Relationships Between Mineral Nutrition, Drought Resistance and Clone in *Populus*

by

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B.Sc. University of Alberta, 1969

A Dissertation Submitted in Partial Fulfilment of the
Requirements of the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Biology

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ABSTRACT

Effects of mineral nutrition on drought and cavitation resistance of poplars were examined in two sets of greenhouse-grown trees. First, 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 sub-irrigation sand-culture system. The trees were subjected to 0, 4, 6, and 8 days of gradual drought stress before measurements of cavitation, anatomical features affecting cavitation, and nutrient mobilization during drought. High foliar concentrations of N increased cavitation compared to barely adequate concentrations, 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. Vessel pit membrane mean pore diameters were 0.132 μm at low P and 0.074 μm at high P: smaller pores would decrease air-seeding cavitation. No other significant effects of mineral nutrition on vessel dimensions were observed. Scanning electron microscopy showed less damage to pit membranes, suggesting greater membrane strength in drought-resistant clones than in drought-sensitive clones.

In the second experiment, three drought-resistant and three drought-sensitive poplar clones (including *P. trichocarpa*) were grown at two levels of N and three levels of potassium (K) and either well-watered, cyclically droughted, or droughted once. Cavitation, osmotic potential, gas exchange, and nutrient mobilization were measured at each stage of drought and rewatering, and fall nutrient retranslocation was monitored. Cavitation was greater with adequate foliar N than at deficiency levels. Moderate supplies of K increased cavitation, but luxury levels sometimes reduced cavitation by decreasing foliar water loss and thus xylem tension. Preconditioning did not reduce vulnerability to cavitation, but there was some evidence of cavitation reversal in a drought resistant clone at high N supply. Vessel diameters were 36.6 µm at low N but 45.2 µm at high N, so within *Populus*, larger diameter vessels correlated with susceptibility to cavitation.

High N supply increased water stress during the first drought, but also increased instantaneous water use efficiency (WUE) before drought occurred, and osmotic adjustment and hardening after drought. Increased K also increased WUE before drought and decreased water stress (decreasing transpiration and wilting) at luxury levels, but did not influence osmotic adjustment or hardening.

Mobilization of nutrients differed with speed and intensity of drought. Gradual drought led to resorption of N and P. In the second experiment, drought was too rapid for retranslocation. Nutrients became more concentrated; some (e.g., N) facilitated hardening and osmotic adjustment, and some (e.g., K) moved out of the leaves on rewatering. In fall, N, P, Cu and K were resorbed, the latter more proficiently with greater N supply at low levels of K.

Clones which were more productive on dry sites resisted severe, but not moderate cavitation. Cavitation-resistant clones maintained high transpiration rates (and less negative water potentials) in drought, especially after hardening, had more, but smaller, stomata and decreased leaf loss in drought, but did not have increased WUE or osmotic adjustment.

Nitrogen fertilization increased cavitation, greater P supply reduced this effect, and K fertilization may make vessels more vulnerable to cavitation but decrease the tension on the xylem that causes cavitation. Nitrogen fertilization levels should be tailored to site water supplies, and appropriate P, and possibly K additions may increase drought resistance.

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A	assimilation (photosynthetic) rate (μmol m^{-2} s^{-1})
ABA	abscisic acid
Al	aluminum
B	boron
Ca	calcium
Cu	copper
Fe	iron
g_s	stomatal conductance (mol m^{-2} s^{-1})
IAA	indole-3-acetic acid
K	potassium
K1	0 mM K supply
K2	0.26 mM K supply
K3	2.57 mM K supply
LA	leaf area
Mg	magnesium
Mn	manganese
N	nitrogen
N1	0.71 mM N supply
N2	7.14 mM N supply
P	phosphorus
PLC	percent loss of conductivity (cavitation)
RE	retranslocation efficiency
Rubisco	ribulose 1,5-bisphosphate carboxylase oxygenase
S	sulphur
T	transpiration (mmol m^{-2} s^{-1})
T x D	hybrids of *Populus trichocarpa* and *P. deltoides*
WUE	water use efficiency
Zn	zinc
ψ_{xp}	xylem water potential as measured in the leaf petiole
ψ_{π}	osmotic potential
$\psi_{\pi ad}$	osmotic adjustment

ACKNOWLEDGMENTS

Thanks to my supervisors Dr. Robert van den Driessche and Dr. Barbara Hawkins for their patience, advice and support over the years. I wish to thank my committee members Dr. Nigel Livingston and Dr. Nancy Turner and also Dr. John Owens for their time, suggestions and encouragement, and thanks to both Nigel and John for equipment loans. Among staff of the B.C. Ministry of Forests Research Branch, I would especially like to thank David Ponsford for his invaluable assistance and technical ideas, Maria Davradou for her inspiration and time, Dr. Michael Stoehr, W. Bergurud, V. Sit and S. Chan for statistical guidance; Dr. S. Berch for advice on photomicrography; Clive Dawson and his staff for plant tissue analysis, and all the others at Glyn Road for their friendliness and many equipment loans. Among many others in the Department of Biology, University of Victoria, thanks to Dr. C. Singla for SEM instruction and to Tom Gore for guiding me through the mysteries of electronic imaging. I would like to thank Cees van Oosten of MacMillan Bloedel for supplying productivity rankings and poplar cuttings over the years, and Peter McAuliffe of Scott Paper Ltd., New Westminister, B.C. for poplar cuttings in year two.

Finally, I would like to thank my family for their encouragement and support.

Chapter 1

INTRODUCTION

1.1 RATIONALE AND OBJECTIVES

1.1.1 Rationale

An alternative supply of pulpwood is required to meet escalating demands for pulp and paper if diminishing old growth forests are to be preserved. Poplar is an economical source of paper, needing little bleaching. Native poplar stands in B.C., however, are part of the riparian cottonwood ecosystem, one of the most endangered forest ecosystems in North America (Rood and Mahoney 1990). British Columbia's new Forest Practices Code contains new measures to protect riparian areas. Restrictions on harvesting of B.C.'s forests have led to the establishment of trial plantations of high-productivity hybrid poplars (*Populus trichocarpa* x *P. deltoides* or T x D) on eastern Vancouver Island, often on marginal land. This area is subject to summer drought, and in general, plantation sites tend to be drier in North America than in Europe (Blake et al. 1984). In dry conditions, poplar species are vulnerable to drought stress (Braatne et al. 1992), and in particular to cavitation (air bubbles blocking the flow of water up the stem) which reduces their productivity (Tyree et al. 1992). Hybrids must be selected which are drought and cavitation resistant. The proposed planting sites are also deficient in phosphorus and probably other nutrients, and short-rotation poplars have unusually high nutrient requirements (Liu and Dickmann 1996), so it is important to know how nutrition will influence drought and cavitation resistance. The results of this research could facilitate selection of hybrids for specific sites (which may or may not be dry or deficient in various nutrients), and help determine fertilization prescriptions.

1.1.2 Hybrid Poplar Advantages

Hybrid poplars are grown in short-rotation plantations for biomass fuel (pellets or liquid), pulp and paper, and sometimes for lumber (Heilman et al. 1989, Stettler et al. 1991). Given the right conditions, hybrids may be more productive than either parent, displaying hybrid vigour. They often have more efficient water use and crown architecture, higher net photosynthetic rates per unit leaf area and greater leaf area. Clones of T x D, developed at the University of Washington in Seattle, have been especially successful in field trials in the Pacific Northwest. For poplars in general, the greater the leaf area the more productive the tree (Ridge et al. 1986, Stettler et al. 1988); T x D has both more and larger leaves than parental species (Cain and Ormrod 1984), combining the larger epidermal cells of *P. trichocarpa* Torr. & Gray with the more numerous epidermal cells of *P. deltoides* Bartr. *ex* Marsh (Ridge et al. 1986). In one study, up to four times the parental leaf area translated into a seven to 17 times greater stem biomass in hybrid progeny (Roden et al. 1990).

Leaf growth and water potential both decrease more under dry conditions in parental stock than in their hybrids (Roden et al. 1990). *Populus trichocarpa* is often more drought sensitive than *P. deltoides*, partly because *P. trichocarpa* has less sensitive stomata (Pezeshki and Hinckley 1982), although they do not open fully. Hybrid stomata are more open and sensitive than the *P. trichocarpa* parent and sometimes more sensitive than *P. deltoides*. Stomatal insensitivity may be genetically linked to thicker spongy mesophyll and whiter abaxial leaf surfaces (Stettler et al. 1988). Greater ability for osmotic adjustment appears to be inherited from the *P. deltoides* parent, but perhaps adjustment in the fine roots from *P. trichocarpa* (Tschaplinski and Tuskan 1994).

Hybrids may grow for a greater proportion of the day than parental stock, because fast-growing hybrids experience less turgor loss during midday water deficits, and consequently less midday inhibition of leaf growth (Stettler et al. 1986). Hybrids continue growing longer in drought because they maintain their cell wall extensibility, losing only 30% while *P. deltoides* can lose 68% and *P. trichocarpa* 61%. Hybrids may delay normal leaf cell development; leaf cell walls stiffen as they mature. In parents, drought may accelerate the stiffening process.

1.1.3 Objectives

Experimental objectives were to determine:

1. how nitrogen, phosphorus and potassium nutrition affect drought resistance and

vulnerability to cavitation,

2. whether resistances or sensitivities to cavitation are manifested in anatomical differences in the xylem of hybrid poplars,
3. if vulnerability to cavitation correlates with drought resistance in hybrid poplars and may be useful as a selection criterion,
4. the effects of preconditioning on cavitation, by subjecting trees to drought cycles and different levels of potassium,
5. the extent of poplar nutrient retranslocation during drought, and its effect on drought resistance and cavitation (clones which retranslocate more efficiently might be more suitable for dry sites, especially if they are able to restore the nutrients to the leaves when the drought is relieved), and
6. clonal characteristics governing productivity in drought.

1.2 LITERATURE REVIEW

A preliminary review of relevant literature will provide a broad background for the research in the following chapters. The main thrust of that research has been to determine the effect of nutrition on cavitation (Chapters 2 and 3), since it appears that this has not been studied in any species. The review will begin with basic facts about cavitation, especially poplar cavitation and hydraulic architecture. Poplar water relations will be reviewed, including preconditioning (Chapters 4 and 5), followed by poplar nutritional studies on nitrogen (N), phosphorus (P) and potassium (K), along with nutrient effects on water relations. Retranslocation will be discussed (Chapter 5). The final section will be a brief summary of characteristics correlated with poplar drought resistance in the literature (Chapter 6).

1.2.1 Cavitation

Resistance of xylem to cavitation may be the most important parameter determining drought resistance of a tree (Tyree and Ewers 1991). Certainly survival of newly planted seedlings such as western hemlock is threatened when water stress, due to root loss and incomplete root-soil contact, leads to cavitation (Kavanagh and Zaerr 1997).

Cavitation is the formation of gas bubbles in xylem vessels or tracheids. These bubbles interrupt the flow of water through the vessel. The gas bubble is an embolism, which usually expands until contained by pit membranes in the vessel wall. The xylem of a tree trunk can be compared to a collection of thousands of very thin pipes carrying water from soil to leaves. According to the cohesion theory of the ascent of sap, water molecules in small tubes with wettable walls (vessels) have sufficient attraction for each other and for the walls of the pipes or vessels (through hydrogen bonding) to maintain a continuous water column of greater than thirty metres under tensions of three to thirty megapascals. As water is lost through evaporation from leaves, tension is exerted that draws more water up the trunk.

$$\psi_{xp} = 4*T / d \tag{1.1}$$

where ψ_{xp} is xylem pressure potential or tension exerted on the water column, T is surface tension of water (0.072 N m^{-1} at 20°C) and d is meniscus diameter of the water film covering leaf mesophyll cells (continuous with the xylem water column). As the film evaporates, water withdraws further into the cracks between cells, so that meniscus diameters decrease, increasing tension. These mesophyll cells are behind the stomatal pore, so resulting tension depends on speed of stomatal closure (Taiz and Zeiger 1991).

If the soil is too dry to replace the water as fast as it is lost, the water column is "stretched" and can break (Kramer and Kozlowski 1979). This break spreads rapidly through the vessel like the contracting ends of an overstretched and snapped rubber band. The empty space may be a vacuum at first, but air from surrounding solutions and tissues enters (Tyree and Ewers, 1991). Or, the transpirational pull may create negative pressures (the stretch) in the water column so that water is below vapour pressure: it is now in a higher energy or "metastable" state where water vapour is the stable phase (Sperry 1995). The region of vaporized water is the break (embolism or cavity) which then rapidly spreads.

