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Genetic variation of xylem hydraulic properties shows that wood density is involved in adaptation to drought in Douglas-fir (*Pseudotsuga menziesii* (Mirb.))

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Abstract

· *Introduction* Relationships between wood density and hydraulic efficiency and safety (hydraulic specific conductivity and vulnerability to cavitation, respectively) could clarify the physiological process explaining the impact of density on fitness. We have used new, relatively high-throughput phenotyping methods to estimate genetic variation of wood hydraulic specific conductivity (k_s) and vulnerability to cavitation (VC) as an important step toward demonstrating the adaptive value of wood density.

· *Objective* The first aim of this study is to test if, in Douglas-fir, there is a relationship between wood hydraulic properties (k_s and VC) and wood density. The second objective is to estimate genetic variation of wood k_s and VC. These results could aid understanding of the role of wood density in the hydraulic properties of xylem and may clarify the role of wood density in adaptation to drought.

· *Results* Many significant relationships were found between wood density and wood hydraulic properties at clone and tree level, as well as significant genetic variation for k_s and VC. We have also found positive correlations between tree height, specific conductivity and vulnerability to cavitation, but no relation was found between radial growth and hydraulic variables.

· *Conclusions* Our results suggest that wood density has an adaptive value and that microdensity can be used to study adaptation to drought in Douglas-fir. The novel methods used to measure k_s and VC proved to be interesting alternatives for localized measurement of wood hydraulic properties and were compatible with a robust estimation of genetic variation.

Keywords Adaptation to drought · Wood density · Douglas-fir · Hydraulic efficiency · Hydraulic safety

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1 Introduction

Conduit lumen diameter, conduit length and cell wall thickness are known to radically affect sap conduction efficiency and protection against cavitation and wall collapse (Cruiziat et al. 2002; McElrone et al. 2004; Sperry et al. 2008; Tyree et al. 1994). The lumen to cell wall proportion of the xylem is closely related to wood density (Bucci et al. 2004; Hacke et al. 2001a; Stratton et al. 2000). Several authors (Domec and Gartner 2002a, b; Hacke et al. 2001a, b; Mayr and Cochard 2003; Stiller 2009) showed that wood density is closely related to the hydraulic

properties of the xylem. A recent study has shown that Douglas-fir trees surviving the 2003 heat and drought wave in France had a significantly higher wood density than trees that died shortly after the heat wave (Martinez-Meier et al. 2008). Dalla-Salda et al. (2009) found that a Douglas-fir clone more resistant to cavitation had a higher wood density than other, more vulnerable, clones. All these results suggest that wood density may be an adaptive trait involved in resistance to drought.

Phenotypic variation of wood hydraulic properties involved in resistance to drought can affect surviving capacity within a tree population and thus fitness. If the wood hydraulic properties involved in resistance to drought are genetically variable and heritable, then favourable phenotypes can be selected and transmitted to the next generation. While it is well known that between-species variation exists for wood hydraulic properties (Bond and Kavanagh 1999; Cavender-Bares and Holbrook 2001; Domec et al. 2007; Sperry and Tyree 1990) and that there is genetic variation for wood density (Cornelius 1994; Johnson and Gartner 2006), fewer results are available concerning within-species genetic variation of wood hydraulic properties related to hydraulic efficiency and safety (Dalla-Salda et al. 2009; Rosner et al. 2007, 2008; Vander Willigen and Pammenter 1998).

Nowadays, relatively high-throughput phenotyping methods for wood hydraulic properties allow rapid, localized measurement of specific conductivity (k_s) (Dalla-Salda et al. 2009) and resistance to cavitation in the wood of adult trees (Cochard 2002; Cochard et al. 2005). This is in accordance with the number of trees compatible with a robust estimation of genetic variation. The indirect X-ray microdensity method developed by Polge (1966) is largely used in genetic improvement programs for the selection of trees with desirable density characteristics. These profiles allow the description of wood density variation within a tree ring, through the radius, with a precision of several micrometres. Combining these methods, we can measure k_s, vulnerability to cavitation (VC) and wood density in exactly the same portion of wood.

The first aim of this study is to test whether, in Douglas-fir, there is a relationship between wood hydraulic properties (k_s and VC) and wood density. The second objective is to estimate genetic variation of wood k_s and VC. If there is genetic variation of k_s and VC, then it could be possible to select genotypes with desirable hydraulic characteristics within the framework of a Douglas-fir breeding program. If we find that there is a relationship between hydraulic properties (k_s and VC) and certain wood density traits, they could be used to indirectly select genotypes with advantageous hydraulic properties. In all cases, these results could help in the understanding of the role of wood density in the hydraulic properties of xylem,

thus clarifying the role of wood density in adaptation to drought.

2 Materials and methods

Douglas-fir trees used for this study belong to a clonal experimental trial installed in Orléans, France, by INRA. The 18-year-old trees belong to seven clones chosen from a total of 27 clones planted at the same site. Randomly selected clones had at least three healthy trees, not more than 4 m apart, in order to minimize within-clone environmental variation. In each tree, we measured total height (height) and stem circumference at breast height (circ).

