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CAVITATION FATIGUE — THE WEAKENING OF CAVITATION RESISTANCE OF XYLEM AND ITS REVERSIBILITY

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INTRODUCTION : THE PHENOMENON OF CAVITATION FATIGUE

Xylem function is essential for the growth and survival of higher land plants. Xylem must not only be efficient under favorable conditions to facilitate high rates of stomatal conductance and carbon uptake, but it should also remain functional under drought conditions, when water potential (Ψ) drops to low values. Since water in the xylem is lifted up to the leaves by negative pressure (Steudle, 2001; Zimmermann, 1983), it is intrinsically vulnerable to cavitation. This phenomenon is observed in many, if not all plants, and it is induced by soil drought,

high evaporative demand, or winter frost Sperry, 1995. According to the air-seeding hypothesis Zimmermann, 1983, drought-induced cavitation occurs when air enters a functional conduit through pores in the cell wall. There is good evidence that the air enters through pores in pit membranes separating the functional conduit from one that is already embolized or air filled (Jarbeau *et al.*, 1995; Sperry *et al.*, 1996). Resistance to drought-induced cavitation is thus thought to be closely related to the properties of pit membranes (Hacke and Sperry, 2001).

Some plants refill embolized xylem conduits after a drought, either by generating positive xylem pressure (Cochard *et al.*, 1994; Hacke and Sauter, 1996; Sperry, 1993; Sperry *et al.*, 1988) or by means that are yet unknown Salleo *et al.*, 1996. It is currently debated under what conditions xylem refilling can occur (Tyree *et al.*, 1999; Zwieniecki and Holbrook, 1998). In order to benefit from refilling, xylem should not become more susceptible as a result of previous embolism-refilling cycles. However, air-seeding involves a rapid pressure change in the cavitating conduit. The operating pressure in a functional conduit may be as low as -3 MPa (and even substantially lower in some xeric plants),

but it will rise instantly to near 0 MPa when cavitation is triggered. Moreover, once a conduit is embolized and uncoupled from the transpiration stream, pit membranes must seal the conduit for potentially long periods of time to prevent the spreading of the embolus. Pit membranes may thus be regarded as valves that are subjected to severe mechanical challenges and high pressure differences. It seems possible that their microstructure and mechanical properties become altered by cavitation-refilling cycles, thereby leaving the xylem more susceptible to future cavitation events. This issue is of basic importance for understanding how plants cope with repeated drought cycles.

