



# Cavitation fatigue and its reversal in sunflower (*Helianthus annuus* L.)

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Received 3 September 2001; Accepted 17 January 2002

## Abstract

**'Cavitation fatigue' is the increased susceptibility of a xylem conduit to cavitation as a result of its prior cavitation. It was investigated whether cavitation fatigue induced *in vivo* could be repaired in intact plants. Sunflowers (*Helianthus annuus* L.) were subjected to soil drought in the greenhouse. Native embolism and vulnerability to cavitation was measured in well-watered controls and after 5 d and 10 d of controlled drought. A dramatic cavitation fatigue was observed where droughted xylem that was refilled in the laboratory developed up to 60 PLC (percentage loss of hydraulic conductivity) at  $-1$  MPa versus only 5.2 PLC in non-droughted controls. Rewatered plants showed the complete reversal of cavitation fatigue over 4 d. Reversal of fatigue was correlated with the refilling of embolized vessels in the intact plants ( $r^2 = 0.91$ ,  $P < 0.01$ ), suggesting that xylem transport to fatigued vessels was required for their repair. The *in vivo* reversal of fatigue was partially duplicated in excised stem segments by perfusing them with root exudates from droughted (DR) and well-watered (WW) plants. The DR exudate had a greater effect, and this was associated with a greater pH in the DR versus WW saps, but there was no difference in total cation concentration. Perfusions with 2 mM  $\text{CaCl}_2$  and KCl solutions also partially reversed cavitation fatigue as opposed to no effect with deionized water, suggesting a role of ions in addition to a pH effect. It is suspected that fatigue is caused by stretching and partial disruption of linkages between cellulose microfibrils in inter-conduit pit membranes during air seeding, and that the reversal of fatigue involves restoring these linkages by ingredients in xylem sap.**

Key words: Cavitation fatigue, embolism, soil drought, xylem sap.

## Introduction

Cavitation and embolism of xylem conduits by water stress causes the loss of hydraulic conductivity in the xylem. There is substantial evidence that the mechanism for the cavitation is 'air seeding' (Zimmermann, 1983), which most likely occurs at the pit-membranes between air-filled and functional conduits (Crombie *et al.*, 1985; Jarbeau *et al.*, 1995; Sperry *et al.*, 1996). Embolized conduits can refill during nightly or seasonal root pressure (Ewers *et al.*, 1997; Hacke and Sauter, 1996; Pickard, 1989; Sperry *et al.*, 1994). It is also possible that embolized conduits refill while the xylem pressure is substantially negative, although the mechanism is unknown (Holbrook and Zwieniecki, 1999; McCully *et al.*, 1998; Salleo *et al.*, 1996; Tyree *et al.*, 1999). However, until recently it has not been determined whether cavitated and refilled conduits necessarily regain their initial resistance to cavitation by air seeding, or whether their cavitation resistance is altered by the previous cavitation process. This question is of basic importance for understanding how plants respond to repeated drought cycles.

It has been reported that in 'resilient' species, cavitation resistance before and after a cavitation/refilling cycle was not significantly different (Hacke *et al.*, 2001), as was also found earlier (Alder *et al.*, 1997). A larger group of 'weakened' species, however, showed a significant increase in cavitation vulnerability after a cavitation/refilling cycle. This 'cavitation fatigue' was caused by a decrease in the air-seeding pressure, perhaps because of a stretching or rupturing of pit membranes during the initial cavitation event. The fatigue was observed whether stem segments were cavitated by air drying or by centrifugation, and it also occurred *in vivo* in droughted plants. This was a surprising result. If xylem conduits are refilled by the plant after a drought only to re-embolize immediately under modest xylem pressures, the plant will not be able to recover its original hydraulic conductivity without producing new conduits.

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The goal of this study was to determine whether cavitation fatigue induced *in vivo* during soil drought is able to be reversed by the plant once the drought is relieved. In the earlier study (Hacke *et al.*, 2001), the xylem was always refilled with pure water in excised stem segments under laboratory conditions. It is possible that if conduits were allowed to refill with xylem sap under natural conditions, the cavitation fatigue would be reversed. The extent of recovery from cavitation fatigue was determined in droughted and rewatered sunflower plants (*Helianthus annuus*), a species known to suffer considerable fatigue as a result of drought (Hacke *et al.*, 2001). Follow-up experiments examined the role of natural xylem sap and its constituents in the reversal of cavitation fatigue.

