

Mechanisms of xylem recovery from winter embolism in *Fagus sylvatica*

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Received February 21, 2000

Summary Hydraulic conductivity in the terminal branches of mature beech trees (*Fagus sylvatica* L.) decreased progressively during winter and recovered in the spring. The objective of this study was to determine the mechanisms involved in recovery. Two periods of recovery were identified. The first recovery of hydraulic conductivity occurred early in the spring, before bud break, and was correlated with the occurrence of positive xylem pressure at the base of the tree trunk. Active refilling of the embolized vessels caused the recovery. The second recovery of hydraulic conductivity occurred after bud break and was correlated with the onset of cambial activity. Formation of new functional vessels, leading to an increase in xylem diameter, was largely responsible for the increase in xylem conductivity. The two mechanisms were complementary: active refilling of embolized vessels occurred mostly in the root and the trunk, whereas formation of new functional vessels occurred mainly in young terminal shoots.

Keywords: beech, cavitation, growth, hydraulic conductance, root pressure, water relations.

Introduction

Cavitations are likely to occur in xylem conduits, because sap ascends under high tensions (Milburn 1973, Sperry et al. 1988a). Following a cavitation event, conduits become filled with air and are unable to supply water to the leaves. Cavitations are caused by the expansion of air bubbles in the conduit lumens. The two main phenomena responsible for cavitation events are dehydration and frost. Xylem dehydration results in increased sap tension. As the sap tension exceeds the maximum capillary pressure generated by the pit membrane, it pulls air–water menisci in the pit membrane into a functional conduit (Sperry and Tyree 1988). The mechanism of freezing-induced embolism differs from that caused by dehydration: when sap freezes, small air bubbles form between ice crystals that can expand on thawing even under moderate sap tensions (Sperry and Sullivan 1992).

In habitats where sap-freezing temperatures occur, a high degree of embolism has been measured in many species at the end of the winter period (Wang et al. 1992). For ring-porous species such as *Quercus* (Cochard and Tyree 1990) and

Fraxinus (Cochard et al. 1997), one freeze–thaw event may be enough to impair most of the xylem conduits. In diffuse-porous species (*Acer*: Sperry et al. 1988b; *Fagus*: Borghetti et al. 1993), the number of embolized xylem conduits increases progressively over the winter. Freezing events cause little damage to the xylem of coniferous species (Sperry and Sullivan 1992).

For temperate tree species, rapid xylem recovery from winter embolism is essential to support transpiration of new leaves in the spring. Two recovery mechanisms seem to have evolved: replacement of embolized vessels by new, functional ones, and refilling of embolized vessels through an active mechanism. The first mechanism is common to all plant species with secondary cambium for which the production of new vessels increases overall xylem functionality. Recovery from winter embolism is based solely on this mechanism in several tree species, including *Quercus* (Cochard and Tyree 1990) and *Fraxinus* (Cochard et al. 1997) species and the diffuse-porous species *Prunus* (T. Améglio, unpublished observations). Other species are able to produce positive xylem pressures that actively dissolve embolisms (Sperry et al. 1987). Active refilling has also been observed in the presence of high xylem tensions; however, the mechanism underlying this process has not been identified (Holbrook and Zwieniecki 1999, Tyree et al. 1999).

The purpose of this study was to analyze mechanisms of xylem recovery from winter embolism in *Fagus sylvatica* L. This species is known to develop a significant amount of winter embolism and to recover during spring (Borghetti et al. 1993). We followed the seasonal progression of embolism in twigs of mature trees and correlated these variations with xylem pressure potentials, xylem osmotic potentials and cambial activity. The timing of embolism recovery was correlated with the timing of leaf expansion and sap flow.

Materials and methods

Sites and plant material

During 1993 and 1994, experiments were conducted on two mature, 70-year-old *Fagus sylvatica* trees growing in the Arboretum of Amance, near Nancy, in eastern France (Site 1, 48°45' N, 6°20' E, elevation 255 m). Trees were growing at the

periphery of the Arboretum, with southern branches exposed to full sunlight. During 1996, experiments were conducted in a 30-year-old natural stand of the Hesse state forest, near Sarrebourg, in eastern France (Site 2, 48°40' N, 7°05' E, elevation 300 m). During 1997, all measurements were made on one dominant 15.4-m-tall tree at Site 2. A 15-m-high scaffolding tower provided access to the tree.

