Cavitation Fatigue. Embolism and Refilling Cycles Can Weaken the Cavitation Resistance of Xylem[1]

Uwe G. Hacke*, Volker Stiller, John S. Sperry, Jarmila Pittermann, and Katherine A. McCulloh

Department of Biology, University of Utah, Salt Lake City, Utah 84112

Although cavitation and refilling cycles could be common in plants, it is unknown whether these cycles weaken the cavitation resistance of xylem. Stem or petiole segments were tested for cavitation resistance before and after a controlled cavitation-refilling cycle. Cavitation was induced by centrifugation, air drying of shoots, or soil drought. Except for droughted plants, material was not significantly water stressed prior to collection. Cavitation resistance was determined from "vulnerability curves" showing the percentage loss of conductivity versus xylem pressure. Two responses were observed. "Resilient" xylem (*Acer negundo* and *Alnus incana* stems) showed no change in cavitation resistance after a cavitation-refilling cycle. In contrast, "weakened" xylem (*Populus angustifolia, P. tremuloides, Helianthus annuus* stems, and *Aesculus hippocastanum* petioles) showed considerable reduction in cavitation resistance. Weakening was observed whether cavitation was induced by centrifugation, air dehydration, or soil drought. Observations from *H. annuus* showed that weakening was proportional to the embolism induced by stress. Air injection experiments indicated that the weakened response was a result of an increase in the leakiness of the vascular system to air seeding. The increased air permeability in weakened xylem could result from rupture or loosening of the cellulosic mesh of interconduit pit membranes during the water stress and cavitation treatment.

There have been a number of reports suggesting that cavitation and emptying of xylem conduits can be followed at close intervals by their refilling (Tyree et al., 1986; Salleo et al., 1996; McCully et al., 1998; Holbrook and Zwieniecki, 1999; Tyree et al., 1999). The implication is that xylem conduits of at least some species undergo frequent (daily) cycles of cavitation and refilling. This possibility leads to a question: What effect does this cycling have on the cavitation resistance of the xylem? Can vessels be recycled without compromising their ability to withstand negative pressures? Or does the act of cavitation fatigue the vessels in some way to make them more prone to cavitate in the future? If refilling is to be effective in restoring xylem conductance, the xylem should not become weakened in the process.

There are reasons to suspect that refilled vessels might not be as resistant to cavitation as when fresh from the vascular cambium. There is substantial evidence that cavitation by water stress occurs by the "air seeding" mechanism (Zimmermann, 1983) operating at pits between cavitated and functional conduits (Crombie et al., 1985; Jarbeau et al., 1995; Sperry et al., 1996). Although it has usually been thought that the air seeding occurs through pre-existing pores in the pit membranes, it is possible that in some cases the pit membrane ruptures before admitting the air (Sperry and Tyree, 1990) or becomes damaged as a result of the rapid energy release upon cavitation. Even if membranes do not irreversibly rupture, the meshwork of microfibrils could become stretched as a result of the great pressure difference (several MPa for many species) separating a water-filled vessel from an already embolized one. If a vessel with prestressed and more porous pit membranes would be refilled, it should be more susceptible to cavitation. Even if stress and cavitation were to have no effect on pit membrane function, refilling may leave small microbubbles behind that could nucleate cavitation prematurely during subsequent stress.

There is only one instance that we know where the cavitation resistance of refilled vessels has been tested. In this experiment, stems of *Betula occidentalis* were used to generate a "vulnerability curve" showing the loss of xylem conductivity from cavitation (percentage loss of conductivity [PLC]) with decreasing xylem pressure. The stems were then refilled in the laboratory and the curve repeated. The second curve was identical to the first, demonstrating that cavitation did not weaken the vessels (Alder et al., 1997; Fig. 1C).

In this paper we surveyed six species to determine the effect of a cavitation and refilling cycle on cavitation resistance. We used stem or petiole segments that had not been significantly water stressed prior to collection. These segments were subjected to water stress and refilling cycles under controlled laboratory conditions. Additional experiments were conducted in selected species to compare laboratory results with controlled soil drought in intact plants and to investigate the mechanism for the surprising differences

[1] This work was supported by the National Science Foundation (grant no. IBN–9723464), by the U.S. Department of Agriculture (grant no. 97–37100–2649 to J.S.S.), and by the Alexander von Humboldt-Foundation, Germany (Feodor-Lynen fellowship to U.G.H.).

* Corresponding author; e-mail hacke@biology.utah.edu; fax 801–581–4668.

