

Nitrogen and potassium effects on xylem cavitation and water-use efficiency in poplars

H. P. HARVEY and R. VAN DEN DRIESSCHE

Centre for Forest Biology, University of Victoria, P.O. Box 1700, Victoria, B.C. V8W 2Y2, Canada

Received January 12, 1999

Summary Effects of N and K nutrition on drought and cavitation resistance were examined in six greenhouse-grown poplar clones: *Populus trichocarpa* (Torr. & Gray) and its hybrids with *P. deltoides* Bartr. and *P. euramericana* (Dole) Guinier, before and after preconditioning to water stress. Both tendency to cavitate and water-use efficiency (WUE) increased when N supply was increased, whereas K supply had little impact on cavitation. Mean xylem vessel diameters increased from 36.6 μm at low-N supply to 45.2 μm at high-N supply. Drought-hardy clones, which were relatively resistant to cavitation, had the smallest mean vessel diameters. Vulnerability to cavitation had a weakly positive relationship with vessel diameter, and a negative correlation with transpiration. Drought hardening offered no protection against cavitation in a subsequent drought. Under drought conditions, increasing N supply increased leaf loss and decreased water potentials, whereas increasing K supply decreased leaf loss. Drought-resistant clones exhibited similar WUE to drought-susceptible clones, but had smaller, more numerous stomata and greater leaf retention under drought conditions.

Keywords: cottonwood, embolism, *Populus deltoides*, *P. euramericana*, *P. trichocarpa*.

Introduction

Poplars are potentially the fastest growing trees in North America (McLennan and Mamias 1992), particularly some hybrids of *Populus trichocarpa* Torr. & Gray with *P. deltoides* Bartr. (T \times D) (Stettler et al. 1988). Thus, poplars could help satisfy the heavy demand for paper pulp. However, rapid growth depends on the availability of water and nutrients, and both may be suboptimal on planting sites in the Pacific Northwest (DeBell et al. 1990). Little is known about nutrient effects on drought hardiness, and how these effects vary by clone. Among woody plants, poplars are exceptionally vulnerable to drought-induced cavitation (Tyree et al. 1994). Cavitation reduces potential productivity on dry sites by blocking the flow of water in xylem vessels (Tyree and Ewers 1991, Tyree et al. 1992, Sperry et al. 1994). Nitrogen (N) fertilization increased drought-induced cavitation in a small field study (Harvey 1997), and in greenhouse-grown hybrid poplars (Harvey and van den Driessche 1997), but the mechanism underlying this N effect was not identified. Increased phosphorus (P) supply de-

creases cavitation, possibly because it reduces pit membrane pore size (Harvey and van den Driessche 1997). The effect of potassium (K) on cavitation has not been determined, although, after N and P, K is the nutrient most limiting to tree growth (Clancy et al. 1995). Foliar K concentration was correlated with productivity in a *P. trichocarpa* clone, but not in a D \times T clone (Harrington et al. 1997).

It is possible that increasing K supply would improve drought hardiness of poplars, in part by decreasing cavitation. Potassium serves as an enzyme cofactor, an osmoticum, and an electrolyte at membranes, and is necessary for stomatal control (Taiz and Zeiger 1991). Potassium fertilization increased drought resistance of wheat (*Triticum aestivum* L.) (Pier and Berkowitz 1987) and Scots pine (*Pinus sylvestris* L.) (Christersson 1976). In one experiment, K also affected vessel wall thickness and vessel length of poplar (Cutter and Murphey 1978), indicating that it may affect xylem vessel conductivity. Because high N supply increases cavitation and lowers foliar K concentrations (van den Driessche and Ponsford 1995, Harvey and van den Driessche 1997), whereas high P supply decreases cavitation and increases foliar K (Houman et al. 1991), it is possible that the N and P effects on cavitation are mediated through their effects on foliar K concentration.

We hypothesized that increasing K supply would decrease cavitation and increase water-use efficiency. We also hypothesized that increased N supply would increase vessel diameters, making them more vulnerable to drought-induced cavitation.

Materials and methods

Plant material

Six poplar clones were used. Clones 1, 2, 4 and 5 were supplied by MacMillan Bloedel, Ltd., Vancouver, Canada, and Clones 3 and 6 were from Scott Paper Ltd., New Westminster, Canada. Clones 1 and 2 are diploid *Populus trichocarpa* \times *P. deltoides* (T \times D) hybrids, productive on dry plantation sites. Clone 3, Robusta, *P. euramericana* (Dole) Guinier, is a well-characterized hybrid of *P. nigra* L. and *P. deltoides*, and was the most drought resistant of five clones compared by Havaux et al. (1988). Clones 4 and 5 are triploid T \times T \times D hybrids that are not productive on dry sites. Clone 6, *P. trichocarpa* (Torr. & Gray) (Blom), is more drought-sensitive

than *P. deltoides*, many T × D hybrids (Pezeshki and Hinckley 1982), and Robusta (Havaux et al. 1988). The clones were numbered in estimated order of decreasing drought resistance. Drought resistance was not tested directly. Rather, clones known to be drought-resistant (Clones 1 to 3) were contrasted with clones known to be drought-sensitive (Clones 4 to 6).

Plant culture and nutrition

Dormant first-season poplar stems were sliced into 23-cm cuttings, each with several nodes from which new trees could potentially develop. On April 4, 1996, these cuttings were planted in clean sand, one per 8-liter plastic pot. Two open-ended polyethylene greenhouses were used so that water supplies could be manipulated, but otherwise trees were grown under ambient conditions. Inside each greenhouse, 324 trees were randomly arranged. Light averaged 80% of available PAR, and temperatures ranged from 0.5 °C in April to 29.2 °C in July and 27.5 °C in August. Unless drying was scheduled, water was supplied at least once a day. The two adjoining greenhouses provided fixed time blocks. Because the drought treatments were separated by 10 days between the two greenhouses, plants in Greenhouse 1 were harvested by August 10, and plants in Greenhouse 2 by August 20. Most plants developed at least two main stems per cutting, but Clone 5 often had only one main stem. Any extra stems beyond the first two were removed as they appeared.

