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Vulnerability to cavitation in populations of two desert species, *Hymenoclea salsola* and *Ambrosia dumosa*, from different climatic regions

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Abstract

Seeds from different populations of two desert species, *Hymenoclea salsola* Torr. and A. Gray and *Ambrosia dumosa* (A. Gray) Payne, were collected along a climatic gradient, germinated in a greenhouse and the plants tested for their vulnerability to cavitation by the air-pressure method. Differences among populations were evident in *A. dumosa*, but not in *H. salsola*. Greenhouse treatments simulating regimes in temperature and relative humidity encountered in different desert environments did not cause appreciable changes in vulnerability to cavitation. It is suggested that a homeostatic mechanism may have helped in maintaining a constant water potential drop in the xylem with little need for an adjustment in the resistance to cavitation. Different plant organs had different vulnerabilities to cavitation, with roots being the most and woody stems the least susceptible. Young green twigs were intermediate. A simulation model confirmed that low water potentials are most likely to cause runaway cavitation in the roots, not in the other organs. It is hypothesized that green twigs are adapted to the favourable water conditions of the growing season, while woody stems are adapted to endure prolonged periods of drought stress.

Key words: Cavitation, xylem embolism, hydraulic conductance, *Hymenoclea salsola*, *Ambrosia dumosa*.

Introduction

Regulation of xylem water pressure potential Ψ_{px} (MPa) is an important component of plant adaptation to different climatic conditions. Low values of Ψ_{px} may cause stomatal closure and reduce photosynthetic carbon fixation through a negative feedback loop (Ludlow, 1980). Although some traditional evidence for a role of leaf water potential on stomatal behaviour has been questioned (Davis and Zhang, 1991), recent experiments have shown that stomata operate in order to keep leaf water potential within rather narrow limits (Saliendra *et al.*, 1995).

Stressful levels of Ψ_{px} can be caused by critical levels of soil water potential (Ψ_s), low atmospheric humidity (leaf–air humidity gradient Δw, mmol mol^{-1}) or low plant hydraulic conductance (k, mmol m^{-2} s^{-1} MPa^{-1}) as can be seen by the following form of the Ohm's Law analogy:

$$\Psi_{px} = \Psi_s - \frac{g_{tot}\Delta w}{k} \quad (1)$$

where g_{tot} is leaf conductance to water vapour (mmol m^{-2} s^{-1}) and liquid flow through the xylem is equated to transpiration rate. Plant hydraulic conductance k cannot be considered a constant and, in fact, normally declines during water stress (Blizzard and Boyer, 1980). One important mechanism of reduction of plant k is xylem cavitation and embolism, i.e. the processes by which a functional vessel loses its conductive capacity after the formation of an air embolus (Tyree and Sperry, 1989). Many authors agree that the most likely mechanism of drought-induced xylem cavitation is the so-called *air-seeding mechanism* (Zimmermann, 1983), by which air entry from a cavitated vessel into a functional one is caused by the failure of the inter-vessel pit membrane to withstand an excessive pressure difference, $\Delta\Psi_{px}$, across the air–water meniscus.

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This pressure difference is related to the diameter of the largest pore in the pit membrane according to the capillary equation, modified according to Sperry and Tyree (1988):

$$\Delta\Psi_{px} = \frac{4\tau}{d} \quad (2)$$

where τ (N m^{-1}) and d (μm) are the surface tension of the xylem water and the diameter of the largest pore, respectively. Therefore, variability in vulnerability to drought-induced cavitation among species, populations or individuals should be related to variations in anatomical properties. However, anatomical properties can vary greatly also within each individual, both because of the differences among organs (e.g. roots, stems, twigs) and because of the degree of their ontogenetic development. Considering only the above-ground organs, young twigs of *Ambrosia dumosa* (A. Gray) Payne and *Hymenoclea salsola* Torr. and A. Gray bear protoxylem and metaxylem vessels, while the basal woody stems bear secondary xylem vessels.

Both *A. dumosa* and *H. salsola* are sub-shrub, drought deciduous species, which shed their leaves during the prolonged droughts typical of the North-American deserts. Most of the terminal twigs and the woody basal stems survive drought stresses and constitute the canopy structure for the next generation of twigs. However, after extremely severe drought periods and/or heavy reproduction events, the entire above-ground canopy can die back (Comstock *et al.*, 1988).

This paper reports results of some experiments aimed at documenting the variation in (a) anatomical characters and (b) vulnerability to cavitation of different populations of the two species *H. salsola* and *A. dumosa*. The hypothesis that these two sets of characters may vary in response to the climate of the site of origin, as well as in response to experimental changes in the climatic growing conditions (temperature and relative humidity), was tested. Both species have a wide natural range, extending as far north as southern Utah (*A. dumosa*) and California (*H. salsola*) and as far south as Baja California in Mexico (Turner *et al.*, 1995). Besides the obvious differences in latitude and altitude, the different deserts are characterized by a range of thermal and pluviometric regimes, varying from one extreme of hot, dry summers and cold, wet winters to another of hot, wet summers and mild, wet winters. The hypothesis that the degree of ontogenetic development of above-ground organs and the anatomical differences between above- and below-ground organs influenced their vulnerability to cavitation was also tested.

Materials and methods

Seed population selection

Three populations of each species were selected to represent a broad range of climatic conditions during the growing season (Table 1). Seeds from population 1 were collected at the northern fringes of the Mojave Desert, south-east of Goldfield, NV; the site is close to the point where *A. dumosa* and other species typical of the warm deserts disappear towards the higher elevations of the Great Basin. Sub-zero winter temperatures are common and summers are very hot and dry. Annual precipitation is very low and mainly concentrated in the winter and spring seasons. During the main growing season (spring), temperatures tend to be mild and leaf-air humidity gradients (Δw, mmol mol^{-1}) are low.

Seeds from population 2 were collected at Beaverdam, UT, at the junction of the Colorado Plateau with the south-eastern part of the Mojave Desert. At this site, winter sub-zero temperatures are not frequent, while summers are very hot and dry. During spring temperatures are mild and Δw are medium to low.

Seeds from population 3 were collected at Organ Pipe Cactus National Monument, AZ, in the Sonoran Desert. The precipitation regime is greatly affected by summer monsoonal storms which make up a variable but, on average, large fraction of the total precipitation. Temperatures can be very high in the summer and mild in the winter. Values of Δw during the growing season tend to be high.

Greenhouse plant propagation

Seeds were germinated in greenhouse seed trays under a shade cloth; after germination, seedlings from each of the three populations were transferred into 25 l, 25 cm diameter cylindrical PVC pots, randomly assigned to a treatment (see later) and placed in each of three greenhouse bays. Soil was prepared by thoroughly mixing fritted clay, pasteurized soil and silica sand (3:1:1, by vol.) amended with dolomitic lime, gypsum, superphosphate and Micro-max. Plants were kept well-watered and fertilized throughout the whole experiment.

Table 1. *Geographic and climatic characteristics of the study sites*

Climate data from nearby meteorological stations at Mina, Joshua Tree National Area and Organ Pipe Cactus National Monument (OPCNM), respectively, for the study sites 1, 2 and 3. Numbers in parentheses give seasonal percentages of rainfall. Mean leaf–air humidity gradients were calculated as the average of monthly leaf–air humidity gradients weighted by the ratio of monthly precipitation to potential evapotranspiration (Comstock and Ehleringer, 1992).

