

Developmental control of xylem hydraulic resistances and vulnerability to embolism in *Fraxinus excelsior* L.: impacts on water relations

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Abstract

The hydraulic properties and leaf gas exchanges of *Fraxinus excelsior* L. branches differing by their age and their vertical crown position, but in comparable ambient air conditions (vapour pressure deficit and global radiation) were compared. The variations in leaflet water potential ψ_{leaflet} , leaflet stomatal conductance and transpiration rate, E , were small between different branches of the same crown. Whole branch hydraulic resistances (r_{branch}), and partitioning between leaf (r_{leaf}) and xylem resistance (r_{xylem}) were assessed with a high pressure flowmeter. r_{leaf} represented 90% and 10% of r_{branch} for upper and lower crown branches, respectively. The changes resulted from increases in r_{xylem} caused by the formation of short shoot internodes mostly located in secondary axes. However, leaf area-specific branch resistances ($r_{\text{branch}}^* = r_{\text{branch}} \times LA$) were nearly constant throughout the crown. This was consistent with the vertical variations in ψ_{leaflet} because $r_{\text{branch}}^* \times E$ represents the water potential drop from the trunk to the leaves. Because r_{xylem} was higher, lower ψ_{xylem} values were predicted in lower crown rachises. However, rachises from lower crown branches were less vulnerable to embolism than in upper branches (ψ_{xylem} at onset of embolism, ψ_{cav} , were -3 and -2 MPa, respectively). It was concluded that r_{xylem} increased with branch age, but r_{branch}^* remained constant because LA decreased. As a consequence, E was maximized and ψ_{xylem} remained above ψ_{cav} . This suggested that, in *Fraxinus*, leaf gas exchanges and leaf areas were coupled with xylem hydraulic capacities probably through a control of bud activity.

Key words: Water relations, cavitation, regulation, crown, growth, Ash.

Introduction

In plants, hydraulic resistances along the sap pathway, from the soil to the leaves, induce negative water potentials (ψ , MPa) proportional to water loss under steady-state conditions (van den Honert, 1948). As a result, when water loss is high, very low ψ are generated in xylem conduits (ψ_{xylem}). When ψ_{xylem} values are very low and drop below a threshold value, ψ_{cav} , cavitations occur and embolisms form thus increasing hydraulic resistances (Sperry and Tyree, 1988; Tyree and Sperry, 1989).

Over the past decades, much attention has been paid to the study of xylem vulnerability to cavitation (see Tyree *et al.*, 1994, for a recent review). From these studies, it can be concluded that most species operate close to their point of xylem dysfunction (Tyree and Sperry, 1988). However, trees cope with this precarious condition by regulating their water loss precisely (Jones and Sutherland, 1991; Cochard *et al.*, 1996).

Reductions in photosynthetic and transpiration rates in old trees have recently been ascribed to increases in xylem resistance (Yoder *et al.*, 1994; Waring and Silvester, 1994; Panek and Waring, 1995). Resistances were not directly measured in these studies, but the results suggest that xylem resistances may vary during branch development with significant implication for branch water relations. Hydraulic resistances and xylem vulnerability may both, therefore, limit water loss in plants.

Little is known about the localization of the main resistances along the sap pathway and the potential changes caused by developmental or environmental factors (Yang and Tyree, 1994). The objectives of this study were to analyse the changes in xylem hydraulic properties occurring during the ontogenic development of branches

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and to estimate the possible implications for leaf gas exchange and the control of xylem embolism. The age-related changes in whole-branch hydraulic resistance, stomatal conductance, leaf water potential, and xylem vulnerability were assessed for branches of mature *Fraxinus excelsior* trees. This species was chosen because it forms very open crowns with low leaf area index and thus exhibits relatively low within-crown variations in environmental conditions.

Materials and methods

Site and plant material

Experiments were conducted during 1994 and 1995 in a forest stand located in the Amance forest near Nancy, France (6°20'E; 48°45'N, elevation 250 m). The site was clear-cut in 1970 and let to regrow by natural regeneration and coppicing. Ash trees (*Fraxinus excelsior* L.), 21–25 years old, 15–18 m tall, occurred mixed with other broad-leaved trees in the stand (mostly beeches, birches, and poplars). Measurements were made on representative dominant or co-dominant ash trees. Four trees were chosen in 1994 for stomatal conductance estimates. Five trees were selected in 1995 and used as follows: three were equipped with sap flowmeters and sampled only for leaflet water potential measurements; one was sampled for xylem embolism time-course, construction of vulnerability curves and positive xylem pressure measurements; the last one was entirely sampled for whole branch hydraulic analysis. Crowns were divided into three equal vertical layers that were called the 'upper', 'middle' and 'lower' layers. Branches were less than 3 years old and more than 6 years old in the upper and lower layers, respectively. Two scaffolding towers allowed measurements and sampling in all crowns. Following Joyce and Steiner (1995) a lateral shoot of the main stem (trunk) was called the 'primary branch system'. A primary branch is composed of a primary and secondary branch axes (when present). Primary branch age (years) was defined as its number of annual growth increments.

