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Xylem dysfunction during winter and recovery of hydraulic conductivity in diffuse-porous and ring-porous trees

Abstract Xylem embolism in winter and spring as well as the occurrence of positive xylem pressure were monitored in several diffuse-porous and one ring-porous tree species *(Fraxinus excelsior)*. In *Acer pseudoplatanus* and *Betula pendula* embolism reversal was associated with positive (above-atmospheric) xylem pressures that frequently occurred during a 2-month period prior to leaf expansion. In *Acer* high stem pressures were occasionally triggered on sunny days after a night frost. The other species investigated showed no positive xylem pressure during the monitoring period in 1995. *Populus balsamifera* exhibited a complete embolism reversal in 1994, but, like *Fagus sylvatica,* recovery was slow and incomplete in 1995. *Fraxinus* did not refill embolized vessels, but relied entirely on the production of new earlywood conduits in May. *Populus x canadensis* Moench *"robusta"* did not recover from embolism during the monitoring period. Under a simulated root pressure of 20 kPa however, excised branches of *Populus x canadensis* restored maximum hydraulic conductance within 2 days, illustrating the great influence of even small positive pressures on conductivity recovery in spring. In the absence of positive pressure there was no substantial refilling of embolized vessels within a rehydration period of 9 days.

Key words Embolism · Freezing stress · Water relations · Root pressure · Xylem

Introduction

Bubbles forced out of solution during freezing pose a threat for water transport in tall plants (Scholander et al. 1955; Zimmermann 1983). If xylem sap is under tension after thawing, bubbles may expand and cause cavitation. Vulnerability to freezing-induced cavitation increases with xylem tension (Oertli 1971; Sauter 1984; Sperry

and Sullivan 1992) and in particular with conduit volume (Ewers 1985; Sperry and Sullivan 1992).

Depending on xylem structure, there are different strategies to cope with winter frost. Water transport in conifers is apparently not restricted by freeze-thaw events (Hammel 1967; Sucoff 1969; Sperry et al. 1994). This is probably due to the small volume of tracheids which limits bubble expansion. In contrast, temperate ring-porous trees irreversibly lose their efficient earlywood vessels due to winter frost (Zimmermann 1983; Ellmore and Ewers 1986; Cochard and Tyree 1990; Sperry et al. 1994). This can be tolerated because new megaporous (>100 µm diameter) vessels are produced prior or parallel to leaf expansion. The loss of hydraulic conductance in winter may also be extensive in diffuse-porous trees, even though embolism usually increases more gradually than in ring-porous trees (Sperry and Sullivan 1992; Sperry et al. 1994).

In species of *Acer, Alnus* and *Betula* embolism reversal was associated with root pressure (Sperry et al. 1988; Sperry 1993; Sperry et al. 1994). Trees showing little or no root pressure did not recover from winter embolism (Sperry 1993). A positive (above atmospheric) xylem pressure does not occur commonly in all woody species (Scholander et al. 1955; Kramer and Kozlowski 1960). If positive pressure was a prerequisite for rapid bubble dissolution, then non-bleeding species should not be able to maintain maximum hydraulic conductance in older growth rings, and tall trees should not be able to reverse embolism in upper parts of the crown where xylem pressure is always sub-atmospheric based on a 10-kPa/m pressure drop with height.

In this study we quantified the loss of hydraulic conductance in several diffuse-porous trees and one ring-porous species in winter and monitored spring recovery. Trees were growing under relatively mild winter conditions in northern Germany. Our intention was to determine if dissolution of freezing-induced air bubbles is restricted to trees producing positive xylem pressures in late winter and spring. We also simulated a constant root pressure to study if and how fast maximum hydraulic conductance could be reestablished in excised branches

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collected in winter. Another objective here was to test if embolism reversal in microporous vessels is easier to achieve than in megaporous (>100 μm diameter) conduits.

