

Effects of summer drought and winter freezing on stem hydraulic conductivity of *Rhododendron* species from contrasting climates

ROBERTO A. CORDERO^{1–3} and ERIK T. NILSEN¹

¹ Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

² Present address: Smithsonian Tropical Research Institute, P.O. Box 2072, Balboa, Republic of Panama

³ Author to whom correspondence should be addressed (corderor@bci.si.edu)

Received June 28, 2001; accepted February 3, 2002; published online August 1, 2002

Summary We studied the limits to maximum water transport in three diffuse-porous evergreen shrubs exposed to frequent winter freeze–thaw events (*Rhododendron maximum* L. and *R. catawbiense* Michaux from the Appalachian Mountains) and to a severe summer drought (*R. macrophyllum* G. Don. from the Oregon Cascades). Percent loss of hydraulic conductivity (PLC), vulnerability curves to xylem embolism and freezing point temperatures of stems were measured over 2 years. Controlled freeze–thaw experiments were also conducted to determine the effect of thaw rate on PLC. During both years, native PLC was significantly higher in winter than in summer for *R. macrophyllum*. Seasonal changes in PLC were variable in both *R. catawbiense* and *R. maximum*. Only *R. maximum* plants growing in gaps or clearings showed higher PLC than understory plants. A rapid (2–4 day) natural recovery of high native PLC during the winter was observed in both *R. maximum* and *R. macrophyllum*. Compared with the bench-dehydration method, vulnerability curves based on the air-injection method consistently had less negative slopes and greater variation. Fifty percent PLC (PLC₅₀) obtained from vulnerability curves based on the dehydration method occurred at –1.75, –2.42 and –2.96 MPa for *R. catawbiense*, *R. maximum* and *R. macrophyllum*, respectively. Among the study species, *R. macrophyllum*, which commonly experiences a summer drought, had the most negative water potential at PLC₅₀. In all species, stem freezing point temperatures were not consistently lower in winter than in summer. A single fast freeze–thaw event significantly increased PLC, and *R. catawbiense* had the highest PLC in response to freezing treatments. Recovery to control PLC values occurred if a low positive hydraulic pressure was maintained during thawing. *Rhododendron macrophyllum* plants, which commonly experience few freeze–thaw events, had large stem diameters, whereas plants of *R. catawbiense*, which had small stem diameters, suffered high embolism in response to a single freeze–thaw event. Both drought-induced and winter-induced embolism caused a significant reduction in hydraulic conductivity in all species during periods when drought or freeze–thaw events occurred in their native habitats. However, rapid recovery of PLC follow-

ing freezing or drought maintained the species above their relatively low margins of safety for complete xylem dysfunction.

Keywords: Appalachians, cavitation, evergreen shrubs, freezing temperature, Oregon Cascades, xylem diameter.

Introduction

Plant adaptation to summer drought, winter freezing or both may include mechanisms that minimize the potential for embolism formation and its detrimental consequences. Vulnerability to xylem cavitation and embolism has been proposed as a major factor in the reduction of plant productivity (Tyree and Sperry 1988). The immediate consequence of embolism formation is a reduction in hydraulic conductivity of the xylem, which results in an increase in resistance to the vertical flow of water in stems and to leaves (Yang and Tyree 1993, 1994).

According to the air-seeding hypothesis, induction of cavitation resulting in embolism formation occurs during drought, and depends mostly on surpassing a critical water potential at the interface between the air and water meniscus in the pores of the pit membranes (Sperry 1995). Although vessel size is unrelated to drought-induced cavitation across taxa (Sperry and Sullivan 1992), there is a correlation within an individual (Sperry 1995). On the other hand, it is generally accepted that freeze–thaw-induced embolism is related to conduit volume because a greater amount of gas can be dissolved in a large vessel than in a small vessel, and larger embolisms can develop (Sperry 1995). Thus, small xylem conduits are less vulnerable to embolism caused by freeze–thaw events than large xylem conduits (Cochard and Tyree 1990, Wang et al. 1992). Similarly, the rate of thawing has been shown to affect the intensity of cavitation (Langan et al. 1997).

Independently of the cause of embolism, plants that naturally experience relatively high leaf water potentials are more likely to reach the point at which 100 percent loss of conductivity occurs than plants that experience more negative leaf water potentials (Sperry 1995). In addition to changes in leaf and stomatal traits, rapid recovery of hydraulic conductivity by refilling of embolized vessels must represent a viable strat-

egy for plants living close to catastrophic xylem dysfunction (Field and Holbrook 1989). However, detailed mechanisms for embolism repair are still unclear (Holbrook and Zwieniecki 1999, Tyree et al. 1999).

We studied a group of closely related species of evergreen *Rhododendron*, section *Pontica*, Ericaceae (Davidian 1992) as a representative system for evaluating the occurrence of cavitation in stems caused both by water stress and by freeze–thaw events during winter. We tested five hypotheses in an attempt to identify possible adaptations associated with xylem structure and vulnerability to xylem dysfunction. (1) *Rhododendron macrophyllum* G. Don (from a region prone to summer drought) is more resistant to water-stress-induced embolism than *R. maximum* L. or *R. catawbiense* Michaux (species from mesophytic habitats). (2) *Rhododendron* species from regions subjected to a high frequency of winter freezing (*R. maximum* and *R. catawbiense*) are more resistant to freezing-induced embolism than *R. macrophyllum*, which is from a region with mild winters. (3) Native loss of hydraulic conductivity will be higher in plants growing in open environments than in plants growing in the shaded understory. (4) Stem tissue freezing points will decrease with increasing frequency of natural freeze–thaw events. (5) Mean xylem diameter of *Rhododendron* species is inversely correlated with the frequency of winter freezing events in their native habitats.

Materials and methods

Study sites

The study was conducted at two sites: the H.J. Andrews Experimental Forest, in the Willamette National Forest, near Blue River, Oregon (44°12′ 43″ N, 122°15′ 21″ W; 410–1630 m a.s.l.), and the Coweeta Hydrological Laboratory, near Otto, North Carolina (35°02′ 29″ N, 83°27′ 16″ W; 679–1592 m a.s.l.). The H.J. Andrews Experimental Forest has a maritime climate with wet mild winters and dry cool summers (Van Cleve and Martin 1991). Mean monthly temperature ranges from 1 °C in January to 18 °C in July. Mean annual precipitation varies with elevation from 2300 mm at the base to 3550 mm at the upper elevations. Precipitation occurs mainly in November to March and low rainfall coincides with moderately high summer temperatures, creating a prominent seasonal water deficit from May to October. The mean annual freezing temperature of the *R. macrophyllum* stems and the daily minimum air temperature between October and April (1994–1998, Primary Meteorological Station, 430 m a.s.l., H.J. Andrews Experimental Forest, Oregon) were used to calculate the percentage of days with freeze–thaw cycles. The mean percentage of days with freeze–thaw events was 10.0% (21 events), and ranged from 6.5 to 12.1%.