**Materials and methods**

*Drought/rewatering experiment*

The protocol for the drought/rewatering experiment is summarized in Fig. 1A. Sunflower plants (*Helianthus annuus* L., variety ‘wilting, DeKalb, Illinois) were grown in a greenhouse in 4.5 l pots under natural light. The soil consisted of 22% topsoil, perlite, and wood mulch each, 17% vermiculite, 11% peat mulch, and 6% sand. During growth, plants were watered frequently to avoid water stress and embolism. At 6–7 weeks of age the plants were subjected to a soil drought/rewatering cycle (Fig. 1A). Water was withheld for 10 d at which point the plants were extremely wilted. Then watering was resumed. At various drought and rewatering stages (0, 5, and 10 d of drought, and 1, 2, and 4 d after rewatering) a subset of plants was harvested for the measurement of native embolism and the resistance of the xylem to cavitation.

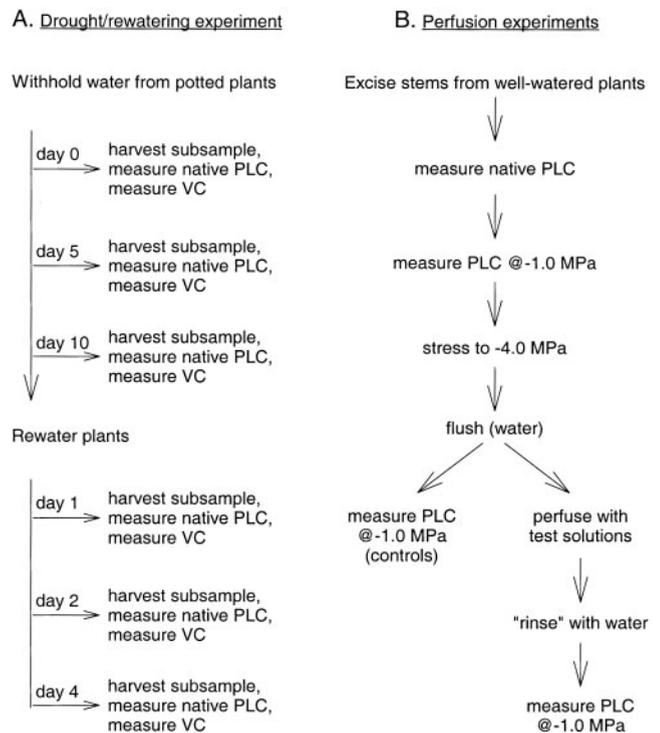
*Cavitation resistance and native embolism*

The resistance of the xylem to cavitation was determined from ‘vulnerability curves’ (VC) which show the relationship between xylem pressure and the percentage loss of hydraulic conductivity (PLC). The vulnerability curves were measured using the centrifugal force method (Alder *et al.*, 1997) on 0.14 m long stem segments. Four to six plants were measured for each drought/rewatering period. One stem segment was cut from each plant under water. Hydraulic conductivity ( $K_{\text{native}}$ ) was measured before the segments were flushed at 100 kPa for 60 min with deionized and filtered (0.2  $\mu\text{m}$ ) water to reverse any native embolism. After flushing the maximum hydraulic conductivity ( $K_{\text{max}}$ ) was measured. The percentage that  $K_{\text{native}}$  was below  $K_{\text{max}}$  gave the segment’s native PLC (= native embolism). The flushed segments were exposed to progressively more negative pressures by spinning them in a custom-built centrifuge rotor (3 min at each pressure). The  $K$  was remeasured between each pressure and the PLC (relative to  $K_{\text{max}}$ ) associated with each pressure was recorded in a vulnerability curve. All  $K$  measurements were made with a pressure head of *c.* 5 kPa, which was sufficiently small to prevent the displacement of gas from embolized vessels running through the segment. To estimate the pressure causing 50 PLC ( $P_{50}$ ) a Weibull function was fitted (Neufeld *et al.*, 1992) to each segment’s vulnerability curve, and solved for the  $P_{50}$ .