Microclimatic data

Rainfall and minimum daily temperature data were those from Champenoux (about 300 m from the site, at the same elevation). A pyranometer (Cimel, Paris, France) measured the incident global radiation, R_g , 2 m above the canopy. Two ventilated psychrometers (INRA) measured the wet and dry bulb temperatures 2 m above the canopy and 10.5 m above ground, i.e. in the lower crown layer. Leaflet surface temperature and relative air humidity values measured by a porometer (see below) were also combined to assess vertical gradients of air vapour pressure deficit (VPD) in the crown. In 1995, nine linear thermopyranometers (INRA) were installed at bud break in the crown of three trees (one per layer) to monitor the changes in global radiation interception due to leaf development.

Positive xylem pressure potential

To investigate the occurrence of positive xylem pressure potential, an Electronic pressure transducer (model PDCR 1000, Druck LTD, Leicester, England) was tightly fitted to a 2 cm deep hole drilled at the base of one Ash trunk and connected to a data logger (model 21X, Campbell Scientific LTD, Logan, Utah, USA). The hole was filled with water at the beginning of the experiment. The system ran from March

to May 1995. For comparison, ψ_{xp} was also measured at the base of one *Fagus sylvatica*, one *Betula pendula*, one *Quercus petraea*, and one *Carpinus betulus*, all from the same stand.

Sap flux densities, leaflet water potential and stomatal conductances

Sap flux densities (F_d , $\text{dm}^3 \text{dm}^{-2} \text{h}^{-1}$) were monitored throughout the two growing seasons with 0.02 m long radial flowmeters inserted in the trunk at breast height (Granier, 1987). Preliminary experiments showed that more than 90% of the whole sap flux occurred within the outer most 0.01 m (Granier, unpublished results). Leaflet water potential (ψ_{leaflet}) was measured with a pressure chamber on detached leaflets chosen from the middle part of the leaf. Diurnal time-courses and vertical crown variations of minimum ψ_{leaflet} were assessed during sunny days. On 19 July 1995, from 03:00 h to 20:00 h, ψ_{leaflet} was measured each hour on three leaflets randomly sampled from the upper canopy layer. On 27 June and 10 July 1995, ψ_{leaflet} was measured between 11:30 h and 13:30 h on six leaflets detached from two branches in each canopy layer. Vertical variation of midday leaflet stomatal conductance, g_s ($\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$), and transpiration rate, E ($\text{mmol m}^{-2} \text{s}^{-1}$), were assessed with a steady-state porometer (model LI-1600, Li-cor Inc., Lincoln, NE). Measurements were made between 11:00 h and 13:00 h (when E is maximal and ψ_{leaflet} minimal). Leaflets were randomly sampled on eight to nine branches in each crown from different layers and cardinal orientations. Approximately 500 different leaflets were measured.

Seasonal progression of embolism and dye coloration

The seasonal progression of embolism in the xylem was measured periodically from February to December 1995 following the hydraulic technique of Sperry *et al.* (1988) and described elsewhere (Cochard *et al.*, 1991). In short, the technique measures the hydraulic conductance of 0.02–0.03 m long excised xylem segments prior to and after water refilling. Although the segments were probably shorter than the length of the megaporous vessels, the conductances were correctly estimated because vessel lumens are more limiting to water flow than are vessel ends (Chiu and Ewers, 1993). On each sampling day, one entire primary branch system (3–6 years old, 1.2–2 m long) was cut from the middle crown layer and brought to the laboratory in a black plastic bag to limit leaf evaporation. On average, four leaves (two rachis segments per leaf), four current (1995), two 1994, and two 1993 shoot internodes were measured. The 1994 and 1993 internode segments were cut from the secondary axes. The samples were excised and manipulated under tap water to avoid air entry into the vessels. In a preliminary experiment the maximum vessel length in primary branch systems were determined with the air perfusion method (Ewers and Fisher, 1989). No vessels were found cut open at both ends in the leaf rachis nor in the current year shoots and only few vessels were cut open in the secondary 1994 and 1993 axes. The measured degrees of embolism were thus close estimates of the *in vivo* values. For each segment, the mean diameter (without bark) was measured and the total distal leaf area (LA) connected to the sample. For dye coloration, Phloxine B (SIGMA, ref P-2759) was used which is more freely transported by the water stream than other dyes (e.g. Safranin or basic Fuchsin, Granier *et al.*, 1994). Segments were perfused for 10–15 min with a 0.001 g g^{-1} solution and a positive pressure difference of 3 kPa. The localization of the sap pathway

was visually assessed on cross-sections taken in the middle of the sample segments.

Vulnerability curve (VC)

VCS were established by dehydrating intact excised branches in an 0.8 m long pressure chamber (Cochard *et al.*, 1991, 1992). A detailed description of the technique and a comparison with the more traditional air dehydration method can be found in Cochard *et al.* (1992). At the end of August 1995, ten upper and eight lower crown layer primary branch systems were sampled and inserted into the pressure chamber. Air pressure in the chamber was set to a predefined value (between 2 and 5 MPa) and kept constant for 1–3 h until sap exudation ceased from the cut end. The degree of xylem embolism was then measured on four randomly sampled leaves (two rachis segments per leaf) and 1 to 3 current year shoot internodes. Current year shoot internodes on lower branches were too short to be measured.