Coweeta has a humid, maritime climate with warm summers, mild winters, and rainfall during all seasons. Annual precipitation is variable, with a mean of 1780 mm at lower elevations to over 2500 mm on upper slopes. Normal rainfall exceeds evapotranspiration all year, and droughts are rare (Helvey and Hewlett 1962). Mean monthly air temperature is

3.3 °C in January and 21.6 °C in July. Mean annual frequency of freeze–thaw events during the winter months is 54, ranging from 28 to 76 (Lipp and Nilsen 1997). *Rhododendron maximum* and *Kalmia latifolia* L. (Monk et al. 1985) dominate the Coweeta understory.

Species

The three *Rhododendron* study species are relatively large, broadleaf subcanopy shrubs, with similar leaf sizes. The rank order from largest to smallest stature is *R. maximum*, *R. catawbiense* and *R. macrophyllum*. *Rhododendron maximum* and *R. catawbiense* were from populations found at Coweeta. Shrubs of *R. maximum* are shade tolerant and form dense thickets at Coweeta that inhibit the establishment of other species (McGee and Smith 1967, Monk et al. 1985, Phillips and Murdy 1985, Clinton et al. 1994). In this study, *R. maximum* was from a population growing near Grady Trail at an elevation of 550 m a.s.l. Plants were marked in places in light gap conditions and in adjacent understory conditions. Total daily photon flux density (PFD) on a typical summer day in the light gap and shaded environments was 5.49 and 0.705 mol m⁻² day⁻¹, respectively. *Rhododendron catawbiense* does not form dense thickets and is located in high elevation regions of Coweeta in more exposed habitats. At Coweeta, we studied an *R. catawbiense* population found at the summit of Picken's Nose Trail, at an elevation of 1495 m a.s.l.

Rhododendron macrophyllum G. Don grows at all elevations in the Andrews Experimental Forest, and is common throughout the understory (Zobel et al. 1976). We studied a population in and adjacent to a 5-year-old clear-cut at 1500 m a.s.l., where we marked plants growing in the clear-cut (high light), the margin of the forest (intermediate light) and under mature forest (low light). Total PFD for a typical day during the summer was 34.88 and 0.517 mol m⁻² day⁻¹ in the high- and low-light environments, respectively.

Percent loss of conductivity

Native percent loss of conductivity (PLC), calculated from the reduction in stem hydraulic conductivity (K_H), was determined as described by Sperry et al. (1988). We collected branches of *R. maximum* and *R. catawbiense* from plants growing in the forest understory and in canopy light gaps. For *R. macrophyllum*, we collected branches from the clearing, intermediate and understory locations. We used three or more branches per measurement period. Mass flow rate (F ; kg s⁻¹) of solution (oxalic acid at 5 to 10 mM) under low hydraulic pressure (P ; 12–15 kPa) was measured in stem sections 8–12 cm in length (L) excised from collected branches (5–10 mm diameter). Vessel length ranged between 2 and 8 cm in all three species. After flushing with oxalic solution for 1 h at about 150 kPa, maximum flow rate was reached and maximum K_H was measured again at the initial hydraulic pressure. We calculated K_H as FL/P (kg m s⁻¹ MPa⁻¹).

Vulnerability curves to embolism

The relationship between xylem water potential and the reduction in K_H is a vulnerability curve to embolism (Sperry and

Saliendra 1994). We obtained vulnerability curves by measuring K_H after pressurizing stem sections with nitrogen gas (Cochard et al. 1992) in a cavitation chamber (PMS Instruments, Corvallis, OR). Stems segments were notched (5–7 cuts) to facilitate gas penetration. We measured K_H as described for PLC. No obvious leakage of notched segments was observed. Vulnerability curves were also constructed by means of the dehydration method (Sperry and Sullivan 1992), in which branches were allowed to dehydrate on the laboratory bench until a given leaf water potential was reached. Branch water potential equilibration was ensured by enclosing the branches in plastic bags for at least 2 h. Stem sections were then cut and the initial and final (maximum) hydraulic conductivity was measured. The vulnerability curve for *R. maximum* based on the dehydration method was obtained from Lipp and Nilsen (1997). The exponential sigmoid equation (ES), $PLC = 100/(1 + e^{a(\psi - b)})$, where ψ is injected pressure and a and b are constants, was fitted to the vulnerability curves (Pammenter and Vander Willigen 1998). Coefficient a is related to the gradient of the curve and coefficient b indicates the water potential at 50% PLC (PLC_{50}) (Pammenter and Vander Willigen 1998). The vulnerability curves were also fit to a least square linear regression (LSR). Both models produced the same value for PLC_{50} ; thus, only linear regressions are presented.

Stem freezing point temperature

The first freezing exothermal temperature (the temperature when apoplastic water freezes) is hereafter referred to as the freezing point temperature (Lipp and Nilsen 1997). Seasonal variation in freezing point temperature was measured over 2 years in three to six stems of detached branches from plants growing in the two (*R. maximum* and *R. catawbiense*) or three (*R. macrophyllum*) forest light environments. Temperature was measured with a copper-constantan thermocouple, which was attached to the stem surface with surgical tape (without damaging the tissue), connected to a data logger (Campbell CR-21X, Campbell Scientific, Logan, UT). To dampen the rate of temperature decline to simulate a freezing event in the field, entire branches were placed inside a plastic cooler that was located inside a domestic freezer.

Freeze–thaw experiments

A temperature-controlled chamber was constructed for the freeze–thaw experiments designed to test Hypothesis 2 (see Introduction). This system allowed us to apply constant freezing and thawing rates to three branches simultaneously, and to avoid the desiccation effect of freezing (Figure 1). The chamber consisted of a Styrofoam box with three isolated copper tubing coils connected in series. These copper coils were connected to an external cooling bath/circulator (RTE 140, Neslab, Portsmouth, NH) possessing a microprocessor controller for programming freezing and thawing rates. Only a 15-cm section of the stem was kept inside the chamber. The branch base protruded below the chamber and was connected to plastic tubing filled with water, which was connected to a water source that provided a low hydraulic pressure (~2 kPa).

