

Long-term acclimatization of hydraulic properties, xylem conduit size, wall strength and cavitation resistance in *Phaseolus vulgaris* in response to different environmental effects

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ABSTRACT

Phaseolus vulgaris grown under various environmental conditions was used to assess long-term acclimatization of xylem structural characteristics and hydraulic properties. Conduit diameter tended to be reduced and 'wood' density (of 'woody' stems) increased under low moisture ('dry'), increased soil porosity ('porous soil') and low phosphorus ('low P') treatments. Dry and low P had the largest percentage of small vessels. Dry, low light ('shade') and porous soil treatments decreased P_{50} (50% loss in conductivity) by 0.15–0.25 MPa (greater cavitation resistance) compared with 'controls'. By contrast, low P increased P_{50} by 0.30 MPa (less cavitation resistance) compared with porous soil (the control for low P). Changes in cavitation resistance were independent of conduit diameter. By contrast, changes in cavitation resistance were correlated with wood density for the control, dry and porous soil treatments, but did not appear to be a function of wood density for the shade and low P treatments. In a separate experiment comparing control and porous soil plants, stem hydraulic conductivity (k_h), specific conductivity (k_s), leaf specific conductivity (LSC), total pot water loss, plant biomass and leaf area were all greater for control plants compared to porous soil plants. Porous soil plants, however, demonstrated higher midday stomatal conductance to water vapour (g_s), apparently because they experienced proportionally less midday xylem cavitation.

Key-words: hydraulic conductivity; embolism; soil texture; moisture; shade; phosphorus; wood density.

INTRODUCTION

Long-term structural acclimatization is an important mechanism that enables plants to modify the water supply to the canopy (Ewers, Oren & Sperry 2000; Magnani, Mencuccini & Grace 2000; Mencuccini 2003; Li, Xu & Cohen 2005). While recent work has highlighted the adaptive significance

of variation in cavitation resistance and water transport capacity between species (Maherali, Pockman & Jackson 2004), much less is known about whether variation in cavitation resistance and water transport capacity exists in individual plants because of long-term acclimatization. If species can optimize hydraulic conductivity (k_h) for a given investment in transport tissue (McCulloh & Sperry 2005), investing only as much as what is necessary for their particular water use niche (Sperry *et al.* 2002), it may also be possible that individual plants can acclimatize to optimize their water transport. Recent studies looking specifically at long-term hydraulic acclimatization are few. Magnani *et al.* (2000) highlighted long-term structural acclimatization of foliage, conducting sapwood and fine roots in ageing forest stands. The importance of structural acclimatization was reviewed by Mencuccini (2003) who concluded that structural acclimatization to environmental factors which increased the availability of a resource tended to result in a less efficient (per unit leaf area) hydraulic system. Most recently, Li *et al.* (2005) compared the effect of soil texture and evaporative demand on structural acclimatization. They found that sand-grown plants developed greater root length in response to lower root conductivity, and plants under higher evaporative demand also developed more absorptive roots.

If long-term acclimatization by individual plants can work to optimize their water transport, a second question is whether the same kinds of trade-offs that constrain hydraulic optimization at the species level would also constrain long-term acclimatization of individuals. Recent evidence has shifted attention from the 'safety versus conducting efficiency' trade-off (Baas 1976; Carlquist 1982; Zimmermann 1983) towards a trade-off between construction cost and hydraulic conductance (k_h). This 'energy minimization' principle states that xylem resistance should be the least possible for a given investment in vascular tissue (West, Brown & Enquist 2000; McCulloh & Sperry 2005). Environmental effects that increase stem mechanical support, for example, may come at the expense of a decrease in k_h .

This study focuses on three traits (i.e. conduit diameter, inter-conduit pore diameter and conduit reinforcement)

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important to k_h , cavitation resistance and mechanical stability. Changes in conduit diameter have the potential to dramatically affect k_h because of the Hagen–Poiseuille law (Zimmermann 1983; Tyree, Davis & Cochard 1994). Changes in inter-conduit pore size can affect cavitation resistance through ‘air seeding’, the aspiration of an air bubble into a water-filled conduit through inter-conduit pores (Zimmermann 1983; Sperry & Tyree 1988; Tyree *et al.* 1994). A change in conduit reinforcement would affect both mechanical stability and cavitation resistance (Hacke *et al.* 2001). Hacke *et al.* (2001) found that greater wood density and conduit wall reinforcement, the ratio of conduit wall thickness (t) to conduit wall span (b), were necessary to support against conduit implosion because of the negative pressures within the xylem.