Crown hydraulic architecture

Whole branch hydraulic resistance r_{branch} was measured as described by Tyree *et al.* (1993a) with a high pressure flow meter (HPFM). Our HPFM apparatus was identical to the one described by Tyree *et al.* (1995). The temperature of the capillary tube and of the branch was measured to account for changes in water viscosity. Values were standardized to 20 °C. The capillary tubes were 0.177 and 0.053 m long HPLC tubing having a 250 μm internal diameter. Data were logged (30 s sampling rate) and computed with a Campbell 21X data logger.

Forty-four entire primary branch systems, 1–7 years old, were cut from the main stem of one tree with hand pruners in August 1995. This represented more than 95% of the total tree leaf area. In the laboratory, the amount of embolism caused by air entry into the cut xylem vessels was first reduced by successive vacuum infiltrations. The branch was then sprayed with water, covered with a black plastic bag, connected to the HPFM and perfused at a pressure $P=0.4$ MPa until water was dripping from leaf blades and water flow F (mmol s^{-1}) entering the branch became constant (after 1–2 h). The entire branch resistance, r_{branch} (MPa s mmol^{-1}) was then estimated as P/F and the entire branch leaf area (LA) specific conductance, r_{branch}^* ($\text{MPa s mmol}^{-1} \text{m}^2$) computed as $P \times LA/F$. Leaf blades, leaf rachises, current-year shoots, and secondary axes were then successively removed, and the hydraulic resistance of each of these components determined as above. r_{leaf} was defined as the sum of blade and rachis hydraulic resistances and r_{xylem} as the difference between branch hydraulic resistance and r_{leaf} . When leaf blades and other components were cut, F was usually immediately constant.

The drop of water potential along the whole branch sap pathway was computed as:

$$\Delta\psi_{\text{branch}} = r_{\text{branch}}^* \times E \quad (1)$$

where E is the mean midday branch transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$). $\Delta\psi_{\text{branch}}$ was computed only when E is maximal because $\Delta\psi_{\text{branch}}$ is then maximal and ψ_{xylem} is more likely to approach ψ_{cav} . Similarly, the drop in water potential from trunk insertion to mid-leaf-rachis was computed as:

$$\Delta\psi_{\text{rachis}} = (r_{\text{branch}} - r_{\text{blade}} - 0.5r_{\text{rachis}})E \times LA \quad (2)$$

Crown main stem hydraulic conductivities, K_{stem} , were not measured, but estimated by extrapolating the diameter versus conductivity relationship, established on branch segments, to the main stem diameter values. The main stem was divided in segments of length L (0.2–0.5 m) and the drop in water

potential across each segment computed as:

$$\Delta\psi_{\text{stem}} = L/K_{\text{stem}} \times LA \times E + 0.01L \quad (3)$$

where LA is the total leaf area distal to the stem segment and $0.01L$ the pressure drop due to gravitational forces. Individual segment potential drops were then summed to obtain the whole potential drop along the main stem axis, starting at the base of the crown.

Xylem anatomy

Vessels diameter were measured in upper and lower branch leaf rachises, and, to a lesser extent, in shoot internodes. Five leaves were randomly taken from two branches aged 2 years and 7 years. For each leaf, one thin cross-section was taken from the middle of the rachis and diameter of 200 vessels were measured. Measurements were made with an ocular-mounted light chamber and a digital table (Océ Graphics model G6450). The resolution was about 0.5 μm .

Results

Crown microclimate

R_g was similar in the upper and middle crown layers, but significantly lower in the lower layer (about 45% and 30% incident R_g , respectively, Fig. 1b). Air VPD was identical in all crown layers (Fig. 3b) and no differences were detected in dry and wet bulb temperatures above and below canopy.

Positive xylem pressure potential

No sap exudation and no positive pressure were detected during the studied period in *Fraxinus* and *Quercus* (Table 1). However, if these species made a rapid wound response to isolate the drilled area, positive pressures may not have been detected by the pressure transducer. For comparison *Carpinus* and *Betula* exhibited high positive pressure (up to 0.2 MPa) until leaf expansion. Only weak pressures were recorded in *Fagus*.

Seasonal progression of embolism

At the beginning of the study and until bud break (beginning of May 1995), approximately 100 PLC values were measured in all shoot internodes on a tree (Fig. 1c). When leaves developed (see increase in light interception, Fig. 1b), and the new xylem rings started to form, the PLC in 1994 and 1993 internodes rapidly decreased down to c. 40% and 80%, respectively. The decrease in PLC was only apparent and corresponded to the formation of new vessels, not to a re-activation of older ones because only current year early-wood vessels were coloured after dye infiltration. The PLC was higher in the 1993 internodes because the high pressure perfusion reactivated the vessels in two older annual rings. The mean PLC then remained constant all through the growing season although with large variation because of the small sample size. In current year internodes and leaf rachises, PLC