Materials and methods

Trees and study site

Experiments were conducted on *Acer pseudoplatanus* L., *Betula pendula* Roth, *Fagus sylvatica* L., *Fraxinus excelsior* L., *Populus balsamifera* L., and *Populus x canadensis* Moench "robusta". Trees were 4–10 m tall, and were growing in the Botanical Garden of the University Kiel in northern Germany. Measurements were carried out from April 1994 to June 1995. Weather data were recorded in the Botanical Garden.

Monitoring of seasonal occurrence of embolism

Embolism was quantified following the method described in detail by Sperry (1993). The method was slightly modified (Hacke and Sauter 1995). In the morning, 3- to 5-year-old branches, 1–2 m long and 1.0–1.9 cm in basal diameter, were collected, brought to the laboratory and recut under water. Branches of *Acer* and *Betula* were cut from the same trees that were chosen to measure positive xylem pressure (see below). Six segments about 0.15 m long were cut from a branch under water, shaved at both ends with a razor blade, and were then fitted to numbered plastic tubes at the basal end. Segments were located >0.4 m from the original cut end of the branch to avoid including vessels embolized during collection. The segments were perfused with filtered (0.2 μm) deionized water, and hydraulic conductivity (k_h) as defined by Tyree and Ewers (1991) was measured, using a pipette and stopwatch. Gas bubbles in the water were removed by agitating it vigorously with an electric shaker under vacuum. After the initial k_h had been determined, six segments were flushed at once at a pressure of 0.1 MPa using a pressure pump. Segments were repeatedly flushed for 5–10 min followed by a brief vacuum perfusion in the opposite direction of flow until hydraulic flow could not be elevated further. Maximum conductivity was usually achieved after one or two flushes. The initial k_h was then expressed as a percentage of the maximum.

Special care has to be taken when the initial k_h is measured in ring-porous species (Cochard and Tyree 1990), because air bubbles might be flushed out of "open" megaporous vessels, giving erroneous conductivity readings. Therefore, the maximum pressure was only 3 kPa (0.3 m water head) when conductivity was measured in *Fraxinus*. Preliminary dye perfusions had shown that functional megaporous vessels in *Fraxinus* are restricted to the most recent growth ring. In order to ensure that earlywood vessels in older growth rings had not been artificially refilled during the first k_h measurement, we used 0.1% (w/v) aqueous safranin (which was degassed and filtered through a 0.2-μm membrane filter) as the measuring solution in *Fraxinus*. After the initial k_h and been determined, the efflux end of each branch segment was recut and immediately viewed using a binocular microscope. In a few instances there were stained earlywood vessels in older growth rings, and the initial k_h of such a segment was extraordinarily high. These k_h values were regarded as artefacts and were not included.

Staining

Branch segments were stained with 0.1% (w/v) aqueous safranin under a pressure of 3 kPa to identify functional vessels.

Simulation of root pressure

Long (1.5–2 m) branches of *Populus x canadensis* and *Fraxinus* were sampled in March, recut under water, and connected to a 2-m water column to simulate a constant root pressure of 20 kPa. Branches were 3–5 years old. The water that was fed into the branch base had been degassed, filtered (0.2 μm), and acidified with citric acid (pH=3) to reduce microbial growth. After various periods of pressurization (0–72 h), segments were cut from the shoots under water, and conductivity was measured as described above.

Prior to pressurization, several branches of *Populus x canadensis* had been dehydrated in the laboratory for 10 days in order to induce additional embolism. During this period leaf flush had been triggered by warm temperatures in the laboratory. In an attempt to study embolism reversal in the absence of positive pressure, the basal ends of branches which had been dehydrated for 6 days were placed in a water-filled bucket. Branches had been recut under water several times in order to remove vessels embolized during dehydration. After various periods of rehydration, during which leaves had begun to unfold, conductivity was measured as described above.