Many researchers prefer the air seeding hypothesis: when xylem pressure potential is too great, air bubbles are drawn into the vessel through a pore in the pit membrane adjoining another cavitation site (Cochard and Tyree 1990). The original embolism may form as above, or when leaf loss or insect damage allows air into the vascular system. However formed, these air-filled bubbles or embolisms block the flow of water up the xylem. In nature, this damage could become permanent as the cavitated vessels form nonconducting heartwood. In ring-porous trees they become blocked with tylose (Kramer and Kozlowski 1979).

1.2.1.1 Freezing-Induced Cavitation

Cavitation may also be induced by freeze-thaw cycles, and susceptibility to this depends on vessel diameter according to the formula:

$$P_{bubble} = 2T / r_{bubble} + P_{xylem} \tag{1.2}$$

where P_{bubble} is internal pressure in the gas bubble (a negative value), T is the surface tension of water, r_{bubble} is bubble radius (vessel radius) and P_{xylem} is xylem pressure relative to atmospheric (Sperry 1995).

Populus x *canadensis* Moench Robusta exhibited cavitation-induced losses in hydraulic conductivity upon freezing, when air became less soluble in water as it turned from liquid to ice (Just and Sauter 1991). This type of embolism seemed to dissolve readily on thawing in some species, especially with positive root pressure in spring. The clone Robusta did not recover in this trial (Hacke and Sauter 1996b).

Populus are diffuse-porous trees, meaning they have almost equally large, cavitation-prone vessels in spring and summer wood (Mauseth 1991). Embolisms in ring porous species, with their larger, more vulnerable spring vessels and smaller diameter summer vessels, are usually irreversible (Sperry 1995). Most conifers, with tiny tracheids instead of vessels, are much less vulnerable to freezing-induced cavitation than angiosperms, a selective advantage which may partially explain their preponderance in northern regions.

Repeated freeze-thaw cycles increase the opportunities for bubble formation, and fast thaws allow less time for bubble dissolutions (Sperry 1995). Winter cavitation in Sweden causes branch die-back of *Picea abies*, and even determines presence or absence of the species in mixed forests according to long-term climate cycles (Kullman 1996). Freezing-induced cavitation is a less important consideration on Vancouver Island because of its milder climate, and this dissertation will focus on drought-induced cavitation.

1.2.1.2 Anatomical Features Affecting Drought-Induced Cavitation

Tyree and Sperry (1989) contend that, although large-vessel species are usually more vulnerable to cavitation, diameters of the pores in pit membranes determine vulnerability to cavitation in drought (with large vessels often having larger pores). In spring, when carbohydrate demands are high, fast-growing cells have less primary and secondary cell wall formation and thus larger pores, while slower growing cells in summer have smaller pit-membrane pores and are less vulnerable to cavitation. Pit membranes (adjoining pairs of primary cell wall and middle lamella located between two cells at areas where secondary walls are missing) are on the side walls of vessels, and provide lateral transport of water from ray parenchyma, tracheids or other vessels (Mauseth 1991). In *Populus*, vessels end in radial vessel groups or radial multiples which share tangential walls covered in a "honeycomb" design of bordered pits (Zimmermann 1978). There may be a genetic component to rates of wall formation and cell expansion (Tyree and Sperry 1989). Also, cavitation occurred sooner in older vessels of *P. tremuloides* (trembling aspen) due to degradation of pit membranes as seen with the scanning electron microscope (Sperry et al. 1991). In older pit membranes, pore sizes were 0.5 μm compared to a maximum of 0.08 μm in young pit membranes, and embolisms would more easily pass through these larger holes. Thus cavitation was not only a stress response, but also a natural component of sapwood senescence and its conversion to heartwood. In fast-growing branches only the newest xylem conducts water in the spring. This loss of functional xylem limits the leaf area a tree can support.

Cells of the same size from different species may have different vulnerabilities. Vulnerability was not correlated with conduit type (vessel versus tracheid) or diameters, but rather with xylem pressures experienced in nature (Tyree and Sperry 1989). In contrast to the above generality, vessel diameters of *Populus balsamifera* correlated positively with cavitation, with more vulnerability (and larger vessels) in roots than stems and the least in petioles. This pattern was not observed in an *Alnus* species (Hacke and Sauter 1996a). Both species had narrow safety margins of xylem pressure potential (ψ_{xp}) for cavitation, perhaps because their natural habitats are moist. Vulnerability to cavitation in poplars may limit stomatal conductance and therefore growth in hot dry situations.

Conductivity in *P. deltoides* decreased sharply below water potentials of -1.0 MPa with 100% loss of conductivity at -2.0 MPa compared to -4.0 MPa for *Quercus rubra* L. (red oak) (Tyree et al. 1992). There was a 50% loss of hydraulic conductivity in one-year-old stem segments at ψ_{xp} of -0.7 MPa for *P. deltoides* and -1.7 MPa for *P. balsamifera* L. (balsam poplar) and *P. angustifolia* James (narrowleaf cottonwood) (Tyree et al. 1994b). This means *P. deltoides* is more vulnerable to cavitation than any other North American tree investigated to date. Further studies on the genetic variability in xylem vulnerability are needed. (Tyree et al. 1994b).

Leaf specific conductivity (rate of flow per gram of fresh weight of leaves supplied) within a tree may be determined by vessel diameters. Conduit diameters decrease from base to tip, with smaller values in branches than in the main stem (Zimmerman 1978). Tyree and Ewers (1991) found that a single junction from stem to leaf or from stem to branch had the same drop in water potential as a 3.4 or a 2.5 meter increase in stem length respectively. There is a constriction in vessels at the base of branches, so that with transpiration, pressure must fall more quickly in the xylem of lower branch leaves than in the topmost leaves of the leader. These constrictions are due to more small-diameter and fewer large-diameter vessels in the nodes compared to internodal regions (Salleo et al. 1982). Sometimes, as in *P. deltoides*, there is also a constriction as the xylem enters the leaf petiole (Zimmerman 1978). Small branches or individual leaves may die back when drought is severe, reducing leaf area and thus transpirational loss, conserving water for the surviving parts of the tree. Leaf specific conductivities of T x D were twice as high in the upper branches as in the lower ones (Hinckley et al. 1994). Perhaps this unequal conductivity explains why poplars shed their leaves (especially the lower ones) more readily in drought than some other tree species (Rood and Mahoney 1990), a survival mechanism to decrease drought-induced cavitation by reducing leaf area and relieving xylem tension (Tyree and Ewers 1991).

1.2.1.3 Cavitation Reversal

Recently there has been interest in reversal of drought-induced embolisms. Edwards et al. (1994) demonstrated cavitation reversal in *Pinus sylvestris* L. (Scotch pine) stem segment tracheids at negative water potentials. This was possibly due to diffusion of water out of the tracheid and mass transport away in surrounding functional tracheids. The de-aerated solution used to measure conductivity might hasten the dissolution. *Acer grandidentatum* Nutt. (bigtooth maple) roots were more vulnerable than stems to embolisms, and vulnerability varied with the site, but root embolisms were more easily reversed with rewatering (Alder et al. 1996). Roots of *Populus balsamifera*, *Alnus glutinosa* (L.) Gaertn. (black alder) (Hacke and Sauter 1996a) and *Betula occidentalis* Hook. (water birch) (Sperry and Saliendra 1994) were also more vulnerable than stems. *Laurus nobilis* L. (laurel) stems recovered from embolisms except at > 60% cavitation, probably through the influence of indol-3-acetic acid (IAA), which may have induced solute accumulation in the phloem (Salleo et al. 1996). Solutes could be transferred to vessels via the ray parenchyma, decreasing osmotic potential and causing vessels to refill without positive pressures. The possibility of cavitation reversal after rewatering in intact poplar stems is examined in this thesis (Chapter 3).

1.2.1.4 Cavitation Benefits

Embolisms may have some positive functions. Cavitated vessels may provide space for expansion of water into ice in the winter (Sperry et al. 1991). Once filled with tyloses, cavitated areas become heartwood which may serve as a water storage area (Sperry et al. 1991). Root cavitation can even prevent backflow of water from lateral roots of desert plants such as cactus and agave when pressure potential of the soil is more negative than that of the plants (Sperry 1995). Small branch dieback due to selective cavitation may lessen water requirements (Tyree and Ewers 1991). Finally, moderate cavitation, especially reversible cavitation as in roots, may enhance drought survival by decreasing water use (Neufeld et al. 1992, Alder et al. 1996).

1.2.2 Poplar Water Relations

To be economically feasible (and conservation minded) in North America, poplar plantations must be located on marginal land, which is often dry (Blake et al. 1984). This has happened in the Mississippi River flood plain (Farmer 1970), the Pacific Northwest east of the Cascades (Bassman and Zwier 1991), and eastern Vancouver Island in British Columbia. Poplar productivity is limited by stress tolerance (Heilman et al.1989, Tschaplinski and Blake 1989a). For instance, *P. trichocarpa* has only slight to moderate capability of surviving low leaf water potentials (Pezeshki and Hinckley 1988). Selecting suitable cultivars for planting on such dry sites means finding poplars that have not only high productivity, but also high water use efficiency and drought tolerance (Bassman and Zwier 1991, Farmer 1970, Tschaplinski et al. 1994, Blake et al. 1984). Fortunately, high productivity often correlates with drought resistance (Tschaplinski and Blake 1989a).

1.2.2.1 Stomatal Characteristics

Not all poplars are equally responsive to environmental changes. One stand of T x D was poorly coupled to the atmosphere (Hinckley et al. 1994), although individual leaves were not (Hinckley, pers. comm.). Unresponsiveness to environmental change seems to be a phenomenon common to some clones of *P. trichocarpa* and their hybrids (Dickmann et al. 1992). Stomatal opening/closing of *P. euramericana* and *P. tristis* x *P. balsamifera* was well coupled to the atmosphere (drought caused stomatal closure), which is usually the case for poplar.

Water use efficiency (WUE or transpiration per unit dry matter produced), drought tolerance and stomatal characteristics all interact and vary widely, and WUE may vary by genotype (clone) rather than by larger groupings of the genus such as section or even by species. Among 17 species or hybrids, WUE could not be explained by one physiological or morphological factor (Blake et al. 1984).

Clones of European species have more stomata, and American clones have larger ones. American clones have stomata on both surfaces, although more on the abaxial side, except *P. trichocarpa* clone Columbia River has no adaxial stomata. Of all stomatal characteristics among ten clones, the only ones correlated with yields were abaxial stomatal length and mean stomatal length (Ceulemans et al. 1984).

Patterns of stomatal conductance have been observed. Leaf conductance increased quickly at low light in the morning for *P. trichocarpa*, then levelled off as stomata became light saturated at 400 μmol m^{-2} s^{-1} (Pezeshki and Hinckley 1982). Often stomata open and evaporative demand increases, then water content decreases. Whether hybrids are drought sensitive or drought tolerant, both ψ_{xp} and water content of the leaves are highest at daybreak and lowest at midday (Wang et al. 1983). Leaf conductance was 15% greater before than after solar noon (Magnussen 1985) and stomata opened faster than they closed, remaining open in the late afternoon at irradiances at which they were closed in the morning (Ceulemans et al. 1988). *Populus trichocarpa* had an especially weak closing response to declining light: stomata remained open until the photosynthetically active radiation fell to 80 μmol m^{-2} s^{-1} in the late afternoon (Pezeshki and Hinckley 1982). In fact, stomata of *P. trichocarpa* clone Columbia River and *P. koreana x P. trichocarpa* hybrid Peace were open even at night (Furukawa et al. 1990). Stomatal conductance of Peace was very little affected by light, water stress, ABA or ozone: leaves turned brown in drought, but the trees survived in the field. These aberrant clones are useful because their differences in structure and function can sometimes explain differences in WUE and drought resistance.

Light and temperatures affect stomatal conductance. Usually increasing light and/or temperature (to an optimum level) caused increases in stomatal conductance in six

Ontario clones (Magnussen 1985). Clonal differences were seen only at the two highest light intensities at 32°C. Water use efficiency was negatively correlated with light intensity at 20°C, but was positively correlated with light level between 10°C and 20°C.

Leaf age affects conductance. Stomatal control decreased with age for T x D and two *P. maximowiczii* hybrids (Reich 1984). Well before senescence, at 48 days, leaves exhibited more random stomatal oscillations (i.e., more conductance in the dark and after excision and less conductance in the light). Lower conductance in the light decreased CO_2 uptake and thus photosynthesis. This loss of stomatal control may be the reason why older leaves are lost first under dry conditions. Young expanding leaves (less than 12 days old) had immature stomata. Optimum leaf age for stomatal control was 12 to 24 days, but there was little decline in function at 36 days (Ceulemans and Impens 1980).