*Perfusion experiments*

Perfusion experiments were conducted to test whether the fatigue reversal observed in intact plants (see Results) could be duplicated by exposing fatigued conduits to various test solutions. Test solutions included native xylem sap and artificial ion solutions.

Figure 1B summarizes the protocol. Stem segments were cut (underwater) from well-watered sunflower plants. As in the drought/rewatering experiment,  $K$  was measured before and after plants were flushed at 100 kPa with deionized and filtered water to obtain  $K_{\text{native}}$  and  $K_{\text{max}}$  values. Only segments with < 5% native PLC were used to insure the segments had not experienced significant water stress. The cavitation resistance was measured as the PLC induced by a single pressure of –1.0 MPa via centrifugation (Fig. 1B, PLC @ –1.0 MPa). The segments were spun to –4.0 MPa in a centrifuge to create an average of  $92.8 \pm 1.8\%$  embolism and induce cavitation fatigue. The segments were again flushed at 100 kPa to refill embolized conduits. The  $K_{\text{max}}$  and PLC at –1.0 MPa for controls was measured directly after the water flush. Other segments were perfused with a test solution. The flow-through was collected and its volume measured to ensure complete perfusion of the segment xylem. After perfusion, segments were ‘rinsed’ by flushing with de-ionized and filtered water at 100 kPa for



**Fig. 1.** Schematic diagram of the (A) drought/rewatering experiment and (B) the perfusion experiments. In the drought/rewatering experiment water was withheld from potted sunflower plants. Plants were harvested at various drought and irrigation times and the native percentage loss of hydraulic conductivity (native PLC) was measured on stem segments as well as complete vulnerability curves (VC). In the perfusion experiments native PLC and the PLC at –1.0 MPa (PLC @ –1.0 MPa) was first measured in sunflower stem segments cut from well-watered plants. The segments were then stressed to induce > 90 PLC and flushed with deionized water to refill embolized conduits. The PLC @ –1.0 MPa was measured either immediately or after perfusion with various test solutions including water controls.

sufficient time to replace the estimated xylem volume five times. This ensured that  $K$  was always measured with water, eliminating potential ion effects on  $K$  (van Ieperen *et al.*, 2000; Zwieniecki *et al.*, 2001). The  $K_{\max}$  and PLC at  $-1.0$  MPa were then measured to determine the extent of fatigue reversal caused by the test perfusion. The  $K_{\max}$  was a function of segment diameter, and for a given diameter it did not differ between cohorts of plants or experimental treatments.

Two of the test solutions were native xylem saps collected from well-watered (WW) versus droughted (DR) sunflower plants. To provide xylem sap, plants were grown in a greenhouse. The WW plants were watered daily. The DR plants were watered once per week, which caused wilting between waterings and smaller plant size. At 6–7 weeks of age root exudate of 7 WW and 12 DR plants was collected. The night before collection, all plants were thoroughly watered. On the following morning their intact and potted root system was put in a root pressure chamber. About 0.1–0.2 MPa of air pressure was applied to the soil–root system and the stem was cut 2 cm above the chamber. Root exudate was collected for 6–12 h into 50 ml centrifuge tubes (Life Science Products, Denver, CO) and frozen until used in the perfusion experiment. The slow rate of root exudation was chosen to minimize dilution of the xylem sap. The smaller DR plants did not yield enough root exudate within a 12 h collection period, and the sap of two DR plants was required for the perfusion of one stem segment.

Before the perfusion, root exudates were thawed and centrifuged for 4 min at 4000 r.p.m. (Centra CL2, IEC, Needham Heights, MA) and decanted to obtain particle-free sap that would minimize the clogging of xylem during perfusion. Segments were perfused with sap for 15 h at a pressure head of 5 kPa; controls were perfused for the same time and pressure with deionized and filtered water (Fig. 1B, 'perfuse with test solution'). Despite sap filtration, some clogging occurred at the upstream ends of the segments after 15 h. To remove clogged xylem, 0.055 m was removed from both ends of the segments. Thus, the original segments were 0.25 m long, resulting in a final trimmed length of 0.14 m (centrifuge rotors were available that could accommodate either length). If  $K_{\max}$  after perfusion was less than 90% of the  $K_{\max}$  before perfusion despite the trimming, the segments were discarded.