Geographical characteristics	Goldfied	Beaverdam	OPCNM
Site number	1	2	3
Latitude	37° 29′	37° 03′	31° 90′
Longitude	117° 12′	113° 52′	112° 47′
Elevation (m)	1650	945	512
Climatic characteristics			
Average temperature (°C)			
Winter (Oct–Feb)	4.6	7.7	14.8
Spring (Mar–May)	10.7	14 5	18 9
Summer (Jun–Sept)	22.7	25.1	29.2
Annual	12.1	15.2	20.6
Average rainfall (mm)			
Autumn-Winter (Oct-Feb)	39.1 (49.6)	120.4 (42.3)	93.2 (40.0)
Spring (Mar–May)	28.7 (22.3)	54.2 (31.1)	25.7 (11.0)
Summer (Jun–Sept)	24.6 (28.1)	68.9 (26.6)	114.0 (49.0)
Annual	92.5	242.8	232.2
Mean leaf–air humidity gradient during growing season (Δw, mmol mol^{-1})	16.17	22.67	33.23

To simulate the climatic differences encountered by the different ecotypes during the growing season, three treatments of different daytime temperature T (°C) and relative humidity RH were imposed in the three greenhouse bays. The treatments comprised: a cold-dry regimen (CD, $T=22.6\pm0.10$ °C, $RH=42.5\pm0.96\%$), a hot-dry regimen (HD, $T=31.5\pm0.15$ °C, $RH=26.9\pm0.61\%$) and a hot-humid regimen (HH, $T=32.6\pm0.13$ °C, $RH=64.4\pm0.44\%$) in an incomplete factorial design. This resulted in about a 2-fold difference in Δw between growth treatments ($\Delta w=17.65$ (±0.31), 35.20 (±0.31) and 20.41(±0.33) mmol mol^{-1} for treatments CD, HD and HH, respectively). The temperature range was chosen so that photosynthetic rates were within 90% of those at the species temperature optimum (about 28 °C) (Comstock and Ehleringer, 1988).

Adequate ventilation was obtained by using a number of fans in each bay and natural light was supplemented using a combination of Na-vapour and metal halide lamps. Photoperiod was 14 h and night-time temperatures approximately around 18 °C in all bays. CO_2 concentration, relative humidity and temperature in all bays were continuously monitored.

Measurement of vulnerability to cavitation

Vulnerability to cavitation was measured using the air pressure method (Cochard *et al.*, 1992; Salleo *et al.*, 1992). The whole canopy (Experiment 1, see below) or sample shoots (Experiments 2 and 3) were cut at their base, immediately immersed in a sink filled with water and twigs were repeatedly cut under water to a final length of about 20 cm.

In all three experiments, the sample stems were then perfused with distilled, filtered (to 0.1 μm), degassed, acidified (with HCl, pH = 2) water at a pressure of about 140 kPa for 1 h, the time required to obtained stable, maximum flow rates. Stems were then placed inside custom-built pressure sleeves (Sperry and Saliendra, 1994) with both ends protruding, and connected to a water source line of a known hydrostatic pressure ($\approx$10 kPa). The pressure sleeves were then connected to two pressurized gas cylinders, which were used to create either low or high gas pressures inside the sleeves. Maximum saturated conductivity was measured by collecting water into pre-weighed vials filled with absorbent dry paper while a low air pressure ($\approx$70 kPa) was maintained in the sleeve. This pressure level was chosen in order to drain all the open vessels, to avoid refilling under the hydrostatic gradient while at the same time minimizing additional cavitation during the measurements.

Pressure gradients necessary to cause air-seeding in xylem vessels were obtained by holding the gas pressure inside the sleeves at predefined constant levels for 20 min. At the end of each pressurization period, pressure was gradually decreased to the low background level and, after 5 min, water flow rates were measured again. This procedure was repeated until the stems were almost completely cavitated. Tests showed that multiple notching facilitated gas entry into woody stems and saturated the cavitation response during the 20 min of pressurization. Notching was not necessary in young twigs and in roots.

The air pressure, or air-injection, method has been shown to give results closely comparable to the traditional dehydration system (Sperry and Tyree, 1990; Cochard *et al.*, 1992; Jarbeau *et al.*, 1995; Sperry and Saliendra, 1994), the reason being that the pressure difference between air and the water phase required to drain an inter-conduit pit is the same, whether the air is being pushed through the pit by air pressures greater than atmospheric, or being pulled in by water pressures less than atmospheric.

Experimental set-up

Experiment 1: Vulnerability to cavitation tests were performed on individuals grown in the three different greenhouse bays to test for the effect of growth environment. All three populations of both species were represented to test for differences among ecotypes. This experiment was part of a larger one, intended to investigate aspects of gas-exchange regulation in desert species and the relationships between climate and plant water-use characteristics.

Plants were brought to the laboratory for gas-exchange measurements. One day later, three stems were selected and used for vulnerability to cavitation tests. Stems were stratified according to their position in the canopy. One stem was selected from the basal woody trunk and two stems from the young unlignified twigs in the crown region.

Experiment 2: Results from Experiment 1 suggested an effect of the type of organ (woody stems with secondary xylem versus twigs with primary xylem) on the vulnerability to cavitation, but the small size of the plants used in the gas-exchange experiment prevented adequate replication. Therefore, Experiment 2 was devised to improve detection of these differences. Larger plants were grown under the same conditions and, for each plant, three to six stems were sampled from each of the following categories: woody trunks, young twigs and primary roots. To restrict the sample size, samples were taken from only two populations of *H. salsola* (populations 1 and 2) and *A. dumosa* (populations 1 and 3).

Experiment 3: A third experiment was designed to test the hypothesis that the degree of ontogenetic development in the canopy twigs influences their vulnerability to cavitation. A set of mature plants ($n=23$) were cut at ground level to generate resprouting from the cut stumps. Five resprouts per plant were marked and their shoot length was measured. Sequential sampling for vulnerability to cavitation ($n=3$ for each sampling date) started after the shoots had reached the minimum length required to fit into the pressure sleeves and continued until shoot elongation ceased (about 3 months later). At that time, sprouts were completely woody along a large portion of their length and conducting elements were mainly secondary xylem vessels. The three sample stems for each date were always obtained from three different plants.

Complete canopy destruction in *H. salsola* can be considered similar to the canopy dieback that occurs when washbeds are flooded during the rainy season or during severe drought.

Anatomical measurements

From most of the stems used in Experiments 1 and 2 thin (about 30–40 μm thick) sections were manually cut, stained in a solution of Saffron dye mixed with EtOH, washed and mounted in glycerine on microscope slides. Slides were viewed under high magnification (400×) with a light microscope, and the vessel perimeter ($n=330\pm47$ for each section) traced on a Micro-Plan II bit-pad interfaced with an image-analysis software (Don Santo Corp., Natick, Mass., USA). Since vessels were elliptical in cross-section, the vessel hydraulic diameter (i.e. the diameter of the capillary of circular cross-section having the same flow rate under the same pressure gradient, Nonweiler, 1975; Zimmermann and Jeje, 1981) was estimated using the formula for ellipses (Lewis, 1992).