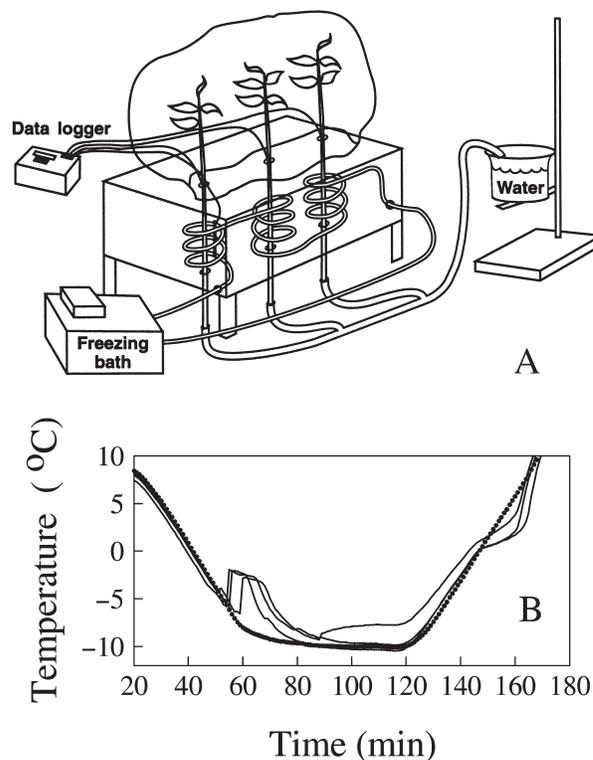


Figure 1. (A) Experimental setup for the freeze–thaw experiments. (B) Example of the temperature variation during a typical fast/fast freeze–thaw experiment on *Rhododendron* stems (solid line) and a dowel (dotted line) inside the freezing chamber.

The branch foliage emerged above the chamber and was kept inside a moistened plastic bag. Stem temperature was measured as above to ensure that stems reached freezing and thawing temperatures. The water connection was maintained only during the freezing phase of the experiments. The following experiments were performed: a control treatment in which the freezing and thawing rates were $6\text{ }^{\circ}\text{C h}^{-1}$ (slow/slow treatment, S/S); a treatment with a freezing rate of $6\text{ }^{\circ}\text{C h}^{-1}$ and a thawing rate of $25\text{ }^{\circ}\text{C h}^{-1}$ (slow/fast treatment, S/F); a treatment with a freezing rate of $25\text{ }^{\circ}\text{C h}^{-1}$ and a thawing rate of $6\text{ }^{\circ}\text{C h}^{-1}$ (fast/slow treatment, F/S); and a treatment with freezing and thawing rates of $25\text{ }^{\circ}\text{C h}^{-1}$ (fast/fast treatment, F/F). The minimum freezing temperature was $-10\text{ }^{\circ}\text{C}$ and was maintained for at least 60 min in all treatments to ensure complete freezing of stems. The natural cooling rate of *R. maximum* stems under field conditions is $3\text{ }^{\circ}\text{C h}^{-1}$ (Lip and Nilsen 1997). Initial tests at freezing rates of 3 and $6\text{ }^{\circ}\text{C h}^{-1}$ resulted in PLC similar to native unstressed PLC. A freeze–thaw rate of $6\text{ }^{\circ}\text{C h}^{-1}$ was chosen because it reduced the total time for a single freeze–thaw experiment. This rate is comparable to the natural ($0.1\text{--}0.2\text{ }^{\circ}\text{C min}^{-1}$) and experimental ($0.13\text{--}0.35\text{ }^{\circ}\text{C min}^{-1}$) warming rates used by Pockman and Sperry (1997). The leaf water potential of branches in the longest treatment (S/S, with the largest probability of dehydration effects) was always higher than -0.15 MPa just after the end of the thawing period. To test whether rapid refilling occurred, we included a fifth

treatment in which the water source was left attached during the thawing period of the F/F treatment, hereafter called the F/F/W (recovery) treatment. After each of these experiments, stem segments were recut under water and immediately connected to the manifold for PLC measurements.

Vessel diameters

Stem segments used for K_H measurements were preserved in 50% ethanol. These segments were sectioned (20–25 μm thick), dehydrated, stained with safranin and fixed on slides with Permunt. Five sections each from five plants were analyzed per light environment per species. Vessel diameter was determined for all vessels contained between two adjacent rays and from the vascular cambium to the pith. Series of printed photographs along the cross section were obtained (Video Graphic Printer, Sony, UP-890MD). Cell lumen diameter was measured directly from the photographs in two directions (the distance along the longest axis and the distance perpendicular to that axis), and mean vessel diameter ($n > 400$ per species) was calculated. Frequency distributions of vessel diameters were analyzed in four classes. The percentage contribution of each diameter class to total stem hydraulic conductivity was calculated as a proportion of the sum of the fourth power of all radii (Σr^4) and the hydraulically weighted diameter calculated as described by Sperry et al. (1994). Serial cross sections of entire branches stained with a natural pigment were studied to determine maximum vessel lengths. No vessels longer than 10 cm were found in *R. catawbiense* or *R. macrophyllum*. Lipp and Nilsen (1997) reported similar lengths for *R. maximum*, with only a few vessels longer than 10 cm.

Statistical analyses

A Wilcoxon rank-sum test (WSR) was used to compare mean native PLC and freezing point temperatures between microhabitats and between seasonal periods among species. The WSR test was also used to compare means between freeze-thaw treatments. Analyses of covariance (ANCOVA) were used to compare the relationships between PLC and ψ of the vulnerability curves by species, using the method as a categorical variable. Vessel diameters were tested by analysis of variance, and a post-hoc Scheffe test was used to compare means.

Results

Seasonal variation of native PLC

Mean values (\pm SD) of maximum K_H varied little among species. Gap plants of *R. maximum* had a maximum K_H of $1.96 \times 10^{-5} \pm 0.9 \times 10^{-5} \text{ kg m s}^{-1} \text{ MPa}^{-1}$ during September 1998, whereas *R. macrophyllum* had a maximum K_H of $9.94 \times 10^{-6} \pm 2.8 \times 10^{-6} \text{ kg m s}^{-1} \text{ MPa}^{-1}$ during January 1999. *Rhododendron maximum* did not show any marked seasonal variation in PLC. However, understory plants seemed to be more resistant to loss of K_H in winter than plants from light gap environments (Figure 2A). *Rhododendron catawbiense* showed a seasonal increase in PLC in winter. Although there were no consistent