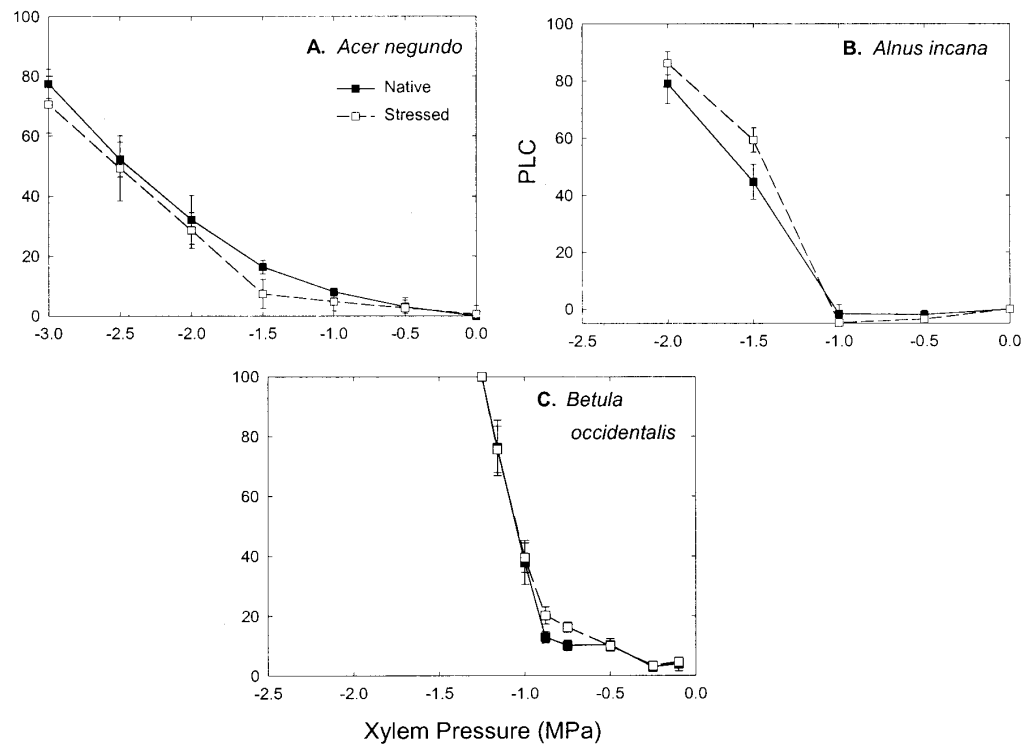


Fig. 1. Native (solid squares) vs. stressed (open squares) vulnerability curves showing the resilient response of stem xylem in *Acer negundo*, *Alnus incana* and *Betula occidentalis*. Curves show the per cent loss of xylem conductance (PLC) with decreasing xylem pressure. Native and stressed curves were similar (t test, $P > 0.05$). From Hacke *et al.* (2001).

Hacke *et al.* (2001) were the first to systematically determine how the process of cavitation alters the cavitation resistance. They identified several “resilient” species, whose cavitation resistance before and after a cavitation-refilling cycle was not different (Fig. 1). Cavitation resistance was expressed with vulnerability curves, showing the percent loss of hydraulic conductance (PLC) of stem segments as a function of xylem pressure. Generally, as the xylem pressure gets more negative, higher values of PLC are recorded. A large data set of these curves has been accumulated over the last

2 decades. It has become clear that cavitation resistance varies tremendously among species and plant organs, and that this variation is correlated with the xylem pressure that is associated with the typical habitat of a species (Davis *et al.*, 1999; Pockman and Sperry, 2000; Sperry, 1995; Sperry and Hacke, 2002). Not surprisingly, *Betula occidentalis*, a riparian tree growing along rivers and creeks, showed the most susceptible xylem of all the species shown in Fig. 1. What matters most for this review, however, is the fact that the native curve was identical to the stressed curve, which was measured after

