of qualitative attribute was included in the model, as follow:

$$Y_i = a + bX_i + a_1 D + b_1 DX$$

Where Y_i and X_i are the independent and dependent variable, respectively. Categorical variable D (0,1) represent the dummy variable having two levels that can be in our case the genotype group as Hybrid and Native poplar. Dummy variable assumes arbitrarily two values 0 and 1 so that, for example of a simple linear model we have:

We assign D=0, so that equation is:

$$Y_i = a + bX_i$$

When D=1, we obtain the following equation:

$$Y_i = (a + a_1) + (b + b_1)X_i$$

The parameters a_1 and b_1 represent the variation, which can be negative or positive; than a and b parameters of the linear equation. The introduction of the variation of parameters into the model was tested via *t*-test with a level of significance of 0.05. To test the hypothesis about equality of two genotype groups (hybrid and native) in slope coefficients, the Student's t-test was used (Zar 2010).

A bootstrap ordinary non-parametric resampling procedure was applied to quadratic basal diameter and height of shoots to estimate the standard deviation of the corresponding size inequality indexes (Efron and Tibshirani 1993; Dixon et al. 1987; Damgaard 2005), with a bootstrap sample size of 500. All analysis were performed in R statistical software (R Core Team 2016) with a p-value considered significant when lower than levels $\alpha = 0.05$.

Results

Size inequality indices

Basal diameter

Time-trend for size inequality indices for basal diameter, quadratic basal diameter and total height is reported in Figure 10. The estimated coefficients indicated an increasing time-trend of both CV and $\hat{G}$

for basal diameter in hybrid clones. While, black poplars showed a decreasing time-trend. Indeed, intercepts (a, a_1) and slopes (b, b_1) are statistically different (Table 10). In particular, intercept was higher whereas slope was lower in black poplars than hybrid clones.

Table 10 -Last squares coefficient estimates of linear regression describing the time-trend of size inequality indices for poplar hybrid and black poplar genotypes in a first 3-year rotation coppice (2010-2012). Group of genotypes is encoded as dummy variable where the two levels are Hybrid and Native. SE represent standard error of estimated. Intercept a and slope b are coefficients for hybrid group, a_1 and b_1 for black poplar group.

	Basal diameter (cm)				**Quadratic basal diameter (cm^2)**				**Total height (m)**			
	Estimate	SE	*t*-value	*p*-value	Estimate	SE	*t*-value	*p*-value	Estimate	SE	*t*-value	*p*-value
Coefficient of variation												
a	0.349	0.080	4.363	0.000	0.713	0.177	4.022	0.001	0.270	0.045	5.961	0.000
b	0.106	0.037	2.870	0.009	0.168	0.082	2.041	0.053	0.065	0.021	3.115	0.005
a_1	0.304	0.139	2.195	0.038	0.413	0.307	1.345	0.192	-0.038	0.079	-0.485	0.632
b_1	-0.134	0.064	-2.091	0.048	-0.108	0.142	-0.761	0.454	-0.010	0.036	-0.278	0.784
Gini coefficient												
a	0.201	0.038	5.362	0.000	0.389	0.059	6.559	0.000	0.156	0.026	6.063	0.000
b	0.053	0.017	3.073	0.005	0.068	0.027	2.463	0.022	0.036	0.012	3.031	0.006
a_1	0.167	0.065	2.564	0.017	0.186	0.103	1.810	0.083	-0.038	0.044	-0.849	0.405
b_1	-0.079	0.030	-2.616	0.015	-0.079	0.048	-1.653	0.112	-0.001	0.021	-0.055	0.957
Pearson’s asymmetry												
a	0.590	0.333	1.769	0.090	1.152	0.451	2.556	0.018	0.189	0.311	0.606	0.550
b	0.052	0.154	0.336	0.740	0.074	0.209	0.353	0.727	0.009	0.144	0.062	0.951
a_1	-0.074	0.578	-0.128	0.899	0.202	0.781	0.259	0.798	0.937	0.539	1.739	0.095
b_1	0.290	0.267	1.084	0.289	0.475	0.361	1.316	0.201	0.086	0.250	0.347	0.732
Lorenz’s asymmetry												
a	0.966	0.067	14.341	0.000	0.930	0.044	21.215	0.000	0.910	0.074	12.315	0.000
b	-0.017	0.031	-0.548	0.589	-0.018	0.020	-0.883	0.386	-0.010	0.034	-0.299	0.768
a_1	-0.152	0.117	-1.305	0.205	-0.118	0.076	-1.554	0.134	0.208	0.128	1.626	0.118
b_1	0.079	0.054	1.461	0.157	0.069	0.035	1.976	0.060	-0.072	0.059	-1.219	0.235

