

Winter xylem embolism and spring recovery
in *Betula cordifolia*, *Fagus grandifolia*,
Abies balsamea and *Picea rubens*

J. SPERRY

Department of Biology, University of Utah,
Salt Lake City, UT 84112, USA.

SUMMARY

Xylem embolism was monitored from mid-winter to mid-summer in four co-occurring species: *Betula cordifolia* (Reg.) Fern., *Fagus grandifolia* Ehrh., *Abies balsamea* (L.) Mill., *Picea rubens* Sarg. The study site was a west-facing slope in the northern Green Mountains of Vermont, U.S.A.; *Betula* and conifers were sampled at 914 m; *Fagus* was collected at 827 m near its local altitudinal limit. Embolism was quantified by the percent the hydraulic conductivity of branch segments was below the maximum obtained following removal of air embolism in xylem conduits. Between early February and early May, the deciduous species averaged 60 to 84% embolism compared to 15 to 60% for the conifers. From April 24 to May 25, embolism in *Betula* dropped from 81 to 8%; this recovery was associated with root pressures up to 86 kPa as measured with manometers at the lower trunk. *Betula* trees in which root pressure was eliminated by overlapping saw cuts still showed 75% embolism in June; only 4% was present in control trees cut in a similar fashion after leaf flush. Root pressure was weak (3 kPa) and uncommon in *Fagus*, and trees remained 80% embolized through June showing considerable dieback. *Fagus* at lower elevations (60 m) were 33% embolized in June with no dieback. Embolism in the conifer species decreased from 40% embolism in late April to 6% in late June despite no detected positive xylem pressures. The mechanism for conifer recovery is unknown.

INTRODUCTION

Xylem embolism occurs as a result of water stress and freezing of xylem sap and it has the immediate consequence of reducing the hydraulic conductivity of the xylem. Longer-term consequences could include reduced growth and dieback. Seasonal studies on deciduous temperate trees and vines have shown that during the winter up to 100% of the hydraulic conductivity can be eliminated by freezing-induced embolism; growing season values may be considerably lower (Sperry *et al.*, 1987; Sperry, Donnelly & Tyree, 1988; Cochard & Tyree, 1990). This suggests

xylem is more vulnerable to freezing than to water stress in these species. This may be a result of very different mechanisms for embolism formation by the two stresses.

Experimental evidence indicates embolism caused by water stress can be explained by entry of air through inter-conduit pit membranes (Fig. 1, lower arrows; Crombie, Hipkins & Milburn, 1985; Sperry & Tyree, 1988; Sperry & Tyree, 1990). Perhaps the most compelling observation is that the relationship between embolism and xylem tension in water-stressed stems is identical to that between embolism and air-injection pressure in hydrated stems. Furthermore, the embolism threshold for both air injection and water stress can be varied by treatments altering permeability of inter-conduit pit membranes to air-water menisci (Sperry & Tyree, 1990). If even a few air-filled xylem conduits are present (*e.g.*, because of leaf-abscission) the continuity of water conduction depends on capillary forces preventing passage of an air-water interface between conduits.

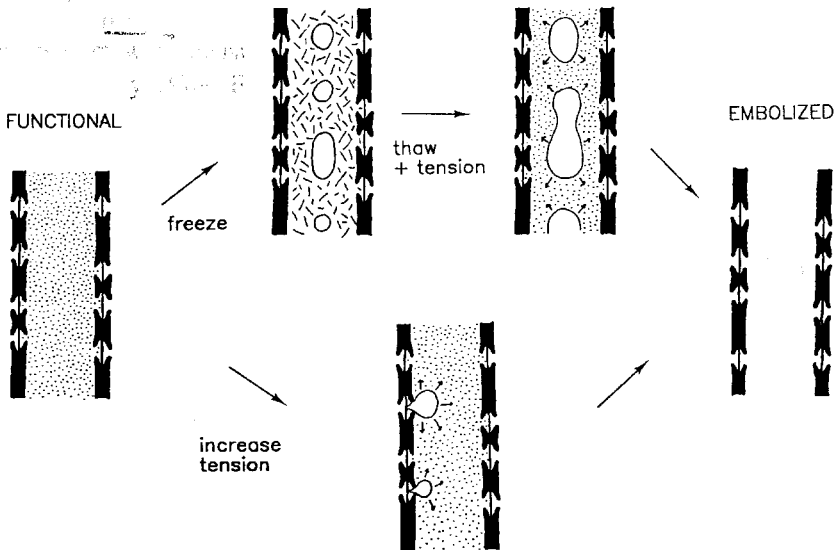


FIG. 1. Embolism formation by freezing (upper arrows) and by water stress (lower). Vulnerability to freezing may be a function of conduit volume; vulnerability to water stress depends on permeability of inter-conduit pit membranes to air-water interfaces.

