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The mechanism of water-stress-induced embolism in two species of chaparral shrubs

J. A. JARBEAU[1], F. W. EWERS[2] & S. D. DAVIS[1]

[1]Natural Science Division, Pepperdine University, Malibu, CA, 90263, USA, and [2]Department of Botany and Plant Physiology, Michigan State University, E. Lansing, MI 48824, USA

ABSTRACT

The mechanism of water-stress-induced embolism of xylem was investigated in *Malosma laurina* and *Heteromeles arbutifolia*, two chaparral shrub species of southern California. We tested the hypothesis that the primary cause of xylem dysfunction in these species during dehydration was the pulling of air through the pores in the cell walls of vessels (pores in pit membranes) as a result of high tensions on xylem water. First, we constructed vulnerability-to-embolism curves for (i) excised branches that were increasingly dehydrated in the laboratory and (ii) hydrated branches exposed to increasing levels of external air pressure. Branches of *M. laurina* that were dehydrated became 50% embolized at a xylem pressure potential of –1·6 MPa, which is equal in magnitude but opposite in sign to the +1·6 MPa of external air pressure that caused 50% embolism in hydrated stems. Dehydrated and pressurized branches of *H. arbutifolia* reached a 50% level of embolism at –6·0 and +6·4 MPa, respectively. Secondly, polystyrene spheres ranging in diameter from 20 to 149 nm were perfused through hydrated stem segments to estimate the pore size in the vessel cell walls (pit membranes) of the two species. A 50% or greater reduction in hydraulic conductivity occurred in *M. laurina* at perfusions of 30, 42, 64 and 82 nm spheres and in *H. arbutifolia* at perfusions of 20 and 30 nm spheres. Application of the capillary equation to these pore diameters predicted 50% embolism at xylem tensions of –2·2 MPa for *M. laurina* and –6·7 MPa for *H. arbutifolia*, which are within 0·7 MPa of the actual values. Our results suggest that the size of pores in pit membranes may be a factor in determining both xylem efficiency and vulnerability to embolism in some chaparral species. *H. arbutifolia*, with smaller pores and narrower vessels, withstands lower water potentials but has lower transport efficiency. *M. laurina*, with wider pores and wider vessels, has a greater transport efficiency but requires a deeper root system to help avoid catastrophically low water potentials.

Key-words: *Heteromeles arbutifolia*; *Malosma laurina*; chaparral; embolism; hydraulic conductivity; pit membrane; water stress; xylem.

Correspondence: Stephen D. Davis, Natural Science Division, Pepperdine University, Malibu CA, 90263, USA.

INTRODUCTION

Transpiration at the leaf surface pulls liquid water from the soil, through the plant, and into the atmosphere as a water continuum (Van den Honert 1948). The evaporative pull of transpiration creates a tension (negative pressure) on the xylem water of a plant. To move up the stem, water must travel not only through the lumens of xylem vessels but also between adjoining vessels via pit membranes. The pit membrane is a degraded primary cell wall containing pores that facilitate the passage of water between vessels while preventing air bubbles and pathogens from passing between them (Crombie, Hipkins & Milburn 1985; Van Alfen *et al.* 1983). If the water in the xylem conduits comes under severe tension as a result of water stress, the water column may cavitate, resulting in an air embolism (blockage) in the xylem vessel. The result is an overall reduction in hydraulic conductivity in the stem, further exacerbating plant water stress (Tyree & Sperry 1988,1989).

One proposed mechanism for water-stress-induced embolism, known as the 'air-seeding hypothesis', holds that air penetration through pores in the walls of vessels and tracheids is the cause of xylem dysfunction (Oertli 1971; Zimmermann 1983). Air-seeding is thought to occur when air is pulled into the lumen of a conducting vessel either from an adjacent vessel which was previously embolized or from the surrounding intracellular spaces filled with air (Fig. 1). When air enters the conducting vessel, the water column under tension from transpirative pull breaks, causing the vessel to fill with air. Subsequent cavitations in surrounding vessels will occur until the air reaches pores small enough to prevent the passage of an air bubble through them. At that point, an air–water meniscus will form in the pores between embolized and non-embolized vessels and will remain intact as long as the tension on the water column does not increase further (Tyree & Ewers 1991).