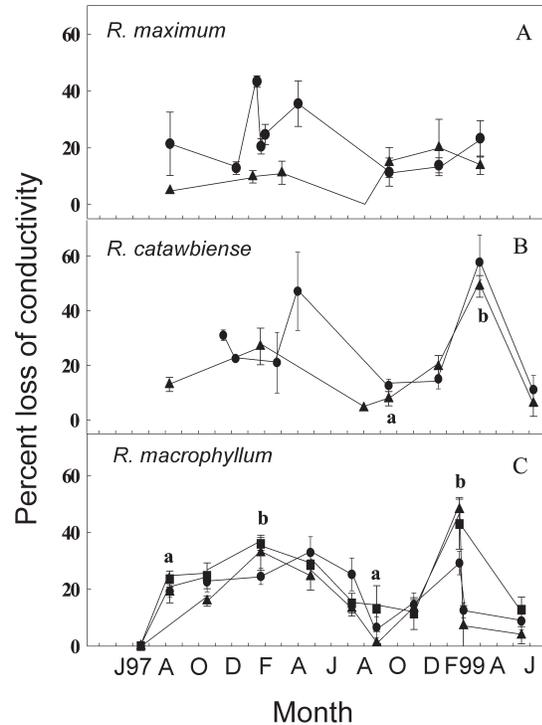


Figure 2. Seasonal variation in native percent loss of conductivity (\pm SEM) in three *Rhododendron* species. Habitats with different light environments are included: ● = environment with light gaps; ■ = intermediate shade; and ▲ = forest understory. Letters indicate statistically significant differences between seasonal means. A Wilcoxon rank-sum test was used to compare means when the assumptions for a two-sample test were invalidated.

differences in PLC between forest microenvironments, understory *R. catawbiense* plants tended to have lower PLC values than plants growing in canopy gaps (Figure 2B). Maximum PLC for *R. catawbiense* was as high as 70% at the end of the winter. *Rhododendron macrophyllum* had PLC values between 0 and 20% during non-winter months, and PLC increased to 40–50% in midwinter (Figure 2C). *Rhododendron macrophyllum* did not show strong differences in PLC between light microenvironments (Figure 2C). Values of PLC varied greatly within several days in winter, changing from 30 to 10% in *R. maximum* and from 50 to 10% in *R. macrophyllum* over a few days (Figures 2A and 2C).

Vulnerability curves

Because parameters obtained from the ES and LSR models and applied to the vulnerability curves constructed by means of the air-injection method did not differ significantly between species, the simplest model is presented. In both *R. maximum* and *R. macrophyllum*, the treatment effects and their interaction were all significant (ANCOVA, Table 1; Figure 3). For *R. catawbiense*, the treatment effect was not significant, but the interaction term was significant (Table 1, Figure 3). In all species, the air-injection method yielded PLC₅₀ values consistently lower than those obtained by the dehydration method. This difference varied from -0.5 MPa in *R. macrophyllum* to

Table 1. Summary of ANCOVA used to compare vulnerability curves generated by two methods for three *Rhododendron* species from the Appalachians and the Oregon Cascades. Sources of variation: Ψ = water potential; Method = dehydration or air-injection. Abbreviations and symbols: r^2 = coefficient of determination; ns = not significant; and ** = $P < 0.01$.

Source	<i>R. maximum</i>	<i>R. catawbiense</i>	<i>R. macrophyllum</i>
Ψ	**	**	**
Method	**	ns	**
Interaction	**	**	**
r^2	0.74	0.70	0.74

–1.35 MPa in *R. catawbiense*. The PLC values derived with the dehydration method from the LSR model were significantly different between species, and the coefficients of determination were generally higher than with the ES model (Table 2). The PLC₅₀ showed that drought susceptibility to embolism decreased in the order *R. catawbiense* > *R. maxi-*

Table 2. Parameters obtained from the least squares regression fit of the vulnerability curves (leaf Ψ versus PLC) of three *Rhododendron* species from the Appalachians and the Oregon Cascades. Data for *R. maximum* (dehydration method) were obtained from Lipp and Nilsen (1997). Water potential (Ψ) at PLC₅₀ is also shown.

Species	<i>R. maximum</i>	<i>R. catawbiense</i>	<i>R. macrophyllum</i>
No. of branches	11	7	10
<i>Air-injection method</i>			
Slope	–9.83	–10.8	–9.47
Intercept	19.32	13.26	16.5
r^2	0.65	0.74	0.66
P	0.0001	0.0001	0.0001
Ψ at PLC ₅₀	–3.1	–3.4	–3.5
<i>Dehydration method</i>			
Slope	–18.4	–18.6	–19.0
Intercept	5.45	17.4	–6.2
r^2	0.80	0.61	0.95
P	0.0001	0.0001	0.0001
Ψ at PLC ₅₀	–2.42	–1.75	–2.96

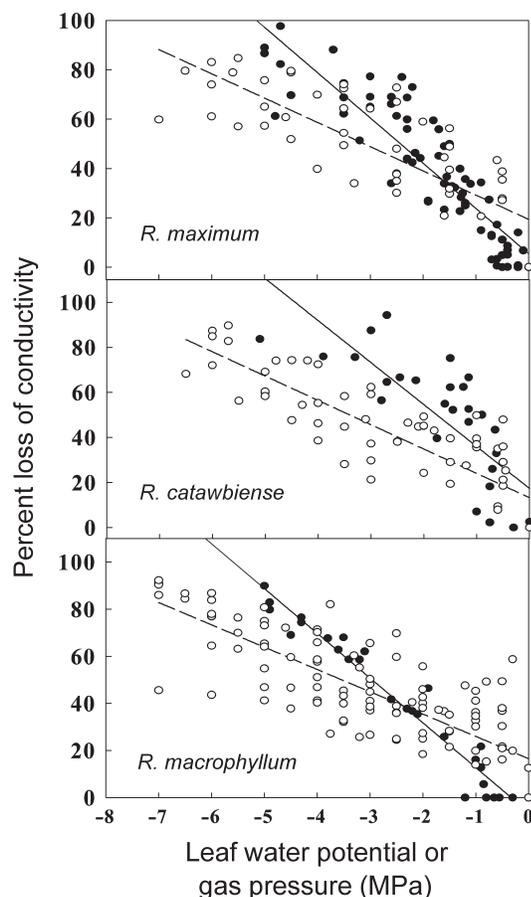


Figure 3. Vulnerability curves to xylem cavitation in three *Rhododendron* species. Linear regression fitting is included for the data sets obtained by means of the air-injection method (○) and the dehydration method (●).

mum > *R. macrophyllum*, with PLC values of –1.75, –2.42 and –2.96, respectively (Table 2). However, the slopes of PLC versus stem water potential relationships were similar among species (Table 2). Both Appalachian species yielded positive intercept values.

Freezing point temperatures

There was little evidence for significant seasonal variation in stem freezing point temperatures in any of the species (Figure 4). In *R. maximum*, freezing points varied from –1.6 °C during August in gap-grown stems to –3.3 °C during February in understory stems. In high light, *R. catawbiense* tended to have lower stem freezing points in winter than in summer (–4.6 versus –1.4 °C; Figure 4B). However, the patterns did not hold in the second year in these two species. Variation in stem freezing temperatures of *R. macrophyllum* did not show consistent differences in response to light environment. The lowest mean freezing point temperature observed was –4.3 °C in May 1999 in plants grown in intermediate light.