Freezing-induced embolism can occur because of the low solubility of gases in ice (Fig. 1, upper arrows; Hammel, 1967). Gases dissolved in xylem sap freeze out to form bubbles that potentially nucleate cavitation if they are large enough and if xylem tensions are high enough (Oertli, 1971). Evidence suggests larger-volume conduits are more vulnerable than small ones; presumably because larger bubbles form in higher-volume conduits (Ewers, 1985; Cochard & Tyree, 1990). Conifer tracheids are apparently resistant, although the reason for this is unclear (Hammel, 1967; Sucoff, 1969; Robson, McHardy & Petty, 1988).

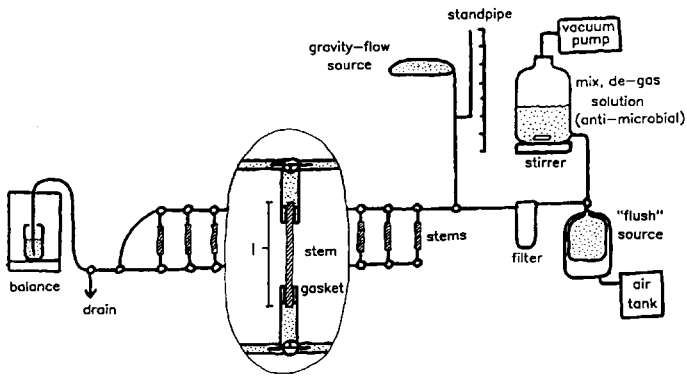


FIG. 2. Apparatus for measuring hydraulic conductivity and embolism. See text.

Given that deciduous temperate trees and vines are vulnerable to winter embolism, it is not surprising that re-filling of embolized vessels has been observed in at least two cases: *Acer saccharum* (Sperry *et al.*, 1988) and *Vitis* species (Scholander *et al.*, 1955; Sperry *et al.*, 1987). Refilling was attributed to root and stem pressures in *Acer* and to root pressure in *Vitis*. Circumstantial evidence suggests extensive embolism occurs daily in some herbs because of water stress (*Plantago major*, *Zea mays*) and that vessels are re-filled at night by root pressures (Milburn & McLaughlin, 1974; Tyree *et al.*, 1986). For these plants, the action of positive pressures in refilling embolized vessels may be critical for their performance and perhaps survival.

The purpose of the present study was to compare winter embolism levels and spring recovery in four co-occurring tree species of the northeastern United States: *Betula cordifolia*, *Fagus grandifolia*, *Picea rubens*, and *Abies balsamea*. The genus *Betula* is well known for its

ability to generate root pressure in spring and fall (Johnson, 1944), but it has not been demonstrated that these pressures act in re-filling of embolized vessels. In contrast, no report of root pressures in *Fagus grandifolia* was found in the literature. As mentioned, conifers are apparently resistant to freezing-induced embolism, and although they have the ability to generate root pressures under experimental conditions (O' Leary & Kramer, 1965; Lopushinsky, 1980), there seem to be no reports of positive pressures occurring in mature trees in the field.

MATERIALS AND METHODS

The study site was the west side of Camels Hump mountain in northern Vermont. The *Betula* and conifer site was ca. 914 m, the *Fagus* site was ca. 823 m near the foot of the conifer zone, and near the altitudinal limit for this species in northern Vermont. Weather data were collected by a station at the 914 m site operated by Timothy Perkins of the University of Vermont.

Measurement of embolism

Embolism was measured every one to five weeks from early February 1989 to July 1989. For each species, 10 branches from the crown were collected with a pole pruner from the same group of mature trees and brought back to the lab in plastic bags. In the laboratory, one segment 10 to 20 cm long and 0.3 to 1.5 cm in diameter was cut from each of these branches giving ten segments per species. Cuts were made underwater and segments were far enough from the original cut end of the branch to avoid including vessels or tracheids embolized by the initial cut. Segments were fitted with rubber gaskets cut from latex tubing, trimmed with a sharp razor blade, and fitted to a tubing apparatus designed to measure the hydraulic conductivity (Fig. 2).