Pearson and Lorenz’s asymmetry exhibited a stable time-trend (Figure 10), and a not statistically significant linear regression coefficients (Table 10). Basal diameter Pearson’s asymmetry is mostly positive (g_1 >0) except in Hoogvorst where assumes a negative value in the third year of rotation (Figure 10), reaching a value of maximum value of 2.0 in the third year of rotation for Badolato. In 83% of the cases, Lorenz’s asymmetry showed a value lower than 1.0 with a minimum of 0.68 value observed for Hoogvorst in last year of coppice rotation. Value of S above 1.0 are exhibited in about 17% of the cases, mostly represented by Vesten clone in each year of the rotation.

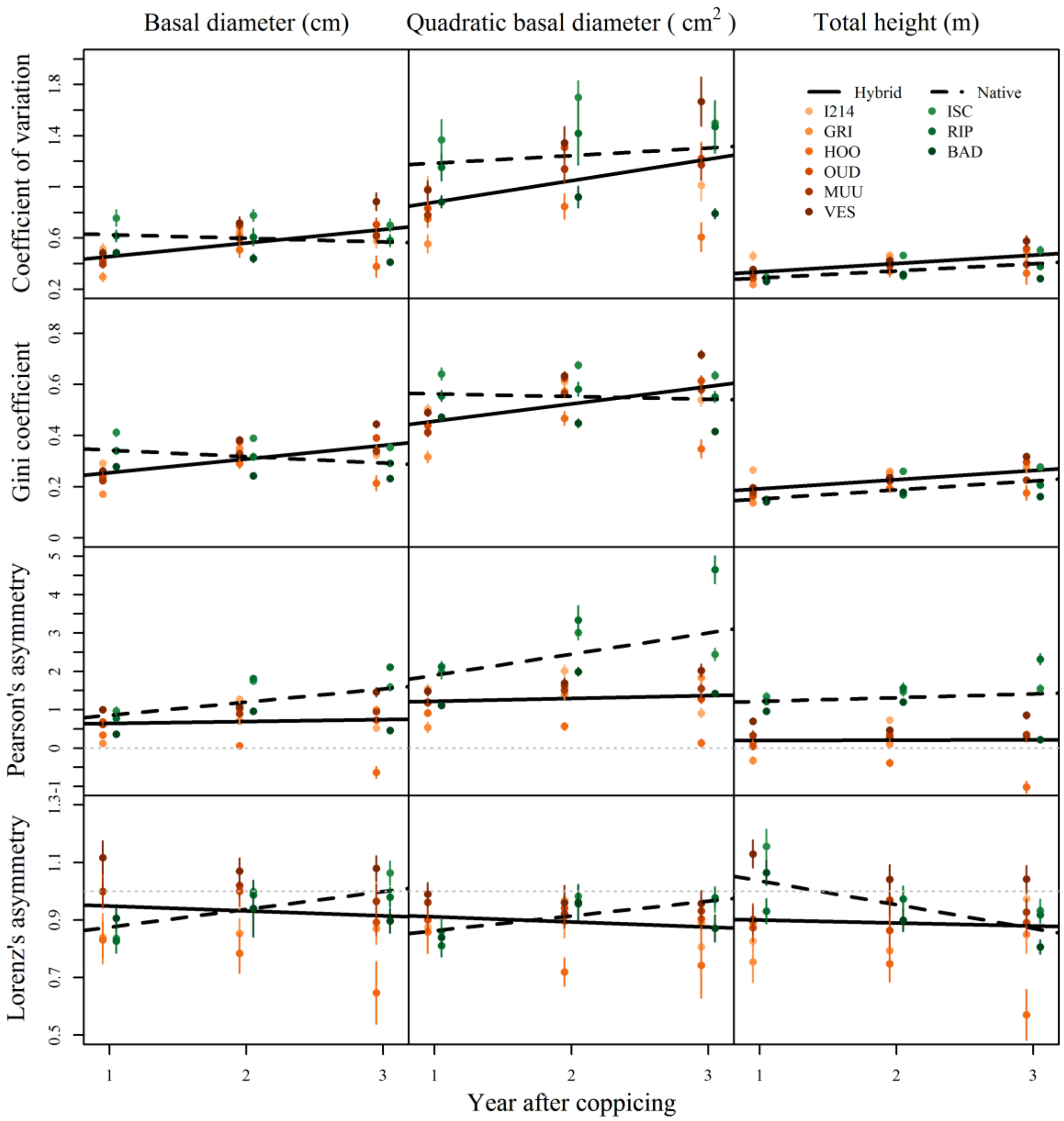

Figure 10 – Size inequality indices for basal diameter, quadratic basal diameter and total height frequency distributions of hybrid clone (orange) and native (green) poplar genotypes during the first 3-years rotation coppice (2010-2012). Solid and dashed lines represent linear trend for hybrid and native poplar genotypes, respectively. Dotted grey lines indicate values of symmetry for Pearson’s asymmetry (g_1=0) and Lorenz’s asymmetry (S=1), respectively. Values are bootstrapped means and error bars are related standard errors. See table 1 (main introduction) for poplar genotype codes.

Quadratic basal diameter

The calculated linear regression coefficients indicate an increasing time-trend of both CV and $\hat{G}$ for quadratic basal diameter in both genotype groups. Indeed, coefficients in hybrid genotypes are significantly different from zero but not statistically different from black poplars (Table 10). Pearson's and Lorenz's asymmetry tends to be stable (constant) along rotation time. Values of g_1 are and S are above and below 0.0 and 1.0 in each year of rotation, respectively.

Height

For total height, the time-trend of CV and $\hat{G}$ increase in all group of genotypes (Figure 10). No significant differences in linear coefficients are observed between hybrid and native poplar genotypes (Table 10), Pearson's and Lorenz's asymmetry show a stable time-trend (Figure 10). Values of g_1 are close to zero in hybrid clones whereas are close to 1.0 in hybrid genotypes along time rotation. Only 17% of the S values are above 1.0, while the 83% are below 1.0, reaching a minimum in Hoogvorst in the last year of rotation.