Field measurements of positive xylem pressure

Bubble manometers were constructed from 1-ml glass pipettes sealed at one end. These were attached to freshly cut branches with a short piece of rigid tubing which was filled with water at the beginning of the experiment. The xylem pressure of bleeding trees compressed the bubble. Xylem pressure was calculated from the volume change of the bubble. Temperature was assumed to be constant during the measurements. Beginning in mid-February 1995, the occurrence of positive xylem pressure was monitored at least once a week. Measurements were conducted between 11:00 h and 15:00 h. We also investigated the diurnal pattern of xylem pressure in *Acer* and *Betula*. Most of the measurements were made at 1 m above ground level.

Results

In October 1994, there was an abrupt loss of hydraulic conductivity in *Populus balsamifera* and *Fraxinus*, after night temperatures had occasionally fallen below 0° C while trees still carried some leaves (Fig. 1a). In contrast to the spring period of 1994 when *Populus balsamifera* showed a complete and rapid embolism reversal, recovery was slow and remained incomplete in 1995. Similarly *Fagus* reached only 85–95% of maximum conductivity in spring. Conductivity in *Populus* and *Fagus* dropped after bud break, probably because bubbles that had not yet dissolved expanded as xylem tensions increased. During the monitoring period in 1995 we failed to detect positive xylem pressures in either species. *Fraxinus* had a conductivity of 1–2% during winter (Fig. 1a). Dye perfusions made in winter showed that only narrow latewood vessels had remained functional. In 1994 and 1995, there was a sharp increase in conductivity parallel to leaf expansion and to the production of new earlywood vessels. Branch segments of *Fraxinus* were never at 100% k_h, which is due to the method of determining the maximum k_h: many embolized vessels in older growth rings were not blocked by tyloses and therefore contributed to the k_h after the flushing procedure.

Conductivity recovery in *Acer* and *Betula* was associated with positive xylem pressures that frequently occurred in March and April (Fig. 1c). Pressures measured 1 m above the ground in a 10-m-tall *Betula* often ranged
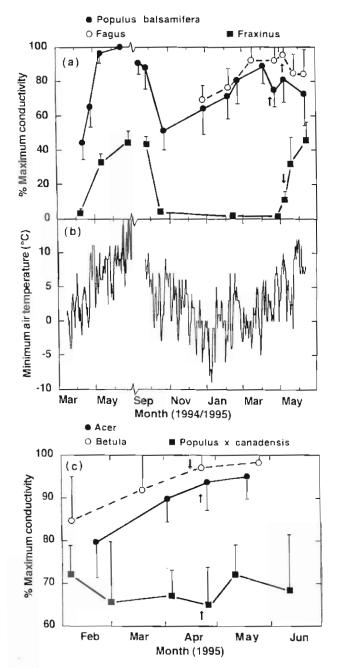
Fig. 1 a Seasonal change in the percentage maximum hydraulic conductivity. Means of $n=6$ branch segments and standard deviations are given. *Arrows* denote time of bud break. Data points recorded in September and October for *Populus balsamifera* are from Hacke and Sauter (1995). b Minimum daily air temperature at the study site. c Seasonal change in percentage maximum hydraulic conductivity. Data points are means of $n=6$ segments with standard deviations. *Arrows* denote time of bud break

Fig. 2 a Diurnal time course of positive xylem pressure after a night frost ($-2°$ C, 29 March). *Numbers* refer to height above ground level at which pressure was measured. b Temperature on the sun facing stem surface of *Acer* ($\square$), air temperature ($\blacktriangle$), and soil (5 cm) temperature ($\triangle$)