The pressure gradient (ΔP in MPa) required to break the air-water meniscus in the pores of pit membranes can be calculated using the capillary equation, modified according to Sperry & Tyree (1988):

$$\Delta P = 4\,(T/D), \qquad (1)$$

where T (N m^{-1}) represents the surface tension of the xylem water and D (μm) is the diameter of a pore in the pit

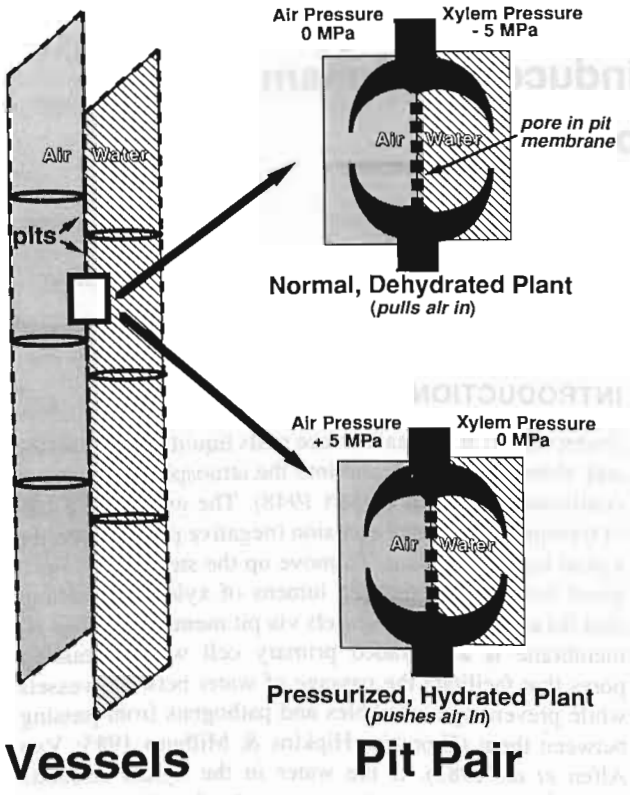

Figure 1. Diagram of two adjacent vessels with a close-up view of pits containing pit membranes with pores. The vessel on the right conducts water and is fully functional while the vessel on the left is embolized with air. A meniscus in the pores of the pit membrane prevents air from entering the functional vessel. According to the air-seeding hypothesis, the pressure required to force air through the largest inter-vessel pore and embolize the water-filled vessel is equal in magnitude but opposite in sign to the tension required to pull air into that same vessel during water stress. The pressure required to break the meniscus can be calculated by the capillary equation ($\Delta P = 4\ T/D$) which is a function of the surface tension of xylem water (T) and pore diameter (D).

membrane. This equation indicates that small pores can withstand higher xylem tensions than larger pores without disruption of the air–water meniscus, potentially leading to vessel cavitation. Equation 1 also predicts that the positive pressure required to force air through the largest pore of a water-filled vessel should be equal in magnitude but opposite in sign to the tension required to pull air into that vessel by water stress (Fig. 1).

Various methods have been employed to test this hypothesis. Crombie, Hipkins & Milburn (1985) showed that gas pressure applied to one end of stem segments of *Rhododendron* caused an increase in the expression of sap, an increase in permeability to gas, and an increase in cavitation events detected acoustically. Furthermore, the infusion of stem segments with a butanol solution, which reduces surface tension (T in Eqn 1 above), increased the susceptibility of *Rhododendron* to cavitation. Sperry & Tyree (1988) measured the pressure required to force air through hydrated stems of sugar maple (*Acer saccharum*). They found a close correspondence between air permeability and vulnerability curves to water-stress-induced embolism. Furthermore, pores in pit membranes examined via SEM were found to have diameters in the range predicted by the capillary equation to cause embolism via air-seeding. Cochard, Cruiziat & Tyree (1992) demonstrated for willow (*Salix alba*) and cottonwood (*Populus deltoides*) that cavitation is induced to the same extent by high xylem tension during dehydration as by high external air pressure without xylem tension. Sperry & Tyree (1990) also demonstrated for three species of conifer (*Abies balsamea, Picea rubens* and *Juniperus virginiana*) that air pressure treatments of hydrated branches placed in a chamber could duplicate the degree of xylem embolism caused by water stress. Most recently, Salleo *et al.* (1992) induced embolism in intact branches of *Salix viminalis, in situ*, by injecting air into stems via a pressure collar.

We provide additional evidence for the air-seeding hypothesis through results obtained from two separate experiments. First, we compared the susceptibility of two chaparral shrub species to embolism induced both by water stress and by the application of external air pressure. Secondly, we perfused branches from each species with sub-microscopic spheres (ranging in diameters from 20 to 149 nm) and measured the resulting loss in hydraulic flow at each sphere diameter. This allowed us to estimate indirectly the distribution of pore sizes in pit membranes of the two species and to calculate, by the capillary equation, theoretical susceptibility to embolism by air-seeding.

We chose as our experimental subjects two co-occurring species of chaparral shrubs with potentially very different vulnerabilities to embolism. One species, *Malosma laurina* (= *Rhus laurina*, Hickman 1993), has deep roots and, for a chaparral shrub, high seasonal water potentials (Thomas & Davis 1989; Saruwatari & Davis 1989) and was thus expected to have relatively wide pores in its pit membranes and to be relatively vulnerable to xylem embolism caused by water stress. The other species, *Heteromeles arbutifolia* (Christmas-berry, Hickman 1993), is intermediate in rooting depth and experiences moderately low seasonal water potentials (Hellmers *et al.* 1955; Davis & Mooney 1986), and was thus predicted to have narrower pores in its pit membranes resulting in xylem more resistant to embolism.