A subsample of the perfusate was refrozen for chemical analysis. Concentration of each ion above the detection limit of  $0.005 \text{ mmol l}^{-1}$  was measured with inductively-coupled plasma spectrometry (ICPS) at the soil testing laboratory of Utah State University. The pH of the xylem sap samples was measured with a micro pH electrode (Model S900C, Sensorex, Garden Grove, CA) on a Markson pH meter (Model 4603, Markson, Hillsboro, OR).

Two other test solutions were also used besides WW and DR saps. Stem segments 0.14 m in length were perfused for 2 h with 2 mM  $\text{CaCl}_2$  and 2 mM KCl solutions (pH 5.25) at a pressure head of 17 kPa. No clogging occurred during the short perfusion, and the segments did not require trimming.

### Statistics

Data were analysed with the SPSS 8.0 statistics package for PC (SPSS Inc., Chicago IL) using the 0.05 significance level. Comparisons of native PLC and PLC at  $-1.0$  MPa between treatments (Figs 2, 3b, 5) were made with a one-way ANOVA and the LSD test for *a priori* pairwise comparisons (all treatment means versus control, Figs 2, 3b), or Tukey's HSD test for *a posteriori* comparisons between all means (Fig. 5). Student's *t*-tests (independent sample) were used to compare means of other parameters as noted.

## Results

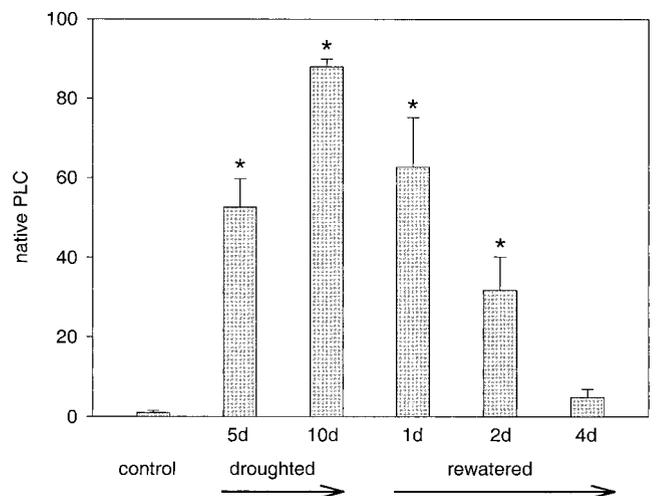
### Drought/rewatering experiment

Drought caused a progressive increase in native PLC in sunflower stems from less than 1 PLC in well-watered plants to nearly 90 PLC after 10 d of no water (Fig. 2). Rewatering the plants caused the gradual reversal of this drought-induced embolism over a 4 d period. The PLC after 4 d of watering was not different from the value prior to drought (Fig. 2).