The overall objective of this study was to determine if various environmental factors result in structural acclimatization of vessel size, wall strength and inter-conduit pore size in the herbaceous *Phaseolus vulgaris*. In addition, this study sought to determine the effect of these changes on hydraulic properties and cavitation resistance, and to determine whether changes in structural traits correlated with each other, reflecting possible trade-offs.

MATERIALS AND METHODS

Experiment 1

Experimental material and propagation

Between May and July 2000, seeds of *P. vulgaris* (common bean) cv. Othello and Durango race (Mencuccini & Comstock 1999) were grown in a GC-16 plant growth chamber (ENCONAIR Ecological Chambers, Inc., Winnipeg, MB, Canada) on the campus of Augustana College, Sioux Falls, SD, USA. Within the growth chamber, temperatures were set at 25 °C during the day and 20 °C at night. Light levels averaged at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the level of the canopy. Humidity levels fluctuated with outside conditions ranged between 26.5 and 78.6%. Plants were grown in 3 L pots, with 2 bean plants per pot. Treatment pots (9 replicates per treatment) were exposed to reduced light levels (‘shade’), reduced moisture levels (‘dry’), reduced phosphorus nutrition (‘low P’) and increased soil porosity (‘porous soil’), and compared with the control treatment (‘control’). Treatments were imposed within 1 week of seed germination, and continued for the duration of the experiment. A shade cloth was used for the shade treatment, and light levels averaged at 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The porous soil treatment served as control for the reduced low P treatment. The porous soil was chosen for the low P treatment because it is chemically inert, and therefore gives better control for nutrient deficiency studies than using regular potting soil. The soil medium for the control, dry and shade treatments was composed of a potting soil, black dirt, peat moss, sand and perlite (1:1:1:0.5:0.5, by volume) mixture with 0.70 kg L^{-1} bulk density and 0.74 total porosity (calculated assuming 2.65 kg L^{-1} particle density). The soil medium for the porous soil and low P treatments was composed of

fritted clay with 0.67 and 2.5 kg L^{-1} bulk density and particle density, respectively, and 0.73 total porosity (Dririte, Crestwood, IL, USA; van Bavel, Lascano & Wilson 1978). Although similar in bulk density and total porosity, the saturated hydraulic conductivities (k_{soil}) of the two soils were quite different, with the porous soil having a k_{soil} of $8.2 \times 10^{-4} \text{ kg m s}^{-1} \text{ MPa}^{-1}$ (SE = 2.4×10^{-5}), and the control soil mixture having a k_{soil} of $3.4 \times 10^{-5} \text{ kg m s}^{-1} \text{ MPa}^{-1}$ (SE = 3.6×10^{-6}). The control, shade and porous soil treatments were watered daily, alternating between a one-tenth strength nutrient solution (Johnson *et al.* 1957) and deionized water. The dry treatment plants were kept between 50 and 75% of field capacity. Pot moisture level was monitored using a balance (Weight-Tronix, Fairmont, MN, USA); when pots neared 50% of field capacity, water was added until they were close to 75% of field capacity. The low P treatment was watered daily, alternating between deionized water and a modified one-tenth Hoagland’s solution containing 0.01 mM phosphorus, compared to the 0.2 mM concentration received by all the other treatments. Additional slow-release supplemental nutrients (~ 100 g) were given to the porous soil treatments in the form of Osmocote (14-14-14 NPK; Scotts-Sierra, Marysville, OH, USA).

Vulnerability curves

Stems, at least 14.5 cm in length with one or more nodes, were harvested between June and July 2000. Stem segments of *P. vulgaris* were cut under water following the protocols described by Sperry & Saliendra (1994). The segments were flushed with a 0.22 μm filtered water solution for at least 15 min to refill any embolized vessels and ensure maximum conductance. After flushing, the stems were perfused with 0.22 μm filtered water solution, adjusted to pH < 2.0 with hydrochloric acid. k_h was calculated based on stem exudate measurements. Xylem cavitation was induced using the centrifugal method (Alder *et al.* 1997). The amount of cavitation was quantified by measuring k_h after applying tensions of 0.0, -0.3, -0.6, -1.0 and -1.4 MPa. k_h measured at 0.0 MPa was expressed as the maximum conductance; all conductance measurements at the more negative tensions were expressed as a per cent loss from this initial conductance (PLC).

