Age-related evolution of water use efficiency in poplar clones

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AGE-RELATED EVOLUTION OF WATER USE EFFICIENCY IN POPLAR CLONES

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Acknowledgements

Abstract

Growth pattern, intra and inter annual variability of $\delta^{13}C$ in the poplar clones (*Populus* x *euramericana* hybrids) at two different sites were investigated. High growth variation was found among the clones present at Migron as no definite growth pattern was followed by the clones. BAI for the clones went on increasing till the rotation time. At Begaar the trees followed the same pattern of growth, in which there was a first slow growth period, then a rapid growth period, which then stabilizes and finally a mature growth period. However, there were some marker years found in which the growth was suppressed due to drought like 1998, 2003 and 2005. Intra annual variations for $\delta^{13}C$ was dominated by $\delta^{13}C$ depleted early wood and $\delta^{13}C$ enriched latewood at both sites with little clonal variations. *Fritzi Pauley* at Migon and *I 214* at Begaar were found to be more water use efficient than others. There was a significant clonal difference found at both sites for WUE. A moderate positive relationship was found between the BAI_{rel}l and $\delta^{13}C$ at Begaar, in which the $\delta^{13}C$was positively correlated with BAI_{rel}. *I 45/51* was the most productive clone with highest tree diameter at the rotation time and was found to be highly water use efficient after *Fritzi Pauley*.

Key words: Poplar clones, growth pattern, intra and inter annual variation of water use efficiency

Introduction

Populus sp. are tree species that normally occur in flood plains, on the riparian strips (Eckenwalder 1996). In temperate regions, it is one of the fastest growing tree species (Zsuffa *et al.*, 1996). The genus *Populus* includes about 30 species. It belongs to the family *Salicaceae*. It is a complex genus and has been divided into 6 botanical sections. Hybridizations within or between these sections have produced many natural and artificial hybrids (Altman 1999). The natural range of the genus *Populus* covers almost all Northern hemisphere (USA, Canada, Europe, Russia, China, Japan and North Africa) but representatives of the section *Aigeiros* are especially present in the Eastern USA (like *P. deltoides*) and around the Mediterranean (like *P. nigra*). In the world, 25 million hectares of poplars are currently grown. The only poplar species spontaneous in southern France is *P. nigra* whose widespread variety 'Italica' has been imported from Asia. It is often blended with *P. alba*. In North America, the cottonwood (*P deltoides*) covers a large range. The area under poplar cultivation is constantly increasing in countries of the northern hemisphere. In France, plantations increased from 100 000 ha in 1939 to nearly 250 000 ha these recent years. Poplar is the third widely grown species after oak and beech. Between 1995 and 1998, poplar plantations have increased by 1 to 2% per annum. Poplar occupies an important place in the timber industry. Unlike oak, mainly sawn, 70 to 75% of poplar wood is used for the unwinding manufacture of matches, plywood and light packaging, the latter being the bulk of the use of poplar wood. The packages made from poplar wood are much lighter in weight and France is the first producer. The rest of the production of poplar wood is used for the production of pulp and lumber. It will then make crates, furniture, beams and frames.

The American cottonwood and the European black poplar were frequently crossed to provide fast growing *Populus X euramericana* hybrids for Poplar cultivation (Ceulemans *et al.*, 1992; Stettler *et al.*, 1996). In France many cultivars of Poplar are cultivated. Most of these cultivars are *Populus X euramericana* hybrids. These cultivars are showing a large diversity in their productivity in the natural environment (table 1).

Table 1. Top six Poplar cultivars (*Populus* x *euramericana*), hybrids of *P. deltoides* x *P. nigra*) and their biomass productions, expressed in g d.wt d^{-1}. (Foret-entreprise n° 177- Nov 2007 and Monclus *et al.*, 2005).

Six most important Poplar cultivars of 2007 and their productivity		
	2007	**Production (g d.wt d^{-1})**
1	I 45/51	360
2	Triplo	271
3	Dorskamp	240
4	I 214	217
5	Koster	193
6	Flevo	187

Poplar is highly dependent on water for its productivity (Tschaplinski *et al.*, 1994). In order to extend poplar cultivation from the flood plains to areas with low water availability or where water availability varies from year to year and seasonally, more water-use efficient clones of poplar are required. In this context, one functional trait that could be used as index for high productivity under low water availability is intrinsic water-use efficiency.

Water use efficiency at integrated crop level has been defined as the net accumulated biomass in return to the water used by the crop (see Richards *et al.,* 2002, for a synthesis). At integrated plant level it has been defined as the ratio between accumulated biomass and transpired water (Briggs and Shantz 1913). At instant level it is measured as the ratio of net CO_2 assimilation rate vs. transpiration rate (Condon *et al.,* 2004) but at intrinsic level (Wi) it is defined as the ratio instantaneous rate of CO_2 assimilation (A) and conductance to water vapour (g_w) through stomata (Condon 2002). This trait can be estimated using a proxy, which is the difference between the carbon isotope composition of plant material and of atmospheric CO_2 (Farquhar *et al.,* 1982).

According to Farquhar 1989, stable carbon isotope composition of a naturally occurring material can be expressed as δ, which is the relative deviation of this compound from the internationally accepted standard PDB.

$$\delta 13C = \left(\frac{^{13}C/^{13}C_{sample}}{^{13}C/^{12}C_{PDB}} - 1 \right) \times 1000 \tag{1}$$

where the standard commonly used, is the carbon from the PDB limestone (Craig 1957). Carbon isotope discrimination is the measure of $^{13}C/^{12}C$ ratio in the plant relative to the value of the same ratio in the air on which the plant has fed (Farquhar and Richards, 1984).

As a result of human activities, the ratio of $\delta^{13}C$ in the atmosphere has decreased from −6.69‰ to −7.24‰ between 1956 to 1978 (Keeling *et al.,* 1979) and has further declined to −8.00‰ in 2003 (Direct air measurement at Assekrem, Algeria part of the Global Atmosphere Watch). $\delta^{13}C$ of atmospheric CO_2 has the current value of -8‰. Carbon isotope discrimination by the plant is expressed as:

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \tag{2}$$

where δa and δb are the concentration of $\delta^{13}C$ in the atmosphere and in the plant respectively. The factors that determine Δ are illustrated in the model proposed by Farquhar, O' Leary & Berry (1982):

$$\Delta = a + (b - a)\left(\frac{C_i}{C_a} \right) \tag{3}$$

There are two types of discrimination that occurs while the CO_2 enter through stomata and is fixed by carboxylases:

- **a** is the fractionation caused by the diffusion through boundary layers and stomata, which is -4.4 % (Farquhar & Richards 1984; Condon, 2002).

- **b** is the discrimination by Rubisco, which takes place while the CO_2 is changed to the first product of photosynthesis after the process of carboxylation (b = -27 % O'Leary 1982).

C_i and C_a are the concentrations of CO_2 in the intercellular spaces and in the ambient air respectively. Net photosynthesis which is, in this case is measured as CO_2 uptake, and the leaf conductance to CO_2, g_c, both are linked by Flick's law:

$$A = g_c (C_a - C_i)$$ (4)

Given that $g_w = 1.6 g_c$ Δ can be related to the ratio A / g_c

Δ can be related to A / g_w (intrinsic water-use efficiency) using equations 3 and 4:

$$\Delta = a + (b - a) \left[1 - \frac{1.6}{C_a} \left(\frac{A}{g_w} \right) \right]$$ (5)

where A / g_w is the intrinsic water-use efficiency so:

$$W_i = \frac{C_a}{1.6} \left(\frac{b - \Delta}{b - a} \right)$$ (6)

Where C_a is the concentration of CO_2 in the atmosphere at that time. Due to the post-industrial increase of the CO_2 levels (from 270 to 360 µmole mole-1); the stable carbon isotopic composition of the wood cellulose has been greatly affected primarily due to the anthropogenic activities (Porté and Loustau, 2001). Values taken for CO_2 mole fraction and carbon isotopic composition of the air for this study were 360µmol mole[-1] and –8‰.