A randomized complete block factorial design was chosen (6 clones × 3 K × 2 N × 3 drought regimes) with three replications within each of the two adjoining greenhouses (blocks). Trees were spaced 30 cm apart, in 30 rows of 12 trees per greenhouse.

The six nutrient treatments were designated: N1 (0.71 mM N supply as NH₄NO₃), N2 (7.14 mM N), K1 (no K added to the sand), K2 (0.26 mM K as K₂SO₄), and K3 (2.57 mM K). Other essential nutrients were supplied as: P, 0.33 mM; Ca, 1.00 mM; Mg, 0.82 mM; Cu, 0.47 mM; Mo, 0.03 mM; Zn, 0.47 mM; B, 0.02 mM and Mn, 0.36 mM (van den Driessche 1990). Nutrient solutions, 400 ml per pot, were added by hand once a week after bud flush for four weeks, then twice weekly, except during drought.

Drought treatments and cavitation measurement

Trees were assigned one of three watering treatments: control, one drought cycle, and two drought cycles. The two-drought-cycle treatment (hardening + drought treatment) was designed to test the effects of drought hardening on response to a subsequent drought, because some poplar clones have greater drought resistance after a first episode of drying (Gebre and Kuhns 1993, Liu and Dickmann 1993, Tschaplinski and Tuskan 1994).

Trees in the control treatment were kept well watered throughout the 18-week experiment. Trees in the one-drought-cycle treatment were kept well watered until Week 18 when water was withheld for 6 days. Trees in the two-cycle-drought treatment were kept well watered until Week 16 when water was withheld for 3 days. The trees were then re-watered for 10 days before water was again withheld for 6 days at

Week 18. Control trees were hand-watered daily when water was withheld from other trees. Cavitation was measured in trees of all treatments at Week 18 and it was also measured in trees in the hardened + subjected to drought treatment after the 3-day drought at Week 16 and again after the 10-day period of re-watering. Xylem water potential (Ψ_{xp}) was assessed by measuring leaf water potential with a pressure chamber before testing stems for cavitation. Cavitation was measured as percent loss of conducting xylem (PLC), by the dye perfusion method (Harvey and van den Driessche 1997). A solution of 0.1% safranin dye was passed through 10-cm stem segments at a pressure of 3.4 kPa for 10 min. Excess dye was then removed during a 2-min rinse with deionized distilled water. Stem segments perfused with dye were sectioned with a sliding microtome (Model 860, American Optical Corp., New York) and examined with the aid of a microscope. Unstained (cavitated) and stained (functioning) vessels were counted to calculate PLC and vessel number (Zimmermann 1978, Sperry et al. 1988, Sellin 1991).

Leaf physiology

At Weeks 14 and 18, gas exchange of the eighth fully expanded leaf was measured with an LI-6400 portable photosynthesis system (Li-Cor, Inc., Lincoln, NE). Cuvette temperature was maintained at 25 °C and PAR was set at 1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Gas exchange was measured from stomatal opening in the morning until mid-afternoon, on clear days when air temperatures were ≥ 25 °C. Instantaneous WUE was calculated from gas exchange measurements as net photosynthesis (*A*) divided by stomatal conductance (*g_s*). Stomatal closure was determined as the Ψ_{xp} below which mean *g_s* is less than 0.1 $\text{mol m}^{-2} \text{s}^{-1}$.

Growth

Cutting diameters were measured on the day of planting. Because growth in the first season is proportional to cutting size (C. van Oosten, former Poplar Manager, MacMillan Bloedel, pers. comm.), cutting diameter was used as a covariate in statistical analyses of growth. Heights and diameters of all trees were measured before drought was imposed. Dry weight and biomass allocation to root, shoot and leaf were measured at week 18. Each part of each tree was harvested, dried separately at 80 °C, then weighed (*n* = 648 leaves, stems, and roots). Leaf area was measured on a subsample of leaves from each tree in Greenhouse 1, with a leaf area meter (Delta-T Devices, Cambridge, U.K.). The measured leaves were then weighed to obtain a weight to area conversion factor for final leaf area calculations from whole-tree leaf weights.

Nutrient analysis

Leaf, stem and root samples were dried, weighed, ground and analyzed for macro- and micronutrients at week 18 as described by Harvey and van den Driessche (1997). For each plant part, samples from three trees per treatment were combined before analysis.

Plant anatomy

Vessel diameters were measured in 40 μm cross-sectional slices cut from the center of each stem segment. The slices were examined with a Zeiss microscope (Carl Zeiss, Inc., Oberkochen, Germany) equipped with a Cohu camera (Cohu, Inc., San Diego, CA). The image was digitized and vessel diameters recorded with the Bioscan Optimus 3.14 program (BioScan, Inc., Edmonds, WA). All vessels more than 20 μm in diameter were measured vertically and horizontally for two views per slide. Views were chosen randomly from the central area of the xylem ($n = 216$).

Stomatal anatomy was examined at week 18 by making leaf prints with clear nail polish (Ceulemans et al. 1988, 1995). Abaxial and adaxial prints were from the seventh fully expanded leaf, near the midrib, between the second and third veins. Stomatal length and density were measured as described for vessel diameters ($n = 72$).