Weighted averages of hydraulic diameters (d_h, μm) for each section were calculated using the formula:

$$d_h = 2\frac{\sum r^5}{\sum r^4} \quad (3)$$

This weights the hydraulic diameters of the single vessels according to their contribution to the total hydraulic conductance of the segment.

Maximum vessel length was measured on roots and shoots of at least 10 individuals fom each population of both species by the paint-infusion method (Zimmermann and Jeje, 1981) and by forcing a low-pressure ($\approx$70 kPa) air stream through the xylem under water, and progressively cutting back the stem until bubbles appeared under water. Maximum vessel length averaged about 15 cm in the roots and about 10 cm in the woody stems and young twigs of both species.

Statistical analyses

Average data for each individual were used in the comparison of cavitation thresholds of different organs among populations and growing bays. A Gompertz model was used to fit the relationship between percentage loss of conductivity and applied pressure by nonlinear regression techniques:

$$\%l = ae^{(-be^{-cP})} \quad (4)$$

where $\%\ l$ is the percentage loss of conductivity measured and P (MPa) is the positive air pressure applied in the sleeves. The Gompertz function fitted the dataset better than previously used functions, like the Weibull one (Neufeld *et al.*, 1992). Comparisons among treatments were made in two ways: (a) by calculating the average percentage loss of conductivity at each pressure and comparing the means using a one-way ANOVA and (b) by testing the differences between the mean pressures required to obtain fixed cavitation thresholds (10%, 20%, 50%, and 80% loss of conductivity) in one or two-way ANOVAs. Confidence interval tests were used to compare treatment means.

Results

Vulnerability to cavitation varied among populations, species and plant organs. *A. dumosa* exhibited larger differences among ecotypes, both in the woody stems and in the young twigs, while *H. salsola* showed significant differences among populations only for the first portion of the vulnerability curve of the basal woody stems (until

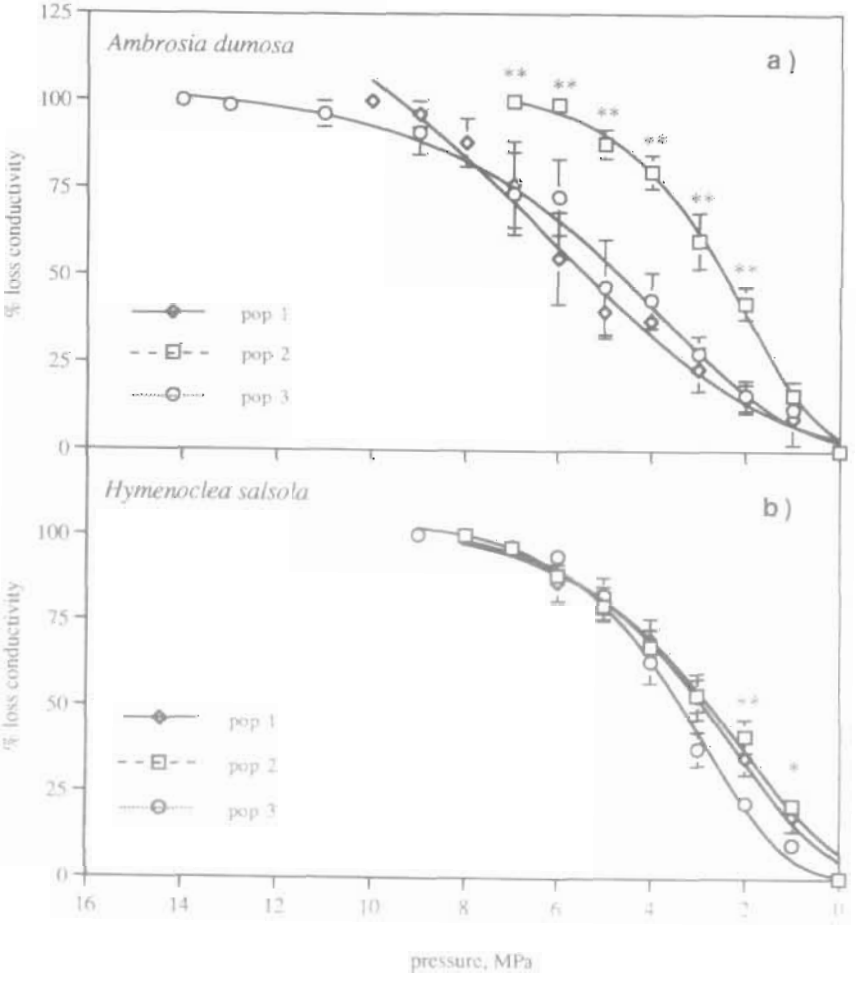

Fig. 1. Vulnerability curves for woody stems of the two desert species *Ambrosia dumosa* (a) and *Hymenoclea salsola* (b). Seeds from population 3 of both species were sampled at Organ Pipe Cactus National Monument, Az, a site characterized by high leaf–air humidity gradients during the growing season, while populations 1 and 2 were sampled at Goldield, Nv, and at Beaverdam, Ut, respectively, which are both characterized by relatively low leaf–air humidity gradients during the growing season. Woody stems were sampled in the basal trunk in the canopy. Stars denote significant differences among populations: *, $P<0.05$; **, $P<0.01$. For *A. dumosa*, population 3 was significantly different from 2, but not from 1. For *H. salsola*, population 3 was significantly different from 1 and 2 only for the lower range of pressures.

a pressure of 2 MPa, Figs 1a, b, 2a, b). In both species, differences among populations corresponded only partially to the climatic differences among sites, with population 3 being more resistant to cavitation than population 2 ($P<0.01$ in *A. dumosa*, $P<0.05$ in *H. salsola*). As a species, *A. dumosa* showed more resistant woody stems than *H. salsola* in populations 1 and 3 (both $P<0.01$), whereas young twigs of all populations were equally susceptible to cavitation in both species. Analyses of the cavitation thresholds (Table 2) substantially confirmed these results.

In no case was growth environment (i.e. greenhouse bay) found to be a significant factor in determining cavitation thresholds (Table 2).

Variations among populations in the cavitation thresholds of woody tissues were only partially paralleled by variations in vessel diameters (Table 3). Weighted hydraulic diameters d_h were significantly different between *H. salsola* populations 2 and 3, but not among *A. dumosa* populations. In population 3 of both species distributions of vessel d_h^4 showed that small vessels were responsible for a larger proportion of total stem conductance (Fig. 3a, b). The two species did not differ from each other either for the mean d_h or for the diameter distributions ($P>0.05$). Differences among organs in vulnerability to cavitation were very clear in both species (Fig. 4a, b), with the greatest susceptibility in the roots followed by the young twigs, while the woody stems proved to be the most resistant organ.