Xylem embolism

The seasonal progression of embolism in the xylem was measured periodically from August 1993 to September 1994 at Site 1 and from January 1996 to July 1997 at Site 2, by the hydraulic technique of Sperry et al. (1988a) as described by Cochard et al. (1991). The technique involves measuring the hydraulic conductance of 0.02–0.03-m-long excised xylem segments before and after water refilling. On each sampling day, one branch (2–10 years old, 1–2 m long) was cut from the crown of one tree and transported to the laboratory in a black plastic bag to limit leaf evaporation. In 1993 and 1994, branches were sampled in the sun-exposed part of the crown of each tree. In 1996, branches were excised with a pruning pole from the crowns of randomly chosen trees at Site 2 (mean tree height was 12.7 m). In 1997, randomly selected branches were cut with a hand pruner from the middle crown of the intensively studied tree at Site 2.

The degree of xylem embolism was measured on eight to 16 shoot internodes excised from each sampled branch. Shoot internodes were 1 to 6 years old. Samples were excised and manipulated under tap water to avoid air entry into the vessels. The degree of embolism was calculated as percent loss of hydraulic conductivity (PLC) as described previously (Cochard et al. 1991).

Sap exudation and xylem pressure potentials

A visual assessment of sap exudation at the base of trees at Site 1 was made during spring 1994. In each tree, a 4-mm-wide and 20-mm-deep hole was drilled into the bole 30 cm aboveground. After several weeks, a new hole was made to avoid the possible effect of a plugging reaction.

To determine the occurrence of positive xylem pressure potentials, electronic pressure transducers (Model PDCR 1000, Druck Ltd., Leicester, U.K.) were tightly fitted in 2-cm-deep holes drilled at the base of three trees in 1996 and one tree in 1997 at Site 2. Transducers were connected to a data logger (Model 21X, Campbell Scientific Inc., Logan, UT). The holes were filled with water at the beginning of the experiment. The transducers were installed in February and the measurements ended in May when the leaves were fully developed.

We also tried to connect sensors to the cut ends of branches of the same trees, but the measurements were unreliable because the connectors rapidly became filled with air and so the transducers were no longer hydraulically coupled to the xylem vessels.

Sap osmotic potentials

The seasonal progression of sap osmotic potential (MPa) was measured periodically from mid-February until the end of

May 1997. Fifty-cm-long branches were excised from the mid-crown of the selected tree at Site 2 and brought to the laboratory. Xylem sap was immediately collected by vacuum extraction (Bollard 1953) and stored at –20 °C in 1.5-ml test tubes until measurement. Sap osmotic potential was determined with a dew point osmometer (Wescor 5500, Logan, UT).

Sap flow density

Sap flux densities (F_d , $\text{dm}^3 \text{dm}^{-2} \text{h}^{-1}$) were monitored throughout the 1997 growing season with a 20-mm-long radial flow meter (Granier 1987) inserted in the trunk of the selected tree at breast height.

Shoot growth

During 1997, variations in branch diameter were continuously monitored with LVDT linear displacement transducers (Model DF2.5, Solartron Metrology, Massy, France). Two transducers were mounted with an Invar holder on the bark of two mid-crown branches of one tree at Site 2. Initially, branch diameters were about 2 cm. Wood temperature was measured in the vicinity of the transducers by two thermocouples inserted a few mm into the wood. The outputs of the LVDT and thermocouples were recorded by a data logger at 30-min intervals.

Cambial activity was assessed in the same shoot internodes used to measure xylem embolism. A thin cross section was taken from the middle of the sample and observed with the aid of a light microscope. During 1994 and 1996, only the onset of cambium growth was determined. During 1997, the radius of each annual ring was measured with a micrometric ocular at six evenly spaced locations. Relative ring growth was calculated as the ratio of current (1997) ring area to total xylem area. The data were used to compute the theoretical increase in conductivity resulting from new vessel formation (see Discussion).