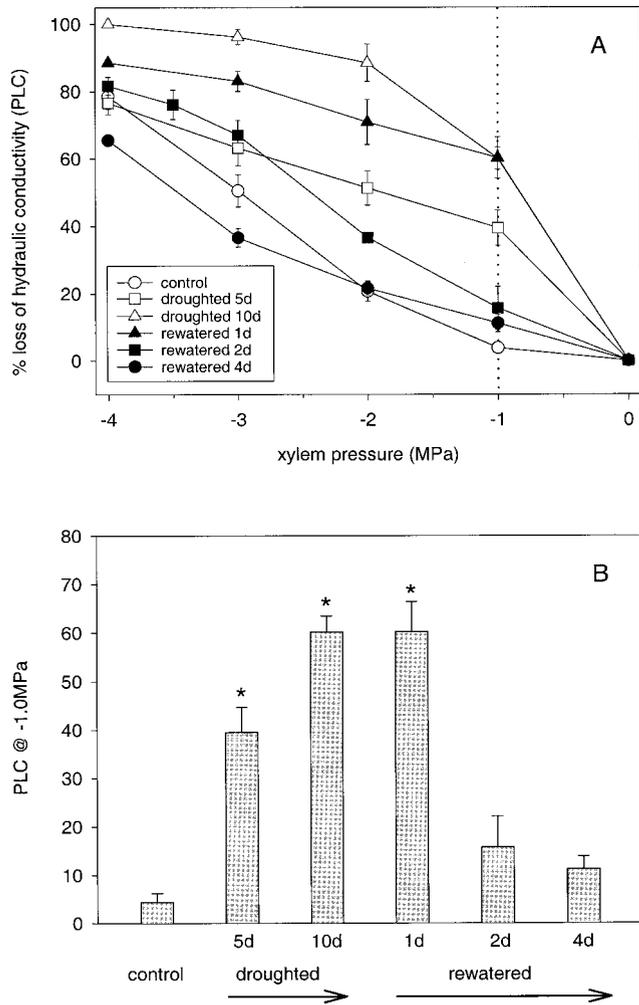
Cavitation fatigue also increased with increasing days of drought. The shape of the vulnerability curve changed dramatically from the typical concave or sigmoidal curve in well-watered plants with a  $P_{50}$  at  $-3.0 \pm 0.1$  MPa (Fig. 3a, open circles) to a convex shape in the droughted plants, with a  $P_{50}$  of  $-1.7 \pm 0.3$  MPa after 5 d of drought (Fig. 3a, open squares), and  $-0.8 \pm 0.1$  MPa after 10 d of drought (Fig. 3a, open triangles).

Interestingly, the fatigue *decreased* in rewatered plants. The vulnerability curve after 1 d of rewatering had the same  $P_{50}$  ( $-0.7 \pm 0.3$  MPa) as after 10 d of drought, but showed less PLC for pressures below  $-1$  MPa (Fig. 3a, compare solid versus open triangles). After 2 d of rewatering, there was a substantial shift in the vulnerability curve to a more sigmoidal shape accompanied by a decrease in the  $P_{50}$  to  $-2.4 \pm 0.1$  MPa (Fig. 3a, solid squares). After 4 d of rewatering, the curve was similar in shape to the original well-watered curve, except that the  $P_{50}$  was even lower at  $-3.3 \pm 0.1$  MPa (Fig. 3a, compare solid versus open circles).

To illustrate the rise in cavitation fatigue with drought and its reversal with rewatering, Fig. 3b compares just the PLC at  $-1.0$  MPa across treatments (i.e. at the



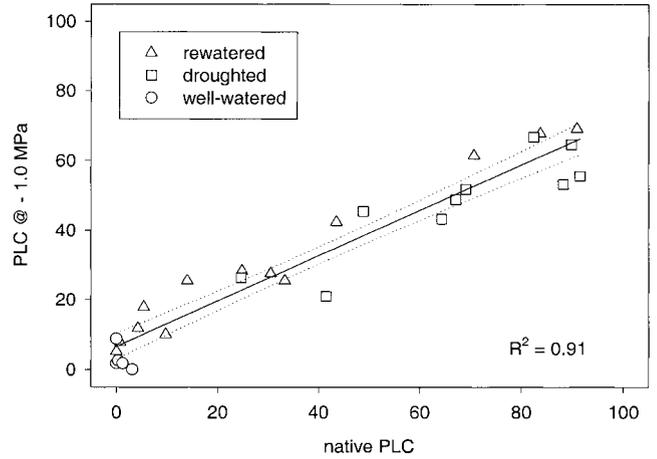
**Fig. 2.** Percentage loss of hydraulic conductivity (PLC) measured in stem segments of *Helianthus annuus* during a soil drought/rewatering experiment. The values reflect the xylem embolism developed within the intact plant, hence the term 'native' PLC. Arrows indicate the temporal sequence during the experiment. Means and SE,  $n=4-6$ . Asterisked means are different from well-watered controls (LSD test).