1.2.2.2 Preconditioning

Preconditioning, which may include osmotic adjustment, often improves the drought resistance of poplars. Osmotic adjustment is a drought stress tolerance mechanism, defined as increase in cell solute concentration in order to lower water potential without decreasing cell turgor. Potassium is an important osmoticum, but sugars and organic acids such as polyamines are others (Taiz and Zeiger 1991). For a xeric and a mesic clone (Gebre and Kuhns 1991), dehydration tolerance seemed at least partly due to osmotic adjustment. Adjustment involved changes in the dry weight fraction (ratio of dry weight to turgid weight), the proportion of bound water and the accumulation of solutes. Other suggested hardening mechanisms were decreased electrolyte leakage, substances in stress leaves lingering after drought, drought-increased cuticular development, or drought stressed cell walls retaining water.

Several clones of *P. deltoides* were tested for organic solute buildup and accompanying dehydration tolerance (Gebre et al. 1994). Results were sometimes ambiguous, with considerable clonal variation. One clone exhibited more drought stress and wilted more easily (perhaps because of higher stomatal conductance) but had a lower injury index (more stable membranes) when preconditioned, but another had the highest sucrose concentration level. All three exhibited osmotic adjustments of 0.23 to 0.48 MPa with sucrose, malic acid, glucose, fructose, myoinositol and salicin contributing at different levels for different clones (Gebre et al. 1994). Solutes accounted for only 50% of adjustment, but inorganic ions and primary amino acids had not made important contributions in earlier studies. Rewatering decreased total solutes (probably translocated out of the leaf) predawn but not at midday, but increased salicin, and adjustment was maintained for several days. Adjustment was greater when measured predawn than at midday, but differences between clones were not related to time of measurement (Gebre et al. 1997).

Hybrids of T x D appeared to inherit water stress tolerance from *P. deltoides* (Tschaplinski and Tuskan 1994). However, some hybrids had even more sucrose, glucose and salicin than either parent. In a study of water stress tolerance and late-season organic solutes (Tschaplinski and Blake 1989b), tolerance and solute concentrations varied by clone and treatment. In the field, one clone combined high productivity with drought stress tolerance (due to low saturated osmotic potential and turgor loss points). The main solutes were salicyl alcohol, salicin, sucrose and an unidentified compound. Repeated drying cycles increased osmotic adjustment in the leaves but not the roots. Greenhouse-grown plants sometimes reacted differently than in the field.

For poplars in general, carbohydrates and organic acids seem to be important components of osmotic adjustment, whereas amino acids contribute more to the adjustment of agricultural crops (Tschaplinski and Tuskan 1994). Of six preconditioned clones, only one hybrid had increased solutes in the fine roots. The same hybrid had a 1.45 fold increase in leaf metabolites and ions, including salicin, myoinosital and amino acids. This clone and *P. deltoides* (which had a 1.5 fold increase in metabolites and ions), also had increased K, malic acid, sucrose and glucose. High levels of malic acid were suggested as an indicator of water stress tolerance (Ceulemans and Impens 1980).

1.2.2.3 Water Content

Plant water content (percent of dry weight or $cm^3\ cm^{-3}$) is one measure of drought stress (Kramer and Kozlowski, 1979). For *P. euramericana* (Robusta), a poplar leaf at 78% water content had water potentials of -1.4 MPa and was fully turgid (Pieters and Zima 1975). Lowering the water content reduced photosynthesis whether light was saturated or limiting.

The yearly flux in whole tree water content for *P. tremuloides* was extremely variable (Gibbs 1939). There was a significant drop from December to April as stored reserves were used up, an increase just before leaf opening and a drop after leaf opening. The top young branches continued to increase in water content longer than the rest of the tree. Water content declined all summer to September, when there was a fast autumn refilling (Gibbs 1939, Sauter 1966). The heartwood of black poplar stores water in surplus times and donates it to the sapwood in dry periods (Sauter 1966).

For aspens, water content of the wood was greatest in winter, with more seasonal change in the outer sapwood (Bendtsen and Rees 1962). Water made up about 50% of the green weight in summer. In all seasons, water content decreased with height, varied with wood density, and was greatest in the outer sapwood, but there was more in the pith than in the heartwood. Site factors had little effect on moisture content. Time domain reflectometry can be used to measure the instantaneous water content of a plant (Constantz and Murphy 1990). Cottonwood and aspen had water contents of 0.65 cm^3 cm^{-3} compared to only 0.2 or 0.3 cm^3 cm^{-3} for conifer species tested.

The amount of soil water used by *P. tremuloides* depended on aspect (west most) and elevation of the site (sprout stands needed more water at lower elevations), and tree size (Tew 1967). A T x D hybrid (8-15 cm in diameter) used 20 to 51 kg of water per day per plant (Hinckley et al. 1994). Four poplar clones were most productive at soil water contents of 70% to 85% of field capacity (Naidenova-Yaneva 1974). *Populus euramericana* (Robusta) required irrigation when soil moisture contents fell below 50% of field capacity and transpiration was less than 928 mg g^{-1} h^{-1} (Varfolomeev 1984).

Water balance models sometimes help with poplar plantation site selection. The SWATRER model (a computer package for modelling field water balance) was useful at one site in Belgium (van Slycken and Vereecken 1990). Girth growth (since at this site girth growth correlated linearly with transpiration and dry matter production) was used for estimating the growth-site relationship. Various components of the model such as sinks, evapotranspiration, and interception of light need fuller investigation before recommending it for wider use.

1.2.3 Poplar Nutrition, and the Influence of N, P and K Nutrition on Water Relations

The factor most limiting to forest tree growth worldwide is water, followed by N, P and K in that order (Clancy et al. 1995 Kramer and Kozlowski 1979). There is a complex interaction between the effects of water and nutrients on growth (Oren and Sheriff 1995), with water supply influencing nutrient uptake, nutrient supply affecting water uptake, and availability of one nutrient affecting the need for and uptake of other nutrients. Examining the effects of N, P and K nutrition on forest tree water relations, then, will involve a brief review of nutrient interactions and of the impact of water stress on nutrients and root growth, and a detailed study of the influence of each nutrient on water balance.

Excess N caused K deficiency symptoms in *Picea glauca* (Moench) Voss (white spruce) and *Picea engelmannii* Parry (Engelmann spruce) (van den Driessche and Ponsford 1995), and is known to increase the need for P (Marschner 1986). Luxury levels of P lead to Zn and Cu deficiencies in poplars (Timmer and Teng 1990), but also increase K uptake (Houman et al. 1991). Potassium is antagonistic to Ca and Mg uptake in poplars (Diem and Godbold 1993), but K increased P uptake in *Picea sitchensis* (Bong.) Carr (Sitka spruce) (Bradbury and Malcolm 1977). Therefore, any effect of N, P or K on water relations could potentially be indirect, caused by changes in another nutrient.

1.2.3.1 Effects of Drought on Nutrients

Drought increases requirements for certain nutrients, both by decreasing uptake and increasing optimum concentrations (Oren and Sheriff 1995). Nutrients are obtained from soil solutions through mass flow and diffusion. With low soil moisture both processes are reduced, along with root extension (Marschner 1986), although increased nutrient solution concentration in drier soil ameliorates the situation slightly by increasing diffusion (Oren and Sheriff 1995). Mass flow of dissolved mobile ions is positively related to transpiration, and diffusion of ions into roots depends on the number of water-saturated pores in the soil; both transpiration and soil water are decreased by drought. Furthermore, the rhizosphere may dry faster than the bulk soil, so that contact may be lost between soil and roots, further decreasing diffusion (Oren and Sheriff 1995). Uptake of P and K are especially reduced when soils are dry (Marschner 1986).

Clancy et al. (1995) reviewed drought effects on plant nutrient contents. Depending on nutrient availability, drought-stressed plants tend to increase internal K and soluble N concentrations, perhaps to lower osmotic potential (Mattson and Haack 1987). For instance, *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir) had higher foliar P and K in drought (Kemp and Moody 1984). In another study, three-year-old Douglas-fir needles had greater concentrations of soluble N, especially proline, in drought (van den Driessche and Webber 1975). However, there have also been reports of reductions in foliar N content with drought for current year Douglas-fir needles (Clancy et al. 1995 citing Cates et al. 1983). Ponderosa pine (*Pinus ponderosa* Laws.) needle N was unchanged by drought (Clancy et al. 1995). Drought response might vary with drought level, tree or needle age, genetic variation in drought-induced nutrient retranslocation, mycorrhizal infection rates, soil nutrient limitations, or rooting habit.

Mattson and Haack (1987) explained that deep roots may reach the water table and supply more water during a drought. This would maintain growth, which along with the low-concentration nutrient solution supplied by the subsoil, would lead to dilution of tissue nutrients. Plants with shallow roots might contact less water, so cell expansion, stomatal opening and growth would be less. However, shallow roots would access a richer nutrient solution in the topsoil, more concentrated by drought. Increased diffusive nutrient uptake, along with decreased growth dilution, might lead to greater plant nutrient concentrations.

1.2.3.2 Drought Effects on Root Growth

Drought tends to favour root growth compared to shoot growth, an adaptation which enhances water-locating abilities (Ferrier and Alexander 1991). Nutrient effects on root growth also have important implications for water relations (below). Water uptake is optimized with increased new unsuberized roots, long root hairs to bridge the gap between dry soil and root, and deep roots (extension of the main root) to access a falling water table (Marschner 1986). Nutrient effects on roots may be mediated by nutrient effects on hormones: abscisic acid (ABA) (from root cap cells) inhibits root extension while increasing lateral roots and root hairs, and ethylene has a similar effect. Indole-3-acetic acid (IAA) (an auxin from shoots) promotes lateral root growth, and cytokinin (from the apical meristem) inhibits it, with both inhibiting main axis elongation and increasing ethylene production (Marschner 1986). Water stress may also be induced by freezing or flooding (Marschner 1986).

1.2.3.3 Nitrogen

Nitrogen fertilization increases total biomass, and often leaf biomass and leaf area. Leaf area is directly related to poplar productivity. Maximum biomass is expected at leaf N concentrations of 2% to 3% (Blackmon 1976; National Poplar Commission 1987) or 3.8% (McLennan 1996). Recommended levels of fertilization vary by site and poplar species. One hundred kilograms per hectare, alone or with lime or P, improved growth of both *P. trichocarpa* and a *P. deltoides* x *P. trichocarpa* hybrid in the Pacific Northwest (DeBell et al. 1990). In some poplar studies, total N in the aboveground biomass (but not necessarily in the foliage) was related to clonal productivity and varied widely by clone, crown position (more in the upper canopy), date, and the year of sampling (Heilman 1985, Heilman and Stettler 1986)

Leaching of nitrates and other nutrients into the groundwater can sometimes follow fertilization. However, for one study in Russia, fertilization rates which matched plant needs increased growth and soil nutrients without contaminating the groundwater (Czepinska-Kaminska and Janowska 1991).

Nitrogen-fixing alders included in mixed stands can supply additional N. When *Alnus glutinosa* was interplanted with a hybrid poplar, the poplar exhibited more growth and a higher N content than in pure poplar stands, and whole plantation yields were higher than separate pure plantings (Cote and Camire 1984). When ^{15}N labelled alder leaf litter was incorporated in the soil, poplars recovered 10 to 15% (Kurdali et al. 1990). Black cottonwood with red alders had a higher N content and more growth than poplars grown alone, but the alders were smaller and had lower N content than usual due to shading and competition (Radwan and DeBell 1988, Stettler et al. 1986).

If all weeds were eliminated, N fertilization was not needed for poplars on a moderately fertile site, but an infertile site did require it, especially until crown closure (Hansen et al. 1988). For *Populus maximowiczii* x *P. trichocarpa* over ten years, yearly mowing greatly increased survival and growth of the poplars compared to unmowed plots (Czapowskyj and Safford 1993). However, for a stand of T x D, at the end of the fourth year (McLaughlin et al. 1987) a cover crop became an N source instead of a sink. Nitrogen-fixing trefoil ground cover seemed more effective than native plants at improving growth of fertilized trees and increasing N recovery by the ecosystem. Nitrogen deficiency symptoms can indicate the need for fertilization (Heilman et al., 1989). These symptoms are smaller, uniformly pale green or yellowish leaves, with the lowest leaves affected first. Foliar N concentrations of less than 2.7% in the upper crown in mid-August are another indication of the need for N augmentation.

