

# Investigation of drought reaction in juvenile aspen wood (*Populus tremula* L.)

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## Introduction

The decline of the world's resources of fossil fuels and climate change in the foreseeable future are two major reasons for a persistent demand for renewable energy research in forestry and agriculture in Germany. One option for the future energy supply mix will be the production of liquid fuels from woody biomass (Kaltschmitt 2007). Short rotation forestry in coppices of fast growing tree species - in Central and Northern Europe mainly poplar (*Populus* spp.) or willow (*Salix* spp.) - on farmland is a management system that combines the advantages of a low energy input forestry system with agricultural cropping. The energy input to output ratio (1:21) is the best compared with other bio-energy purposes (Schneider 2007) because the intensity of the agricultural activities can be reduced to the initial plantation. Furthermore, the established poplar root system can be used for an additional rotation period after the first harvesting step. Finally this management system increases the ecological value of the agricultural ecosystem for breeding bird populations, for mammals and for accompanying vegetation.

The major problem concerning short rotation coppices of poplar or willow is their low profit due to a high water demand for a satisfactory growth. Recent studies showed that there are considerable differences in intrinsic water use efficiency (WUEi) represented by  $\delta^{13}\text{C}$  between current licensed cultivars (Dillen et al. 2008, Monclus et al. 2005).

In our study tree ring investigations were used to describe the performance in WUEi and drought tolerance of two contrasting aspen populations - a natural and a planted crossbred - in their juvenile stage. We discuss the usefulness of mean annual  $\delta^{13}\text{C}$ , mean X-ray density and other tree ring specific / wood anatomical traits for selection of drought tolerant genotypes.

## Material and Methods

### *Plant material*

The aim of our study is providing data for genetic mapping of quantitative trait loci (QTL) using an aspen F1-mapping population (*Populus tremula* x *P. tremula*). This population was derived from a controlled cross between two elite aspen trees collected in the "Elbsandsteingebirge", a protected mountainous area near the Elbe river, 50 km south of Dresden, Germany. The 103 even aged F1-offspring (full-sib) were established in the greenhouse and planted in the field of the tree nursery of the Saxonian state-owned forest enterprise, Graupa near Dresden in 1998 on well drained sandy soil (1.0 m groundwater level).

A second population - 35 not even aged genotypes reference population - was selected from autochthonous material growing in natural succession on sandy dry or contrasting wet riparian sites between Berlin and Dresden, Germany. This population was used to obtain information about the typical growth and tree ring performance representative for the natural spectrum of autochthonous aspen sites under the continental climate conditions of Central and East Germany. Wood discs from stem were collected from the F1-population about 10 cm above ground and from the reference population about 30 cm above ground.

### *Cell length of vessels and fibres*

Tree ring specific wood samples representing the complete growing period were obtained as described in the paragraph "Tree ring isotopes" of this chapter. The wood samples were incubated with Jeffrey's maceration solution (Trendelenburg & Mayer-Wegelin 1955) for five hours at room temperature. After three washing steps the macerates were stained with safranin dye (1% aqueous solution) followed by additional three washing steps, careful manual cell separation in a droplet of water and fixation on a microscope slide with glycerine gelatine. Microphotos were taken at 50-times magnification. Cell length was measured for at least 10 vessels and 50 fibres per tree ring. The length of each intact cell (vessel VL and fibre FL) was measured (software package AxioVision, Carl Zeiss AG, Germany) in order to avoid bias of tree ring mean, due to subjective selection of cells with a specific length. All available tree rings were investigated.

### *X-ray wood densitometry*

The measurements of mean tree ring wood density (XD) were performed as initially described for purposes of dendrology (Eschbach et al. 1995, Schweingruber 1988) on a Dendro Workstation (Walesch Electronics GmbH, Switzerland) except some modifications described by Günther (2004). Wood extractives had not to be removed before taking the X-ray radiography because of delayed heartwood formation in aspen (Wagenführ 2007). All tree rings of the respective tree populations were investigated.

