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No trade-off between hydraulic and mechanical properties in several transgenic poplars modified for lignins metabolism

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A B S T R A C T

Wood provides water transport and mechanical support of trees. Sap is transported under negative pressure in plant xylem conduits, which can be subject to embolism during severe drought. Typically, denser woods show thicker cell walls and stronger mechanical properties. Ten transgenic poplar lines modified for expression of genes involved in lignin metabolism were produced from the female clone 717-1B4 of *Populus tremula* × *Populus alba* to test the hypothesis of a possible trade-off between hydraulic and mechanical functions. Poplar lines underexpressed genes encoding for cinnamoyl alcohol dehydrogenase (CAD), cinnamoyl CoA reductase (CCR) and caffeic acid 3-O- methyltransferase (COMT), while new poplar lines underexpressed the CAD genes or overexpressed the MYB308 gene, encoding for a transcription factor repressing the phenylpropanoid metabolism. To maximize the contrast between line behaviors, these plants were grown under two different water regimes, and the impact on their hydraulic traits and xylem properties was analyzed to test for a link between water condition and mechanical and hydraulic properties. Our results show that the resistance to xylem cavitation was lower for the transgenic lines than for the control line 717-1b4 while they show neither a positive nor a negative tendency for the longitudinal Young's modulus between the transgenic lines and the control line. ASOMT10b and ASOMT2b, which possessed a down-regulated expression for all the genes, showed a lower value of the resistance to implosion index $(t/b)^2$. No difference for xylem hydraulic conductivity between the lines was found. The changes in lignin metabolism in these transgenic lines did not affect the water transport, despite the change in the lignin content. Our data on the transgenic poplar lines do not therefore support the mechanical vs. hydraulic trade-off hypothesis and we point out that angiosperm trees have numerous ways to acclimate their internal structure in order to adjust their mechanical properties without hydraulic coupling. Moreover, we observed an acclimation to water stress for P_{50} but not for the Young's modulus. MYB308-25.1 showed better mechanical properties and vulnerability to cavitation than the control line 717-1b4. Finally, we present evidence that lignins are involved in the vulnerability to cavitation, probably through modifications of pit structure and behavior.

Keywords:

Water stress
Woody plants
Forest trees
Embolism
Lignin
Mechanical properties
Hydraulic properties
Trade-off
Xylem cavitation
Hydraulic efficiency
Young's modulus

1. Introduction

Wood performs several functions in trees, the two most specific being water transport and mechanical support. Sap is transported under negative pressure (tension) in plant xylem conduits (Zimmermann, 1983). Under drought conditions, if the water potential in a xylem vessel drops below a threshold limit of pressure, cavitation events occur and the vessel is embolized and can

no longer transport water (Tyree and Dixon, 1986). Xylem cavitation can affect plant productivity and plant survival (McDowell et al., 2008; Brodribb and Cochard, 2009), and xylem vulnerability to cavitation is correlated with drought tolerance (Maherali et al., 2004; Pockman and Sperry, 2000; Tyree et al., 2003). However, lower vulnerability to cavitation is correlated with lower hydraulic conductivity (Cochard et al., 1992; Hacke et al., 2006). This trade-off between safety and efficiency will depend on the pit area and the pit membrane structure. Intervessel pits, which are composed of a small and thin porous membrane with overarching secondary cell walls, are the main pathway for the water flow, and they prevent the passage of air bubbles from one embolized vessel to a conducting vessel. In addition to cavitation events, xylem vessels have to

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resist implosion under negative pressure. The structural parameters that control this collapse involve the transversal mechanical cell wall properties and the shape of the vessel: the higher the ratio of the vessel wall thickness (t) to the lumen diameter (b), the more mechanically resistant the cylinder is to transverse buckling (Hacke et al., 2001; Cochard et al., 2004; Brodrribb and Holbrook, 2005; Awad et al., 2010). The fibre matrix surrounding the vessels also appears to play a key role (Hacke et al., 2001; Jacobsen et al., 2005). In support of this, drought resistance is positively correlated with wood density, which is a mainly a function of wall thickness and lumen diameter, actually their ratio. Drought resistance may thus be associated with stronger wood (Hacke et al., 2001; Niklas, 1997).

Mechanical support can be enhanced at the tissue level by a decrease in wood porosity, i.e. principally an increase in wood density, and at the cell wall level, by microfibril angle changes, arrangement of the cell wall layers or cell wall chemistry (Gindl, 2001; Mencuccini et al., 1997; Jagels et al., 2003; Jagels and Visscher, 2006). Stems have also to be mechanically resistant to cope with external forces such as wind, and at this scale, their resistance depends mainly on microfibril angles and wood density (Meylan and Probine, 1969; Lindström et al., 1998; Lichtenegger et al., 1999). An increasing number of studies indicate that there may be interrelations between hydraulic and mechanical stress acclimation. These interactions are positive or, on the contrary, trade-offs. Several studies have found a trade-off between hydraulic conductivity and mechanical strength (Gartner, 1991a,b,c; Wagner et al., 1998; Jagels et al., 2003; Christensen-Dalsgaard et al., 2007), while others have failed to find any trade-off (Woodrum et al., 2003; Pratt et al., 2007; Rosner et al., 2008, 2007; Utsumi et al., 2010). However, xylem safety (resistance to cavitation and implosion) and mechanical strength have still been found to be positively correlated (Rosner et al., 2008; Utsumi et al., 2010; Pratt et al., 2007), possibly because both functions depend on wood density. According to Pratt et al. (2007), the stem mechanical strength appears to be important in maintaining xylem transport under negative pressure and this could be a strategy both to prevent vessel collapse and to withstand mechanical stresses caused by gravity or wind. However, insights into the genetic control of both vulnerability to cavitation and resistance to implosion are still lacking, which hinders the assessment of possible trade-offs between the hydraulic and mechanical functions of wood.

Lignins are a characteristic feature of secondary cell walls and account for 20–30% of the dry mass of wood. In angiosperms, they consist of complex phenolic polymers resulting from dehydrogenative polymerization (*via* radical coupling reactions) mainly of three primary phenylpropanoid monomers, *p*-coumaryl (4-hydroxy-cinnamyl), coniferyl (4-hydroxy-3-methoxy-cinnamyl), and sinapyl (3,5-dimethoxy-4-hydroxy-cinnamyl) alcohols, analogs varying in their degrees of methoxylation (Harkin, 1967; Freudenberg and Neish, 1968). Lignins deposition is restricted to certain cell types, such as tracheid elements (gymnosperms), fibres, vessels (angiosperms) or ray cells in the xylem and sclerenchyma. In these cells, lignins are mainly, in proportion, deposited in the middle lamella and the primary wall, and to a lesser extent in the secondary wall (Fromm et al., 2003). Ubiquitous in vascular plants, lignins play important roles in structural support, providing additional rigidity and compressive strength (Niklas, 1992; Chabannes et al., 2001). Transgenic tobacco plants with a lower cinnamoyl alcohol dehydrogenase (CAD) activity produced lignins with a reduced crosslink density and xylem with a reduced tensile stiffness (Hepworth and Vincent, 1999). These plants compensated for a reduced Young's modulus by producing more xylem tissue that ensures stem rigidity, suggesting that lignins are involved in mechanical properties. The enzymes of lignins biosynthesis have been studied for many years and several genes of the biosynthesis pathway were cloned many years ago (Boudet et al., 1995; Whetten

and Sederoff, 1995). Many attempts at biotechnological modification of lignification have been made to decrease the total quantity of lignins in plant tissues, or modify its monomer constitution, for the pulp and paper industries (Whetten and Sederoff, 1991; Dean and Eriksson, 1992).

In this study, we used transgenic poplars with modified lignins composition to evaluate the involvement of lignins in wood hydraulic and mechanical properties, and to verify the hypothesis of a trade-off between them. We used different poplar lines underexpressing different genes from the monolignol biosynthesis pathway either by antisense strategy or by co-suppression, encoding for cinnamoyl alcohol dehydrogenase (CAD) (Baucher et al., 1996; Lapierre et al., 1999; Pilate et al., 2002), cinnamoyl CoA reductase (CCR) (Leplé et al., 2007) or caffeic acid 3-*O*-methyltransferase (COMT) (Lapierre et al., 1999; VanDoorselaere et al., 1995; Pilate et al., 2002). Transgenic poplars strongly down-regulated for CAD activity had a slightly reduced lignin content (up to 10%), but no major differences in S/G (syringyl/guaiacyl) ratio; however, their lignins contained more free phenolic groups and also indene derivatives resulting from the incorporation of cinnamaldehydes (mainly sinapaldehydes) within the polymer (Lapierre et al., 1999; Ralph et al., 2001a,b; Kim et al., 2002; Lapierre et al., 2004). CCR down-regulated transgenic poplars exhibited a patchy orange coloration in the outer xylem: the patchy colored wood appeared associated with up to 50% reduced lignin content, an increased S/G ratio and the incorporation of ferulic acid in the lignin polymer. The cohesion of the wall appears affected particularly at sites richer in S units (Leplé et al., 2007). COMT down-regulated transgenic poplar lines appeared more condensed with a lower S/G ratio with fewer phenolic groups on G units. This results from the significant incorporation of 5-hydroxy-coniferyl alcohol monomers in place of sinapyl alcohol (VanDoorselaere et al., 1995; Lapierre et al., 1999; Ralph et al., 2001a,b; Pilate et al., 2002). All these changes have indeed potential effects on the alteration in the physical wood properties. In addition, we produced new poplar lines overexpressing the AmMYB308 gene, encoding for a transcription factor known to repress the phenylpropanoid metabolism: indeed, transgenic tobacco plants over-expressing this transcription factor exhibited an inhibition of monolignol production resulting in plants with at least 17% less lignins in their vascular tissue (Tamagnone et al., 1998). The transgenic plants used in our study exhibited a reduced secondary growth. To maximize the contrast between lines, these plants were grown under two different water regimes, and the impact on their hydraulic traits and xylem properties was analyzed. This enabled us to test the hypothesis that the effects of water conditions on the mechanical and the hydraulic properties were linked.