Statistical analysis

Within a greenhouse (block), harvests were randomized. During drought treatments, larger trees can deplete the limited supply of water from the pots, becoming more stressed within the allotted drying time. To check for this effect, cavitation was analyzed with and without Ψ_{xp} as a covariate, and adjusted if necessary.

Because block, clone, treatment and drought regime are all fixed effects, it was a fixed model, so the error term for all main effects and interactions was the experimental error (Anderson and McLean 1974), and SAS (Statistical Analysis System, Cary, NC) general linear models procedure was used for most tests. Where more than one measurement was needed per tree (as in hardening trees, before and after re-watering), different stems were used. Repeated measures statistics were followed by ANOVAs on the differences to assess changes.

Linear and nonlinear regressions were used to determine effects of different parameters on cavitation. The cumulative (sigmoid) equation from the Slidewrite program (see Harvey and van den Driessche 1997) was used to create clonal vulnerability curves of PLC at a given Ψ_{xp} .

Means were compared with Duncan's Multiple Range Tests and pairwise multiple comparisons. Planned contrasts were made between drought-resistant and drought-sensitive clones, controls versus trees subjected to drought once, and linear and quadratic K. Normality of the residuals of data sets was tested with the univariate procedure in SAS (Steel and Torrie 1980).

Results

Nutrient treatment effects on cavitation and gas exchange

In trees subjected to a single drought, increasing N supply led to a more negative Ψ_{xp} , and more than doubled PLC (Table 1). The use of Ψ_{xp} as a covariate reduced the difference between the two N treatments (Figure 1), but cavitation was still significantly greater in high-N trees than in low-N trees (62 versus 38%, $P = 0.001$). Hardening eliminated the effect of N on Ψ_{xp} , but not on PLC (Table 1). In the N2 treatment, hardened trees

subjected to a second drought had higher PLC than trees subjected to a single drought, which in turn cavitared more than well-watered controls ($P = 0.001$). There was no clear effect of K on cavitation.

Photosynthetic rates of well-watered trees were significantly higher in the N2 treatment than in the N1 treatment. Because g_s was not affected by the N treatments, WUE (A/g_s) was significantly higher in well-watered high-N trees than in well-watered low-N trees (Table 1). However, in trees subjected to a single drought, the N treatments had no effect on WUE, and A , g_s and E were lower in high-N trees than in low-N trees, whether expressed per unit area or for the whole tree at a given Ψ_{xp} . When not adjusted for Ψ_{xp} , whole-tree E was not affected by the N treatments. In response to the second drought, the negative effects of the N2 treatment on gas exchange were decreased, so that E , A and g_s were again greater in high-N trees than in low-N trees, as in the well-watered control trees. Hardening did not increase WUE for any of the treated trees.

Among the K-treated trees at Week 14, transpiration of well-watered trees was least in the K3 treatment ($P = 0.014$) (5.31 ± 0.14 , 5.24 and $4.76 \text{ mmol m}^{-2} \text{ s}^{-1}$ at K1, K2 and K3 respectively). A high-K supply increased WUE at Week 14 ($2.23 \pm 0.139 \times 10^{-5}$, 2.36×10^{-5} and 2.67×10^{-5} at K1, K2, and K3, respectively; $P = 0.026$), but not at Week 18.

Clonal effects on cavitation and gas exchange

On average, drought-resistant clones were less vulnerable to cavitation than drought-sensitive clones (Table 2), even when Ψ_{xp} was used as a covariate (Figure 2). In particular, diploid Clones 1 and 2 (T \times D hybrids) cavitared less than triploid Clones 4 and 5. *Populus trichocarpa* (Clone 6) did not cavitate significantly more than Clone 1.

Drought-resistant clones also maintained higher A and E under drought conditions than drought-sensitive clones, especially when hardened ($A = 13.46 \pm 1.40$ versus $9.27 \pm 1.11 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$, $P = 0.025$; $E = 6.37 \pm 0.76$ versus $3.93 \pm 0.60 \text{ mmol m}^{-2} \text{ s}^{-1}$, $P = 0.017$) (Figures 3a and 3b). However, clonal drought resistance was not correlated with WUE or with stomatal closure before hardening. After hardening, drought-resistant clones and *P. trichocarpa* did not close their stomata in response to the drought conditions imposed.

Effects of nutrient treatments on growth, anatomy, and nutrient concentrations

Increasing N supply increased tree dry weight and leaf area (Table 3), but leaf loss was also proportionately greater in the N2 treatment under drought conditions (27% at N2 versus 5% at N1). The N2 treatment also increased stem volume, leaf area ratio, and vessel diameter (especially at K3) but decreased root:shoot ratio (Table 4). Stomatal numbers per unit area and per tree were greater in the N2 treatment than in the N1 treatment, although stomata were smaller.

Potassium fertilization affected tree morphology ($P \leq 0.05$). Increased K supply decreased leaf areas and leaf area ratios (Tables 3 and 4). The K3 + N2 treatment increased tree weight

Table 1. Effects of N fertilization and drought on photosynthesis (A), transpiration (E), water use efficiency (WUE), percent loss of conducting xylem (PLC) and leaf petiole water potential (Ψ_{xp}).

Variable	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	WUE ($A/g_s \times 105$)	PLC	Ψ_{xp} (MPa)
<i>Well watered: Week 14</i> ¹					
N1	8.7	5.15	1.78	–	–
N2	13.4	5.05	3.06	–	–
<i>P</i> -value	0.001	0.550	0.001	–	–
<i>Well watered: Week 18</i> ²					
N1	10.7	6.72	2.07	28	–0.76
N2	15.5	7.01	3.61	26	–0.92
<i>P</i> -value	0.001	0.453	0.001	0.402	0.017
<i>Drought treated: Week 18</i> ²					
N1	10.3	6.08	4.85	33	–0.96
N2	4.28	1.76	8.00	71	–1.91
<i>P</i> -value	0.001	0.001	0.228	0.001	0.001
<i>Hardened + drought treated: Week 18</i> ²					
N1	8.78	5.21	4.02	34	–1.09
N2	12.06	4.31	7.69	82	–1.03
<i>P</i> -value	0.008	0.139	0.219	0.001	0.721

¹ For each N treatment, $n = 54$.