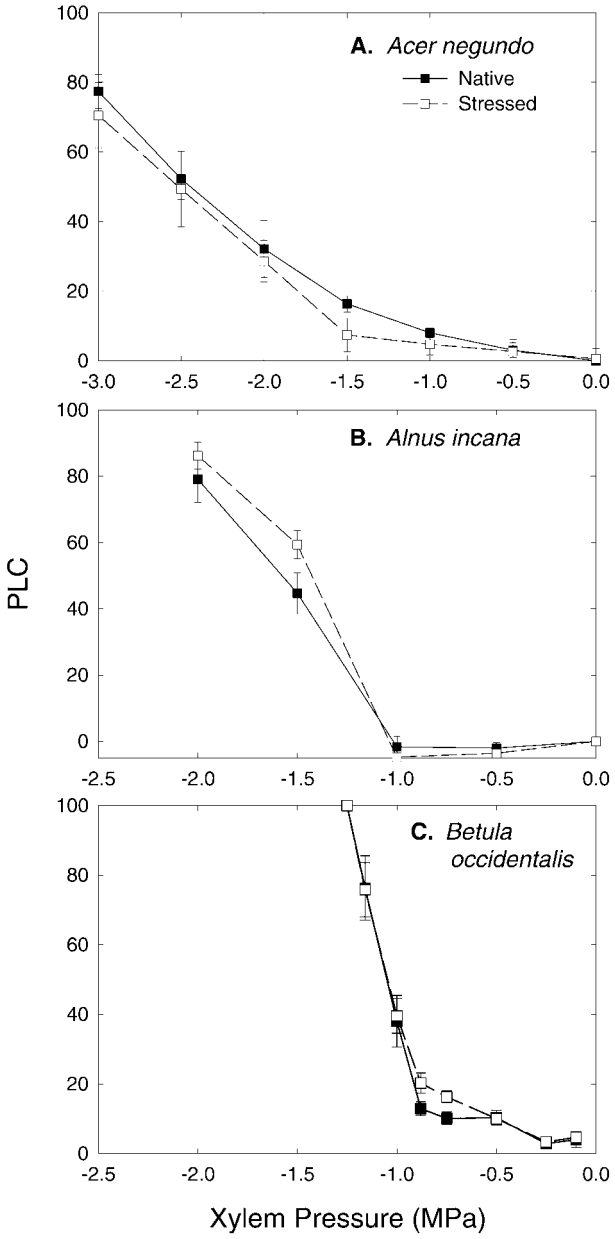

Figure 1. Native (■) versus stressed (□) vulnerability curves showing the resilient response of stem xylem in *Acer negundo, Alnus incana,* and *B. occidentalis.* Curves show the PLC with decreasing xylem pressure. Native and stressed curves were similar (*t* test, $P > 0.05$). Means and SE, $n = 6$. *B. occidentalis* curves are from Alder et al. (1997).

that we observed in the response of xylem to the cavitation and refilling cycle.

RESULTS

Native Versus Stressed Vulnerability Curves

All native vulnerability curves possessed a similar shape, with little PLC occurring until xylem pressures dropped below some threshold value, which caused the PLC to increase steeply (Figs. 1 and 2, solid squares). Material used for these experiments was unlikely to have experienced significant drought-induced embolism. For *Helianthus annuus* and *Aesculus hippocastanum* we only used segments with less than 10% native PLC. The remaining material was from a riparian Red Butte Canyon site that does not experience soil drought or xylem pressures below the embolism thresholds for the native curves in Figures 1 and 2.

Stressed vulnerability curves revealed two different responses to the cavitation-refilling treatment. In the "resilient" response (*Acer negundo* and *Alnus incana*), the stressed curve was identical to the native curve (Fig. 1, compare open versus solid squares), as had been found previously for *B. occidentalis* (Fig. 1C; Alder et al., 1997). In a converse manner, in the "weakened" response (*Populus angustifolia, P. tremuloides, A. hippocastanum,* and *H. annuus*), the second curve was substantially more vulnerable to cavitation (Fig. 2, compare open versus solid squares). For example, in *P. angustifolia*, PLC at −0.5 MPa was 9% in the native curve versus nearly 50% in the stressed curve (Fig. 2A). In *H. annuus*, PLC at −1.0 MPa was 9% versus 81% in the native versus stressed curves, respectively (Fig. 2D). The shape of the weakened curve was also different from the native curve. Rather than having an approximately sigmoidal shape with an embolism threshold as in a native curve, weakened curves resembled more of an exponential increase in PLC to a maximum and showed no pressure threshold (e.g. Fig. 2D).

Air Dehydration Versus Centrifuge Comparison and Soil Drought Experiments

The weakening response was identical whether xylem was stressed to >70 PLC on the centrifuge or by air drying. The PLC at test xylem pressures of −0.5 and −1.0 MPa for *P. angustifolia* and *A. hippocastanum*, respectively, was not different for material that had been previously stressed by air drying or by centrifugation. (Fig. 4, compare spun versus air-dried PLC in stressed stems). This indicated that the weakening response was not caused by mechanical stress associated with centrifuging.

The weakening effect was also observed in *H. annuus* stems that had been stressed in the intact plant during controlled soil drought. In stems where native PLC exceeded 95% as a result of the drought, the vulnerability curve showed an even greater weakening effect than curves from stems stressed in the centrifuge (Fig. 2D, compare open circles and open squares). The greater weakening in the droughted plants was associated with their having been stressed to greater PLC (>95%) than the centrifuged stems (75%–95%).

Further results from *H. annuus* confirmed that the extent of weakening depended on the extent of native embolism induced by the previous stress treatment.