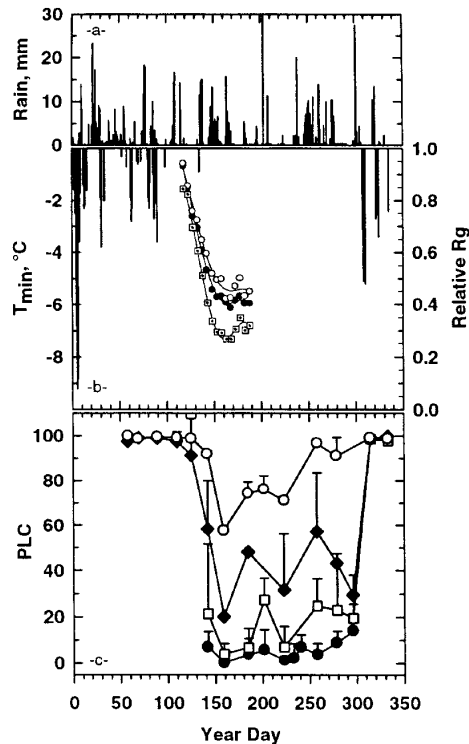


Fig. 1. Microclimate and seasonal changes in xylem embolism. (a) Time-courses of rainfall, mm d^{-1} . (b, left y-axis) Daily minimum air temperature, only sub-zero temperatures are shown. (b, right y-axis) Vertical attenuation of global radiation, R_g , in the crown of three trees. R_g was continuously measured in the upper (\circ), middle (\bullet) and lower (\square) crown layers. Each point represents the daily mean R_g values in each crown layer ($n=3$) relative to the mean incident R_g value 2 m above tree canopy. Seasonal changes reflected leaf development. (c) Time-course of xylem embolism in current year (1995) leaf rachises (\bullet), current year shoot internodes (\square), 1994 shoot internodes (\blacklozenge), and 1993 shoot internodes (\circ). Embolism was quantified by the percentage loss of hydraulic conductance (PLC , y-axis). Error bars represent standard deviation SD ($n=8$ for rachises, $n=2-4$ for shoot internodes).

was constant throughout the growing period (<20% and 10%, respectively) because no drought developed (see rainfall Fig. 1a). At the end of October, leaflets then rachises started to abscise, but no significant increase in PLC was noted in the internodes and rachises that

remained attached to the shoots. The first below freezing temperatures were recorded at the beginning of November (Fig. 1b) which resulted in a rapid and extensive loss of hydraulic conductivity in all internodes.

Vulnerability curves

Shoot internodes were as vulnerable as leaf rachises (Fig. 2, upper branches only). Leaf rachises from the lower canopy layer were clearly less vulnerable than rachises from the upper layer (onset of embolism at -3 and -2 MPa and 50 PLC at -3.5 and -2.5 MPa, respectively).

Xylem anatomy

On average, rachis vessels were significantly wider ($P < 0.001$) in a 2-year-old branch ($22.6 \mu\text{m}$, ± 5.4 SD, $n = 1016$) than in a 7-year-old one ($19.5 \mu\text{m} \pm 6.1$ SD, $n = 1016$). A positive relationship ($r^2 = 0.74$, $n = 10$) was found between the rachis diameter and the mean vessel diameter. Mean vessel diameter in a current year terminal shoot was $28.4 \mu\text{m}$ (± 7.0 SD, $n = 132$).

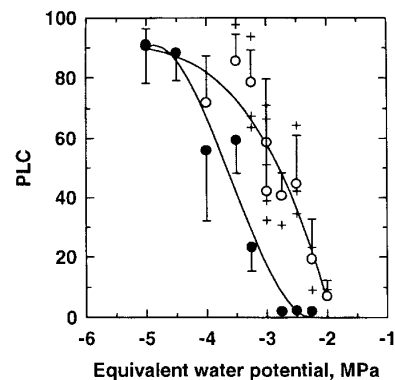


Fig. 2. Vulnerability to embolism of upper crown branch leaf rachises (\circ) and shoot internodes ($+$) and lower crown branch leaf rachises (\bullet). Different branches were pressurized in a large pressure chamber at different positive air pressures. The equivalent water potential (x-axis) is the opposite value of the air pressure. Bars represent SD ($n=8$). For shoot internodes, each symbol represent one measurement.

Table 1. Positive xylem pressure potential in *Fraxinus excelsior* and four other co-occurring species

Species	Anatomy	Maximum ψ_{xp} (MPa)	Onset	End	Bud break
<i>Fraxinus excelsior</i>	Ring porous	nd	–	–	Early May
<i>Quercus petraea</i>	Ring porous	nd	–	–	End April
<i>Fagus sylvatica</i>	Diffuse porous	0.02	Mid-March	Mid-April	End April
<i>Carpinus betulus</i>	Diffuse porous	0.19	(*)	Early April	End March
<i>Betula pendula</i>	Diffuse porous	0.20	Mid-March	Mid-April	Mid-April

nd: Not detectable.

*Pressures were already positives in *Carpinus* at the onset of the measurements.