² For each N \times drought treatment, $n = 36$ for gas exchange measurements and $n = 72$ for PLC and Ψ_{xp} measurements.

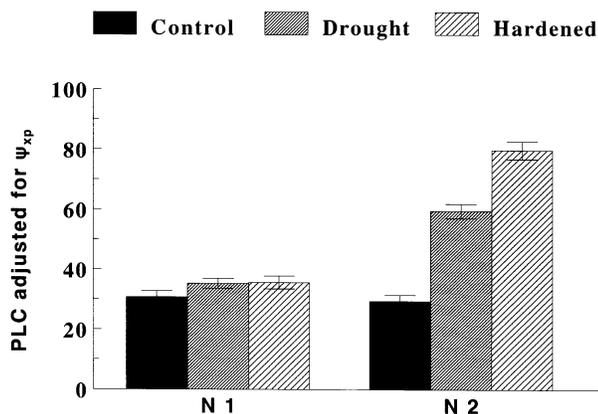


Figure 1. Percent loss of conducting xylem (PLC) adjusted for water potential (MPa) at low- and high-N supply (N1 = 0.71 mM and N2 = 7.14 mM) at the Week 18 harvest in August ($n = 72$ per N per drought treatment). Error bars represent standard errors.

and leaf thickness (not shown), and decreased leaf loss during drought.

The N2 treatment increased N and decreased K and P concentrations in all plant parts ($P = 0.0001$). Leaf N increased from $7.93 \pm 0.08 \text{ g kg}^{-1}$ (\pm SE) at N1 to $10.02 \pm 0.08 \text{ g kg}^{-1}$ at N2. Leaf P decreased from $2.00 \pm 0.03 \text{ g kg}^{-1}$ at N1 to $1.34 \pm 0.03 \text{ g kg}^{-1}$ at N2. Leaf K was $17.04 \pm 0.11 \text{ g kg}^{-1}$ at N1 but only $8.97 \pm 0.11 \text{ g kg}^{-1}$ at N2. Increased K supply increased K in all plant parts, especially the leaves, which had concentrations of $10.44 \pm 0.13 \text{ g kg}^{-1}$ at K1, 11.93 g kg^{-1} at K2 and 16.64 g kg^{-1} at K3.

Effect of clonal drought resistance on growth and anatomy

Drought-resistant clones had smaller vessel diameters than drought-sensitive clones (Table 2), but on average trees of the drought-resistant clones were larger and had greater root:shoot ratios than the trees of the drought-sensitive clones (Table 5). Drought-resistant T \times D hybrids experienced less leaf loss under drought conditions than the drought-sensitive T \times D hybrids. Stomata were smaller in drought-resistant clones than in drought-sensitive clones, and there were generally more adaxial stomata and total stomata per tree. *Populus trichocarpa*, purportedly the least drought-resistant clone (Clone 6) had no adaxial stomata.

Relationships of gas exchange and vessel diameters to PLC

Just before the final harvest, E was negatively correlated with cavitation ($r^2 = 0.59$, $P = 0.001$, intercept 77.10 and slope -7.31), as was A ($r^2 = 0.52$). The relationship between PLC and E was weaker for Clone 6 ($r^2 = 0.30$) than for the clonal mean. Clonal PLC means were positively related to vessel diameters ($r^2 = 0.73$, $P = 0.030$, intercept -74.23 and slope 2.81), although the relationship between vessel diameter and PLC over all data was weak ($r^2 = 0.16$, $P = 0.001$, intercept -7.73 ($P = 0.26$) and slope 1.23).

Discussion

Nutrient effects on cavitation

Trees in the high-N treatment were more vulnerable to drought-induced cavitation and had vessels with larger diameters than trees in the low-N treatment. Vessel diameter and susceptibility to cavitation are also positively correlated in *Betula occidentalis* Hook (Sperry and Saliendra 1994), *Ceratonia siliqua* L. (carob) (Salleo and Lo-Gullo 1989) and *Populus*

Table 2. Clonal differences in percent loss of conducting xylem (PLC) and leaf petiole water potential (Ψ_{xp}) for well-watered trees and trees subjected to a single drought. Measurements were made at Week 18 on 144 trees per treatment ($n = 24$ per clone). Vessel diameters (μm) are also presented ($n = 36$ per clone). Means followed by the same letter are not significantly different by Duncan's Multiple Range Test.

Variable	Clone 1 (T × D)	Clone 2 (T × D)	Clone 3 (Robusta)	Clone 4 (T × T × D)	Clone 5 (T × T × D)	Clone 6 (Blom)	P-value
<i>Well watered</i>							
PLC	23	30	24	32	28	24	0.120
Ψ_{xp}	-0.91	-0.91	-0.93	-0.73	-0.76	-0.80	0.329 ¹
<i>Drought treated</i>							
PLC	35 c ¹	59 a	51 ab	66 a	63 a	40 bc	0.001 ²
Ψ_{xp}	-1.11 a	-1.68 ab	-1.07 a	-1.99 b	-1.38 a	-1.38 a	0.017
Vessel diameter	39.0 b	41.8 a	37.8 b	42.5 a	42.8 a	38.2 b	0.001

¹ Drought sensitive > drought resistant $P = 0.02$.

² Drought resistant > drought sensitive $P = 0.02$.