Size attribute differences in classified shoots

The table 11 shows the result of a general linear regression model used to predict differences in size attributes among classified shoots as a function of year of first multi stem rotation 2010-2012 . Linear regression was significant for each variable at 0.05 level of significance with an *adj*R^2 ranged from 0.62 to 0.67 (Table 11).

Table 11 – Fit statistics for general linear regression model describing the differences in size attributes of classified shoots as a function of time, for poplar genotypes in a first 3-year rotation coppice (2010-2012). Where MSE is mean square error obtained from cross validation with k-fold=5; R^2 and adjR^2 are multiple and adjusted coefficient of regression, respectively; F and *p*-value are Fisher's statistic and significance level.

Variable	**MSE**	**R^2**	***adj*R^2**	**$F_{(3,23)}$**	***p*-value**
Basal diameter (cm)	1.56	0.66	0.62	15.19	<0.001
Quadratic basal diameter (cm^2)	1.96	0.69	0.65	17.36	<0.001
Quadratic mean basal diameter (cm)	1.77	0.68	0.63	15.41	<0.001
Height (m)	0.68	0.71	0.67	18.50	<0.001
Dry weight (kg)	2.23	0.71	0.67	18.88	<0.001

Annual increment in diameter and height of each poplar genotype (Table 8 of chapter 2) does not full describe growth dynamic in shoot cohorts. Instead, when increments are calculated separately according to dominant and intermediate shoot crown classes (Figure 11) emerges that difference in radial growth is significantly different between genotype groups (quadratic basal diameter (cm): *t*= -2.271, *p*= 0.033). In particular, linear regression slopes in both genotype groups are positive and significantly higher in hybrid clones than black poplars (Table 12). Basal diameter (D, *t* = - 1.822, p = 0.082), quadratic mean diameter (Dg, *t* = -1.862, p = 0.081) and longitudinal growth (total height (m): *t*= -1.627, *p*= 0.117) not significantly differed over the time in the two crown classes of hybrid and black poplar genotypes. More relevant are the differences in aboveground dry biomass increment, which diverges significantly among hybrid and black poplar genotypes (comparisons of the slope (kg): *t*=-2.146, *p*=0.043).