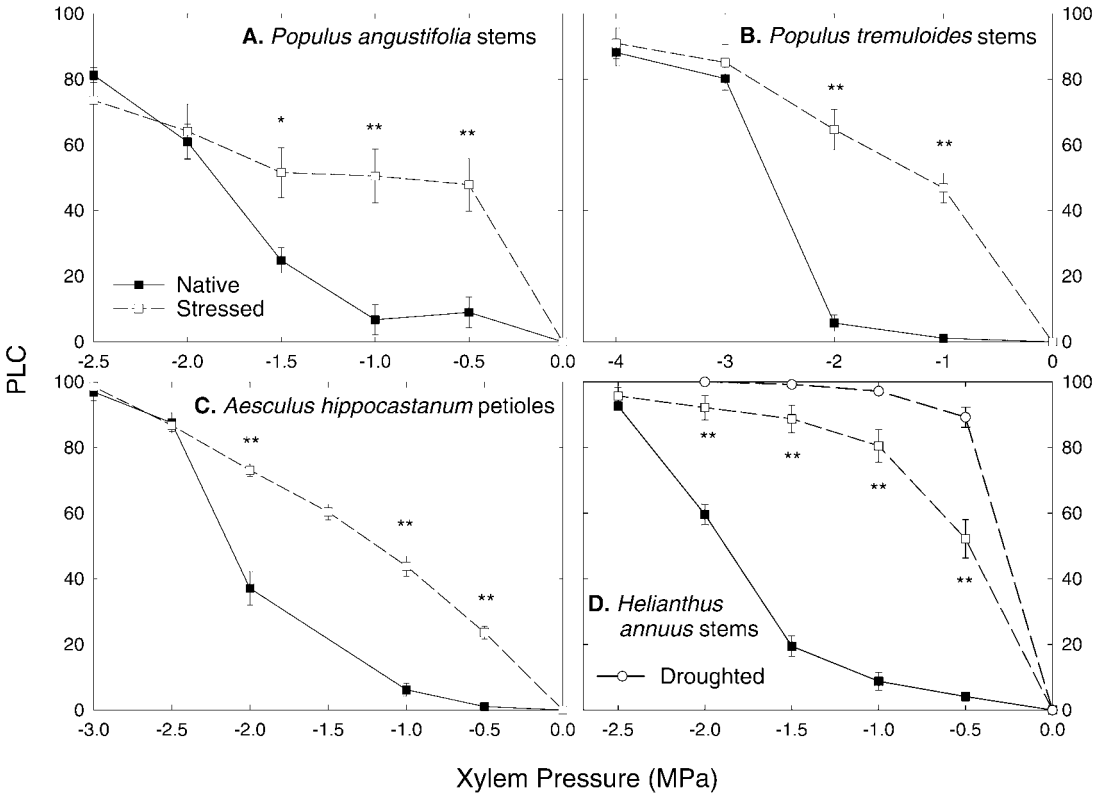

Figure 2. Native (■) versus stressed (□) vulnerability curves showing the weakened response seen in xylem of *P. angustifolia, P. tremuloides, A. hippocastanum,* and *H. annuus*. All data is for stem xylem except for petioles in *A. hippocastanum*. Means and SE, $n = 6$. Stressed xylem was significantly 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).

When native PLC caused by the stress treatment was plotted versus the PLC induced by a −1 MPa test pressure, a strong linear relationship ($P < 0.0005$, $r^2 = 0.95$) was found across stems from all well-watered and droughted plants, and from excised shoots that were air-dried to various PLC values from <30% to >95% (Fig. 3).

The Bubble Versus Air Seeding Hypotheses for the Weakening Response

Experiments on *P. angustifolia* and *H. annuus* did not support the hypothesis that weakening resulted from bubbles remaining in xylem conduits after the refilling treatment. *P. angustifolia* stems stressed to >70 PLC and refilled showed the same PLC at a test pressure of −0.5 MPa ($P > 0.05$) whether they were measured immediately after refilling (47.8 ± 19.8 PLC) or following the overnight soaking and pressurizing treatment (56.6 ± 3.7 PLC). Stems of *H. annuus* droughted to >70 PLC and refilled with degassed water exhibited the same PLC ($P > 0.05$) from a test pressure of −1.0 MPa (72.3% ± 2.8%, $n = 3$) as stems refilled in the normal manner (73.1% ± 3.9%, $n = 3$). If bubbles were causing the weakening response, either treatment should have promoted their dissolution, thereby reducing the weakening response. Stem segments of *P. angustifolia* that were heavily embolized (81.9 ± 10.2 PLC) independent of water stress (via air entry at cut ends, see "Materials and Methods") did not exhibit any weakening response relative to native material ($P > 0.05$) when refilled and subjected to a test pressure of −0.5 MPa (3.3 ± 16.8 versus 9.0 ± 11.6 PLC, respectively). The refilling treatment apparently eliminated any microbubbles that would have caused a weakening effect even in these non-stressed stems, according to the bubble hypothesis.

Results did support the air seeding hypothesis. In the weakened species *P. angustifolia* and *A. hippocastanum*, there was significantly more PLC by air injection at a test pressure for stressed versus native material (Fig. 4, white bars, compare native versus stressed). In contrast, the resilient stems of *A. negundo* (Fig. 1A) did not show any difference in PLC by air injection in native versus stressed stems (Fig. 5, white bars, native versus stressed).