1.2.3.3.1 Amino Acids

Glutamine is the major N compound in the xylem sap of *P. deltoides* (Dickson et al. 1985, Schneider et al. 1994). In ^{14}C studies (Dickson et al. 1985) glutamine moved from xylem to phloem in the upper stem, then was translocated to developing tissues. It accumulated at the internodes near recently mature leaves. When amino acid fluctuations were followed for a year (Schneider et al. 1994), the highest concentrations were seen at budbreak and leaf expansion. At this time storage proteins were broken down to their amino acid constituents in the ray parenchyma. Arginine appeared to be the storage amino acid. Low temperatures stimulated arginine accumulation in one poplar species: after September it comprised more than 90% of α-ketoglutarate in bark and xylem. At bud break and during the peak growing season, arginine decreased rapidly and glutamine and glutamate increased to 90% of α-ketoglutarate (Sagisaka 1974).

Other labelled amino acids have been supplied in solution to cut stems of *P. deltoides* (Vogelmann et al. 1985). Alanine was taken up most, followed by threonine, then glutamic acid, then aspartic acid. Glutamic acid and aspartic acid went directly into the lamina of mature leaves. Threonine went to the developing leaves or was used in the stem, but little alanine got to the leaves because it was used so quickly. Most labelled amino acids were incorporated into organic acids and proteins. Uptake and distribution of amino acids appeared to be selective in this species.

1.2.3.3.2 Nitrogen Effects on Water Relations

It is important to realize that N fertilization may sometimes be counterproductive on dry sites. The effects of N supply on plant water balance have been described as positive, negative or nonexistent in different experiments. After reviewing the literature, van den Driessche (1984) concluded drought resistance is often decreased by high N levels and unchanged or increased by moderate N, so results could depend on the authors' definitions of high N. Foliar N concentrations are often not reported, and N fertilization levels can be difficult to compare among solution cultures, pot, or field.

Drought greatly offset the positive effects of high N supply on leaf biomass, size, chlorophyll and Rubisco content, N concentration and stem biomass for two contrasting poplar clones (Liu and Dickmann 1992a). Nitrogen fertilization was much less effective on droughted than on well-watered stands of *Pinus radiata* D. Don (Monterey pine) (Raison and Myers 1992), although *Pinus resinosa* Ait. (red pine) has shown a better growth response to N on a dry site after drought hardening (Miller and Timmer 1994). Nitrogen level was inversely related to tropical tree drought resistance characteristics, such as a thicker cuticle and smaller leaves (Kramer and Kozlowski 1979). Nilsen (1995) noted that N fertilization decreased growth of *Picea abies* (L.) Karst. (Norway spruce) on a dry site (citing Spiecker 1987), and might cause imbalances of other nutrients from growth dilution.

Nitrogen effects on plant drought hardiness have been attributed to influences of N on WUE (including stomatal control, possibly mediated by effects on ABA supply), root growth, root:shoot ratio, hydraulic conductivity, osmotic potential, or cell wall elasticity, so these effects will all be reviewed. Inconsistent experimental results could be due to species differences, failure to specify N concentration levels, developmental stages of the plant (Tan and Hogan 1995), or N sources. Degree of water stress and criteria used to determine drought hardiness may both affect the decision as to whether N has a positive or negative effect on plant water status.

1.2.3.3.2.1 Nitrogen and Water Use Efficiency (WUE)

Water use efficiency is often used as a criterion for measuring productivity in drought. It may be calculated as grams CO_2 fixed in photosynthesis divided by grams of water lost in evapotranspiration. Carbon dioxide is taken in, and water lost, through the stomata. In drought, these pores are at least partially closed to prevent water loss (and cavitation). With moderate drought, transpiration is reduced more than photosynthesis by stomatal closure, so WUE is increased, but in severe drought, WUE is decreased. This is analogous to Ohm's Law which states flux = gradient/resistance:

$$Flux = \frac{C_{air} - C_{mesophyll}}{r_{air} + r_{leaf(stoma+mesophyll)}} \tag{1.3}$$

where C is gas concentration and r is resistance (Kramer and Kozlowski 1979). For gas flow through the stomata, the gradient is gas concentration difference between the air and the leaf mesophyll. Resistance is mainly air or boundary layer resistance plus the leaf resistances, mainly stomatal and mesophyll. Mesophyll resistance is large for CO_2, about 6 sec/cm, but close to zero for water. Adding stomatal resistance when the pores close, then, has a relatively bigger effect on water vapour exchange than on CO_2 exchange

(Kramer and Kozlowski 1979). Mesophyll resistance to CO_2 molecules is greater than to water because CO_2 diffuses more slowly (a bigger molecule), and must cross the plasma membrane, the cytoplasm and the chloroplast envelope before assimilation in the chloroplast (Taiz and Zeiger 1991). Resistances to uptake of different carbon isotopes are detectable. A method for measuring long-term WUE by carbon isotope discrimination was proposed by Farquhar et al. in 1982.

Photosynthesis is also decreased by mesophyll dehydration. In severe drought, metabolism is altered enough that photosynthesis decreases more than transpiration, and WUE is decreased. The damage may be partly due to reduced activity of membrane-bound enzymes (Taiz and Zeiger 1991).

Abscisic acid is probably the primary signal mediating the pumping of K out of the guard cells for stomatal closure. ABA is synthesized in both the leaf mesophyll and the root, and there are leaf messenger or root messenger schools of thought (Taiz and Zeiger 1991). Passioura (1988) showed that a chemical signal independent of leaf water potential was sent to the shoot if the soil was dry. When only a portion of a root system was dried, ABA was exported to the leaves and stomata closed even though the plant received ample water and leaf water potentials remained high.

As a leaf messenger, ABA would be translocated from the mesophyll. Normally, the mesophyll chloroplast is more alkaline than the cytosol, so weakly acid ABA is dissociated into H+ and ABA ions which accumulate in the chloroplast. With drought, chloroplast pH is lowered and apoplast pH raised. ABA reassociates and passes through the membrane into the apoplast. Here the transpiration stream carries it to guard cells. Next, ABA synthesis in the mesophyll increases to maintain the stomatal closure. Experimentally, leaf ABA and stomatal opening can be achieved if leaves but not roots are desiccated (Taiz and Zeiger 1991).

Nitrogen has been shown to increase WUE in *Pinus* species (Guehl et al. 1995, Brix and Mitchell 1986, Sheriff et al. 1986), decrease it in cotton (Radin and Parker 1979), or have no effect on well-watered Douglas-fir (Mitchell and Hinckley 1993). In the last case, however, foliar N concentrations were only 1.25% at low N and 1.58% at high N, levels which are barely adequate (van den Driessche 1992) to adequate (Marschner 1986). These seeming contradictions may be genetic, but some plants can have differing WUE under different degrees of stress (Mitchell and Hinckley 1993). Hybrid poplars (*Populus x euramericana* and *P. tristis* x *P. balsamifera*), for example, had low WUE in drought, intermediate in flood, and high WUE with no stress (Liu and Dickmann 1993). Water use efficiency of American elm (*Ulnus americana* L.) increased with moderate drought but decreased with severe drought (Reich et al. 1989). Douglas-fir and lodgepole pine (*Pinus contorta* Dougl.) both had greater WUE on dry than well-watered soil, with large genotype differences in Douglas-fir WUE (Smit and van den Driessche 1992).

Nitrogen can also have different effects at different levels of water stress. Excess N increased WUE of poplars under normal conditions, somewhat in flooding, but decreased WUE in moderate drought, although speeding recovery. High N increased ABA in drought for these clones, causing stomatal closure (Liu and Dickmann 1992b), but for *P. trichocarpa*, ABA increased with drought without stomatal closure, perhaps due to lack of extensibility of guard cells or lack of ABA during leaf development (Schulte and Hinckley 1987). However, for some species, low rather than high N stimulates ABA production (Marschner 1986). In American elm, unlike the poplar, high N increased WUE at any drought level. However, only high N trees reached the most severe drought levels (-2.2 MPa), making comparison difficult with low N plants (Reich et al. 1989).

Water use efficiency may not always be a good indicator of drought resistance. In competition for limited water, stomatal closure decreases growth, and water-consuming competitors will take all the available moisture. For instance, drought tolerant *Artemisia tridentata* Nutt. did not have high WUE, while *Pinus ponderosa* Laws., which has a higher WUE, did not compete as well (DeLucia and Heckathorn 1989). Lodgepole pine had lower WUE and greater productivity than Douglas-fir, because efficient root water uptake was more critical than WUE to growth in drought (Smit and van den Driessche 1992). Sugarcane varieties that grow best in drought endure lower water potentials and delay stomatal closure until the point of runaway cavitation (Neufeld et al. 1992).

Stomatal control and hydraulic conductivity, both potentially improved by increasing

N level, are both positively correlated with water potential, but better stomatal control will decrease water use while greater hydraulic conductivity will increase it (Oren and Sheriff 1995). Again, there are conflicting reports on N effects on stomata and conductivity. Foliar N concentration did not affect stomatal control of Douglas-fir (Mitchell and Hinckley 1993), but rather increased mesophyll conductance and Rubisco content without altering WUE. Stomatal control at high N levels improved in Scots pine (*Pinus sylvestris* L.) (although less so at the most negative water potentials of -1.4 MPa) (Hillerdal-Hagströmer et al. 1982), but decreased along with drought resistance in cotton (also to -1.4 MPa) (Radin and Parker 1979). High N levels led to late summer stomatal closure in droughted birch, followed by early leaf loss (Wendler and Millard 1996).

Low N sometimes reduces root hydraulic conductivity of nonconiferous species (Oren and Sheriff 1995). However, N level had no influence on root hydraulic conductivity per unit root surface area of two oak species (Steudle and Meshcheryakov 1996).

Xylem water potential at a given degree of drought may be used to indicate drought resistance, and is related to WUE. Nitrogen had varied effects on water potential, perhaps due to different drought-induced influences of N level on ABA, and thus on stomatal control. It increased predawn water potential of *Pinus radiata* with ample water (Myers and Talsma 1992) and at a given water content (Raison and Myers 1992). Water potential of Douglas-fir decreased at high N in two experiments (Brix 1972, Nilsen 1995). However, N had no effect on predawn leaf water potentials of birch (*Betula pendula* Roth.) (Wendler and Millard 1996) or carob (*Ceratonia siliqua* L.) (Correia and Martins-Loucao 1995). Sometimes N fertilization speeds recovery of normal water potentials after drought (Myers and Talsma 1992).

1.2.3.3.2.2 Nitrogen and Root:Shoot Ratio

Root growth patterns alter at different levels of N. Nitrogen increases rooting density when applied with P, but at high N, extension of the main root axis is inhibited and lateral root growth increased. This may be due to more ABA or more IAA (Marschner 1986). High N in the nursery doubled root growth capacity of Douglas-fir, lodgepole pine and white spruce (van den Driessche 1992). Nitrogen and irrigation increased fine roots and root branching in a mixed hardwood forest (Pregitzer et al. 1993). However, fertilization of Scots pine reduced fine root tips to a third, perhaps due to less mycorrhizae (Oren and Sheriff 1995), and droughted Norway spruce (*Picea abies* (L.) Karst.) had fewer living small roots with N fertilization (Clemensson-Lindell and Asp 1995).

Root:shoot ratios have more effect on water balance than leaf area alone (Kramer and Kozlowski 1979). Roots of droughted trees tend to grow more than shoots, increasing the root:shoot ratios (Ferrier and Alexander 1991). On the other hand, N often increases leaf area more than root area (Marschner 1986, Imo and Timmer 1992), leading to less water uptake per unit transpiring surface, which can add to water stress (Oren and Sheriff 1995). For instance, high N decreased root:shoot ratios of Sitka spruce (Ferrier and Alexander 1991) and Norway spruce (Nilsen 1995), which led to increased transpiration and decreased water potentials. High N (2.5%) birch could not withstand drought as well as at "low" N (1.8%), suffering midsummer stomatal closure and leaf abscission, even though there was no difference in predawn leaf water potential or osmotic adjustment between N levels (perhaps because the "low" N was not low, but moderate). There was increased transpirational loss of water from the larger high N canopy (Wendler and Millard 1996). Sugar maple (*Acer saccharum* Marsh.) seedlings also experienced greater water stress with large leaf areas than when partly defoliated (Kramer and Kozlowski 1979), but Brix and Mitchell (1986) found that N increased Douglas-fir leaf area 50% without increasing water stress.

1.2.3.3.2.3 Nitrogen and Osmotic Potential

At full turgor, N did not affect osmotic potential of jack pine (*Pinus banksiana* Lamb.). However, low N plants were more successful at maintaining turgor, mainly due to increased cell wall elasticity (Tan and Hogan 1995). This seems to counter the hypothesis of Radin and Parker (1979) that low N cotton, with increased cell wall material, performed better in drought due to more rigid cell walls. Low N plants tend to have increased concentrations of the osmoticum, K, so might sometimes have improved osmotic potentials.