For each clone, three trees were felled during March 2008. Pieces of stem of 30 cm were cut at 0.10, 1.30 and 3 m from the base (hereafter "positions"). From each tree, we also cut a 60-cm piece next to the base of the stem (Fig. 1). All samples were soaked in plastic tanks filled with water in order to prevent water loss. Samples were sawn to: three discs per tree of 15 cm thickness and a piece of stem (initially 60 cm between 0.10 and 1.30 m) of 40 cm. All discs were used to measure specific hydraulic conductivity (k_s) and microdensity variables. k_s was measured in earlywood of tree rings 2005, 2006 and 2007, and in the same tree rings, microdensity profiles were obtained using the X-ray indirect method developed by Polge (1966) (Fig. 1). The 40-cm piece of stem was kept to make the vulnerability curves. Samples were kept wet during the cutting process and were immediately placed in large plastic containers filled with fresh water. All plant material was maintained in wet condition and in a cold chamber at 2°C to prevent cavitation.

2.1 k_s measurements

Immediately before doing the k_s measurements, the 15-cm discs were sawn under water jet in a humid atmosphere in order to obtain a 5-cm-thick disc that was kept in water.

To measure localized axial hydraulic conductivity in tree rings (k_s) (Dalla-Salda et al. 2009), we used 2-mm-diameter veterinary needles, connected individually to the end of a water column using flexible plastic tubes. Three needles were carefully inserted with a rubber hammer into the earlywood of each ring, under water. The operation was repeated for three successive sapwood rings (2005, 2006 and 2007) in the three discs cut at three heights in each tree. k_s was thus measured 27 times per tree (3 needles × 3 rings × 3 discs). To prevent air bubbles, the needles were filled with water using a thinner needle and a syringe.

After inserting and filling the nine needles of a disc, we took the disc out of the water and connected only one of the

Fig. 1 Scheme showing the cutting method and sample's destination. Three discs were taken from each tree (at 0.10, 1.30 and 3 m). In each disc, we measured specific hydraulic conductivity (k_s) locally matching microdensity measurements. k_s measurements were conducted in 2005, 2006 and 2007 tree rings. Vulnerability curves were constructed using 2007 tree ring from a piece of stem taken between the 0.10-m and the 1.30-m discs
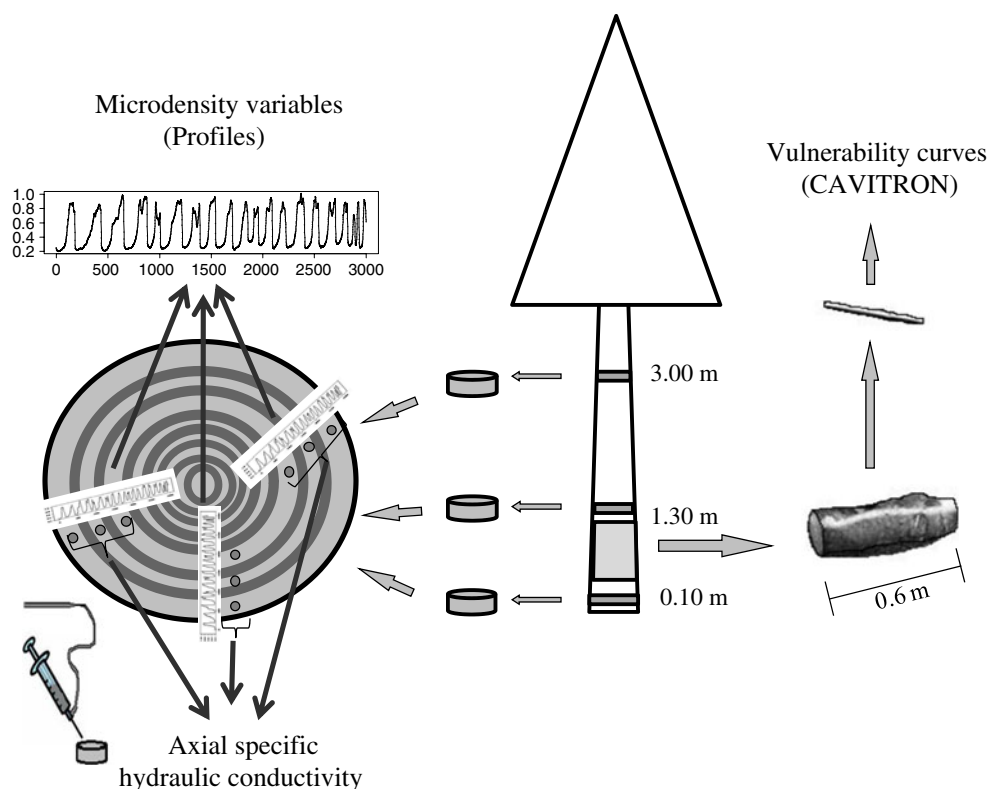
needles to the water column through a stopcock and laboratory tubing. The other extremity of the 120-mm-long column was connected to a micro-pipette (placed horizontally) allowing reading of the variation in water volume with time.

The system was filled with distilled Phloxine coloured water, making reading easier and permitting permanent verification of dripping and absence of air bubbles. In addition, we could verify that coloured water really passed through the sample and arrived exactly at the other side, in the same portion of the ring where the needle was inserted. In all the cases, needles were installed avoiding compression of the wood. We assume that the samples were near water saturation, thus presenting a k_s near maximum conductivity at the moment of measurement. This assumption was not only because all precautions were taken during sample manipulation but also because the trees were cut at the very beginning of a humid, cool, rainy 2008 spring following the 2007 growing season in Orléans, France, which was also especially humid and cool.