Hydraulic conductivity was defined as mass flow rate (kg s^{-1}) of measuring solution through a segment divided by the pressure gradient (MPa m^{-1}). The solution used was 10 mol m^{-3} oxalic acid in deionized water; the low pH (ca. 1.8) of this solution inhibited microbial growth. Solution was filtered (220 nm) immediately before use (see Fig. 2). Mass flow rate was measured by routing efflux from a stem segment to a reservoir on a balance; solution flowed into the stem from a source reservoir under a pressure gradient determined by the difference in height between the source and balance reservoirs, and length of stem (Fig. 2).

Typical pressure differences across stems were about 7 kPa. Ten stems at a time were mounted in parallel on the apparatus so conductivity measurements could be made sequentially on each one by directing the flow with stopcocks. After the initial conductivity measurement was completed on all ten segments, flow was routed through all stems at once under a higher pressure of about 100 kPa from a pressurized tank of solution. The purpose of this "flush" was to promote dissolving of air in embolized vessels. Following a flush of 1 to 2 hrs, conductivity of each stem segment was measured again, and the process repeated until conductivity reached a stable maximum value. The percentage by which the initial conductivity was below the maximum gave the "percentage loss in hydraulic conductivity", or a measure of the reversible embolism in each stem segment. This method has been described in detail in Sperry, Donnelly, & Tyree (1987).

Initial measurements on conifers showed declines in conductivity following the flush. This was prevented by de-gassing the solution by agitating it (with magnetic stirrer; Fig. 2) for one hour under vacuum. A better method used in subsequent studies is to draw water into an evacuated container through a jet (e.g., a pipette; Robson pers. commun.). Previous work on conifers (Sperry & Tyree, 1990) has shown that complete recovery of maximum conductivity may be prevented by persistent aspiration of pit membranes. However, this was observed when embolism had been induced by tensions over 7 MPa. These are much higher than typical biological values (less than 2 MPa) for these species. Regardless of whether the torus remained aspirated or not, the maximum conductivities determined for conifers from their native condition would represent the maximum extent of recovery if it occurred.

Stem segments were perfused with 0.05% (w/v) safranin dye to see which xylem conduits were functional. Filtered (220 nm) dye was siphoned through stems for about 30 minutes with 10 kPa sub-ambient pressure.

For *Fagus*, the percentage loss of hydraulic conductivity and the cross-sectional area of the conductivity segment were related to the dry weight of leaves distal to the segment. "Huber values" (Ewers, 1985) were computed as the cross-sectional areas of the stem (estimated from stem diameters) divided by the weight of oven-dried leaves.

Measurement of positive xylem pressures

Manometers were constructed from glass tubes (inner diam. 1 mm) sealed at one end. Tubes were partially vacuum-infiltrated with a weak dye

solution, and connected with a minimum of dye-filled tubing to a freshly severed lateral branch near the base of a tree exhibiting positive pressure. Pressure caused by attachment was released by venting the manometer to atmosphere. With the vent closed, the length of the air bubble in the glass tube was measured when it stabilized. The vent was re-opened, and the length of the bubble at atmospheric pressure was recorded. The ratio of bubble lengths gave the root pressure relative to atmospheric assuming bubble length was proportional to volume, and temperature was constant. Results were expressed in kPa above atmospheric (ca. 91 kPa at 914 m).

Before root pressure was observed in spring, overlapping saw-cuts were made on five trees of both *Fagus* and *Betula* in order to minimize root pressures above the cuts. Cuts went halfway into the bole at approximately chest height and were made from opposite sides of the tree, one approximately 0.3 m above the other. A second set of trees for each species was cut in the same way after the leaves had flushed out. Embolism recovery in cut trees was compared to non-cut ones to evaluate the role of root pressure in embolism recovery. The effect of overlapping cuts on root pressure was evaluated with manometers.

RESULTS

Deciduous species

Betula and *Fagus* averaged between 65 and 85% embolism from late January to early May (Fig. 3a). Within each species, embolism did not correlate with segment diameter (0.2 to 1.2 cm), or height above ground (1 to 10 m; data not shown). Fig. 4 shows typical distributions of embolized vessels in transverse sections of branches. In *Betula*, embolism was concentrated in the outer-most xylem of the branch, and was often distributed around the entire circumference. Branches in this species tended to be vertical. In *Fagus*, embolism was also in the outermost xylem, but generally more on the upper side of the branches, most of which were horizontal.