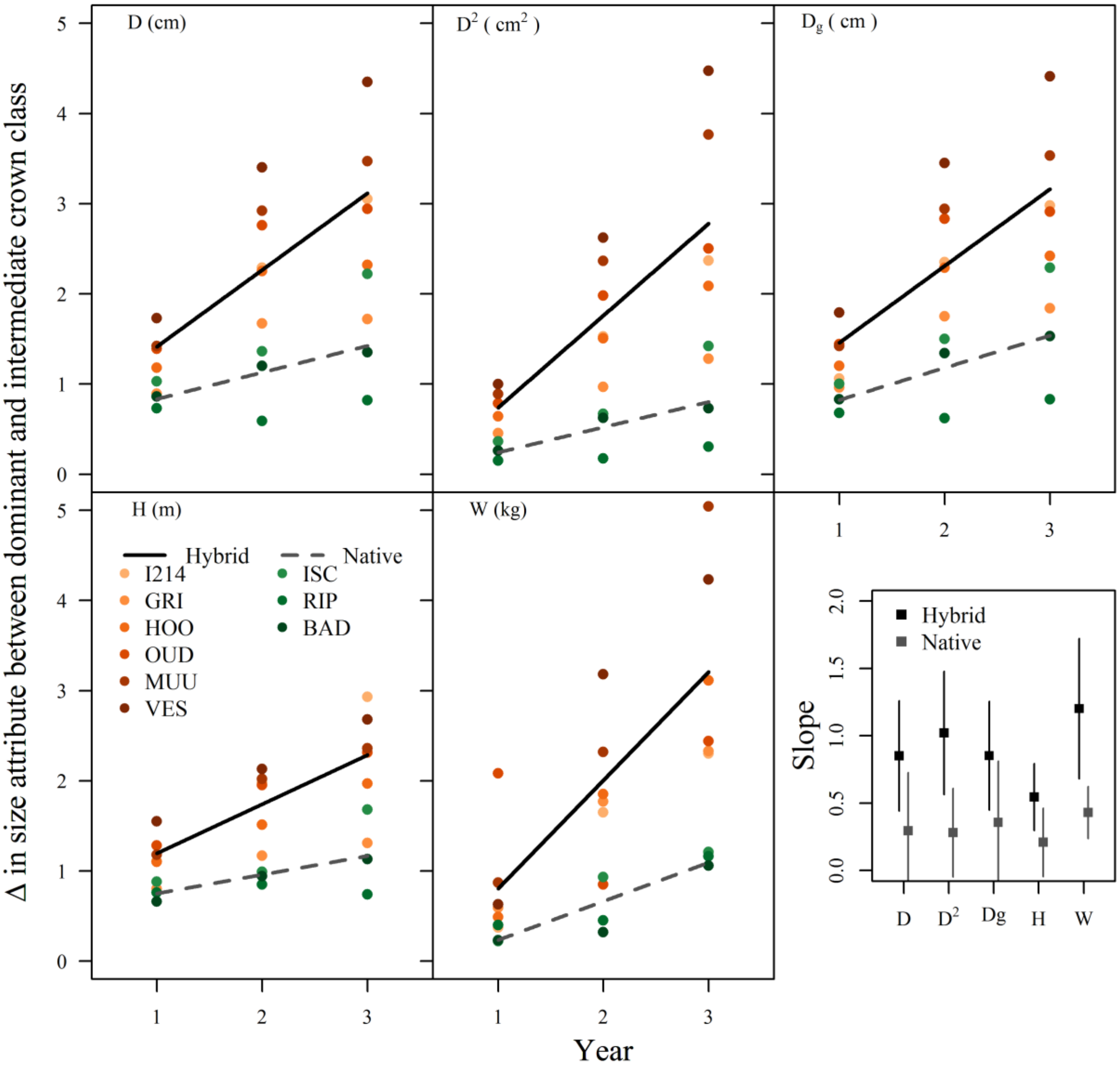

Figure 11 - Differences between dominant and intermediate crown strata in mean values of basal diameter (D, cm), quadratic basal diameter (D^2, cm^2),quadratic mean basal diameter (Dg, cm), total height (H, m) and woody dry weight (W, kg) during each three years of the first coppice cycle (2010–2012) for hybrid clones and native black poplar provenances. The right lower panel show the mean and 95% confidence interval of the estimated slope values of the linear regression between strata differences in D, D^2, Dg, H and W. See Table 1 (Main Introduction) for poplar genotype codes.