Freeze–thaw experiments

The freezing chamber produced precise and constant variation in freezing and thawing rates (Figure 1B). With the exception of *R. catawbiense*, a single slow freeze–thaw event produced a PLC similar to the native PLC observed in summer in these plants (Figure 5). The PLC of plants in the intermediate treatments (F/S and S/F) did not differ from that of plants in the extreme treatments in either *R. maximum* or *R. macrophyllum*. However, the F/F treatment increased mean PLC significantly compared with the control treatment (S/S) in all three species (Figure 5). *Rhododendron catawbiense* had a mean PLC of 45.2% in the control treatment (similar to natural rates), which contrasted significantly with mean PLC values of 14.4 and 23.0% in *R. maximum* and *R. macrophyllum*, respectively

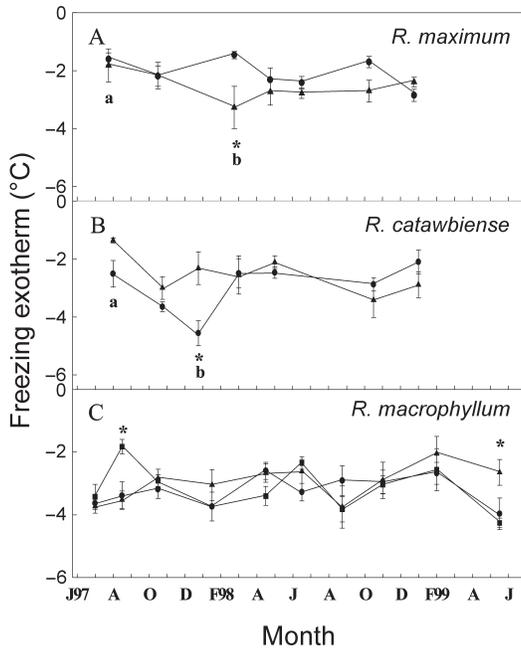


Figure 4. Freezing point temperatures (\pm SEM) for stem segments from three *Rhododendron* species. Habitats with different light environments are included: ● = light gaps; ■ = intermediate shade; and ▲ = forest understory. Letters indicate differences between seasonal means. Asterisks indicate statistically significant differences between light environments. A Wilcoxon rank-sum test was used to compare means when the assumptions for a two-sample *t*-test were invalidated.

(Figure 5). All species showed a significant reduction in PLC in the F/F/W treatment compared with the F/F treatment. Percent loss of conductivity in the two Appalachian species recovered to values similar to those in the control treatment. However, *R. macrophyllum* had a significantly smaller mean PLC in the F/F/W treatment than in the control treatment (Figure 5). Among species, *R. catawbiense* showed the greatest variation in PLC. In this species, PLC was 57% in the recovery treatment.

Vessel diameter

Mean vessel diameter differed significantly between species (in ascending order: *R. catawbiense* < *R. maximum* < *R. macrophyllum*; Table 3, Figure 6). Our hypothesis that species from habitats with a higher frequency of freeze–thaw events have smaller vessel sizes was confirmed. In *R. macrophyllum*, mean vessel diameters were smaller in plants growing in shady environments than in open environments (Table 3, Figure 6). In *R. catawbiense* and *R. maximum*, 30 and 38% of vessels were larger than 16 μ m in diameter, which contributed 70 and 74.2% of the total hydraulic conductivity, respectively. In *R. macrophyllum*, 20, 70 and 75% of vessels were larger than 16 μ m in the shady, intermediate and open microenvironments, respectively, and they contributed 85, 94 and 95% of total hydraulic conductivity (Figure 6).

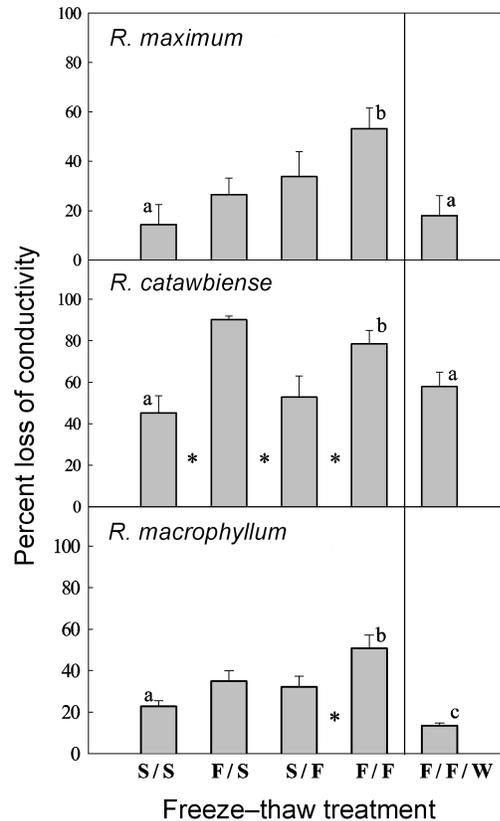


Figure 5. Mean percent loss of conductivity \pm SEM following several freeze–thaw experiments on branches of three *Rhododendron* species. Abbreviations: S/S = slow (6°C h^{-1}) freezing and thawing rates (control treatment); F/S = fast (25°C h^{-1}) freezing and slow thawing rate; S/F = slow freezing and fast thawing rate; F/F = fast freezing and thawing rates; and F/F/W = fast freezing and thawing rates, with water source attached during thawing period. Asterisks indicate significant differences between adjacent columns. Different letters indicate significant differences between treatments. The Wilcoxon rank-sum test was used to compare means.

Discussion

Analysis of methods used to construct vulnerability curves

The two techniques used to construct vulnerability curves yielded different results. We conclude that the dehydration method yielded data that best represent the vulnerability of the vascular system to drought-induced embolism for two reasons. First, plants experiencing relatively high minimum leaf water potentials (such as the study species) live relatively close to the point of 100 percent loss of conductivity (PLC_{100}), representing a low margin of safety (Sperry 1995). Our data from the dehydration method indicated that the study species had a mean safety margin of -2.8 MPa, which is similar to other species that experience high minimum water potentials (Sperry 1995). Second, if we consider the water potential at PLC_{100} from the vulnerability curves produced by the air-injection method, a safety margin of -6.25 MPa is predicted. Such high safety margins characterize species that maintain

Table 3. Mean (\pm SD) and maximum diameters of xylem vessels and hydraulically weighted diameter (see Materials and methods for a description of the calculation of hydraulically weighted diameters) for three *Rhododendron* species. Letters indicate a significant difference between means (Scheffe test) (n = sample size).