2. Materials and methods

2.1. Plant materials and culture conditions

The study was performed on ten transgenic poplar lines modified for expression of genes involved in lignin metabolism. All the lines were obtained from the female clone 717-1B4 of *P. tremula* × *P. alba* using the procedure described in Leplé et al. (1998) and using a disarmed *Agrobacterium tumefaciens* strain (C58pMP90). In the SCAD1 line, the CAD (cinnamoyl alcohol dehydrogenase) activity is down-regulated (Lapierre et al., 1999). In the CCR-FS3 and CCR-FAS13 lines, the CCR (cinnamoyl CoA reductase) activity is down-regulated (Leplé et al., 2007). In the ASOMT10b and ASOMT2 lines, COMT (caffeic acid 3-*O*-methyltransferase) activity is down-regulated (Lapierre et al., 1999).

Two other poplar transgenic lines (p70-ASCAD25.1 and p70-ASCAD34.2), strongly down-regulated for CAD activity, were obtained after cocultivation with a binary vector carrying the entire

cad cDNA in antisense orientation under the control of the double 35S promoter from CaMV (Lapierre et al., 2004). Likewise, two independent transformants overexpressing the AmMYB308 gene under the control of the cauliflower mosaic virus 35S promoter were produced using pJAM495 construct described in Tamagnone et al. (1998). This transcription factor is known to inhibit both phenolic acid metabolism and monolignol production, and transgenic plants expressing MYB308 exhibit decreased lignin content in their vascular tissues (Tamagnone et al., 1998).

The plants were multiplied clonally *in vitro* on Murashige and Skoog medium (Murashige and Skoog, 1962). When they reached 4 cm in height, they were gradually acclimatized on a hydroponic solution (Morizet and Mingeau, 1976) and grown in a controlled environment room: 16 h daylight, at 21–22 °C, 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (day) and 18–19 °C (night) with 70 \pm 10% relative humidity. After they reached 57 days of age and 30 cm in height, they were transferred into 10-l pots filled with a commercial soil (40% black, 30% brown and 30% blond peat moss, pH 6.1, DUMONA-RN 75–3851 Arandon, Holland) and grown for 90 days in a greenhouse: 16 h daylight, at 22 (\pm 1) °C, 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (day) and 19 (\pm 1) °C (night) with 70 \pm 10% relative humidity.

Two experiments with different water regimes were successively conducted on the poplar lines, with 3–4 plants per line and per experiment. In a first experiment (Exp. 1), plants were grown with a soil water content ranging between 40% and 75% of field capacity (FC). In a second experiment (Exp. 2), plants were grown under a water-stressed condition so that the soil water content ranged between 25% and 45% of FC. At the start of the experiment, the soil water content was 100% of FC, so that each plant was set up with an optimal water status. The soil water was evaluated by the measurement of the weight of the pots, and the plants were then watered every 3, 4 or 5 days depending on their assigned watering regime.

2.2. Leaf water potentials

The midday and predawn leaf water potentials (ψ_m and ψ_p) were measured using a pressure chamber (Scholander et al., 1965). Two leaves per plant were removed to gauge ψ_m and ψ_p . Predawn water potential was recorded between 04:00 and 06:00 am solar time and ψ_m was recorded between 12:00 and 14:00 pm solar time. The measurements were made for Exp. 2 after 85 days from the beginning of the experiment, just before watering and one day after watering.

2.3. Vulnerability curves

Vulnerability to xylem cavitation was assessed with the Cavitation technique (Cochard, 2002; Cochard et al., 2005). Its principle is as follows: a centrifugal force progressively increases the water tension in a xylem segment, and the resulting loss of hydraulic conductance is measured at the same time. The curve of percentage loss of xylem conductance (PLC) vs. xylem water tension represents the sample's vulnerability to cavitation. Vulnerability curves were determined on one 0.28 m long sample per plant; i.e. four samples for each transgenic line. Xylem pressure (P) was first set to a reference pressure (–0.5 MPa) and the maximal conductance (K_{max}) of the sample was determined. The xylem pressure was then set to a more negative value for 3 min and the conductance (K) of the sample was re-measured. The percent loss of conductance of the sample was then computed as:

$$\text{PLC} = 100 \times \left(1 - \frac{K}{K_{\text{max}}} \right)$$

The procedure was repeated for more negative pressures (with –0.25 or –0.5 MPa step increments) until PLC reached at least

90%. Rotor velocity was monitored with an electronic tachymeter (10 rpm resolution). The PLC curves were fitted using the following sigmoid function (Pammenter and Van Der Willigen, 1998):

$$\text{PLC} = \frac{100}{1 + e^{s(P-P_{50})/25}}$$

where P_{50} is the xylem pressure causing 50% loss of conductance and s is the slope of the curve at its inflection point.

2.4. Wood infradensity

Wood xylem density is defined as

$$\rho = \frac{M_0}{V_s}$$

A 20 mm long segment was cut from the basal end of each sample used for analyses of vulnerability to cavitation. Its fresh volume (V_s) was measured according to the Archimedes principle by water displacement with an analytical balance. Its dry mass (M_0) was determined after drying to constant weight for one night in an oven at 104 °C.

2.5. Mechanical behavior

Mechanical tests were performed on the same fresh samples that had previously been characterized for their vulnerability to cavitation. The bark was peeled to focus on the xylem properties. The longitudinal Young's modulus E_L was measured by the three-points bending method on a mechanical testing machine (INSTRON 5565). The distance between the external support points was set at 140 mm. The experiment was controlled by the displacement of the central force application point. Its speed was set at 5 mm min^{-1} . A preload, controlled by a force limit (0.5 N), ensured the first contact with the sample. Four load–unload cycles were then performed in such a way that the strain never exceeded the elastic limit: the maximum displacement was set at 1.8 mm. Force and displacement were simultaneously recorded by Bluehill® software. The last three load cycles were used for the evaluation of the linear slope α of the displacement–force curves. The sample was assumed to be a regular cylindrical beam, and its diameter was measured with a laser beam micrometer for three points. The mean values were used for the slope and the diameter to compute the longitudinal Young's modulus E_L from:

$$E_L = \frac{4L^3}{3\pi D^4} \alpha$$

where L is the distance between the external support points and D is the mean diameter of the sample.

The specific Young's modulus E_ρ is defined as the elastic modulus per unit of mass density.

2.6. Anatomical traits

Anatomical traits were characterized for all the samples used to measure the vulnerability to cavitation and mechanical properties. Sections were prepared from the basal part of the sample and stained with Astra blue and Safranin dyes. Sections were examined under an optical microscope (Zeiss Axioplan 2, Zeiss, Jena, Germany), and data were recorded using a digital camera (Axio-Cam HR, Zeiss) with AxioVision digital imaging software. Images were recorded for six sections per sample. After spatial calibration, anatomical measurements were performed by image analysis using ImageJ software (Rasband, 2009). Automatic segmentation enabled us to isolate the vessels, and their diameters (μm), their numerical areal density (mm^{-2}) and the area fraction they occupied (%) were measured. We then measured the vessel wall thickness t (μm)

Table 1
Gene ID, accession numbers and primer sequences used in QPCR analyses.

Gene ID	Name	Primer sequences	Accession number (Genbank)
Caffeic acid 3-O- methyltransferase	COMT	Forward 5'TGATGCGCCTGTTCATG3' Reverse 5'CAAGGCCTATGGGATG3'	CF234721
Pectin methylesterase	PME1	Forward 5'GAGATGGTTAGGGAGG3' Reverse 5'AATTACAGTTCGCCGAG3'	AI164340
	PME2	Forward 5'GAGTGGATGTCAGTAGC3' Reverse 5'GCTAGCATGTCACACTC3'	AI165089
Polygalacturonase	PG 1	Forward 5'GGCAATCTCGAAATTGAG3' Reverse 5'TGAGGTTTTGACCATGG3'	AI163516
	PG 2	Forward 5'CAGTCTTACCAGCCAC3' Reverse 5'AAAGCTCCCTGGCCG3'	AI164358
UDP glucose dehydrogenase	UGDH1	Forward 5'ACTGAAGTGCCAGAC3' Reverse 5'CCCAATAAGCTAGGTCC3'	AI163328
	UGDH2	Forward 5'GCTTTCAAGAAAGACAGC3' Reverse 5'TAACTTGCTCTATAGTAC3'	AI162135
	UGDH3	Forward 5'ACTCAAGTGCCGGAT3' Reverse 5'CAGCACTCTCCAGTAT3'	AI166238
Xylan 1,4-β-xylosidase	XYL 1	Forward 5'GAGAGGCCAGGAACTCCAGGG3' Reverse 5'GACACCAGAGAGGAGTCTGGG3'	AI164515
	XYL 2	Forward 5'GGGAGGACTTGTGAATGAGC3' Reverse 5'GAACGATACGTCATGCC3'	AI163643

between joining vessels and the conduit wall span b (μm) separating them to calculate the transversal reinforcement index of the bordered pit field $(t/b)^2$, according to Hacke et al. (2001).