Identifying clones with low discrimination and high water-use efficiency could be very useful for drought stressed areas (Braatne *et al.,* 1992). High intrinsic water-use efficiency can be achieved either by lower stomatal conductance or by higher photosynthetic capacity or by a combination of both. In spite of the fact that poplar is highly depending on water availability, some degree of variability in drought tolerance has been found among clones (Monclus 2006). There are certain features like arly stomatal closure (Marron *et al.,* 2002) osmotic potential changes (Gebre *et al.,* 1994) and other physiological changes (Marron *et al.,* 1995) that are responsible for highly variable clonal responses to drought stress.

Measuring Δ, that eventually enables us to assess the water use efficiency of the plant, is an indirect method of measurement (Farquhar et al., 1989). In trees, Ponton *et al.,* 2001 evidenced a linear and negative correlation between Δ and W_i. Δ is highly heritable in trees (Brendel *et al.,* 2002). In the experiment done by Brendel on Maritime pine, classical quantitative genetics and quantitative trait dissection (QTL) were used in order to investigate the genetic determinism of (i) carbon isotope composition in wood cellulose ($\delta^{13}C$, a time

integrated estimate of water use efficiency) and (ii) diameter growth and their relationship on adult trees (15 years). Considerable variation was found for $\delta^{13}C$ (range of over 3‰) and for ring width (range of over 5 mm) and significant heritability (narrow sense 0.17/0.19 for $\delta^{13}C$ and ring width, respectively). Four significant and four suggestive QTLs were detected for $\delta^{13}C$, the first for $\delta^{13}C$ in a forest tree species. Two significant and four suggestive QTLs were found for ring width. No co-location of QTLs was found between $\delta^{13}C$ and growth. This heritability of $\delta^{13}C$ was also shown to be correlated with the productivity; a positive relationship has been found (Johnson *et al.,* 1999) and for *Populus davidiana* (Dode) Schneider (Zhang *et al.,* 2004)

Among trees, high variability at family level (Brendel *et al.,* 2002), at provenance level (Lauteri *et al.,* 1997; Roupsard *et al.,* 1998) and at interspecific level (Guehl *et al.,* 1998; Bonal *et al.,* 2000; Ponton *et al.,* 2001) have been found.

In commercial poplar clones, productivity was found to be tightly correlated with stem and leaf traits (Ceulemans, 1990). When comparing 31, 3 months-old poplar clones of *Populus X canadensis* (Moench) in a glasshouse under constant light for productivity and discrimination, productivity was found to be tightly correlated with leaf traits like total leaf area and maximum individual leaf area was found to be the estimator of the clones with high productivity. Large clonal diversity for $\delta^{13}C$ and no correlation was found between discrimination and productivity traits (Marron *et al.,* 2005). In this persuite, studies done by Rae *et al.,* 2004; Marron *et al.,* 2005 and Monclus *et al.,* 2005 showed that there was no correlation found between the productivity and discrimination among *P. × interamericana* Brockh. and *P. × euramericana Guinier*.

This lack of correlation between Δ and productivity opens a road for the selection of clones with low discrimination and high water-use efficiency but at the same time without affecting the productivity.

In the open field study done on the 29, 3 years-old poplar clones of *Populus X euramericana*, high productivity was evident in the clones showing large total leaf area and nitrogen contents was negatively correlated with discrimination. No correlation was found between productivity and discrimination at field level, which confirmed the prospects of selecting clones with high water-use efficiency without affecting the productivity (Monclus *et al.,* 2005).

Figure 1. PCA of poplar clones (*Populus X euramericana* hybrids) for the productivity and Δ, showing genetic diversity among the poplar clones and lack of correlation between productivity traits and Δ (from Monclus *et al.,* 2005).
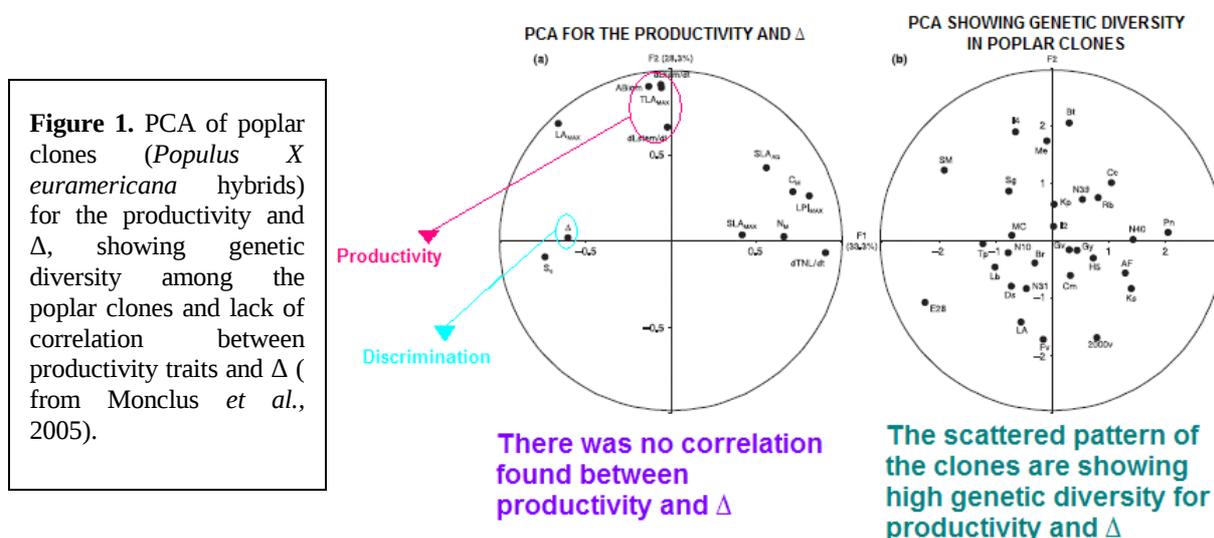
During the same experiment, the effects of moderate water stress were assessed. Moderated drought had a significant impact on productivity but the clonal ranking was maintained. Once more no correlation was found between discrimination and productivity and the same clone I 45-51 was found to be highly productive and water use efficient (Monclus *et al.*, 2006)

All these above stated results were obtained from young poplar plants of 5 years or less and cannot be extrapolated to the long term and to adult trees plantation. In particular, this juvenility is accompanied by higher discrimination and lower water use efficiency in early growth stage (Freyer 1979; Francey and Farquhar 1982), which has been shown in *Abies alba (see* Bert *et al.*, 1997). Here the question is, does the poplar clones show juvenility in their early stages of life? If that the young plants show "juvenile effect" in which the water-use efficiency is low in the early stages of plant life, does the clonal ranking of poplar clones set by the studies on the young poplar for discrimination (an estimator of water-use efficiency) remains the same? To test this hypothesis there is need to test the older individuals of the poplar clones (*Populus X euramericana* hybrids) for discrimination.

Keeping this important objective in mind this study was designed to measure

1. the inter-annual growth pattern of different Poplar clones in two different sites;

2. the temporal evolution of water-use efficiency among the poplar clones over several years of growth;

3. the intra-annual variation in $\delta^{13}C$ in the annual ring;

4. and to test that whether clonal ranking found in earlier poplar studies for Δ and W_i remains the same in the older individuals or not.

Many studies have been done to measure the temporal evolution of water-use efficiency in tree species other than Poplar. In this connection, Bert *et al.*, 1997 measured the variations of water-use efficiency in *Abies alba* over the last century using the annual rings. In his study he found that apart from the yearly fluctuation of Δ from 1860 to 1940 there was a general decreasing trend in Δ till1980. As a result of which the water-use efficiency of the *Abies alba* has increased dramatically from 1940 to 1980. This was due to the fact that the concentration of isotopic carbon in the atmosphere has decreased to a large extent in this period due to industrialization and motorization. In the above stated research, cellulose fraction of wood in each annual ring was extracted and then discrimination was calculated. Here the question is why to extract cellulose? Cellulose is used due to the facts that

it is an immobile compound in wood;

it remains confined in the annual ring in which it is formed;

its isotopic signature is rather close to that of carbohydrates in leaves and phloem, while that of lignins differs significantly due to discrimination during the first steps in the metabolic pathway.