Species	Light environment	n	Mean diameter (μm)	Maximum diameter (μm)	Hydraulic diameter (μm)
<i>R. maximum</i>		525	15.20 (3.77) a	27.40	18.70
<i>R. catawbiense</i>		565	14.40 (3.88) b	28.32	18.40
<i>R. macrophyllum</i>	Understory	540	16.48 (4.08) c	32.14	20.50
<i>R. macrophyllum</i>	Intermediate	464	18.70 (4.75) d	33.90	23.14
<i>R. macrophyllum</i>	Clearing	432	18.80 (4.45) d	30.26	22.41

natural water potentials of -4 to -6 MPa (Sperry 1995). Therefore, the dehydration method more closely predicts the safety margin to catastrophic embolism in the studied *Rhododendron* species, which is only slightly less negative than those predicted for many other species. Small safety margins are not restricted to plants in mesic environments. For example, in

Mediterranean environments, some congener species with contrasting leaf habit and xylem vessel sizes are also living close to their safety limit (Tognetti et al. 1998).

Three aspects of the air-injection technique may have contributed to the production of vulnerability curves with consistently less negative slopes than those obtained by means of the dehydration method. First, rapid refilling can occur during the time between pressurization and the actual flow measurements (Jarbeau et al. 1995), which can produce a false K_H reading, resulting in a decrease in the slope of the vulnerability curve. The time between pressurization and flow measurements in our study increased with increasing pressure, from 5 min to almost 40 min, and some refilling may have occurred during these periods given the high tendency of these branches to refill following embolism. Second, it is possible that the pressurization technique has a large wound effect on pressurized stems (Rood et al. 2000, M.A. Zwieniecki, Harvard University, Cambridge, MA, personal communication) that may cause an increase in flow during the flushing period as a result of the loss of tissue and membrane integrity. And third, although more than five notches were made in each pressurized stem, they may not have been completely effective, favoring relatively smaller cavitations as pressures increased.

Comparison of vulnerability curves among species

Rhododendron macrophyllum, which grows in habitats with a distinct summer drought period, was less susceptible to drought-induced cavitation than species from regions characterized by seasonally constant precipitation, as predicted by Hypothesis 1. *Rhododendron macrophyllum* also had the largest vessel diameters, which supports Hypothesis 5 and confirms that drought-induced embolism is unrelated to vessel size (Tyree and Sperry 1989). The relatively low vulnerability to drought-induced cavitation of *R. macrophyllum* may account for the maintenance of native PLC between 10 and 30% during the summers of both years. The positive intercept values obtained for the Appalachian species suggest that refilling was more common in these species than in *R. macrophyllum*.

Seasonal and light effects

Maximum K_H values in *Rhododendron* species are similar to those of *Acer saccharum* Marsh. stems 2–5 mm in diameter, and *Thuja occidentalis* L. stems 5–10 mm in diameter (Tyree

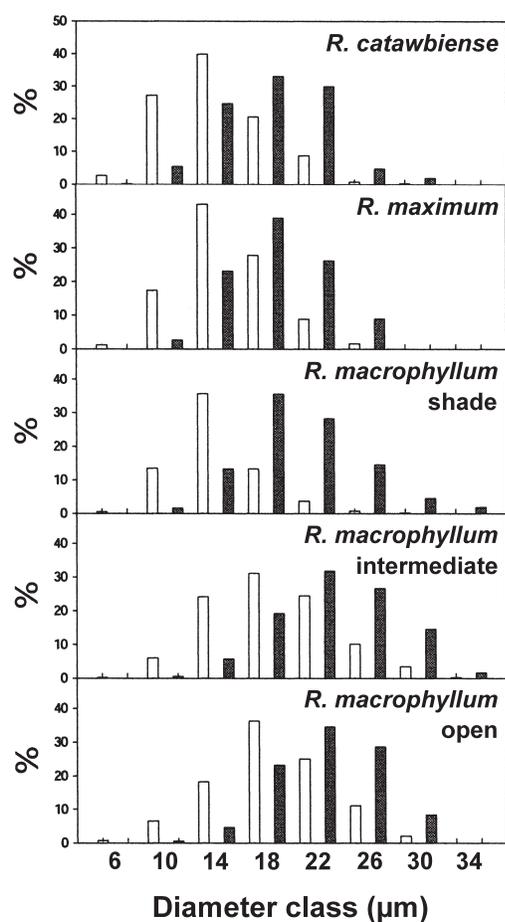


Figure 6. Lumen diameter and hydraulically weighted volume frequency distribution of vessels in three *Rhododendron* species from eastern and western USA. See Materials and methods for a description of the calculation of hydraulically weighted diameters. Table 3 shows the corresponding mean diameters and hydraulically weighted diameters per species.

et al. 1991). Similar values have been reported for 5–10 mm diameter stems of several tropical tree species (Zotz et al. 1998).

Winter-induced embolism could have an important effect on water relations of the *Rhododendron* species. Embolism was highest during winter (February–March) in all species in both years of the study, with PLC values reaching more the 60%.

Light availability significantly affected PLC, but the effects were not consistent between species or years. During the first winter, *R. maximum* and *R. catawbiense* plants in high-light sites had greater PLC than plants in low-light sites, supporting Hypothesis 3. However, light-acclimated shoots of *Fagus sylvatica* L. are less vulnerable to embolism than shoots acclimated to 90% shade (Cochard et al. 1999).

There were no seasonal or light effects on PLC of *R. macrophyllum* in either year or on the other two species during the second year of the study. Winter PLC was consistently high in *R. macrophyllum*, whereas winter PLC varied greatly in *R. maximum* and *R. catawbiense*, suggesting that *R. macrophyllum* had a greater sensitivity to freeze–thaw-induced embolism, supporting Hypothesis 2. Moreover, *R. macrophyllum* plants had the largest mean vessel diameters, supporting Hypotheses 2 and 5.

Hypothesis 4 was supported by the data from *R. maximum* and *R. catawbiense* showing a significant reduction in mean stem freezing temperature in winter compared with summer, but only in shade and open-canopy stems, respectively. In addition, as predicted by Hypothesis 4, we found no seasonal or microsite variation in stem freezing temperatures in *R. macrophyllum*.