Results

Figure 1a shows the seasonal progression of xylem embolism, measured as the percentage loss of hydraulic conductance (PLC), of two 70-year-old trees at Site 1 during 1993 and 1994. Because both trees exhibited similar patterns, the data were pooled. The basal level of embolism was high in 1-year-old shoot internodes (about 50 PLC) during summer 1993 and remained high until spring 1994. Xylem embolism recovery was conspicuous at the end of May 1994 and PLC continued to decrease during the growing season until PLC values of about 15 were reached in August 1994. Current-year shoot internodes exhibited low PLC values during summer 1993 (about 10 PLC). Values progressively increased up to 40 PLC during the fall when temperatures dropped below freezing (Figure 1b), and thereafter the progression of xylem embolism paralleled that of the 1-year-old internodes. Sap exudation at the base of the trees was observed at the end of March and the beginning of April 1994. Leaf flush occurred at

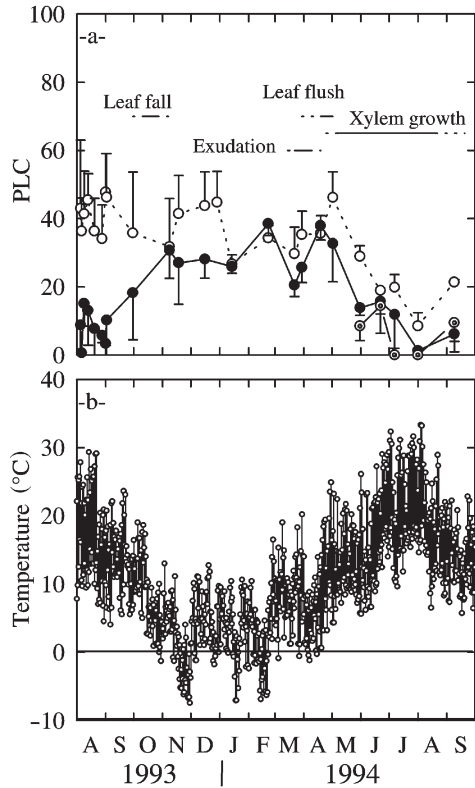


Figure 1. (a) Progression of the percentage loss of hydraulic conductivity (PLC) in the xylem of two 70-year-old beech trees from Site 1 during the 1993 and 1994 growing seasons. The PLC values are for shoot internodes developed during the 1994 (○), 1993 (●) and ≤ 1992 growing seasons (○). The error bars represent 1 SD. (b) Progression of minimum and maximum air temperatures during 1993 and 1994.

the end of April 1994, and new vessels were formed at the beginning of May.

Figure 2a shows the time course of embolism in the xylem of 30-year-old trees from Site 2 during 1996. The amount of embolism was 40–50 PLC in January; it then increased during the winter and reached 85 PLC in March. The youngest apical shoot internodes (closed symbols) exhibited significantly higher PLC values than the older internodes. Trees started to recover from xylem embolism at the end of March. The large variations in PLC values observed during April may have arisen because the trees were not at the same phenological stage when sampled, or may have been caused by late spring frosts (Figure 2c). Significant xylem pressures were measured at the base of the trees during April 1996 (Figure 2b). Xylem pressures were higher during the day than at night, reaching a maximum of 45 kPa. Xylem pressures were positively correlated with air temperature (not shown). Continuous positive xylem pressures were measured for one week in mid-April 1996. Leaf flush occurred at the end of April–beginning of May and the onset of xylem growth began in May.