Betula showed an abrupt decrease from 74% embolism on May 1 to 8% on May 25 (Fig. 3a); during this time root pressures were observed and measured. On May 1 no root pressure was seen at the 914 m site, although it was observed at 457 m. By May 8, *Betula* at the site averaged 62% embolism and all trees were showing root pressure. Pressures measured in four trees at noon had maximum values between 15 kPa for a 3 m sapling and 52 kPa on a 7 m tree. The onset of root pressure

corresponded with the first above-freezing soil temperatures at 5 cm depth (Fig. 3b). On May 12, *Betula* averaged 35% embolism, and root pressure was still present. Maximum pressures in four trees ranged from 42 to 86 kPa. On May 25, *Betula* was only 8% embolized, buds had broken, and leaves were expanding; root pressure was absent. On June 19, *Betula* was 4% embolized and showing no root pressure.

Betula trees treated with overlapping saw cuts prior to May 1 did not recover from embolism (Fig. 3a, cut), nor did sap flow from wounds made above the cuts. The experiment shown in Fig. 5 demonstrated the effectiveness of overlapping cuts in eliminating root pressure.

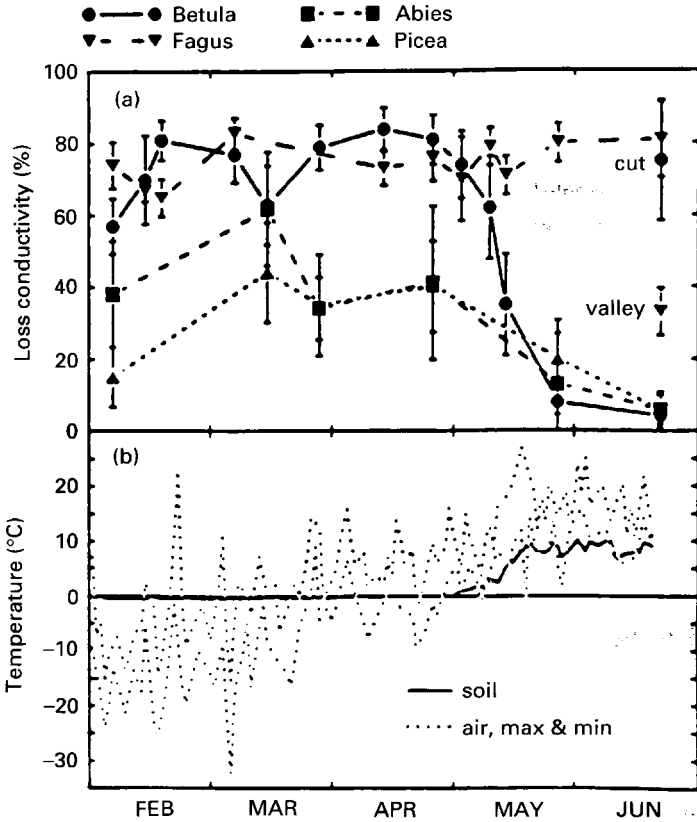


FIG. 3. (a) Embolism (percentage loss hydraulic conductivity) vs. month; means with 95% confidence limits. "Cut": *Betula* with overlapping saw cuts made before May 1. "Valley": *Fagus* from 60 m site; (b) Air and soil (5 cm) temperatures.

Trees given the overlapping cut after leaf flush showed the same embolism levels as non-cut controls.

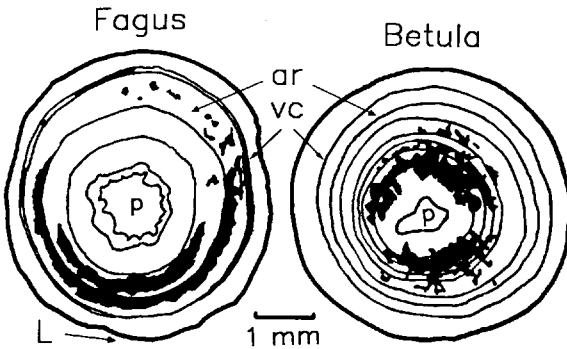


FIG. 4. Transverse sections with dye-stained, functional xylem in black; ar, annual ring; vc, vascular cambium, p, pith. *Fagus*, from June 19 collection; narrow outer ring is current year growth; L, lower side of horizontal branch. *Betula* from a December 12 collection; vertical branch.