Vessel diameters

Sections of stem segments used for measurements of cavitation resistance were frozen and used later for measurements of vessel diameter. The stems were sectioned with a hand microtome and stained with toluidine blue O (TBO) solution (1 mL 0.5% TBO:20 mL distilled water). Stained stem sections were viewed under an Olympus BH-2 light microscope (Olympus America, Inc., Melville, NY, USA), and pictures of xylem conduits were taken with a SPOT insight digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI, USA). An image analysis programme (SPOT Advanced; Diagnostic Instruments, Inc.) was used to calculate the cell areas of the irregularly shaped xylem

vessels, while diameter was calculated as the diameter of an equivalent circle.

Conduit wall strength

Sections of stem samples were used to measure 'wood' density. Following the protocols described by Hacke, Sperry & Pittermann (2000), 3-cm-long sections were placed in a vial of deionized water and equilibrated under a vacuum for 24 h. The sections were longitudinally cut, and the pith, phloem and epidermis were removed with a razor blade. Fresh volume of the stem sample was determined by Archimedes' principle. The stems were submerged, and displacement weight was converted to stem fresh volume by dividing by the density of water (in grams per millilitre). The samples were then dried at 70 °C for 48 h, and wood density was calculated as the ratio of dry weight (DW) to fresh volume.

Estimates of conduit wall strength $(t/b)_h^2$, the ratio of conduit wall thickness (t) to wall span (b) based on the hydraulic mean diameter (h), were obtained for the control and porous soil treatments. The $(t/b)_h^2$ was calculated as described by Kolb & Sperry (1999). Values of $(t/b)_h^2$ were calculated as described by Hacke *et al.* (2001). Values of t and b were measured on conduit pairs where at least one of the vessels was within 3 mm of the $(t/b)_h^2$ (rather than both). This was because of the vessel arrangement in *P. vulgaris*, which made it extremely unlikely for two larger-sized vessels to be directly adjacent to each other. Values of t and b were measured using an image analysis software (SPOT Advanced; Diagnostic Instruments, Inc.).

Experiment 2

Experimental material and propagation

During the last week of May and the first week of June 2004, seeds of *P. vulgaris* cv. Othello (same as in experiment 1) were germinated and grown in 5 L pots with 1 bean plant per pot (15 replicates per treatment) in a glasshouse on the campus of Augustana College. The soil medium was the same as in the experiment for the control and porous soil treatments. Both treatments were watered daily and given 250 mL one-fourth strength nutrient solution (Johnson *et al.* 1957) 3 times per week. As in experiment 1, slow-release supplemental nutrients were also given to both treatments in the form of Multicote controlled release fertilizer (14-14-14 NPK; Haifa NutriTech, Inc., Altamonte Springs, FL, USA). Plants were harvested during the second week of July 2004.

Water use, conductance and xylem water potential measures

Stomatal conductance to water vapour (g_s) measurements were made on upper canopy leaves between 1000 and 1400 h (solar time) with a Li-Cor 1600 steady-state porometer (Li-Cor, Lincoln, NE, USA) between 24 June and 2 July 2004. Plant water use was measured over the same time

period by watering plants to field capacity, bagging to prevent evaporation and monitoring water loss gravimetrically using a balance (Weight-Tronix, fairmont, MN, USA). The plants were maintained near 95% of their field capacity. Midday xylem water potential (Ψ_{mid}) was measured for plants watered to field capacity with a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) on 9 July 2004.

Stem k_h measures were made on ~15.0-cm-long stems, with at least one node. The stem segments were flushed with 0.22 μm filtered water for at least 15 min to refill any embolized vessels. After flushing, the stems were perfused with 10 mM potassium chloride solution (pH between 6.0 and 7.0). k_h , defined as the mass flow rate through the stem divided by the pressure gradient across the stem segment (in kilograms per metre per second per megapascal), was calculated based on stem exudate measurements. For leaf specific conductivity (LSC) (k_h values were divided by plant leaf area), leaf area was measured using Li-Cor 3000 leaf area meter (Li-Cor) or estimated based on the relationship between leaf area and leaf DW.