Figure 3a shows the time course of embolism in the xylem of one tree at Site 2 during 1997. The PLC values increased in the 1-year-old shoot internodes during the winter, significantly decreased at the beginning of April after positive xylem

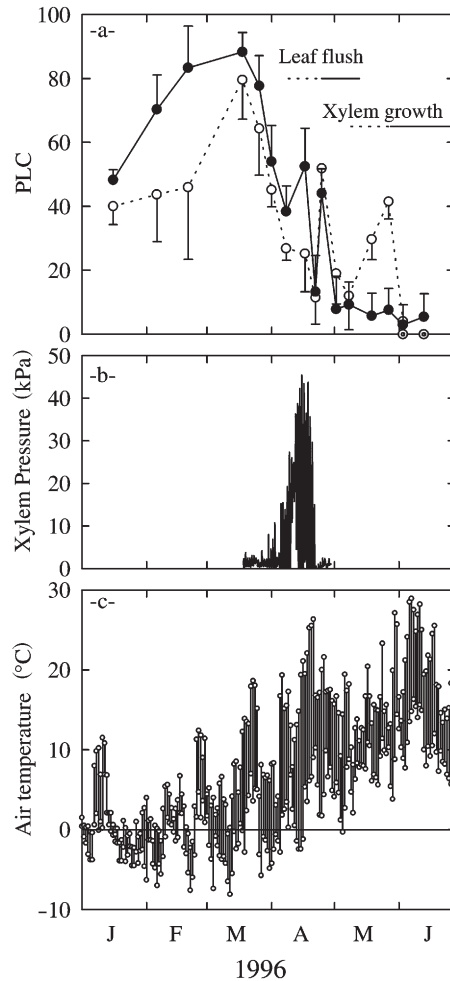


Figure 2. (a) Progression of the percentage loss of hydraulic conductivity (PLC) value in the xylem of 30-year-old beech trees from Site 2 during the 1996 growing season. The PLC values are for shoot internodes developed during the 1996 (○), 1995 (●) and ≤ 1994 growing seasons (○). (b) Progression of mean hydrostatic xylem pressure measured at the base of three trunks during the 1996 growing season. (c) Progression of minimum and maximum air temperatures for the 1996 growing season. The error bars represent 1 SD.

pressures were measured (Figure 3b) and then increased again after a period of below-freezing temperatures (Figure 3d). Xylem pressures and air temperatures were much lower in 1997 compared with 1996. The recovery of xylem functionality occurred at the end of April, after leaves had flushed, and was concomitant with the onset of xylem growth (Figure 3c) and sap flow (Figure 3b). The pattern of cambial reactivation was clearly basipetal. Before the onset of xylem growth, we observed small daily fluctuations in branch diameter that were positively correlated with branch temperature (stem shrinkage at night). On three occasions we noted a pronounced branch constriction that was concomitant with a below-freezing wood temperature. Once cambial activity and sap flow started, stem constrictions were observed during the day. Over the entire period that stem diameter variations were recorded (February–October 1997), the fluctuations were closely synchro-