1.2.3.4 Phosphorus

After N, P is the nutrient most limiting to plant growth, and P deficiencies are often encountered in alkaline soils where they decrease protein metabolism and auxin synthesis (Clancy et al. 1995). Phosphorus application often increases productivity of poplars on dry sites more than on wet sites (DeBell et al. 1990). Phosphorus is an essential macronutrient, needed for sugar phosphates, nucleic acids, nucleotides, coenzymes, phospholipids and ATP (Taiz and Zeiger 1991). Critical foliar nutrient levels (below which growth may be severely restricted) of P for poplar may be from 0.17 to 0.57% (McLennan 1996). Moderate to high amounts of P increased growth on dry sites, but not with heavy irrigation (DeBell et al. 1990). In Britain, few soils which are suitable for poplars need N fertilization, but soils are sometimes short of phosphate (Tabbush 1993).

Populus balsamifera achieved three times more growth with high compared to low P in Alaskan taiga seedlings (Chapin et al. 1983). *Populus tremuloides* had 1.6 times the growth at high P, while alders and conifers did not respond to P. This meant that P level determined succession, because on nutrient poor sites the alder and conifers grew just as fast as the poplar. Root to leaf ratios were not affected by P on the taiga.

Zero P decreased the biomass of roots and shoots, and low P decreased K in roots and leaves of *P. maximowiczii* (Houman et al. 1991). Phosphorus affects polyamide levels: zero P decreased spermine in leaves and roots (when K was low); increased putrescine in leaves and decreased it in roots, and in roots spermidine decreased with decreasing P.

1.2.3.4.1 Excess Phosphorus

Negative effects of P fertilization on poplars are sometimes evident. Excess P caused zinc (Zn) and copper (Cu) deficiencies, depending on the ability of each clone to maintain P:Zn and P:Cu balance (Teng and Timmer 1990a). At low P supply, lack of Zn was the primary deficiency with a secondary Cu deficiency, but at higher P level, Zn and Cu were equally limiting. Healthy growth was achieved at P:Cu ratios below 800 and P:Zn ratios below 70 (Teng and Timmer 1990b). The symptom of P-induced Zn deficiency was rosetting of small leaves at the ends of dwarfed shoots (probably because gibberellic acid synthesis is reduced at low Zn supply) (Teng and Timmer 1993). A P:sulfur antagonism in poor soil was also demonstrated in Italy (Liani 1967).

1.2.3.4.2 Phosphorus Effects on Water Relations

Since drought increases the need for P, part of the positive effect of P on growth in drought would be relief of drought-induced deficiencies. Moderate to high amounts of P increased cottonwood growth on dry sites (DeBell et al. 1990). Sheriff et al. (1986) also found P increased water use efficiency of *Pinus radiata*. Hybrid poplars have an optimum concentration of foliar P beyond which there is no further growth improvement, but the optimum is higher in dry conditions (van den Driessche, pers. comm.). This indicates an effect on drought resistance beyond relief of deficiencies. A flood-induced moisture stress and nutrient imbalance in loblolly pine was decreased by P fertilization (Hook et al. 1983). Phosphorus (and vesicular arbuscular mycorrhizae or VAM) increased growth of drought sensitive (*Leucaena leucocephala* (Lam.) de Wit) but not drought resistant (*Acacia nilotica* (L.) Willd.) arid tropical plants (Michelsen and Rosendahl 1990).

Many plants adapt to P deficiencies by either symbiotic relationships with mycorrhizae to increase P uptake, more efficient extraction or utilization of P, or by reduced growth (Marschner 1986). Phosphorus is usually described as having either a positive or neutral effect on water relations, and positive effects are attributed to improved root growth and hydraulic conductivity.

1.2.3.4.2.1 Phosphorus, Root Growth and Conductivity

Effects of P on root growth are variable. High levels of P, unlike N, may increase root growth more than shoot growth, increasing the root:shoot ratios and resulting in more water uptake per unit transpiring surface (Clancy et al. 1995). However, Marschner (1986) reports the opposite: P deficiency increases root:shoot ratios. The longer the P deficiency lasts, the greater the proportionate increase in root dry weight, root length, and fine roots, although rooting density may decrease. *Phaseolus vulgaris* L. roots branched more when P supply was low, and genotypes efficient at P uptake were inherently well-branched (Lynch and Beebe 1995).

There is more agreement on the effect of P on root conductivity, with many studies showing that greater P concentrations increase hydraulic conductivity (Radin and Eidenbock 1984, Skinner and Radin 1994). Radin and Matthews (1989) suggested three possible reasons for this increase: alterations in root morphology or in conductivity of root cortex cells, or increased root apoplastic bypass flow. However, in 1994 Skinner and Radin discovered that bypass flow was decreased only slightly by P deficiency, and could not explain how P affects root conductivity. Phosphorus may have protected the root plasma membrane structure of *Pinus taeda* L. (loblolly pine), improving absorption (Hook et al. 1983).

Although the reason for depression of root conductivity at low P remains obscure, the result of this decrease was inhibition of leaf cell expansion of cotton during the day (Radin and Eidenbock 1984). Phosphorus deficiency also reduces leaf cell division as well as respiration and photosynthesis (Marschner 1986). Some authors believe the effect of P on conductivity may be indirect, mediated by decreases in leaf weight ratios (leaf dry weight/total plant dry weight), as for cotton (Skinner and Radin 1994) and green ash (*Fraxinus pennsylvanica* Marsh.) (Andersen et al. 1989).

1.2.3.4.2.2 Phosphorus and Stomatal Conductance

Photosynthesis and stomatal conductance of Douglas-fir seedlings decreased slightly at high P, but many conflicting reports may mean P has little direct effect on stomatal control (Dosskey et al. 1993). The tendency of P to increase K uptake might lead to an indirect enhancement of stomatal control via K effects on the guard cells.

1.2.3.4.2.3 Phosphorus and Mycorrhizae

Although poplars are considered selective toward mycorrhizae, they can host endotrophic, ectotrophic or occasionally ectendotrophic infections (Shultz et al. 1983). At least eight poplar clones are autotrophic (no mycorrhizae), even when soil P is low. Eugenie and Tristis clones exposed to ecto- and endomycorrhizae showed no height growth differences the first year, but stem diameters were greater with mycorrhizae than without.

Ectotrophic infection of *P. deltoides* was greatest in very moist but not waterlogged conditions, and almost nonexistent in very dry soils, with less in the top soil horizon (Lodge 1984). Endotrophes exceeded ectotrophes at most levels, but were least at the driest and wettest sites. In the field, there were less endomycorrhizae where ectomycorrhizae were present, perhaps a competitive interaction. In another report, *P. trichocarpa* was ectotrophic and *P. deltoides* ectotrophic, endotrophic and autotrophic (Shultz et al. 1983). The bulk of recent literature on P and water relations deals with mycorrhizae. Low soil P usually increases mycorrhizal infection, although in one experiment VAM was not correlated with P supply to *Thuja, Sequoia, Calocedrus* or *Sequoiadendron* species (Kough et al. 1985). A deficiency of P (or K) increases root exudation of reducing sugars and amino acids, which encourage mycorrhizal development (Marschner 1986).

Some researchers claim that mycorrhizal improvements in plant water status are due solely to increases in tissue P concentration, which in turn may increase root hydraulic conductivity, as in *Pseudotsuga menziesii* (Coleman et al. 1990), *Picea sitchensis* (Lehto 1992), and *Fraxinus pennsylvanica* (Andersen et al. 1988). Others believe increased absorptive area entirely explains mycorrhizal enhancement of drought resistance, as in *Pinus radiata* (Reid and Bowen 1979), and loblolly pine (Sands et al. 1982). VAM has little effect on P uptake for species with highly branched roots and long root hairs (Marschner 1986). However, many trees, including *Pinus*, do not have extensive root branching. Root length and root number of *Pinus taeda* seedlings were only 7.5% compared to *Cornus florida* L. (flowering dogwood) of the same age (Kramer and Kozlowski 1979). Pine would benefit more from mycorrhizal increases in absorptive surface areas than poplars, which have many fine roots.

Some ectomycorrhizae can also make organic N, which could not normally be assimilated, available to the host plant. This is important, since coniferous forest soils may have 250 times more organic than inorganic N in the litter layer (Trofymow and van den Driessche 1991).

1.2.3.5 Potassium

Potassium is another essential macronutrient which is a cofactor to more than forty enzymes, helps control stomatal opening and closing, and balances the electric charge at cell membranes (Taiz and Zeiger 1991). The poplar genus has a greater requirement for K (higher concentrations) than *Platanus, Alnus, Betula* or *Fraxinus* species (sycamore, alder, river birch or ash) in Kentucky (Wittwer and Immel 1980). Critical foliar levels may be 1.2 to 2.64% (McLennan 1996) in poplars. The fresh weight of shoots and roots of *P. maximowiczii* was much less without K. For *P. deltoides* and its hybrids, K deficiency correlates with *Melampsora* rust susceptibility, whereas poplars deficient in N and P were more resistant to the rust (Suzuki 1973). Normally K is the main cation in the cell, so if K is deficient, the plant may produce other cations such as polyamines to maintain the ionic balance (Houman et al. 1991). The polyamines putrescine, spermine, spermidine and agmatine are important in controlling plant growth and reaction to stress. Low K supply greatly increased putrescine levels in the root and shoot. Low K in combination with zero P decreased spermine in leaves and roots, but spermidine was negatively correlated with K in leaves.

Wood anatomy of poplar is affected by K. At low K levels, *P. deltoides* had shorter, narrower fibres, narrower annual rings in the lower stems, and narrow vessel elements in the upper stem (Cutter and Murphey 1978). Longest fibre length was at 17 ppm N and 53 ppm K. For *P. maximowiczii* x *P. berolinensis,* wood characteristics including percent of wood and pith (but not bark) varied erratically with K level. There was higher specific gravity with low K, and fibres were longest at 40 ppm K. Vessel cell walls were thickest at 78 ppm K, and maximum ray spacing and minimum vessel lengths were observed at 90 ppm K.

1.2.3.5.1 Excess Potassium

Adverse effects, or at best lack of response, sometimes follow addition of K. Mean stem height, weight and diameter were greatest at two to 50 ppm K for *P. maximowiczii* x *P. berolinensis* grown in solution (Cutter and Murphey 1978). There was a decrease in growth at 100 and 150 ppm K, with seedlings ceasing to grow after six weeks at 150 ppm. *Populus trichocarpa* and a D x T hybrid showed no effect from added K the first year, and growth decreased the second year (DeBell et al. 1990).

When *P. trichocarpa* seedlings were supplied with a range of nutrients, K content of all parts increased with K supply (with greatest root increases in the fine roots, and most leaf increases in the older leaves). A growth response occurred only in the more productive clone (Diem and Godbold 1993). There was an antagonism between K and both calcium (Ca) and magnesium (Mg) ions, with an inverse relationship between contents of K and of Ca and Mg. Potassium and Mg have similar cell functions, and are at high concentration in the cytoplasm. High Ca concentrations might precipitate inorganic P. In the vacuoles, K+Ca+Mg totals are usually similar in quantity, but not charge. If Ca^{++}+Mg^{++} levels were higher, and K^{+} lower, the vacuole would be more positively charged. This would upset the electrochemical balance across the vacuole/cytoplasm membrane, unless cationic polyamines like putrescine, which may increase with K deficiency, equilibrate charges. Regulation of K, Mg and Ca in the leaves is likely by selective loading and transport in the roots, not competition for uptake.

Thus, there may be Ca and Mg deficiencies if K is in excess, and Mg and Ca requirements were higher for a pure stand of *P. deltoides* than for a mixed forest (Nelson et al. 1987). At less than 50 µmol of Mg per gram leaf dry weight, the photosynthetic rate of *P. euramericana* was decreased (Dorenstouter et al. 1985).

1.2.3.5.2 Potassium Effects on Water Relations

Low K levels can decrease drought resistance of pine, although not spruce (Christersson 1976), and lead to loss of turgor and wilting with drought (Marschner 1986). Optimal K concentrations differ between normal and drought conditions, with six times more K sometimes needed to improve photosynthesis in severe drought than with ample water (Pier and Berkowitz 1987). As mentioned previously, K uptake is sometimes decreased by drought as well.

Several mechanisms have been suggested for K increasing drought hardiness, such as improved stomatal control (1.2.3.1.2.1) or osmotic potential in leaf or root (Marschner 1986). Faba beans had more than 20 times as much K per stoma and nearly doubled guard cell volume and osmotic pressure when stomata were open compared to closed (Humble and Raschke 1971).