According to the air-seeding hypothesis, the PLC in native or stressed xylem should be the same whether it was induced by air injection or dehydration. This

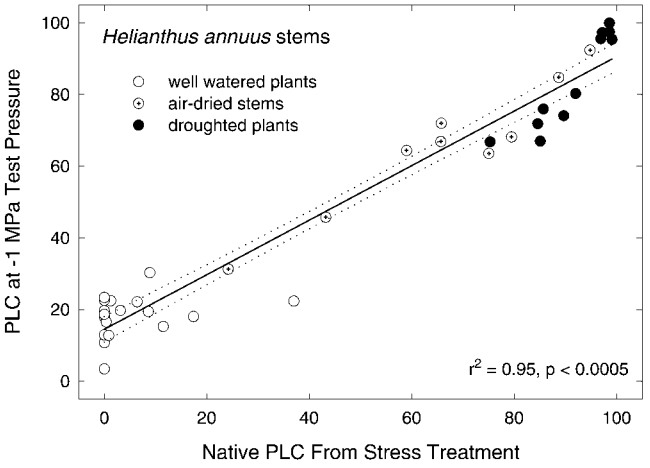

Figure 3. Relationship between Native PLC from a water-stress treatment versus the PLC at test pressure of −1.0 MPa in *H. annuus* stems. The greater the PLC caused by stress, the more extensive the weakening response as indicated by greater PLC at the test pressure. ○, Stems from well-watered plants where water stress was minimal and native PLC was generally below 20%. A subset of these stems with <5 PLC were used to generate the native and stressed curves in Figure 2D. ●, Stems from droughted plants where water stress caused >70 PLC. A subset of these stems with PLC >95% were used to generate the droughted stem curve in Figure 2D. ⊕, Excised stems that were air dried to varying PLC values as shown. Dotted lines show 95% confidence intervals for the regression.

was the case in all instances (Figs. 4 and 5) except stressed stems of *P. angustifolia.* In these stems the air injection caused less PLC than expected from dehydration results (Fig. 4A, compare white and black bars for stressed material). This may have resulted from the applied test pressure being less than the actual injection pressure (see "Materials and Methods"). This was supported by results of the pulsed injection applied to *A. hippocastanum* petioles. Whereas normal injection at the test pressure of 1 MPa caused 31.7 ± 4.4 PLC in stressed petioles, the pulsed method (same test pressure) resulted in a higher value of 51.9 ± 7.4 PLC, which was not significantly different from stressed petioles centrifuged to the test xylem pressure as shown in Figure 4B ("stressed, injected" versus "stressed, spun"). Applying the pulsed procedure to native petioles of this species caused no difference ($P > 0.05$) in the test pressure PLC versus the steady injection (7.6 ± 4.2 versus 7.4 ± 4.1 PLC, respectively).

DISCUSSION

The results revealed two responses to controlled cavitation-refilling cycles. In the resilient response there was no change in cavitation resistance (Fig. 1), as seen previously for *B. occidentalis* (Alder et al., 1997). In the weakened response, cavitation resistance was substantially reduced following a cavitation-refilling cycle (Fig. 2), indicating a "cavitation fatigue" by analogy with the stress-induced weakening of metals. The weakened response was detected regardless of whether cavitation was induced by centrifugation, air dehydration, or soil drought in intact plants (Figs. 2–4). The air injection experiments indicated that the weakening response was a result of an increase in the leakiness of the vascular system to air seeding (Figs. 4 and 5). The extent of weakening was proportional to the extent of native PLC induced by stress (Fig. 3).

It is presumable that the increased leakiness occurred at the inter-conduit pit membranes where air seeding has been shown to occur in other studies (Crombie et al., 1985; Jarbeau et al., 1995; Sperry et al., 1996). It is unknown whether the leaky xylem was caused by pit membrane rupture in association with cavitation or by a stretching and weakening of the cellulose mesh of the membrane. If actual rupture had occurred, the weakening effect would probably

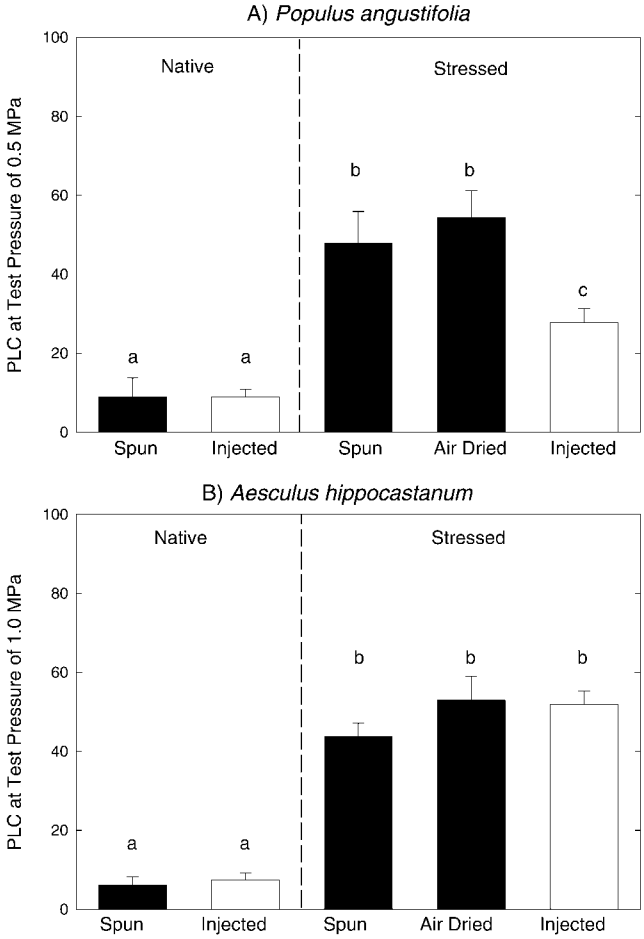

Figure 4. PLC in stem (*P. angustifolia*, A) and petiole (*A. hippocastanum*, B) segments at the indicated test pressure (see Fig. 6, procedure 2) for the centrifuge method (black bars) and the air injection method (open bars, injected). Results shown for native segments (left) and separate stressed segments (right). Stressed segments were dehydrated to a stress pressure of −2.5 MPa and refilled. Dehydration was achieved by centrifugation (spun, injected) or air drying (air dried). Treatments with same letters were not different ($P > 0.05$; LSD test). Means and SE, $n = 5$ to 12.