### *Tree ring isotopes*

Stem discs, approximately 1.0-1.5 cm thick, were sampled and stored at -20°C to avoid metabolic isotope fractionation or microbial activity. Cross and radial sections (20 µm and 100 µm), were dissected on a sliding microtome beginning at the same angle of the wood sample. Cross sections were stained with safranin dye (1% aqueous solution) in order to visualize tree ring boundaries positions which were transferred to the radial sections. Tree ring wood samples representing the whole growing period were separated by cutting the radial sections with a scalpel at the tree ring boundary lines. The  $\alpha$ -cellulose was extracted from the annual ring slice samples following a procedure described in detail by Wiesberg (1974). All samples were processed using sodium hydroxide for chemical disintegration and sodium chlorite for bleaching. Homogenized cellulose samples were pyrolised to obtain either CO<sub>2</sub> for carbon isotope analyses or CO for oxygen isotope analyses. These combustion gases were separately analysed for <sup>13</sup>C or <sup>18</sup>O isotope contents using an element analyser linked with a mass spectrometer (Helle 1996, Treydte et al. 2006). Only the tree rings of 2002 (rather regular weather conditions at the sampling sites) and 2003 (drought year, but increased groundwater level following the big "2002 flood") were investigated. Carbon isotope ( $\delta^{13}\text{C}$ ) and oxygen isotope ratios ( $\delta^{18}\text{O}$ ) were calculated according to Schleser (1995).

## **Results**

Significant differences between the two populations were obtained for the performance of the young trees concerning all investigated traits. For the reference population, VL and FL mean tree ring values show the expected development (Zobel & van Buijtenen 1989) resulting in an increase approximately by 50 % (FL) and 40 % (VL) with cambium age from juvenile to adult tree rings (Fig. 1). XD is more variable in the first three tree rings than in the following ones. The reference population starts growing with a high mean value of 582 kg/m<sup>3</sup> in the first tree ring (cambium age 1) and subsequently lowers the wood density in the next two tree rings to 525 kg/m<sup>3</sup>. The later tree rings have mean XD values around 550 kg/m<sup>3</sup> and show a slightly downward tendency of XD with cambium age. The trend of the radial increment ( $i_r$ ) is shown in figure 1. It follows a typical growth pattern of pioneer forest trees with a comparatively early culmination at cambium age 6. Only the first six years of growth were investigated in the F1-population (Fig. 1). Unlike the reference population, the even aged F1-population starts with higher mean values for VL and FL (cambium

age 1, 1998). During the next two years VL and FL decrease, most probably due to a plant water deficit in the absence of a well developed root system. After the third year, tree rings have higher FL and VL with a tendency to increase. In the drought year 2003 the FL follows this tendency, while mean VL remains stagnant.