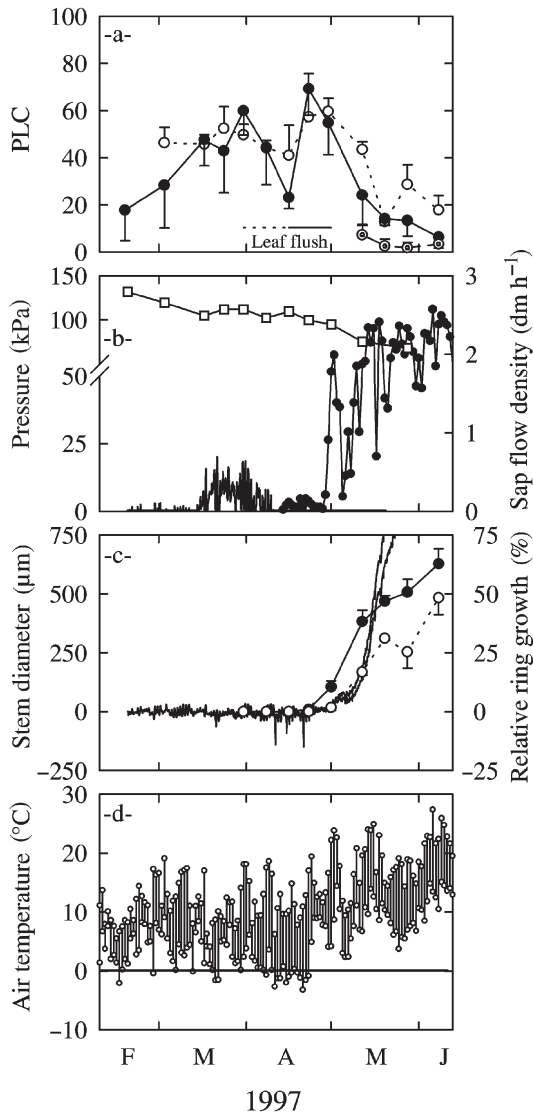


Figure 3. (a) Time course of percent loss of hydraulic conductivity (PLC) in xylem of one beech tree from Site 2 during the 1997 growing season. The PLC values are given for shoot internodes developed during the 1997 (○), 1996 (●) and ≤1995 growing seasons (○). (b) Progression during the 1997 growing season of hydrostatic xylem pressure measured at the base of the trunk (solid line, left axis), sap osmotic potential (□, left axis) and sap flow density in the trunk (●, right axis). (c) Diameter variation for two branches (solid lines) and relative increase in xylem area for the 1996 (●) and 1995 (○) shoots measured during the 1997 growing season. (d) Progression of minimum and maximum air temperatures during the 1997 growing season.

nized with photoperiod, which is contrary to the findings of Sorbetti-Guerri et al. (1998).

Sap osmotic potential (Figure 3b) remained low (about 0.1 MPa) throughout the study period and tended to decrease with time.

Discussion

We confirmed that the xylem of *Fagus sylvatica* is vulnerable

to freezing-induced embolism. The degree of xylem embolism increased progressively over the winter, with the maximum amount varying between 40 and 80 PLC from winter to winter. A gradual increase in xylem embolism during the winter is typical of diffuse-porous species (Sperry and Sullivan 1992). For ring-porous species, the degree of embolism increases markedly after the first frosts (Cochard and Tyree 1990). The mechanism of embolism formation following a freeze-thaw event has been well documented (Sperry and Sullivan 1992, Lo Gullo and Salleo 1993, Améglio et al. 1995). An analysis of this mechanism for *Fagus sylvatica* was found to agree with current theory (Lemoine et al. 1999). At the beginning of spring, we measured significant increases in xylem hydraulic conductivity. Recovery was almost complete in 1-year-old shoot internodes, but many vessels remained embolized in older internodes. Two independent mechanisms may have resulted in the overall increase in hydraulic conductivity in spring: (1) production of new, functional conduits, and (2) refilling of air-filled conduits (Figure 4). The first mechanism has rarely been quantified. The second mechanism implies an active dissolution of the air trapped in embolized vessels. Until recently, it was thought that embolism could be removed only when the xylem water potential was near or above atmospheric value (Tyree and Yang 1992). However, recent observations indicate the occurrence of embolism removal even in the presence of negative pressures in the xylem (Holbrook and Zwieniecki 1999, Tyree et al. 1999) by a mechanism not yet identified.

We analyzed our data according to current paradigms (refilling by xylem pressure and xylem growth) to determine if new paradigms are needed to account for xylem recovery from winter embolism. Although refilling by xylem pressure and xylem growth can be distinguished by direct observation of frozen samples (Utsumi et al. 1996) or dye coloration (Sauter 1984), we used indirect correlative and quantitative approaches to assess the contributions of the two mechanisms to xylem recovery from winter embolism.

Our correlative approach involved comparing the timing of embolism removal, xylem pressure and xylem growth. The two mechanisms were not synchronous; the occurrence of xylem pressures always occurred before the onset of xylem growth. It was therefore possible to determine qualitatively the impact of each mechanism on the recovery of xylem embolism. In 1996, the degree of xylem embolism decreased markedly during the period of positive pressure and before the occurrence of xylem growth. In contrast, in 1994 and 1997, positive xylem pressure occurred before the final recovery of conductivity and most of the recovery was observed after xylem growth was initiated. However, a significant, but partial, recovery of xylem embolism occurred at the shoot apices only when the xylem was under positive pressures. We conclude that xylem pressure alone was not sufficient to explain the patterns of xylem recovery in our study and that at least two mechanisms were qualitatively responsible for the recovery.