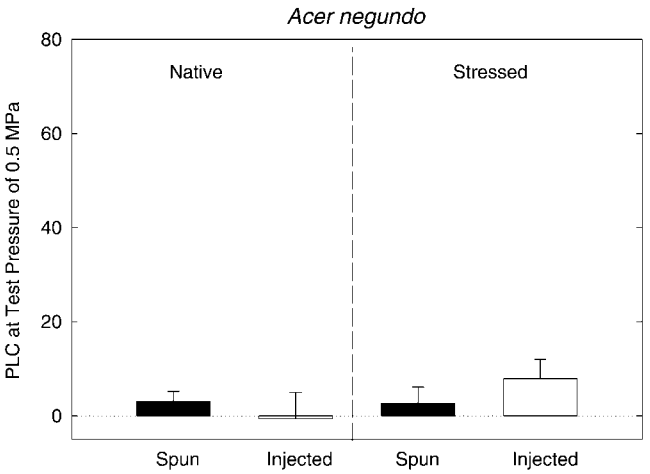

Figure 5. PLC in stem segments of *A. negundo* at a test pressure of −0.5 MPa for the centrifuge method (solid bars, spun) and the air injection method (open bars, injected). Results shown for native segments (left) and separate stressed segments (right). Stressed segments were dehydrated in the centrifuge to −3 MPa and refilled. Treatments were not different ($P > 0.05$; LSD test).

be permanent. If leakiness was caused by stretching of the complex linkages between cellulose microfibrils, it is possible that the plant could restore these linkages and eliminate the weakening effect in vivo. The large weakening response seen in both *Populus* species (Fig. 2, A and B) is consistent with results of Sperry et al. (1991) who observed degradation of intervessel pit membranes in older growth rings of *P. tremuloides*. Our experiments suggest that this degradation may have been caused by previous stress events.

Our results were obtained from contrived, often severe, stress events in the laboratory and the greenhouse, and all material was refilled under artificial conditions. How relevant are the results to cavitation resistance of intact plants in the field? Weakening in *H. annuus* was proportional to native PLC (Fig. 3), suggesting that whenever cavitation occurs weakening would follow for a non-resilient species. With the exception of the droughted *H. annuus*, our material showed no evidence of substantial weakening in the native state: all native curves possessed a roughly sigmoidal shape with a threshold embolism pressure (Figs. 1 and 2, native curves). However, we purposely selected young material that was unlikely to have experienced significant cavitation in situ. The naturally growing plants in Red Butte Canyon live in a consistently moist riparian habitat where minimum stem xylem pressure is not likely to vary during the growing season (e.g. Sperry and Sullivan, 1992). They do not experience much cavitation, and their native curves did not show much evidence of weakening from prior stress events.

In contrast, a different type of native vulnerability curve is often seen in stem xylem of species that experience strong seasonal variation in soil moisture. For example, upland species from the Sonoran desert possess very high native PLC (>50%) even under favorable water availability, and the native curves can have a shape very similar to that of the weakened xylem in our study (Pockman and Sperry, 2000). The same was found for cold desert shrubs of the Great Basin (Hacke et al., 2000a). These results suggest that unlike the riparian species we examined here, species subjected to large fluctuations in xylem pressure and that experience significant cavitation in situ may also exhibit a permanent weakening response as indicated by high native PLC and the shape of the native vulnerability curve.

Root xylem of woody plants often exhibits the same symptoms of weakening. Roots can have high native PLC (>50%; Sperry and Saliendra, 1994; Alder et al., 1996; Kolb and Sperry, 1999) even under favorable water availability, and their native vulnerability curves can have a shape typical of the weakening response that we observed under laboratory conditions. Root xylem is commonly more vulnerable to cavitation than stem xylem as evidenced by less negative xylem pressures required to cause 100% embolism. During soil drought, xylem pressures in roots can drop to cavitation-inducing levels (thus inducing weakening), whereas stem xylem experiences no cavitation (Alder et al., 1996; Kolb and Sperry, 1999; Hacke et al., 2000b).

In our study we only addressed weakening caused by water stress-induced cavitation. It is possible, but not documented, that weakening may also be caused by freezing-induced cavitation. However, this is unlikely in our species since some of the native material we used had been subjected to at least one winter, and previous studies have indicated that extensive cavitation by freezing is likely in our species (Sperry and Sullivan, 1992; Sperry et al., 1994). The mechanisms of freezing- versus water stress-induced cavitation are quite different, so it cannot be predicted how the two forms of cavitation might differ with respect to the weakening phenomenon.

Further experiments are needed to test whether weakening is a permanent condition, or whether processes associated with natural refilling in intact plants can restore the initial cavitation resistance. Weakening could compromise the functioning of species experiencing cavitation and refilling cycles in nature. These species may possess resilient xylem or may have some means of repairing the weakening effect. The phenomenon of cavitation fatigue complicates the interpretation of vulnerability curves because it means that they can represent the inherent properties of the original xylem and its subsequent stress history. The weakening effect explains the large differences seen in some vulnerability curves when performed on flushed versus non-flushed native material (Sperry et al., 1991; Pockman and Sperry, 2000): weakened xylem that is embolized under field conditions will only show up on a vul-

nerability curve if the xylem is first refilled by flushing. For curves obtained from flushed material (via centrifuge or air injection methods), the more resistant, low pressure end of the vulnerability curve may reflect the original xylem properties, whereas the high pressure end of the curve may be much more variable to the extent it is modified by permanent weakening effects.