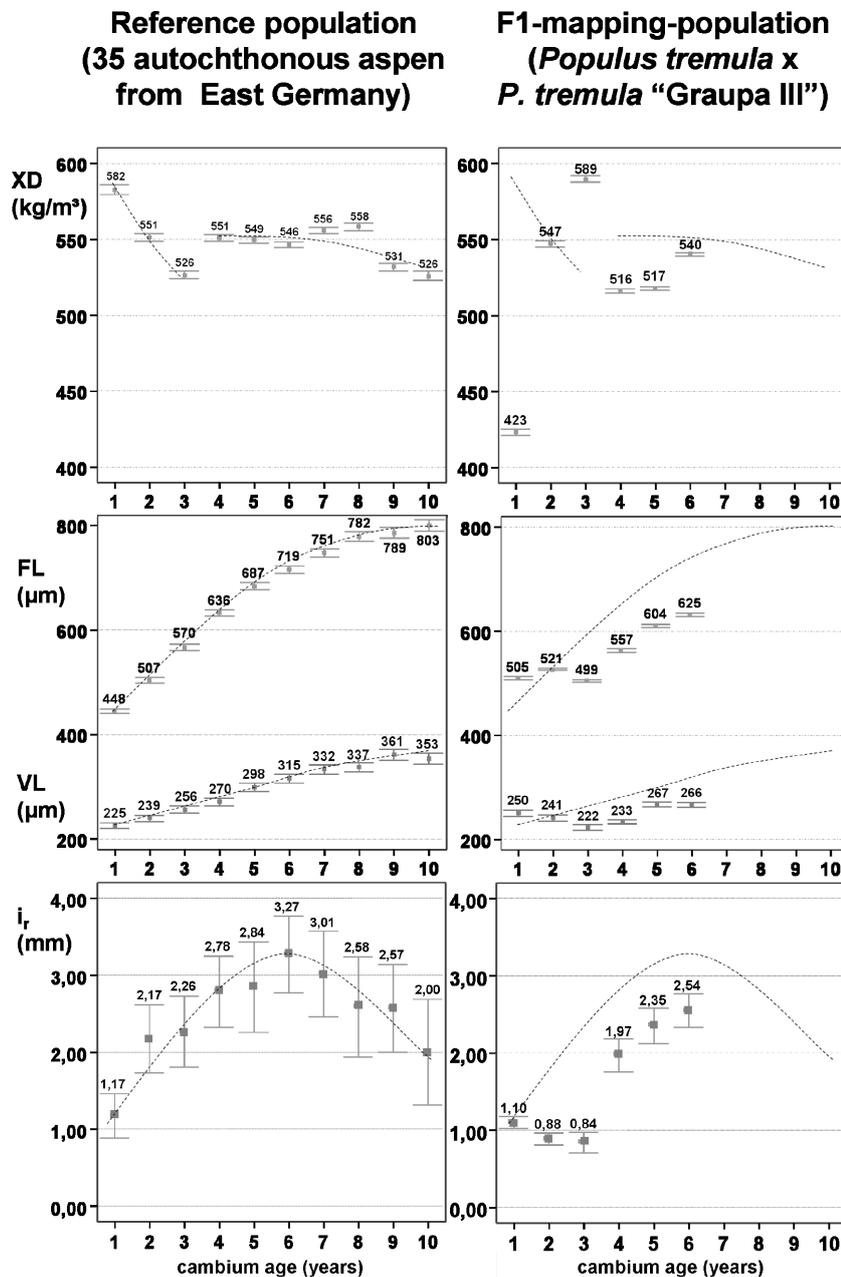


Figure 1: Mean X-ray wood density (XD), fibre length (FL), vessel length (VL) and radial increment ( $i_r$ ) of the tree rings of two contrasting aspen (*Populus tremula*) populations – one not even aged from natural succession in East Germany on the left-hand side and one even aged F1 full-sib crossbred offspring on the right-hand side. Dotted lines (curve manually fitted) show the juvenile trend pattern of the respective trait in the reference population.

A pattern of adaptation to a water deficit after the transfer from the greenhouse to the field – combined with the absence of a root system – becomes also evident in the XD data for the F1-Population. In contrary to the XD values of the reference population, mean XD is very low in the first tree ring (423 kg/m<sup>3</sup>), and increases within the next two growing seasons to 588 kg/m<sup>3</sup>. The following two tree rings have nearly the same mean XD (517 kg/m<sup>3</sup> or 518 kg/m<sup>3</sup>) while an increase

of wood density was evident in the drought year of 2003, even though the trees had water supply from ground water. The mean  $i_r$  performed similar to FL. It starts with a value comparable to that of the first tree ring in the reference population. Unlike the reference population, the F1-population shows decreasing  $i_r$  in the next two vegetation periods and increasing values after successful root establishment.