MATERIALS AND METHODS

Plant material

Branches from 7-year-old *M. laurina* (Nutt.) Abrams and *H. arbutifolia* (Lindley) specimens that regenerated from root crowns after a 1985 wildfire in the Santa Monica Mountains (Thomas & Davis 1989) were collected from a natural stand on the Pepperdine University campus in Malibu, California (34 ° 02' 30' N, 118 ° 43' 30' W), at an elevation of 280 m. All branches in the field were cut longer

than the maximum vessel length to prevent air being artificially introduced into xylem vessels. The maximum vessel lengths of *M. laurina* (1·1 m ± 0·09) and *H. arbutifolia* (0·8 m ± 0·07) were measured on 10 individuals of each species by methods previously described by Zimmermann & Jeje (1981). We sampled 10 individual shrubs of *M. laurina* and *H. arbutifolia* and collected a total of between 61 (*M. laurina*) and 91 (*H. arbutifolia*) branches from each species to determine the relationship between xylem embolism formation and water stress. Similarly, we collected a total of 48 (*M. laurina*) to 69 (*H. arbutifolia*) branches from each species to determine the relationship between xylem embolism and the application of external air pressure (see explanation given below). At the time of collection, the cut end of each branch was immediately wrapped in parafilm and the entire branch covered in plastic bags to prevent excessive evaporation. The bagged branches were returned to the laboratory within 15 min after collection.

Vulnerability to embolism induced by water stress

Curves of vulnerability to water-stress-induced embolism were determined by measuring the relationship of water potential to percentage loss of hydraulic conductivity. Branches brought to the laboratory were uncovered and allowed to dehydrate on a bench-top for 1 to 6 d to achieve increasing levels of water stress. The night before final measurements, 12 branches were tightly bagged so that the water content stabilized throughout the branch. The following morning, the water potential for a leaf of each branch was measured with a pressure chamber (Scholander *et al.* 1965). The branches were then cut under water, alternately at each end, to produce a stem segment 10 cm in length and 6–8 mm in diameter.

The percentage loss of hydraulic conductivity due to embolism (% loss in K_h) of stem segments was measured by comparing the hydraulic conductivities (K_h) of a stem segment before and after a series of high-pressure (175 kPa) perfusions to remove air emboli (Sperry, Donnelly & Tyree 1988). For all experiments, a degassed 10 mol m^{-3} citric acid solution was initially filtered through a 0·1 μm filter. This solution was then passed through stem segments under 3 kPa of hydrostatic pressure and collected in a container on the pan of an analytical balance. We empirically demonstrated that the low-pressure treatment (3 kPa) was not sufficient to push a meniscus through a vessel that might be open at both ends of our stem segments. We did this by increasing pressures in 1 kPa steps from 1 to 6 kPa, finding that calculated K_h remained constant. The flow rate was measured as the mass increase per unit time, but was subsequently converted to the volume flow rate ($m^3\ s^{-1}$), correcting for the effects of pressure and temperature. After the initial reading, the stem segments were perfused with citric acid solution for two 1-h-long periods under 175 kPa of pressure. This process removed air emboli from stem segments and resulted in an increase in K_h. Hydraulic conductance per unit pressure gradient (= hydraulic conductivity, K_h, $m^4\ MPa^{-1}\ s^{-1}$), as defined by Tyree & Ewers (1991), was calculated as the volume flow rate of citric acid solution (q, $m^3\ s^{-1}$) through a given stem segment (dx, m) divided by the pressure gradient (dP/dx, MPa m^{-1}):

$$K_h = q / (dP/dx). \quad (2)$$

To determine the percentage loss of conductivity, the difference between the initial and final values for K_h was divided by the final value and multiplied by 100. The methods for measuring K_h are fully described by Sperry, Donnelly & Tyree (1988). The terminology follows that of Tyree & Ewers (1991).