Freeze–thaw-induced cavitation

Between the Appalachian species, Hypothesis 2 predicted that susceptibility to winter-induced embolism was more likely in *R. maximum*. Lipp and Nilsen (1997) found significant seasonal variation in PLC during the warm winters at Coweeta, North Carolina and less variation during the cool winters in Virginia, suggesting that plants from Coweeta had greater sensitivity to freeze–thaw events than plants from Virginia. However, a comparison of the two species from Coweeta indicated that *R. catawbiense* was more susceptible to freeze–thaw-induced embolism than *R. maximum*. This effect might be associated with the difference in elevation (> 1000 m a.s.l.) between sites, which resulted in the studied *R. catawbiense* population experiencing more freeze–thaw events per year than the studied *R. maximum* population. In addition, in the freeze–thaw experiments, PLC of control *R. catawbiense* plants was higher than in the other two species. Moreover, the reduced capacity of *R. catawbiense* to recover from a single freeze–thaw event, even with a small positive pressure, further indicates that it is a highly vulnerable species. *Rhododendron catawbiense* also had the smallest safety margin to catastrophic embolism among the species.

Davis et al. (1999) found that species with xylem diameters larger than 30 mm were extremely sensitive to cavitation by freezing at a xylem pressure of –0.5 MPa. The studied *Rhododendron* species had a mean vessel diameter less than 30 mm,

but PLC was significantly increased by a single fast freeze–thaw event. *Rhododendron catawbiense* was the most sensitive among the study species and also had the smallest mean vessel diameter. Thus, our results do not strictly conform with the findings of Davis et al. (1999), even though the freezing and thawing rates were similar in both studies and we used larger branch structures attached to a water source so that our freeze–thaw experiments would be more realistic than those performed in a centrifuge with cut stems.

Low rates of freezing and thawing produced cavitation percentages similar to native PLC, indirectly suggesting that a high rate of stem cooling or warming to a constant minimum temperature will significantly increase cavitation in these species. Studies on *Larrea tridentata* (DC.) Cov. (Pockman and Sperry 1997) did not reveal any relationship between cooling or warming rates and embolism formation; however, the minimum freezing temperature was below –11 °C. Some embolisms have been obtained in other species with higher freezing temperatures (Langan et al. 1997, Davis et al. 1999). Davis et al. (1999) warmed and cooled stem sections under a constant pressure of –0.5 MPa, which tends to overestimate cavitation associated with a single freeze–thaw cycle by favoring bubble expansion and thus cavitation. In contrast, the method we used favors rapid dissolution of bubbles and hence decreases the likelihood of cavitation. It is possible that the unfrozen sections above and below the frozen section buffer the pressure changes occurring during the freezing and melting of xylem sap. Although we do not know how this enhances cavitation, the unfrozen stem areas could provide the pressure release necessary during freezing to avoid bubble shrinking. In addition, water released to the rest of the xylem could compensate pressures and extend the dissolution times of bubbles (Sucoff 1969). We have no explanation for the significant effect of the faster freezing rate on *R. catawbiense*.

We interpreted the observed recovery of K_H as evidence of refilling of embolized vessels, which seems to be a plausible mechanism for repair of xylem dysfunction in *Rhododendron*. Rapid recovery of hydraulic conductivity after significant embolism events has been observed in *R. ponticum* L. and *R. maximum* (Crombie et al. 1985, Lipp and Nilsen 1997). In *Populus*, refilling of embolized elements after one freeze–thaw event occurred after 2 days when cut branches were stored without pressure (Just and Sauter 1991). In *Pinus sylvestris*, reversal of embolism occurred even in the presence of some xylem tension, but the rate of recovery was greater when xylem tension was small (Sobrado et al. 1992). Also, percent flux recovery was greater when the initial relative water content of stems was high (Sobrado et al. 1992). Moreover, root pressure can remove air from winter embolized vessels in grapevine in spring (Sperry et al. 1987). Atmospheric pressures of 0.10 MPa can result in recovery of freeze-induced cavitated vessels in *Acer rubrum* L. (Sauter 1984). Among possible refilling mechanisms, root pressure may occur in *Rhododendron* (Lipp and Nilsen 1997). Many other diffuse-porous species are known to exhibit root pressure in the spring (Sperry and Sullivan 1992, Sperry et al. 1994, Davis et al. 1999). We found that a small positive pressure, even lower

than the root pressures reported for shrubs (Pickard 1989), was sufficient for K_H to fully recover in two of the *Rhododendron* species.

The studied *Rhododendron* species had small vessel diameters and were relatively sensitive to drought-induced embolism. However, the species with the largest vessels had the greatest tolerance to drought-induced cavitation and came from a drought-prone environment. Vulnerability to cavitation was closely associated with the relatively high water potentials that these species experience. The study species maintain a safety factor that is similar to that of other species with high water potentials, but unlike that of other species with small vessels. Although there was only a slight difference in freeze-thaw susceptibility among study species, the species with the smallest diameter vessels was the most susceptible to freeze-thaw-induced cavitation. This finding indicates that small vessel diameters did not prevent cavitation and suggests that refilling following freeze-thaw-induced cavitation is the main mechanism by which these species overcome stress caused by drought and freeze-thaw events in their native ranges. We found only slight evidence for adaptation of xylem hydraulic properties to habit condition. This could mean that phylogenetic constraints on vascular structure are strong, preventing adaptation of hydraulic properties to regional climate. However, microsite, seasonal and yearly differences in drought and winter intensity seem to influence individual plant performance and may explain a portion of the variability found. Alternatively, these species may utilize other physiological properties to adjust water relations to the specific environment. Further studies on a wider diversity of *Rhododendron* species and habitats will help distinguish among these scenarios.

Acknowledgments

We thank B. Gartner and R. Spicer for their help with the logistics, support, and their invaluable assistance with wood microtechniques. Thanks to B. Engelbrecht for detailed reviews of previous versions of this manuscript. We are grateful to the Coweeta Hydrological Lab (North Carolina) and the Andrews Experimental Forest (Oregon) for facilities provided during the fieldwork, and to the Smithsonian Tropical Research Institute at BCI for facilities provided during data analysis and preparation of this paper. T. Lei and S. Semones helped us in the field. This study was supported by NSF grant IBN 9630791.