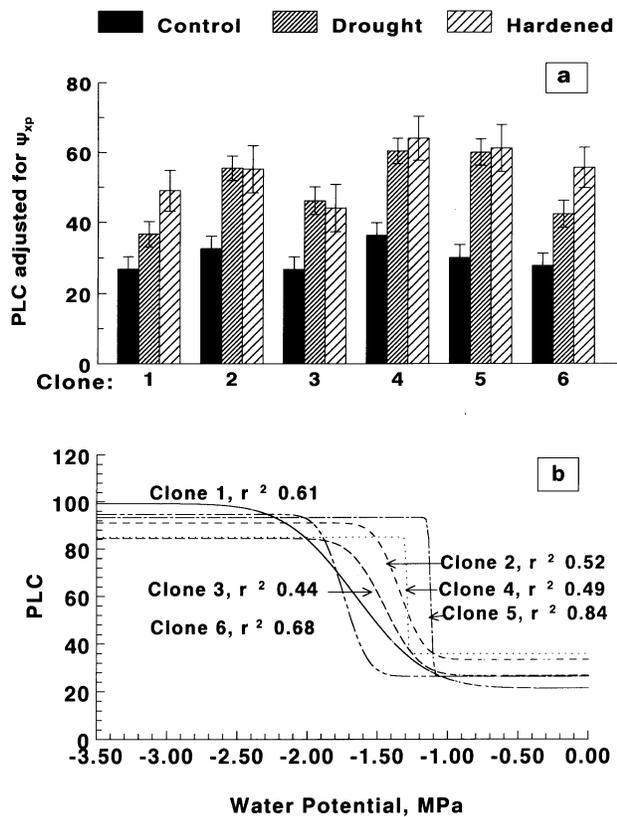


Figure 2. (a) Percent loss of conducting xylem (PLC) adjusted for water potential (Ψ_{xp}) ($n = 24$ per clone per drought treatment). Error bars represent standard errors. (b) Vulnerability curves (PLC versus Ψ_{xp}) for unhardened clones ($n = 48$ per clone). Six clones were studied: Clones 1 (T × D), 2 (T × D), 3 (Robusta), 4 (T × T × D), 5 (T × T × D) and 6 (*P. trichocarpa*).

balsamifera L. (Hacke and Sauter 1996), but no such relationships were found in conifer species (Alder et al. 1996, Hacke and Sauter 1996, Sperry and Ikeda 1997).

There were other N effects that might make high-N trees more vulnerable to cavitation than low-N trees. For example, N fertilization resulted in increased leaf area and stomatal den-

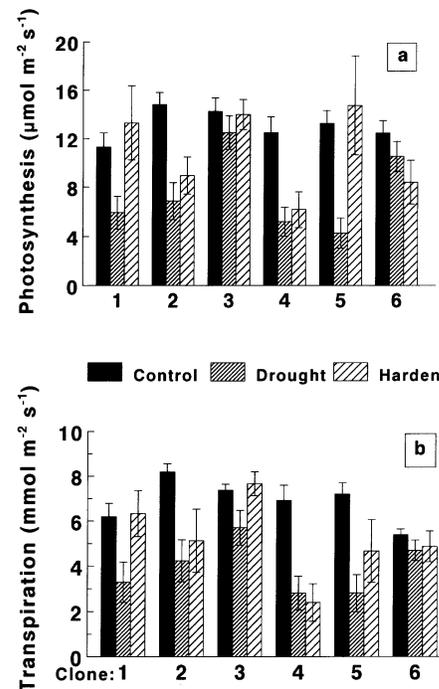


Figure 3. Clones 1 (T × D), 2 (T × D), 3 (Robusta), 4 (T × T × D), 5 (T × T × D) and 6 (*P. trichocarpa* Blom), (a) Net photosynthetic rates ($n = 12$ per clone per drought treatment), and (b) transpiration rates ($n = 12$). Error bars represent standard errors. The six clones were either well watered (controls), subjected to drought once at Week 18, or hardened and then subjected to a second drought at Week 18 in August.

sity, thereby predisposing the trees to greater water loss and thus greater tensions in the xylem. Nitrogen fertilization also decreased the concentration of P, which is needed to maintain small pores in the xylem vessel pit membranes (Harvey and van den Driessche 1997). After hardening, N fertilization no longer increased water stress, perhaps because drought-induced abscission of leaves reduced the surface area for transpirational water loss, which would compensate for de-

Table 3. Effects of N and K fertilization and drought on tree dry weight (g), leaf area (m²), and leaf loss (g lost/g total leaf weight) at 18 weeks. Measurements were made on 216 trees for each drought treatment, 108 trees for each N treatment and 72 trees for each K treatment. Means followed by the same letter are not significantly different by Duncan's Multiple Range Test.

	N1	N2	P-value	K1	K2	K3	P-value
<i>Well-watered</i>							
Tree weight	25.46	95.67	0.001	59.70	59.42	62.57	0.063
Leaf area	0.105	0.435	0.001	0.276	0.261	0.273	0.387
<i>Drought treated</i>							
Tree weight	23.83	93.65	0.001	58.66	58.34	59.20	0.864
Leaf area	0.099	0.419	0.001	0.260 ab	0.270 a	0.248 b	0.046
<i>Hardened + drought treated</i>							
Tree weight	22.36	76.26	0.001	49.65	48.74	49.53	0.791
Leaf area	0.097	0.359	0.001	0.244 a	0.222 b	0.218 b	0.016
Leaf loss	0.055	0.267	0.001	0.176	0.183	0.122	0.048 ¹

Table 4. Morphological responses to N and K fertilization. Abbreviations: LAR = leaf area ratio, SD = number of stomata per tree × 10⁷, SL = stomatal length and R:S = root mass:shoot mass ratio. For each N × K treatment, n = 108 for the first four variables, n = 12 for the stomatal data, and n = 36 for vessel diameters. Means within a row followed by the same letter are not significantly different by the Least Squares Means test.

