Nutrition, xylem cavitation and drought resistance in hybrid poplar

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Summary Effects of mineral nutrition on susceptibility to cavitation were examined in four hybrid poplar clones. Two drought-sensitive and two drought-resistant hybrid clones of black cottonwood *(Populus trichocarpa* Torr. & Gray) and eastern cottonwood (*P. deltoides* Bartr.) were grown at three concentrations of nitrogen (N) applied factorially with two concentrations of phosphorus (P) in a greenhouse, and subjected to varying degrees of drought stress before measurement of cavitation and of anatomical features that might affect cavitation. Mean vessel pit pore diameters were 0.132 μm at low P, and 0.074 μm at high P, but no other significant effects of mineral nutrition on vessel dimensions were observed. Vessel diameter and specific conductivity were greater in the drought-resistant clones than in the drought-susceptible clones. Drought-resistant clones did not reach such low water potentials as drought-sensitive clones during the cavitation induction experiments, suggesting better stomatal and cuticular control of water loss. Scanning electron microscope observations showed less damage to pit membranes, also suggesting greater membrane strength in drought-resistant clones than in drought-sensitive clones. High concentrations of N increased cavitation, whereas high concentrations of P decreased cavitation as measured by both hydraulic flow apparatus and dye perfusion techniques. For one test, cavitation was 48% at high N and low P, but only 28% at high N and high P. We consider that N fertilization may make poplars more susceptible to cavitation on dry sites, but P fertilization may reduce this effect.

Keywords: cottonwood, embolism, hydraulic conductivity, nitrogen, phosphorus, Populus.

Introduction

The demand for wood products is rivalled by the protest against cutting old growth forests in coastal British Columbia. One solution to the cut or conserve dilemma is the establishment of plantations of fast-growing hybrid poplar on marginal land. Given the right conditions, native poplars are the fastest growing trees in North America and are easily propagated (McLennan and Mamias 1992), and many hybrids have been developed that are more vigorous than the parental stock. Hybrids of black cottonwood (*Populus trichocarpa* Torr. & Gray, female) and eastern cottonwood (*Populus deltoides* Bartr., male) (T × D) have been successful in field trials in the Pacific Northwest (Stettler et al. 1988), and extensive commer-

cial plantations of these hybrids have been established in the region. However, some plantations are subject to summer drought (DeBell et al. 1990), including sites on eastern Vancouver Island. Vulnerability to cavitation limits growth of *Populus* species on dry sites (Tyree et al. 1992, 1994*b*), and threatens survival more than leaf dehydration tolerance (Braatne et al. 1992). Cavitation means xylem vessels become blocked by air bubbles or embolisms, reducing hydraulic conductivity and thus productivity (Tyree and Ewers 1991, Tyree et al. 1992, Sperry et al. 1994). The planting sites also tend to be deficient in nitrogen (N) (National Poplar Commission 1987, DeBell et al. 1990, Heilman and Fu-Guang 1993) and phosphorus (P) (DeBell et al. 1990) and probably other nutrients, but little is known about nutrient effects on drought hardiness, including cavitation resistance.

Cavitation resistance could be at the leaf level, because stomatal control can preclude development of tension that leads to breaks in the water column (Braatne et al. 1992, Sperry et al. 1994), or at the xylem level, because both large conduits (Zimmermann 1978, Hargrave et al. 1994) and large pit membrane pores (Sperry et al. 1994) have been positively correlated with embolism formation. Although direct effects of nutrients on cavitation are unknown, there have been studies on the effect of fertilization on drought resistance. The effects of high concentrations of N on drought resistance of poplar vary with availability of water. With ample water, N fertilization increased leaf ABA in two poplar clones, so that stomata closed and leaf water stress was reduced, but the positive effects of N were much reduced under drought conditions (Liu and Dickmann 1992*a*, 1992*b*). Phosphorus concentration showed a positive correlation with root hydraulic conductivity in *Fraxinus pennsylvanica* Marsh. (Andersen et al. 1989), indicating that P nutrition might affect susceptibility to cavitation.

In this study, the effects of N and P nutrition on percent loss of hydraulic conductivity were measured under drought conditions to determine whether these nutrients would affect cavitation, and therefore growth and survival, of hybrid poplar on sites subject to seasonal water deficit. Xylem anatomy was examined to test the further hypothesis that effects on vulnerability to cavitation were due to anatomical differences in the xylem associated with differing availabilities of these nutrients. Finally, both drought-resistant and drought-sensitive poplar clones were compared to determine whether cavitation resistance is a component of drought resistance.