After resprouting, shoots underwent a phase of rapid longitudinal growth (Fig. 5a) which slowed down after 2 months and almost ceased after 3 months. During the same time period, the shoots also showed a gradual shift from being very susceptible to being very resistant to cavitation (Fig. 5b, c). The final woody stage replicated the results already obtained for the same populations (cf. Figs 1, 2), whereas the first stages of young resprouts showed a much greater susceptibility to cavitation com-

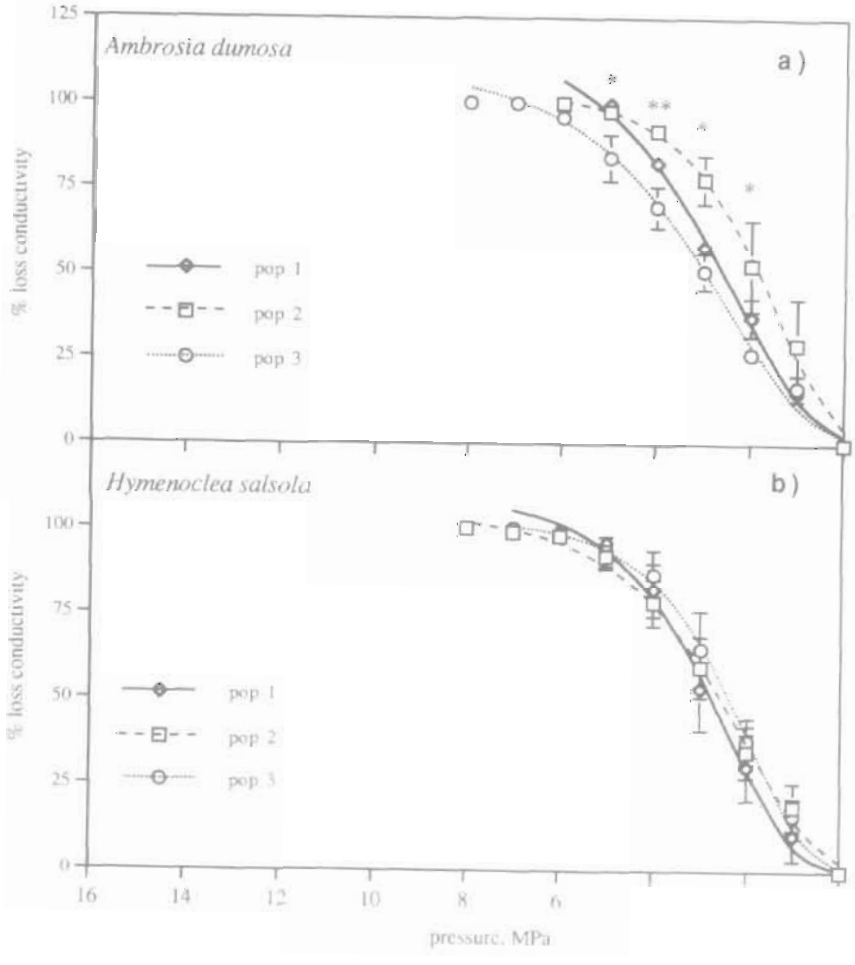

Fig. 2. Vulnerability curves for green stems of the two desert species *Ambrosia dumosa* (a) and *Hymenoclea salsola* (b). Seeds from population 3 of both species were sampled at Organ Pipe Cactus National Monument, Az, a site characterized by high leaf–air humidity gradients during the growing season, while populations 1 and 2 were sampled at Goldield, Nv, and at Beaverdam, Ut, respectively, which are both characterized by relatively low leaf–air humidity gradients during the growing season. Green twigs were sampled from the terminal shoots in the canopy, basal to the leafy portion. Stars denote significant differences among populations: *, $P<0.05$; **, $P<0.01$. For *A. dumosa*, population 3 was significantly more resistant than 2, but not than 1. For *H. salsola*, no difference was detectable among populations.

Table 2. *Comparison of vulnerability thresholds (±1 s.e.) of woody stems and young twigs for different populations of* Hymenoclea salsola *and* Ambrosia dumosa[a]

Populations	Thresholds (MPa)				Range of maximum pore diameter (μm)
	10%	20%	50%	80%	
Hymenoclea salsola. woody stems ($n=30$)[b]					
1	0 78 (0.15) ab	1.30 (0.14) a	2 87 (0.30) a	5.32 (0.57) a	0.054–0.369
2	0.48 (0.12) a	1 09 (0.12) a	2.66 (0.24) a	5.20 (0.51) a	0 055–0.600
3	1.25 (0.15) b	1 95 (0.14) b	3.53 (0.29) a	5.04 (0.39) a	0 057–0 230
Bay	ns	ns	ns	ns	
Hymenoclea salsola. young twigs ($n=23$)[b]					
1	1.14 (0.25) a	1 52 (0.25) a	2.38 (0.37) a	3.38 (0.52) a	0.085–0 253
2	1.17 (0.17) a	1.70 (0.17) a	2 80 (0.25) a	3.89 (0.35) a	0.074–0.246
3	0.56 (0.21) a	1.14 (0.21) a	2.41 (0.31) a	3.56 (0.44) a	0.081–0.514
Bay	ns	ns	ns	ns	
Ambrosia dumosa woody stems ($n=15$)[b]					
1	1.62 (0.42) a	2.69 (0.42) ab	5.52 (0.56) b	8.24 (1.04) b	0.035–0.0178
2	1.05 (0.34) a	1.54 (0.35) a	2.53 (0.46) a	4.13 (0.85) a	0.070–0.274
3	1.62 (0.37) a	2.87 (0.38) b	5.40 (0.51) b	7.75 (0.93) b	0.037–0 0178
Bay	ns	ns	ns	ns	
Ambrosia dumosa: young twigs ($n=14$)[b]					
1	0.74 (0.22) a	1.35 (0.23) a	2.64 (0.26) ab	3.93 (0.35) ab	0.073–0.389
2	0.70 (0.22) a	1.01 (0.23) a	1.87 (0.26) a	2.93 (0.35) a	0.098–0.411
3	0.82 (0.24) a	1.55 (0.26) a	3.15 (0.30) b	4.83 (0.39) b	0.60–0.351
Bay	ns	ns	ns	ns	

[a] Significance levels refer to two-way ANOVA tests. Confidence intervals tests were used for multiple comparisons of treatment means. Means within a column not followed by the same letter are significantly different ($P<0.05$). Estimates of maximum wall pore diameters made using Equation (1) and the 10% and the 80% thresholds.

[b] n refers to number of individuals tested.

Table 3. *Comparison of weighted vessel hydraulic diameters* d_h *in μm (±1 s.e.) in woody stems of different populations of* Hymenoclea salsola *and* Ambrosia dumosa (n=*23)*

Population	*Hymenoclea salsola*		*Ambrosia dumosa*	
	d_h	*F*	d_h	*F*
1	41.62 (1.61) ab		34.90 (2.62) a	ns
2	43.35 (1.39) a	*	35.97 (2.42) a	
3	36.80 (1.68) b		30.61 (2.03) a	
Bay		ns		ns

Weighted d_h calculated according to Equation (2). *F*-test refers to two-way ANOVA. Multiple comparisons of treatment means made with confidence intervals tests. Means within a column not followed by the same letter are significantly different ($P<0.05$), ns, not significantly different.

pared with the young twigs in Experiments 1 and 2. Data collected over different days showed that young resprouts had significantly higher (i.e. less negative) xylem water potentials than terminal twigs of mature canopies (always $P<0.01$).