MATERIALS AND METHODS

Plant Material

Stem segments were collected from *Populus angustifolia* James, *Populus tremuloides* Michx., *Alnus incana* Moench, and *Acer negundo* growing along Red Butte creek in Red Butte Canyon near Salt Lake City (111°47′W, 40°47′N, elevation 1,750 m). This is the same site from which *Betula occidentalis* Hook. stems of the Alder et al. (1996) study were collected. Stem material was collected between late March and July of 1999. The moist riparian habitat was selected because previous studies have indicated that stems would experience pressures above −1.0 MPa, with native embolism being less than 15% in *B. occidentalis* and *A. incana* during the growing season (Dina, 1970; Sperry and Sullivan, 1992; Dawson and Ehleringer, 1993; Sperry et al., 1994).

Petiole segments of *Aesculus hippocastanum* were collected in July 1999 from a single tree on the campus of the University of Utah where it received frequent irrigation. We also collected stem segments from greenhouse-grown *Helianthus annuus*. Plants were grown in 4.5-L pots under natural light and well-watered conditions until the incipient flowering stage when they were used for the experiments. The soil consisted of 22% each of topsoil, perlite, and wood mulch, 17% vermiculite, 11% peat mulch, and 6% sand.

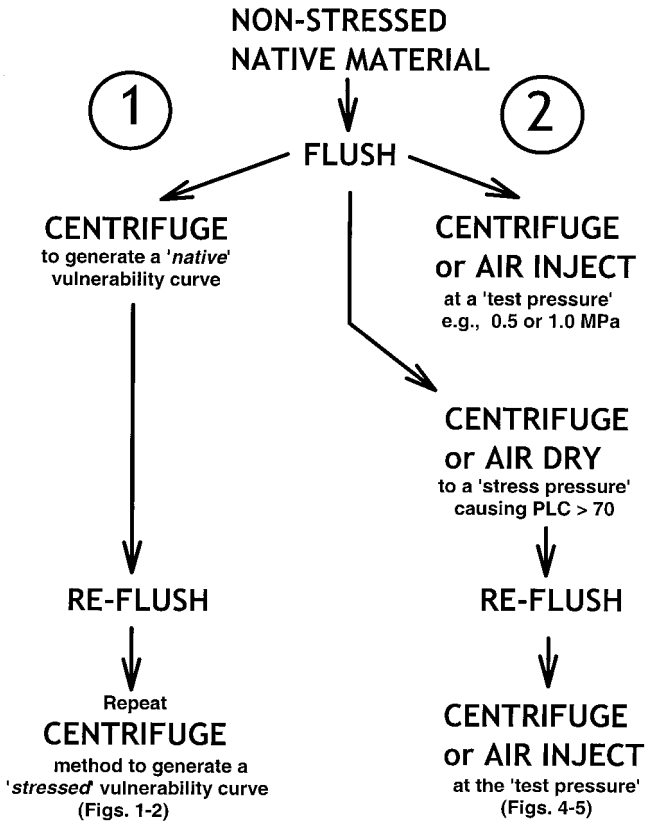

Figure 6. Summary of procedures used to assay the effect of water stress on cavitation resistance. Both began with non-stressed native material that was flushed to remove any native PLC. In procedure 1 (e.g. results in Figs. 1 and 2), the centrifuge method was used to generate a "native" vulnerability curve, material was re-flushed, and then a second "stressed" vulnerability curve was completed. In procedure 2 (e.g. results in Figs. 4 and 5), PLC at a single "test" pressure (usually 0.5 or 1.0 MPa) induced by centrifugation or air injection was measured with or without the stems having prior exposure to a "stress" pressure sufficient to cause >70 PLC. The stress pressure was applied using the centrifuge method or by air drying.

Native Versus Stressed Vulnerability Curves

The cavitation resistance of the xylem was determined from vulnerability curves that show the relationship between xylem pressure and the PLC caused by cavitation and subsequent embolism of the xylem conduits. Comparison was made between the "native" curve measured on branch segments after collection versus the "stressed" curve measured on segments previously stressed in the laboratory to a xylem pressure causing >70 PLC (Fig. 6, procedure 1) and then refilled.

Native and stressed vulnerability curves were measured using the centrifugal force method (Alder et al., 1997). For the native curves, stem segments (petioles in *A. hippocastanum*) 1- to 2-years-old and 0.14 m long were flushed at a mild pressure of 75 kPa for 30 min with deionized and filtered (0.2 μm) water to reverse any native embolism. Native PLC was measured for those species where we had no knowledge of water potentials in the intact plants (*H. annuus* and *A. hippocastanum*). After flushing, the maximum hydraulic conductivity (K_{max}) was measured. The segments were spun in a centrifuge (>2 min at speed) to induce a known negative xylem pressure calculated from the angular velocity and length of the segment (Alder et al., 1997). The PLC caused by centrifugation was calculated from the hydraulic conductivity after spinning relative to K_{max}. The same segments were spun to progressively lower negative pressures until PLC reached 70% to 85%. Curves were based on mean embolism of a minimum of six segments.