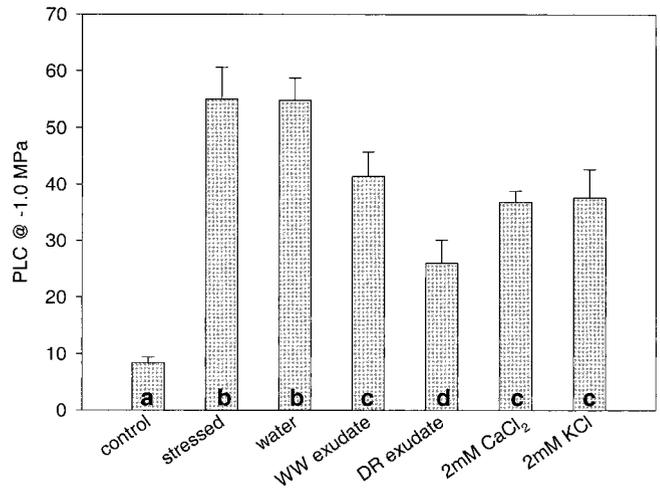
**Fig. 3.** (A) Vulnerability curves of stem xylem in *Helianthus annuus* during a soil drought/rewatering experiment. Stems were flushed prior to the vulnerability curve to remove native embolism. Curves show percentage loss of xylem conductivity (PLC) with decreasing xylem pressure for well-watered control plants (open circles), plants droughted for 5 d (open squares), and 10 d (open triangles), rewatered for 1 d (solid triangles), 2 d (solid squares), and 4 d (solid circles). Means and SE,  $n=4-6$ . (B) Percentage loss of hydraulic conductivity (PLC) of all treatments at  $-1.0$  MPa. This value was used to quantify the extent of cavitation fatigue in subsequent experiments. Arrows indicate the temporal sequence during the experiment. Means and SE,  $n=4-6$ . Asterisked means are different from well-watered controls (LSD test).

vertical dotted line in Fig. 3a). This increased from about 5 PLC in well watered controls to over 60 PLC in the 10 d droughted plants. It went back down from 60 PLC after 1 d of rewatering to about 10 PLC after 4 d, a value no different from the initial one. In subsequent experiments, the PLC at  $-1$  MPa was used in flushed stems as a simplified measure of the fatigue effect.

There was a strong linear relationship between the amount of fatigue, quantified by the PLC at  $-1.0$  MPa, and the native PLC of the plants during the drought and rewatering cycle (Fig. 4). This relationship suggested that



**Fig. 4.** Relationship between native PLC in stem segments during the drought/irrigation treatment (% native embolism) and the PLC at  $-1.0$  MPa in these stems after they were flushed and spun on the centrifuge. The greater the native PLC, the more extensive the fatigue as indicated by greater PLC at  $-1$  MPa. Circles are well-watered controls, squares are droughted plants, and triangles are rewatered plants. Dotted lines show 95% confidence intervals for the regression.



**Fig. 5.** Percentage loss of hydraulic conductivity (PLC) at  $-1.0$  MPa for previously flushed stem segments. 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 CaCl<sub>2</sub>, 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 homogeneous sets of means determined with a Tukey's HSD test after a one-way ANOVA tested significant.

the reversal of fatigue required the *in vivo* refilling of embolized conduits. In other words, if the conduits were not refilled naturally by the plant with its own xylem sap, but only with deionized water in the laboratory, they remained fatigued. This observation suggested testing the effect of sunflower xylem sap versus deionized water on the reversal of fatigue.

### Perfusion experiments

In these experiments, fatigue was induced by spinning stem segments to  $-4$  MPa on the centrifuge before perfusion with a test solution. The extent of fatigue was measured by the PLC at  $-1$  MPa in flushed stems (Fig. 1B).

Stems that were not exposed to  $-4$  MPa on the centrifuge had a PLC at  $-1$  MPa that was below 10 (Fig. 5, control). Stressing the stems to  $-4$  MPa and testing them after flushing with water showed the expected fatigue effect with a PLC at  $-1$  MPa of  $55.0 \pm 5.6$  (Fig. 5, stressed). This was not different from the 10 d droughted plants ( $60.1 \pm 3.3$  PLC at  $-1$  MPa, *t*-test), indicating that centrifugation was equivalent to drought in producing fatigue. Long-term (15 h) perfusion with deionized water had no effect on the fatigue (Fig. 5, water). Interestingly, however, perfusion with root exudate caused a significant decline in cavitation fatigue, with the exudate from droughted plants being especially effective (Fig. 5, WW and DR exudate). A similar decline in fatigue could be produced by perfusing with  $\text{CaCl}_2$  and  $\text{KCl}$  solutions (Fig. 5,  $\text{CaCl}_2$ , and  $\text{KCl}$ ), but note that the perfusion time for these solutions was 2 h versus the 15 h for the exudates. No perfusion was able to duplicate the complete reversal of fatigue that was seen after 4 d of rewatering the intact plants (Fig. 3b).