2.7. RNA isolation and cDNA synthesis

A 10 cm long stem segment was collected above the one used for analysis of vulnerability to cavitation. The bark was peeled off and the xylem was immediately frozen in liquid nitrogen and then stored at -80°C . The xylem from each plant was ground. Equal quantities of ground xylem from each plant were then pooled for RNA extraction. RNA was isolated using CTAB extraction buffer as described by Chang et al. (1993) and stored at -80°C . First strand cDNAs were synthesized by reverse transcription using $2\ \mu\text{g}$ of total RNA in a final reaction volume of $20\ \mu\text{l}$ using the Super Script™ III first strand synthesis system for RT-PCR (Invitrogen, Paisley, UK) according to the manufacturer's instructions. PCR amplifications were performed as described by Mai et al. (2009) using the primers indicated in Table 1. The PCR products were ligated into PGEM-Teasy vector (Promega, Charbonnières, France) and transformed into DH5 α *Escherichia coli* strain, and the sequences were checked.

2.8. Expression analysis by quantitative real-time PCR

The single strand cDNA was used as a template in quantitative real time PCRs (QPCRs) as described in Mai et al. (2009). The cDNA was normalized in dependence of the level of constitutively expressed 18S rRNA (Nualpun et al., 2005). QPCRs were carried out with gene specific primers (Table 1) in the following $25\ \mu\text{l}$ mixture: $2\ \mu\text{l}$ diluted cDNA, $0.25\ \mu\text{l}$ of diluted SYBR Green (Sigma, St. Louis, USA; 1:1000 in 25% dimethyl sulfoxide), 1 U of platinum Taq DNA polymerase (Invitrogen, Paisley, UK) and 200 nM of each gene-specific primer. QPCRs were carried out in a iCycler iQ™ (Bio-Rad, Hercules, CA, USA). The PCR program was as follows: incubation for 5 min at 95°C , followed by 40 cycles of denaturing at 94°C for 15 s, annealing at 55°C for 20 s and extension at 72°C for 30 s, followed by a melting curve analysis to check the specificity of fragment amplification. For each condition, three replicates were performed for QPCR analysis. The method to calculate the relative expression ratio is described by Pfaffl (2001):

$$\text{Ratio} = \frac{(E_{\text{target}} + 1)^{\Delta C_{\text{P}}^{\text{target}}(\text{control-sample})}}{(E_{\text{ref}} + 1)^{\Delta C_{\text{P}}^{\text{ref}}(\text{control-sample})}}$$

2.9. Statistical analysis

We carried out an analysis of variation (ANOVA) to determine whether the means of P_{50} , Young's modulus, wood density, specific Young's modulus, leaf water potential and anatomical parameters were significantly different. When we found a significant difference, we referred to Tukey's multiple range test at $P < 0.05$ to compare the mean values between the control line 717-1b4 and the other lines. All the measured and derived data underwent statistical analysis using the Statgraphics plus 4.1 software package.

3. Results

The transgenic poplar lines were compared with the non-transformed line in two experiments with different water regimes. To check whether the water status in the transgenic poplar was modified, a survey of leaf water potentials was conducted in Exp. 2, for which the water regime was the more restrictive (Table 2). Before and after irrigation, i.e. when the soil water content was respectively at its lowest and highest levels, the nine transgenic lines showed no significant difference between them and the control line 717-1b4 for the ψ_m and ψ_p values. At the end of the two experiments, stem samples were collected and several physiological, anatomical and molecular biological tests were performed. Table 3 shows the P_{50} and Young's modulus mean values for each transgenic line in both Exp. 1 and Exp. 2. P_{50} values of transgenic lines were higher (lower resistance to cavitation) than the control line 717-1b4 in the two experiments (Table 3). The difference became significant between the control line 717-1b4 and the six transgenic lines ASOMT10b, ASOMT2b and MYB308-25.1, CCR-52.3fs, 70ASCAD25.1, 70ASCAD34.2 in Exp. 2 and only CCR-52.3fs in Exp. 1. For the longitudinal Young's modulus we found neither a positive nor a negative trend between the transgenic lines and the control line. Nevertheless, the lines 70ASCAD25.1, CCR-62.13fas and CCR-52.3fs showed statistically much lower stiffness than the control line in Exp. 1, whereas only the line MYB308-25.1 showed a higher Young's modulus in Exp. 2. The results from Fig. 1 show no correlation between P_{50} and Young's modulus.

To explain variations in the Young's modulus, we investigated the two factors that could influence the mechanical property of the wood: the specific Young's modulus (Fig. 2A), which is a property of the wall, and the wood density (Fig. 2B). The specific Young's modulus was lower in the CCR-62.13fas and 70ASCAD25.1 lines than in the control line in Exp. 1, whereas it was higher in 70ASCAD34.2 and

Table 2
 Predawn and midday leaf water potentials (ψ_p and ψ_m) of transgenic poplars in Exp. 2. The ψ_p and ψ_m values were measured on the 10 transgenic poplar lines at the end of the experiment. The measurements were made just before irrigation, when the water soil content was minimal and the day after irrigation, when the water soil content was maximal. Each data point represents the mean value (\pm S.E.) from 4 to 5 leaves. There was no significant difference between lines, according to an ANOVA analysis ($P < 0.05$).

Line	717-1b4	CCR-62.13fas	CCR- 52.3fs	70ASCAD 25.1	70ASCAD 34.2	ASOMT 10b	ASOMT 2b	MYB308- 25.1	MYB308-12.3	SCAD1
Before irrigation										
ψ_p (MPa)	-0.86 \pm 0.25	-0.88 \pm 0.36	-0.97 \pm 0.17	-0.86 \pm 0.48	-0.86 \pm 0.35	-0.91 \pm 0.12	-0.86 \pm 0.14	-0.51 \pm 0.06	-0.93 \pm 0.34	-0.80 \pm 0.18
ψ_m (MPa)	-1.18 \pm 0.24	-1.22 \pm 0.14	-1.27 \pm 0.10	-1.37 \pm 0.07	-1.22 \pm 0.08	-1.16 \pm 0.13	-1.24 \pm 0.11	-1.15 \pm 0.11	-1.27 \pm 0.16	-1.20 \pm 0.15
After irrigation										
ψ_p (MPa)	-0.02 \pm 0.01	-0.03 \pm 0.01	-0.03 \pm 0.02	-0.04 \pm 0.01	-0.04 \pm 0.00	-0.03 \pm 0.02	-0.02 \pm 0.02	-0.03 \pm 0.01	-0.01 \pm 0.00	-0.05 \pm 0.02
ψ_m (MPa)	-0.36 \pm 0.08	-0.34 \pm 0.09	-0.38 \pm 0.11	-0.30 \pm 0.02	-0.40 \pm 0.07	-0.38 \pm 0.13	-0.27 \pm 0.04	-0.48 \pm 0.21	-0.34 \pm 0.01	-0.27 \pm 0.04

Table 3
 Vulnerability to cavitation and mechanical properties of the transgenic poplars in Exp. 1 and Exp. 2. The vulnerability to cavitation was evaluated by scoring the P_{50} values, xylem pressure inducing 50% loss of conductance, while the mechanical properties were evaluated by the Young's modulus. Each data represents the mean value (\pm S.E.) from 4 to 5 leaves. Data that are significantly different from the control line (T89) according to an ANOVA ($P < 0.05$) are indicated with an asterisk (*).