Sapwood's specific conductivity (k_s) was calculated according to Darcy's law:

$$k_s = \frac{V \times L \times \eta}{t \times A \times \Delta P}$$

where V is the volume of water that went through the sample (cubic metres), L is the sample length (metres), η is

the viscosity of water at the temperature at which the experiments were conducted (newton second per *square metre*), t is the time (seconds), A the cross-sectional area (square metre) of the sample (in this case it corresponds to the needle's internal area) and ΔP is the pressure difference (pascals) between ends of the sample. k_s was expressed in square metre as a consequence of separating the viscosity (η) from the driving agent in terms of a pressure difference. Thus, k_s is determined only by wood structure and is independent of the nature of the fluid (Domec and Gartner 2002b).

2.2 Vulnerability to cavitation curves (VC)

In order to obtain the pieces of wood compatible with the measuring equipment, we extracted four to six longitudinal portions from each trunk, splitting the trunk with an axe and a hammer, following the wood grain (Fig. 1). The 2007 tree ring was large enough to comprise the entire VC sample that is a 0.7×0.7×26.5-cm vertical stick. Samples were always manipulated in humid conditions using a shallow pan filled with water and kept in a cold chamber (2–3°C) when not in use. Three samples were cut and measured per tree.

To establish the vulnerability curves, we used a CAVITRON (Cochard 2002; Cochard et al. 2005). This technique uses centrifugal force to lower xylem pressure in the sample and to induce a positive pressure gradient

between sample ends and thus a flow of water through the sample. By measuring this flow, it is then possible to determine how the sample hydraulic conductance varies as the centrifugal force is increased (Cochard et al. 2005).

In our case, samples were not saturated to restore maximum conductivity in order to avoid cavitation fatigue. Refilling may also leave behind small micro-bubbles that could nucleate cavitation during subsequent stress (Hacke et al. 2001b).

Once the vulnerability curves were constructed, a non-linear sigmoidal Boltzmann function was fitted to compute the percentage loss of conductivity (PLC) at tree level. Four parameters were calculated to describe PLC variation: the P12, P50 and P88 (value of the x pressure-axis of the curve with 12%, 50% and 88% loss of conductivity) and the maximum slope of the VC curve (located at the inflexion point), calculated according to Domec and Gartner (2001).

The P12 value is termed the air entry point (Sparks and Black 1999) and is an estimate of the xylem tension at which pit membranes are overcome within the conducting xylem when cavitation starts. P50 is the point of 50% loss of hydraulic conductivity. Likewise, P88 represents the full embolism point and is interpreted as an approximation of the actual maximum tension of the xylem before failing and becoming non-conductive (Domec and Gartner 2001). As suggested by Sperry (1995), the slope can be considered as a measure of the xylem safety margin between P12 and P88.

2.3 Microdensity variables

The discs from 0.10, 1.30 and 3 m, after being used to measure k_s, were sawn with a double-blade saw in order to obtain wood stripes precisely located next to the k_s measurements. These were about 8 mm wide and 1.5 mm thick and as long as the sample radius.

They were then oven-dried up to moisture equilibrium at 60°C and subsequently analysed by indirect X-ray micro-densitometry (Polge 1966). The resulting X-ray films were scanned at 1,000 dpi resolution with 8 bits per pixel. The digitalized images were processed using WinDENDRO, Regent Instruments Inc. (Guay et al. 1992), obtaining a microdensity profile with a spatial resolution of 25 μm. The last step of the data process used a computer routine written in R language (R Development Core Team 2009) to assess the following ring variables: ring width (RW), mean ring density (MRD), minimum ring density (MID), maximum ring density (MAD), earlywood density (EWD), latewood density (LWD), earlywood proportion (EWP) and the mean density of the first 100 within-ring microdensity values (SEQ, i.e. the mean density of the first 2.5-mm-wide part of the earlywood). This last parameter was calculated from the point where the veterinary needle used to measure k_s was inserted into the first 2.5 mm of the earlywood (Fig. 2).
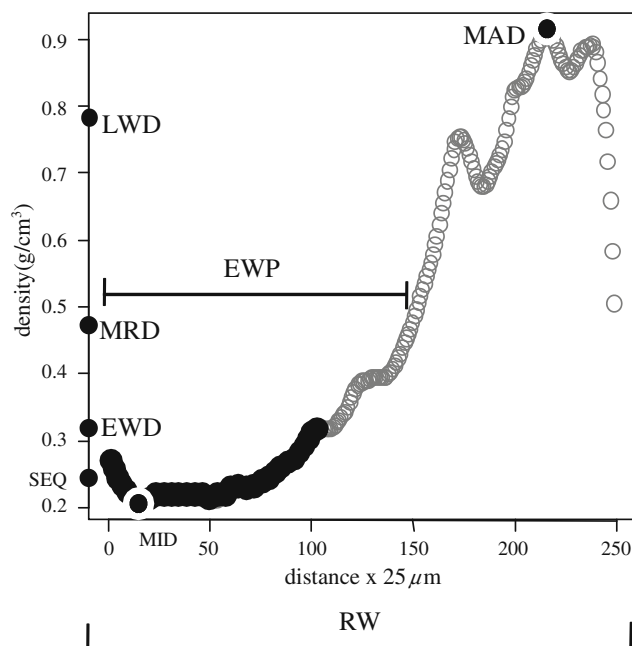