Vulnerability to embolism induced by external air pressure

We used positive air pressure to induce embolism artificially in hydrated branches to investigate whether the pattern of embolism coincided with the pattern of embolism observed in branches subjected to water stress (Fig. 1). The method used was similar to that described by Cochard, Cruiziat & Tyree (1992). Up to 12 hydrated branches (longer than the maximum vessel length) were collected the night before an experiment, wrapped in plastic bags, and placed in large buckets of water. The next morning, six hydrated branches were retained in the buckets until measurement of the % loss of K_h later in the day (controls), while six additional branches were loosely wrapped with wet cheesecloth and artificially pressurized (treated). The cheesecloth prevented water loss during pressurization. The distal ends of the six treated branches were inserted, three at a time, into a manifold of three pressure chambers, 1 m in length (Fig. 2). The proximal end of each branch was shaved with a new razor blade to reduce clogging of

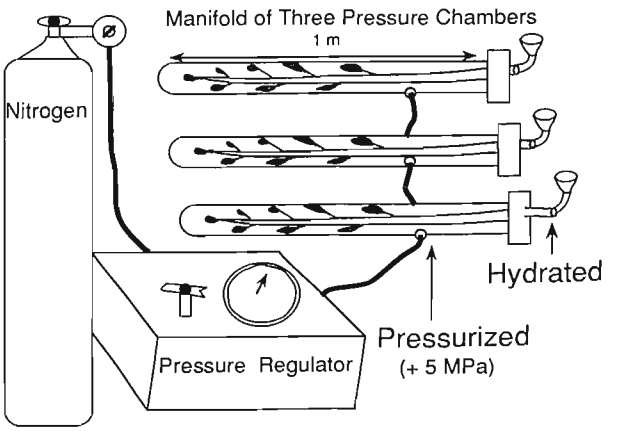

Figure 2. Diagram of the apparatus used to apply high external air pressures to the distal ends of three long branches while keeping the cut ends outside the chambers under atmospheric pressure. The branches were maintained in a fully hydrated state by wrapping them in wet cheese cloth and attaching water-filled funnels to the exposed, cut ends outside the chamber.

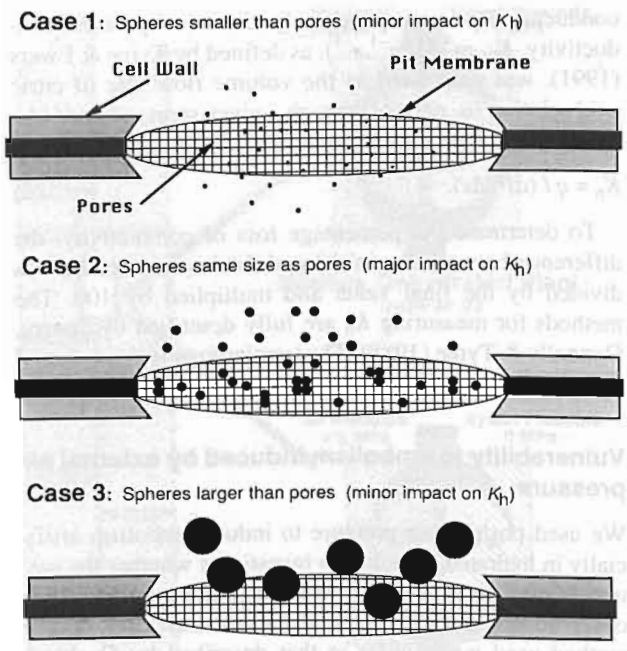

Figure 3. Diagram of the proposed effects that different sizes of polystyrene spheres would have on pores in pit membranes of xylem vessels. In case 1, spheres smaller than the pores would pass through, having little effect on K_h. In case 2, because of the similar diameters of pores and spheres, spheres would wedge into pores causing a large reduction in K_h. In case 3, spheres would be too large to form a complete seal over the pores, and thus K_h would be slightly impacted.

vessels by cellular debris. The proximal cut surface was secured to a water-filled reservoir to insure hydration during the entire treatment. The nitrogen pressure in the chamber was then gradually increased (<1 MPa min^{-1}) to the desired value (between 0·5 and 8·5 MPa). During pressurization, small bubbles emerged from the cut surfaces of stems into the reservoir. The pressure was held at the prescribed value for 20 min. After this time, the chamber pressure was gradually released (<1 MPa min^{-1}) and water from the reservoirs was allowed to re-enter the stems. The branches were removed from the chambers, cut under water to 10-cm-long segments, and inserted into a manifold of tubing to measure % loss of K_h by the methods described above. The manifold could accommodate 12 stem segments, six from the control branches (unpressurized) and six from the treated branches (pressurized). In all cases, the six control branches were collected from the same individuals as the six pressurized branches.

In a preliminary experiment, we placed six branches completely inside the chambers so that the proximal ends were at the same pressure as the distal ends during pressurization. We found the % loss of K_h for the six branches pressurized at 5 MPa to be not significantly different from that of six control, non-pressurized branches ($t=0·63$, $P>0·55$). This indicated that it was the pressure gradient between the inside of the chamber (distal end of branch) and the outside of the chamber (proximal end of branch) that caused embolism formation in the usual pressurization experiments (Figs 2 & 4), not a 'bends' effect. We also compared the mean water potentials (–0·32 ± 0·19 MPa) of five hydrated branches immediately prior to our pressurization treatment to the mean water potentials (–0·24 ± 0·10 MPa) of the same branches immediately after the release of pressure. They did not differ significantly ($t=0·81$, $P>0·45$). This indicated that our pressurization treatment did not cause xylem embolism via tissue dehydration (water-stess-induced embolism).