Line	717-1b4	CCR-62.13fas	CCR-52.3fs	70ASCAD 25.1	70ASCAD 34.2	ASOMT 10b	ASOMT 2b	MYB308- 25.1	MYB308-12.3	SCAD1
Exp. 1										
P_{50} (MPa)	-2.05 \pm 0.04	-1.97 \pm 0.11	-1.73 \pm 0.08*	-1.87 \pm 0.13	-1.92 \pm 0.07	-1.92 \pm 0.08	-1.94 \pm 0.08	-2.03 \pm 0.08	-1.84 \pm 0.19	-1.90 \pm 0.02
Young's modulus (MPa)	10,634 \pm 571	8025 \pm 119*	8691 \pm 251*	7614 \pm 679*	10,369 \pm 683	9773 \pm 304	9472 \pm 501	10,729 \pm 675	10,181 \pm 960	11,545 \pm 159
Exp. 2										
P_{50} (MPa)	-2.55 \pm 0.09	-2.35 \pm 0.12*	-2.46 \pm 0.01	-2.36 \pm 0.06*	-2.33 \pm 0.06*	-2.23 \pm 0.02*	-2.18 \pm 0.04*	-2.21 \pm 0.03*	-2.43 \pm 0.05	-2.42 \pm 0.08
Young's modulus (MPa)	9909 \pm 408	8706 \pm 575	9872 \pm 624	8841 \pm 359	10,357 \pm 354	10,196 \pm 312	9004 \pm 440	11,417 \pm 607*	10,809 \pm 981	40,432 \pm 497

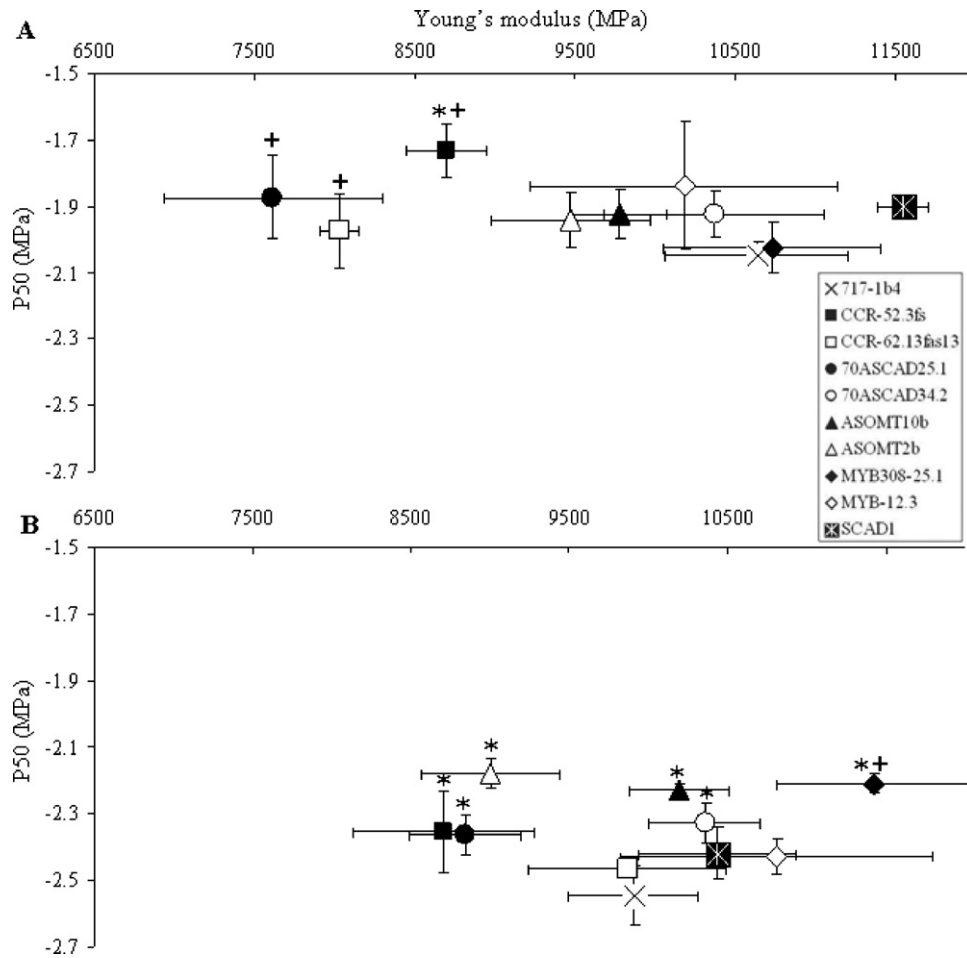


Fig. 1. Correlation between Young's modulus and P_{50} (xylem pressure causing 50% loss of hydraulic conductance) in the transgenic poplar lines, in Exp. 1 (A) and Exp. 2 (B). Each dot represents data for one transgenic line for both the Young's modulus and the P_{50} values. Data are mean values (\pm S.E.) from 4 to 5 plants. The linear regression was not significant at $P < 0.05$. Data significantly different between transgenic lines and the control 717-1b4 line are indicated by an asterisk (*) for P_{50} and a cross (+) for Young's modulus.

MYB308-12.3 in Exp. 2 (Fig. 2A). No difference was found between the transgenic and the control lines for wood density, except in Exp. 1 for the SCAD1 line (Fig. 2A and B).

The difference found for P_{50} between ASOMT10b and ASOMT2b and the control line 717-1b4 for P_{50} in Exp. 2 prompted us to study their anatomical features and the regulation of their wall metabolism at the gene expression level. The vessel diameters, the area fractions they occupied and the vessel density showed no significant difference between the three lines (Table 4). However, ASOMT10b and ASOMT2b showed a lower value of the resistance to implosion index evaluated by $(t/b)^2$ in both Exp. 1 and Exp. 2 (Table 4).

To explain this difference in $(t/b)^2$, the expression of ten genes involved in the wall metabolism (Table 1) was studied in xylem tissue for the ASOMT10b and ASOMT2b lines and compared with the control line 717-1b4 (Fig. 3). Since the difference in $(t/b)^2$ was found in both Exp. 1 and Exp. 2, we investigated the gene expression only for one experiment, Exp. 1. The ASOMT10b and ASOMT2b lines showed a down-regulated expression for all the genes compared with the control line, and these differences were significant in an ANOVA test (Fig. 3).

4. Discussion

We used several lines of transgenic poplars to investigate trade-off and causal links between hydraulic safety and the mechanical

properties of the wood. These different transgenic lines were differently affected as regards the lignin metabolism: briefly, ASOMT transgenic poplars exhibit a strongly decreased S/G ratio and more condensed lignins (VanDoorselaere et al., 1995; Lapierre et al., 1999); ASCAD transgenic poplars appear to be modified in the structure of the lignin polymer, which is likely to be more fragmented than in control plants (Lapierre et al., 1999). CCR down-regulated plants exhibit a patchy phenotype with orange spots where lignin content appears to be strongly reduced (up to 50%), whereas lignin content does not appear altered in wood areas without this colored phenotype. Finally, MYB expressing transgenic poplars have not been characterized for their altered lignin metabolism, but they exhibited a reduced growth, which we could tentatively relate to the reduced lignin content generally observed in tobacco expressing this same AmMYB308 gene (Tamagnone et al., 1998). Although these transgenic lines are not fully characterized for their xylem features, the different modifications in the lignins composition and structure offer many possibilities to test the role of the lignins in the mechanical and hydraulic properties, and especially the trade-off between the two traits.

Transgenic poplars with severe reductions in xylem lignin contents were previously shown to have a reduced efficiency in water transport (Coleman et al., 2008; Kitin et al., 2010). Here, no transgenic line showed any difference in water status, no difference for ψ_m and ψ_p being detectable before and after watering. Likewise, we found no difference for xylem hydraulic conductivity between

Table 4

Anatomical parameters of the xylem from the ASOMT10b and ASOMT2b lines. Each data point represents a mean value (\pm S.E.) from 4 to 5 plants, with six stem sections per plant. For each data line, values followed by different letters are significantly different according to an ANOVA analysis at $P \leq 0.05$. Vessel diameter, area fraction occupied by vessels (A_v), vessel density and implosion resistance index $(t/b)^2$.

Parameter	Exp. 1			Exp. 2		
	717-1b4	ASOMT 10b	ASOMT 2b	717-1b4	ASOMT 10b	ASOMT 2b
Vessel diameter (μm)	21.39 \pm 0.36 ^a	21.03 \pm 0.59 ^a	21.42 \pm 1.08 ^a	21.10 \pm 0.35 ^a	21.46 \pm 1.04 ^a	22.00 \pm 1.07 ^a
A_v (%)	7.90 \pm 2.02 ^a	8.23 \pm 1.58 ^a	8.59 \pm 1.33 ^a	7.76 \pm 0.77 ^a	7.76 \pm 0.77 ^a	8.77 \pm 1.63 ^a
Vessel numerical density (mm^{-2})	12.30 \pm 3.02 ^a	13.10 \pm 2.39 ^a	13.57 \pm 2.01 ^a	12.14 \pm 1.22 ^a	12.26 \pm 2.80 ^a	13.86 \pm 2.18 ^a
$(t/b)^2$ (10^{-3})	9.7 \pm 3.3 ^a	7.0 \pm 1.7 ^b	7.0 \pm 2.2 ^b	8.7 \pm 2.9 ^a	4.9 \pm 1.6 ^b	3.9 \pm 0.9 ^b

the lines (data not shown). Thus it seems that the changes in lignin composition in our transgenic lines did not affect the water transport, despite a drastic change in the lignin content (Coleman et al., 2008; Kitin et al., 2010). If lignin metabolism changes are able

to modify the conductivity, the impact in our transgenic lines is not strong enough that we can enlighten it. Moreover, we can exclude any variation in the water availability between the poplars lines in the course of the experiment, and thus any difference in