Fig. 2 Tree ring's density values calculated from a microdensity profile of one typical Douglas-fir tree ring, showing ring width (*RW*), mean ring density (*MRD*), minimum ring density (*MID*), earlywood density (*EWD*), earlywood proportion (*EWP*), mean density of the first 100 within-ring microdensity values (*SEQ*) corresponding to the dark portion points, maximum ring density (*MAD*) and latewood density (*LWD*)

2.4 Statistical analysis

2.4.1 Relationship between hydraulic properties and microdensity variables and growth traits (circumference and height)

Circumference (circ) and tree height (height) were measured in all trees before tree felling. For each k_s value, we have a microdensity value measured exactly next to it. All the k_s measurements were used to study the association between k_s and microdensity variables. To study the association between VC and microdensity variables, we used the microdensity variables computed in the 2007 tree ring sampled at breast height. In order to assess the relationships between growth traits and hydraulic variables, each hydraulic variable was averaged at tree level. Associations were computed at tree and clone levels. We used Pearson correlation coefficients that were computed using R software (R Development Core Team 2009).

2.4.2 Phenotypic and genetic variation of wood hydraulic properties: k_s, P12, P50, P88 and slope

The following model was used to assess the significance of the k_s variation in positions (0.10, 1.30 and 3 m), years (tree rings) and clones. Logarithm of k_s was used for normality

assumption:

$$y_{ijh} = \mu + \alpha_i + \beta_j + \chi_h + (\alpha\beta)_{ij} + (\alpha\chi)_{ih} + (\beta\chi)_{jh} + \varepsilon_{ijh}$$

where y_{ijh} is the ijhth logarithm of k_s observed variable; μ is the overall mean; α_i is the fixed effect of the ith position (0.10, 1.30 and 3 m); β_j is the fixed effect of the jth tree ring, χ_h is the fixed effect of the hth clone; $(\alpha\beta)_{ij}$, $(\alpha\chi)_{ih}$ and $(\beta\chi)_{jh}$ are the corresponding interaction effects between position and tree ring, between position and clones and between tree ring and clone, respectively; and ε_{ijh} is the random error.

Note that for PLC variables and slope, measurements were taken only in the 2007 tree ring in a 40-cm-long sample taken below the breast height region. Thus, position and tree ring effects are not present as sources of variation. The following model was used to assess significant differences between clones for the parameters of the vulnerability curves (P12, P50, P88 and slope):

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where y_{ij} = ijth PLC observed variable, μ = overall mean, τ_i = fixed effect of the ith clone and ε_{ij} = random error.

2.4.3 Phenotypic and genetic variation of microdensity variables: RW, MRD, MID, EWD, EWP, SEQ, MAD and LWD

To quantify phenotypic variation in microdensity parameters, we separated the different sources of variation: position, tree ring and genetics. Through R (R Development Core Team 2009), we estimated tree ring (2005, 2006 and 2007), position and clone effects for all microdensity variables.

To assess the significance of the variation in the microdensity variables, the following model was used:

$$y_{ijh} = \mu + \alpha_i + \beta_j + \chi_h + (\alpha\beta)_{ij} + (\alpha\chi)_{ih} + (\beta\chi)_{jh} + \varepsilon_{ijh}$$

where y_{ijh} is the ijhth observed microdensity variable; μ is the overall mean; α_i is the fixed effect of the ith position; β_j is the fixed effect of the jth tree ring; χ_h is the fixed effect of the hth clone; $(\alpha\beta)_{ij}$, $(\alpha\chi)_{ih}$ and $(\beta\chi)_{jh}$ are the corresponding interaction effects between position and tree ring, between position and clones and between tree ring and clone, respectively; and ε_{ijh} is the random error.

3 Results

3.1 General results on the study characters

Table 1 shows general mean, coefficient of variation, and minimum and maximum values for growth traits, wood hydraulic properties and microdensity variables.

Table 1 Mean, phenotypic coefficient of variation (CV_p), and minimum (min) and maximum (max) of analysed variables

	Mean	CV_p (%)	Min	Max
Growth traits				
Circ (cm)	34.2	13.2	24	42
Height (m)	6.3	7.8	5.8	7.3
Hydraulic variables				
k_s (m²)	4.56^{-12}	35.36	1.56^{-12}	1.03^{-11}
P12 (MPa)	−1.36	27.28	−1.80	−0.89
P50 (MPa)	−2.48	30.32	−3.11	−1.91
P88 (MPa)	−3.22	30.17	−3.93	−2.76
Slope	72.70	32.30	45.85	132.30
Microdensity variables				
RW (mm)	8.41	28.80	1.34	16.51
MRD (g/cm³)	0.43	14.19	0.30	0.69
MID (g/cm³)	0.22	21.09	0.15	0.49
EWD (g/cm³)	0.32	17.38	0.19	0.68
EWP (%)	73.31	17.04	34.72	94.06
SEQ (g/cm³)	0.26	24.31	0.17	0.69
MAD (g/cm³)	0.84	8.02	0.47	0.97
LWD (g/cm³)	0.70	9.97	0.36	0.85

The date of measurement does not have any significant effect on k_s nor on VC (data not shown), indicating that harvesting, storage and measurement methods can be accepted.