Variable	N1			N2			N × K P ≤
	K1	K2	K3	K1	K2	K3	
Tree mass (g)	24.30 c	24.15 c	23.18 c	87.70 b	86.85 b	91.03 a	0.012
Stem volume (cm ³)	9.90 c	10.50 c	10.45 c	30.14 a	27.57 b	30.42 a	0.046
R:S	0.413 a	0.463 a	0.468 a	0.253 b	0.246 b	0.293 b	0.001 ¹
LAR (m ² g ⁻¹)	0.004 b	0.004 b	0.004 b	0.005 a	0.005 a	0.004 b	0.001
SD	1.870 b	1.841 b	1.888 b	9.277 a	10.13 a	10.57 a	0.001 ¹
SL (μm)	39.77 a	38.59 ab	38.7 ab	36.27 c	36.74 c	37.28 bc	0.001 ¹
Vessel diameter (μm)	37.21 c	37.38 c	35.55 c	43.86 b	44.86 b	47.05 a	0.001

¹ Significantly different by N level instead of N × K.

Table 5. Clonal differences in physical characteristics: stomata per tree × 10⁷ (SD), stomatal length (μm; SL), leaf area ratio (m² g⁻¹; LAR), drought-induced leaf loss (leaf loss) (g lost/g total), tree mass (g) and root mass:shoot mass ratios (R:S). Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test. For each clone, n = 12 for the stomatal data and n = 108 for all other variables.

Variable	Clone 1 T × D	Clone 2 T × D	Clone 3 Robusta	Clone 4 T × T × D	Clone 5 T × T × D	Clone 6 Blom	P ≤
SD	7.066 a	7.174 a	7.086 a	4.487 b	4.334 b	5.932 a	0.001 ¹
SL	37.53 b	34.00 c	25.17 d	47.10 a	48.03 a	35.39 c	0.001 ²
LAR	0.0043 b	0.0040 a	0.0054 d	0.0039 a	0.0043 b	0.0049 c	0.001
Leaf loss	0.048 c	0.072 bc	0.066 bc	0.105 a	0.095 ab	0.061 bc	0.011 ²
Tree mass	67.10 a	59.16 b	49.50 c	59.20 b	57.40 b	44.85 d	0.001 ¹
R:S	0.371 b	0.502 a	0.333 bc	0.415 b	0.286 c	0.230 c	0.001 ¹

¹ Greater in drought resistant clones (P ≤ 0.05)

² Less in drought resistant clones (P ≤ 0.05)

creased water uptake through embolized vessels. Osmotic adjustment also occurred in high N trees after hardening (Harvey 1997). However, because vessels had not refilled after the first drought, cavitation was greater in hardened trees after the second drought than in un-hardened trees after the first drought.

No clear effects of K on cavitation were observed. At high-N supply, K increased vessel diameters, which may increase cavitation; however, high-K supply also tended to re-

duce transpiration and leaf area ratios, which may decrease cavitation.

Nutrient effects on water-use efficiency

Both N and K increased instantaneous WUE under well-watered conditions but not under drought conditions. High-K supply also increased WUE in well-watered Sitka spruce

(*Picea sitchensis* (Bong.) Carr.) (Bradbury and Malcolm 1977). Larger vessels, greater E , and smaller leaf area ratios may have enabled the trees to maintain water status and reduce leaf loss in the K3 treatment.

Increasing the N supply of well-watered trees increased A without increasing g_s , thus increasing WUE. However, A decreased in trees in the high-N treatment during the first drought, suggesting desiccation damage to the photosynthetic apparatus (Taiz and Zeiger 1991). Chlorophyll concentrations sometimes decrease in response to drought in poplar leaves (Ibrahim et al. 1997), and for some *Populus* clones, ABA increases in response to a high-N supply and accelerates stomatal closure (Liu and Dickmann 1992). After hardening, N supply was positively related to A in trees subjected to a second drought, perhaps because of greater osmotic adjustment at high N (Harvey 1997).

Pre-drought increases in WUE might help conserve soil water. Long-term productivity and WUE of a Douglas-fir stand on an intermittently dry site was increased by N fertilization (Brix and Mitchell 1986). However, in several other studies, long-term WUE decreased in response to N fertilization (Allen et al. 1990, Raison and Myers 1992, Nilsen 1995). The effect of N fertilization on WUE may depend on the degree and frequency of drought. If droughts are short and not too severe, N fertilization might speed recovery (Liu and Dickmann 1992) and allow rapid growth at times when water is ample.

Indicators of drought-resistant clones

On average, the drought-resistant clones were larger than the drought-sensitive clones, yet they maintained similar Ψ_{xp} during drought, and were less vulnerable to cavitation. These drought responses might be partly associated with the small mean vessel diameters of the drought-resistant clones.

Clones which were known to be productive on dry sites also had the ability to maintain E under drought conditions. High E under drought conditions indicates that stomata are open, allowing trees to photosynthesize and maintain growth. Stomatal opening without excess water loss in the drought-resistant clones may have been facilitated by the small but numerous stomata. The high-N trees also had small, numerous stomata, and grew well, but they were not drought resistant. The drought-resistant clones had greater root:shoot ratios than the drought-sensitive clones, which should make the internal water balance more positive, because there is more root surface area for water uptake compared with leaf area for water loss. The T \times D clones that are productive on dry sites showed less leaf loss during drought, which is probably an important indicator of productivity on dry sites. Clonal drought resistance was not related to either osmotic adjustment (Harvey 1997) or WUE in this study, although Liu and Dickmann (1996) found a productive *Populus* clone that exhibited increased WUE in response to severe drought.