Except for *A. hippocastanum*, the stressed curve was measured on the same segments used for the native curve. The segments were flushed to refill embolized conduits and restore hydraulic conductivity to within 10% of the original K_{max}. In a few heavily embolized segments (PLC > 90%), the initial K_{max} could not be restored after multiple flushes. The reason for this is unknown, and these segments were discarded. The stressed vulnerability curve was measured on the refilled segments with a minimum sample size of six.

Air Versus Centrifuge Dehydration Comparison

To test whether the weakened resistance to cavitation that we observed in stressed stems of some species (the "weakened" response, see "Results") was a function of the xylem pressure versus mechanical stress in stems during centrifugation, we compared the effect of stressing the xylem in the centrifuge with comparable water stress induced by air dehydration (Fig. 6, "air dry" option in procedure 2). Two species showing the weakening response were chosen (*A. hippocastanum* and *P. angustifolia*). Unstressed, native material was flushed and centrifuged (n = 6–8) or air-dried (n = 5–8) to a stress pressure inducing >70 PLC. Segments were reflushed, and then centrifuged at a moderate test pressure of −0.5 or −1.0 MPa. This test pressure was chosen because significant PLC at such high (less negative) pressure was indicative of the weakening response. Water potential during air dehydration was measured with stem psychrometers (PWS Instruments, Guelph, Ontario, Canada) in *P. angustifolia* or on detached leaves using the pressure chamber in *A. hippocastanum.*

Air drying experiments were also conducted on detached shoots of *H. annuus.* Nine shoots were dried, and PLC was measured on stem segments that had been excised from the shoots under water to avoid causing embolism during collection. Stems were refilled, centrifuged to a test pressure of −1 MPa, and measured for PLC to determine the magnitude of the weakening response.

Controlled Soil Drought Experiments

We used the greenhouse-grown *H. annuus* plants (see "Plant Material") to compare the extent of weakening caused by soil drought in intact plants with that caused by centrifugal or air-drying stress on excised stems. Plants were well-watered until the incipient flowering at which time water was withheld for >1 week. Stem segments (n = 11 from 11 plants) were excised from droughted plants under water to avoid causing embolism during collection, were measured for native embolism, and were refilled. Segments with >95% native PLC (n = 5) were used to generate a complete vulnerability curve using the centrifuge method. The other six segments were centrifuged to a test pressure of −1 MPa and were measured for PLC.

Experiments on the Mechanism of the Weakening Response

We tested two hypotheses proposed to account for the weakening response. The first was the bubble hypothesis: After flushing a stressed and embolized segment, residual bubbles remained in the vessels to nucleate cavitation at mild xylem pressures in the stressed stems. The second was the air-seeding hypothesis: The xylem conduits become more leaky to air as a result of the stress episode, thus lowering the air-seeding threshold and increasing the vulnerability to cavitation.

We tested the bubble hypothesis on *P. angustifolia* with two types of experiments. In one experiment, a set of stems (n = 3) was exposed to a stress pressure causing >70 PLC and flushed to within 10% of K_{max}. Stems were submerged in water and pressurized at 2 MPa overnight to promote bubble dissolution, and the PLC at a −0.5 MPa test pressure was measured the next day. PLC at the test pressure was compared with stems not given the overnight treatment to determine if weakening had been reduced by soaking the stems. In a second experiment, native stems (n = 4) were flushed and artificially embolized without subjecting the stems to water stress-induced cavitation. This was done by blowing air into one end at low pressure (0.1 MPa) or by spinning them to a modestly negative pressure (−0.5 MPa) with both ends exposed to air. We refilled these stems and measured the PLC at the test pressure to evaluate the extent of weakening. If the bubble hypothesis was correct, it should not matter how the embolism was formed (whether artificially or by cavitation) and these non-stressed stems should show weakening.

We also tested the bubble hypothesis on *H. annuus* stems that had been naturally embolized during the soil drought. One set of embolized stems was flushed with the normal non-degassed water supply, and a second was flushed with degassed water. Flushing lasted >30 min. Stems were then embolized at a test pressure of −1.0 MPa and evaluated for weakening. If microbubbles were causing the weakening response, the use of degassed water should have promoted their dissolution and reduced the weakening response.

We tested the air seeding hypothesis with a series of air injection experiments on stem segments of *P. angustifolia* and *A. negundo* and petiole segments from *A. hippocastanum*. The air injection procedure determined the PLC caused by pushing air into xylem vessels through air seeding sites. It is normal that the PLC caused by air pressure is the same as that caused by xylem pressure of equal, but opposite, magnitude (Sperry et al., 1996). In the injection procedure, 0.14-m-long segments were inserted through a double-ended pressure chamber with both segment ends protruding (see also Salleo et al., 1992; Sperry and Saliendra, 1994). The mid-section of the segment was subjected to 10 min of the test air pressure (= the opposite of the test xylem pressure by centrifugation for the same species) inside the chamber. Hydraulic conductivity of the segments was measured before and after the injection treatment.