Materials and methods

Plant material, nutrient treatments, and experimental design

Cuttings of four T × D clones were collected from MacMillan Bloedel's Yellow Point, BC poplar stool beds in February 1995. Two of the clones were ranked as drought resistant (49-177 and 15-29) and two as drought sensitive (52-237 and 52-226, a triploid) according to productivity on dry sites in field trials in the Pacific Northwest (C. van Oosten, MacMillan Bloedel, Yellow Point, BC, personal communication).

In a greenhouse, 192 30-cm cuttings were planted in a sub-irrigation sand-culture system similar to that described by van den Driessche (1978). Supplemental mercury vapor lighting provided a 16-h photoperiod. During the experiment, temperatures ranged from 20 to 32 °C, and PAR ranged up to 1400 μmol m^{-2} s^{-1} (Model LI-189 quantum radiometer photometer, Li-Cor, Inc., Lincoln, NE).

Nutrient treatments consisted of three concentrations of N (0.36, 1.43 and 7.14 mM as NH_4NO_3) in combination with two concentrations of P (0.10 and 0.65 mM as KH_2PO_4) to give six fertilization treatments. Other essential nutrients were supplied at the following concentrations: K, 1.28 mM; Ca, 1.00 mM; Mg, 0.82 mM; S, 1.16–1.41 mM; Fe chelate, 0.04 mM; Cu, 0.31 μM; Mo, 0.03 μM; Zn, 0.31 μM; B, 0.02 mM; and Mn, 0.36 μM (van den Driessche 1990).

Each treatment was replicated twice, with each replication randomly assigned to harvest one or two (time blocks). The result was a randomized block split-plot factorial design, with nutrient treatment as the whole-plot experiment unit and clone as the split-plot factor. Within each tank, four ramets of each of the four clones were randomly arranged.

Growth and nutrient content measurements

Cutting diameter at planting was recorded. After three months of growth, measurements were made of stem height and diameter; dry weights of leaf, stem and root; and leaf area (Li-Cor Model 3100 leaf area meter).

Chemical analyses of leaf macro- and micronutrients were carried out by digesting Ca, K, Mg, P, S, Fe, Cu, Mn, Zn, and B in a microwave digester (CEM MDS-81D, SCP Science, St. Laurent, Canada) with 30% hydrogen peroxide and nitric acid, followed by atomic emission spectroscopy (ARL 3560 ICP spectrometer, Thermco Instruments Inc., Mississauga, Canada). Nitrogen and S contents were determined by combustion elemental analysis (Fisons NA-1500 analyzer, Iso-Mass Scientific Inc., Calgary, Canada).

Cavitation measurements

Sensitivity to cavitation was assessed by vulnerability curves: percent loss of conductivity (PLC) versus xylem water potential (Ψ_{xp}) causing the loss. To obtain different Ψ_{xp} values, the trees were droughted for 0, 4, 6 and 8 days (four drought regimes by withholding irrigation). Midday Ψ_{xp}, as measured with a pressure chamber on excised leaves, was lower for controls than for trees droughted for four days, but predawn Ψ_{xp} was higher for controls. To determine which set of readings was most representative of cavitation, Ψ_{xp} of controls was measured in the greenhouse at midday the day before cavitation testing, at predawn in the greenhouse, after being brought to the laboratory, and immediately before cavitation testing.

Degree of cavitation was ascertained by two methods. The first was a physical measurement of the conductivity of water through stem segments before and after removal of embolisms. The apparatus was similar to that described by Sperry et al. (1988) and trees were prepared as described by Tyree et al. (1992). After cutting a stem segment from near the base of a tree, the top of each tree was dried on the laboratory bench to wilting or for 18 h, whichever came first. Stems were recut under water to excise embolisms induced by cutting stems in the air, and all segments tested were 0.4 to 0.9 cm in diameter. The conductivity-testing solution was 10 mM oxalic acid (to inhibit microbial growth) filtered to 0.1 μm, then deaerated by sparging with helium to avoid further embolism and blockage of the xylem with particles (Sperry et al. 1988). Vessel length distribution tests (Zimmermann and Jeje 1981, Sperry et al. 1991) showed that, on average, 98.5% of vessels were less than 7 cm, and this stem segment length was therefore used for cavitation determinations.

Water flow, which was measured by a balance monitored by a computer, was used to calculate specific conductivity (K_s in kg s^{-1} m^{-1} MPa^{-1}) with Darcy's equation (Edwards and Jarvis 1982, Tyree and Ewers 1991). Xylem area for the equation was determined by color photocopying stem cross sections beside a known 5-mm square, enlarging, then cutting out the xylem and the square and weighing for comparison. Leaf specific conductivity (LSC) was obtained by substituting leaf area above the test segment for xylem area (Zimmermann 1978). Percent loss of conductivity (PLC) was determined as:

$$PLC = 100(K_{max} - K_{initial})/K_{max}, \qquad (1)$$

where K_{max} is conductivity with embolisms removed, and $K_{initial}$ is conductivity before flushing (Sperry et al. 1988, Tyree et al. 1994b). Note that PLC is independent of xylem or leaf area (Equation 1).