Measured at three different positions (0.10, 1.30 and 3 m), k_s was found to decrease significantly ($p<0.05$) from the base of the stem to the crown. All microdensity variables related to the first part of the tree ring, SEQ, MID, EWD and EWP, increased significantly in this direction ($p<2.2^{-16}$), while LWD and MAD decreased significantly ($p<2.2^{-16}$).

3.2 Relationships between wood hydraulic properties, microdensity variables and growth traits

In Table 2, we show the significant phenotypic correlations and their associated probability at tree and clonal levels between microdensity variables, growth traits and wood hydraulic properties.

Non-significant correlations were found between RW and wood hydraulic properties, while many significant correlations were found between wood hydraulic properties and microdensity variables. Strong positive correlations were found between k_s and EWP at the clonal level. Moderate positive correlations were found between k_s and EWP, MAD and LWD at the individual level, and between slope and MAD at the clonal level. Strong negative correlations were found between k_s and MID, k_s and SEQ

Table 2 Pearson's coefficient of phenotypic correlations and their associated probability (between brackets) between microdensity variables and hydraulic variables at tree and clonal levels

	Wood hydraulic variables									
	k_s		P12		P50		P88		Slope	
	Tree	Clonal	Tree	Clonal	Tree	Clonal	Tree	Clonal	Tree	Clonal
Growth traits										
Circ	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Height	0.47*	ns	ns	ns	0.45*	0.89**	0.48*	0.76*	ns	ns
Microdensity variables										
RW	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
MRD	−0.51*	ns	ns	ns	−0.56**	−0.76*	−0.70***	−0.84*	ns	ns
MID	−0.61**	−0.77*	ns	ns	−0.57**	−0.76*	−0.71***	−0.89***	−0.48*	ns
EWD	ns	ns	ns	ns	−0.57**	−0.77*	−0.73***	−0.85*	ns	ns
EWP	0.64***	0.79*	ns	ns	ns	ns	ns	ns	ns	ns
SEQ	−0.64**	−0.85*	ns	ns	−0.71***	−0.85*	−0.84***	−0.97***	−0.43*	ns
MAD	0.52*	ns	−0.65***	−0.85*	ns	ns	ns	ns	0.54*	ns
LWD	0.45*	ns	−0.54*	−0.78*	ns	ns	ns	ns	ns	ns

Only significant correlation values (α=5%) are shown

RW ring width, *MRD* mean ring density, *MID* minimum ring density, *EWD* earlywood density, *EWP* earlywood proportion, *SEQ* sequence, *MAD* maximum ring density, *LWD* latewood density, *ns* not significant

* Associated probability between 0.01 and 0.05

** Associated probability between 0.001 and 0.01

*** Associated probability between 0 and 0.001

ns=>0.5

(Fig. 3), P12 and MAD (Fig. 3), P12 and LWD, P50 and MID, EWD, SEQ (Fig. 3) and finally P88 and MID, EWD and SEQ (Fig. 3) at the clonal level. Strong negative correlations at the tree level were found between P50 and SEQ and between P88 and MID, EWD, SEQ. Moderate negative correlations at the tree level were found between k_s and MID and k_s and SEQ, P12 and MAD, P12 and LWD, P50 and MID and P50 and EWD, slope and MID and slope and SEQ. MRD is moderately and negatively related to k_s and to P50 at the tree level and is strongly and negatively correlated to P50 at the clonal level and to P88 at both tree and clonal level.

No correlation was found between circ and wood hydraulic properties. At the individual level, moderate positive correlations were found between height and k_s, P50 and P88. At the clone level, we also found strong positive correlations between tree height and P50 and P88.

3.3 Genetic variation of growth traits, wood density and wood hydraulic properties

3.3.1 Growth traits

Our results show that clones are significantly different for circumference and height (Fig. 4).

3.3.2 Microdensity variables

A significant clonal effect was found for all microdensity variables (Table 3). Position and tree ring effects were also significant for all variables except for MRD and MAD, respectively.

In Fig. 5, we show MRD, MID, SEQ and MAD variation with respect to clone and position.

3.3.3 Hydraulic properties

A significant effect of clone, position and of their interaction was found for the logarithm of k_s, whereas the effect of tree ring was not found significant (Table 4).

The VC curves at clone level are shown in Fig. 6. A significant genetic effect was found for slope, P12, P50 and P88 (see Table 5).

4 Discussion

Our results concerning k_s and VC are consistent with those of our previous study with three different clones (Dalla-Salda et al. 2009) and those found in Douglas-fir and other species by several authors for similar, younger and older trees (Domec and Gartner 2001, 2002a, b; Mayr and Cochard 2003; Sperry and Ikeda 1997). This demonstrates the effectiveness of the methods developed, tested and used to measure these variables. These results could not only help to facilitate studies on hydraulic properties (accurate, localized and faster measurements) but could also favour to the development of selection criteria based on microdensity profiles, attainable with wooden cores. In the present context of global climatic change, associating genetic and ecophysiological studies is of particular importance.