References

- Clinton, B.D., L.R. Boring and W.T. Swank. 1994. Regeneration patterns in canopy gaps of mixed-oak forests of the southern Appalachians: influences of topographic position and evergreen understory. *Am. Mid. Nat.* 132:308–319.
- Cochard, H. and M.T. Tyree. 1990. Xylem dysfunction in *Quercus*: vessel sizes, tyloses, cavitation, and seasonal changes in embolism. *Tree Physiol.* 6:393–407.
- Cochard, H., P. Cruziat and M.T. Tyree. 1992. Use of positive pressures to establish vulnerability curves. *Plant Physiol.* 12:445–453.
- Cochard, H., D. Lemoine and E. Dreyer. 1999. The effects of acclimation to sunlight on the xylem vulnerability to embolism in *Fagus sylvatica* L. *Plant Cell Environ.* 22:101–108.
- Crombie, D.S., M.F. Hipkins and J.A. Milburn. 1985. Gas penetration of pit membranes in the xylem of *Rhododendron* as the cause of acoustically detectable sap cavitation. *Aust. J. Plant Physiol.* 12: 445–453.
- Davidian, H.H. 1992. The *Rhododendron* species. Vol. III. Elepidotes continued (Neriiflorum-Thomsonii, Azaleastrum and Camtschaticum). Timber Press, Portland, OR, 429 p.
- Davis, S.D., J.S. Sperry and U.G. Hacke. 1999. The relationship between xylem conduit diameter and cavitation caused by freezing. *Am. J. Bot.* 86:1367–1372.
- Field, C.B. and N.M. Holbrook. 1989. Catastrophic xylem failure: tree life at the brink. *Trends Ecol. Evol.* 4:124–126.
- Helvey, J.D. and J.D. Hewlett. 1962. The annual range of soil moisture under high rainfall in the Southern Appalachians. *J. For.* 60: 485–486.
- Holbrook, N.M. and M.A. Zwieniecki. 1999. Embolism repair and xylem tension: do we need a miracle? *Plant Physiol.* 120:7–10.
- Jarbeau, J.A., F.W. Ewers and S.D. Davis. 1995. The mechanism of water-stress-induced embolism in two species of chaparral shrubs. *Plant Cell Environ.* 18:189–196.
- Just, J. and J.J. Sauter. 1991. Changes in hydraulic conductivity upon freezing of xylem of *Populus × canadensis* Moench “Robusta.” *Trees* 5:117–121.
- Langan, S.J., F.W. Ewers and S.D. Davis. 1997. Xylem dysfunction caused by water stress and freezing in two species of co-occurring chaparral shrubs. *Plant Cell Environ.* 20:425–437.
- Lipp, C.C. and E.T. Nilsen. 1997. The impact of subcanopy light environment on the hydraulic vulnerability of *Rhododendron maximum* to freeze-thaw cycles and drought. *Plant Cell Environ.* 20: 1264–1272.
- McGee, C.E. and R.C. Smith. 1967. Undisturbed rhododendron thickets are not spreading. *J. For.* 65:4334–4336.
- Monk, C.D., D.T. McGinty and F.P. Day, Jr. 1985. The ecological importance of *Kalmia latifolia* and *Rhododendron maximum* in the deciduous forest of the southern Appalachians. *Bull. Torrey Bot. Club* 112:187–193.
- Pammenter, N.W. and C. Vander Willigen. 1998. A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree Physiol.* 18:598–593.
- Phillips, D.L. and W.H. Murdy. 1985. Effects of rhododendron (*Rhododendron maximum* L.) on the regeneration of southern Appalachian hardwoods. *For. Sci.* 31:226–233.
- Pickard, W.F. 1989. How might a tracheary element which is embolized by day be healed by night? *J. Theor. Biol.* 141:259–279.
- Pockman, W.T. and J.S. Sperry. 1997. Freezing-induced xylem cavitation and the northern limit of *Larrea tridentata*. *Oecologia* 109: 19–27.
- Rood, S.B., S. Patiño, K. Coombs and M.T. Tyree. 2000. Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. *Trees* 14:248–257.
- Sauter, J.J. 1984. Detection of embolization of vessels by a stain technique. *J. Plant Physiol.* 116:331–342.
- Sobrado, M.A., J. Grace and P.G. Jarvis. 1992. The limits of xylem recovery in *Pinus sylvestris* L. *J. Exp. Bot.* 43:831–836.
- Sperry, J.S. 1995. Limitations on stem water transport and their consequences. *In* Plant Stems, Physiology and Functional Morphology. Ed. B.L. Gartner. Academic Press, San Diego, pp 105–124.
- Sperry, J.S. and N.Z. Saliendra. 1994. Intra- and interplant variation in xylem cavitation in *Betula occidentalis*. *Plant Cell Environ.* 17: 1233–1241.
- Sperry, J.S. and J.E.M. Sullivan. 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, J.S., N.M. Holbrook, M.H. Zimmermann and M.T. Tyree. 1987. Spring refilling of vessels in wild grapevine. *Plant Physiol.* 83:414–417.
- Sperry, J.S., J.R. Donnelly and M.T. Tyree. 1988. A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Environ.* 11:35–40.
- Sperry, J.S., K.L. Nichols, J.E.M. Sullivan and S.E. Eastlack. 1994. Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. *Ecology* 75:1736–1752.
- Sucoff, E. 1969. Freezing of conifer xylem sap and the cohesion tension theory. *Physiol. Plant.* 22:424–431.
- Tognetti, R., A. Longobucco and A. Raschi. 1998. Vulnerability of xylem to embolism in relation to plant hydraulic resistance in *Quercus pubescens* and *Quercus ilex* co-occurring in a Mediterranean coppice stand in central Italy. *New Phytol.* 139:437–447.
- Tyree, M.T. and J.S. Sperry. 1988. Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? *Plant Physiol.* 88:574–580.
- Tyree, M.T. and J.S. Sperry. 1989. Vulnerability of xylem to cavitation and embolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40:19–38.
- Tyree, M.T., D.A. Snyderman, T.R. Wilmot and J.L. Machado. 1991. Water relations and hydraulic architecture of a tropical tree (*Schefflera morototoni*). *Plant Physiol.* 96:1105–1113.
- Tyree, M.T., S. Salleo, A. Nardini, M.A. Lo Gullo and R. Mosca. 1999. Refilling of embolized vessels in young stems of Laurel. Do we need a new paradigm? *Plant Physiol.* 120:11–21.
- Van Cleve, K. and S. Martin. 1991. Long-term ecological research in the United States. LTER Network Office, Univ. Washington, Seattle, WA, 178 p.
- Wang, J., N.E. Ives and M.J. Lekowics. 1992. The relation of foliar phenology to xylem embolism in trees. *Funct. Ecol.* 6:469–475.
- Yang, S. and M.T. Tyree. 1993. Hydraulic resistance in *Acer saccharum* shoots and its influence on leaf water potential and transpiration. *Tree Physiol.* 12:231–242.
- Yang, S. and M.T. Tyree. 1994. Hydraulic architecture of *Acer saccharum* and *A. rubrum*: comparison of branches to whole trees and the contribution of leaves to hydraulic resistance. *J. Exp. Bot.* 45:179–186.
- Zobel, D.B., A. McKee, G.M. Hawk and C.T. Dyrness. 1976. Relationship of environment to composition, structure, and diversity of forest communities of the central western Cascades of Oregon. *Ecol. Monogr.* 46:135–156.
- Zotz, G., M.T. Tyree, S. Patiño and M.R. Carlton. 1998. Hydraulic architecture and water use of selected species from a lower montane forest in Panama. *Trees* 12:302–309.