The second method of measuring embolism involved cutting 7-cm stem segments adjoining those tested by water conduction, and perfusing them with 0.1% safranin dye (in 10 mM oxalic acid). The segments were frozen, and later 40 μm cross-sectional slices were cut with a sliding microtome (Model 860, American Optical Corp., New York) from the midpoint of the frozen stem for immediate study under a light microscope (binocular dissection microscope, MEIJI.EMT, Tokyo, Japan). Only xylem vessels that conduct water become stained, so counting the percent of unstained to stained sapwood provided another measure of embolism (Zimmermann 1978, Sperry et al. 1988, Sellin 1991). Permanent slides were made and photographed for anatomical comparisons.

Anatomical differences

Size and condition of pores in the xylem vessel-to-vessel pit membranes were examined with an SEM (JEOL JSM-35 scanning electron microscope, Soquelec, Montreal, Canada) on microtomed 40 μm transverse sections. Samples from 16 trees

were dehydrated in an ethanol series, critical point dried and gold coated (Sperry et al. 1991). Measurements were made on the SEM screen and from photographs taken at 13,000× or greater.

Pore size relationships to critical Ψ_{xp} at which cavitation might occur were calculated according to the capillary equation (Sperry et al.1991):

$$p = 4T/D, \tag{2}$$

where p is pressure in MPa needed to displace an air–water interface from a circular pore, D is diameter of pore in μm, and T is the surface tension of water (0.072 N m^{-1} at 20 °C). The contact angle between sap and pit membrane is assumed to be 0°.

Vessel diameters of 96 trees were measured by projecting slides of dyed stem cross sections onto a screen, measuring all vessels in a xylem segment, and averaging for each tree. Largest and smallest stained (conducting) and unstained (embolized) vessels were also recorded.

Photomicrographs of leaf prints made with clear nailpolish were used to count and measure abaxial stomata (Ceulemans et al. 1988). Samples were taken from 16 trees per block (one of each clone at low and high N and P) from the eighth fully expanded leaf by the midrib, between veins.

Statistical analysis

Analyses of variance and covariance were performed with the SAS software package (SAS Institute, Cary, NC), Version 6.0. The randomized block split-plot design was described previously ($2 \times 3 \times 2 \times 4$ with four replications). The ANOVA model was mixed, with time block (harvest) random and all other factors fixed, so each main effect and interaction was tested by its interaction with block (i.e., N was tested by N ×

Block) (Anderson and McLean 1974). Regressions were used to determine effects of different parameters on retranslocation and cavitation. Multiple regressions using the MAXR and RSQUARE methods were used to find which factors most affected PLC (Pimentel 1979).

Curve fitting of data showed the relationship between Ψ_{xp} (MPa) and cavitation (PLC) was described best by the cumulative (sigmoid) equation from the computer graphing program SlideWrite Plus 3.00 (Daniele 1995). Nonlinear cumulative regressions were used to create and compare S-shaped vulnerability to cavitation curves for the different clones and nutrient treatments. Analysis of variance was then used to test the four unknown parameters of the cumulative equation: "a" (minimum PLC), "b" (PLC drop), "c" (Ψ_{xp} at 50 PLC), and "d" (transition width) for treatment effects (Daniele 1995, W. Bergerud, B.C. Ministry of Forests, Victoria, BC, personal communication).

Planned contrasts were performed between drought-resistant and drought-sensitive clones, and linear and quadratic contrasts for N. Normality of the residuals of data sets were tested, and means were compared with Duncan tests and least square means (Steel and Torrie 1980).

Results

Growth and nutrient content

High concentrations of N fertilization increased leaf area, dry weights of tree and leaf, and leaf:root ratio ($P \leq 0.05$), but P had no statistically significant effects on growth (Table 1). Drought-resistant clones (49-177 and 15-29) had larger stem volumes than drought-sensitive clones (52-237 and 52-226) (Table 2), and greater leaf, stem and root dry weights ($P \leq 0.05$).

Table 1. Growth and nutrient concentration responses to N and P fertilization, all clones.