The deposition of lignin and lipids are not confined to a single year.

Material and methods

1) Site description:

Two study sites Begaar and Migron, located in Southwestern France, were selected. On both study sites, trees were growing at a distance of 7 m from each other, at a density of 204 stems/ha. The soil of at Begaar can be characterized as clayey from 0-40 cm and silty clay from 40-200 cm, with a pH = 5.70 and presence of Fe/Mg in the second horizon. The soil of Migron is characterized as silty clay from 0-110 cm and sandy from 110-120 cm, with a pH of 7.50. The two sites are submitted an oceanic climate with a mean annual temperature of 12-13°C and a mean annual precipitation of 1130mm for Begaar and 800 mm for Migron. During a normal year, Begaar receives maximum precipitation during the months of June, July and August but on the contrary for Migron, receives comparatively low precipitation during these months. Two sites were located at an elevation of 14 and 17 m form the sea level respectively (E Paillassa, PC).

Table 2. Description of the two study sites Begaar and Migron, showing different climatic and geographical and plantation characteristics.

	BEGAAR	MIGRON
Precipitation (mm)	1130 mm	800 mm
Mean Temperature	13°C	12°C
Soil texture	Clayey Silt	Silty Clay
Plantation pattern and Density	7X7 m 204 St/ha	7X7 m 204St/ha
Elevation a.s.l (m)	14	17

2) Clone distribution and sample selection:

Distribution of clones at each site is given in the table below. It is evident from the table that all clones are unevenly distributed in the two study sites except *Flevo* and *I 214*, which are present at both sites.

A total of 27 tree disks from 8 poplar clones were sampled. There were 10 disks from Begaar, sampled in June 2006, comprising *Dorskamp, Flevo, I 214* and *Robusta* and 17 disks from Migron, sampled in June 2007, comprising 6 Poplar cultivars Blanc du Poitou, *Flevo, Fritzi Pauley, Ghoy, I 214* and *I45/51*.

The clones present were all hybrids of *P. deltoids* x *P. nigra* (*Populus* x *euramericana* hybrids).

Table 3. Distribution of clones and number of trees/clones present at the two sites

CLONES	BEGAAR	MIGRON
Blanc du Poitou		3
Dorskamp	2	
Flevo	3	3
Fritzi Pauley		2
Ghoy		3
I 214	2	3
I 45/51		3
Robusta	3	

3) Growth measurement:

Stem cross-sections were dried and polished progressively with finer grades of sandpaper to enhance ring boundaries and to make them more visible (see Maingi, 1998; Tarhule and Hughes, 2002). Three radii were selected at right angles to each other in order to cover the growth variations around the disk. Wood splits and dead knots were avoided while selecting the radii for growth measurements. Disks were cross-dated and ring-widths were measured under 1.25X magnification (Leica MZ 6; Tokyo, Japan). Software used was LINTAB 1.08© 2002. This equipment can measure width of rings on various forms of samples. The accuracy of the measurements is the hundredth of a millimeter (for details see ANNEX I). Ring width measured along the three radii was used to compute Radial increment (RI), which was expressed in mm y^{-1} and basal area increment (BAI) was calculated in cm² y^{-1}. BAI is more directly related to wood production of the tree than the radial increment (Visser 1995). This approach helped us to remove the temporal decreasing trend of ring width, which is generally visible in trees as their circumference increases (Kolb and McCormick 1993). BAI was calculated from RI using the simple equation for the area of a circle (Hogg and Schwarz 1999). Additionally, we calculated relative basal area increments (BAI_{rel}) for each tree,

$$BAI_{rel} = \frac{BAI_n - BAI_{n-1}}{BAI_{n-1}}$$

This represents the fractional change in basal area of a tree. Relative basal area increment standardizes growth relative to initial tree basal area, and thus reduces the influence of tree diameter on the growth index.

4) Annual ring separation and grinding:

Three wood slices (Width = 3cm) were excised from each disk at right angle, to take into account the circumferential isotopic variability (Leavitt and Long 1984). Annual rings were separated using a sharp razor blade and a hammer. Care was taken in determining the boundaries of the annual rings. Ring boundaries were demarcated using razor blade under the microscope on both sides of the wood slice before giving the final cut to separate the annual

ring. The annual rings were carefully cross-dated. In order to be sure of the cross dating, annual ring separation was started from the bark towards the core. As the date of tree felling was known and large and reasonably distinct annual rings formed by poplar, it was easy to cross date each annual ring. Each annual ring was further sliced into small matchsticks in order to grind them into fine homogeneous wood powder using a ring grinder (SODEMI).

Using the growth data, the year of maximum growth was selected for the analysis of intra annual $\delta^{13}C$ variability. For this purpose, annual ring formed during the selected year was further subdivided into three segments; early wood, middle wood and late wood.

5) Cellulose extraction:

At the laboratory, we followed the draft (mentioned in Quality Assurance Research INRA) MOPE-002.2 ANALYSIS: Extraction of the cellulose fraction (via the sodium chlorite) from plant material. Extraction of cellulose was done following the procedure given by Leavitt and Danzer (1993), which refers to Green (1963). This procedure consists of several stages of sample purification that are:

- Phase 1: Elimination of extractable solvents

- Phase 2: Elimination of hot water-soluble compounds

- Phase 3: Digestion of lignin by oxidizing agents

- Phase 4: Reduction of the presence of hemicelluloses

5-1 / Sample preparation

The powder samples of wood were dried in an oven at 50° C for 24 Hours. They were then, crushed through a shredder for 3 minutes. Each sample consisted of 60 mg powder wood placed in a membrane Teflon (PTFE membrane 47 mm Filter 0.45 µ m) closed with Teflon tape to form small packets. The colored beads were strung on Teflon ribbon for identification of samples.

5-2 / Elimination of extractables

It is to extract and eliminate non-structural components of wood such as tannins, fats and resins. This step is carried out in 2-stroke (double extraction) in an extractor type Soxhlet. Beforehand, samples (small packets) were counted and 40 samples were placed in the extractor of Soxhlet. The ball reservoir was filled with extraction solvent, respectively: toluene-absolute ethanol (2: 1; vv) to make up the volume to 250 ml in the case of the first extraction and then with absolute ethanol, 250 ml at the second extraction. The samples are washed successively 3 times for each extraction. The samples are then left the Soxhlet over night and left in the air under Sorbonne to remove residual solvent.

5-3 / Elimination of water-soluble compounds

This step can further purify cellulose fraction from all types of compounds like inorganic salts, polysaccharides low molecular weight, starch, some carbohydrates

5-4 / Elimination of lignins

After wax and water-soluble compounds removal, samples were de-lignified. The oxidizing agent used was sodium chlorite ($NaClO_2$) under acidic condition at 70°C. In 1000 ml of distilled water and in the presence of samples, this step proceeds in a succession of repeated additions of equal quantities of sodium chlorite (2.46 g $NaClO_2$ previously weighed / g dry weight) in a solution acidified with $C_2H_4O_2$ (p^H 3.8) depending on the time sequence that follows:

- D-Day: $C_2H_4O_2$ + $NaClO_2$

- D-Day +1:   9 pm: $C_2H_4O_2$ + $NaClO_2$

    - 11 hours: $C_2H_4O_2$ + $NaClO_2$

    - 13 hours: $C_2H_4O_2$ + $NaClO_2$

    - 15 hours: $C_2H_4O_2$ + $NaClO_2$

    - 17 hours to exit samples of $NaClO_2$ bath.

The samples were rinsed several times with distilled water and placed under magnetic agitation in a large volume of distilled water all night.

5-5 / Reducing the presence of hemicelluloses

A final purification step was done to reduce the proportion of hemicelluloses in the samples. The samples were placed in 1 liter of soda solution in an ultrasonic bath at room temperature for 45 minutes. The samples were rinsed in distilled water and neutralized in 1 liter of solution HCl 0.06N at room temperature for 30 minutes. The samples were thoroughly rinsed under running water distilled and put to dry in the air under Sorbonne ventilated hood.