Statistics

Differences in the vulnerability of dry, shade, porous soil and low P treatments to xylem cavitation were analysed using repeated measures analysis of variance (ANOVA). k_h measurements on stems from individual pots at different levels of tension were the repeated factor. Values of the PLC for -0.3, -0.6 and -1.0 MPa were transformed using a square-root logarithmic transformation to meet normality assumptions. The -1.4 MPa data were excluded from the statistical model because of missing data points (i.e. stem segments were prone to being damaged by centrifugation at higher tensions). Differences in the percentages of vessels in different size classes were also analysed using repeated measures ANOVA. The percentage of vessels in different size classes on individual stems was the repeated factor. Values of the percentage of vessels in different size classes were transformed using a square-root transformation to meet normality assumptions. Effects of treatments on basal diameter, vessel diameter, hydraulic mean diameter, wood density and P_{50} were evaluated using ANOVA; specific comparisons between treatments were made using contrasts. The t -tests were used to compare estimates of $(t/b)_h^2$ between the porous soil and control treatments, and for comparisons of conductance, plant mass, leaf area and water potential between the control and porous soil treatments in experiment 2.

RESULTS

Experiment 1

Exposing plants to various environmental factors caused significant alterations in plant and xylem structural characteristics. The control plants had the greatest above-ground DW biomass with all other treatments reduced in size

Table 1. Effects of environmental factors on xylem structural characteristics in *Phaseolus vulgaris*

Characteristic	'Control'	'Dry'	'Shade'	'Porous soil'	'Low P'
Stem basal diameter (mm)	5.7 A (0.41)	3.6 B (0.17)	3.4 B (0.26)	3.6 B (0.35)	3.2 B (0.17)
Average vessel diameter (μm)	61 A (2.28)	49.3 B (1.16)	58.9 A (2.01)	56.1 A (2.78)	49.3 B (1.25)
Mean hydraulic diameter (μm)	106 A (6.15)	82.64 B (3.20)	107.19 A (7.55)	99.56 A (6.46)	93.55 A (4.52)
'Wood' density (g mL^{-1})	0.2243 A (0.0238)	0.2681 A (0.0174)	0.1712 A (0.0245)	0.3574 B (0.0270)	0.2999 AB (0.0233)
P_{50} (MPa)	-0.4013 A (0.0419)	-0.4984 A (0.0692)	-0.5888 B (0.0625)	-0.6616 B (0.0496)	-0.3771 A (0.0626)
$(t/b)_h^2$	0.0025 A (0.0003)	0.0028 A (0.0003)			

Each number represents the mean ($n = 9$, except for shade where n ranged from 5 to 7). In addition, $(t/b)_h^2$ averages were based on 16 vessel pairs for the control, and 19 vessel pairs for the dry treatment from eight different control stems and four dry stems. SE values are in parentheses. P_{50} is the xylem tension resulting in 50% loss in hydraulic conductivity (k_h); $(t/b)_h^2$ is a measure of conduit reinforcement. Letters next to means indicate significant differences between treatments.

compared to the controls. The next largest were the dry treatment plants (62% of controls), followed by porous soil, shade and low P treatment plants (49, 41 and 10% of the controls, respectively). In addition, the shade plant stems tended to have elongated internodes while the dry and porous soil stems tended to have short internodes. The various treatments caused significant alterations in plant stem basal diameter ($P < 0.0001$), one measure of plant size. In addition, the various treatments significantly affected average xylem vessel diameter ($P < 0.0002$), wood density ($P < 0.0001$) and P_{50} ($P < 0.005$, Table 1). Plant stem basal diameter was smaller for low moisture (dry), low light (shade) and increased soil porosity (porous soil) treatments (all $P < 0.001$), compared with the control. In general, vessel size tended to decrease for all treatments, but the decrease was only significant for the dry treatment ($P < 0.0001$; compared with the control) and for low P ($P < 0.02$; compared with porous soil) (Table 1). The hydraulic mean diameter was also significantly lower for the dry treatment compared with the control ($P < 0.0006$). Wood density tended to increase for most treatments (except shade), but only significantly increased for porous soil ($P < 0.0002$; compared with the control) (Table 1). There was a significant decrease in P_{50} (increased cavitation resistance, Table 1, Fig. 1) for the shade ($P < 0.03$) and porous soil ($P < 0.003$) treatments, compared with the control, and a significant decrease in resistance (increased P_{50}) for the low P treatment ($P < 0.001$) compared with the porous soil treatment (Table 1, Fig. 1). Note that porous soil was the control for low P.