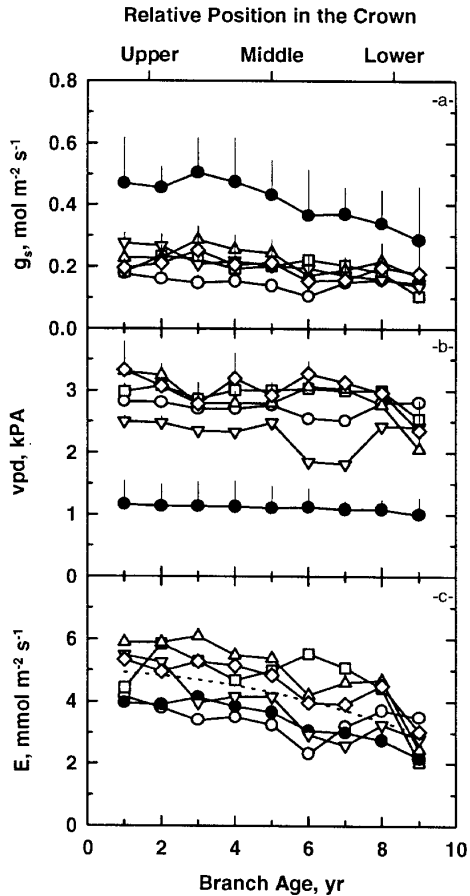


Fig. 3. Mean leaf stomatal conductance, g_s (a), air vapour pressure deficit, VPD (b) and leaf transpiration rate, E (c) for branches of different age (x -axis). Young branches being closer to tree apex, the x -axis expresses also the distance to the apex. Different symbols and different lines represent different days. The dotted line in graph (c) is a second order polynomial fitting. Error bars represent SD ($n=4-29$ with an average of 9). Error bars are not shown for graph (c).

Water relations

Daily variations in F_d and ψ_{leaflet} were symmetrical for all trees (Fig. 4b, c). As a result F_d had a negative linear relationship to ψ_{leaflet} ($r^2=0.93$ to 0.96). The decrease in ψ_{leaflet} and the increase in F_d after sunrise clearly levelled off after 08:00 h whereas incident R_g and air VPD continued to increase above the canopy (Fig. 4a). ψ_{leaflet} remained nearly constant between 08:00 h and 17:00 h.

No consistent vertical variations in ψ_{leaflet} could be detected between the two uppermost crown layers of the three studied trees (Fig. 5a). In all but one instance, the highest ψ_{leaflet} values were found in the lower crown layer. g_s varied little within the crown (Fig. 3a), but was much higher when ambient air VPD was low (closed circles in Fig. 3). As a result, E was nearly constant in

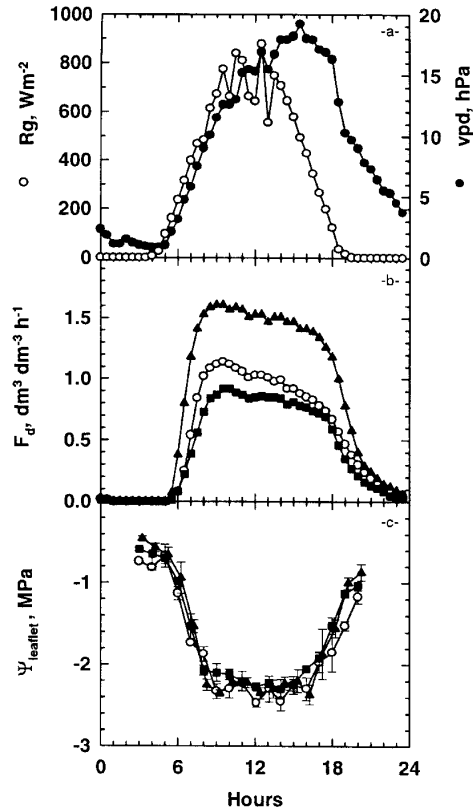


Fig. 4. Daily time-courses in air VPD (●) and global radiation (○) above the canopy (a), sap flux density (b) and leaflet water potential (c). For (b) and (c), different symbols represent different trees. Error bars in graph (c) represent SD ($n=3$).

the crown and similar for different ambient air VPD (Fig. 3c).

Crown hydraulic architecture

On a leaf area basis, rachis segments and current year primary axis internodes (axial) showed higher K_i values than secondary axis internodes (Fig. 6). In other words, the LSC were higher in the rachises and the 1995 internodes. Leaf rachises from the lower crown layer exhibited lower LSC values than in upper crown leaves.

There was a considerable variation of r_{branch} between branches of a same crown (Fig. 7a). However, r_{branch}^* was remarkably constant all through the crown (0.29 ± 0.04 SD, $n=37$) (Fig. 7b), and independent of branch basal diameter, length or leaf area. Only seven branches, out of 44, exhibited much higher r_{branch}^* values. Although r_{branch}^* was constant, the distribution of resistances within a branch varied with age (Fig. 7c). For young apical branches, more than 90% of the resistance was found in the leaf whereas, in the oldest branches, most of the resistance occurred in the stem xylem. The development