We related changes in PLC to changes in saturated wood-area specific hydraulic conductivity (K_w , $\text{mmol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) after embolism removal. If embolisms are removed by

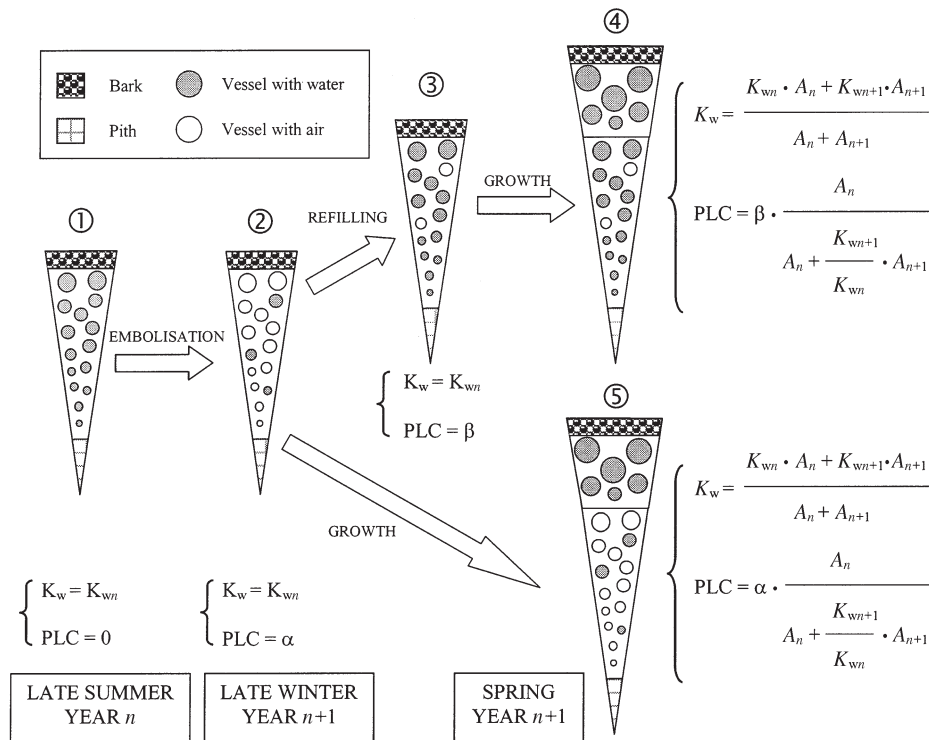


Figure 4. Schematic representation of progression and recovery of embolism for a current-year shoot (formed in year n). In (1), a cross section of the shoot is shown in late summer when cambial activity has ceased and all the xylem vessels are filled with water (no embolism, $PLC = 0$, xylem area = A_n). The saturated wood-area specific hydraulic conductivity (K_w) is equal to K_{wn} . Following freeze-thaw events, some vessels become filled with air and PLC increases up to α in late winter (Step 2): K_w is still equal to K_{wn} . Two mechanisms can explain shoot hydraulic recovery in the spring. Positive xylem pressures may refill some embolized vessels with water (Step 3), thus reducing PLC to $\beta < \alpha$. In this case, K_w remains equal to K_{wn} . Later on, the cambial reactivation further decreases the PLC value (Step 4). The second possibility

is that vessels do not refill before the onset of new ring development (Step 5, new ring area A_{n+1}). The decrease in PLC will then be caused only by the formation of new vessels and will be a function of A_{n+1} and K_{wn+1} (K_w value for the new ring). In this case, K_w increases. Therefore, the paths (2) \rightarrow (3) and (2) \rightarrow (5) can be differentiated by comparing concurrent changes in PLC and K_w values (see results in Figure 5).

refilling air-filled vessels, then PLC values should decrease and K_w should remain constant. If new vessels are produced, then PLC values should still decrease, but K_w should increase (see Figure 4), because K_w increases with branch age in beech as a result of xylem conduit diameters tending to increase with age (Cochard et al. 1999). Figure 5 shows that the progression of xylem embolism from February to March 1997 occurred at a constant K_w value (\square) as expected, because no vessels were formed during the period. The transient recovery that was observed at the beginning of April 1997 when xylem pressures were positive also occurred at a constant K_w value (\circ), confirming an active refilling of the air-filled vessels. The final recovery was associated with an increase in K_w values, suggesting new vessel production. Therefore, both active refilling and xylem growth contributed qualitatively to the recovery from xylem embolism in the studied trees.