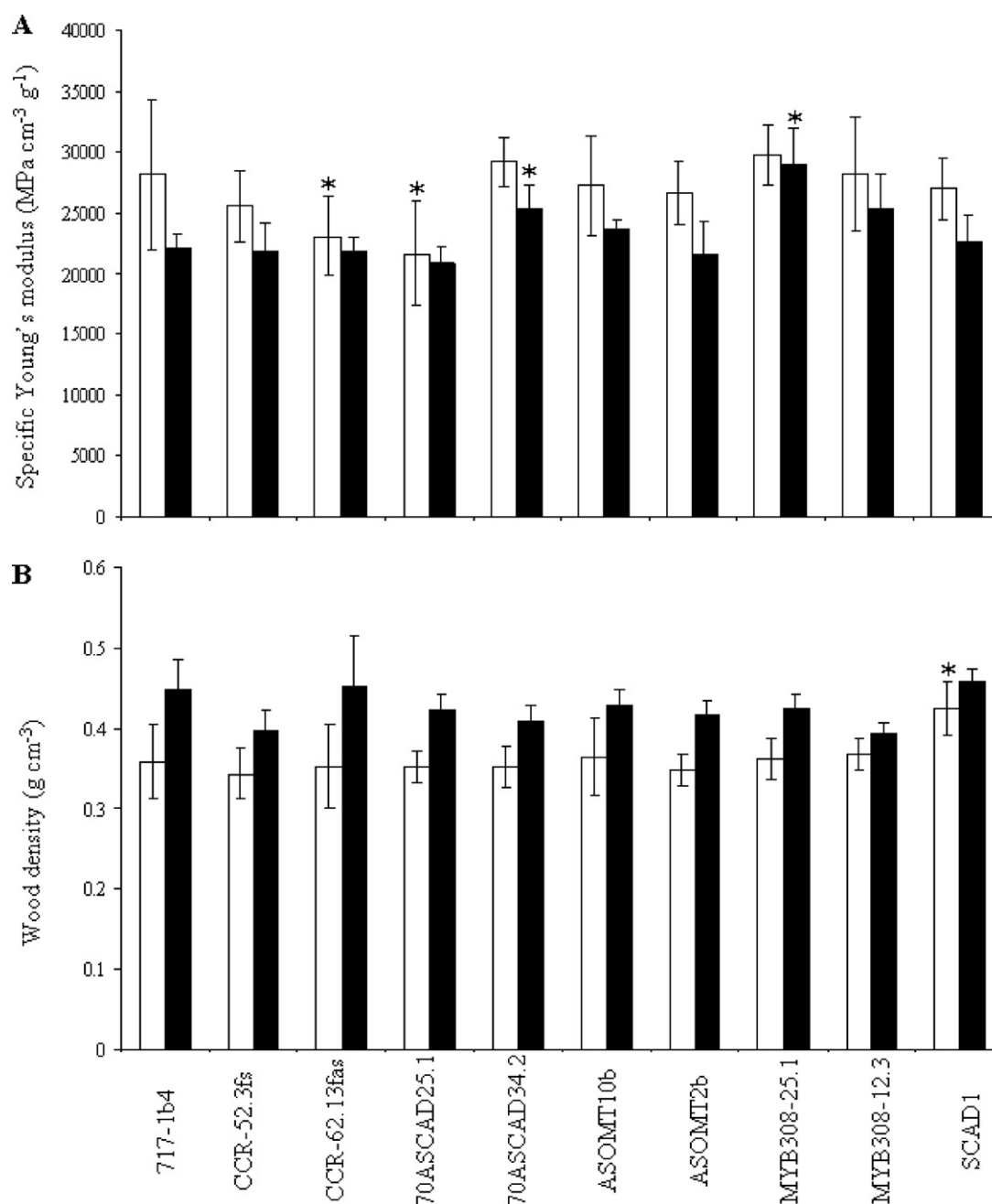


Fig. 2. Specific Young's modulus (A) and wood density (B) in transgenic poplar lines. Open bars represent Exp. 1 and closed bars Exp. 2. Data are mean values (\pm S.E.) from 4 to 5 samples. Data significantly different between transgenic lines and the control 717-1b4 line are indicated by an asterisk (*).

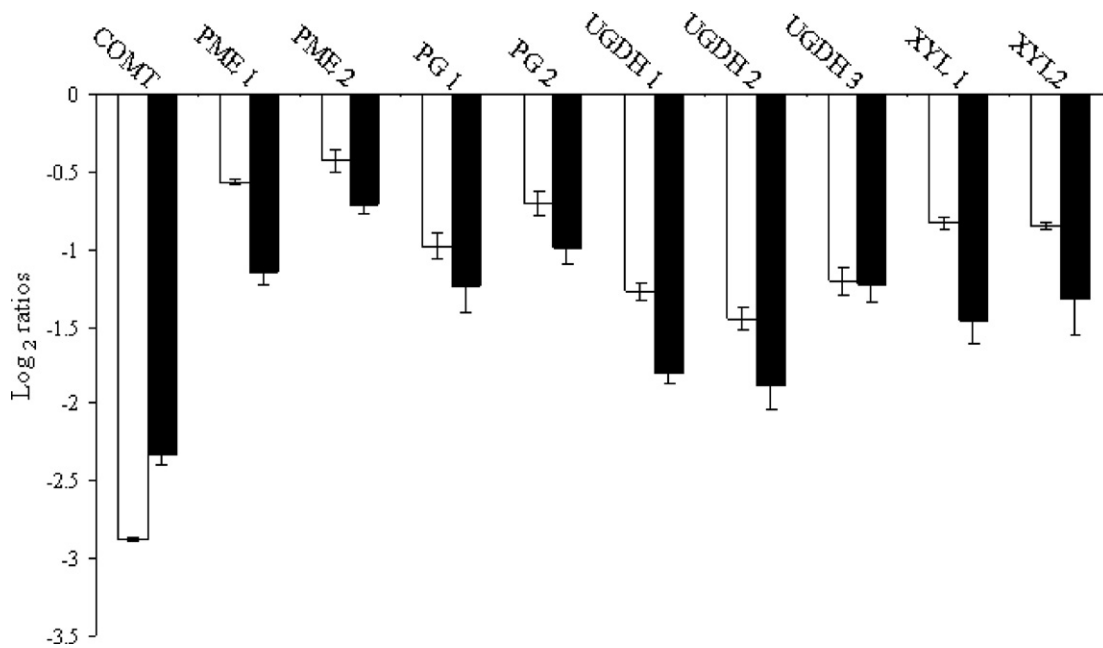


Fig. 3. Quantitative real time PCR (QPCR) analysis of the relative transcript abundance of genes from wall metabolism in ASOMT10b and ASOMT2b lines. Relative transcript abundance was expressed as the ratio of gene expression level in ASOMT10b (open bars) or in ASOMT2b (closed bars) compared with the control line 717-1b4 in Exp. 1. The names of the genes investigated, with their accession numbers and the sequences primers are given in Table 1. Each data point represents a mean value (\pm S.E.) from 3 replicates.

acclimation to the water status between transgenic lines for vulnerability to cavitation. This had to be checked, since we previously showed that vulnerability to cavitation varied according to the water status of the poplar tree (Awad et al., 2010).

It has been hypothesized that stronger woods show higher resistance to drought and to vessel implosion (Hacke et al., 2001; Jacobsen et al., 2005). Also many studies have addressed the relationship between the hydraulic and mechanical stress adaptations, especially the trade-off between hydraulic conductivity (efficiency) and mechanical strength. Nevertheless, it can easily be demonstrated that this simple trade-off cannot be explained by physical considerations: according to the Hagen–Poiseuille law, the hydraulic conductance scales with the fourth power of the vessel radius and leads to large vessels. On the other hand, for a given biomass production (the amount of cell walls), the best way to increase the stem rigidity is to increase its diameter and not to increase the local Young's modulus of the material by way of thicker cell walls. Thus on the contrary, the theoretical optimization of the anatomical structure would lead to an increase in the stem diameter by way of a very porous xylem made of very large conduits and thin cell walls. It is clear that the key point cannot be this hydraulic conduction – Young's modulus trade-off (Kern et al., 2005) and research work is accordingly now focused on the hydraulic safety function (Rosner et al., 2008; Utsumi et al., 2010; Pratt et al., 2007). Seeking a relation between resistance to cavitation (and/or implosion) and mechanical strength, researchers found a positive correlation at both within- and between-species levels. They also found that the two parameters were positively correlated with the wood density. However, our data on the transgenic poplar lines do not support this trade-off, although we observed variations for both traits at a similar range. They showed no relationship, neither positive nor negative, between resistance to cavitation (P_{50}) and longitudinal mechanical properties (Young's modulus, Fig. 1). In the first experiment (Exp. 1), we found significant differences between lines for the mean of Young's modulus, such as 70ASCAD25.1, CCR-62.13fas13 and CCR-52.3fs, which showed lower elastic properties than the control line, but only the CCR-52.3fs showed a

statistically higher P_{50} value. By contrast, in the second experiment (Exp. 2), the six lines that were significantly more vulnerable to cavitation compared with the control line showed both more or less favorable mechanical properties and clearly not necessarily the worst mechanical properties. Only line MYB308-25.1 showed better mechanical properties and vulnerability to cavitation than the control line 717-1b4, at variance with the positive correlation described by others (Rosner et al., 2008; Utsumi et al., 2010; Pratt et al., 2007). To support the lack of trade-off between the two traits, we observed an acclimation for P_{50} but not for the Young's modulus between the two experiments (Fig. 1A and B). The P_{50} mean value decreased significantly ($P < 0.001$) from -1.91 MPa in Exp. 1 to -2.35 MPa in Exp. 2, whereas the Young's modulus mean values in Exp. 1 (9495 MPa) and in Exp. 2 (9934 MPa) were not significantly different ($P = 0.158$). The correlation between P_{50} and Young's modulus was also tested per plant for each experiment (data and figures not shown). There was no correlation, and the linear regression was not significant. However, we have to distinguish between the wood of conifers and that of angiosperms. In conifers, the same cells have to deal with both mechanical and hydraulic considerations and the cells that compose the surrounding material of a conducting tracheid are similar. In angiosperms, cells are specialized, with vessels that support hydraulic function and surrounding cells that are responsible for most of the macroscopic wood mechanical behavior. Hence the angiosperm xylem structure has many possibilities and it is easy to conceive that a change of lignins structure in the cell wall can affect hydraulic properties without modifying the mechanical properties, and *vice versa*.