1.2.3.5.2.1 Potassium and Stomatal Control

Increased stomatal control with increased K has been described in a review as improving drought hardiness by either increasing or decreasing transpiration (Christersson 1976). For instance, correlation between foliar K concentration and transpiration was negative in Sitka spruce (Bradbury and Malcolm 1977) but positive in Scots pine and Norway spruce (Christersson 1976). Previously, Christersson (1973) had reported an inverse relationship between K and transpiration for drought-hardened but not for unhardened Scots pine seedings. Therefore, conflicting results might be due to degree of hardening of seedlings, or Ca antagonism (Christersson 1976). Perhaps K (and P, which often increases with K) effects of decreasing transpiration and increasing WUE of Sitka spruce are physiological, not morphological, because changes are too rapid after addition of these nutrients to allow physical alteration (Bradbury and Malcolm 1977). Van den Driessche (1984) found both high and very low K decreased transpiration except in dormant seedlings, a quadratic effect which could explain some contradictions.

1.2.3.5.2.2 Potassium and Osmotic Potential

In spite of the prominent role generally assigned to stomatal control via K in drought resistance, several studies have found non-stomatal K-induced improvements in drought hardiness. For instance, K did not change the water potential at which stomata closed; instead it increased cytoplasmic desiccation tolerance (Pier and Berkowitz 1987).

Potassium accumulated outside the chloroplast and could be exchanged for stromal H^+ with water stress, maintaining stromal pH and restoring photosynthesis even at lower water potentials.

Potassium is one of the main osmotica in plants, leading also to higher levels of proline, and thus maintaining tissue water levels in drought (Marschner 1986). Osmotic adjustment is usually in the leaf in poplars, but one hybrid showed adjustment in the fine root (Tschaplinski and Tuscan 1994). High osmotic potential in the root stele is needed for "turgor-pressure-driven solute transport" in the xylem, and thus maintenance of plant water balance (Marschner 1986).

1.2.3.6 Poplar Rooting Habit

The roots of two T x D hybrids had a wide horizontal spread (Friend et al. 1991). Extent and degree of branching varied by clone, with coarse roots more concentrated at the surface than fine roots. Total root biomass was about six megagrams per hectare. Of this, 31% was the original cutting, 26% coarse roots, 35% fine roots, and 8% in small roots. Special features of *Populus* include vertical sinker roots, fine roots which are long and thin and can grow 10 mm per day, and both strong genetic control of root growth and plasticity in response to environmental change (Pregitzer and Friend 1996). Unfertilized *Populus* exhibited long lateral roots, whereas the same clone with NPK fertilization had shorter lateral roots and more root hairs (Pantos-Derimova 1977).

1.2.3.7 Poplar Nutrient Sampling Protocol

Foliar elements of *P. trichocarpa* which can be translocated at senescence, including the macronutrients, were in higher concentrations in the upper canopy (McLennan 1990). To attain statistically significant results, the less variable elements (N, P, K, S, Mg, and Ca) required at least 15 to 20 foliar samples from recently matured leaves on lateral branches in the top third of dominant trees. For the more variable elements (SO_4 sulphur, Cu, Zn, Mn, B and Fe), 21 to 56 samples were needed for allowable errors of 10% and α significance of 0.95, with $\beta = 0.95$ at the higher sample numbers. Composite samples required more replications. Heilman (1985) suggested similar sample sizes for measuring N in *Populus deltoides* (23 to 55 trees, or six to 37 if no first year trees are included).

1.2.3.8 Steady State Requirements

Poplars are known to have unusually large nutrient requirements to achieve their full growth potential, but this is only true when a plantation is first established or for very short rotations (Nelson et al. 1987). Vogelmann et al. (1985) concluded that *P. deltoides* has an "efficient uptake and transfer system." In a mature poplar stand, nutrients are recycled via the litter and retranslocation from senescing leaves.

In the seventh year, a plantation of *P. deltoides* was at 60% of carrying capacity (maximum potential productivity for the site) but approaching steady-state levels of nutrients in the canopy, and only needed to replace nutrients used in annual wood production (Nelson et al. 1987). Most nutrients were then obtained from nutrient cycling, whether biochemical (i.e., internal retranslocation) or biogeochemical (i.e., weathering and decomposition). Less than half the N (which was most limiting) would have to be supplied by fertilizer or soil organic matter mineralization. Turnover rate was fastest for K and slowest for N. Per unit biomass produced, N and P requirements were about equal to an average mixed forest, but K, Ca and Mg needs were higher. Efficiency of use was in this order: P>Mg>K>N>Ca. Soil nutrient requirements were considered relatively modest at this point, so although short rotation poplar plantations often place heavy demands on the soil, long rotation stands may not.

1.2.3.9 Retranslocation

1.2.3.9.1 Fall Retranslocation

Fall resorption of nutrients from senescing leaves is a significant nutrient-conservation tactic, and affects productivity (Killingbeck 1996). Abscisic acid initiates the senescence process, probably by stimulating ethylene production in the leaves. Ethylene is the main regulator of leaf senescence and abscission (aging and loss).

Normally, auxins produced in the apex inhibit encoding of genes for hydrolytic enzymes. Ethylene causes a breakdown of auxins, so hydrolytic enzymes are produced and break down proteins, nucleic acids and chlorophyll. These breakdown products are retranslocated out of the leaves via the phloem. Eventually, specialized cells in the petiole called the abscission layer are also hydrolyzed, and the leaf falls off, but normally not before many nutrients have been recovered (Taiz and Zeiger 1991).

Poplars generally have high levels of canopy N, efficiently retranslocated and stored in the fall (Pregitzer et al. 1990). Leaves of two hybrid poplar clones contained 75% of the total tree N in August. After leaf fall, 70% to 80% of the tree N was stored in large roots. Mean resorption levels of *P. tremuloides* were 43% for N, 51% for P and 10% for copper (Cu), although older stems resorbed less N and Cu. Aluminum, boron, calcium, iron, magnesium, manganese and zinc were not resorbed. Premature leaf loss reduced retranslocation. (Killingbeck et al. 1990). *Populus deltoides* mobilized 23% of leaf P to the bark (Cote and Dawson 1991).

Resorption proficiency refers to terminal foliar nutrient content, and highly proficient trees have final concentrations of only 0.7% N and 0.05% P (0.067% P in deciduous trees, and 0.045% P in evergreens, with equal proficiency in N resorption). Resorption may not be possible below 0.3% foliar N and 0.01% P (about 20 and one $\mu g/cm^2$) (Killingbeck 1996). Resorption proficiency may be more meaningful than resorption efficiency, because the latter may increase with nutrient availability and decrease if nutrients are resorbed during drought before fall (both change the apparent maximum nutrient level). Both are useful concepts, since efficiency shows relative conservation strategies, while proficiency shows how selection has reduced waste of nutrients. When measuring resorption, it is usually better to look at changes per unit leaf area instead of in concentration, especially for comparisons at different times (Killingbeck 1996).

Resorbed nutrients may be stored in the root, bark, branches and wood, and (supplemented by fall and winter root uptake) provide a nutrient pool for periods of heavy demand such as bud break and leaf expansion, (van den Driessche 1984). More soluble N was found in the inner bark and more arginine in roots of Douglas-fir (van den

Driessche and Webber 1977). Starch and sugar are stored in poplar roots, including long-lived fine roots, and the original cutting also serves as a storage taproot, but N and water treatment had no effect on these reserves in one study (Nguyen et al. 1990). In the Pacific Northwest, softwoods can supply more of their nutrient needs by retranslocation from old to new tissue than hardwoods, giving them an advantage on nutrient poor sites (van den Driessche 1984). A *Pinus radiata* stand was still retranslocating applied N 12 years later (Crane and Banks 1992). Hardwoods often withdraw N, K, Mg, Fe, S and sometimes P, but not Ca and Na (van den Driessche 1984).

Storage proteins have attracted considerable interest recently, as they are likely the sinks for N retranslocated from senescent leaves (Coleman et al. 1994). *Populus deltoides* accumulated a 32-kilodalton (kD) bark storage protein (BSP) in vacuoles of the phloem parenchyma and xylem ray cells of the inner bark in autumn and winter. BSPs were associated with short day photoperiod, and quantities were influenced by N availability. In *P. euramericana*, protein bodies were more numerous as 32 kD proteins increased (Sauter and van Cleve 1990). Both 32 kD and 36 kD proteins were induced in short days in three poplar hybrids (Langheinrich and Tischner 1991), probably derived from Rubisco, light harvesting proteins, and chlorophyll.

There is also a great deal of genetic variation in the short-day requirement to reach maximum storage protein content: from 24 days to nine weeks (Langheinrich 1993). Critical photoperiod for growth cessation usually increases with latitude and elevation of place of origin of the clone, and is associated with the photoperiod needed to produce storage proteins. Perhaps the amount of BSP in autumn could be one selection criterion for efficient clones. Storage proteins appear to be encoded by a multigene family of about five genes (Langheinrich 1993). Bark storage proteins are similar to the gene product of win4, a wound inducible gene not associated with short days. Win4's products may control accumulation and translocation of leaf N (Coleman et al. 1994).

1.2.3.9.2 Drought Retranslocation

If N is retranslocated during drought from the leaf to the stem and root, efficiency of retranslocation might improve productivity of some clones under dry conditions, and could be a screening criterion as well. For barley, nutrient resorption efficiency (g m^2 resorbed / g m^2 maximum) was greater at low than at high nutrient availability, and decreased with drought (Pugnaire and Chapin 1992). For perennial C_4 grasses, N was retranslocated in drought (mainly to roots), adversely affecting photosynthesis after drought was relieved (Heckathorn and DeLucia 1994). There must be a balance between photosynthetic losses and N conservation: perhaps fast growing species which retain their leaves at low water potentials can then return the retranslocated nutrients from the stem to the leaves with rewatering. Nitrogen was retranslocated in the fall for some poplar clones, especially in a more productive clone (Pregitzer et al. 1990).

For an understorey shrub *Viburnum acerifolium* L., P was always and N sometimes resorbed less in drought than in fall, so more nutrients were lost when leaves dropped prematurely due to dry conditions (Minoletti and Boerner 1994). Poplar clones which react to drought by abscising leaves may be more productive if they retranslocate their nutrients to the stem and root before abscission.

1.2.3.10 Summary of Nutrient Effects on Drought Resistance

Nutrient effects on plant water relations may vary with species, plant part, stages of development, concentration of the nutrient, degree of water deficit, and other factors such as nutrient interactions. Nitrogen influences on water balance are controversial. Nitrogen often increases WUE and recovery from drought, but may decrease drought tolerance and survival. Phosphorus and K probably tend to increase drought resistance of many sensitive trees, especially in the absence of mycorrhizal infection. Some mycorrhizae may increase drought hardiness of vulnerable species by improving N, P and K nutrition or by increasing root absorptive surfaces. Resorbed nutrients from senescent foliage also play an integral role in a tree's nutrient balance. Research is still needed on the physiological explanations for many of these nutrient effects.

With the increasing interest in improving forest tree growth to shorten rotation time, it is important to understand the effects of nutrients on plant water relations before investing in fertilizers, and possibly negatively affecting the environment. Optimum nutrient concentrations should not be exceeded, and in any case, N fertilization could be detrimental on dry sites. The research contained in this dissertation should contribute to this understanding.

1.2.4 Characteristics of Drought Resistant Poplar

A summary of suggested indicators of either drought-stress resistance or WUE (or both) reveals many inconsistencies and clonal differences. A selection criterion that works for one group of poplar clones does not necessarily hold true for another group. Indicators of drought tolerance and WUE which various researchers have proposed are:

1) decreased transpiration (Blake et al. 1984, Farmer 1969),
2) high stomatal resistance, either abaxial resistance for WUE under normal conditions (Blake et al. 1984) or adaxial to indicate drought resistance (Kelliher et al. 1980),
3) low total stomatal resistance under normal conditions (Tschaplinski et al. 1994) or during drought for another set of clones (Kelliher and Tauer 1980),
4) stomatal opening at lower light levels in the morning under well-watered conditions (Blake et al. 1984),
5) high numbers of adaxial stomata (Magnussen 1985),
6) high total stomatal number (Ceulemans et al. 1988),
7) increased productivity as an indicator of WUE (Blake et al. 1984) and drought resistance (Tschaplinski and Blake 1989a) in combination with other factors,
8) high root:shoot dry weight ratios under well-watered conditions to indicate WUE (Blake et al. 1984) and in drought to indicate drought-stress tolerance (Magnussen 1985),
9) low leaf area:root dry weight ratios, especially in drought (Tschaplinski and Blake 1989a),
10) leaf shedding in drought (Blake et al. 1984),
11) leaf growth cessation at lower stomatal resistances (Kelliher and Tauer 1980),
12) increased specific leaf area, which is *leaf area to leaf weight* ratio (Magnussen 1985),
13) increased *leaf weight* to *leaf area* ratios (Furukawa 1972),

14) high ratios of mesophyll cell surface to leaf area (Bassman and Zwier 1991),
15) osmotic adjustment (solute accumulation to lower osmotic potential) (Gebre and Kuhns 1993, 1991), including high malic acid levels (Tschaplinski and Tuskan 1994),
16) maintenance of cell wall extensibility with drought (Roden et al. 1990),
17) decreased electrolyte leakage with drought (Gebre and Kuhns 1991) and
18) decreased chlorophyll fluorescence quenching (Havaux et al. 1988).