Estimating effective pore diameters in pit membranes

Five vials of certified polystyrene spheres were purchased from the Duke Scientific Corporation (Palo Alto, CA; lot numbers 11883, 12053, 12057, 12232, 12301 and 14126). The mean diameters of the spheres in each vial were 20, 30, 40, 64, 82 and 149 nm. The range of uncertainty for these diameters was certified as ±1·5, ±1·3, ±1·8, ±2·8, ±3·2 and ±4 nm, respectively.

A 1% formalin, 1 kmol m^{-3} potassium phosphate buffer solution in deionized water (pH = 6·7) was degassed, passed through a 0·1 μm filter, and used in all subsequent perfusions. The solution inhibited fungal growth (Sperry, Donnelly & Tyree 1988) and, unlike the citric acid solution, also prevented spheres from clumping by maintaining a neutral pH.

Stems from *H. arbutifolia* and *M. laurina* that had few side branches and were much longer than the maximum vessel length were collected in the field and bagged. Collected branches were cut under water to a length of 1·2 m (still longer than the maximum vessel length) and inserted into a tubing manifold which allowed measurements of K_h. Branches were then subjected to a series of 1-h-long, high-pressure perfusions (175 kPa) until K_h readings stabilized, indicating that all emboli had been removed. At this point, 3 cm^3 of a 2% by volume suspension of spheres was introduced into the manifold via hydrostatic pressure. Once the spheres had entered the manifold and mixed with the formalin solution, they were perfused into the branch at 175 kPa for 3–5 h. During this time, spheres were kept in suspension by manually vibrating the tubing manifold. By comparing the post-sphere conductivity (measured with a sphere-free solution) to the initial-maximum conductivity, the % loss of K_h resulting from clogging by spheres of a specific diameter was determined. Between three and five experiments were performed at each sphere diameter.

Van Alfen *et al.* (1983) demonstrated for alfalfa (*Medicago sativa*) that flow rates through pores in pit membranes of vessel cell walls are affected by a narrow size range of dextran molecules. They suggested three possible outcomes of sphere and pore interaction in functional xylem (Fig. 3). Briefly, if the spheres are smaller than the pores in pit membranes, they pass through the pores with little impact on K_h. If the spheres are similar to the size of pores they wedge into pores to form a tight seal, greatly