In our quantitative approach we sought to determine: (1) if the active refilling was explained by the magnitude of the xylem pressure at the bottom of the tree, and (2) how much reduction in embolism can be expected from the formation of a new functional ring. For the first analysis, we based our approach on current understanding of embolism dissolution (Tyree and Yang 1992), namely, that air trapped in the vessel lumen can dissolve when the air pressure exceeds the atmospheric pressure. This occurs when the hydrostatic tension in the liquid phase becomes less (in absolute values) than the capillary pressure of the air bubble. Vessel diameter in the current-year shoots averages 20 μm for our beech trees (Cochard

et al. 1999). The critical hydrostatic xylem tension required to dissolve the air trapped in such vessels would therefore be -15 kPa (Jurin's law). The tension along the sap pathway is

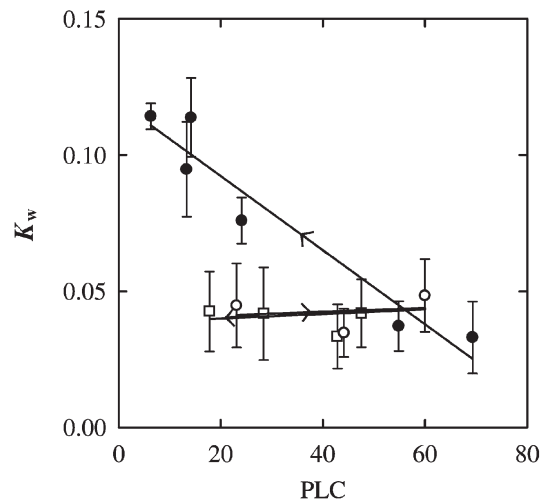


Figure 5. Change in saturated wood-area specific hydraulic conductivity (K_w , $\text{mmol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) versus measured PLC values for the 1997 season. From February to March 1997, PLC values increased at constant K_w values (\square), suggesting that vessels were emptying. In April 1997, PLC values decreased at constant K_w values (\circ), suggesting that vessels were refilling. From mid-April on (\bullet), PLC values decreased again but K_w increased, suggesting new vessel production. The error bars represent 1 SD.

caused by a gravimetric pressure gradient (about 10 kPa m^{-1}) and a hydrodynamic gradient induced by resistance to sap flow. Because tree water losses are negligible before leaf expansion, the latter gradient can be neglected. Thus, we computed that the xylem pressure at the base of the 14.5-m-high study tree must exceed 130 kPa to remove air bubbles at the apex of the trees. The maximum pressure we measured in this study was 45 kPa, which would correspond to an active refilling up to a height of 6 m only. Hence, the xylem pressure that we measured does not explain the apparent active refilling that we observed to a height of 14.5 m. We offer two explanations to account for this discrepancy. First, we cannot exclude the possibility that, several weeks after the pressure inducers were installed on the trees, they became partly hydraulically disconnected because of a plugging reaction. This would have resulted in an underestimation of the actual xylem pressure. A second explanation is that the mechanism generating the xylem pressure originated from the stems themselves instead of the root system. Stem pressures are known to occur in several species such as *Acer* (Johnson et al. 1987) or *Juglans* (Améglio and Cruiziat 1992; F.W. Ewers et al., Michigan State University, unpublished observations). The pressure buildup is usually associated with an increase in sap osmolarity caused by a buildup of sucrose in the apoplast. However, the sap osmotic pressure remained constant while the xylem conduits were refilling in our study. Therefore, until accurate pressure measurements are obtained during the active refilling period, we cannot determine if the xylem pressures measured at the base of the trunk caused the active refilling. Although the xylem pressures were relatively weak compared with the height of the trees, we suggest that more precise techniques should be used to determine these pressures before looking for a hypothetical new mechanism.