Comparisons of $(t/b)_h^2$ between the porous soil and control treatments were not significantly different. The values observed in *P. vulgaris* were an order of magnitude lower than values reported for woody species (Hacke *et al.* 2001).

Except for the low P curve, the shapes of the PLC curves were similar for the different treatments (Fig. 1). Typically, k_h decreased rapidly with increased tension, with P_{50} occur-

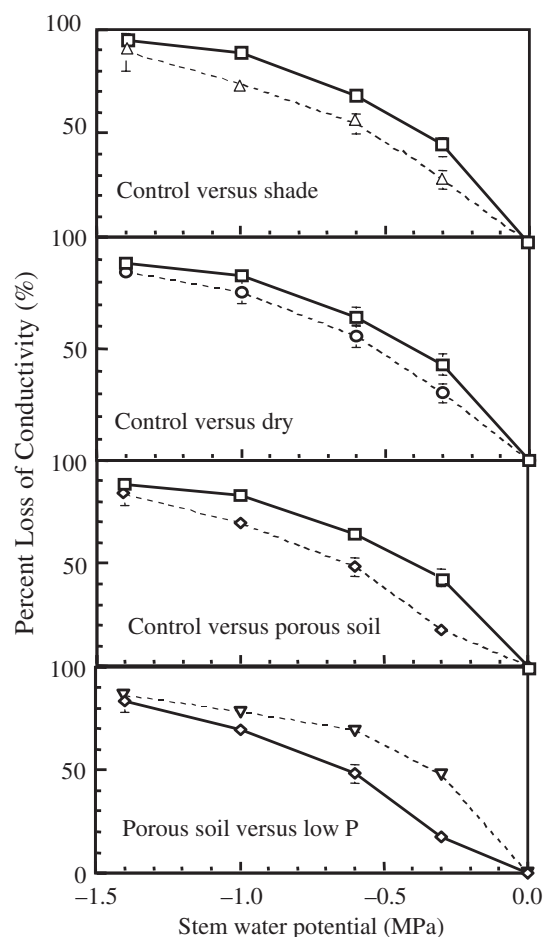


Figure 1. Effect of applied tension (in megapascals) on the per cent loss of conductivity (PLC) in *Phaseolus vulgaris* for 'control' (\square), 'dry' (\circ), 'shade' (\triangle), 'porous soil' (\diamond) and 'low P' (∇) treatments. Each point represents the mean (\pm SE, $n = 9$).

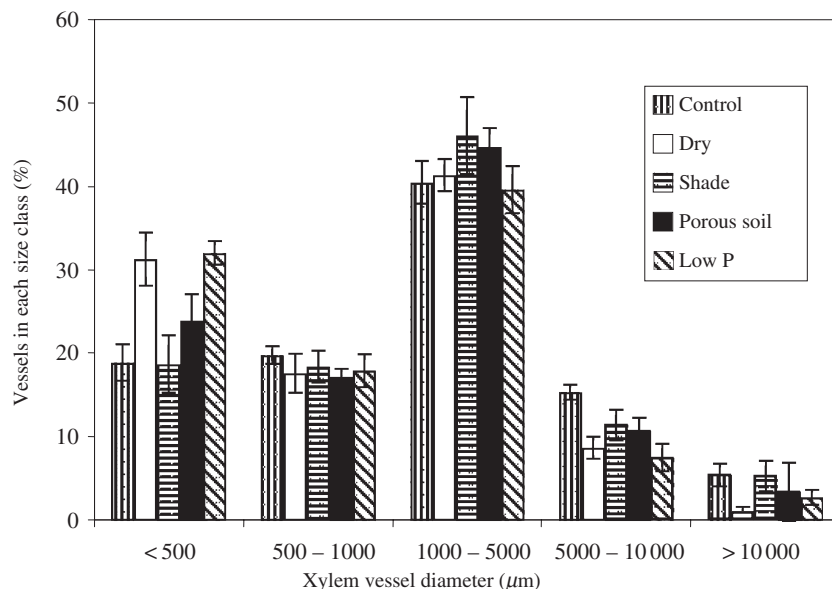


Figure 2. Average percentage of xylem vessels (\pm SE) in different size classes (ranging between <500 and $>10\,000$ μm in diameter) for *Phaseolus vulgaris*. For each treatment, all the vessels from seven to nine different stems were measured. There were ~ 50 – 150 vessels per stem. SE values are in parentheses.