This list will be added to, and in some cases contradicted by, the findings in Chapter 6 on characteristics of drought resistant clones.

1.3 OUTLINE

The next five chapters will summarize the results of three years of research on poplar drought resistance. Chapter 2 contains recently published experimental results (Harvey and van den Driessche 1997) on the effects of N, P and drought resistance on cavitation in hybrid poplars. Moderate to high levels of N interacted with low and high P supply in four commercially important clones. To our knowledge, this is the first study of nutrient effects on cavitation, and we found that in our greenhouse sub-irrigation culture experiment, high levels of N increased cavitation but the high P supply could counteract this effect. There was more variation in the field, but some results were confirmed. Scanning electron microscopy revealed that P may have decreased cavitation by decreasing pore size in the xylem intervessel pit membranes, increasing the tension needed for inspiration of embolisms into vessels. Drought resistant clones also had less damaged pit membranes. Nitrogen and P effects may have been indirect, caused by their effects on K concentration, with a higher N supply decreasing K concentration, but a higher P supply increasing it in most clones.

Chapter 3 explores the effects of preconditioning, N (at low to adequate concentrations) and K on cavitation in poplars. In a greenhouse pot experiment, increasing foliar N concentrations from low to moderate levels led to a highly significant increase in cavitation. Of three levels of K supply, the moderate level increased cavitation compared to no addition or ten times as much. Nitrogen effects on cavitation were more fully explained both because N was more limiting and because more measurements were made. The controversial question of whether cavitation is reversible in poplars was tested, and a mid-season cycle of drought showed that for one clone, cavitation may have been partially reversible.

Chapter 4 concentrates on the effects of mineral nutrition on other parameters of drought resistance of poplars: water potential, gas exchange (including water use efficiency), osmotic potential and preconditioning. This led to new insights into the advantages and disadvantage of fertilizing poplars on dry plantations.

Chapter 5 examines another aspect of drought resistance and productivity, the ability of trees to mobilize nutrients from the leaves into the stems and roots for storage before leaf loss from drought stress or autumn abscission. In one set of trees, drought was slow, and in another, extremely fast. Nutrient mobilization responses were quite different in these two situations, being more related to autumn retranslocation with slow drought, while nutrients accumulated as part of the drought hardening process in the rapid drought. Clonal differences in drought and fall nutrient retranslocation are interesting, and help explain their productivity on dry and wet sites.

Chapter 6 attempts to find selection characteristics for clones which will be productive on dry sites. Poplar clones were ranked by field performance in drought by Cees van Oosten of MacMillan Bloedel's poplar division, so we could contrast characteristics of drought resistant and drought sensitive clones. Gas exchange (with water use efficiency), osmotic adjustment, and stomatal anatomy were considered, and other aspects such as vessel anatomy, cavitation and retranslocation are drawn in from earlier chapters. We hope this chapter will help plantation managers and poplar breeders as they search for the "ideal" clone for each site.

This dissertation should contribute to the body of knowledge needed to make informed decisions on poplar plantation site selection, suitable clones for each site, and fertilization prescriptions. It should also increase our understanding of factors affecting growth, and their effects on cavitation and drought resistance.

Chapter 2

MINERAL NUTRITION AND CAVITATION IN HYBRID POPLAR I: EFFECTS OF MODERATE TO HIGH NITROGEN, PHOSPHORUS AND DROUGHT RESISTANCE [1]

2.1 INTRODUCTION

Many 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, 1994b), and threatens survival more than leaf dehydration intolerance (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 N (National Poplar Commission 1987, DeBell et al. 1990, Heilman and Fu-Guang 1993) and P (DeBell et al. 1990) and probably other nutrients, but little is known about nutrient effects on drought hardiness, including cavitation resistance.

This 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 bigger 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 are studies on the impact of fertilization on drought resistance. The effects of high concentrations of N on drought resistance of poplars 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 1992a, 1992b). Phosphorus concentration showed a positive correlation with root hydraulic conductivity in *Fraxinus pennsylvanica* (Andersen et al. 1989), indicating that P nutrition

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[1] This chapter is essentially the same as Harvey and van den Driessche 1997, except for field data.

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 poplars on sites subject to seasonal water deficits. 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.

2.2 MATERIALS AND METHODS

2.2.1 Plant Material, Nutrient Treatments, and Experimental Design

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

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 Hg vapor lighting provided a 16-h photoperiod. During the experiment, air 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.

2.2.2 Growth and Nutrient Content

Cutting diameters at planting were 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 micro nutrients 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, Thernco Instruments (Canada) Inc., Mississauga, Canada). Nitrogen and S contents were determined by combustion elemental analysis (Fisons NA-1500 Analyzer, Iso-Mass Scientific Inc., Calgary, Canada).

2.2.3 Cavitation

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 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 bringing 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). A stem segment was cut from near the base of a tree for cavitation measurement. Then the top of each tree was dried on the laboratory bench to wilting or for 18 hours, whichever came first, and a second stem segment removed and tested. 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 de-aerated 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 (Mettler PJ300) 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):

$$K_s = F*L*\eta / (A*dP), \qquad (2.1)$$

where F is flow rate (kg s^{-1}), L is length (m), A is cross sectional area of conducting xylem (m^2), η adjusts for viscosity (Weast 1976), and dP is pressure difference (MPa) between the menisci at the top of the water column and at outflow.

. Xylem area for the equation was determined by colour 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:

$$PLC = 100\ (K_{max}-K_{initial}) / K_{max}, \qquad (2.2)$$

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 2.1).

The second method of measuring embolisms involved cutting 7-cm stem segments adjoining those tested by water conduction, and perfusing with 0.1% safranin dye (also 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 embolisms (Zimmermann 1978, Sperry et al. 1988, Sellin 1991). Permanent slides were made and photographed for anatomical comparisons.

2.2.4 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 13000x or greater.

Pore size relationships to critical ψ_{xp} at which cavitation might occur were calculated according to the capillary equation (equation 1.1) where ψ_{xp} is now pressure (MPa) needed to displace an air-water interface from a circular pore and d is pore diameter (μm). The contact angle between sap and pit membrane is assumed to be 0° (Sperry et al. 1991).

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 nail polish were used to count and measure abaxial stomata (Ceulemans et al. 1978, 1995). 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.

2.2.5 Statistical Analysis

Analyses of variance and covariance were performed on SAS Version 6.0. The randomized block split-plot design was described previously (2x3x2x4 with four replications) (Appendix 1). The ANOVA model was mixed, with time blocks (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 x 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 (SAS Institute Inc. 1985, 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 (SAS Institute Inc. 1985) 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, Bergerud pers. comm.).

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).

2.2.6 Field Trial

To test consistency of the greenhouse results with plantation stock, a field component was undertaken. MacMillan Bloedel established Morningstar Plantation near Qualicum Beach, Vancouver Island. Here, the B.C. Ministry of Forests (van den Driessche and Brown 1995) had set up a large fertilization experiment. From each of four blocks, four plots were randomly chosen at two levels each of N (0 and 200 kg per hectare as NH_4NO_3), and P (0 and 250 kg per hectare as TSP or DAP) (randomized block split-plot design). Surrounding each plot was a buffer strip of clone 49-177 (clone one of the greenhouse experiment). August 21, 1996, one branch was chosen from each of three buffer strip trees per plot for a total of 48 branches (middle branch, green wood, westward orientation, identical plot positions). First, ψ_{xp} was measured with a pressure chamber. Branches were cut and bagged (2.2.3), then returned to the laboratory. Next morning, one branch per plot was tested for cavitation by the dye perfusion method (2.2.3), immediately after remeasuring ψ_{xp}. A second branch was dried on the bench 15 minutes and a third branch to wilting. After remeasuring ψ_{xp}, branches were rebagged and stored in the dark for five hours (to equilibrate) before cavitation testing.

2.3 RESULTS

2.3.1 Growth and Nutrient Content

High levels of N fertilization increased leaf area, dry weights of tree and leaf, and leaf:root ratios ($p \leq 0.05$), but P had no statistically significant effects on growth (Table 2.1). Drought-resistant clones (49-177 and 15-29) had larger stem volumes than drought-sensitive clones (52-237 and 52-226) (Table 2.2), and greater leaf, stem and root dry weights ($p \leq 0.05$) (not shown).

Nitrogen concentrations in leaves, stems and roots increased with N treatment, but leaf, stem and root K concentration decreased at the highest level of N fertilization (Table 2.1). Phosphorus treatment increased leaf and stem P concentrations, but stem P concentration was inversely related to N treatment level (Table 2.1). Nitrogen increased concentrations of S in all plant parts, and decreased Zn (not shown) and Mn in the stem (Table 2.1). Phosphorus increased Cu in the stem, Mg in the leaf and root, and Zn in the leaf ($p \leq 0.05$) (not shown). Drought-resistant clones had significantly lower concentrations of many nutrients than drought-sensitive clones: 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 more stem K, root Mn, and root P $(p \leq 0.05)$ (not shown).

2.3.2 Conductivity and Cavitation, Greenhouse Experiment

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 level, with high P increasing conductivity at high N (p=0.0283) (Figure 2.1). 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 2.2).

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 decreases in 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 2.2) (PxCl p=0.0326). 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 2.3a)(p=0.001). Percent loss of conductivity calculated using counts of dyed versus undyed vessels (conducting versus embolized), showed qualitatively similar nutrient interactions (Figure 2.3b). Cavitation at high N (7.14mM) was 48% at low P (0.1mM) but only 28% at high P (0.65mM) (p=0.021).

Drought-resistant clones, hydraulically measured, lost more than 99% of conductivity significantly less often than drought-sensitive clones, and reached less negative ψ_{xp} (Table 2.2), although their vulnerability curves indicated they were not more resistant to cavitation at moderate ψ_{xp} than the drought-sensitive clones (Figure 2.4). For instance, ψ_{xp} at 50% loss was less negative, meaning 50% loss was more quickly reached, for the drought-resistant clones (Table 2.2). 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).

2.3.3 Anatomical Differences

Measuring only natural-looking, spherical pores on SEM photomicrographs (Figure 2.5a,b), pit membrane pores were larger at low P than at high P (Table 2.3). Clonal differences in pore diameter were not statistically significant, but were ranked in order of drought resistance (Table 2.3), along with the ψ_{xp} at which cavitation might occur (equation 2). Regression analyses suggested that pore diameter was also influenced by concentrations of stem Mn and leaf K, along with tree size (Table 2.4).

Drought-sensitive clones appeared to have many more ripped and damaged pit membranes than drought-resistant clones. For hardy clones, averages of the largest hole (considering both natural looking pores and more obviously damaged areas) per pit were 0.151 µm and for sensitive clones 0.438 µm (p=.0373) (Figure 2.5c,d). 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 2.3) (p=.077).

Vessel diameters 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 the drought-sensitive clones (Table 2.2) (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 levels of fertilization (29μm versus 33μm) ($p=0.0303$) (not shown). However, the β level of significance was low (≈ 0.5) for these anatomical measurements because of the small sample sizes relative to variation.

2.3.4 Field Trial

Higher levels of N`supply led to more negative ψ_{xp} in the field at a given P level, and within an N level, ψ_{xp} was less negative with increased P supply ($p=0.05$) (Figure 2.6). Cavitation was greater for branches dried longest in the laboratory (38.9%) than for those dried 15 minutes (21.0%) or not at all (15.4%) ($p=0.001$). There was NSD in PLC overall, although N fertilization tended to increase PLC, but considering only the two least stressed drought levels, which had less variation, there was significantly more cavitation at higher N supply (20.9% compared to 15.5% at low N) ($p=0.047$).