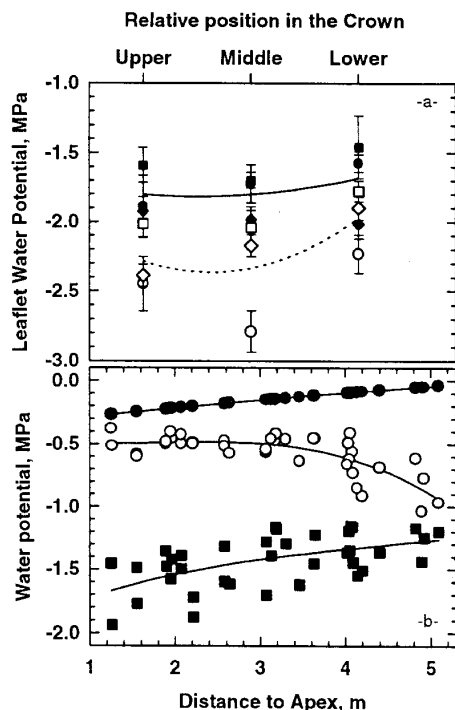


Fig. 5. (a) Midday ψ_{leaflet} values for branches from different vertical crown layer (upper x-axis). Different symbols represent different trees, and different colours two different days. Error bars in graph represent SD ($n=6$). (b) Computed water potentials at the trunk insertion (\bullet), in the leaf rachises (\circ) and the leaf blade (\blacksquare) of 44 primary branches in the crown of one tree. The water potential is arbitrarily set to 0.0 MPa at the base of the crown. x-axis is the distance from the branch insertion on the trunk to the tree apex. The correspondence between the two x-axes is approximate.

of secondary axes was mostly responsible for the increase in xylem resistance (data not shown).

The computed vertical variation in leaflet water potential were minimal in the crown, which was consistent with the actual variation (Fig. 5b). The drops in water potential along the crown sap pathway reflected the variation in branch resistances. As a result, the rachis water potential was much lower in leaves from the lower crown layer (Fig. 5b).

Discussion

The life history of *Fraxinus* vessels was very similar to what has previously been observed for temperate *Quercus* species (Cochard and Tyree, 1990; Cochard et al., 1992) and may be common to many temperate ring-porous species. New vessels were formed before leaf expansion, remained functional throughout the summer and rapidly embolized with the first fall frost. Xylem conduits were very prone to frost because of their large internal lumen volume (Sperry and Sullivan, 1992). *Fraxinus* lacked the

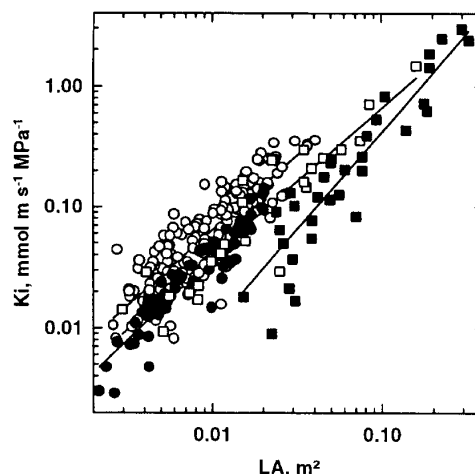


Fig. 6. Xylem segment native hydraulic conductivity (y-axis) versus total leaf area distal to the segment (x-axis). Segment were excised from upper (\circ) and lower branch (\bullet) leaf rachises, current year internodes from primary branch axes (\square) and previous years internodes from secondary branch axes (\blacksquare). Lines are first order regressions through the logarithmic values.

refilling mechanism (Cochard et al., 1994) so early-wood vessels were conductive only during the year they were formed. However, smaller late-wood vessels and tracheids can remain functional for many years (Granier et al., 1994). As a result, for these species, PLC values are not correct estimates of the degree of xylem dysfunction in >1-year-old shoot internodes because they depend on the number of inactivated annual rings.

In eastern France, temperatures below sap freezing are common during the winter. Winter embolism can be seen as a natural phenomenon in the life history of ash vessels, which means that it should not prejudice the tree water relations during the growing season. However, frost events during spring, after the current early-wood vessels are formed, will considerably lower shoot hydraulic conductances if new vessels cannot be formed. The results suggest that, in this case, leaf gas exchange and tree growth are likely to be depressed (see below). Late spring frosts (and early fall) are probably major limitations to the functioning of large vessel-bearing species and may compromise their success under high altitudes or latitudes.

Leaf rachises from different positions in the same crown exhibited contrasting vulnerabilities. The increase in xylem safety was associated with a decrease in xylem efficiency (lower LSC values and smaller vessel sizes). Decreased xylem efficiency may thus be a trade-off for increase safety in *Fraxinus* (Tyree et al., 1994). This suggests that branch developmental conditions influence xylem vulnerability. Similar observations have been made on *Fagus* branches and saplings for which shade-grown branches were more vulnerable than sun-exposed ones