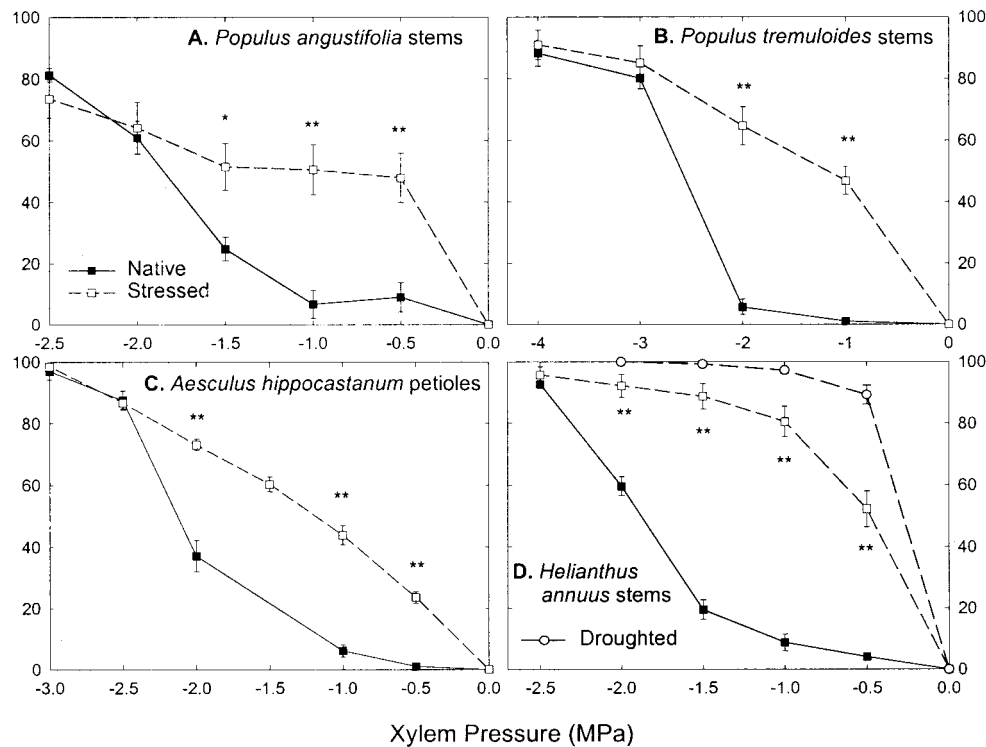


Fig. 2. Native (solid squares) vs. stressed (open squares) vulnerability curves showing the weakened response seen in xylem of *Populus angustifolia*, *P. tremuloides*, *Aesculus hippocastanum*, and *Helianthus annuus*. All data is for stem xylem except for petioles in *A. hippocastanum*. Means and SE, $n=6$. Stressed xylem was significantly more vulnerable to embolism than the non-stressed native xylem (t test; * = $P<0.05$; ** = $P<0.01$). Stems of *H. annuus* that were stressed in droughted plants to a native PLC >95% also exhibited a weakening response (D, compare open circles for droughted stems with open squares for centrifuged stems). From Hacke *et al.* (2001).

stems had experienced 80 PLC and had subsequently been refilled with deionized water in the laboratory. This resilient response was also observed in *Alnus incana* and *Acer negundo*. In contrast, in a number of “weakened” species (*Populus angustifolia*, *Populus tremuloides*, *Aesculus hippocastanum*, *Helianthus annuus*), the second (stressed) curve was substantially more vulnerable to cavitation (Fig. 2). For example, in *H. annuus*, PLC at -1 MPa was only 9% in the native curve versus 81% in the stressed curve. The shape of the weakened curve was also different from the native one: while native vulnerability curves were usually sigmoidal with a more or less defined embolism threshold (e.g. Figs. 1 B,C), weakened curves showed an exponential increase in PLC and high levels of embolism even at mild pressures.

The weakening response occurred independently of how embolism was induced. There was a similar weakening response regardless whether embolism was induced by centrifugal force, air dehydration, or soil drought in intact plants. Moreover, observations on *H. annuus* showed that the amount of weakening was proportional to the embolism rate induced by stress. In other words, there was a strong relationship between native PLC and the extent of fatigue.

2. WHAT CAUSES CAVITATION FATIGUE?

Hacke *et al.* (2001) tested two hypotheses proposed to account for the weakening response. After flushing a stressed and embolized segment with water in the laboratory, small air bubbles could be left behind. These bubbles could then prematurely trigger cavitation the next time xylem pressures drop. However,

this hypothesis was ruled out by a series of experiments. First, stems were submerged in water and pressurized overnight to promote bubble dissolution, and the PLC was then measured the next day. There was no influence on the weakening response. Second, introducing air into the xylem at very mild pressure (avoiding water-stress induced cavitation) did not cause a fatigue effect. The second hypothesis was the air-seeding hypothesis: xylem conduits become leakier to air as a result of the stress episode, thereby lowering the air-seeding threshold. This was tested by injecting air into the xylem by using a double-ended pressure chamber Sperry and Saliendra, 1994. Usually the PLC caused by air pressure is the same as that caused by xylem pressure of equal, but opposite, magnitude. Stressed stems of weakened species showed indeed a greater PLC by air injection than native stems, supporting the hypothesis that the fatigue is associated with an increased susceptibility to air seeding.