Consistent with the cavitation data, roots had significantly larger vessels than woody stems in both species ($P<0.01$, Fig. 6a, b). When all the data from different organs were pooled together, significant inverse relationships were found between vessel d_h and cavitation thresholds (Table 4). However, when cavitation thresholds were related to vessel diameters for each organ separately, no significant relationship was found (always $P>0.05$).

Discussion

There are no published vulnerability curves for species of the North-american deserts. The resistance to cavitation was compared for the two sub-shrubs with species of different ecosystems (Californian chaparall and coastal sage), which are characterized by a roughly similar degree of summer drought stress. Two shallow-rooted perennial shrubs of chaparral of southern California, *Heteromeles arbutifolia* (Lindley), and *Ceanothus megacarpus* Nutt., reached 50% cavitation rate at about 6.2 and 11 MPa, respectively (Kolb and Davis, 1994; Jarbeau *et al.*, 1995), well beyond the values for our two species. By contrast, *Malosma laurina* (Nutt.) Abrams, a deeply-rooted perennial shrub of chaparral, reached 50% loss of conductivity at about 1.6 MPa (Jarbeau *et al.*, 1995), a figure much lower than the data for *H. salsola* and *A. dumosa*. The only shrub with a habit similar to that of the two species used here, *Salvia mellifera* Greene, a drought deciduous, shallow-rooted perennial shrub of coastal sage, reached 50% embolism at a water potential of about 4.5 MPa (Kolb and Davis, 1994), a value larger than the data for *H. salsola*, which typically grows inside or on the border of washes, but smaller than for *A. dumosa*, more frequent on gravelly plains and rocky hillslopes. For woody stems, *H.salsola* showed greater velnerability to cavitation in two out of three populations than *A. dumosa*. However, green stems were equally susceptible, suggesting that the

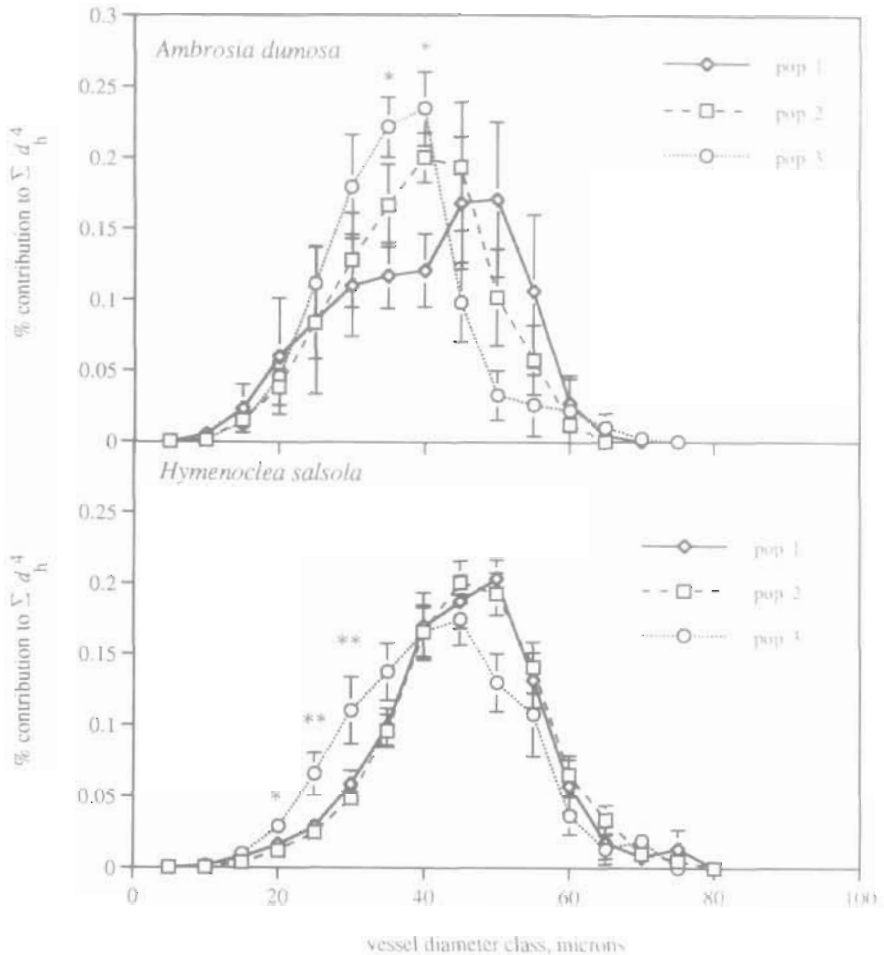

Fig. 3. Distribution of vessel hydraulic diameters d_h according to their contribution to the total conductance of the woody stems (assumed proportional to $\sum d_h^4$). Symbols for populations as in Figs 1 and 2. Stars denote significant differences among populations. *, $P<0.05$; **, $P<0.01$ For both species, population 3 had larger frequencies in the smaller vessel classes.

two species may respond very similarly to a reduction in soil water potential at the beginning of a drought. The greater vulnerability of peripheral twigs compared with basal stems contrasts with the opposite pattern found for the canopy of *Q. ilex* by LoGullo *et al.* (1995), where 1-year-old twigs had greater resistance to cavitation and narrower and shorter vessels, than 2- and 3-year-old stems. In both species used in this experiment, leaves are progressively shed during a drought, whereas woody stems normally survive for at least two seasons in *H. salsola* and for many years in *A. dumosa*, constituting the structure of the canopy for the new regrowth. On the other hand, while some of the terminal twigs can be shed during a drought, those that endure the stress period become hardened and more lignified. It is possible therefore that the vulnerability curves for the young twigs represent a specialization for the rather mild conditions prevailing during the growing season while the woody stems are specialized to sustain the greater water stress levels of drought periods.

In *H. salsola*, population 3, which originated in a warm dry site, showed a slightly more resistant xylem in the woody tissues than the other two populations, but no difference was evident among the young twigs. In *A. dumosa*, woody stems of population 1 sampled at a site characterized by lower temperatures and low Δw, had a vulnerability curve similar to those of population 3, originated at a much warmer and drier site. Moreover, differences did not clearly match with any single climatic parameter (e.g. Δw). The anatomical differences among populations were generally minor and only partially consistent with the climatic differences among sites.