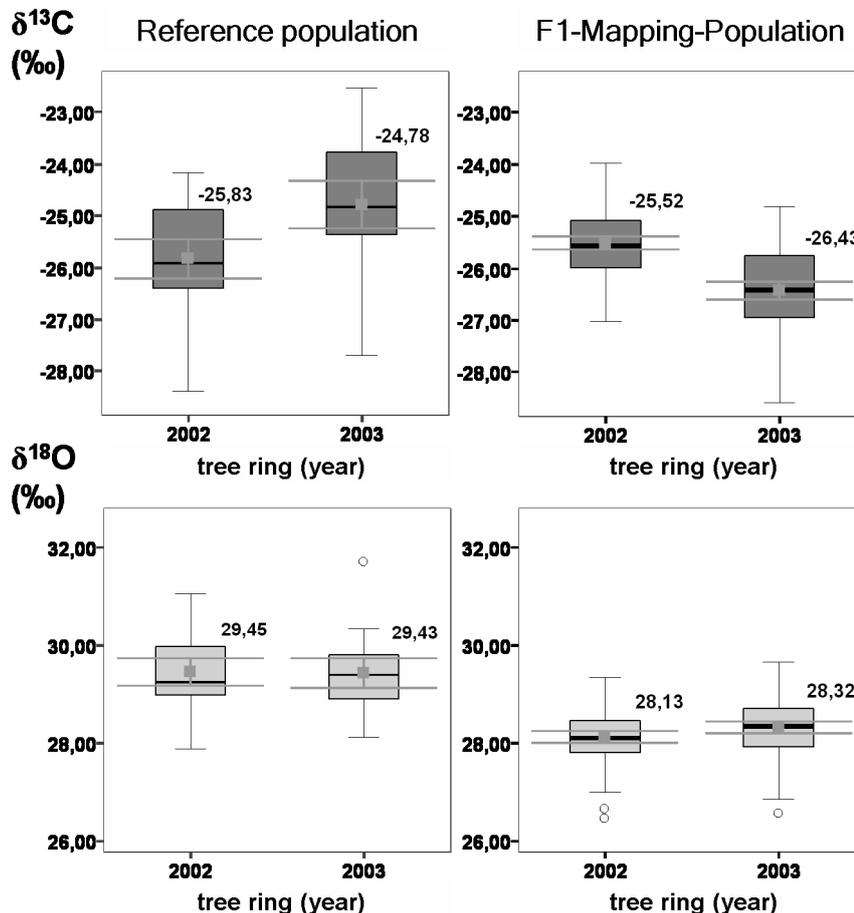


Figure 2: Mean  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  of the 2002 and 2003 tree rings of two contrasting aspen (*Populus tremula*) populations – one not even aged from natural succession in East Germany on the left-hand side and one even aged F1 full-sib crossbred offspring from a tree nursery without irrigation but good water supply via groundwater during 2003 summer drought vegetation period on the right-hand side.

$\delta^{13}\text{C}$  (Fig. 2) of the reference population, is significantly higher in 2003 (drought year) than in 2002 (rather normal year). The opposite behaviour is shown for  $\delta^{13}\text{C}$  in the F1-population in 2003 compared with 2002. No changes between 2002 and 2003 were obtained for mean  $\delta^{18}\text{O}$  in the reference population whereas a slightly increased mean  $\delta^{18}\text{O}$  was detected in the 2003 tree rings of the F1-population (Fig. 2, T-test:  $P \leq 0,001$ ; Wilcoxon-test:  $P \leq 0,002$ ).

## Discussion

The investigation showed that classical dendrological tree ring analysis can be used to compare the drought reaction of tree populations. With respect to the behaviour of the F1-population our results indicate that the effects of juvenility and drought interfere each other in young poplar because the root system has to be developed first. Compared with the reference trees which mainly originate from root sprouting, the F1-population trees suffer from water deficit in the first three years until root establishment. In the following years the F1-trees show the typical juvenile

trend of FL, VL and  $i_r$ , but never reach the mean value level of the reference population. Therefore, our results support the assumption that the adaptation of trees during the first years can be essential for the performance of the poplar culture during the whole rotation period. This might be the major drawback for the establishment of poplar plantations on set-aside agricultural land, where the yield of the trees is markedly lower under suboptimal water supply (Röhle et al. 2008, Röhle et al. 2005, Wolf & Bönisch 2004).