If we assume that air seeding occurs at pit membranes, the process causing cavitation fatigue will involve the organization of microfibrils and mechanical properties of pit membranes. A possible scenario for the effect of embolism-refilling cycles on pit membrane properties of angiosperms is shown in Fig. 3. According to this hypothesis, the microfibril meshwork becomes stretched upon air seeding. This loosening of the microfibril network could result from the pressure difference between an embolized and functional vessel or it could be associated with the rapid pressure increase in a cavitating vessel (potentially several MPa within a fraction of a second). The breaking of hydrogen and ionic bonds between microfibrils in the pit membrane could allow the membrane to stretch more, resulting in wider pores that will admit air more easily (Fig. 3C). Alternatively, a

rupturing of pit membranes could cause the weakening response. In that case, it would be irreversible. However, as we shall see in the next section, current evidence suggests that the fatigue effect is reversible under certain conditions (Fig. 3D), indicating that it is not caused by the tearing of pit membranes, but rather by a reversible slippage of microfibrils.

3. CAVITATION FATIGUE IS REVERSIBLE IN SUNFLOWER

If cavitation-fatigue is caused by a loosening of the microfibril meshwork of pit membranes, could it be reversed? And if so, what are the conditions necessary for this reversal? This question is of considerable significance. Sunflower for instance frequently develops root pressure given there is sufficient soil moisture. This root pressure will refill embolized vessels

quickly (Stiller and Sperry, 2002). However, when we look at the stressed vulnerability curve of sunflower (Fig. 2D), it becomes obvious that the vast majority of vessels would re-cavitate even at mild pressures if the fatigue were permanent. Sunflower and other species that invest metabolic energy in the refilling of embolized xylem would thus hardly benefit from this effort.

Stiller and Sperry (2002) showed that the cavitation fatigue increased with increasing days of drought. In Fig. 4, cavitation fatigue is expressed as PLC at a xylem pressure of -1 MPa. Interestingly, the fatigue *decreased* after intact potted plants had been rewatered. The reversal of fatigue shown in Fig. 4 required the *in vivo* refilling of embolized vessels, i.e. vessels were refilled with xylem sap, not with deionized water as in the initial study by Hacke *et al.* (2001). Apparently,

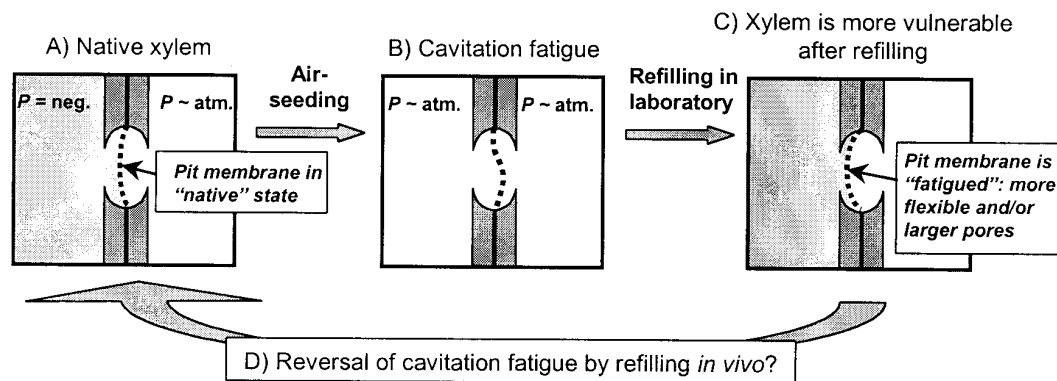


Fig. 3. Illustration of hypothetical changes in pit membrane structure which may underlie the process of cavitation fatigue. Pressure differences associated with air seeding (A,B) may loosen the microfibril network of pit membranes, making them more flexible with greater pores in the stretched state (C). This could result in greater susceptibility to air-seeding after an initial embolism-refilling cycle. Refilling of embolized xylem by root pressure *in vivo* partially reversed the fatigue in sunflower (D).

components of the xylem sap promoted the reversal of fatigue. Long-term perfusion with deionized water had no effect on the fatigue (Fig. 5, water). In contrast, perfusion with root exudate caused a significant reversal of cavitation fatigue. Exudate from droughted plants was particularly effective (Fig. 5, DR exudate). CaCl_2 and KCl solutions also promoted a decline in fatigue. A chemical analysis of the root exudate showed no difference in the total cation concentration between droughted and well-watered xylem saps. In both saps, potassium was the most abundant cation. However, xylem sap of droughted plants had a higher pH (8.2) than the sap of well-watered plants (6.6). There is also evidence that higher temperatures promote the reversal of cavitation fatigue, with a temperature of

35°C showing the greatest effect in *Aesculus hippocastanum* petioles (V. Stiller and J. Sperry, unpubl.).