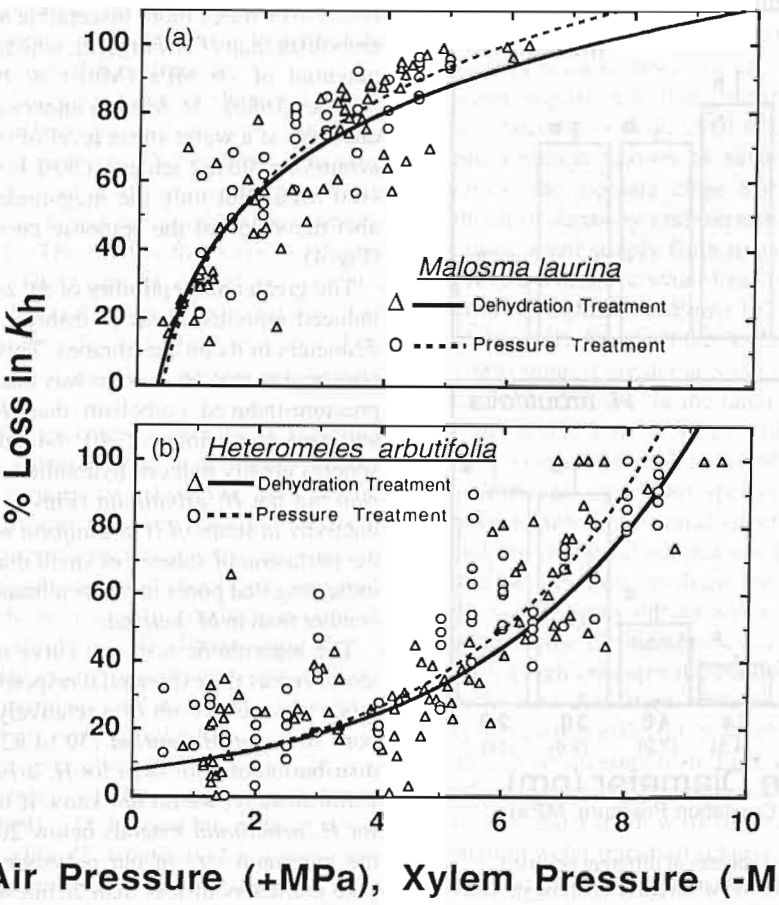

Figure 4. Comparison of the percentage loss of hydraulic conductivity induced by water stress (open triangles) to that induced by pressure treatment (open circles) in (a) *Malosma laurina* and (b) *Heteromeles arbutifolia*. The abscissa represents positive pressures in the pressure treatment and negative pressures (tensions) in the dehydration treatment. The data were fitted with a logarithmic equation for water stress ($y = 35{\cdot}2 + 75{\cdot}6 \log(x)$, $r^2 = 0{\cdot}74$) and for pressurization ($y = 33{\cdot}2 + 86{\cdot}7 \log(x)$, $r^2 = 0{\cdot}74$) for *M. laurina* but by an exponential equation for water stress ($y = 7{\cdot}74 \log(0{\cdot}1263x)$, $r^2 = 0{\cdot}41$) and for pressurization ($y = 7{\cdot}61 \log(0{\cdot}1356x)$, $r^2 = 0{\cdot}42$) for *H. arbutifolia*.

reducing K_h. Finally, if spheres are larger than the pores, they have a minor impact on K_h because the sphere cannot wedge into pores. This model was empirically verified by Van Alfen *et al.* (1983) using membrane filters of known pore diameter.

Statistical analysis

Mean values of % loss of K_h resulting from the presence of spheres in each diameter class were compared by one-way ANOVA followed by a Scheffe multiple range test.

RESULTS

In *Malosma laurina*, the pattern of percentage loss of hydraulic conductivity (% loss of K_h) induced by water stress in dehydrated stems coincided with the pattern of % loss of K_h induced by pressure treatments on hydrated stems (Fig. 4a). At a water pressure potential of −1·6 MPa, the mean loss of conductivity in dehydrated branches was 50%. This was identical in magnitude but opposite in sign to the +1·6 MPa of air pressure required to induce a 50% loss of conductivity in hydrated plants. In *Heteromeles arbutifolia* there was also a close correspondence between embolism induced by water stress and embolism induced by external air pressure (Fig. 4b). However, in this species the mean loss of 50% conductivity occurred at −6·0 MPa for dehydrated branches but at +6·4 MPa for pressurized branches. In both species, decreasing water pressure potentials and increasing air pressures resulted in an increase in % loss of K_h. However, *M. laurina* was more sensitive than *H. arbutifolia* and the pattern of change in % loss of K_h with respect to pressure was logarithmic for *M. laurina*, but exponential for H. *arbutifolia* (Fig. 4).

Polystyrene spheres that were either small (20 nm) or large (149 nm) had little effect on hydraulic conductivity in *M. laurina* (Fig. 5a). In contrast, spheres with intermediate diameters (30, 40, 64 and 82 nm) caused greater than 50% loss of K_h (Fig. 5a). The pattern was very different for *H. arbutifolia*. Only spheres in the two smallest categories

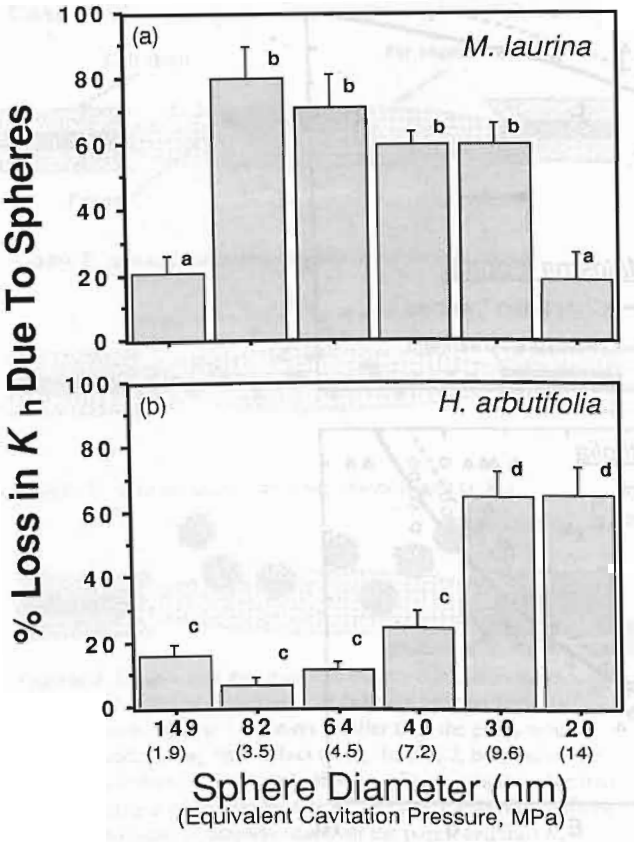

Figure 5. Effect of polystyrene spheres of different certified diameters on hydraulic conductivity when perfused through stems of (a) *Malosma laurina* and (b) *Heteromeles arbutifolia*. The equivalent cavitation pressure was calculated using the capillary equation ($\Delta P = 4T/D$) where the surface tension of water (T) was 0·072 N m^{-1} at 20 °C and D the sphere diameter. Bars represent + 1 SE, n = 3 to 5. Letters adjacent to bars represent a significant difference by one way ANOVA followed by a Scheffe multiple range test at $P<0.05$.