The chemical analysis of the root exudate showed no difference in total cation concentration between DR and WW saps (pooled average of  $13.3 \pm 3.3$  mmol  $\text{l}^{-1}$ ). In both saps, the principal cation was  $\text{K}^+$  followed by  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . The DR sap had a higher pH ( $8.2 \pm 0.2$ ) than the WW sap ( $6.6 \pm 0.4$ , *t*-test), suggesting that this could have been involved in the greater reversal of fatigue with the DR sap.

### Discussion

Five conclusions were reached from these results: (1) intact sunflower plants develop extensive embolism during drought and reverse this embolism within days after drought relief (Fig. 2), (2) substantial cavitation fatigue accompanies the drought-induced embolism (Fig. 3), (3) the fatigue is reversed in association with the natural refilling of embolized conduits in rewatered plants (Figs 3, 4), (4) the reversal of fatigue is promoted by ingredients in the xylem sap (Fig. 5), and (5) ionic composition and pH may be involved in the repair of fatigue.

The strong relationship between native PLC and the extent of fatigue (Fig. 4) might suggest that fatigue is an artefact of small air bubbles left behind after the flush used to remove native embolism. These small bubbles could prematurely nucleate cavitation. However, a series

of experiments (reported in Hacke *et al.*, 2001) have ruled out this explanation. Briefly, introducing air into the xylem independently of cavitation followed by a flush did not cause a fatigue effect; treatments designed to promote gas dissolution did not influence the fatigue response; and the fatigue was associated with increased susceptibility to cavitation by air seeding.

Assuming that the air seeding is occurring at the pit membrane, the mechanisms that cause cavitation fatigue and reversal depend on how the structure of the pit membrane is related to air seeding and to the composition of the xylem sap. The pit membrane is a modified compound middle lamella of the adjoining conduits. It has a different microfibril organization and porosity relative to regular primary cell walls, and it is bathed in xylem sap rather than being adjacent to a protoplast. Nevertheless, its properties could be similar, in principle, to those of a mature primary cell wall.

These results suggest that in sunflower, at least, the fatigue is not caused by complete rupture of these membranes. If the membranes were torn across the cellulose microfibrils, it seems unlikely that they could be knitted back together in the absence of a protoplast for new wall synthesis. The fact that sunflower can repair the weakened pit membranes, and even make them less vulnerable to air seeding than originally (Fig. 3A, compare well-watered plants with 4 d rewatered plants), suggests that the weakening is the result of a more subtle change in membrane properties than an irreparable tear.

It is suspected that the complex system of hydrogen and ionic bonds that link microfibrils together via hemicellulose and pectin molecules is altered during the air-seeding process. These bonds may become partially broken while the membrane is under mechanical stress caused by the large pressure difference between air on one side and water under negative pressure on the other. The breaking of these bonds could allow the membrane to stretch more, widening its pores to cause air seeding. If these bonds are not re-established after cavitation, the membrane will be mechanically weaker and more porous when subjected to subsequent mechanical stress.

These results suggest a novel significance for the composition of the xylem sap: the repair of cavitation fatigue. Sap components may be necessary to create the proper conditions for restoring interfibrillar bonds and the original membrane elasticity. Although  $\text{KCl}$  and  $\text{CaCl}_2$  solutions promoted repair, the cation concentration was no greater in DR versus WW exudates, suggesting that something more than just cation concentration caused the more effective repair by the DR exudate (Fig. 5). Other experiments with sunflower also report no increase in cation concentration with drought (Gollan *et al.*, 1992). The observed increase in sap pH with drought, however, is a general response in sunflower (Gollan *et al.*, 1992) and many other species

(Hartung *et al.*, 1988; Wilkinson *et al.*, 1998; Wilkinson and Davies, 1997), where it is commonly discussed with respect to the compartmentation and redistribution of abscisic acid (ABA) (Daeter and Hartung, 1995; Wilkinson, 1999). These results suggest it may also play a role in fatigue repair. None of this study's perfusion experiments were able to repair the fatigue completely, as was the case in the rewatered plants. It is not known whether this was because the intact and rewatered plants had a different sap composition from that obtained from the root system exudate, or whether the longer 4 d time interval in the intact plants played a role.