4. WHAT IS THE MECHANISM FOR RECOVERY FROM CAVITATION FATIGUE?

The results of Stiller and Sperry (2002) indicate that in sunflower, at least, cavitation fatigue is not caused by an irreparable rupture of pit membranes. It seems unlikely that torn pit membranes could be repaired in the absence of a protoplast. We believe that the complex system of hydrogen and ionic bonds that link microfibrils together is altered during the air seeding process. The partial breaking of these bonds is probably caused

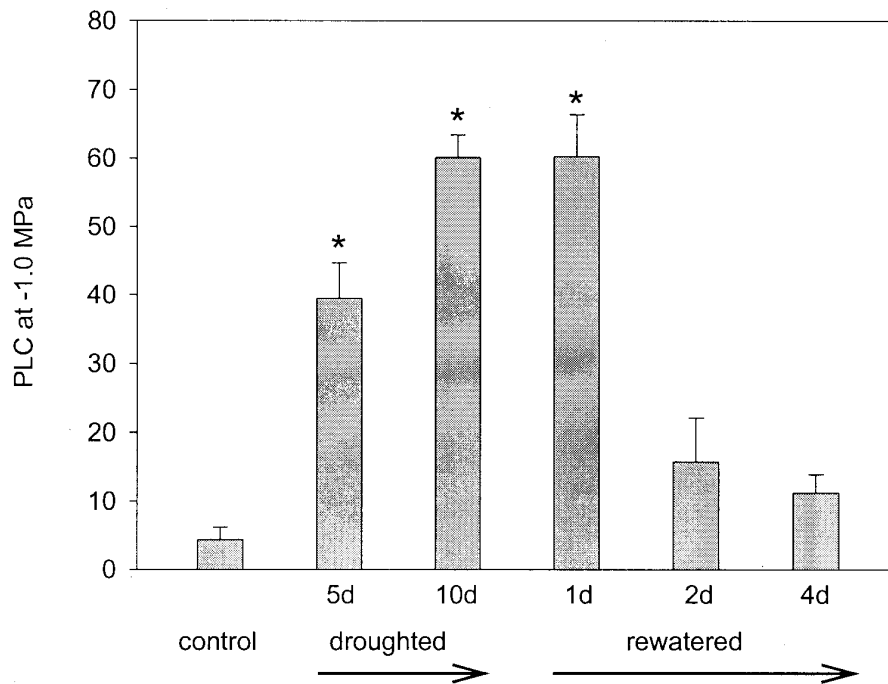


Fig. 4. Per cent loss of hydraulic conductance (PLC) of droughted and rewatered sunflower stems at a test pressure of -1.0 MPa. This value was used to quantify the extent of cavitation fatigue. Note that the fatigue was reversed after rewatering. Arrows indicate the temporal sequence during the experiment. Means and SE, $n=4-6$. Asterisked means are different from well-watered controls (LSD test). From Stiller & Sperry (2002).

by the mechanical stress associated with drought-induced cavitation. How can the original membrane properties be reestablished? Current evidence suggests that cations (both calcium and potassium) as well as a higher pH may promote at least a partial reversal of the fatigue. The phenomenon of cavitation fatigue and its reversal could also be related to the stimulating effect of potassium on hydraulic conductivity, which was first described by Zimmermann (1978; 1983), and was recently reevaluated by van Ieperen *et al.* (2000) and Zwieniecki *et al.* (2001). According to the study of

Zwieniecki *et al.*, pectins may act as a so-called “hydrogel” (Tanaka, 1981), which shrinks and swells reversibly in response to a variety of external and internal factors. Among them, the ionic strength, polarity, and pH of the surrounding solution, as well as temperature are of major importance. According to this concept, all treatments that lead to the shrinking of a hydrogel would also promote recovery from cavitation fatigue (Stiller and Sperry, 2002). Clearly, we need to find out more about the biochemical and biophysical properties of pit membranes.