While *Betula* was exhibiting consistent root pressure and embolism recovery during May, *Fagus* was showing neither. Embolism remained between 70 and 80%, and root pressures were only occasionally observed. These were weak, measuring up to 3 kPa. Trees with overlapping cuts prior to May 1 had the same embolism levels as non-treated controls and trees given cuts after leafing out (data not shown).

At the June 19 measurement, dieback was observed in *Fagus* at the 823 m site. Leaves were curled and dried on many of the branches of both small and large trees.

This was probably not frost damage because there were no subfreezing temperatures after leafing out in late May (Fig. 3b), and other species in the same locale were healthy. In addition, there was no obvious evidence of a pathogen. Production of new xylem had a minimal impact on conductivity; new xylem was sparse in mid-June (Fig. 4) and was still minimal as late as July 27 when the trees were still 74% embolised (data not shown). *Fagus* in a low elevation forest (60 m) on June 26 was 33% embolized and no dieback was observed (Fig. 3b, valley).

Fagus branches at the site bearing no dead foliage had significantly higher (*t* test, $p=0.05$) Huber values ($1.10 \times 10^{-2} \text{ m}^2 \text{ kg}^{-1}$, $n=9$) than the low-elevation trees (0.38×10^{-2} , $n=9$) in July. This meant that for a given stem diameter, the low-elevation *Fagus* supported more foliage than the

one at the study site. Within the study site there was no correlation between the amount of embolism in a branch and the weight percentage of dead foliage. Branches that bore only dead foliage were either 100% embolized, or their xylem was plugged so the branches had no conductivity either before or after the flushing treatment. The vessels in these stems were occluded with brownish material, and were air-filled. The converse was not necessarily true: at least one branch was 100% embolized and yet showed no dead foliage at all.

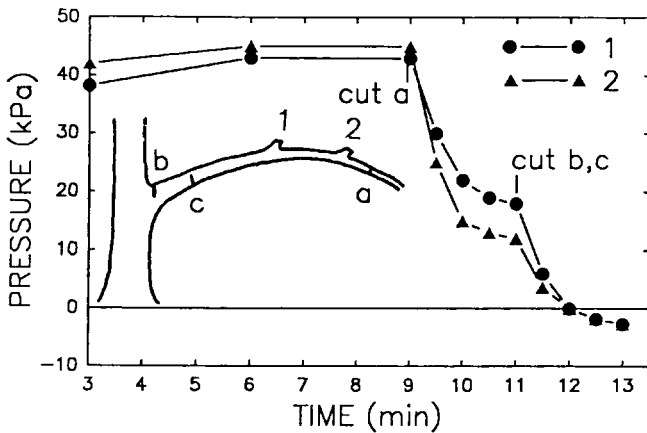


FIG. 5. Xylem pressure (kPa above atmospheric) in *Betula* measured in manometers at branch stubs 1 and 2 (inset). Cutting at a dropped pressures, especially in manometer 2 near the cut. Overlapping cuts at b and c eliminated positive pressures; they became slightly sub-ambient because the cuts were below the manometers.

Conifer species

During the winter, the two conifer species showed considerably less embolism than the deciduous species (Fig. 3a). With the exception of a peak in early March (*Abies*, 60%; *Picea*, 42%) values did not exceed 40%. The March peak corresponded with an exceptionally cold period just preceding collection (Fig. 3b). Between the April and June measurements, there was an almost complete recovery from embolism; in both species it declined from 40% to 6%. This recovery preceded the initiation of new xylem in late May and was not associated with any

detectable positive xylem pressures.

DISCUSSION

Significant levels of embolism were present in all species during the winter months. The highest levels were observed in the deciduous species, *Fagus grandifolia* and *Betula cordifolia* (Fig. 3a). The distribution of embolized vessels near the periphery in these species (Fig. 4) is similar to a previous report for *Acer saccharum* where most winter embolism was found in the southern sides of vertical stems (Sperry *et al.*, 1988). This suggests warming by the sun was important in their formation, perhaps via the freeze-thaw mechanism (Fig. 1). In addition, sublimation and water-stress could contribute. Preliminary experiments with *Betula* suggested sublimation was probably a minor cause of embolism when bark was intact (Sperry, unpublished); water stress in deciduous trees in winter may not create xylem tensions high enough to cause significant embolism in the absence of a freeze-thaw event.