In the third year of cycle, dry biomass was significantly higher in most hybrid clones compared to black poplars (range: 31.3 *vs*. 10.1 Mg ha^{-1} in Muur hybrid clone and Isclero black poplar provenance, respectively).

Table 12 – Last squares coefficients estimates of linear regression describing the differences in size attributes of classified shoots as a function of time, for poplar genotypes in a first 3-year rotation coppice (2010-2012). Group of genotypes is encoded as dummy variable where the two levels are Hybrid and Native. SE represents standard error of estimate.

	Estimate	SE	*t*-value	*p*-value
Basal diameter (cm)				
a [hybrid]	0.564	0.381	1.482	0.152
b (year) [hybrid]	0.851	0.176	4.830	0.000
a_1 (genotype) [native]	-0.025	0.659	-0.038	0.970
b_1 (year x genotype) [native]	-0.556	0.305	-1.822	0.082
Quadratic basal diameter (cm^2)				
a [hybrid]	-0.284	0.407	-0.697	0.493
b (year) [hybrid]	1.021	0.188	5.421	0.000
a_1 (genotype) [native]	0.244	0.705	0.347	0.732
b_1 (year x genotype) [native]	-0.741	0.326	-2.271	0.033
Quadratic mean diameter (cm)				
a [hybrid]	0.606	0.379	1.597	0.124
b (year) [hybrid]	0.852	0.176	4.849	0.000
a_1 (genotype) [native]	-0.139	0.657	-0.212	0.834
b_1 (year x genotype) [native]	-0.495	0.304	-1.627	0.117
Height (m)				
a [hybrid]	0.651	0.230	2.831	0.009
b (year) [hybrid]	0.545	0.106	5.119	0.000
a_1 (genotype) [native]	-0.109	0.398	-0.273	0.787
b_1 (year x genotype) [native]	-0.337	0.184	-1.826	0.081
Dry weight (kg)				
a [hybrid]	-0.398	0.448	-0.887	0.384
b (year) [hybrid]	1.202	0.208	5.789	0.000
a_1 (genotype) [native]	0.202	0.777	0.260	0.797
b_1 (year x genotype) [native]	-0.772	0.360	-2.146	0.043

Discussion

The observed time trend in both CV and $\hat{G}$ for the basal diameter (treated as continuous variable, i.e. not considering variable classified according crown classes) suggests different competition type between hybrid and native black polar genotypes. The increasing time-trend of CV highlighted an asymmetric competition type in hybrid clones, where larger shoots get a disproportionate share of resources relative to their size. Conversely, black poplar genotypes showed a decreases of CV, which in turn should reflect a proportionate share of resources according to symmetric competition type. When the variables analyzed are quadratic diameter and total height, time-trend of CV and $\hat{G}$ increase in both genotype groups, suggesting the existence of an asymmetric competition type. Although the sensibility of CV to tail changes in the frequency distribution, a less robust statistic than $\hat{G}$ (Bendel et al. 1989), it seems important to select the variable that better depicts resources allocation. $\hat{G}$ was currently calculated on quadratic diameter frequency distribution to depict size inequality and competition type in tree stand by Nord-Larsen et al. (2006). In hybrid poplar short rotation coppice plantations, CV and $\hat{G}$ were calculated on shoot diameter distribution, predicting an asymmetric competition (Laureysens, Deraedt and Ceulemans 2005).