5-6 / Cellulose fraction

The bags formed by the Teflon membrane that contains the samples were opened to extract the cellulose fraction. In general, cellulose was a whitish powder sometimes to the appearance of glitter and sometimes compacted.

6) Carbon isotope analysis:

$^{13}C/^{12}C$ ratio was measured with a elemental analyser (Carlo Erba, NA 1500-NC, Milano, Italy) coupled with an isotope ratio mass spectrometer (Finnigan, Delta-S, Bremen, Germany).1 mg of cellulose was combusted to CO_2 at 1800°C in sealed evacuated quartz tubes containing pre-combusted cobalt oxide and chromium oxide as the oxygen source. The column chromatography was used to separate CO_2. The mass spectrometers used for isotopic analysis generally comprises three basic sections; an ion source, a mass analyzer and an ion collection assembly. A simplified diagram is shown below
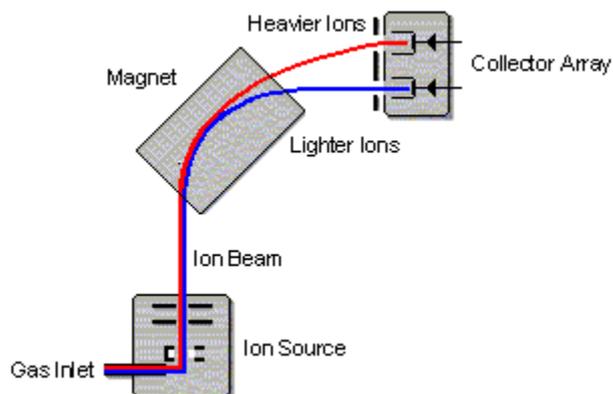
Figure 2. Simplified diagram of mass spectrometer showing three basic sections

Gas molecules are introduced into the ionization chamber where an interaction with a focused electron beam causes electrons to be stripped from the molecules, resulting in the formation of positive ions. The ions are accelerated out of the chamber, down a flight tube which is placed between the poles of an electromagnet. Here, they are separated according to their mass-to-charge ratio (m/z). The ions are typically collected by a simple collector array consisting of three Faraday cup collectors. In order to carry out IRMS analysis, only pure gases e.g. N_2, CO_2 or pure gas, contained within a carrier gas, can be analyzed.

RESULTS

1. Ring anatomy:

Poplar trees have distinct annual ring boundaries that can be easily demarcated by the porous ring structure of the vessels (Fig 3). The fine line of initial parenchyma and the width of the vessel diameter can easily distinguish one annual ring from the other. Vessels of large diameter congregate and make a highly porous line at the beginning of each annual ring. As we progress further, the width of the vessels decreases progressively and at the end of each annual ring there is a distinct band of wood without vessels. Due to this fact, the wood formed at the end of each annual ring is a bit darker in color than the wood formed at the beginning of each annual ring.
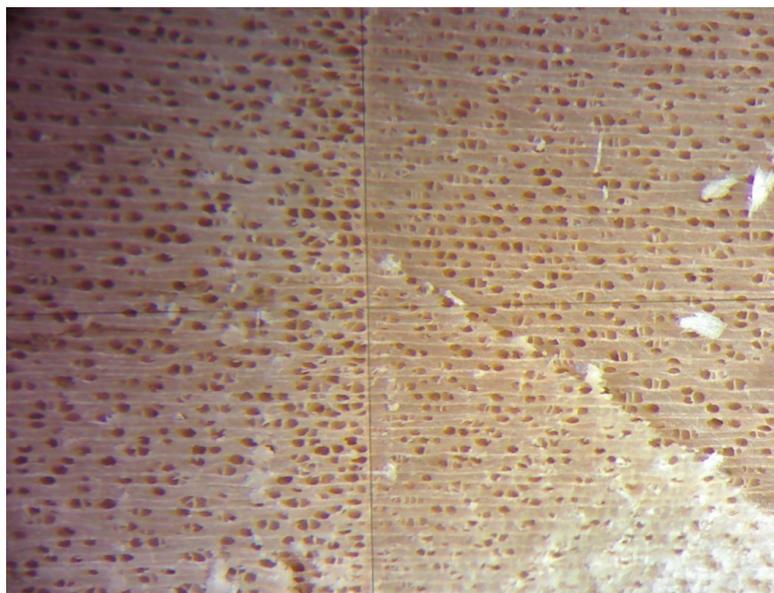
Figure 3. Photograph of Poplar wood, showing distinctively, the ring boundary between the two adjacent annual ring (1.25 x magnifications)

2. Radial and longitudinal growth:

Radial growth of all clones along the two sites followed the same pattern. The annual radial increment can be divided into four phases, first a slow growth for a couple of years, a rapid growth phase of few years which decreases after one or two years of steady growth. At Migron, RI increased to the maximum value after 3-4 years, with minimum and maximum values varying between 1.3-5.25 and 20.4-25.0 mm respectively among clones and then decreased progressively with age. At Begaar radial increment reached maximum values after 6-7 years, two years late than at Migron, with clonal variation for RI between 1.47-2.57 mm for the minimum and 19.9-29.1 mm for the maximum, which then falls down rapidly as compared to the clones at Migron. However, there were some yearly fluctuations in RI at both sites, which can inferred were due to environmental and human activities (heavy pruning done at Migron).

BAI is the total increase in cross sectional area of trunk per year. The developmental pattern for BAI was not consistent between the two sites. At Migron, a mixed pattern for BAI was observed among the clones. BAI decreased for *Fritzi Pauley* after reaching a maximum of 91

cm², remained more or less stable for *Flevo* (max = 159 cm²) and went on increasing for *Blanc du Poitou, Ghoy, I 214* and *I 45/51* till 2004, two years before the trees were felled. A decreasing trend in BAI was found among all the clones present at Begaar after reaching the maximum values in the year 1997. However, the maximum values for BAI were different among the clones, which were 141.3cm² for *Flevo*, 137.7cm² for *Dorskamp*, 138.3cm² for
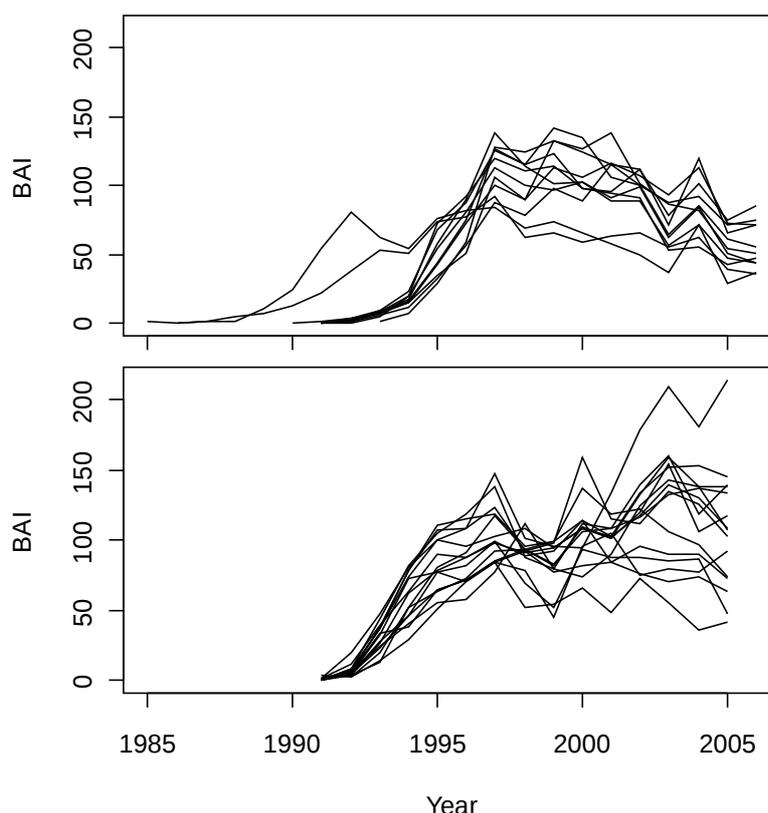
Fig 4. Basal area increment (cm²) for the clones present at Begaar (Above) and Migron (below).Each line represents a single tree.