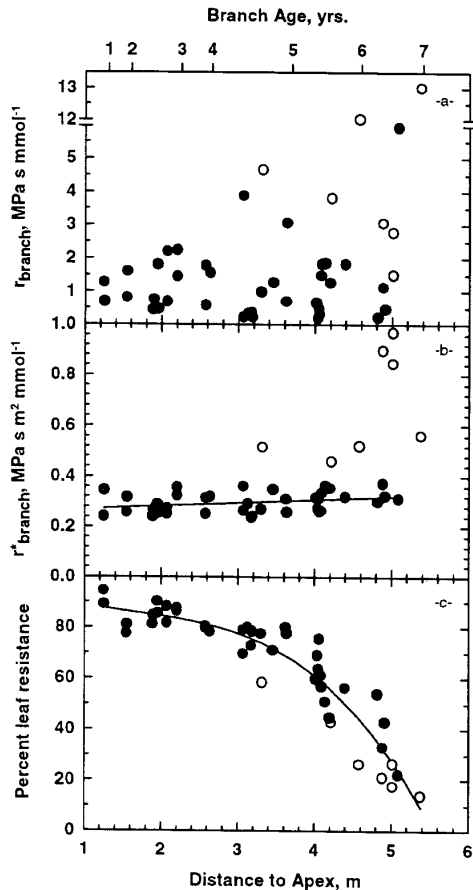


Fig. 7. Vertical crown variation in whole branch hydraulic resistance (a), whole branch leaf specific hydraulic resistance (b) and percentage of whole branch resistance due to leaf resistance (c). Each point represent one primary branch. Open circles indicate seven branches with higher specific resistances.

(Cochard, unpublished results). Within-individual environmental and ontogenic effects on xylem vulnerability may thus be as large as differences between species. Xylem vulnerability has usually been considered as static and species- or organ-specific, but it may actually exhibit a large plasticity and respond to changes in environmental conditions.

To our knowledge, this is one of the first systematic descriptions of the hydraulic architecture of an entire crown of a large tree that includes leaf blade resistances. Previous studies (Tyree, 1988; Zimmermann, 1978; Joyce and Steiner, 1995) only focused on xylem resistances and, according to results from this laboratory and others, the main resistances of the above-ground sap pathway may not have been included. Since the introduction of the HPFM (Tyree *et al.*, 1993a), it is possible reliably to measure whole branch resistance and to determine where resistances are located (see Yang and Tyree, 1994, for

considerations about the validity of the technique). Two major findings resulted from this study: (1) the localization of the main resistances to sap flow changed from leaf blades to xylem conduits as branches aged; and (2) the leaf area-specific r_{branch}^* was a constant value for most branches. The bioclimatical gradients (light, VPD) being rather small in Ash crowns, these vertical hydraulic variations were more likely to be ontogenic than environmental.

Partitioning of r_{branch} into different components has been studied for several species. In these studies, r_{leaf} always represented the highest share of r_{branch} (e.g. 40–60% in *Acer* spp, Yang and Tyree, 1994; 80–90% in *Quercus* spp, Tyree *et al.*, 1993a, 80% in *Juglans regia*, Tyree *et al.*, 1993b). This was also true for *Fraxinus* in this study, but only for branches from the upper crown layer where r_{leaf} represented more than 90% of r_{branch} . However, for the oldest branches, 90% of the resistance was actually located in the xylem itself. Clearly, this was due to an increase in r_{xylem} mainly caused by the formation of short shoot internodes. These short internodes were mostly present in secondary branch axes but also in the terminal primary axes of old branches. Axes bearing short internodes exhibited much lower LSC values. Joyce and Steiner (1995) on a North American *Fraxinus* species and Cochard (unpublished results) on *Fagus sylvatica* made similar observations. Low LSC values in short internodes may result from a decrease in K_i due to a decrease in vessel diameter or current ring xylem area, or to an increase in the number of nodes per unit length (nodes exhibit lower K_i than internodes (Tyree and Alexander, 1993; Cochard, unpublished results)). The physiological implications of an increase in r_{xylem} are discussed below.

Although r_{xylem} increased with branch age, r_{branch}^* ($=r_{\text{branch}} \times LA$) values were nearly identical for most branches irrespective of their lengths, diameters or leaf areas. Yang and Tyree (1994) also observed that whole tree above-ground resistances were constant for trees in the range of 0.02 to 0.2 m basal wood diameter. According to equation [1], $r_{\text{branch}}^* \times E$ is the water potential drop across the branch. As E little varied between branches and as trunk resistances were low, the constancy of r_{branch}^* implies that the water potential drop from the base of the crown to the leaves was similar for all branches. This was confirmed by direct measurements of ψ_{leaflet} .

If ψ_{leaflet} was constant among branches, the fact that r_{xylem} increased with branch age implied that rachises in old branches experienced lower ψ_{xylem} than in young branches (about 0.5 MPa difference). In addition, rachises in old branches exhibited a lower vulnerability to embolism (about 1 MPa difference in ψ_{cav}). Vessels in lower branch rachises appeared to be structurally capable of withstanding higher tensions. Vessel vulnerability seems coupled to the prevailing water potential of a vessel. This

agrees with results on *Betula* where vessel vulnerability decreased along the sap pathway (Sperry and Saliendra, 1994). This suggests that when vessels differentiate during spring, the size of the pores in the pit membrane is adapted to the ψ_{xylem} the vessel will experience days or weeks after.