ring between -0.30 and -0.60 MPa. As tensions increased, the rate of decline in k_h decreased with increasing tension. At about -1.4 MPa, the PLC averaged between 85 and 90%. Comparisons of PLC between treatments showed significant differences ($P < 0.001$). The shade and porous soil treatments significantly increased cavitation resistance ($P < 0.03$ and $P < 0.001$, respectively). The dry treatment exhibited a marginally significant increase ($P < 0.09$; Fig. 1). For the shade, porous soil and dry treatments, the P_{50} was shifted by 0.15 to 0.25 MPa (more negative) (Fig. 1). By contrast, the effect of low P on cavitation resistance when compared with porous soil was a significant decrease in resistance ($P < 0.001$), shifting the P_{50} by 0.30 MPa (more positive) (Fig. 1). There was also a significant treatment \times tension interaction ($P < 0.03$), which reflected a difference in the shape of the curve for the porous soil treatment compared with the curve for the low P treatment.

Environmental factors shifted the size class distribution of vessels (Fig. 2). There was a significant treatment \times vessel size class interaction ($P < 0.02$), which reflected a difference in the percentage of vessels in various size classes for the dry treatment compared with the control ($P < 0.007$). The porous soil treatment exhibited a marginally significant difference ($P < 0.07$). The dry, porous soil and low P treatments had a lower percentage of large vessels and a higher percentage of small vessels compared with the control (low P was not significantly different from porous soil).

Experiment 2

Soil porosity had a dramatic effect on both plant morphology as well as water flow characteristics. In response to the different soil types, above-ground biomass, total plant leaf area and maximum individual leaf size were all significantly lower for the porous soil treatment plants (Table 2, $P < 0.0001$ for all). The porous soil plants were about 66% of the size of the control plants in experiment 2. The k_h

values of the porous soil plants were only 38% of those of the control plants (Table 2, $P < 0.0001$), likely because of a smaller percentage of larger vessels. Despite less leaf area, the porous soil plants also had a lower LSC (Table 2, $P < 0.004$), probably a function of a lower k_h . Because of smaller plant size and less leaf area, plant water loss over a period of 6 d was also lower for the porous soil plants

Table 2. Effect of 'porous soil' treatment on above-ground plant biomass, total plant leaf area, maximum leaf size (length \times width), water potential, pot water loss, hydraulic conductivity (k_h), leaf specific conductivity (LSC), stomatal conductance to water vapour (g_s) and pot water loss per unit leaf area

Characteristic	'Control'	Porous soil
Above-ground plant biomass (g)	33.61 A (1.88)	22.27 B (0.98)
Leaf area (cm^2)	4651.3 A (195.61)	2724.49 B (146.33)
Maximum leaf size (cm^2)	118.7 A (6.33)	87.42 B (3.61)
Water potential (MPa)	-0.79 A (0.05)	-0.76 A (0.04)
Pot water loss (kg d^{-1})	0.41 A (0.01)	0.33 B (-0.01)
k_h ($\text{kg m}^{-1} \text{MPa}^{-1}$)	$1.06\text{E-}04$ A ($9.64\text{E-}06$)	$3.99\text{E-}05$ B ($4.16\text{E-}06$)
LSC ($\text{kg m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$)	0.00025 A (0.00003)	0.00014 B (0.00003)
g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	303.5 A (9.4)	344.3 B (16.8)
Pot water loss per unit leaf area ($\text{kg d}^{-1} \text{cm}^{-2}$)	$8.91\text{E-}05$ A ($2.70\text{E-}06$)	$1.23\text{E-}04$ B ($5.71\text{E-}05$)

Each value represents a mean ($n = 13$ – 15 , except for water potential where $n = 7$) with SE values in parentheses. Letters next to means within each column indicate significant differences between treatments.