I214 and remained lowest at 108.4cm² for Robusta.

The two sites were found to be very different in respect of longitudinal tree growth. It is evident from figure 5 that the clones present at Migron showed maximum tree height, which was measured up to 36.9m for the clone I45/51. The average tree height for this site was 34.28m (range: 30.7-36.9m). At Begaar, the maximum tree was 31.2m high, which is close to the minimum value of tree height at Migron. The minimum tree height was measured for *Flevo*, which was 27.4m. Tree height remained between 27.4-31.2m at this site.
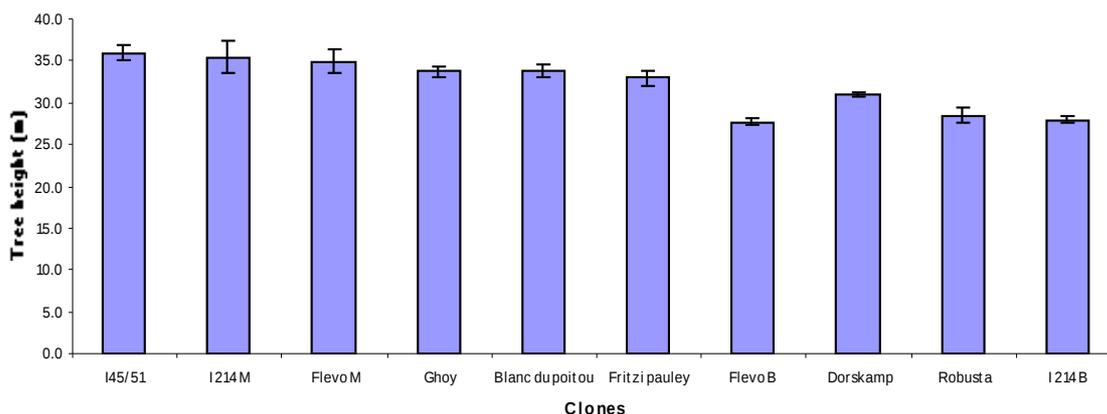
Figure 5. Mean tree height of the clones present at the two sites Migron and Begaar. Error bars represent the standard deviation.

Relative basal area increment:

BAI_{rel} was calculated for ten years (1996 to 2005) in order to detect potential similarities in growth trends among clones with time (Figure 6).
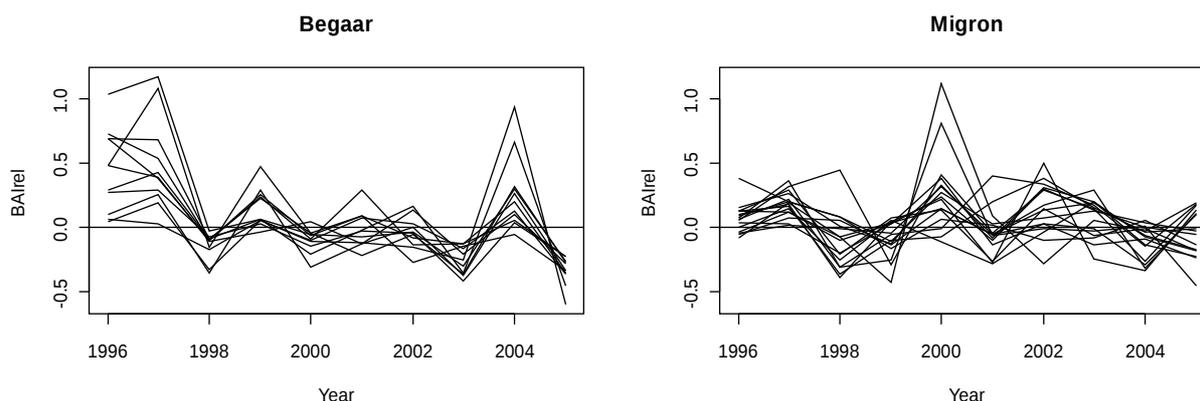
Figure 6. Inter-annual variations in BAI $_{rel}$ for the clones present at the two sites.

BAI_{rel} for all the clones at Begaar followed a well defined pattern over the period of ten years, which is evident from the graph above. Inter annual variations in growth pattern were the same for all the clones from the years 1996-2000 and then from 2002-2005. There was an increase in growth during the years 1997, 1999 and 2004 which is evident from the curve that goes upwards and was lower in 1998, 2000, 2003 and 2005 as a result of which the curve went downwards for all the clones present at this site. Clones present at Migron did not follow the same pattern, which is visible from the BAI rel curves of the clones present at this site.

Cellulose fraction analysis

The yield of cellulose extraction was obtained by weighing the cellulose mass compared to the mass of the sample powder. Mean average yield of cellulose extracted was 32±1 mg (55±8 %) from the two sites.
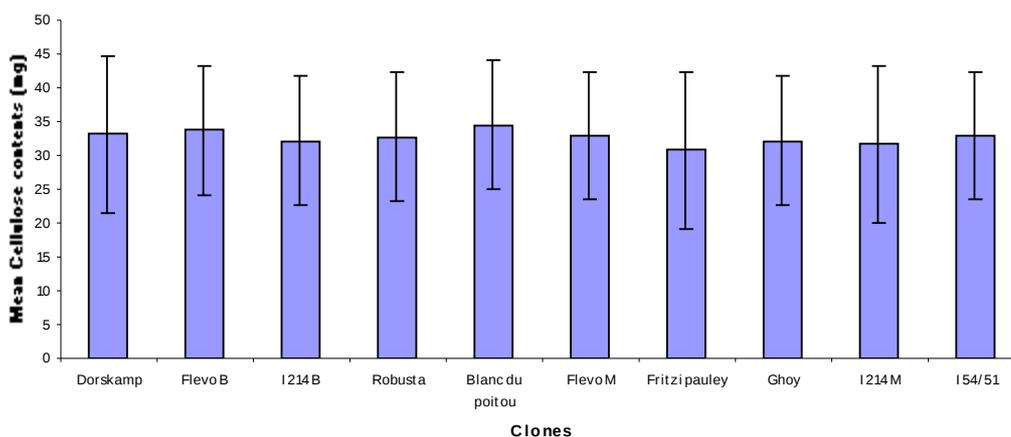
Figure 7. Mean cellulose fraction per clone at each site expressed in percentage (mg), bars are representing the standard error of the measurement. There was no significant difference found among the clones for cellulose contents

At Migron, cellulose contents were found to be highest in Blanc du Poitou, which was 34.5 mg followed by *Flevo, I 45/51, Ghoy* and *I 214* (33.0, 33.0, 32.2 and 31.6 mg respectively). At Begaar, highest cellulose contents were present in *Flevo* (33.76 mg) and lowest contents were found in I214 (32.18mg). No significant difference was found in cellulose content among clones present at Begaar. As a result of Mixed linear model taking cellulose contents as fixed and clones as random effects, significant differences were found between the clones present at Migron, *Blanc du Poitou* being significantly different from *Fritzi Pauley* and *I 214* (p-value= <0.01 and 0.031 respectively) and moderately different from *Ghoy* (p-value = 0.067). There was no significant difference between the other clones. The clones at Migron can be grouped as follows

<p style="text-align:center">Fritzi Pauley[a] < I 214[a] < Ghoy [a] < I 45/51[ab] < Flevo[ab] < Blanc du Poitou[b]</p>

Intra annual $\delta^{13}C$ pattern:

We have analyzed the intra annual carbon isotopic composition for all the clones in cellulose for the year 1997. Year 1997 was chosen because in this year all the clones of the two sites shown a maximum growth. This strengthens the fact that during this year there was no environmental constraint over the growth of the tree.
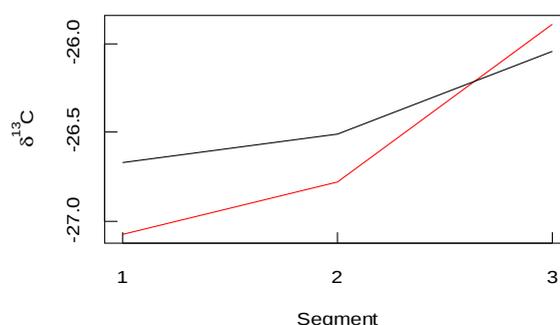
Figure 8. Intra annual variation in $\delta^{13}C$ for the two sites. Red line: Migron and black: Begaar; Early wood (1), summer wood (2) and late wood (3).