In terms of tree breeding, drought resistance is not a directly measurable trait. It can only be determined indirectly by using other traits as proxies which are measurable and related with the trees reaction to water deficits. In cereal crop breeding, the most practicable proxy for drought tolerance of a genotype is its productivity under drought (Fischer & Maurer 1978). Therefore, in terms of tree breeding for agricultural purposes, drought tolerance should not be defined as the ability of a tree to withstand dry conditions or even long lasting drought periods but as the ability of maintaining yield under dry conditions. Drought tolerance of a tree in a narrow sense means that its yield reduction following drought is minimised. But most of the adaptations shown by the possible proxy-traits, result in an undesirable reduction of productivity in *Populus* (Marron et al. 2006, Monclus et al. 2006). To solve this problem in breeding practise, one has to find proxy-traits which are not negatively correlated with biomass yield because a high yield is the primary objective in agriculture. Dillen et al. (2008) and Monclus et al. (2005) investigated  $\delta^{13}\text{C}$  in different poplar genotypes. They found out that the trees substantially differ in their WUEi under drought conditions and that these differences were not correlated with biomass yield as indicated by radial increment. Their conclusion was that this opens a perspective for breeding new stable cultivars with a minimum reduction of their productivity under drought. This conclusion was restricted to conditions of moderate drought, because tree growth is always depending on water availability and the trees ability to water uptake (Monclus et al. 2005).

With respect to the reaction of the F1-population in 2000 (comprehensive drought) and 2003 (atmospheric drought), we could show that some traits seem to answer only to a comprehensive plant water deficit (FL,  $i_r$ ), whereas other seem to represent adaptation to atmospheric drought or water vapour deficit (VL, XD,  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ). In 2003, the radial increment – one parameter determining biomass yield, i.e. carbon gain – was not reduced. Wood density, the other indicator for carbon gain is higher, what points to some adaptation to drought. However, WUEi was reduced, as shown by the lower  $\delta^{13}\text{C}$  value of the F1-population, in average by  $9\mu\text{mol CO}_2 / \text{mol H}_2\text{O}$  ( $\pm 30\mu\text{mol/mol}$ ). The results support some of the conclusions of Dillen et al. (2008) and Monclus et al. (2005). We could show that a high XD is also a trait, which does not necessarily result in a yield reduction if radial increment does not decline unproportionally. Therefore, it might be a useful proxy for drought tolerance in breeding of poplar. It is well known that increased wood density results in a higher resistance to air embolism which is a severe damage to the hydraulic architecture of trees under drought (Cochard et al. 2007, Hacke et al. 2001). However, it is not known from literature if – in addition to the hydraulic safety aspect - a higher wood mean density can help to minimise increment decline under drought. Therefore, the factors influencing wood biomass yield under drought, e.g. vessel diameter, pit diameter and cell wall thickness as expressed by radial increment and density, have to be clarified in more detail in further investigations including stable isotope analyses as indication for water use efficiency.

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### References

Cochard, H., Casella, E., Mencuccini, M. (2007): Xylem vulnerability to cavitation varies among poplar and willow clones and correlates with yield. *Tree Physiology* 27: 1761-1767.