(Table 2, $P < 0.0001$), but it was still 80% of that of the control plants. Although pot water loss was greater for the control plants, midday water potential did not differ between the two treatments (Table 2), but interestingly 'maximum' midday g_s rates were higher for the porous soil plants (Table 2, $P < 0.04$). Xylem tensions at midday (Table 2) in the beans were great enough to induce cavitation based on the results of experiment 1 (see Fig. 1). The higher g_s rates may be a function of the porous soil plants being more resistant to xylem cavitation compared with the control plants. Despite lower LSC and likely a result of a more resistant xylem, pot water loss per leaf area was actually higher for the porous soil plants compared with the control plants (Table 2, $P < 0.0001$).

DISCUSSION

The results of this study confirm that long-term exposure to a number of environmental factors can result in a significant long-term acclimatization resulting in differences in vessel diameter, cavitation resistance and xylem reinforcement. Comparisons between the control and the porous soil treatment plants indicated that these structural differences could significantly affect (or were related to) measures of k_h , g_s , plant water loss, leaf area and plant size.

Comparing the effects of environmental factors on xylem structural characteristics in this experiment on *P. vulgaris* with the literature, there are some patterns that emerge for some of the treatments. Decreased water supply tended to decrease k_h /vessel diameter in container-grown *Vitis* sp. or *Pinus* sp. (Tognetti, Michelozzi & Giovannelli 1997; Lovisolo & Schubert 1998) and/or increase cavitation resistance (Alder, Sperry & Pockman 1996; Sperry & Ikeda 1997; Kolb & Sperry 1999). The study performed by Alder *et al.* (1996), for example, saw an increase in root cavitation resistance in (more xeric) upslope *Acer gradidentatum* compared with (more mesic) downslope trees. This study on *P. vulgaris* also showed the general trend of increased cavitation resistance with greater water stress. In addition, vessel diameter, hydraulic mean diameter and the percentage of large vessels all decreased, while the percentage of small vessels increased for the dry treatment in this study.

In this *P. vulgaris* study, decreased phosphorus levels decreased resistance to xylem cavitation and conduit size. These results are consistent with those of a study performed by Harvey & van den Driessche (1997), which found that glasshouse-grown *Populus* sp. had greater cavitation resistance with increased phosphorus levels because of decreased pore size. The decline in cavitation resistance in the low P treatment does not appear to be influenced by conduit reinforcement as wood density actually increased slightly compared to that in the control treatment. By contrast, a study performed by Ewers *et al.* (2000) on 15-year-old *Pinus taeda* stands resulted in decreased specific conductivity (k_s) and increased cavitation resistance when fertilized with both phosphorus and nitrogen. The combined fertilization, however, made it difficult to evaluate

whether nitrogen or phosphorus was more important in the effect.

The effect of light and soil porosity on *P. vulgaris* was not consistent with those of most of other studies. For *P. vulgaris*, the shade treatment did not change average vessel size or the vessel size distribution compared to the control treatment. Wood density was lower for the shade treatment, but cavitation resistance actually increased. Like the low P treatment, changes in cavitation resistance for the shade treatment do not appear to be a function of conduit reinforcement. By contrast, Cochard, Lemoine & Dreyer (1999) reported an increased resistance in *Fagus sylvatica* (both potted seedlings and sun- versus shade-exposed adults) because of higher light levels, while Lemoine, Cochard & Granier (2002) reported a decreasing resistance with decreasing light in sun- versus shade-exposed adult *F. sylvatica*. It should be noted, however, that water stress may have been a factor in the Cochard *et al.* (1999) study because the high-light treatment plants had more negative water potentials. In two other studies, changes were not seen in conductance for gap versus non-gap *Acer* saplings (Maherali, DeLucia & Sipe 1997) or cavitation resistance (Lipp & Nilsen 1997) in response to light level. The results of increased soil porosity on *P. vulgaris* also did not match with the literature. Increased soil porosity caused a decrease in conduit size and an increased cavitation resistance in *P. vulgaris*. The study performed by Hacke *et al.* (2001) reported that greater soil porosity increased k_h and decreased cavitation resistance in 15-year-old *P. taeda* stands.