At the tissue level, longitudinal wood mechanical behavior is mainly linked to material porosity, i.e. the ratio between the lumen area and the cell wall area. Assuming, as is generally accepted, that the cell wall density is constant, we arrive at the well-known positive relationship between density and wood elasticity that partially contributes to the relationship between latewood percentage and mechanical behavior. To a lesser extent, the density is also correlated with the transversal mechanical properties of wood, but this relationship is much more complex and involves the anisotropy of

the cellular structure (Gibson and Ashby, 1999). Our results show no significant difference between the transgenic lines for the wood density, except for the line SCAD1 (Fig. 2B), nor any correlation between the density and the Young's modulus (data not shown). Consequently, the mechanical behavior of the 70ASCAD-25.1, CCR-62.13fas13, CCR-52.3fs and MYB308-25.1 lines can be explained by a modification of their specific Young's modulus (Fig. 2A), which can be regarded as an intrinsic cell wall property. The cellulose microfibril angle and cell wall chemistry are the main parameters that determine the wall properties (Cave, 1968; Persson, 2000; Gindl, 2001; Mencuccini et al., 1997; Jagels et al., 2003; Jagels and Visscher, 2006). Lignins are randomly oriented in the polymer in the form of a rigid amorphous polymer matrix that is responsible for the stiffening of the cell wall and for the resistance to compression (Gindl, 2002). Modifying lignins should necessarily modify the cell wall properties in an isotropic way, i.e. the xylem properties in the three directions, so leading to changes in longitudinal mechanical properties. In agreement with our results, Hepworth and Vincent (1998) showed that transgenic tobacco with a lower CAD activity modified lignins and reduced the Young's modulus without changing fibre diameter or length. Similarly, Bernard-Vailhe et al. (1996) showed that changes in the type of lignins inter-unit linkage could be responsible for reducing the mechanical properties of the cell wall. Thus we can reasonably assume that the lignins modification in the 70ASCAD-25.1, CCR-62.13fas13, CCR-52.3fs and MYB308-25.1 lines changed the mechanical properties of the cell walls.

Another interesting finding in this work is the increase in xylem vulnerability to cavitation in some transgenic lines with modified lignins composition compared with the control lines, without any change in mechanical properties. Drought-induced cavitation in xylem is thought to occur when an air bubble penetrates a pit wall (Zimmermann, 1983; Cochard et al., 1992; Cochard, 2006). Hence it is well-accepted that the vulnerability to cavitation relies on the pit properties. However, studies on the molecular and genetic basis of cavitation resistance are scarce. It has been demonstrated that the pectin-bound calcium is important for xylem vulnerability to cavitation (Herbette and Cochard, 2010) and a role of lignins has been suggested in xylem vulnerability to cavitation (Coleman et al., 2008). In this last study, a decrease in the xylem lignin content in transgenic poplars resulted in an increase in vulnerability to cavitation. However, also in this work, large areas of the xylem were observed as collapsed in these transgenic plants, and this could probably constitutes a bias in the experimental measures of the vulnerability to cavitation. Indeed, the vulnerability to cavitation is experimentally estimated through the cavitation-induced loss of conductance. And, the collapse of functional conduits geometrically generates a drastic decrease of the conductance behavior. Thus, an apparent loss of conductance can be measured regardless of cavitation events and irreversible cessation of the functionality of the conduits. Further, the xylem collapse can weaken the pit structure and make it more vulnerable. Here, the ASOMT10b and ASOMT2b lines exhibited an increase in vulnerability to cavitation compared with the control line (Fig. 1B), but with no xylem collapse before or after cavitation events (data not shown). This difference can be explained by four non-exclusive hypotheses.

The first hypothesis relates to the pit membrane composition. On the one hand, lignins have been detected in the pit membrane of beech xylem (Fromm et al., 2003). The middle lamella in xylem tissue contains much lignins (more than 60%), and the primary wall has the highest concentration of lignins, the S2 layer containing in proportion the lowest level (around 25%) (Navi and Heger, 2005). On the other hand, there is experimental evidence for a role of the mechanical properties of the pit membrane in the cavitation process (Choat et al., 2004; Sperry and Hacke, 2004). Moreover, lignins polymers are hydrophobic components (Sarkanen and Ludwig,

1971). This property could play a key role in the permeability of the pits. Lastly, changes in the hydrophilic surface properties of vessels could impact the adhesion of water to the wall that would modify the contact angle with the wall at the air–water interface, which influence the speed propagation of the air bubble in the conduit (Kohonen, 2006). Thus it is very plausible that a modification in the lignins structure could affect the pit membrane behavior with or without significant impact on the mechanical properties as for ASOMT2b and ASOMT10b lines.

The second hypothesis relates to the pit membrane thickness. The down-regulation of the COMT genes in these transgenic poplars induced a down-regulation of many other genes involved in the primary wall metabolism, especially in pectin and hemicelluloses metabolism (Fig. 3): two pectin methylsterases (PME), two polygalacturonases, three UDP-glucose dehydrogenases and two xylan synthases. Each of the investigated isoforms has been found to be specifically expressed during the xylogenesis (Hertzberg et al., 2001). We can also assume that this xylem-specific down-regulation of the primary wall components could result in a weakened pit membrane, i.e. one more vulnerable to cavitation events, without affecting the longitudinal mechanical properties, which rely mainly on the prevailing secondary wall. These two hypotheses are the most likely.

The third hypothesis relates to the “rare pit” hypothesis, also called the “pit area hypothesis”. It states that pits with pores of air-seeding size are very rare compared with the great majority of pits with much narrower air-tight pores (Hargrave et al., 1994; Choat et al., 2003; Wheeler et al., 2005). Because of this, the vulnerability of a given conduit is heavily influenced by the area of pit membrane it contains: the larger the pit membrane area, the higher the probability that it contains a vulnerable pit; this leads to the vulnerability of the complete conduit. Evidence supporting this hypothesis includes the often observed rarity of pit membrane pores of air-seeding size, the lack of consistent correlation between indicators of mean membrane pore size and vulnerability to cavitation, and a significant correlation between inter-conduit pit area and vulnerability to cavitation (Hargrave et al., 1994; Choat et al., 2003; Wheeler et al., 2005; Hacke et al., 2006; Jansen et al., 2009). In the context of this “rare pit” hypothesis, we may venture the hypothesis that the ASOMT10b and ASOMT2b lines have longer vessels and/or larger pits, since vessel diameters were unchanged in the poplar lines (Table 4). This would increase the vessel wall area and thus the pit area, which would lead to plants that are more vulnerable to cavitation. Since the impact of lignin metabolism on the vessel length is clearly a hazardous hypothesis we cannot support, this “rare pit” hypothesis is rejected.

The fourth hypothesis involves transversal mechanical properties of the xylem. Hacke et al. (2001) suggest that plants that exhibit a high degree of cavitation resistance and that experience greater negative pressures, show thicker vessel walls relative to their lumen diameter, i.e. a higher $(t/b)^2$ index, which would be a way to resist transverse buckling of the conduits. Considering an isolated vessel, its implosion resistance mechanically increases with the decrease in vessel lumen diameter and/or an increase in wall thickness, and its tangential wall properties. Our anatomical measurements show that a large decrease in the $(t/b)^2$ index for ASOMT10b, ASOMT2b is mainly due to the thinning of the wall and not the increase in the vessel lumen diameter (Table 4). However, at the xylem tissue level, the vessel resistance is related not only to its diameter, but also to the transversal mechanical properties of the surrounding tissues.

Modifications of the lignins composition in a number of transgenic poplars has enabled us to demonstrate that the correlation usually observed between vulnerability to cavitation and the mechanical properties does not rely on a trade-off, or only in part. Further analyses of the macromolecular structure on these

transgenic plants are needed to understand what changes of lignins modifications could affect either the vulnerability to cavitation or the mechanical properties. We report evidence that lignins are involved in the vulnerability to cavitation, probably through modifications of the pits. To date, pit function in xylem hydraulics has been exclusively restricted to the pectin components, and this study opens new avenues of research in pit function related to the lignins. To gain a better understanding of the role of pits in hydraulic efficiency and safety, further research should address the composition of pits in relation to the hydraulic traits, and consider the role of lignins.