For the greenhouse experiment, the environmental differences among bays did not determine variations in susceptibility to cavitation and anatomical characters in either species, despite the fact that the range in air temperature and humidity was large enough to double Δw, potentially causing strong variations in transpiration rates among bays. How could plants growing in environments characterized by largely different transpiration rates have the same vulnerability curves? Larger transpiration rates can be sustained by either lower leaf Ψ_{px} or higher

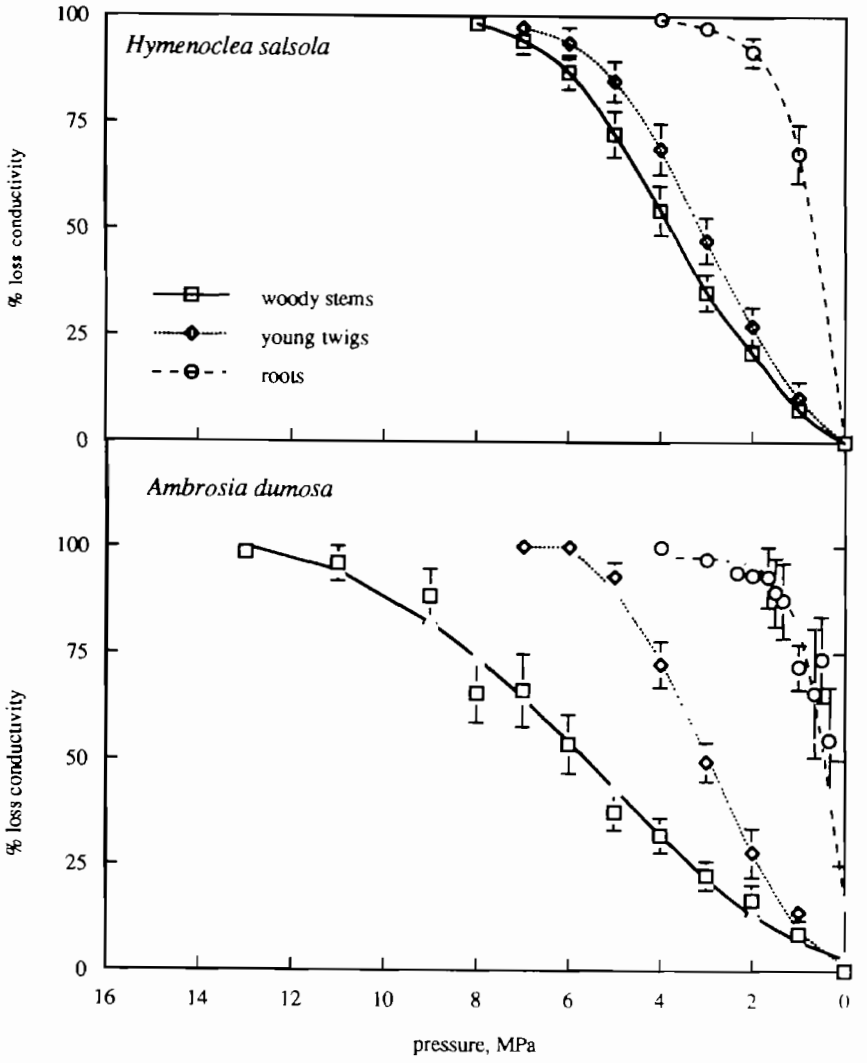

Fig. 4. Vulnerability to cavitation for different organs in (a) *Hymenoclea salsola* and (b) *Ambrosia dumosa*. Roots were very vulnerable to cavitation, while woody stems were very resistant Young twigs had intermediate values. Plants were sampled from two populations (1 and 2 in *A. dumosa* and 1 and 3 in *H. salsola*) of each species and the respective values were aggregated because of the lack of any difference between the two (see Figs 1, 2).

hydraulic conductance per unit of leaf area. In the first case, a higher cavitation rate would be predicted, whereas in the second case the adjustment in the balance between conducting organs and transpiring leaf area would allow a constant water potential and a constant cavitation rate to be maintained. The native cavitation rates for the different populations and the different greenhouse environments were not determined; however, the gas exchange experiments conducted on the same plants (see Experimental Set-up paragraph) suggested that a similar xylem water potential was maintained in all individuals, independently of growing conditions and origin (Comstock and Mencuccini, unpublished results). A constant water potential would probably reduce the selective pressure for the development of a different xylem anatomy and resistance to cavitation among populations and environments. Reductions in leaf area development with increasing Δw have been reported for a number of *H. salsola* populations growing in the field (Comstock and Ehleringer, 1992) and for eight additional desert species (Comstock *et al.*, 1988) and have been explained with the need to accommodate different transpiration rates along the Δw gradient.

The high vulnerability to cavitation of young resprouts compared with the young twigs at the top of the canopy may suggest the existence of a link between vulnerability to cavitation and plant hydraulic conductance. Resprouts could take advantage of an intact root system while a very low leaf area was present (i.e. a high leaf-specific hydraulic conductance). It is possible that a more resistant xylem would have not represented an adaptive character, under this very favourable conditions (as shown by the significantly less negative values of Ψ_{px}).