The fact that CaCl<sub>2</sub> solution was effective in partially reversing fatigue (Fig. 5) was interesting, because of the importance of calcium ions for the structural integrity of the primary cell wall (Carpita and Gibeaut, 1993; Cosgrove, 1997). Calcium ions cross-link the pectin fraction in the wall, which in turn is known to determine the pore size of primary cell walls (Cosgrove, 1999; Matoh and Kobayashi, 1998). The rupture and restoration of calcium cross-links could be partially responsible for fatigue development and reversal. However, the fact that potassium ions were equally effective in reversing fatigue as calcium (Fig. 5), and the possible role of pH suggests there is more to the explanation.

A recent paper (Zwieniecki *et al.*, 2001) explained increases in xylem hydraulic conductivity after ion perfusion as a result of the 'hydrogel-like' behaviour of the pectins in pit-membranes. The authors demonstrate that pectins act as a hydrogel which shrinks and swells reversibly in response to (a) the ionic strength of the solution, (b) the solution pH, and (c) the polarity of the solution (Tanaka, 1981; Zwieniecki *et al.*, 2001). According to this concept, all the perfusion treatments that promoted recovery from fatigue would also have resulted in a shrinking of hydrogels (high ionic strength, high pH). Perhaps the shrinking of the pectin fraction also restores the strength of intermicrofibrillar bonds that had been pulled apart during air seeding.

The fatigue effect is reminiscent of a previously reported 'oxalic-calcium' effect (Sperry and Tyree, 1988, 1990). Perfusing stems of several species (conifers and an angiosperm) with a solution of oxalic acid and calcium caused a striking decrease in cavitation resistance via reduced air-seeding pressure. However, there was no change in the porosity of the membranes as assessed with scanning electron microscopy (SEM). The reduced air-seeding pressure was associated with an increase in membrane flexibility and creep, suggesting that the membranes became more porous only when placed under mechanical stress. The oxalic-calcium effect was immediately reversible when oxalic acid by itself was perfused. Differences in surface tension were negligible, and the mechanism probably involved changes in the strength of the interfibrillar bonds of the pit membrane.

The oxalic-calcium effect provides an example of how sap chemistry can dramatically, but reversibly, alter cavitation resistance via the alteration of membrane mechanics and porosity under stress. It emphasizes that the critical structural feature of the pit membrane is not necessarily its porosity in the unstressed state (potentially visible with SEM), but its porosity when being stretched by an air-water interface.

Although fatigue was not caused by pit membrane rupture in sunflower, this may not be the case in other species. Increased susceptibility to cavitation with xylem age has been documented for *Populus tremuloides* (Sperry *et al.*, 1991), and was associated with dramatic increases in 'resting porosity' as revealed with SEM. Large tears and openings were observed in the older xylem. It is possible that this ageing effect in *P. tremuloides* was also a fatigue effect—the result of prior air seeding—but this is unknown. It is known that fatigue can be induced in this species in the laboratory (Hacke *et al.*, 2001).

It remains to be seen whether cavitation fatigue is reversible in species besides sunflower that can refill their xylem after a drought. This would be expected since the benefit of refilling can only be achieved if fatigue is repaired at the same time. The results imply that species that do not refill would remain permanently weakened. This may be responsible for the considerable amount of extremely vulnerable xylem that is embolized even under non-stressful field conditions in many plants (Kolb and Sperry, 1999; Pockman and Sperry, 2000). When this 'fatigued' xylem is refilled in the laboratory prior to determining a vulnerability curve, the curve shows a large population of very vulnerable xylem (Pockman and Sperry, 2000). This complicates the interpretation of vulnerability curves, because one must distinguish between the 'virgin' curve of newly-developed xylem versus the curve that results from the experience of previous stress events.

### Acknowledgement

This work was funded in part by a grant from USDA (97-3700-2649).

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