Furthermore, no consistent trend in Pearson and Lorenz’s asymmetry of shoots size variable were found during the rotation. Therefore, it would be difficult to use these statistics to depict competition type in coppice stands. g_1 and S are essentially a shape statistics (Knox and Peet 1989) able to give information on which size contribute to variability of size frequency distribution, rather than measure inequality in plant population. Positive skewness ($g_1>0$) was reported for diameter both in tree plantation (Gates 1982; Knox and Peet 1989) and in the first year of hybrid poplar short rotation coppice (Laureysens et al. 2005). Quadratic basal diameter show high value of Pearson’s asymmetry, reflecting the squared transformation of the variable. Skewness for height variable is close to zero in hybrid genotype groups and about 1.0 in black poplars, suggesting that shoot height population are probably normally distributed in hybrid poplars whereas is asymmetrically positive in black poplar. A values of height skewness from -1 to 1 were reported frequently in tree plantation (McGown et al. 2016; Knox and Peet 1989). For all shoot size variables, values of S are almost always less than 1.0, suggest that the size inequality in our coppice plantation is due to a relatively large number of small individuals. In tree plantation were also observed S values that were very close to or below 1.0 (Metsaranta and Lieffers 2008; McGown et al. 2016).

Size inequality analyzed by values of regression slope evidenced that both hybrid and native poplar genotypes are characterized by an asymmetric type of competition. However, it was observed that differences in size attributes diverge more markedly in hybrid than native poplar genotypes. This suggests a different degree of asymmetric competition. Moreover, the degree of competition is much significant for difference in quadratic basal diameter and weight, than basal diameter, mean quadratic diameter and height. This suggests that for detecting the type of competition in coppice plantations, differences in shoot size attributes classified according to crown classes (i.e. treated as discretized variable) are more powerful than diameter and height size attribute, when considered linear continues variable. This is because biomass is better allometrically related to quadratic and cubic size of shoots (quadratic diameter, basal area or volume) than its linear dimension (basal diameter and total height). Not all the size variable are able to highlight inequality in tree plantation (Knox and Peet 1989). In fact, using the difference in basal diameter, quadratic mean diameter and height between dominant and intermediate crown classes no differences in slope where identified between hybrids and black poplar genotypes. Indeed, quadratic basal diameter and weight variables clearly help to detect competition type and corresponding pattern of sharing resources in coppice plantation.

The increasing differences between dominant and intermediate classes along rotation time, observed in hybrid genotypes, can be the results of distinct growth rates of the strata. In contrast, moderate differences between strata in black poplars suggest that both considered crow classes have similar growth rates. This evidence clearly highlights a size inequality much more accentuated in the hybrids than black poplars, which in turn, denote a disproportional resources allocation pattern for hybrids genotypes. Unlike hybrids, the lowest slope values for native black poplars could reflect a quasi-proportional allocation of resources, with small differences between crown classes.

One plausible ecophysiological explanation in the different degree of competition is that the interaction among shoots at high densities are mediate by competition for light (DeMalach et al. 2016). Indeed, in a dense coppice stand, like SRC, dominant trees pre-empt light and shade smaller neighbors (Laureysens et al. 2003). Therefore, light competition exacerbate differences in growth rates among neighbors much more in hybrid clones than black poplars. Additionally, this different degree of competition can be related to differences in light competition among the shoots belonging to the same genotype. As a consequences also the rate of resources acquisition are differed among dominant and intermediate shoots, with a more disproportional rate resources acquisition in dominant than intermediate.

Conclusion

Poplar genotypes growing under short rotation coppices, clearly exhibited an asymmetric competition, albeit hybrid clones showed a higher size inequality than black poplars.

This different degree of size inequality stimulates further research on the competition processes and on the eco-physiology of native poplar genotypes, to find a poplar tree ideotype with reduced asymmetry in competition even under high density of SRC.

From a methodological point of view, a categorization (discrete variable) of size variables is necessary to provide a complete description of size inequality in coppice shoot populations. To obtain a clear indication of a competition type, special attention must be paid in the selection of plant size attributes.

Therefore, we recommend the use of quadratic, cubic or mass variables, which are better related to the biomass because of sharing resources in coppice shoot population.

Future study might attempt to shed light on the competition type of the root system and on the role of the root biomass (and non-structural carbohydrate) in affecting the different size inequality.