Variable	N (mM)			P	P (mM)		P
	0.36	1.43	7.14		0.1	0.65	
Growth							
Tree weight, g	24.63 a[1]	36.83 b	37.38 b	0.027	31.69 a	34.20 a	0.296
Leaf weight, g	9.62 a	15.4 b	16.15 b	0.020	13.38 a	14.05 a	0.171
Leaf area, cm^2	1810 a	2652 b	2721 b	0.021	2345 a	2440 a	0.661
Leaf:root dry wt. ratio	1.51 a	2.01 b	2.10 b	0.046	1.92 a	1.83 a	0.528
Nutrients (g kg$_{dw}$$^{-1}$)							
Leaf N	11.70 a	17.86 b	32.06 c	0.009	20.39 a	20.72 a	0.606
Stem N	4.68 a	6.35 b	12.89 c	0.003	7.69 a	8.27 a	0.400
Root N	9.55 a	17.20 b	27.46 c	0.002	18.54 a	17.61 a	0.203
Leaf P	4.78 a	4.25 a	3.12 a	0.220	2.04 a	6.03 b	0.028
Stem P	2.58 a	2.2 b	2.34 b	0.023	1.51 a	3.22 b	0.003
Root P	3.47 a	3.46 a	3.03 a	0.081	2.33 a	4.29 a	0.110
Leaf K	17.6 ab	19.20 a	14.84 b	0.050	15.96 a	18.43 a	0.107
Stem K	8.83 a	7.87 b	7.24 b	0.021	7.37 a	8.59 a	0.185
Root K	15.83 a	13.24 b	9.08 c	0.004	12.24 a	13.17 a	0.320
Stem Mn	0.021 a	0.012 b	0.011 b	0.037	0.015 a	0.013 a	0.245

[1] Means, within growth and nutrient groups, followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

Table 2. Comparison of tree[1] and vessel sizes versus specific conductivity (K_s), minimum percent loss of conductivity (PLC), water potential (Ψ_{xp}) at 50% loss, and, for each block/clone/treatment combination, mean minimum Ψ_{xp} and average number of cases of > 99 PLC. Clones 49-177 and 15-29 are drought hardy (H), clones 52-237 and 52-226 are drought-sensitive (S).

Factor	Stem volume cm^3	Stem length cm	Vessel diameter μm	K_s max. kg s^{-1} m^{-1} MPa^{-1}	PLC min.	Ψ_{xp} at 50 PLC MPa	Ψ_{xp} min., mean	Cases of 99+ PLC
H 49-177	24.6 a[2]	23.8 a	36.9 a	7.2 ab	33.1 a	−1.24 a	−1.67 a	0.33 a
H 15-29	20.8 b	26.0 a	36.1 a	8.4 a	17.9 b	−1.21 a	−1.88 a	0.33 a
S 52-237	18.0 c	21.2 a	31.1 b	5.5 c	20.6 b	−1.48 b	−2.46 b	1.08 ab
S 52-226	14.5 d	20.2 a	36.6 a	5.9 bc	23.4 ab	−1.34 ab	−2.32 b	1.33 b
Probability	0.002	0.108	0.02	0.0277	0.1	0.0364	0.0049	0.086
Hardy	22.7	24.9	36.5	7.8	25.5	−1.23	−1.78	0.33
Sensitive	16.3	20.7	33.9	5.7	22	−1.41	−2.39	1.21
Probability	0.0006	0.0347	0.03	0.0102	no test	no test	0.0014	0.0246

[1] Stem volume (height/$3\pi r^2$) just before first harvest, when hardy clones compared to sensitive clones were 90.3 cm versus 74.0 cm ($P = 0.0004$) in height and 0.95 cm versus 0.89 cm ($P = 0.0037$) in diameter.

2 Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

Nitrogen concentrations in leaves, stems and roots increased with N treatment, but leaf, stem and root K concentrations decreased at the highest concentration of N fertilization (Table 1). Phosphorus treatment increased leaf and stem P concentrations, but stem P concentration was inversely related to N treatment concentration (Table 1). Nitrogen fertilization increased concentrations of S in all plant parts, and decreased concentrations of Zn (not shown) and Mn in the stem (Table 1). Phosphorus treatment increased concentrations of Cu in the stem, Mg in the leaf and root, and Zn in the leaf ($P \leq 0.05$) (not shown). Compared with drought-sensitive clones, drought-resistant clones had significantly lower concentrations of many nutrients including leaf B and Fe, leaf and stem Ca, Cu, Mg, Mn and Zn, leaf and root N, stem P, and root S ($P \leq 0.05$); however, they had higher concentrations of stem K, root Mn, and root P ($P \leq 0.05$) (not shown).

Conductivity and cavitation

Leaf specific conductivity (LSC), with embolisms removed (maximum LSC), increased with P fertilization ($P = 0.0382$) (not shown). With embolisms, LSC at a given Ψ_{xp} was inversely related to N fertilization concentration, with high P increasing conductivity at high N ($P = 0.0283$) (Figure 1). Specific conductivity (conductivity per unit conducting area of xylem) of cut branches with embolisms flushed out was higher for trees considered drought-resistant than drought-sensitive (Table 2).