Although embolism in the conifer species was less than in the deciduous trees, it was present to a significant degree and increased during the winter (Fig. 3a). If conifers are resistant to embolism caused by freezing and thawing as the literature suggests (Hammel, 1967; Sucoff, 1969; Robson *et al.*, 1988), the observed values may have been a consequence of water stress caused by foliar transpiration and limited uptake from cold and frozen soils. The greater levels exhibited by *Abies balsamea* in February and March (Fig. 3a) could be a reflection of its greater vulnerability to embolism by water stress compared to *Picea rubens* (Sperry & Tyree, 1990). More work is needed to evaluate the mechanism(s) of winter embolism in temperate deciduous trees and conifers.

Recovery of full hydraulic conductivity in *Betula* was associated with root pressures. Not only did root pressure correlate with the abrupt decrease of embolism between May 1 and May 25, but trees in which it was prevented from reaching the crown by overlapping saw cuts showed no recovery from winter levels (Fig. 3a, cut). In addition, *Fagus* showed little or no root pressure and also showed a complete lack of recovery (Fig. 3a). The implication is that root pressure caused recovery by elevating xylem pressures to near or above atmospheric pressure allowing air bubbles in the vessels to dissolve. Although pressures were measured at the bases of trees, in many cases these would have been sufficient to bring crown pressures to positive levels based on the hydrostatic gradient of 10 kPa per meter.

The pattern of recovery in *Betula* resembles that documented for *Acer saccharum* (Sperry *et al.*, 1988) and *Vitis riparia* (Sperry, *et al.*, 1987); both of which showed rather abrupt recovery from winter embolism that was associated with positive xylem pressure. These studies confirm the importance of these pressures for embolism recovery. Positive pressure mechanisms may be critical to the continued success of these species if their growth is sensitive to reduced xylem conductivity.

The fact that *Fagus* did not recover from winter embolism raises the question of whether this influenced its productivity during the growing season. High embolism levels at the high elevation (823 m) site correlated with dieback and high Huber values, and low embolism levels in the valley (60 m) correlated with no dieback and low Huber values. Perhaps embolism influences the elevational range of *Fagus*. However, a much more thorough study would be required to evaluate any cause-and-effect relationship between embolism and dieback, and assess possible pathogen effects. Presumably the trees in the valley exhibited less embolism than higher-elevation ones (Fig. 3a, valley) because of less harsh winter conditions. However, the magnitude of root pressure and consequent recovery may also be a function of habitat in *Fagus*. If Fagaceae in general lack a refilling mechanism, the advantage of the ring-porous condition found in the family (*Castanea*, *Quercus*) is obvious: recovery occurs by production of relatively large diameter earlywood vessels that supply the emerging foliage (Cochard and Tyree, 1990). There is apparently no restoration of conductivity to previously embolized vessels.

In contrast to the deciduous species, recovery in conifers occurred without detectable positive pressures. Refilling in conifers has been demonstrated before (Waring & Running, 1978), and current monitoring in the Wasatch mountains of Utah is showing similar patterns to that seen in Fig. 3a (Sperry, unpublished). The mechanism of this recovery is unknown. Perhaps it occurs at slightly sub-ambient xylem pressures where the gas in embolized tracheids is under positive pressure due to surface tension. Although the mechanism is unclear, it may be operable only in small-volume conduits such as tracheids, because recovery was not observed in *Fagus* in the absence of positive pressures.

The significance of embolism formation and reversal in woody plants depends on how it influences growth. How redundant is the xylem? Models suggest trees have little redundancy when overall hydraulic architecture is considered (Tyree & Sperry, 1988), but this has not been experimentally demonstrated. It is possible that even fairly extensive embolism would not cause rapid decline in health. Interestingly, the *Betula* that were treated with overlapping saw cuts and did not recover

from embolism (Fig. 3a, cut) showed no visual symptoms of decline after one month of being on average 75% embolized. Similarly one *Fagus* branch was found that was 100% embolized and yet supported living foliage. The initial effect of excessive embolism may be a reduction in stomatal conductance followed at some point by leaf senescence and subsequent branch death. The effect of hydraulic conductivity on leaf physiology needs to be quantified before we can evaluate the adaptive advantage of embolism avoidance and recovery mechanisms.

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