CONCLUSIONS AND FUTURE PROSPECTIVES

In a recent economic analysis of a basic SRC supply chain Schweier and Becker (2013) found that a biomass yield lower than 7-8 Mg ha^{-1} yr^{-1} is hardly profitable. A similar minimum biomass yield level was reported also in other previous studies on profitability of poplar SRC (El Kasmioui and Ceulemans 2012; Faasch and Patenaude 2012). Although low, this productivity level is realistic and representative of a SRC established on agricultural marginal soils of lower site productivity class. Based on the threshold it was possible stated that the poplar genotypes with better performance under SRC treatment A (3 years single-stem and 3 years multi-stem) are represented by Muur, Oudenberg and Limatola whereas under SRC treatment B (5 years single-stem and 3 years multi-stem) Hoogvorst, Vesten, Limatola, Isclero, Ripiti and Badolato. Nevertheless, this evaluation is partial because more rotation cycles are necessary to better evaluating field performance of poplar genotypes.

In the past, poplar-breeding activities were aimed to the selection of genotypes having traits to grown under the traditional poplar cultivation system. Reflecting the suitability of its wood for a wide diversity of markets, breeding programs were focused on an equally wide array of improvement criteria. Improvements in agronomic traits such as yield, site adaptability, rooting ability and disease resistance invariably has been the priority in poplar breeding programs (Stanton et al. 2010). In particular, the breeding programs mainly focused on the selection of genotypes resistant or tolerant to foliar diseases, such as rust (*Melampsora* spp.) and spot (*Marssonina* spp.) infections. Quality traits related to stem form (i.e. straightness) are criteria involved in the breeding program for timber productions (Jansson, Bhalerao, and Groover 2010). Selection criteria of poplars useful for SRC system are the same, but quantitative traits such as fast grow, coppicing ability and wood stem specific density are also considered (Ceulemans et al. 1996; Stettler et al. 1996). In addition, physical and chemical traits of lignocellulosic biomass are also important in bioenergy (Anwar et al. 2014) or biopolymers (Ten and Vermerris 2013) productions. Indeed, an alternative and relative recent proposed use of lignocellulosic biomass is into the bio-based industry for the production of biopolymers. This high interest is because the cellulose is considered the strongest potential candidate for the substitution of petroleum-based polymers owing to its eco-friendly properties like renewability, bio-compatibility and bio-degradability (Isikgor and Becer 2015). In addition, also lignin represents a potential candidate to production of biopolymers (Laurichesse and Avérous 2014).

Other important current selection criteria are finalized to generate poplar ideotype able to grow on bed site quality and under low energetic inputs, to assure ecological and economic sustainability of SRC system. It is well know that industrial-scale energy production of lignocellulosic biomass require large amounts of water. Recently, the studies on poplar water use have argued that water use efficiency in SRC systems is substantially higher (Petzold et al. 2011) or similar (Fischer et al. 2013) when compared to conventional agricultural crops. Therefore, to reduce the water use one of the selection criteria of the species for biomass purposes is the efficiency in water use (King et al. 2013). In literature was reported an higher water use efficiency of wild *Populus* spp. than hybrid clone, with wild black poplar genotypes of southern Europe showing high drought tolerance (Viger et al. 2016). Therefore, in the future wild population could be mainly candidate in breeding program for lignocellulosic biomass production purpose.

The use of native species can be represent also a conservation strategies as in the case of *P. nigra*. Indeed, black poplar can be considered on the verge of extinction in a large part of the western Europe of its range distribution (Lefèvre et al. 2001). One of the main causes is the reduction of the natural habitat because of human activity (productive activities and artificial riverside defenses) that prevent the biogeomorphologial processes of river environments (Corenblit et al. 2014). Other important reasons is the widespread of cultivation of poplar hybrid genotypes which represent a potential source of genetic pollution for black poplar native populations. In particular introgression with *P. deltoides* are frequently observed (Cagelli and Lefevre 1995). Therefore, the selection and cultivation of native *P. nigra* provenances in SRC system can represent a strategy of *ex*-situ conservation of species.

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Come closer and see
See into the trees
Find the girl
While you can
Come closer and see
See into the dark
Just follow your eyes
Just follow your eyes

I hear her voice
Calling my name
The sound is deep
In the dark
I hear her voice
And start to run
Into the trees
Into the trees

Into the trees

Suddenly I stop
But I know it's too late
I'm lost in a forest
All alone
The girl was never there
It's always the same
I'm running towards nothing
Again and again and again...

"A Forest". By *The Cure*, Seventeen Seconds, 1980

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