For the second quantitative analysis, we estimated the apparent reduction in the degree of embolism caused by the formation of a new functional ring. To ensure that embolism reduction would be apparent, we chose a case in which the embolized vessels did not refill (Figure 4, Steps 2 \rightarrow 5). The decrease in PLC was calculated based on the area and saturated wood-area specific hydraulic conductivity (K_w) of each ring. Details of the calculations are given in Figure 4. From the data in Figure 5, we determined that K_w before xylem growth (K_{wn}) was about $0.04 \text{ mmol m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ and increased to about $0.11 \text{ mmol m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ after a 63% increase in xylem area (Figure 3c). Therefore, the K_w value for the current ring (K_{wn+1}) was about $0.148 \text{ mmol m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ and K_{wn+1}/K_{wn} was close to 3.7. The results of this calculation are compared with the measured changes in PLC during 1997, shown in Figure 6. Xylem growth had a considerable impact on PLC values and explained all of the observed variation. The impact of xylem growth on the reduction in extent of embolism was less for the older shoots than for the younger shoots because the relative area of the current-year ring decreases with stem diameter and shoot age.

A combination of a late frost and reduced cambial activity may explain why, at the beginning of our field survey, the degree of xylem embolism during summer 1993 was high in

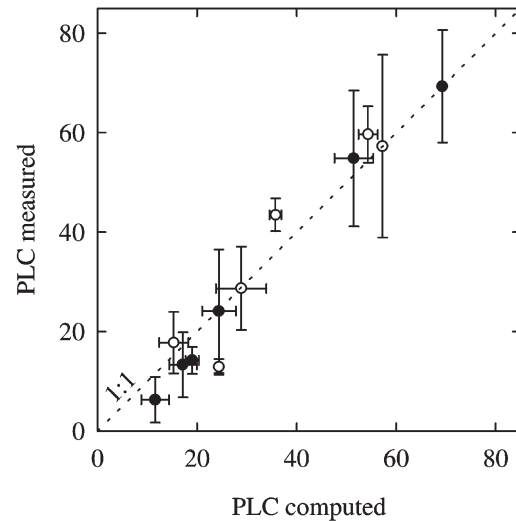


Figure 6. Values of PLC for 1996 (●) and ≤ 1995 (○) shoots measured from mid-April to June 1997 versus predicted PLC values. Predicted PLC values were based on the assumption that emptied vessels did not refill and that the specific hydraulic conductivity per unit xylem area of the new ring was three times greater than the conductivity of the older ring. The error bars represent 1 SD.

1-year-old internodes (about 50 PLC). From year to year, the recovery from winter embolism may be only partial in beech. Further studies are needed to determine if this partial recovery impairs tree functioning during the growing season.

In conclusion, we identified two mechanisms responsible for the restoration of the branch hydraulic conductivity in beech trees in the spring. The first mechanism is the active refilling of embolized vessels and is correlated with the occurrence of root pressures. These pressures were rather weak in beech compared with other tree species (Cochard et al. 1997) and varied from year to year depending on ambient temperature. Furthermore, they occurred early in the season, before leaf expansion. We conclude that, although these low root pressures may significantly contribute to xylem recovery in roots and the trunk, the contribution of this first mechanism to xylem recovery will be small when the temperature is low (such as in 1994 and 1997), and that embolism is likely to form anew if late frosts occur (as in 1997). The second mechanism of xylem recovery resulted from the formation of a new functional ring. We quantified this mechanism and determined that it has a major impact on hydraulic recovery of young shoots. Together, these two mechanisms explained most of the variation in measured PLC values.

Acknowledgments

The French National Forest Service (ONF) supported this work. We are grateful to C. Bodet, B. Clerc, P. Gross and F. Willm for their valuable help in the field. We thank S. Girard for her help with the osmometer. We thank Frank Ewers for his comments on an earlier version of this paper.

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