(20 and 30 nm) caused greater than 50% loss of K_h (Fig. 5b).

In a series of conduction experiments using progressively smaller spheres (from left to right in Figs 5a & b), the 'critical diameter' of spheres was taken as the sphere diameter below which there was an abrupt loss of conductivity (Figs 5a & b). This occurred at 149 nm for *M. laurina* and 40 nm for *H. arbutifolia*, equivalent to a cavitation pressure of 1·9 and 7·2 MPa, respectively (calculated via the capillary equation, Eqn 1). These cavitation pressures approximated the empirically derived 50% loss of conductivity of –1·6 MPa for *M. laurina* and –6·0 MPa for *H. arbutifolia* (Fig. 4).

DISCUSSION

Consistent with a deep-rooting habit and high seasonal water potential (minimum of –2 MPa; DeSouza, Silka & Davis 1986; Thomas & Davis 1989), *M. laurina* was found to be much more susceptible to water-stress-induced embolism than *H. arbutifolia*, which has a minimum water potential of –6 MPa (Miller & Poole 1979; Davis & Mooney 1986). *M. laurina* underwent a 50% loss of conductivity at a water stress level of –1·6 MPa, whereas *H. arbutifolia* did not achieve a 50% loss of conductivity until –6·0 MPa. Not only the magnitude of the response, but also the shape of the response curve, was very different (Fig. 4).

The greater susceptibility of *M. laurina* to water-stress-induced embolism was probably a result of larger pore diameters in its pit membranes. This is consistent with the observation that *M. laurina* was much more susceptible to pressure-induced embolism than *H. arbutifolia* (Fig. 4) and that perfusions of 40, 64 and 82 nm polystyrene spheres greatly reduced hydraulic conductivity for *M. laurina* but not *H. arbutifolia* (Figs 5a & b). Hydraulic conductivity in stems of *H. arbutifolia* was diminished only by the perfusion of spheres of small diameter (30 nm or less), indicating that pores in pit membranes of this species were smaller than in *M. laurina*.

The logarithmic response curve of *M. laurina* to water stress versus the exponential response pattern of *H. arbutifolia* may be a result of a relatively broad distribution of pore sizes for *M. laurina* (30 to 82 nm) versus a narrow distribution of pore sizes for *H. arbutifolia* (20 to 30 nm). Unfortunately, we do not know if the range in pore sizes for *H. arbutifolia* extends below 20 nm because this was the minimum size of our polystyrene spheres. However, pore diameters of less than 20 nm would be equivalent to extremely large cavitation pressures (e.g. 10 nm = 28 MPa) and the approximate pore diameters found in the primary cell walls (not just pit membranes) of higher plants (Nobel 1991).

If sphere perfusion experiments correctly estimate effective pore diameters in pit membranes (Figs 5a & b), they should overestimate resistance to water-stress-induced embolism. This is because cavitation events are determined by the largest pore diameters in a population within a given vessel, not the mean diameter (cf. Sperry & Tyree 1988). When sphere diameters were converted to equivalent cavitation pressures and plotted versus the accumulated loss in conductivity, a 50% loss of K_h was predicted to occur at 2·2 MPa for *M. laurina* and 6·7 MPa for *H. arbutifolia*. This is 0·6 to 0·7 MPa greater than actual values, consistent with the prediction of overestimated resistance to embolism. When the largest pore diameters as estimated by the sphere perfusion experiment (149 nm for *M. laurina* and 40 nm for *H. arbutifolia*; Figs 5a & b) were taken as the 'critical diameters' and then converted to equivalent cavitation pressures, these 'critical pressures' (an indicator of the threshold for increased embolism) corresponded to 60% loss of K_h for *M. laurina* and 70% loss of K_h for *H. arbutifolia* (Figs 5a & b).

These results taken together suggest that perfusion experiments with polystyrene spheres may be useful not only for estimating effective pore sizes in pit membranes (cf. Van Alfen *et al.* 1983), but also in explaining the shape

of vulnerability curves. However, there are a number of uncertainties in using perfusion experiments to predict the tensions at which 50% loss of K_h will occur.

Carlquist (1989) has suggested that, in chaparral shrubs, the pit is a 'compromise between wall strength, conductive efficiency, and conductive safety'. When there is damage to the water continuum, pores in the membranes of pits localize the damage and prevent neighbouring vessels from cavitating (Fig. 1). The smaller the pores, the higher the degree of safety. The trade-off is that as pore size decreases so does the hydraulic conductivity between two vessels. This is presumably due to surface tension and the friction that occurs when xylem water passes through pores.