The intra annual pattern of δ^{13}C showed similar characteristics among clones and sites, with a highly significant increase of δ^{13}C at the end of the season (increase of intrinsic water use efficiency). However, the clones differed slightly for maximum and minimum values. Differences among positions in the annual year ring were tested with a mixed linear model with δ^{13}C as fixed and position as random factor. No difference was evident between the first and the second segment but the third segment was found to differ largely from the first and second ones (see table for p-values).

**Table 4.** Result of statistical analysis done on the segments, where 1, 2 and 3 represents the early, middle and late wood respectively. Significance level is represented by (*).

Segments	Std. Error	z-value	p-value	Significance
2-1	0.158	1.36	0.36	NS
3-1	0.158	5.35	<1e-04	***
3-2	0.158	3.99	0.000219	***

This pattern is consistent with the seasonal pattern of water availability and vapour pressure deficit in the atmosphere of temperate regions, which are characterized by the wet spring reason (i.e., early wood growth period) and the dry end of summer (i.e. latewood growth period).

δ^{13}C analysis

After the test of autocorrelation (whether δ^{13}C value of different year in a clone are independent of each other or not), which was found to be insignificant, variability among the clones present at both sites were tested. A mixed linear model was applied with δ^{13}C as fixed effect and clones as random, significant differences were found among the clones for δ^{13}C at Begaar as well as at Migron (Figure 9, Table 5). At Begaar, *Dorskamp* was significantly different from all other clones with a low δ^{13}C of –27.41‰. δ^{13}C for other clones, *Flevo*, I 214 and *Robusta*, δ^{13}C remained very close at –27.0‰, -26.9‰ and –26.9‰ respectively. The clones can be grouped as:

I 214[a] < Robusta[a] < Flevo[a] < Dorskamp[b]

At Migron, *Fritzi Pauley* was significantly different from all other clones, showing a higher δ^{13}C of –25.51‰. It was followed by *I 45/51, Ghoy, Blanc du Poitou, I 214* and finally *Flevo* (δ^{13}C were –25.94‰, -26.11‰, -26.51‰, -26.52‰ and –26.62‰ respectively). The clones were grouped as follows

Fritzi Pauley[a] > I 45/51[b] > Ghoy[b] > Blanc du Poitou[c] > I 214[c] > Flevo[c]

Table 5. Differences among the clones present at the two site Begaar and Migron, where (.) and (*) showing the significance levels

Clones	Std. Error	z-value	p-value	Sig
BEGAAR				
Dorskamp-Flevo	1.40	-2.96	0.055	.
Dorskamp-I214	1.39	-3.70	<0.01	**
Dorskamp-Robusta	1.39	-3.29	0.019	*
Flevo-I 214	1.25	-0.79	0.996	NS
Flevo-Robusta	1.25	-0.33	1.000	NS
I 214-Robusta	1.24	0.45	1.000	NS
MIGRON				
Flevo.M – Blanc du Poitou	0.12	-0.93	0.989	NS
Fritzi Pauley – Blanc du Poitou	0.13	9.95	<0.01	***
Ghoy – Blanc du Poitou	0.12	3.21	0.025	*
I 214.M – Blanc du Poitou	0.13	-0.10	1.000	NS
I 45/51 – Blanc du poitou	0.12	4.55	<0.01	***
Fritzi Pauley - Flevo.M	0.14	1.69	<0.01	***
Ghoy – Flevo.M	0.12	4.10	<0.01	***
I 214.M - Flevo.M	0.14	0.72	0.0998	NS
I 45/51 - Flevo.M	0.12	5.43	<0.01	***
Ghoy – Fritzi Pauley	0.13	-7.07	<0.01	***
I 214.M – Fritzi Pauley	0.15	-9.18	<0.01	***
I 45/51 – Fritzi Pauley	0.13	-5.88	<0.01	***
I 214.M – Ghoy	0.13	-2.98	0.052	.
I 45/51 – Ghoy	0.12	1.33	0.901	NS
I 45/51 – I 214.M	0.13	4.17	<0.01	***

We tested also whether there was a correlation between years and $\delta^{13}C$. At Migron, there was a decreasing trend in $\delta^{13}C$ shown by the *Flevo, Blanc du Poitou* and *Fritzi Pauley* (slope estimates were –0.042, -0.038 and –0.013 respectively). Increasing trend in $\delta^{13}C$ was shown by Ghoy, I 45/51 and I 214 (estimates 0.055, 0.070 and 0.022 resp). On the other site, Begaar, three clones i.e. Flevo, Dorskamp and I 214 showed a decreasing trend in $\delta^{13}C$ except Robusta, which showed an increasing trend in $\delta^{13}C$ (slope estimates were –0.081, -0.103, -0.073 and *Robusta* with 0.056). There were only two clones at both sites, whose slopes were found to be significantly different from other clones. They were *Dorskamp* and *Flevo* of Begaar (p-values 0.0417 (*) and 0.0585 (**.**) respectively).