The triploid clones, which were susceptible to cavitation, had wide vessels that might be more vulnerable to cavitation, and long stomata that would increase xylem tensions leading to cavitation. *Populus trichocarpa* (Clone 6) is reported to be more drought-sensitive than T \times D hybrids (Braatne et al.

1992). However, in this study *P. trichocarpa* Blom was not especially vulnerable to cavitation even though it had poor stomatal control. It had high leaf K concentrations, and lacked adaxial stomata, like *P. trichocarpa* Columbia River (Ceulemans et al. 1988). This was also the only clone in which E was not well related to PLC, which suggests that there is more than one mechanism by which poplar trees avoid cavitation.

We conclude that N fertilization on dry sites will make poplars more vulnerable to cavitation and drought stress, especially before hardening. However, productivity on a dry site can be optimized by nutrient balance and careful clone selection. For example, improving the P and K status of trees on dry sites should increase water uptake and decrease transpirational water losses and desiccation damage.

Acknowledgments

Among the staff of B.C. Ministry of Forests Research Branch, we thank D. Ponsford and K. Stockberger for technical assistance, W. Bergerud, Dr. M. Stoehr and V. Sit for statistical guidance, and C. Dawson for plant tissue analysis. Among members of the Dept. of Biology, University of Victoria, we thank Drs. B. Hawkins and N. Livingston for facilities, equipment and editorial advice, and T. Gore for assistance with computer imaging. We thank C. Van Oosten, formerly of MacMillan Bloedel, and Peter McAuliffe of Scott Paper for supplying cuttings and clonal rankings. Finally, we thank Dr. J. Sperry of the Dept. of Botany, Duke University for his advice on cavitation measurement, Dr. T. Hinckley of the College of Forest Resources, University of Washington for editorial advice, and Drs. C. Harrington of the Olympia Forestry Sciences Laboratory and S. Rood of the University of Lethbridge for reviewing this manuscript.

References

- Alder, N.N., J.S. Sperry and W.T. Pockman. 1996. Root and stem xylem embolism, stomatal conductance, and leaf turgor in *Acer grandidentatum* populations along a soil moisture gradient. *Oecologia* 105:293–301.
- Allen, H.L., P.M. Dougherty and R.G. Campbell. 1990. Manipulation of water and nutrients—practice and opportunity in southern U.S. pine forests. *For. Ecol. Manag.* 30:437–453.
- Anderson, V.L. and R.A. McLean. 1974. Design of experiments. A realistic approach. Marcel Dekker, Inc., New York, pp 101–103, 137–138.
- Braatne, J.H., T.M. Hinckley and R.F. Stettler. 1992. Influence of soil water on the physiological and morphological components of plant water balance in *Populus trichocarpa*, *Populus deltoides* and their F1 hybrids. *Tree Physiol.* 11:325–339.
- Bradbury, I.K. and D.C. Malcolm. 1977. The effect of phosphorus and potassium on transpiration, leaf diffusive resistance and water-use efficiency in sitka spruce (*Picea sitchensis*) seedlings. *J. Appl. Ecol.* 14:631–641.
- Brix, H. and A.K. Mitchell. 1986. Thinning and nitrogen fertilization effects on soil and tree water stress in a Douglas-fir stand. *Can. J. For. Res.* 16:1334–1338.
- Ceulemans, R., I. Impens and R. Imler. 1988. Stomatal conductance and stomatal behavior in *Populus* clones and hybrids. *Can. J. Bot.* 66:1404–1414.
- Ceulemans, R., L. van Praet and X.N. Jiang. 1995. Effects of CO₂ enrichment, leaf position and clone on stomatal index and epidermal cell density in poplar (*Populus*). *New Phytol.* 131:99–107.