Leaf water potentials were better correlated with percent loss of conductivity (PLC) if measured in the light than in the dark (not shown), and PLC is reported here versus Ψ_{xp} measured in the light. Using the water-conduction apparatus, PLC at similar Ψ_{xp} increased with N supply, with a significant quadratic contrast for N ($P = 0.039$) (not shown). Increased P fertilization, on the other hand, usually led to decreased cavitation. For instance, analysis of clonal vulnerability curves showed the inflexion point, about 50% loss, was usually at a lower Ψ_{xp} with high P than with low P. Statistically, this effect was strongest for Clone 15-29 (Figure 2) (P × Clone, $P = 0.0326$).

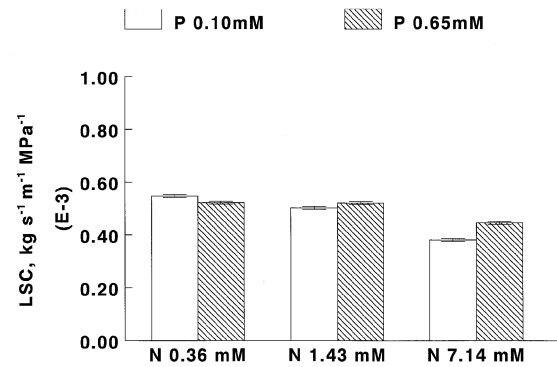Figure 1. Leaf specific conductivity (LSC) with embolisms (kg s^{-1} m^{-1} MPa^{-1}), with water potential as a covariant, shown at low, medium and high nitrogen (0.36, 1.43 and 7.14 mM) and low and high phosphorus (0.10 and 0.65 mM). Bars show standard errors ($n = 32$ per mean).

Only the triploid Clone, 52-226, showed the reverse trend. The interaction between N and P effects was shown by the number of cases of at least 99% loss of conductivity, which increased with higher N supply, but decreased with more P (Figure 3a) ($P = 0.001$). Percent loss of conductivity calculated using counts of dyed versus undyed vessels (conducting versus embolized), showed qualitatively similar nutrient interactions (Figure 3b). Cavitation at high N (7.14 mM) was 48% at low P (0.1 mM) but only 28% at high P (0.65 mM) ($P = 0.021$).

Drought-resistant clones, hydraulically measured, lost over 99% of conductivity significantly less often than drought-sensitive clones, and reached less negative Ψ_{xp} (Table 2), although their vulnerability curves indicated they were not more resistant to cavitation at moderate Ψ_{xp} than drought-sensitive clones (Figure 4). For instance, Ψ_{xp} at 50% loss was less negative, meaning 50% loss was more quickly reached, for the drought-resistant clones (Table 2). There was a small, but statistically significant, positive correlation between maximum conductivity and Ψ_{xp} at which 50% loss occurred ($r^2 = 0.21$, $P = 0.002$).
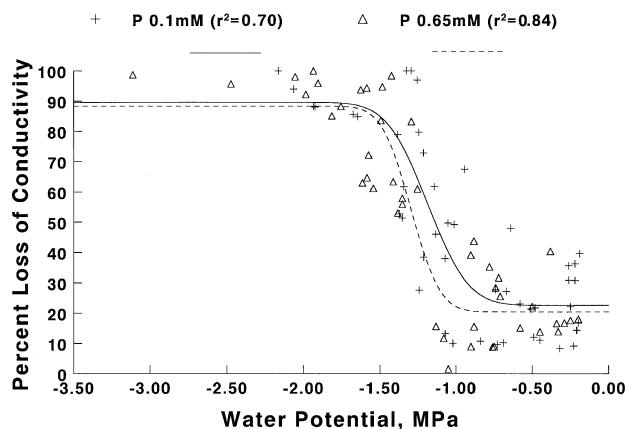
Figure 2. Vulnerability curve (percent loss of conductivity versus water potential in MPa) for Clone 15-29 at 0.1 and 0.65 mM phosphorus. This curve is fitted with a cumulative equation, and analysis of variance of the parameters shows a significantly lower water potential at the inflexion point (approximately 50% loss) for high compared to low phosphorus ($P = 0.033$).
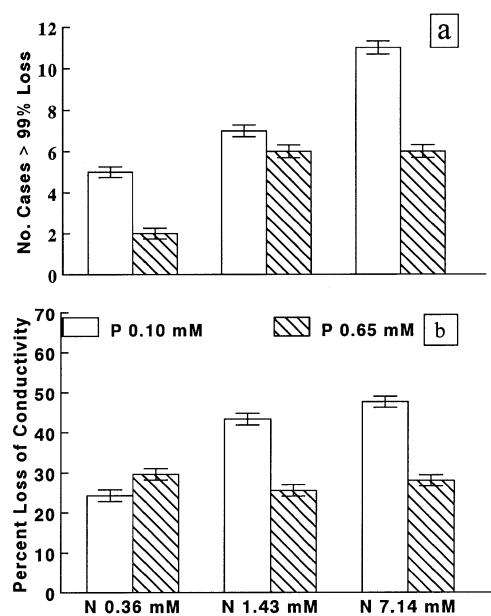

Figure 3. Interaction of nitrogen and phosphorus. (a) Total number of cases of over 99% loss of conductivity, determined hydraulically on 384 stems ($P = 0.001$). Bars show standard errors. (b) Mean percent loss of conductivity determined by dye perfusion, adjusted for water potential ($P = –0.021$). Bars show standard errors ($n = 32$ per mean).