Large differences were found in the vulnerability to

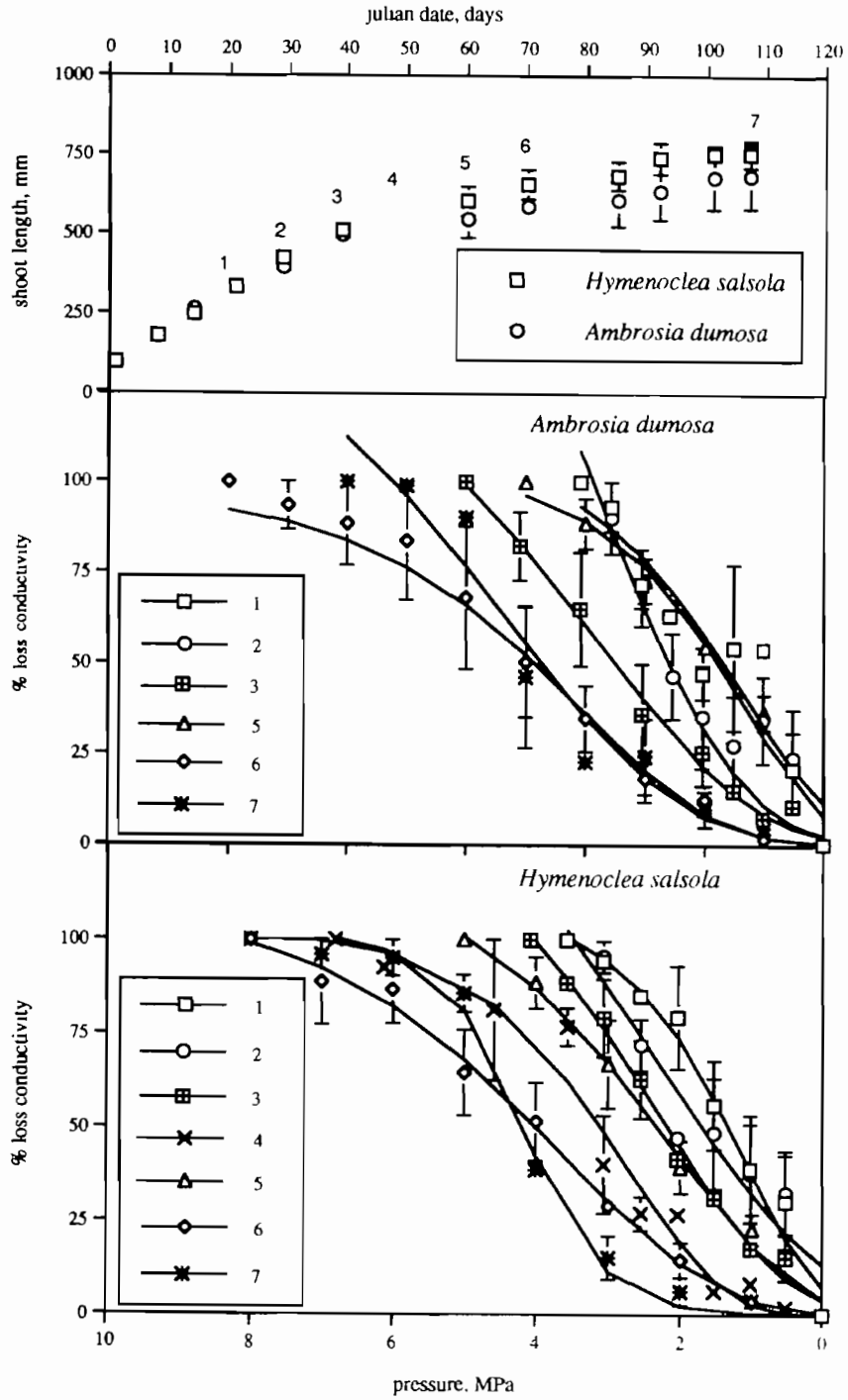

Fig. 5. Effects of the ontogenetic development of canopy shoots on their vulnerability to cavitation. Shoot development was followed after cutting back the whole canopy and subsequent resprouting from the stumps. Xylem in the shoots sampled first was almost entirely composed of protoxylem and metaxylem elements, whereas the last shoots bore mainly secondary xylem elements. (a) Shoot length growth after resprouting for the two species. (b) Developmental sequence of vulnerability to cavitation for *A. dumosa*. (c) Developmental sequence of vulnerability to cavitation for *H. salsola*. Numbers from 1 to 7 mark the sampling dates.

cavitation of different plant organs. Roots exhibited a much greater susceptibility to cavitation than young twigs and woody stems, suggesting that negative water potentials can be more critical in roots than in other organs. However, because they were located at the beginning of the catena of hydraulic resistances, roots experience higher water potentials than other organs. To confirm the suggestion that critical water potentials for runaway cavitation are most likely to happen in the roots, the development of cavitation was simulated using a numerical model of

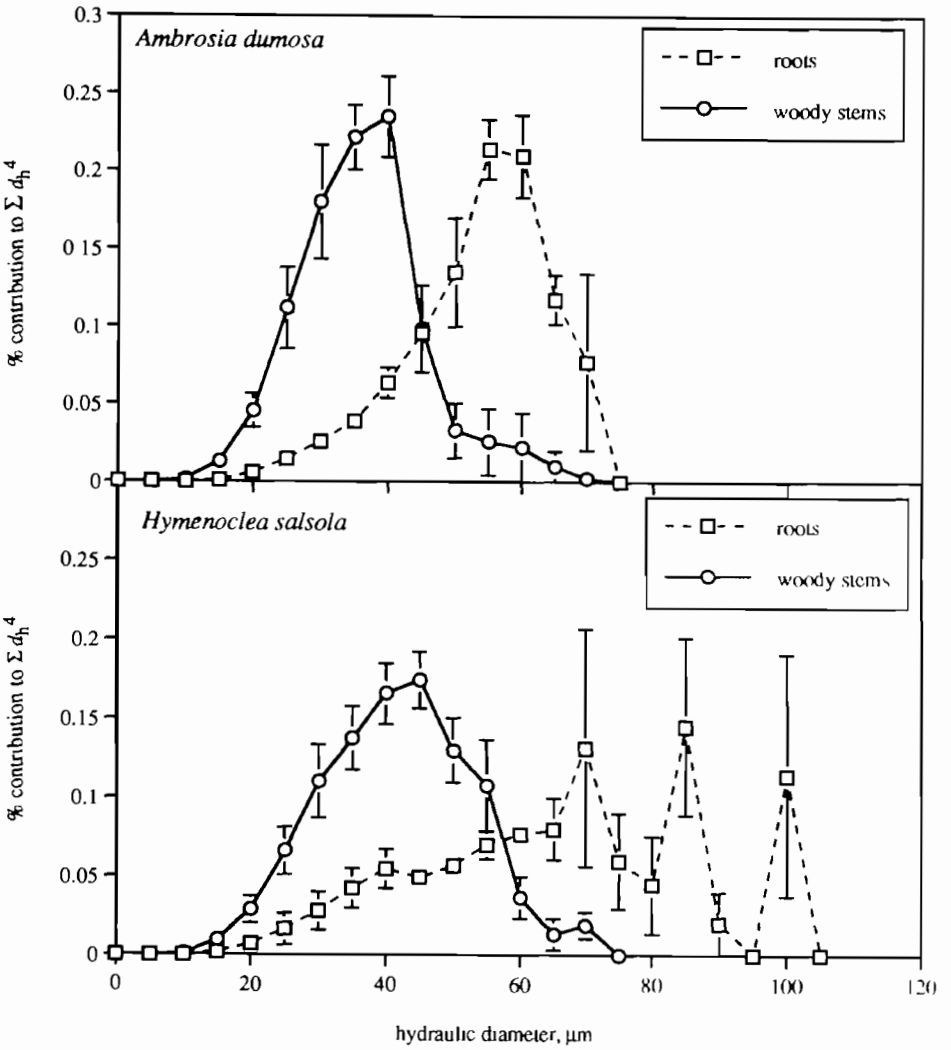

Fig. 6. Distribution of vessel hydraulic diameters d_h according to their contribution to the total conductance of roots or woody stems (assumed proportional to $\sum d_h^4$). (a) Distribution for *A. dumosa*. (b) Distribution for *H. salsola*. For both species, roots showed significantly larger vessel hydraulic diameters than woody stems ($P<0.01$).

Table 4. *Regressions between cavitation thresholds (MPa) and vessel* d_h *(μm) in* Hymenoclea salsola *and* Ambrosia dumosa *(data from all populations and tissue types)*

Thresholds	Equation	R^2
Hymenoclea salsola		
10%	$1/y = -0.627 + 0.054\ d_h$	8.3% ns
20%	$1/y = -1.078 + 0.046\ d_h$	36.5%***
50%	$1/y = -0.458 + 0.021\ d_h$	43.6%***
80%	$1/y = -0.144 + 0.089\ d_h$	42.3%***
Ambrosia dumosa		
10%	$1/y = -2.122 + 0.104\ d_h$	21.6% ns
20%	$1/y = -3.066 + 0.111\ d_h$	70.7%***
50%	$1/y = -1.685 + 0.061\ d_h$	71.3%***
80%	$1/y = -0.964 + 0.035\ d_h$	71.9%***

Vessel weighted hydraulic diameters d_h calculated according to Equation (3). The inverse model fitted to the data is based on Equation (2), assuming vessel diameters and wall pore radii are directly related to each other. R^2, percentage of variance explained by the equation. ***, $P<0.001$; **, $P<0.01$; *, $P<0.05$; ns, not significantly different.