Figure 9. Inter annual variations among the clones for δ^{13}C for the two sites. Higher δ^{13}C is shown by *Fritzi Pauley* at Migron (curve always above than other curves) and lower δ^{13}C is shown by *Dorskamp* at Begaar (curve always below the other curves).
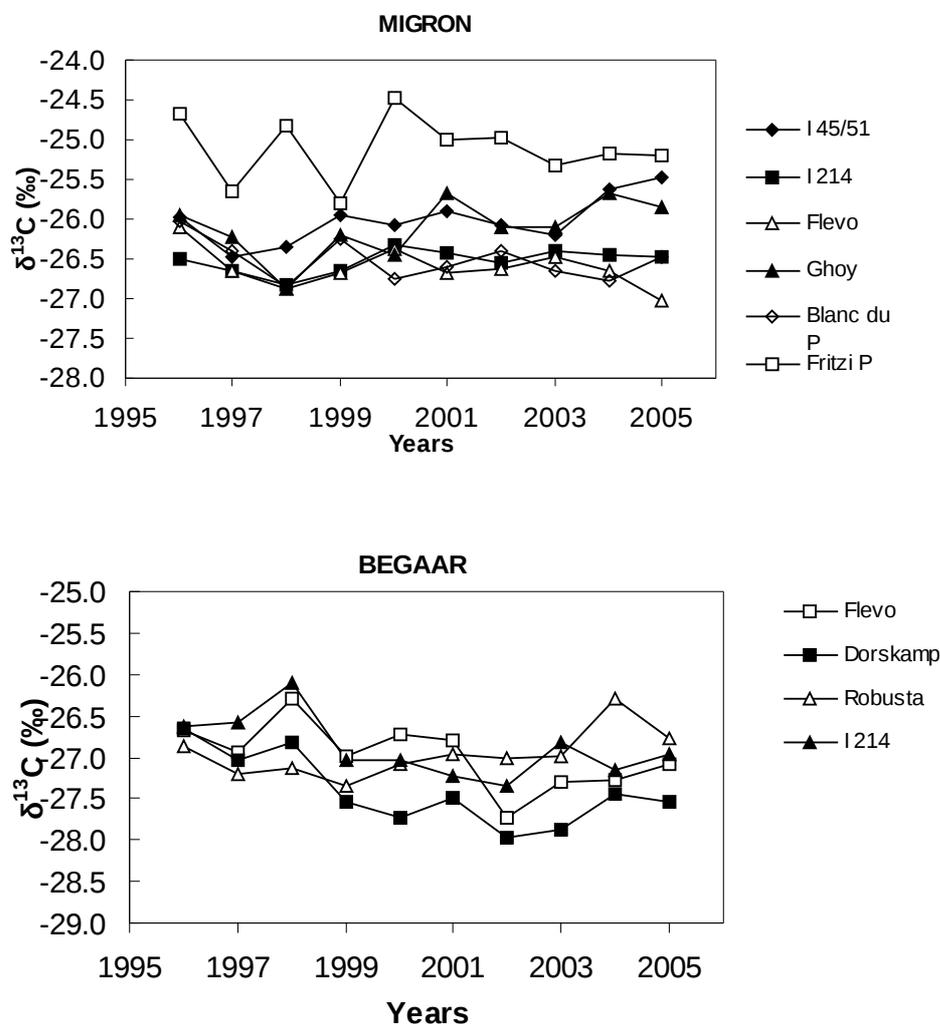
As a result of a mixed linear model with BAI_{rel} as fixed effect and trees as random, a significant relationship was found between δ^{13}C and BAI_{rel} at Begaar (p>0.0225) with a positive slope. The slope was shared by *Dorskamp, Flevo* and *I 214*. There was no correlation found between any of the other parameters like BAI and cellulose weight or BAI and δ^{13}C. There was no correlation was evident between the δ^{13}C and BAI_{rel} at Migron which is evident from the graph below.
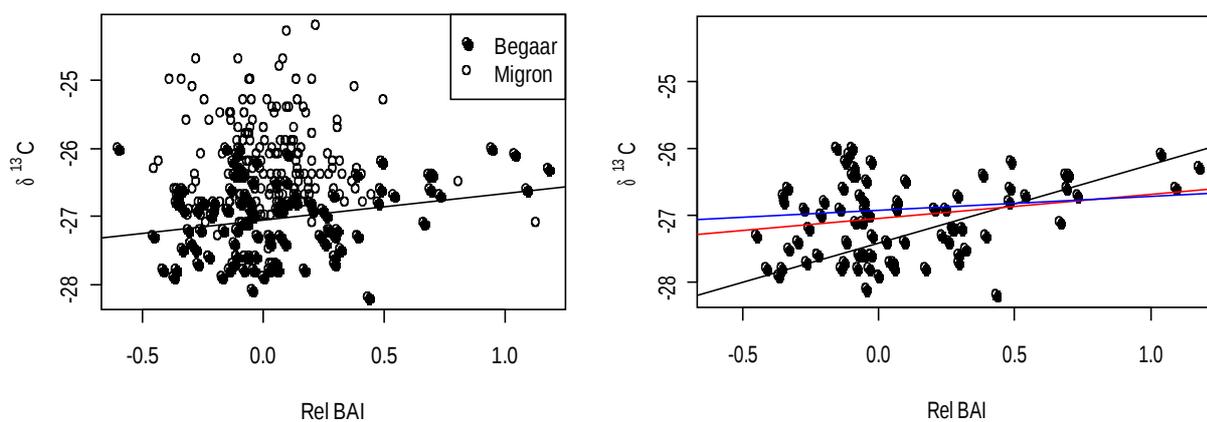
Figure 10. Relationships between δ^{13}C and BAI$_{rel}$ at Begaar and Migron. Solid circles representing Begaar and open circles represent Migron. Positive correlation was found between δ^{13}C and BAI$_{rel}$ at Begaar and the slope was shared by three clones. Black line (*Dorskamp*), blue line (*Flevo*) and red line (I 214).

DISSCUSSION

Growth patterns in poplar clones

Ring width patterns with age were analyzed in many species. These patterns vary from species to species due to the changing growth potential caused by the climatic changes, alteration of the forest community (Z-H. Li *et al.,* 2005). The growth pattern showed by our poplar clones was similar to cottonwood and other species (see Poage and Tappeiner 2002). Poplar clones showed four distinct phases of growth at both the sites, like what had been observed by Willms *et al.,* (2006) on cottonwood. However, the duration of each growth phase was shorter in our case (see Willms *et al.,* 2006). These phases can be related to an age-effect and to a lesser extent to environmental changes. The first establishment phase lasted 1-2 years; RI increased only up to 7 mm y^{-1}. This small growth was due to the fact that the trees spend a considerable fraction of resources for root development in order to generate enough root contact with the moist soil particles to sustain later rapid shoot growth (Mahoney and Rood 1998). After the first establishment phase there was an abrupt transition into an accelerated growth phase. During this phase the trees increased their radius by 20 ± 5 mm y^{-1}. This rapid growth rate was due to the existence of a well-developed root system (Rood *et al.* 1995). This stage lasted for 3-4 years. The third phase was the peak growth phase, which lasted for 1-2 years depending on the environmental conditions, the trees can sustain for long their high growth rate. The last phase was the mature growth phase: radial increment decreased gradually with time, as the tree increase in its diameter which is classical pattern of radial growth.

BAI patterns were very different at both sites. At Begaar BAI was small for the first period, but increased rapidly to reach a maximum and finally decreased thereafter. The clones present at Migron did not follow the same pattern and each clone was found to be different in respect of BAI. This effect on the BAI can be attributed to the removal of branches, which was done to enhance the longitudinal growth of the trees at Migron. The trees present at this site were found to be taller than those at Begaar.

Intra annual variation in $\delta^{13}C$ among the clones.

The carbon isotope signal in tree rings ($\delta^{13}C$) not only showed a considerable inter-annual variability but also seasonal variations (Leavitt and Long, 1991; Li et al., 1996). Seasonal variability for $\delta^{13}C$ commonly ranged form 1‰ to 2‰ and was sometimes as high as 4‰ (Li et al., 2005). This large variability in $\delta^{13}C$ resulted probably from a combination of factors like soil moisture, precipitation and relative humidity of the given site (Leavitt, 2002; Leavitt et al., 2002). Our results match those of Leavitt and Long. (1991) and Li et al (1996) in which there was a negative correlation found between tree ring $\delta^{13}C$ and soil moisture. As poplar is a deciduous species, studies have shown the photosynthetic reserves (starch) of the previous year plays a vital role in forthcoming growth (Helle and Schleser. 2004). Starch is enriched in $\delta^{13}C$ relative to the bulk leaf and stem material (Duranceau *et al.,* 1999; Jaggi *et al.,* 2002; Damesin and Lelarge, 2003). Keeping this fact in mind if the plant tends to utilize the starch reserves, the early wood should be enriched in $\delta^{13}C$. The mechanism regarding the utilization of the photosynthetic food reserve in Poplar is still unclear. A hypothesis can be that the photosynthetic reserves of the previous year are utilized before the commencement of the next year annual ring. Bud break before starting of the annual ring formation. Thus the formation of the early wood of the annual does not include the isotopic signal of the starch of the previous year. Another hypothesis can be that poplar growth does not depend upon the stored

carbon reserve and solely relies upon the current photosynthesis, keeping aside the reserve effect. The seasonal $\delta^{13}C$ pattern was the same in the trees, although there are certain differences in the maximum or minimum values (Leavitt and Long. 1991). These seasonal

Clonal ranking for $\delta^{13}C$ and correlation found between $\delta^{13}C$ and BAI_{rel}:

In this study I 214 was found to be more water use efficient at Begaar, $\delta^{13}C$ remained comparatively high at -26.90‰, followed by *Robusta, Flevo* (–26.95‰ and –26.99‰ resp.). *Dorskamp* was significantly different from all the clones but the WUE was the lowest with a low $\delta^{13}C$ of –27.41‰. At Migron, *Fritzi Pauley* was highly water use efficient and was significantly different from other clones ($\delta^{13}C$ much higher at –25.51‰). I 45/51 was the second with a $\delta^{13}C$ at –25.94‰. They were followed by *Ghoy*, *Blanc du Poitou, I 214* and finally *Flevo* ($\delta^{13}C$ were, -26.11‰, -26.51‰, -26.52‰ and –26.62‰ respectively).

Table 5. Clonal ranking found at Begaat and Migron with mean $\delta^{13}C$ shown by the clones.