Anatomical differences

Measuring only natural-looking, spherical pores on SEM photomicrographs (Figures 5a and 5b), pit membrane pores were larger at low P than at high P (Table 3). Regression analyses suggested that pore diameter was also influenced by concentrations of stem Mn and leaf K, along with tree size (Table 4). Clonal differences in pore diameter were not statistically significant, but were ranked in order of drought resistance (Ta-
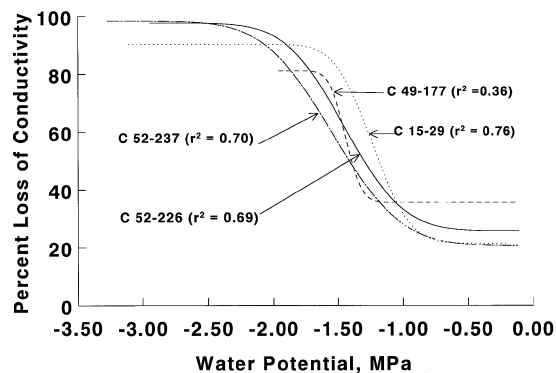

Figure 4. Vulnerability curves for all clones. Clones 1 and 2 are drought resistant (49-177, most resistant, and 15-29), and Clones 3 and 4 are drought-sensitive (52-237 and 52-226). Data points have been removed to avoid confusion.

ble 3), along with the Ψ_{xp} at which cavitation might occur (Equation 2).

Drought-sensitive clones appeared to have many more ripped and damaged pit membranes than drought-resistant clones. Averages of the largest hole (considering both natural looking pores and more obviously damaged areas) per pit were 0.151 and 0.438 μm for hardy and sensitive clones, respectively ($P = 0.0373$) (Figures 5c and 5d). This measurement involved the first ten pits per sample with visible membranes and with pores or holes of any type. Individual clonal averages are shown with corresponding Ψ_{xp} at which cavitation might begin according to the capillary equation (Table 3) ($P = 0.077$).

Vessel diameter showed some tendency to increase as N increased, but this was not statistically significant. However, paired observation t-tests showed that, for all vessels examined, the largest stained (conducting) vessel was significantly smaller than the largest unstained (embolized) vessel ($P = 0.0011$) per tree, and the smallest stained vessel significantly smaller than the smallest unstained ($P = 0.001$), so small vessels appeared to be less vulnerable to cavitation within an individual. Drought-resistant clones had greater vessel diameters ($P = 0.03$) and maximum vessel length ($P = 0.035$) than drought-sensitive clones (Table 2) (which were smaller trees).

At high N, there were more abaxial stomata per mm^2 (158 versus 148 and 146) ($P = 0.0456$) and per tree (44×10^6 versus 38 and 26×10^6) ($P = 0.0127$; $\beta = 0.01$), but stomatal length was less at high N than at low or moderate amounts of fertilization (29 versus 33 μm) ($P = 0.0303$) (not shown). However, the β level of significance was low (≈ 0.5) for these anatomical measurements because of the small sample sizes compared to variation.