a plant composed of hydraulic resistances in series (root endodermis, xylem of the root, stem and young twig, leaf blade). Each organ was given values of hydraulic conductance so that whole-plant hydraulic conductance was equal to 30 mmol $m^{-2}\ s^{-1}\ MPa^{-1}$, similar to the average found for all our plants (Comstock and Mencuccini, unpublished results). To test whether the values attributed to each organ influenced the dynamics of embolism development, a sensitivity analysis was performed by varying the proportion of resistance in the root and the shoot organs. Vulnerabilities to cavitation for the xylem in the roots, stems and young twigs were approximated by analytical functions (see Equation 4 and Fig. 4a, b). Resistances in the root endodermis and leaf blade were assumed to be constant (cf. Jones and Sutherland, 1991). The programme started with a value of soil water potential Ψ_s equal to -0.1 MPa, equilibrium profiles of xylem pressure potentials Ψ_{px} and hydraulic conductances for all the

tissues, and a transpiration rate E (mmol $m^{-2} s^{-1}$) of zero. Increasing E by 1 mmol $m^{-2} s^{-1}$ caused the Ψ_{px} of each tissue to drop with a subsequent drop in the hydraulic conductance because of the onset of cavitation. This further reduced Ψ_{px}. Calculations were continued until values of stable Ψ_{px} and tissue conductances in equilibrium with E were found. E was then increased by another unit and the process repeated until no equilibrium Ψ_{px} could be found and one of the plant tissues collapsed (due to runaway cavitation in the absence of stomatal control of E). Calculations were repeated in subsequent runs by sequentially decrementing Ψ_s, until $\Psi_s = -3.0$ MPa.

In Figure 7 the relationships between each fixed value of E and the corresponding equilibrium value of cavitation rate for the three tissues are given. Three cases, corresponding to three different values of Ψ_s, are plotted. Each curve terminates when a further unit increment in E caused such a large decrease in Ψ_{px} to determine the onset of runaway cavitation (i.e. hydraulic conductance of one tissue dropped to zero). The stars at the end of the curves mark the tissues responsible for runaway cavitation. For both species and at all transpiration rates, simulations predicted large amount of embolism in roots and low amounts in woody stems. The degree of embolism in terminal young twigs varied depending on the extent of hydraulic resistance apportioned to the above-ground, as opposed to the below-ground organs. If the resistance attributed to the roots was medium to large (above 15% of the whole plant value), roots were predicted to be the first to undergo runaway cavitation. Young twigs were predicted to be the first to collapse catastrophically only when root resistance was hypothesized to be very low (below 10% of the whole plant value) and soil water potentials very high (Ψ_s = from 0 to −0.3 MPa) (Fig. 7). Using independent measurements of xylem water potentials at different points along the pathway (Comstock and Mencuccini, unpublished results), it was estimated that root hydraulic resistance always accounted for at least 50% of the total plant value. It was concluded that roots were the most vulnerable organ among those sampled. A soil-to-root resistance in the model was not included, even if it is likely that such a resistance is present during drought development. However, this does not affect the conclusions, because all of the vulnerable 'pipelines' are located after the soil–root interface along the hydraulic pathway. Therefore, the development of a lower Ψ at the soil–root interface would only lower the assumed effective value of Ψ_s.

It could be asked why roots were so susceptible to cavitation. Roots had larger vessel diameters (Fig. 5a, b) and probably higher hydraulic conductivity (i.e. conductance per unit of cross-sectional area), than woody stems. If large vessels are also characterized by large pit pore sizes, then a possible trade-off between conducting efficiency and safety could be speculated, as suggested by some authors (Baas, 1976; Zimmermann, 1983). Using two different staining approaches, LoGullo and Salleo (1993) and Hargrave *et al.* (1994) found clear evidence that, within each individual, larger vessels of *Quercus ilex* L. and of *Salvia mellifera* Greene are more likely to cavitate than narrower ones. In our dataset, when data

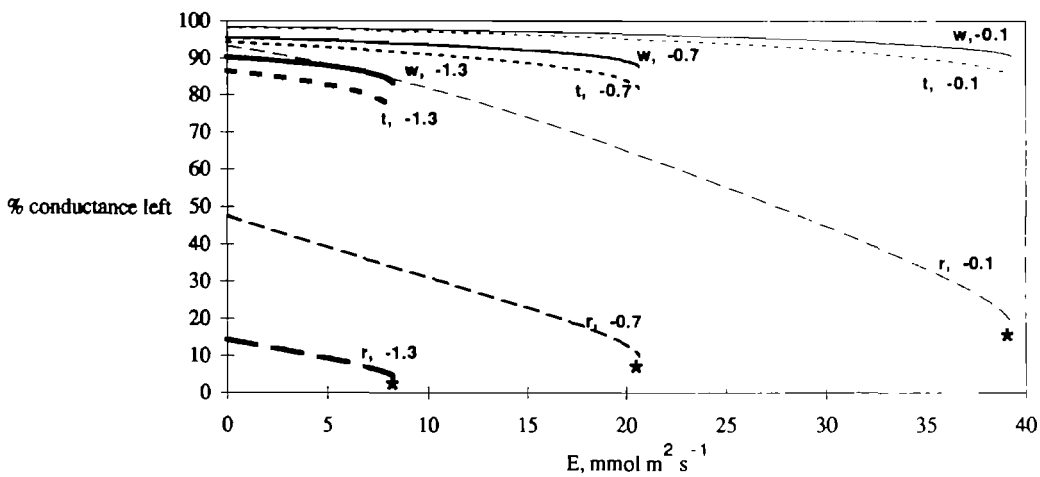

Fig. 7. Simulation of cavitation development in roots (long dashed lines and r), woody stems (continuous lines and w) and green twigs (short dashed lines and t) at different values of transpiration rate (E) and soil water potential, Ψ_s. Three typical simulations corresponding to different values of Ψ_s (−0.1, −0.7 and −1.3 MPa, thin, medium and thick lines, respectively) are shown. The simulation was based on a model of a catena of hydraulic resistances in the plant and assumes no stomatal control of transpiration. Inputs for the model were represented by the vulnerability curves to cavitation taken from Fig. 4, and the assumed Ψ_s. Each curve starts with $E = 0$ and the equilibrium values of hydraulic conductance for each tissue are determined solely by Ψ_s. For each value of E (x-axis) the model calculated equilibrium losses of hydraulic conductance based on water potential gradients within the plant (y-axis), i.e. larger values of E caused progressively larger losses in hydraulic conductance for each tissue. However, the extent of the reduction depended on tissue vulnerability and position along the hydraulic catena. The sudden acceleration of cavitation development at the end of each curve signals the onset of runaway cavitation (end of the model run). A star marks the curve responsible for plant catastrophic collapse. Roots always had the largest cavitation rates and determined the plant collapse, independently of the level of Ψ_s.

were disaggregated according to tissue types, the relationships between vessel d_h and vulnerability thresholds lacked significance in both species. However, when data from different organs were pooled together, significant relationships were found. Therefore variations in vessel diameter among plant organs are strictly linked to variations in pit pore size; it is unlikely that this is the case for variations in vessel diameter among different individuals of the same species. It seems, therefore, that the trade-off between conducting efficiency and safety, if present, may be important only in regulating the functioning of different tissues, not the ecological relations among plants (Tyree *et al.*, 1994).

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