A potential shortcoming of this injection procedure is that the air pressure measured by the gauge on the chamber may not be the same as the pressure at the actual site of air injection in the segment's vascular system when air is flowing from the chamber and through the stem xylem to the outside. To minimize this potential problem we designed a "pulsed" injection procedure that we applied to the petioles of *A. hippocastanum* for comparison with the normal injection. The double-ended injection chamber (with segment) was enclosed within a larger closed pressure chamber. Both chambers were initially pressurized to the test pressure, blocking flow through the segment, and equalizing all pressure at the gauge value of the injection chamber. The pressure in the larger chamber was then quickly reduced to ambient, exposing the segment to a pulse of air equal to the gauge pressure. After 1.5 min of

this pulse, the large chamber was repressurized to block flow and bring all pressures back to the gauge value. By repeating this pressure pulse we could repeatedly expose the segment to an injection pressure close to the gauge pressure. We used three pressure pulses of 1.5 min each for each segment.

As summarized in Figure 6 (procedure 2) PLC caused by air injection at the test pressure was compared for flushed native segments versus separate segments previously stressed to >70 PLC in the centrifuge and flushed. If weakening was associated with a change in air-seeding pressure, it should be detected by greater PLC by air injection in stressed versus native stems. At least five stem segments were used for each air injection treatment.

Statistics

Differences between native and stressed vulnerability curves were based on Student's *t* tests at each test pressure. Comparisons of test pressure embolism between treatments (e.g. Fig. 4) were made with a one-way ANOVA and the LSD test for multiple pairwise comparisons. The SPSS 8.0 statistics package was used to analyze the data (SPSS Inc., Chicago).

Received May 15, 2000; returned for revision August 28, 2000; accepted November 1, 2000.

LITERATURE CITED

Alder NN, Pockman WT, Sperry JS, Nuismer S (1997) Use of centrifugal force in the study of xylem cavitation. J Exp Bot **48:** 665–674

Alder NN, Sperry JS, Pockman WT (1996) Root and stem xylem embolism, stomatal conductance, and leaf turgor in *Acer grandidentatum* populations along a soil moisture gradient. Oecologia **105:** 293–301

Crombie DS, Hipkins MF, Milburn JA (1985) Gas penetration of pit membranes in the xylem of *Rhododendron* and other species. Planta **163:** 27–33

Dawson TE, Ehleringer JR (1993) Gender specific physiology, carbon isotope discrimination, and habitat distribution in boxelder (*Acer negundo*). Ecology **74:** 798–815

Dina SD (1970) An evaluation of physiological response to water stress as a factor influencing the distribution of six woody species in Red Butte Canyon, Utah. PhD Thesis. University of Utah, Salt Lake City

Hacke UG, Sperry JS, Ewers BE, Ellsworth DS, Schäfer KVR, Oren R (2000b) Influence of soil porosity on water use in *Pinus taeda*. Oecologia **124:** 495–505

Hacke UG, Sperry JS, Pittermann J (2000a) Drought experience and cavitation resistance in six shrubs from the Great Basin, Utah. Basic Appl Ecol **1:** 31–41

Holbrook NM, Zwieniecki MA (1999) Embolism repair and xylem tension. Do we need a miracle? Plant Physiol **120:** 7–10

Jarbeau JA, Ewers FW, Davis SD (1995) The mechanism of water stress-induced embolism in two species of chaparral shrubs. Plant Cell Environ **18:** 189–196

Kolb KJ, Sperry JS (1999) Transport constraints on water use by the Great Basin shrub, *Artemisia tridentata*. Plant Cell Environ **22:** 925–935

McCully ME, Huang CX, Ling LEC (1998) Daily embolism and refilling of xylem vessels in roots of field-grown maize. New Phytol **138:** 327–342

Pockman WT, Sperry JS (2000) Vulnerability to xylem cavitation and the distribution of Sonoran Desert vegetation. Am J Bot **87:** 1287–1299

Salleo S, Hinckley TM, Kikuta SB, LoGullo MA, Weilgony P, Yoon TM, Richter H (1992) A method for inducing xylem embolism in situ: experiments with a field-grown tree. Plant Cell Environ **15:** 491–497

Salleo S, LoGullo MA, De Paoli D, Zippo M (1996) Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: a possible mechanism. New Phytol **132:** 47–56

Sperry JS, Nichols KL, Sullivan JEM, Eastlack SE (1994) Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. Ecology **75:** 1736–1752

Sperry JS, Perry A, Sullivan JEM (1991) Pit membrane degradation and air-embolism formation in ageing xylem vessels of *Populus tremuloides* Michx. J Exp Bot **42:** 1399–1406

Sperry JS, Saliendra NZ (1994) Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. Plant Cell Environ **16:** 279–288

Sperry JS, Saliendra NZ, Pockman WT, Cochard H, Cruiziat P, Davis SD, Ewers FW, Tyree MT (1996) New evidence for large negative xylem pressures and their measurement by the pressure chamber method. Plant Cell Environ **19:** 427–436

Sperry JS, Sullivan JEM (1992) Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse-porous, and conifer species. Plant Physiol **100:** 605–613

Sperry JS, Tyree MT (1990) Water stress induced xylem embolism in three species of conifers. Plant Cell Environ **13:** 427–436

Tyree MT, Fiscus EL, Wullschleger SD, Dixon MA (1986) Detection of xylem cavitation in corn (*Zea mays*) under field conditions. Plant Physiol **82:** 597–599

Tyree MT, Salleo S, Nardini A, Lo Gullo MA, Mosca R (1999) Refilling of embolized vessels in young stems of laurel. Do we need a new paradigm? Plant Physiol **120:** 11–21

Zimmermann MH (1983) Xylem Structure and the Ascent of Sap. Springer-Verlag, Berlin