Discussion

For the four hybrid poplar clones examined, an increase in N fertilization increased the tendency to xylem cavitation, whereas an increase in P reduced cavitation. This was seen when either percent loss of conductivity (PLC) at a given water potential (Ψ_{xp}), or number of individuals showing over 99%
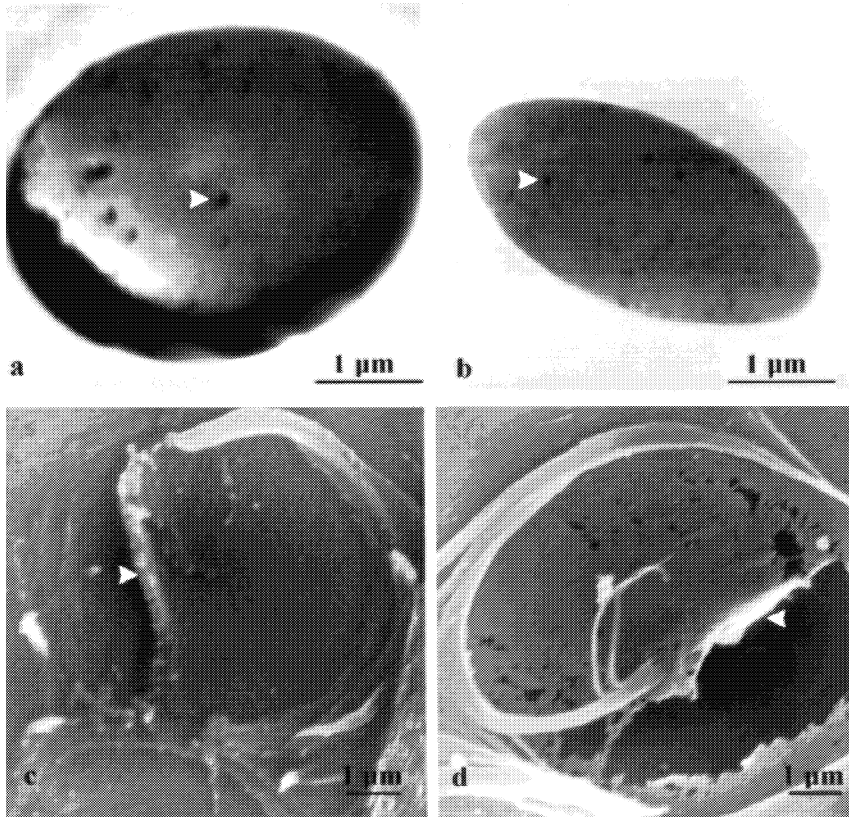
Figure 5. Scanning electron micrographs of bordered pit membranes of Clone 52-237 at low nitrogen and low (a) and high (b) phosphorus. Photographs c and d show entire membranes with secondary walls removed: both membranes have been loosened at the edge by the microtome blade. For drought-hardy Clone 49-177 (c), the membrane looks thick and shows no rips, whereas for drought-sensitive Clone 52-237 (d) the membrane appears fragile and is badly damaged.

Table 3. Mean pit membrane pore size, membrane damage (largest hole diameter) and water potential at which cavitation begins (based on the capillary equation of Sperry et al. 1991, Equation 2), shown for phosphorus fertilizer concentrations (P), clones, and drought-hardy clones (1 and 2) versus sensitive clones (3 and 4). Means averaged for each of 16 trees, at least two samples per tree, from 41 useable photographs of pores and 178 SEM screen measurements of holes.

Factor	Pore diameter μm	MPa to cavitate	Hole diameter μm	MPa to cavitate
P 0.1 mM	0.132	−2.18	0.245	−1.18
P 0.65 mM	0.074	−3.89	0.344	−0.84
Probability	0.022		0.539	
Clone 1: 49-177	0.091 a[1]	−3.2	0.091 a	−3.16
Clone 2: 15-29	0.114 a	−2.88	0.211 a	−1.36
Clone 3: 52-237	0.116 a	−2.62	0.576 b	−0.5
Clone 4: 52-226	0.122 a	−2.4	0.299 ab	−0.96
Probability	0.250		0.077	
Drought-hardy	0.095	3.03	0.151	1.91
Sensitive	0.115	2.5	0.438	0.66
Probability	0.133		0.037	

[1] Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

Table 4. Coefficients for multiple regressions where the dependent variable is pore diameter (μm), and associated coefficients of determination (r^2).

Stem Mn (ppm)	Leaf K (g per 100g)	Leaf K^2	Tree weight (g)	Tree weight[2]	r^2
0.006	–	–	–	–	0.35
0.006	–	–	0.002	–	0.63
–	1.02	−0.34	–	–	0.42
–	0.94	−0.32	−0.02	0.0002	0.77

tension exerted on the xylem (stomatal control and hydraulic conductance), or how well the xylem resists the tension (vessel diameter and xylem pit membrane pore size).

High nitrogen supply probably induced cavitation both by increasing tension on the xylem (including reduced leaf K concentration) and by decreasing xylem resistance to embolism. Nitrogen increased foliage area and leaf weight:root weight ratio, which, together with greater stomatal density at high N, may have increased the rapidity with which a particular Ψ_{xp} was reached during drought. The effect of N treatments could have been through increasing vessel size, assuming that larger vessel size is related to increased probability of cavitation (Zimmermann 1978). Within a species, larger plants may have larger vessel diameters (Aloni 1987), and such a tendency, as size increased with N fertilization, was noted here, but not shown to be significant. Further, the safranin dye test

loss of conductivity was considered. The exception to this was the triploid clone (52-226), which was more vulnerable to 50% loss of conductivity at high P. These nutrient effects on cavitation may be related to how N and P alter either the degree of

showed that embolized vessels tended to be larger than conducting vessels within individual trees. Thus, the possibility that vessel size played a part in the resistance of these hybrid poplars to drought-induced cavitation could not be excluded. Nitrogen treatment increased internal N concentration while decreasing stem P and leaf K, and could therefore have had effects on physiology as well as altering plant size.