Mencuccini (2003) reviewed the importance of structural acclimatization within the literature using a meta-analysis. He concluded that structural acclimatization to environmental factors that increased the availability of a resource tended to result in a less efficient (per unit leaf area) hydraulic system. The shade, low P, dry and porous soil treatments used in this study would all reduce the availability of resources, and based on Mencuccini (2003) should have resulted in a more efficient hydraulic system. For the porous soil treatment, it is interesting to note that LSC is lower, apparently not supporting the idea of Mencuccini (2003). An interesting twist in experiment 2 was that under well-watered conditions, maximum midday g_s measurements were actually higher for the porous soil plants compared with the control plants. Because Ψ_{mid} did not differ between the two treatments (but averaged close to -0.8 MPa), it seems likely that the porous soil treatment plants were able to have higher g_s compared to the control plants because at -0.8 MPa, they should have experienced proportionally less xylem cavitation (See Fig. 1). Using the k_h and LSC values from Table 2 and the PLC curves from Fig. 1, it is possible to estimate conductivity values at water potentials of about -0.8 MPa. The control plants would have been at $\sim 27\%$ of maximum conductance, while the porous soil plants would have been at 41%. This would reduce k_h to 2.87×10^{-5} and 1.63×10^{-5} kg m s $^{-1}$ MPa $^{-1}$ for the control and porous soil treatments, respectively. LSC would have been reduced to

6.18×10^{-5} and 5×10^{-5} kg m⁻² s⁻¹ MPa⁻¹ for the control and porous soil treatments, respectively, and would no longer have been significantly different. In addition, if the amount of water lost per unit leaf area was calculated, the porous soil plants actually lost more water per unit leaf area. Under the water potential conditions to which the plants were exposed, it turned out that by at least one measure, the porous soil treatment plants had a more efficient hydraulic system.

The final objective of this study was to determine if alterations in structural characteristics were correlated. If larger vessels have a tendency to have larger pores, there could be a trade-off between conduit diameter and cavitation resistance ('safety versus conducting efficiency' trade-off; Baas 1976; Carlquist 1982; Zimmermann 1983). Although treatments in this study significantly altered both vessel diameter and P_{50} , these changes were not correlated ($r = 0.02$, $P = 0.93$, $n = 33$). Note that the low P treatment was excluded from the correlation comparisons because Harvey & van den Driessche (1997) reported that low P altered pore size independently of conduit size.

Another possibility is that a stronger relationship exists between conduit reinforcement and conduit size, reflecting a construction cost trade-off. Hacke & Sperry (2001) showed that cavitation resistance correlated much better with conduit reinforcement than with conduit size. They did not show whether conduit reinforcement also correlated with conduit size, but there are good theoretical grounds to assume that greater conduit reinforcement may lead to a decrease in space available for the hydraulic network (Roderick & Berry 2001). A significant negative correlation was observed between conduit diameter and the density of 'woody' bean stems ($r = -0.35$, $P = 0.04$, $n = 33$) perhaps reflecting a weak construction cost trade-off. In addition, the correlation between wood density and cavitation resistance (P_{50}) was close to significant ($r = 0.31$, $P < 0.08$, $n = 33$). These results provide greater support for the idea of a construction cost trade-off than a trade-off between inter-conduit pore size and conduit diameter. Excluding the shade treatment as well (because cavitation resistance for the shade treatment increased despite a decrease in wood density, indicating a different mechanism for changes in cavitation resistance), the correlation becomes even stronger ($r = 0.439$, $P < 0.02$, $n = 27$).

This study clearly shows that long-term acclimatization to various environmental factors can result in altered vessel size, wall strength and inter-conduit pore size in the herbaceous *P. vulgaris*, and that these changes can impact whole plant water use. In general, dry and porous soil treatments tended to decrease vessel size, and increase cavitation resistance and stem density. The shade treatment increased cavitation resistance and decreased stem density, while low P treatment decreased vessel size and cavitation resistance. The results of this study did not support a safety versus efficiency trade-off, but there was evidence that cavitation resistance could be altered by two different mechanisms. For the low P and shade treatments, changes in cavitation resistance appeared to be largely independent of wood

density (a function of inter-conduit pore size), while for the porous soil, dry and control treatments, cavitation resistance was correlated with wood density.

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