Our data on pore diameters and vulnerability show that *M. laurina* is more vulnerable to water-stress-induced embolism than *H. arbutifolia*. However, *M. laurina* appears to be more efficient in xylem transport. A useful measure of xylem transport efficiency is the area-specific conductivity, K_s, or K_h per sapwood area (Tyree & Ewers 1991). In a survey of the area-specific conductivities on 20 species of chaparral shrubs (S. D. Davis, unpublished results), *M. laurina* had over twice the area-specific conductivity of most chaparral species and nearly 3 times the area-specific conductivity of *H. arbutifolia*.

An important factor controlling the area-specific conductivity (xylem efficiency) of plants is the vessel diameter (Tyree & Ewers 1991). *M. laurina* has a mean vessel diameter of 43·9 μm, while *H. arbutifolia* has a mean vessel diameter of 28·5 μm, measured on stem segments similar in diameter to those used in this study (S. D. Davis, unpublished results). Since vessels are not ideal capillaries, flow through tracheary elements could be limited both by lumen resistance (the resistance defined by vessel diameter) and by pit resistance (the resistance defined in part by the size of pores in pit membranes; Zimmermann 1983; Chiu & Ewers 1993). Selective pressure to increase hydraulic conductivity would then tend to favour evolutionary increases both in lumen diameter and in the size of pores in pit membranes (Tyree, Davis & Cochard 1994). Conversely, selective pressure to increase resistance to embolism might favour the evolution of narrow pores in pit membranes and many narrow vessels, to increase redundancy, when certain vessels are embolized (Ewers 1985; Carlquist 1988). Our conclusion, then, is that with large pore and large vessel diameters *M. laurina* has relatively poor safety but increased efficiency in hydraulic conductivity, whereas *H. arbutifolia*, with its small pores and narrow but abundant vessels, has relatively inefficient conductivity but higher safety. It must be acknowledged that this analysis ignores other important selective forces on xylem form and function such as selection for mechanical strength, freezing tolerance, and storage (cf. Tyree *et al.* 1994).

Several studies indicate that plants operate on the 'brink of disaster' relative to water-stress-induced embolism (Tyree & Sperry 1989; Tyree & Ewers 1991). When one vessel cavitates during summer drought due to water stress, the loss of hydraulic conductivity from that embolism increases the xylem tension on other conducting vessels because fewer vessels are available for the same water requirement from transpiration. If subsequent vessels become embolized from the rise in xylem tension, a phenomenon known as runaway embolism may occur unless the stomata close and reduce transpiration. The threat of runaway embolism is normally prevented by an ample water supply from roots and by stomatal closure to prevent excessive water loss. In *M. laurina*, stomata close at –2 MPa (Miller & Poole 1979), and reported root depths of 13 m (DeSouza, Silka & Davis 1986; Thomas & Davis 1989) suggest greater access to soil moisture reserves than for *H. arbutifolia*. In the latter species, stomata close at a water potential of –4 MPa (Miller & Poole 1979) and average rooting depth is 2·2 m (Hellmers *et al.* 1955).

Although these two species of chaparral shrubs may have acquired by natural selection a variety of anatomical and physiological adaptations for survival in a water-limited environment, perhaps the ultimate factor that limits the water supply during water stress in these plants is the pores in the pit membranes of xylem vessels. In *M. laurina*, a high area-specific conductivity is complemented by large pore diameters, wide vessels and deep roots. This system, while efficient in water transport, is highly susceptible to vessel embolism. In *H. arbutifolia*, a low area-specific conductivity is complemented by small pores, narrow vessels and a shallow rooting system. This shrub is inefficient in water transport relative to *M. laurina*. However, it can withstand high xylem tensions before vessels embolize.

CONCLUSION

The results of our study are consistent with the hypothesis of Oertli (1971) and Zimmermann (1983) who state that the mechanism of xylem embolism formation by water stress is related to the pore size in inter-vessel pit membranes. Our results are also consistent with four other studies on the mechanism of embolism formation, although these investigators used a different approach and different plant material (Crombie, Hipkins & Milburn 1985; Sperry & Tyree 1988; 1990; Cochard, Cruiziat & Tyree 1992; Salleo *et al.* 1992). In our study, the strongest evidence for air-seeding as the cause of water-stress-induced embolism is the correlation between pressure and dehydration curves, both for *M. laurina* and for H. *arbutifolia* (Fig. 4). Additional evidence is the pattern of reduction in hydraulic conductivity after perfusion of stems with polystyrene spheres of various diameters (Fig. 5). We conclude that one of the main controlling factors of xylem embolism in these two species of chaparral shrubs is pore size in inter-vessel pit membranes. The occurrence of different susceptibilities to air-seeding in two species of chaparral shrubs which have different physiologies suggests that xylem structure and function have ecological significance.

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