Low K concentrations could have affected many physiological processes, for instance by decreasing stomatal control (Taiz and Zeiger 1991). Stomatal control is considered an important factor in preventing drought-induced embolism formation (Braatne et al. 1992, Sperry et al. 1993). Potassium also influences vessel diameter, length and wall thickness (Foulger et al. 1971, Cutter and Murphey 1978) and is necessary for cell wall extension (Marschner 1986). Thus, some N responses could have resulted from the negative effect of N fertilization on K concentration.

Increases in P supply had no effect on plant or vessel size, so its effect must have been on physiology or xylem vessel pit membranes. Cavitation-resistant plants are expected to have smaller pit membrane pores than vulnerable plants: these require greater negative tensions in vessels before air can enter to cause embolism (Sperry et al. 1994). High P supply halved the mean pit membrane pore size, and hardy clones seemed to have less fragile pit membranes than sensitive clones. The mechanisms by which any of these nutrients might influence cavitation physiologically are not clear, but because membranes consist of primary cell wall material, the influence of P nutrition might be through uridine diphosphate sugars, precursors of the xyloglucan components linking microfibrils of primary cell walls (Hayashi 1989).

Comparison of the vulnerability to cavitation of drought-hardy and drought-sensitive clones may yield insights into the importance of cavitation resistance to drought resistance, and perhaps into which resistance characteristics are more significant. Strong pit membranes, indicated by fewer damage-induced holes, may confer cavitation resistance. The reason for the membrane damage is uncertain. It was once considered to be a result of sample preparation (Liese 1965, Schmid 1965), and some hole development was observed as a result of electron bombardment during SEM viewing. However, membrane degradation was regarded as a natural phenomenon by Barnett (1981), and more recently has been used to explain cavitation vulnerability of older vessels of *Populus tremuloides* Michx. (Sperry et al. 1991) and of roots (Alder et al. 1996). We found that vulnerability to membrane damage was less for drought-resistant clones than for drought-sensitive clones, suggesting that increased pit membrane strength could have conferred some resistance to cavitation. Larger vessels may have larger pit membrane pores than small vessels within a genus or individual (Hargrave et al. 1994), but Tyree et al. (1994*a*) see no reason for such a relationship evolving generally if cavitation is primarily due to air seeding of embolisms. Certainly, our hardy clones had larger vessels, but not larger pores, than sensitive clones. This may reflect their lower stem Mn concentrations, because stem Mn was positively correlated with pore diameter. Manganese increases cell extension (Marschner

1986), leading to thinning of the primary wall (Esau 1965), and perhaps of the pit membrane. However, Mn was also more concentrated at low N than high N, without a corresponding increase in pore size.

Drought-resistant clones (49-177 and 15-29) were no more cavitation resistant than susceptible clones at moderate drought stress, although they performed better under severe drought, as has been found for sugar cane (Neufeld et al. 1992). They had longer and wider vessels and greater hydraulic conductivity (K_s) than drought-susceptible clones. As would be expected from their greater K_s, the drought-resistant clones reached 50% loss of conductivity at less negative Ψ_{xp} than the drought susceptible clones. Larger vessels may increase conductivity and the likelihood of cavitation, if only within a genotype (Sperry et al. 1993). Greater drought resistance of Clones 49-177 and 15-29 in the field may be related to more effective stomatal and cuticular control of water loss in severe drought. Because hardy clones tolerated lower Ψ_{xp} and more cavitation when moderately droughted, stomata presumably remained open to allow continued growth. It has been suggested that moderate amounts of cavitation improve drought resistance by reducing transpiration rates and water use (Neufeld et al. 1992, Alder et al. 1996, J.S. Sperry, University of Utah, Salt Lake City, UT, personal communication). However, drought-resistant clones were less likely to reach critically low Ψ_{xp}, which would lead to $\geq$ 99% cavitation, than susceptible clones, so stomata closed near the point of runaway cavitation. This is considered more adaptive behavior, indicating optimal stomatal control (Tyree and Sperry 1989, Tyree and Ewers 1991).

In conclusion, stomatal control, perhaps coupled with less penetrable pit membranes, seems key to increased drought resistance and avoidance of catastrophic cavitation among these four hybrids. Mineral nutrition can significantly alter cavitation resistance. A low supply of N would lead to a decrease in cavitation for many clones growing on dry sites, but high N accompanied by adequate P might allow sufficient growth to make plantations profitable without an excessive loss of conductivity.

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