Fig. 3 Many significant correlations were found between wood hydraulic properties and microdensity variables. For example, strong negative correlations were found at clone level between: **A** k_s and SEQ (−0.85), **B** P50 and SEQ (−0.85), **C** P88 and SEQ (−0.97) and **D** P12 and MAD (−0.85)
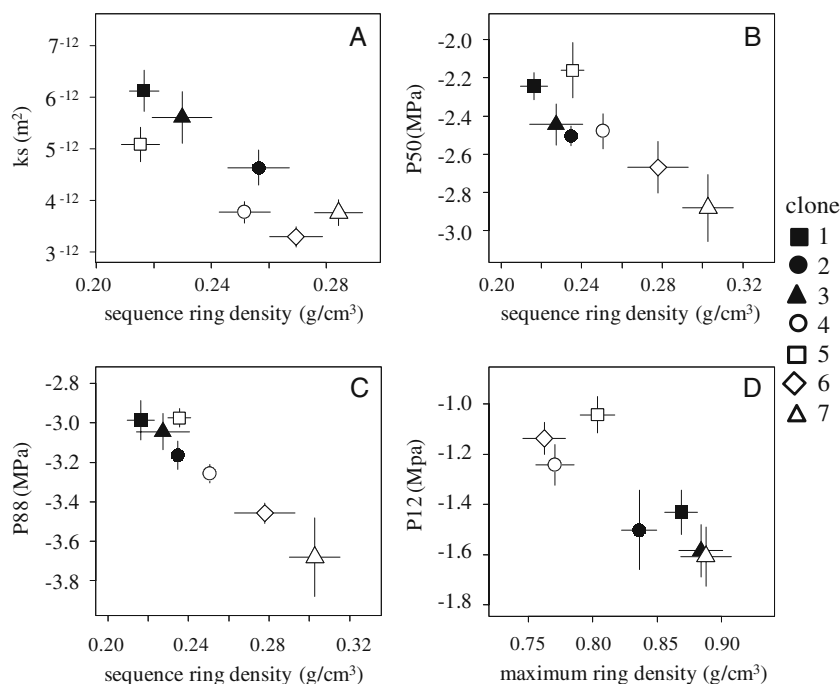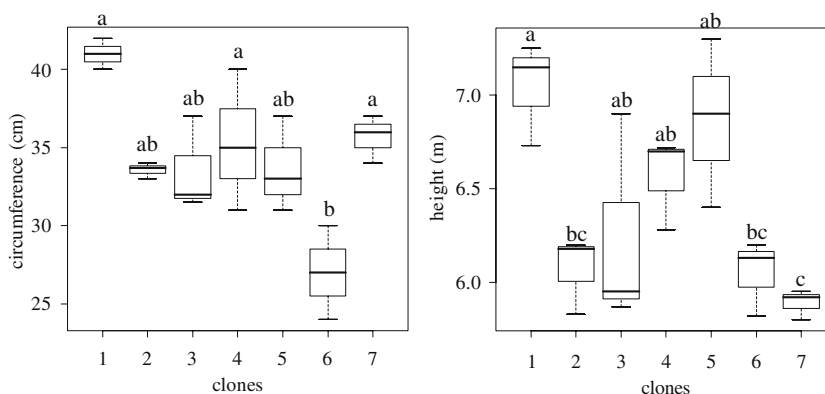
Fig. 4 Circumference and height boxplot (clone's mean and standard deviation). Clones are significantly different for circumference and height. *Similar letters* show no significant differences, Tukey's test ($\alpha = 5\%$)

In contrast to tree circumference, tree height is related to tree hydraulics. Fast height-growing Douglas-fir trees are probably not only more conductive but also more vulnerable to cavitation. We have to take into account that sampling was realised at fixed heights (at 0.10, 1.30 and 3 m) and therefore wood of different cambial ages is being compared. Nevertheless, this difference is never greater than 2 or 3 years. We assume that environmental variation is not relevant since the experimental plot the trees were taken from is a homogeneous terrain in the INRA nursery and the studied clones are separated by less than 30 m.

No correlation was found between ring width (RW) and wood hydraulic properties. Plots of ring width against PLC variables at clone level (not shown here) reveal that at least for the plant material sampled in this study, it may be possible to find fast growing clones with high resistance to drought. Except for RW, each microdensity variable is significantly correlated to at least one hydraulic property. k_s is mostly negatively related to earlywood density variables (MID and SEQ), but not to EWD. This is probably due to the fact that EWD is mean density of a ring segment including not only earlywood but also a large part of transition wood from earlywood to latewood. In contrast, MID and SEQ are completely enclosed in earlywood.

P12 is mostly negatively correlated to latewood density variables (MAD and LWD). The strongest correlation is

found for MAD, which is thus the best candidate for indirectly estimating P12. This is consistent with the results found by Domec and Gartner (2002b). Checking and, if confirmed, trying to understand this relationship will be a subject developed in another study. Here, we emphasize that this correlation can be considered as a method to indirectly estimate P12, at least in similar plant material. P50 and P88 are both principally negatively correlated to earlywood density variables. The strongest correlation is with SEQ, which is here again a good candidate for the indirect estimation of P50 and P88. Higher density values in the first part of the Douglas-fir tree ring imply higher resistance to drought in detriment to k_s.