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References

- Awad, H., Barigah, T., Badel, E., Cochard, H., Herbette, S., 2010. Poplar vulnerability to xylem cavitation acclimates to drier soil conditions. *Physiologia Plantarum*.
- Baucher, M., Chabbert, B., Pilate, G., VanDoorselaere, J., Tollier, M.T., Petit-Conil, M., Cornu, D., Monties, B., VanMontagu, M., Inze, D., Jouanin, L., Boerjan, W., 1996. Red xylem and higher lignin extractability by down-regulating a cinnamyl alcohol dehydrogenase in poplar. *Plant Physiology* 112, 1479–1490.
- Bernard-Vailhe, M.A., Cornu, A., Robert, D., Maillot, M.P., Besle, J.M., 1996. Cell wall degradability of transgenic tobacco stems in relation to their chemical extraction and lignin quality. *Journal of Agricultural and Food Chemistry* 44, 1164–1169.
- Boudet, A.M., Lapierre, C., Grima-Pettenati, J., 1995. Biochemistry and molecular biology of lignification. *New Phytologist* 129, 203–236.
- Brodrribb, T.J., Cochard, H., 2009. Hydraulic failure defines the recovery and point of death in water-stressed conifers. *Plant Physiology* 149, 575–584.
- Brodrribb, T.J., Holbrook, N.M., 2005. Water stress deforms tracheids peripheral to the leaf vein of a tropical conifer. *Plant Physiology* 137, 1139–1146.
- Cave, I.D., 1968. The anisotropic elasticity of the plant cell wall. *Wood Sciences and Technology* 2, 268–278.
- Chabannes, M., Ruel, K., Yoshinaga, A., Chabbert, B., Jauneau, A., 2001. *In situ* analysis of lignins in transgenic tobacco reveals a differential impact of individual transformations on the spatial patterns of lignin deposition at the cellular and subcellular levels. *Plant Journal* 28, 271–282.
- Chang, C., Kwok, S.F., Bleecker, A.B., Meyerowitz, E.M., 1993. Arabidopsis ethylene-response gene ETR1 Similarity of product to two-component regulators. *Science* 262, 539–544.
- Choat, B., Ball, M., Luy, J., Holtum, J., 2003. Pit membrane porosity and water stress-induced cavitation in four co-existing dry rain forest trees species. *Plant Physiology* 131, 41–44.
- Choat, B., Jansen, S., Zwieniecki, M.A., Smets, E., Holbrook, N.M., 2004. Changes in pit membrane porosity due to deflection and stretching: the role of vested pits. *Journal of Experimental Botany* 25, 815–819.
- Christensen-Dalsgaard, K.K., Fournier, M., Ennos, A.R., Barford, A.S., 2007. Changes in vessel anatomy in response to mechanical loading in six species of tropical trees. *New Phytologist* 176, 610–622.
- Cochard, H., 2002. A technique for measuring xylem hydraulic conductance under high negative pressures. *Plant Cell and Environment* 25, 815–819.
- Cochard, H., 2006. Cavitation in trees. *Comptes Rendus Physique* 7, 1018–1026.
- Cochard, H., Cruzat, P., Tyree, M.T., 1992. Use of positive pressures to establish vulnerability curves further support for the air-seeding hypothesis and implications for pressure-volume analysis. *Plant Physiology* 100, 205–209.
- Cochard, H., Froux, F., Mayr, F.F.S., Coutand, C., 2004. Xylem wall collapse in water-stressed pine needles. *Plant Physiology* 134, 401–408.
- Cochard, H., Gaele, D., Bodet, C., Tharwat, I., Poirier, M., Améglio, T., 2005. Evaluation of a new centrifuge technique for rapid generation of xylem vulnerability curves. *Physiologia Plantarum* 124, 410–418.
- Coleman, H.D., Lacey, S.A., Robert, D.G., Shawn, D.M., 2008. Perturbed lignification impacts tree growth in hybrid poplar – a function of sink strength vascular integrity, and photosynthetic assimilation. *Plant Physiology* 148, 1229–1237.
- Dean, J.F.D., Eriksson, K.-E., 1992. Biotechnological modification of lignin structure and composition in forest trees. *Holzforschung* 46, 135–147.
- Freudenberg, K., Neish, A.L., 1968. *Constitution and Biosynthesis of Lignin*. Springer, New York, p. 129.
- Fromm, J., Rockel, B., Lautner, S., Windeisen, E., Wanner, G., 2003. Lignin distribution wood cell walls determined by TEM and backscattered SEM techniques. *Journal of Structural Biology* 143, 77–84.
- Gartner, B.L., 1991a. Is the climbing habit of poison oak ecotypic? *Functional Ecology* 5, 696–704.
- Gartner, B.L., 1991b. Stem hydraulic-properties of vines vs shrubs of western poison oak *Toxicodendron diversilobum*. *Oecologia* 87, 180–189.
- Gartner, B.L., 1991c. Structural stability and architecture of vines vs shrubs of poison oak *Toxicodendron diversilobum*. *Oecologia* 72, 2005–2015.
- Gibson, L.J., Ashby, M.F., 1999. *Cellular Solids, Structure and Properties*, 2nd ed. Cambridge University Press, 510 p.
- Gindl, W., 2001. The effect of lignin on the moisture-dependent behavior of spruce wood in axial compression. *Journal of Materials Science Letters* 20, 2161–2162.
- Gindl, W., 2002. Comparing mechanical properties of normal and compression wood in Norway spruce: the role of lignin in compression parallel to the grain. *Holz-forschung* 56 (4), 395–401.
- Hacke, U.G., Sperry, J.S., Pockman, W.T., Davis, S.D., McCulloh, K.A., 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126, 457–461.
- Hacke, U.G., Sperry, J.S., Wheeler, J.K., Castro, L., 2006. Scaling of angiosperm xylem structure with safety and efficiency. *Tree Physiology* 26, 689–701.
- Hargrave, K.R., Kolb, K.J., Ewers, F.W., Davis, S.D., 1994. Conduit diameter and drought-induced embolism in *Salvia mellifera* Greene (Labiatae). *New Phytologist* 126, 695–705.
- Harkin, J.M., 1967. Ligna natural polymeric product of phenol oxidation. In: Taylor, W.I., Battersby, A.R. (Eds.), *Oxidative Coupling of Phenols*. Marcel Dekker, New York, pp. 243–321.
- Hepworth, D.G., Vincent, J.F.V., 1998. The mechanical properties of xylem tissue from tobacco plants. *Annals of Botany* 81, 751–759.
- Hepworth, D.G., Vincent, J.F.V., 1999. The growth response of the stems of genetically modified tobacco plants (*Nicotiana tabacum* ‘Samsun’) to flexural stimulation. *Annals of Botany* 83, 39–43.
- Herbette, S., Cochard, H., 2010. Calcium is a major determinant of xylem vulnerability to cavitation. *Plant Physiology* 153, 1–8.
- Hertzberg, M., Aspeberg, H., Schrader, J., Andersson, A., Erlandsson, R., Blomqvist, K., BhalariaoR, Uhlén, M., Teeri, T.T., Lundberg, J., Sundberg, B., Nilsson, P., Sandberg, G., 2001. A transcriptional roadmap to wood formation. *PNAS* 98, 14732–14737.
- Jacobsen, A.L., Ewers, F.W., Pratt, R.B., Paddock, W.A., Davis, S.D., 2005. Do xylem fibers affect vessel cavitation resistance? *Plant Physiology* 139, 546–556.
- Jagels, R., Visscher, G.E., 2006. A synchronous increase in hydraulic conductive capacity and mechanical support in conifers with relatively uniform xylem structure. *American Journal of Botany* 93, 179–187.
- Jagels, R., Visscher, G.E., Lucas, J., Goodell, B., 2003. Paleo-adaptive properties of the xylem of *Metasequoia*: mechanical/hydraulic compromises. *Annals of Botany* 92, 79–88.
- Jansen, S., Choat, B., Pletsers, A., 2009. Morphological variation of intervessel pit membranes and implications to xylem function in angiosperms. *American Journal of Botany* 96, 409–419.
- Kern, K., Ewers, F., Telewski, F., Koeler, L., 2005. Mechanical perturbation affects conductivity, mechanical properties and aboveground biomass of hybrid poplars. *Tree Physiology* 25, 1243–1251.
- Kim, H., Ralph, J., Lu, F., Pilate, G., Leple, J.-C., Pollet, B., Lapierre, C., 2002. Identification of the structure and origin of thioacidolysis marker compounds for cinnamyl alcohol dehydrogenase deficiency in angiosperms. *Journal of Biological Chemistry* 277, 47412–47419.
- Kitin, P., Voelker, S.L., Meinzer, F.C., Beeckman, H., Strauss, S.H., Lachenbruch, B., 2010. Tyloses and phenolic deposits in xylem vessels impede water transport in low-lignin transgenic poplars: a study by cryo-fluorescence microscopy. *Plant Physiology* 154, 887–898.
- Kohonen, M.M., 2006. Engineered wettability in tree capillaries. *Langmuir* 2 (7), 3148–3153.
- Lapierre, C., Pollet, B., Petit-Conil, M., Toval, G., Romero, J., Pilate, G., Leplé, J.-C., Boerjan, W., Ferret, V., De Nadai, V., Jouanin, L., 1999. Structural alterations of lignins in transgenic poplars with depressed cinnamyl alcohol dehydrogenase or caffeic acid O-methyl-transferase activity have opposite impact on the efficiency of industrial kraft pulping. *Plant Physiology* 119, 153–163.
- Lapierre, C., Pilate, G., Pollet, B., Mila, I., Leplé, J.-C., Jouanin, L., Kim, H., Ralph, J., 2004. Signatures of cinnamyl alcohol dehydrogenase deficiency in poplar lignins. *Phytochemistry* 65, 313–321.
- Leplé, J.-C., Grima-Pettenati, J., Van Montagu, M., Boerjan, W., 1998. A cDNA encoding cinnamoyl-CoA reductase from *Populus trichocarpa* (accession no AJ224986) (PGR98-121). *Plant Physiology* 117, 1126.
- Leplé, J.-C., Dauwe, R., Morreel, K., Storme, V., Lapierre, C., Pollet, B., Naumann, A., Kang, K.Y., Kim, H., Ruel, K., Lefèbvre, A., Joseleau, J.P., Grima-Pettenati, J., De Rycke, R., Andersson-Gunnerås, S., Erban, A., Fehrle, I., Petit-Conil, M., Kopka, J., Polle, A., Messens, E., Sundberg, B., Mansfield, S.D., Ralph, J., Pilate, G., Boerjan, W., 2007. Downregulation of cinnamoyl-coenzyme A reductase in poplar: multiple-level phenotyping reveals effects on cell wall polymer metabolism and structure. *Plant Cell* 19, 3669–3691.
- Lichtenegger, H., Reiterer, A., Stanzl-Tschegg, S.E., Fratzl, P., 1999. Variation of cellulose microfibril angles in softwoods and hardwoods – a possible strategy of mechanical optimization. *Journal of Structural Biology* 128, 257–269.
- Lindström, H., Evans, J.W., Verrill, S.P., 1998. Influence of cambial age and growth conditions on microfibril angle in young Norway spruce (*Picea abies* (L.) Karst.). *Holzforschung* 52, 573–581.
- Maherali, H., Pockman, W.T., Jackson, R.B., 2004. Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* 85, 2184–2199.
- Mai, J., Herbette, S., Vandame, M., Kositsup, B., Kasemsap, P., Cavaloc, E., Julien, J.L., Améglio, T., Roedel-Drevet, P., 2009. Effect of chilling on photosynthesis and antioxidant enzymes in *Hevea brasiliensis* Muell. Arg. *Trees* 23, 863–874.

- McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G., Yepez, E.A., 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* 178, 719–739.
- Mencuccini, M., Grace, J., Fioravanti, M., 1997. Biomechanical and hydraulic determinants of tree structure in scots pine. *Anatomical characteristics. Tree Physiology* 17, 105–113.
- Meylan, B.A., Probine, M.C., 1969. Microfibril angle as a parameter in timber quality assessment. *Forest Products Journal* 19, 31–34.
- Morizet, J., Mingeau, M., 1976. Effect of environment on water-uptake, as studied on beheaded exuding tomato. 1. Role of nutrients. *Annals of Agronomy* 27, 183–205.
- Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum* 15, 473–492.
- Navi, P., Heger, F., 2005. Comportement thermo-hydrromécanique du bois. *Presses polytechniques et universitaires romandes, Lausanne*.
- Niklas, K.J., 1992. 10 Biomechanics and plant evolution. In: *Plant Biomechanics. An Engineering Approach to Plant Form and Function*. University of Chicago Press, Chicago, pp. 474–573.
- Niklas, K.J., 1997. Mechanical properties of black locust (*Robinia pseudoacacia*) wood. Correlations among elastic and rupture moduli, proportional limit, and tissue density and specific gravity. *Annals of Botany* 79, 479–485.
- Nualpun, S., Pluang, S., Russell, F.D., Wallie, S., 2005. Molecular cloning of a new cDNA and expression of 3-hydroxy-3-methylglutaryl-CoA synthase gene from *Hevea brasiliensis*. *Planta* 221, 502–512.
- Pammenter, N.W., Van Der Willigen, C., 1998. A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree Physiology* 18, 589–593.
- Persson, K., 2000. Micromechanical modelling of wood and fiber properties. Thesis. Lund University Press, p. 213.
- Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* 29 (9), e45.
- Pilate, G., Guiney, E., Holt, K., Petit-Conil, M., Lapiere, C., Leple, J.C., Pollet, B., Mila, I., Webster, E.A., Marstorp, H.G., Hopkins, D.W., Jouanin, L., Boerjan, W., Schuch, W., Cornu, D., Halpin, C., 2002. Field and pulping performances of transgenic trees with altered lignification. *Nature Biotechnology* 20, 607–612.
- Pockman, W.T., Sperry, J.S., 2000. Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *American Journal of Botany* 87, 1287–1299.
- Pratt, R.B., Jacobsen, A.L., Ewers, F.W., Davis, S.D., 2007. Relationships among xylem transport, biomechanical and storage in stems and roots of nine Rhamnaceae species of the California chaparral. *New Phytologist* 174, 787–798.
- Ralph, J., Lapiere, C., Marita, J.M., Kim, H., Lu, F., Hatfield, R.D., Ralph, S., Chapple, C., Franke, R., Hemm, M.R., van Doorselaere, J., Sederoff, R.R., O'Malley, D.M., Scott, J.T., MacKay, J.J., Yahiaoui, N., Boudet, A.M., Pean, M., Pilate, G., Jouanin, L., et Boerjan, W., 2001a. Elucidation of new structures in lignins of CAD- and COMT-deficient plants by NMR. *Phytochemistry* 57, 993–1003.
- Ralph, J., Lapiere, C., Lu, F., Marita, J., Pilate, G., van Doorselaere, J., Boerjan, W., Jouanin, L., 2001b. NMR evidence for benzodioxane structures resulting from incorporation of 5-hydroxyconiferyl alcohol into lignins of O-methyltransferase-deficient poplars. *Journal of Agricultural and Food Chemistry* 49, 86–91.
- Rasband, W.S., 1997–2009. ImageJ. US National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/ij/>.
- Rosner, S., Klein, A., Muller, U., Karlsson, B., 2007. Hydraulic and mechanical properties of young Norway spruce clones related to growth and wood structure. *Tree Physiology* 27, 1165–1178.
- Rosner, S., Klein, A., Muller, U., Karlsson, B., 2008. Tradeoffs between hydraulic and mechanical stress responses of mature Norway spruce trunk wood. *Tree Physiology* 28, 1179–1188.
- Sarkanen, K.V., Ludwig, C.H., 1971. In: Sarkanen, K.V., Ludwig, C.H. (Eds.), *Lignin: Occurrence, Formation, Structure and Reactions*. Wiley-Interscience, New York, p. 916 p.
- Scholander, P., Hammel, H., Bradstreet, E., Hemmingsen, E., 1965. Sap pressure in vascular plants. *Science* 148, 339–346.
- Sperry, J.S., Hacke, U.G., 2004. Analysis of circular bordered pit function I Angiosperm vessels with homogenous pit membranes. *American Journal of Botany* 91, 369–385.
- Tamagnone, L., Merida, A., Parr, A., Mackay, S., Culianez-Macia, F.A., Roberts, K., Martin, C., 1998. The AmMYB308 and AmMYB330 transcription factors from *Antirrhinum* regulate phenylpropanoid and lignin biosynthesis in transgenic tobacco. *Plant Cell* 10, 135–154.
- Tyree, M.T., Dixon, M.A., 1986. Water stress induced cavitation and embolism in some woody plants. *Physiologia Plantarum* 66, 397–405.
- Tyree, M.T., Engelbrecht, B.M.J., Vargas, G., Kursar, T.A., 2003. Desiccation tolerance of five tropical seedlings in panama relationship to a field assessment of drought performance. *Plant Physiology* 132, 1439–1447.
- Utsumi, Y., Bobich, E.G., Ewers, F.W., 2010. Photosynthetic, hydraulic and biomechanical responses of *Juglans californica* shoots to wildfire. *Oecologia* 164, 331–338.
- VanDoorselaere, J., Baucher, M., Chognot, E., Chabbert, B., Tollier, M.T., Petit-Conil, M., Leple, J.C., Pilate, G., Cornu, D., Monties, B., VanMontagu, M., Inze, D., Boerjan, W., Jouanin, L., 1995. A novel lignin in poplar trees with a reduced caffeic acid 5-hydroxyferulic acid O-methyltransferase activity. *Plant Journal* 8, 855–864.
- Wagner, K.R., Ewers, F.W., Davis, S.D., 1998. Tradeoffs between hydraulic efficiency and mechanical strength in the stems of four co-occurring species of chaparral shrubs. *Oecologia* 117, 53–62.
- Wheeler, J.K., Sperry, J.S., Hacke, U.G., Hoang, N., 2005. Intervessel pitting and cavitation in woody Rosaceae and other vesselless plants: a basis for a safety versus efficiency trade-off in xylem transport. *Plant Cell and Environment* 28, 800–812.
- Whetten, R., Sederoff, R., 1991. Genetic engineering of wood. *Forest Ecology and Management* 43, 301–316.
- Whetten, R., Sederoff, R., 1995. Lignin biosynthesis. *Plant Cell* 7, 1001–1013.
- Woodrum, C.L., Ewers, F.W., Telewski, F.W., 2003. Hydraulic, biomechanical, and anatomical interactions of xylem from five species of *Acer* (Aceraceae). *American Journal of Botany* 90, 693–699.
- Zimmermann, M.H. (Ed.), 1983. *Xylem Structure and the Ascent of Sap*. Springer Verlag, Berlin.