from 50 to 100 kPa, while pressure in a 4-m-tall *Acer* usually did not exceed 40 kPa (data not shown). On a bright sunny day following a frosty night however, *Acer* exhibited a much higher pressure than *Betula*. A peak pressure of 154 kPa occurred at about 11:00 h while the stem was warmed by the morning sun (Fig. 2). After that, the stem was shaded by other trees, and pressure
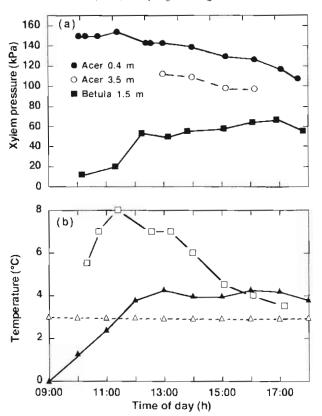
decreased. In contrast, pressure in *Betula* increased steadily until late afternoon. Excised *Acer* branches continued bleeding on that day, indicating that pressure originated in the stem and branches, and not in the roots as in *Betula*. As expected, pressure in *Acer* dropped by approximately 10 kPa/m with height (Fig. 2a, compare closed and open circles). Bleeding stopped by the time of bud swelling *(Acer)* and leaf flush *(Betula)*, respectively.

Populus x canadensis showed no conductivity recovery during the monitoring period (Fig. 1c). In contrast, when branches of *Populus x canadensis* were subjected to a simulated root pressure of 20 kPa, conductivity increased from 65 to 100% within approximately 2 days (Fig. 3a, closed circles). During a 2-day period an almost complete embolism reversal also occurred in branches that had been dehydrated to 10% k_h (Fig. 3a, open circles). In the absence of positive pressure there was no substantial refilling of embolized vessels within a rehydration period of 9 days (Fig. 3b).

When *Fraxinus* branches were subjected to the simulated root pressure, recovery was quick in the beginning, but remained incomplete after 80 h (Fig. 3a, closed squares). Staining indicated that many earlywood vessels of the outermost growth ring were refilled easily while refilling was limited in older growth rings.
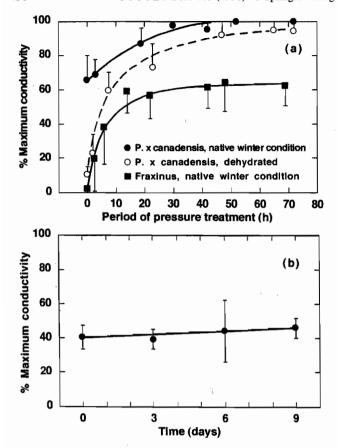
Fig. 3 a Hydraulic conductivity recovery in excised branches under a simulated root pressure of 20 kPa above atmospheric pressure. At the beginning of the experiment branches were in their native winter condition *(closed symbols)* or had been additionally dehydrated in the laboratory *(open symbols)*. Data points represent mean values of $n=4$–6 segments ±standard deviations. **b** Percentage maximum hydraulic conductivity of *Populus x canadensis* branches after various periods of rehydration. The bases of branches that had been dried in the laboratory were placed in water to study refilling of embolized vessels at approximately atmospheric xylem pressure. Means for $n=4$–6 segments are shown with standard deviations. The data were fitted by a linear regression ($y=0.69x+39.43$, $r^2=0.76$)

Discussion

In *Acer* and *Betula* embolism reversal was facilitated by positive xylem pressures that frequently occurred during a 2-month period prior to leaf expansion (Figs. 1, 2). Results shown in Fig. 3a demonstrate that refilling of embolized vessels can be achieved within a few days if xylem pressure is above atmospheric pressure. Species of *Acer* and *Betula* are well known for their ability to generate positive xylem pressures (Wiegand 1906). We found the magnitude of xylem pressure in *Acer* to be considerably variable on different days, indicating that pressure had not always exclusively originated in the roots. Highly increased pressures were measured on bright sunny days after frosty nights. These conditions are known to trigger stem pressure in maple (Wiegand 1906; Sauter 1974; O'Malley and Milburn 1983).