We demonstrate that in Douglas-fir, there is a relationship between wood density and wood hydraulic properties. Therefore, wood density is related to Douglas-fir hydraulic functioning: this is a first outcome tending to reveal wood density as a quantitative trait marker of adaptation in Douglas-fir.

Relationships between density and resistance to cavitation were not observed in other studies (Domec and Gartner 2002a; Dutilleul et al. 1998; Rosner et al. 2007). This may be related to the fact that not only conduit internal dimension but also cell wall pitting plays an important role in resistance to cavitation through embolism initiation and propagation (Pittermann et al. 2006). However, in all of

Table 3 *F* value and probability (Pr) associated to the ANOVA fixed effects for the microdensity variables

Variable	Clone		Position		Tree ring	
	F value	Pr (>*F*)	*F* value	Pr (>*F*)	*F* value	Pr (>*F*)
RW	25.92	$<2.2^{-16}$	8.03	0.004	144.75	$<2.2^{-16}$
MRD	69.04	$<2.2^{-16}$	1.80	ns	14.05	1.7^{-06}
MID	29.27	$<2.2^{-16}$	63.31	$<2.2^{-16}$	6.43	0.002
EWD	46.33	$<2.2^{-16}$	35.88	2.6^{-14}	56.83	$<2.2^{-16}$
EWP	27.29	$<2.2^{-16}$	6.01	0.003	55.10	$<2.2^{-16}$
SEQ	32.46	$<2.2^{-16}$	93.49	$<2.2^{-16}$	12.35	8.0^{-06}
MAD	23.85	$<2.2^{-16}$	54.94	$<2.2^{-16}$	0.18	ns
LWD	25.28	$<2.2^{-16}$	68.11	$<2.2^{-16}$	9.40	0.001

ns not significant

Fig. 5 Mean ring density (**A**), minimum ring density (**B**), sequence ring density (**C**) and maximum ring density (**D**) at the three positions (0.10, 1.30 and 3 m high) for the seven clones. Each data are a mean of the 2005, 2006 and 2007 tree rings
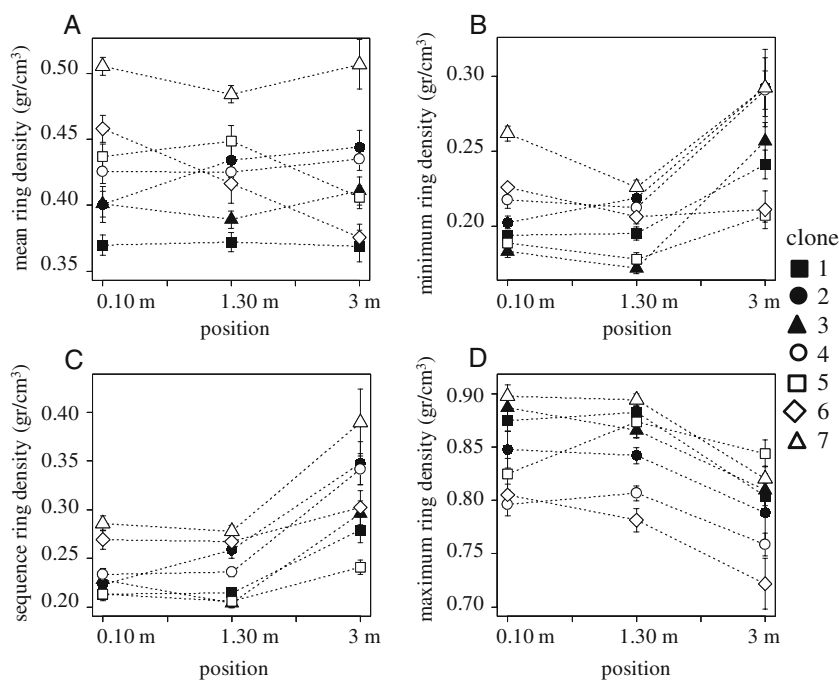
these cases, wood density was measured using gravimetric methods on solid wood samples. In this study, we used the indirect X-ray microdensity method (Polge 1966), which is more precise than the former and which takes into account spatial distribution of the wood material (Decoux et al. 2004), and we were able to differentiate and separate the segments of the microdensity profiles matching the wood volumes used for measuring hydraulic properties inside the rings. Therefore, we increased the precision of the relationship between density and hydraulic properties. In Douglas-fir, there is a strong relationship between within-tree ring density increase and both cell wall thickness increase and tracheid diameter diminution in radial and tangential directions (Rathgeber et al. 2006). Thus, through this study, we can conclude that k_s is mainly correlated to earlywood tracheid internal dimensions. This is consistent with already published results (Cruiziat et al. 2002; McElrone et al. 2004; Stiller 2009; Tyree et al. 1994). Therefore, in Douglas-fir, for indirect measurement of k_s, the best putative microdensity variable would be SEQ.