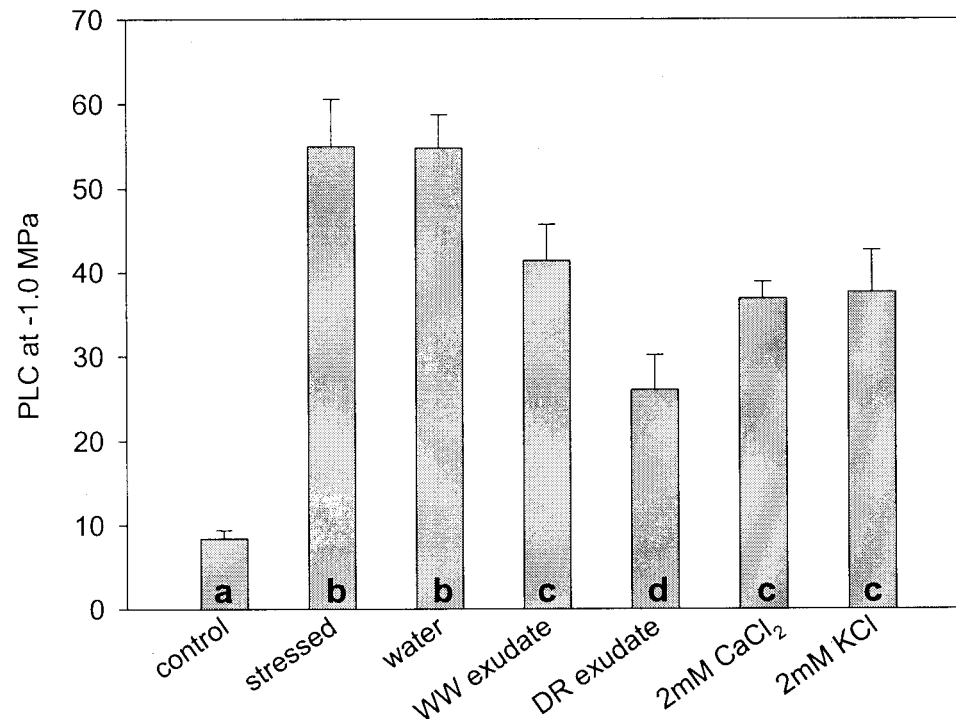


Fig. 5. Per cent loss of hydraulic conductivity (PLC) at -1.0 MPa for previously flushed stem segments of sunflower. All but the “control” had been exposed to -4.0 MPa on the centrifuge prior to flushing to induce cavitation fatigue. All but “control” and “stressed” were perfused with the indicated solution after flushing but prior to the -1.0 MPa PLC measurement. Perfusion solutions were deionized and filtered water (water), root exudate from well-watered plants (WW), root exudate from droughted plants (DR), 2 mM CaCl₂, and 2 mM KCl. Means and SE, $n=6$ for all perfusion treatments, $n=5$ for stressed stems, and $n=35$ for controls. Different letters indicate homogenous sets of means determined with a Tukey’s HSD test after a one-way ANOVA tested significant. From Stiller & Sperry (2002).