- Christersson, L. 1976. The effect of inorganic nutrients on water economy and hardiness of conifers. II. The effect of varying potassium and calcium contents on water status and drought hardiness of pot-grown *Pinus silvestris* L. and *Picea abies* (L.) Karst. seedlings. *Stud. For. Suec.* 136:1–23.
- Clancy, K.M., M.R. Wagner and P.B. Reich. 1995. Ecophysiology and insect herbivory. In *Ecophysiology of Coniferous Forests*. Eds. W.K. Smith and T.M. Hinckley. Academic Press, Inc., San Diego, CA, pp 125–180.
- Cutter, B.C. and W.K. Murphey. 1978. Effects of potassium on growth and wood anatomy of a *Populus* hybrid. *Wood Fiber Sci.* 9:282–288.
- DeBell, D.S., M.A. Radwan, C.A. Harrington, G.W. Clendenen, J.C. Zasada, W.R. Harms and M.R. McKevelin. 1990. Increasing the productivity of biomass plantations of cottonwood and alder in the Pacific Northwest. Annual Tech. Rep., USDA For. Serv., Olympia, WA, pp 29–51.
- Gebre, G.M. and M.R. Kuhns. 1993. Effects of water stress preconditioning on gas exchange and water relations of *Populus deltoides* clones. *Can. J. For. Res.* 23:1291–1297.
- Hacke, U. and J.J. Sauter. 1996. Drought-induced xylem dysfunction in petioles, branches and roots of *Populus balsamifera* L. and *Alnus glutinosa* (L.) Gaertn. *Plant Physiol.* 111:413–417.
- Harrington, C.A., M.A. Radwan and D.S. DeBell. 1997. Leaf characteristics reflect growth rates of 2-year-old *Populus* trees. *Can. J. For. Res.* 27:1321–1325.
- Harvey, H.P. 1997. Relationships between mineral nutrition, drought resistance and clone in *Populus*. Ph.D. Diss., University of Victoria, Canada, pp 113, 160.
- Harvey, H.P. and R. van den Driessche. 1997. Nutrition, xylem cavitation and drought resistance in hybrid poplar. *Tree Physiol.* 17:647–654.
- Havaux, M., M. Ernez and R. Lannoye. 1988. Tolerance of poplar (*Populus* sp.) to environmental stresses. I. Comparative study of poplar clones using the *in vivo* chlorophyll fluorescence method. *Acta Ecol. Plant.* 9:161–172.
- Houman, F., H.M. Fei., D.L. Godbold, A. Majcherczyk, W. Shasheng and S. Wang. 1991. Polyamines in leaves and roots of *Populus maximowiczii* grown in differing levels of potassium and phosphorus. *Can. J. For. Res.* 21:1748–1751.
- Ibrahim, L., M.F. Proe and A.D. Cameron. 1997. Main effects of nitrogen supply and drought stress upon whole-plant carbon allocation in poplar. *Can. J. For. Res.* 27:1413–1419.
- Liu, Z. and D.I. Dickmann. 1992. Abscisic acid accumulation in leaves of two contrasting hybrid poplar clones affected by nitrogen fertilization plus cyclic flooding and soil drying. *Tree Physiol.* 11:109–122.
- Liu, Z. and D.I. Dickmann. 1993. Responses of two hybrid *Populus* clones to flooding, drought, and nitrogen availability. II. Gas exchange and water relations. *Can. J. Bot.* 71:927–938.
- Liu, Z. and D.I. Dickmann. 1996. Effects of water and nitrogen interaction on net photosynthesis, stomatal conductance, and water-use efficiency in two hybrid poplar clones. *Physiol. Plant.* 97:507–512.
- McLennan, D.S. and A. Mamias. 1992. Cottonwoods in British Columbia. Problem analysis. B.C. FRDA Report No. 195, B.C. Ministry of Forests, 50 p.
- Nilsen, P. 1995. Effect of nitrogen on drought strain and nutrient uptake in Norway spruce (*Picea abies* (L.) Karst.) trees. *Plant Soil* 172:73–85.
- Pezeshki, S.R. and T.M. Hinckley. 1982. The stomatal response of red alder and black cottonwood to changing water status. *Can. J. For. Res.* 12:761–771.
- Pier, A.P. and G.A. Berkowitz. 1987. Modulation of water stress effects on photosynthesis by altered leaf K^+ . *Plant Physiol.* 85:65–661.
- Raison, R.J. and B.J. Myers. 1992. The biology of forest growth experiment: linking water and nitrogen availability to the growth of *Pinus radiata*. *For. Ecol. Manag.* 52:279–308.
- Salleo, S. and M.A. Lo-Gullo. 1989. Different aspects of cavitation resistance in *Ceratonia siliqua*, a drought-avoiding Mediterranean tree. *Ann. Bot.* 64:325–336.
- Sellin, A. 1991. Hydraulic conductivity of xylem depending on water saturation level in Norway spruce (*Picea abies* (L.) Karst.). *Plant Physiol.* 138:466–469.
- Sperry, J.S. and T. Ikeda. 1997. Xylem cavitation in roots and stems of Douglas-fir and white fir. *Tree Physiol.* 17:275–280.
- Sperry, J.S. and N.Z. Saliendra. 1994. Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant Cell Environ.* 17:1233–1241.
- Sperry, J.S., J.R. Donnelly and M.T. Tyree. 1988. A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Environ.* 11:35–40.
- Sperry, J.S., K.L. Nichols, J.E.M. Sullivan and S.E. Eastlack. 1994. Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. *Ecology* 75:1736–1752.
- Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics. A biometrical approach. McGraw-Hill Publishing Co., New York, pp 172–210.
- Stettler, R.F., T.M. Hinckley and P.E. Heilman. 1988. Short-rotation intensive culture of Northwest hardwoods: highlights of an integrated research program. Report for U.S. DOE Short Rotation Woody Crops Program, College of Forest Resources, Univ. Washington, Seattle, WA, 86 p.
- Taiz, L. and E. Zeiger. 1991. *Plant physiology*. Benjamin/Cummings Publishing Co., Inc., Redwood City, CA, pp 68–70, 88–89, 114, 353.
- Tschaplinski, T.J. and G.A. Tuskan. 1994. Water-stress tolerance of black and eastern cottonwood clones and four hybrid progeny. II. Metabolites and inorganic ions that constitute osmotic adjustment. *Can. J. For. Res.* 24:681–687.
- Tyree, M.T. and F.W. Ewers. 1991. The hydraulic architecture of trees and other woody plants. *Tansley Review No. 34*. New Phytol. 119:345–360.
- Tyree, M.T., J. Alexander and J. Machado. 1992. Loss of hydraulic conductivity due to water stress in intact juveniles of *Quercus rubra* and *Populus deltoides*. *Tree Physiol.* 10:411–415.
- Tyree, M.T., K.J. Kolb, S.B. Rood and S. Patino. 1994. Vulnerability to drought-induced cavitation of riparian cottonwoods in Alberta: a possible factor in the decline of the ecosystem? *Tree Physiol.* 14:455–466.
- van den Driessche, R. 1990. Paclobutrazol and triadimefon effects on conifer seedling growth and water relations. *Can. J. For. Res.* 20:722–729.
- van den Driessche, R. and D. Ponsford. 1995. Nitrogen induced potassium deficiency in white spruce (*Picea glauca*) and Engelmann spruce (*Picea engelmannii*) seedlings. *Can. J. For. Res.* 25:1445–1454.
- Zimmermann, M.H. 1978. Hydraulic architecture of some diffuse-porous trees. *Can. J. Bot.* 56:2286–2295.