Table 4 F value and probability (Pr) associated to the ANOVA fixed effects for the logarithm of k_s

Source of variation	F value	Pr $(>F)$
Clone	19.61	$<2.2^{-16}$
Tree ring	1.82	0.162
Position	14.45	8.04^{-7}
Tree ring × position	2.05	0.113
Tree ring × clone	0.84	0.602
Clone × position	2.91	0.006

In a preliminary study, Dalla-Salda et al. (2009) measured k_s and VC of another three Douglas-fir clones. These variables were measured in wood that was embolized as a consequence of the 2003 heat wave and drought in France. In this new study, we collected wood samples at the beginning of the 2008 growing season, reducing the possibility of studying wood samples with embolized tracheids. It is interesting to emphasize that this previous study could partially demonstrate the role of wood density in maintaining part of the Douglas-fir xylem functional during the 2003 heat wave and drought in France: the clone
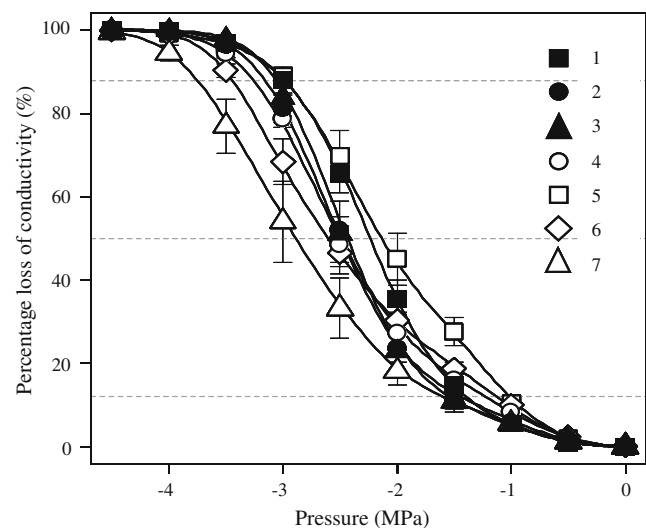

Fig. 6 Mean vulnerability to cavitation (VC) curves at clone level: percentage loss of conductivity (means and standard deviation) as a function of the xylem pressure for the seven Douglas-fir clones

Table 5 F value and probability (Pr) associated to the ANOVA genetic fixed effects for the parameters of the vulnerability curves measured in three different samples per tree (nine curves per clone) for the 2007 tree ring

Variable	F value	Pr ($>F$)
P12	3.61	0.022
P50	3.19	0.034
P88	5.22	0.005
slope	7.20	0.001

most resistant to cavitation (according to P50 measurements) was the clone with the highest MRD and MID. This is consistent with the significant negative correlation we found between MRD, MID and P50 in this study.

Our results also demonstrate that there is significant genetic variation for k_s and VC curve variables in Douglas-fir stem wood. These results are consistent with those found in three Douglas-fir clones in the previous study (Dalla-Salda et al. 2009). Rosner et al. (2007, 2008) also found significant differences between clones for specific hydraulic conductivity in Norway spruce. Other authors (Neufeld et al. 1992; Vander Willigen and Pammenter 1998) showed that there is a significant genetic variation for VC curve variables measured in leaves, branches and seedlings. In our work, the significant genetic variation for VC curve variables was measured directly in the same wood sample where k_s and density were also measured. Our results also show a strong significant clone variation for all microdensity variables. These results highlight the significance of wood density as a quantitative trait marker of Douglas-fir adaptation in the context of global climate change. These findings are a second step toward complete demonstration of the adaptive potential of wood density. More extensive direct or indirect measurements of wood hydraulic properties are necessary to explore the genetic variation of these properties on a larger scale. The strong correlations found between hydraulic and microdensity variables make microdensity profiles promissory not only for the selection of trees with desirable wood density in the framework of breeding programs but also for the indirect study of genetic variation of hydraulic properties and the selection of individuals by these traits.

Measuring k_s in the same tree rings at three different heights (at 0.10, 1.30 and 3 m) enabled us to take the cambial age effect into account. From the base of the stem to the crown, k_s variable decreases while earlywood density variables (especially SEQ) increase: this is further indirect evidence of the well-known relationship between conduit internal dimensions and k_s. Density increase and the k_s decrease with height is considered a cambial age effect (Zobel and Sprague 1998; Spicer and Gartner 2001). The physical relationship between tracheids internal dimensions and k_s is confirmed at tree level and at clone level by the strong positive correlation found between k_s and earlywood density variables.

In this study, we found that there are significant relationships between wood microdensity variables and wood hydraulic properties, as well as significant genetic variation for k_s and VC. This indicates that wood density may have an adaptive value and that it could be used to study adaptation to drought in Douglas-fir. Indirect screening for genetic differences in wood hydraulic properties is possible using the relationship between wood density and xylem hydraulic efficiency and resistance. Through this study, the novel methods used to measure k_s and vulnerability to cavitation proved to be promising alternatives for localized measurement of these variables. Nevertheless improvement is still needed in order to increase the number of samples measurable per time unit.

The relationship found between density and hydraulic properties does not prove that the target of a selective drought event is one of these traits. Other traits like pit characteristics could be involved. In order to be sure, it may be possible to choose extreme phenotypes and submit them to an artificial selective event, or to decompose the physiological process involved. Bordered pit structure will be a research issue in our future studies.

The next step in our study of the adaptive value of wood and the consequences for tree fitness will be the estimation of broad and narrow-sense heritability and of genetic coefficients of variation. Similar studies of the same species in other environmental conditions and of other species with different types of wood (i.e. broadleaves) are also necessary.

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