The inconsistent behaviour of *Populus* species (Fig. 1) is in agreement with observations of Sperry et al. (1994) who found some embolism reversal in *Populus* spp. one year but none the next. Dye perfusions made in mid-winter revealed that there were only a few non-stained (fully embolized) conduits in the outermost growth ring of the *Populus* species and *Fagus* while conductivity was still significantly reduced. This could be explained tentatively by the presence of many freezing-induced gas bubbles that had not expanded to fill the entire conduit lumen. Bubbles, located in the overlap area between two vessels, could limit hydraulic flow without completely inhibiting water (and dye) transfer between conduits. Bubble dissolution requires atmospheric or at most only slightly subatmospheric xylem pressures (Tyree and Yang 1992; Yang and Tyree 1992). Therefore, there is usually no embolism reversal in trees during the growing season (Sperry et al. 1988; Hacke and Sauter 1995). During winter and early spring however, when there is no water loss through transpiring leaves, water potentials may be high enough to allow a slow but steady embolism reversal. This is supported by the fact that the slow increase of conductivity in *Populus balsamifera* and *Fagus* started long before the beginning of cambial activity (Fig. 1a).

An anatomical feature that might contribute to embolism reversal in *Populus* is that vessels often contact with another, so there is a high probability for an embolized conduit to be located beside several water-filled vessels. This argument also holds for *Fagus*, in which vessels are embedded in a matrix of water-filled tracheids. In contrast, embolized vessels in *Fraxinus* are usually not in contact with other water-filled conduits.

The low hydraulic conductivity of *Fraxinus* branches in winter is in agreement with previous observations of ring-porous trees (e.g. Sperry and Sullivan 1992). Cochard and Tyree (1990) observed extensive embolism resulting from a relatively brief frost in *Quercus*. Megaporous vessels are extremely vulnerable to freezing-induced cavitation. In this regard there is a trade-off between efficiency and safety (Zimmermann 1983; Sperry and Sullivan 1992; Tyree et al. 1994). The recovery of hydraulic conductivity in *Fraxinus* resulted clearly from the production of new earlywood vessels in May. Staining showed that older megaporous vessels had not been refilled.

To our knowledge, refilling of vessels in intact branches has not been studied before, although there are numerous reports on recovery of hydraulic conductivity in isolated stem segments (Just and Sauter 1991; Edwards et al. 1994, and literature cited therein). A pressure of 20 kPa led to a sharp increase in k_h. We found no evidence for an expulsion of gas through "leaks" in *Populus* and *Fraxinus* branches which was observed in *Vitis* (Sperry et al. 1987). Thus, refilling was probably promoted by dissolution of gas into the vessel water and the diffusion of gas to the outside surface of the branch (Yang and Tyree 1992). Refilling remained incomplete in *Fraxinus* (Fig. 3a). This is probably due to tyloses that blocked water flow in basal parts of the branches, there-

by "conserving" air pockets in some vessels. The fact that the dye moved further in outer growth rings indicates that tyloses were more frequent in inner annual rings. Results shown in Fig. 3a suggest that many megaporous vessels in *Fraxinus* could be refilled in spring if there was a sufficient root pressure. Refilling of megaporous vessels via root pressure does occur in *Vitis* species (Scholander et al. 1955; Sperry et al. 1987), but ring-porous trees apparently do not take advantage of this possibility. Refilling might not have been a selection benefit in these trees, probably because hydraulic conductance is sufficiently restored by newly formed megaporous vessels in spring.

In conclusion, spring recovery of hydraulic conductivity was either associated with positive xylem pressure *(Acer, Betula)*, or was accomplished by the production of new earlywood vessels *(Fraxinus)*. *Populus balsamifera* did restore maximum conductivity in 1994, but, like in *Populus x canadensis* and *Fagus*, embolism reversal remained incomplete in 1995. The inconsistent behaviour of *Populus balsamifera* suggests that the amount of recovery from embolism in diffuse-porous trees not exhibiting positive xylem pressure is influenced by additional factors, i.e. the severity of water stress created during longer frost periods when water uptake is excluded due to frozen soil and by the occurrence of late frosts in spring.

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