5. ECOLOGICAL IMPLICATIONS

Many plants undergo more or less frequent embolism-refilling cycles when periods of water stress alternate with conditions permitting xylem refilling (Cochard, Ewers and Tyree, 1994; Ewers *et al.*, 1992; Hacke and Sauter, 1996; Holbrook *et al.*, 2001; Salleo, Lo Gullo, De Paoli and Zippo, 1996; Sperry *et al.*, 1994; Stiller and Sperry, 2002; Utsumi *et al.*, 1998). In many cases refilling is associated with metabolic activity to generate positive water pressure in the xylem (Ewers *et al.*, 1997; Sperry *et al.*, 1987). A prerequisite for refilling to be a longer-term benefit is that the xylem maintains its original cavitation resistance. As shown by Hacke *et al.* (2001), this is not always the case. Some species that undergo strong seasonal variation in soil moisture experience significant cavitation in situ, and may permanently remain weakened. For example, portions of the xylem of desert plants are often surprisingly vulnerable to cavitation after the xylem had been refilled in the laboratory (Hacke *et al.*, 2000; Kolb and Sperry, 1999; Pockman and Sperry, 2000; Sperry and Hacke, 2002). There was a substantial PLC after stem and root segments of cold desert shrubs were subjected to a modest xylem pressure of only -0.5 MPa, a pressure to which plants were routinely exposed in the field. Presumably, this represented vessels in older growth rings long since gone out of function and no longer able to sustain significant negative pressure. Cold desert shrubs do not seem to refill embolized vessels, which explain why the weakening effect is not reversed in the stems of these species.

Root xylem often exhibits the same symptoms of weakening. Roots can show high native embolism rates, and vulnerability curves showing high PLC at only mild pressure (Alder *et al.*, 1996; Kolb

and Sperry, 1999; Sperry and Hacke, 2002). During soil drought, the pressure in roots often drops to cavitation-inducing levels, thus inducing weakening. Roots are usually more susceptible to cavitation than stems (Hacke, Sperry and Pittermann, 2000), and many root vulnerability curves may reflect previous drought episodes. The phenomenon of cavitation fatigue therefore complicates the interpretation of vulnerability curves. For curves obtained from flushed material, the more resistant (more negative) end of the vulnerability curve may reflect the original xylem properties, whereas the high pressure (less negative) end of the curve may change dramatically as a result of previous stress exposure, confounding attempts to compare the true cavitation resistance between species. Therefore, Sperry and Hacke (2002) expressed the cavitation resistance of desert species by using the xylem pressure causing 75 PLC (P_{75}) instead of the often used P_{50} , i.e. the pressure causing 50 PLC.

6. UNRESOLVED ISSUES

So far, only weakening caused by drought-induced cavitation has been documented. However, there is preliminary data indicating that it is also caused by freezing-induced cavitation (E. Rubio, L. Castro, S. Davis and J. Sperry, unpubl.). This is intriguing, because pit membranes are not directly involved in the mechanism of freezing-induced embolism Zimmermann (1983). If their mechanical and structural properties are affected by freeze-thaw cycles, the mechanism is unknown.

Another question is why some species exhibit weakening, while others do not. We have found no systematic basis for this distinction. Within a family or even a genus, species may show contrasting responses. In Betulaceae, for instance,

Betula occidentalis, *Betula nigra*, and *Alnus incana* showed resilient xylem, whereas *Carpinus caroliniana* exhibited weakening (U. Hacke and J. Pittermann, unpubl.). Even more surprising is the fact that normally growing *Acer negundo* stems were resilient (Hacke *et al.* 2001) while rapidly growing reiteration sprouts of this species showed the fatigue response (V. Stiller, unpubl.). It is possible that the pit membranes in rapidly growing xylem are mechanically weaker. Progress in understanding the physiological basis for cavitation fatigue and its reversal should resolve these questions.

An often-asked question is why nobody has shown weakened pit-membranes in SEM pictures before, as the resolution of SEM images should be sufficient to show the porosity of the membranes. Age-related degradation of pit membranes has been observed in *Populus tremuloides*, a

species known to exhibit fatigue (Sperry *et al.*, 1991). However, it is not known whether the degradation was caused specifically by previous cavitation events. In those species where cavitation fatigue is reversible, as in *H. annuus*, it is unlikely that any change in pit structure would be observed for two reasons. First, the critical structural feature of the pit membrane is its porosity when being stretched by the pressure gradient at an air-water interface. It is not the membrane porosity in its unstressed state, which is what is visible with SEM. Second, dehydration steps during sample preparation often use ethanol, a substance known to cause shrinking of hydrogels and to promote recovery from cavitation fatigue (V. Stiller, unpubl.). New techniques need to be developed for assessing pit membrane mechanics and structure in relation to cavitation and the fatigue phenomenon.

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