- Dillen, S.Y., Marron, N., Koch, B., Ceulemans, R. (2008): Genetic variation of stomatal traits and carbon isotope discrimination in two hybrid poplar families (*Populus deltoides* 'S9-2' x *P. nigra* 'Ghoy' and *P. deltoides* 'S9-2' x *P. trichocarpa* 'V24'). *Ann Bot-London* 102: 399-407.
- Eschbach, W., Nogler, P., Schär, E., Schweingruber, F.H. (1995): Technical advances in the radiodensitometrical determination of wood density. *Dendrochronologia* 13:155-168.
- Fischer, R.A., Maurer, R. (1978) Drought Resistance in Spring Wheat Cultivars.1. Grain-Yield Responses. *Aust J Agr Res* 29: 897-912.
- Günther, B. (2004): Untersuchungen an historischen Fichtenholzproben zur Entwicklung einer röntgendensitometrisch-multivariaten Datierungsmethode. Institut für Forsttechnik und Forstnutzung. Technische Universität Dresden, Tharandt.
- Hacke, U.G., Sperry, J.S., Pockman, W.T., Davis, S.D., McCulloch, K.A. (2001): Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126: 457-461.
- Helle, G. (1996): Hochauflösende intra-annuelle Kohlenstoff-Isotopenuntersuchungen an Baumjahresringen. Westfälische Wilhelms-Universität, Münster.
- Kaltschmitt, M. (2007): Stand und Potenziale der Biomassenutzung in Deutschland. In: Fachagentur Nachwachsende Rohstoffe e.V. (FNR) (ed) Symposium Energiepflanzen 2007. Verlag Th. Mann GmbH, Gelsenkirchen, 19-34.
- Marron, N., Maury, S., Rinaldi, C., Brignolas, F. (2006): Impact of drought and leaf development stage on enzymatic antioxidant system of two *Populus deltoides* x *nigra* clones. *Annals of Forest Sciences* 63: 323-327.
- Monclus, R., Dreyer, E., Delmotte, F.M., Villar, M., Delay, D., Boudouresque, E., Petit, J.M., Marron, N., Brechet, C., Brignolas, F. (2005): Productivity, leaf traits and carbon isotope discrimination in 29 *Populus deltoides* x *P. nigra* clones. *New Phytologist* 167: 53-62.
- Monclus, R., Dreyer, E., Villar, M., Delmotte, F.M., Delay, D., Petit, J.M., Barbaroux, C., Thiec, D., Brechet, C., Brignolas, F. (2006): Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides* x *Populus nigra*. *New Phytologist* 169: 765-777.
- Röhle, H., Böcker, L., Feger, K.H., Petzold, R., Wolf, H., Ali, W. (2008): Anlage und Ertragsaussichten von Kurzumtriebsplantagen in Ostdeutschland. *Schweizerische Zeitschrift für Forstwesen* 159: 133-139.
- Röhle, H., Hartmann, K.U., Steinke, C., Wolf, H. (2005): Wuchsleistungen von Pappeln und Weiden im Kurzumtrieb. *AFZ / Der Wald* 60: 745-747.
- Schleser, G.H. (1995): Parameters determining carbon isotope ratios in plants. In: Frenzel B (ed) *Problems of stable isotopes in tree rings, lake sediments and peat-bogs as climatic evidence for the Holocene*. Gustav Fischer Verlag, Stuttgart, Jena, New York.
- Schneider, B.U. (2007): Agroforstsysteme als Option der Biomasseerzeugung. In: Fachagentur Nachwachsende Rohstoffe e.V. (FNR) (ed) Symposium Energiepflanzen 2007. Verlag Th. Mann GmbH, Gelsenkirchen, 206-222.
- Schweingruber, F.H. (1988): *Tree Rings - Basics and Applications of Dendrochronology*. Reidel, Dordrecht.
- Trendelenburg, R., Mayer-Wegelin, H. (1955): *Das Holz als Rohstoff*. Hanser, München.
- Treydte, K.S., Schleser, G.H., Helle, G., Frank, D.C., Winiger, M., Haug, G.H., Esper, J. (2006): The twentieth century was the wettest period in northern Pakistan over the past millennium. *Nature* 440: 1179-1182.
- Wagenführ, R. (2007): *Holzatlas*. Carl Hanser Fachbuchverlag, 6th edition
- Wolf, H., Bönisch, B. (2004): Anbau schnellwachsender Gehölze auf stillgelegten landwirtschaftlichen Flächen. <http://www.genres.de/IGRREIHE/IGRREIHE/DDD/23-15.pdf>. In: Begemann F (ed). *Informationszentrum Biologische Vielfalt des ZADI*, Bonn, pp 122-132.
- Zobel, B.J., van Buijtenen, J.P. (1989): *Wood Variation - Its Causes and Control*. Springer Verlag, Berlin, Heidelberg.