As usually observed for most tree species studied (Tyree and Sperry, 1988) midday ψ_{leaflet} values in *Fraxinus* approached ψ_{cav} values measured in upper crown rachises. Because trees are operating very close to the point of xylem dysfunction, some authors (e.g. Jones and Sutherland, 1991; Cochard et al., 1996) proposed that stomata may actively control xylem embolism by limiting E and hence the drop in ψ_{leaflet} . However, ψ_{cav} must be compared with ψ_{xylem} because the drop of potential within the leaf is mainly caused by extra-vascular resistances (Yang and Tyree, 1994; Tyree et al., 1993b). ψ_{leaflet} and ψ_{xylem} may be formulated as:

$$\psi_{\text{leaflet}} = \psi_{\text{soil}} - E \times LA (r_{\text{root}} + r_{\text{xylem}} + r_{\text{leaf}}) \quad (4)$$

$$\psi_{\text{xylem}} = \psi_{\text{soil}} - E \times LA (r_{\text{root}} + r_{\text{xylem}}) \quad (5)$$

where ψ_{soil} is the soil water potential (MPa) and r_{root} the hydraulic resistance along the extra-vascular water pathway from the soil to the root xylem. The difference between ψ_{xylem} and ψ_{leaflet} depends on how r_{leaf} compares to $(r_{\text{xylem}} + r_{\text{root}})$. For upper branches, it was found that r_{xylem} was low so ψ_{xylem} was much higher than ψ_{leaflet} . For the same reason, changes in E will not translate to large changes in ψ_{xylem} . This means that E will have little effect on the control of ψ_{xylem} and embolism. But this also means that upper branches can tolerate rapid changes in E (during cloudy days) without exhibiting cavitation events. For older branches, however, the risk of embolism is potentially higher, but this might be compensated for by increased xylem safety. When r_{root} increases (because of low soil water content) the situation is different because ψ_{xylem} will reach lower values for all branches. Thus, the control of E will have higher impact on the control of ψ_{xylem} and xylem embolism (Cochard et al., 1996; Lu et al., 1996). Moreover, in the case of cavitation events, the change in r_{branch} will be much more pronounced in old branches (Tyree and Cochard, 1996). For instance, if 50 PLC is measured in the xylem then the increase in r_{branch} will be of 10% and 90% in upper and lower crown branches respectively. In conclusion, the lower xylem resistance in the upper branches of Ash trees increases the xylem safety because it increases ψ_{xylem} , buffers its variation, and lowers the impact of embolism on whole branch resistance. However, a trade-off of xylem efficiency for xylem safety (Tyree et al., 1994) may lower the benefit of low r_{xylem} . This might be the case when, within a plant, larger vessels are found more sensitive to cavitation (Salleo and Lo Gullo, 1986; Sperry and Saliendra, 1994). Clearly, the understanding of tree water relations must account for the xylem hydraulic safety and efficiency (Tyree, 1988).

The results suggest that changes in bud activity during the ontogenic development of branches may have strong hydraulic implications. Buds are responsible for both shoot internode length and branch LA. Thus, they control xylem transport capacities and evaporative surfaces. If buds form short internodes then branch r_{xylem} values are likely to increase. However, the data demonstrate that r_{branch}^* remains constant because LA decreases. It is interesting to note that LA plays two antagonist roles: increasing LA decreases r_{leaf} (leaves are organs in parallel), which could in theory compensate for the increase in r_{xylem} , but LA also increases the evaporative surfaces. The functional limitation to this hydraulic system is that ψ_{xylem} must be kept above ψ_{cav} . This means that if LA increases then E must decrease to lower the whole branch transpiration and increase ψ_{xylem} (Meinzer and Grantz, 1990). As a result g_s , and probably the assimilation rate A , are likely to be depressed which will diminish the leaf efficiency for carbon assimilation (in terms of carbon fixed per carbon used to construct and maintain the leaves). It can therefore be concluded that, in *Fraxinus*, LA is balanced against r_{xylem} in order to maximize g_s and A and maintain ψ_{xylem} above ψ_{cav} . As recently suggested by Joyce and Steiner (1995), the balance between r_{xylem} and LA probably results from a positive feedback mechanism. If buds form short internodes then r_{xylem} increases which will lower E and A in the newly formed leaves. It is then plausible that the number of leaf primordia in the new buds or the amount of reserve available for their development during the next growing season will be lowered. In this study, the increase in r_{xylem} became drastic only for branches in the lower crown layer. This might have been caused by the significant decrease in R_g in this crown layer which would further lower the leaf carbon gain and enhance the feedback mechanism. For several branches (open circles in Fig. 7) it was found that r_{xylem} was not balanced with LA which resulted in very high r_{branch}^* . Although E or PLC were not measured in such branches, it is likely that E was lower than for same aged branches, if not, PLC must have been high. The number of such branches increased with branch age and 50% of the 7-year-old branches were in this group. As all 8-year-old branches were dead, uncoupling between r_{xylem} and LA might have strong consequences on branch survival. It is also common in *Fraxinus*, that old branches remain alive and become leaders or form forks (Cluzeau et al., 1994). Branches forming leaders might exhibit low r_{xylem} and r_{branch}^* which would allow them high growth rates. The relationships between hydraulic architecture and crown development await further studies.

In conclusion, *Fraxinus* branches developed short shoot internodes when ageing which increased the xylem resistance to sap flow. However, branch leaf areas and xylem vulnerabilities were adjusted in such ways that leaf gas exchanges were maximized, leaf water potentials remained

constant, and cavitation events were minimized. Leaf areas were thus balanced against xylem hydraulic transport capacities.

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