MIGRON		
Clones	**$\delta^{13}C$ (‰)**	**Ranking**
Fritzi Pauley	-25.51	1
I 45/51	-25.94	2
Ghoy	-26.11	3
Blanc du Poitou	-26.51	4
I 214	-26.52	5
Flevo	-26.62	6
BEGAAR		
I 214	-26.90	1
Robusta	-26.95	2
Flevo	-26.99	3
Dorskamp	-27.41	4

There was a high variability among the clones present at Migron for $\delta^{13}C$ (determinant of WUE) , which can be explained by the model of Farquhar for the carbon isotope discrimination, which explains that as the photosynthetic capacity of the plant increases, Δ decreases accordingly. At Migron, as the branches of the trees were removed in order to enhance the longitudinal growth of stem, the photosynthetic capacity of the trees became highly variable (decreased in different intensities per tree) and thus the overall discrimination rate and hence the WUE of the trees were highly variable and thus no correlation was found between $\delta^{13}C$ and BAI_{rel} among the clones. $\delta^{13}C$ trend which was decreasing for the clones *Flevo, Blanc du Poitou, Fritzi Pauley* and *Dorskamp* suggests that at these two sites there are other strong environmental factors than a general downward trend of atmospheric $\delta^{13}C$ that has implied a strong impact on $\delta^{13}C$ composition of the wood (Tarhule and Leavitt 2004). Among trees in the same environmental conditions, the positive correlation found between BAI_{rel} and $\delta^{13}C$ suggests that an increased growth was related to a higher WUE. Stomatal conduction and carbon assimilation rate can be used in determining this correlation. As predicted by the Farquhar model of carbon isotope discrimination (Farquhar, Ehleringer & Hubick 1989) an increasing photosynthetic capacity will decrease Δ. Positive as well as negative correlations have been found between photosynthesis and growth (Johnsen & Major

1995); however, when assuming a positive correlation between photosynthetic capacity and growth, a positive correlation between $\delta^{13}C$ and growth could suggest a predominantly assimilation rate-based control of $\delta^{13}C$. This is in agreement with results for black spruce: differences among families were found to be mainly determined by differences in photosynthesis (Johnsen & Major 1995). If $\delta^{13}C$ were to be controlled by stomatal conductance, the Farquhar model predicts a negative correlation between $\delta^{13}C$ and growth. Therefore, the positive correlation between $\delta^{13}C$ and growth suggests that the variation of WUE among the measured trees is rather controlled by assimilation than by stomatal conductance. This also suggests for growth and water use efficiency a high non-genetic plasticity to adjust to environmental conditions. Isotopic $\delta^{13}C$ patterns may match with the seasonal relative humidity graphs or with the seasonal precipitation patterns (Li, 1994).

Conclusion

In this study we measured the growth pattern, the intra and inter annual variability of $\delta^{13}C$ in the poplar clones at two different sites. There was a high growth variation found among the clones present at Migron and no definite pattern followed by the clones. BAI for the clones went on increasing till the rotation time. This was due to the pruning (removal of branches), which was done to enhance the longitudinal growth of the trees. At Begaar the trees followed the same pattern of growth, in which there was a first slow growth period, then a rapid growth period, which stabilizes and finally a mature growth period. However, there were some marker years found in which the growth was suppressed due to drought like 1998, 2003 and 2005. Intra annual variations for $\delta^{13}C$ was dominated by $\delta^{13}C$ depleted early wood and $\delta^{13}C$ enriched latewood at both sites with little clonal variations. This pattern of $\delta^{13}C$ changes are consistent with the moisture curves of the temperate regions which are characterized by the wet early season which becomes dryer later in the year. There was a significant difference found among the clones for $\delta^{13}C$ both at Migron and Begaar. *Fritzi Pauley* at Migon and I 214 at Begaar were found to be more water use efficient than others. There was a moderate positive relationship found between the BAI_{rel} and $\delta^{13}C$ at Begaar, in which the $\delta^{13}C$ increased as the BAI_{rel} increased. This can be explained by the Farquhar model, which shows that as the photosynthetic capacity increases, delta decreases and thus the WUE increases. When assuming a positive correlation between photosynthetic capacity and growth, a positive correlation between $\delta^{13}C$ and growth could suggest a predominantly assimilation rate-based control of $\delta^{13}C$. This also suggests for growth and water use efficiency a high non-genetic plasticity to adjust to environmental conditions. *I45/51* was the productive clone with highest diameter at the rotation time and highly water use efficient after *Fritzi Pauley*. However, due to the constraints of site difference, non availability of all clones(*Populus* x *euramericana* hybrids) at the same site, and the silvicultural practice (removal of branches) done at Migron, we were unable to put a solid conclusion. Comparison of all the poplar clones (*Populus* x *euramericana* hybrids) in a common garden will be the best solution for checking the clonal ranking in the older individuals.

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ANNEXES

1) "LINTAB"

Using the measurement chain dendrometric "LINTAB"

This equipment can measure width of rings on samples various forms: carrots polls radial, discs and radial all other forms of samples. The accuracy of the measurements is the hundredth of a millimeter.

The operating principle of the table position is as follows: crank causes a rod micrometric which, itself, moving a tray on which the sample is placed to measure.

Preparatory Phase the measure:

Positioning of the sample on the shelf: it is absolutely necessary following the axis on which the measures will, well parallel to the axis of travel of the plateau. Setting the binocular microscope is essential for a good vision quality.

Start-up measures:

Establish sample if this was not done, the wedge horizontally through a level and ensure that the transect whereby iron on the measures follows much the axis of displacement plateau. Place the centre of the spider on the edge of the ring of departure, either heart or later bark. Go to the menu "Options" and choose the direction of movement of plateau, he will go to either right or it will go to the left. It just easily verify a direction validated by looking at the small red triangle in the bar indication registration rings, which appear to relate small green confirming recording the impulse. If the triangle is right, the board will move from right to left, and conversely if the triangle is left, the board will move on left to right. This indication is a good benchmark.

Click once on the pictogram 1 (blank sheet "New Measurement" Click once on the pictogram 5, (green button), "start measurement "then the information window opens, Fields then validate concerned. Click on the pictogram 7 "turn motor power" to establish connecting the engine and test it by controlling the alignment of the measurement axis, for example. If the rings are broad measure, it is to use the engine; otherwise the crank command is well more practical measures for low widths. In this case, disconnect the engine before the start of the measures. Set the meter to zero by clicking on the pictogram 10. "Set Origin " Before starting measurements, you must click on Trigger throttle control; only at that moment boxes "Position", "distance" and "radius" start from scratch. Make sure the menu option ", the sense of displacement Shelf is the one you want.

The measures can begin, and the display registers in boxes "Position, distance and radius." Boxes "position" and "radius" increment of the width of identifies read earlier, while the case "distance" indicates the width identifies the current measurement. In the last major case appear successively measure identifies each. You can see up to a dozen measures corresponding to the ring last measured. In the case of a mistake being measure; it is possible to erase all rings already measured by clicking successively on the pictogram 11 "Delete last measurement." They also add a little comment in the fields provided for this purpose in

clicking on the pictogram 12 "edit measurement header that also well-being as if these measures are completed but not recorded.

Registration measures:

If you have already created a file, just click on pictogram 4"append measurement" and backs up files automatically without any window open. By clicking on the icon 3 "save measurement," we can create a file "text". When the window is open, choose the menu contextual (right-click), choose "new" and then "Text document" and give a name to this file. Select the file type in which you want to convert your measures: "INRA data file" for the lab "Growth" It is possible to create other file types, such as "excel", for example, it Simply choose the time of saving, knowing that the file "Excel" includes only the width of rings placed in columns and non - combined. Save the measures by clicking on "register" Close all windows except the program LINTAB. To move to the series of measures following the return process from the point of Chapter 1 "starter measures". To save new measures to create the file, click on the icon 4 "Append measurement." If you want to save your data, click on the icon 3 "Save measurement, previous data will be automatically deleted. It is possible to create other types of files. It is recommended at the end of each sequence position to make a additional safeguard in "briefcase", for example, compressing the previous file. It is imperative to close all windows other than "LINTAB" failing which the data will not be registered and will be lost. In the menu "Options", one can choose the years of slaughter of the tree, which allows, at a glance, to see if it is well aligned on ring features previously identified. You can also choose the sense of measure, or the heart to bark, or bark to the heart. Whatever the choice, files measures are always present in the form from the heart to bark.