2.4 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 (in the greenhouse) when either percent loss of conductivity (PLC) at a given water potential (ψ_{xp}), or number of individuals showing more than 99% loss of conductivity was considered. (In the field, sample size was too small for the degree of variation, and drought stress too mild to induce much cavitation, but trends were similar). An exception 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 xylem tension (stomatal control and hydraulic conductance), or xylem resistance to tension (vessel diameter and xylem pit membrane pore size).

High N supply probably induced cavitation both by increasing tension on the xylem (including reduced leaf K concentration) and by decreasing xylem resistance to embolisms. 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 with an increase in N fertilization was noted here, but not shown to be significant. Further, the safranin dye test 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 fertilization 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 embolisms (Sperry et al. 1994). Mean pore diameter of pit membranes was halved by increasing P supply, and hardy clones seemed to have less fragile pit membranes than sensitive clones. The mechanism by which any of these nutrients might influence cavitation physiologically is not clear, but because membranes consist of primary cell wall material, influence of P nutrition might be through uridine diphosphate (UDP) 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 due to 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. (1994a) 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 diameters. 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 cavitation improves drought resistance by reducing transpiration rates and water use (Neufeld et al. 1992, Alder et al. 1996, Sperry pers. comm.). However, drought-resistant clones were less likely to reach critically low ψ_{xp}, which would lead to $\geq$ 99% cavitation, than the susceptible clones, so stomata closed near the point of runaway cavitation. This is considered more adaptive behaviour, indicating optimal stomatal control (Tyree and Sperry 1989, Tyree and Ewers 1991).

In conclusion, stomatal control, perhaps coupled with less penetrable pit membrane, may be the 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.

Table 2.1. Growth and nutrient concentration (g kg^{-1} dry weight) responses to N and P fertilization, all clones.

Variable	N(mM)				P(mM)		
	0.36	1.43	7.14		0.1	0.65	
Growth							
Tree Weight (g)[1]	24.63 b	36.83 a	37.38 a	p=.027	31.69	34.20	p=.296
Leaf Weight (g)	9.62 b	15.4 a	16.15 a	p=.020	13.38	14.05	p=.171
Leaf Area (cm$^{2)}$	1810 b	2652 a	2721 a	p=.021	2345	2440	p=.661
Leaf:Root wt	1.51 b	2.01 a	2.10 a	p=.046	1.92	1.83	p=.528
Nutrients							
Leaf N	11.70 c	17.86 b	32.06 a	p=.009	20.39	20.72	p=.606
Stem N	4.68 c	6.35 b	12.89 a	p=.003	7.69	8.27	p=.400
Root N	9.55 c	17.20 b	27.46 a	p=.002	18.54	17.61	p=.203
Leaf P	4.78	4.25	3.12	p=.220	2.04 b	6.03 a	p=.028
Stem P	2.58 a	2.2 b	2.34 b	p=.023	1.51 b	3.22 a	p=.003
Root P	3.47	3.46	3.03	p=.081	2.33	4.29	p=.110
Leaf K	17.6 ab	19.20 a	14.84 b	p=.050	15.96	18.43	p=.107
Stem K	8.83 a	7.87 b	7.24 b	p=.021	7.37	8.59	p=.185
Root K	15.83 a	13.24 b	9.08 c	p=.004	12.24	13.17	p=.320
Stem Mn	0.021 a	0.012 b	0.011 b	p=.037	0.015	0.013	p=.245

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[1] Means followed by the same letter are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range test.

Table 2.2. Comparison of tree[1] and vessel size (length (cm) and diameter (μm)) versus maximum specific conductivity (K_s) (kg s^{-1} m^{-1} MPa^{-1}), minimum PLC, water potential (ψ_{xp}) at 50% loss (MPa), and, for each block x clone x treatment combination, mean minimum ψ_{xp} (MPa) and average number of cases of >99 PLC. Clones 49-177 and 15-29 are drought- hardy (H), 52-237 and 52-226 drought-sensitive (S).

Factor	Stem vol	Vessel length	Vessel diam	K_s max	PLC min	ψ_{xp} at 50 PLC	ψ_{xp} min	99 + PLC
H 49-177	24.6 a	23.8 a[2]	36.9 a	7.2 ab	33.1 a	-1.24 a	-1.67 a	.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	.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	23.4ab	-1.34 ab	-2.32 b	1.33 b
Pr > F	0.002	0.108	0.02	0.0277	0.1	0.0364	0.0049	0.086
Hardy	22.7 a	24.9 a	36.5	7.8 a	25.5	-1.23	-1.78 a	0.33 a
Sensitive	16.3 b	20.7 b	33.9	5.7 b	22	-1.41	-2.39 b	1.21 b
Pr > F	0.001	0.0347	0.03	0.0102	no test	no test	0.0014	0.0246

[1] Stem volume (cm^3) (ht/3 (πr^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 ($p \leq 0.05$) according to Duncan's Multiple Range test.

Table 2.3. Pit membrane pore size and membrane damage (hole), with average pore and largest hole diameter and water potential at which that pore/hole will begin to cavitate (based on the capillary equation of Sperry et al. 1991, equation 2), shown for P fertilizer concentrations, clones, and drought-hardy clones (1 and 2) versus sensitive clones (3 and 4). Means averaged for each of 16 trees, at least 2 samples per tree, from 41 useable photographs of pores and 178 SEM screen measurements of holes.

Factor	Pore diam. (µm)		MPa to cavitate	Hole diam. (µm)		MPa to cavitate
P 0.1 mM	0.132	a[1]	- 2.18	0.245	a	- 1.18
P 0.65 mM	0.074	b	- 3.89	0.344	a	- 0.84
Pr > F	p=0.022			p=0.539		
Clone 1: 49-177	0.091	a	- 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
Pr > F	p=0.250			p=0.077		
Drought Hardy	0.095	a	-3.03	0.151	a	-1.91
" Sensitive	0.115	a	-2.5	0.438	b	-0.66
Pr > F	p=0.133			p=0.037		

[1] Means followed by the same letter are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range test.

Table 2.4. 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/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

Figure 2.1. Leaf specific conductivity with embolisms (kg s^{-1} m^{-1} MPa^{-1}), with water potential as a covariate, is shown at low, medium and high N (0.36, 1.43 and 7.14mM) and low and high P (0.1 and 0.65mM). Bars show standard errors (n=32 per mean).

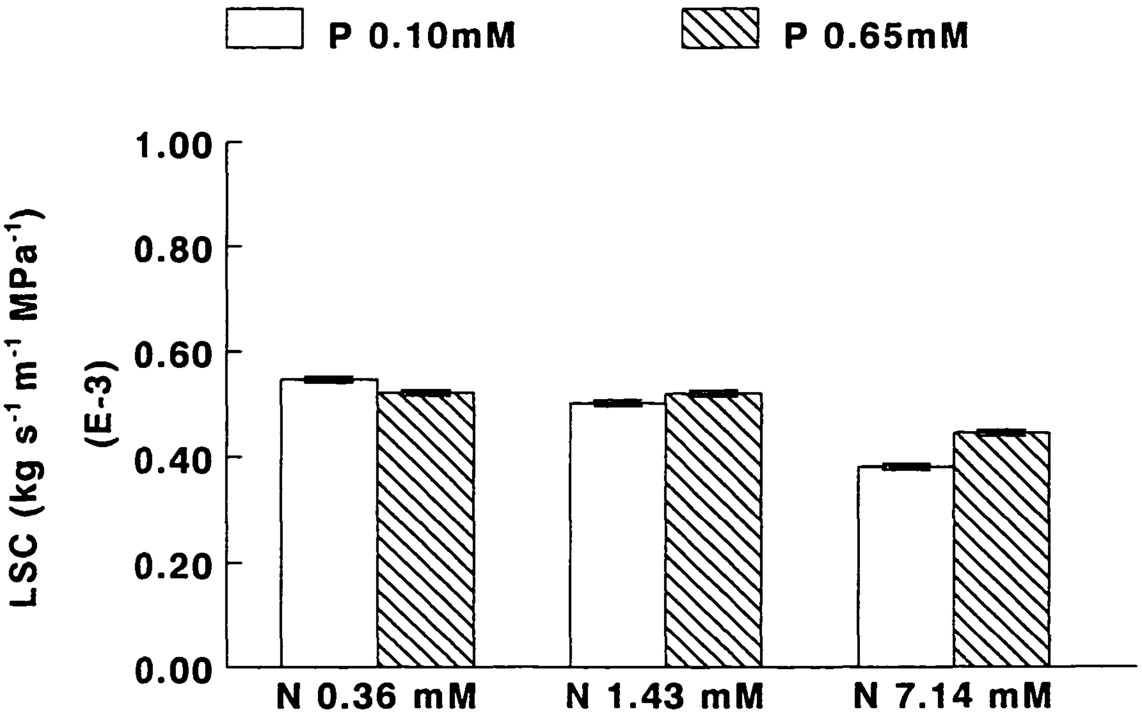

P 0.10mM
P 0.65mM
LSC (kg s^{-1} m^{-1} MPa^{-1})
(E-3)
1.00
0.80
0.60
0.40
0.20
0.00
N 0.36 mM
N 1.43 mM
N 7.14 mM

Figure 2.2. Vulnerability curve (percent loss of conductivity versus water potential in MPa) for Clone 15-29 at 0.1mM P and 0.65mM P. 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 P ($p=0.033$).

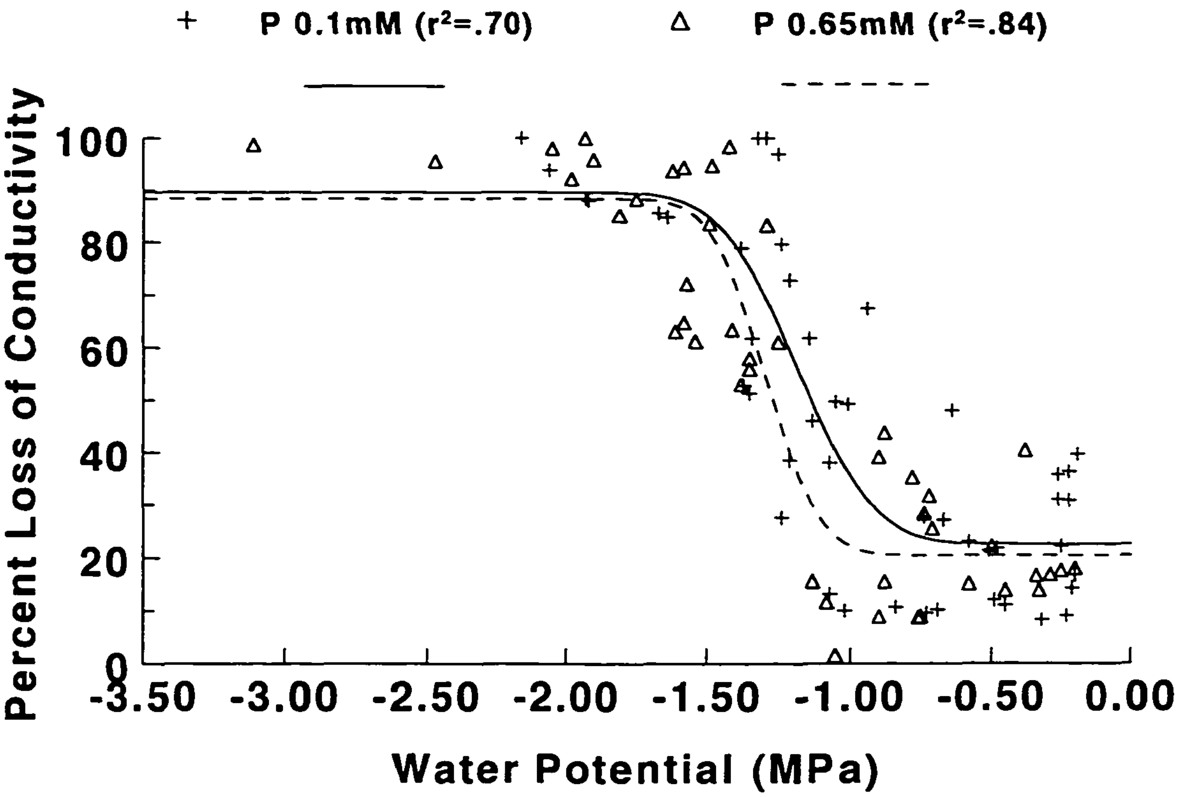

+ P 0.1mM (r^2=.70)
△ P 0.65mM (r^2=.84)
Percent Loss of Conductivity
100
80
60
40
20
0
-3.50
-3.00
-2.50
-2.00
-1.50
-1.00
-0.50
0.00
Water Potential (MPa)

Figure 2.3.Interaction of N and P level. (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=-.021). Bars show standard errors (n=32 per mean).

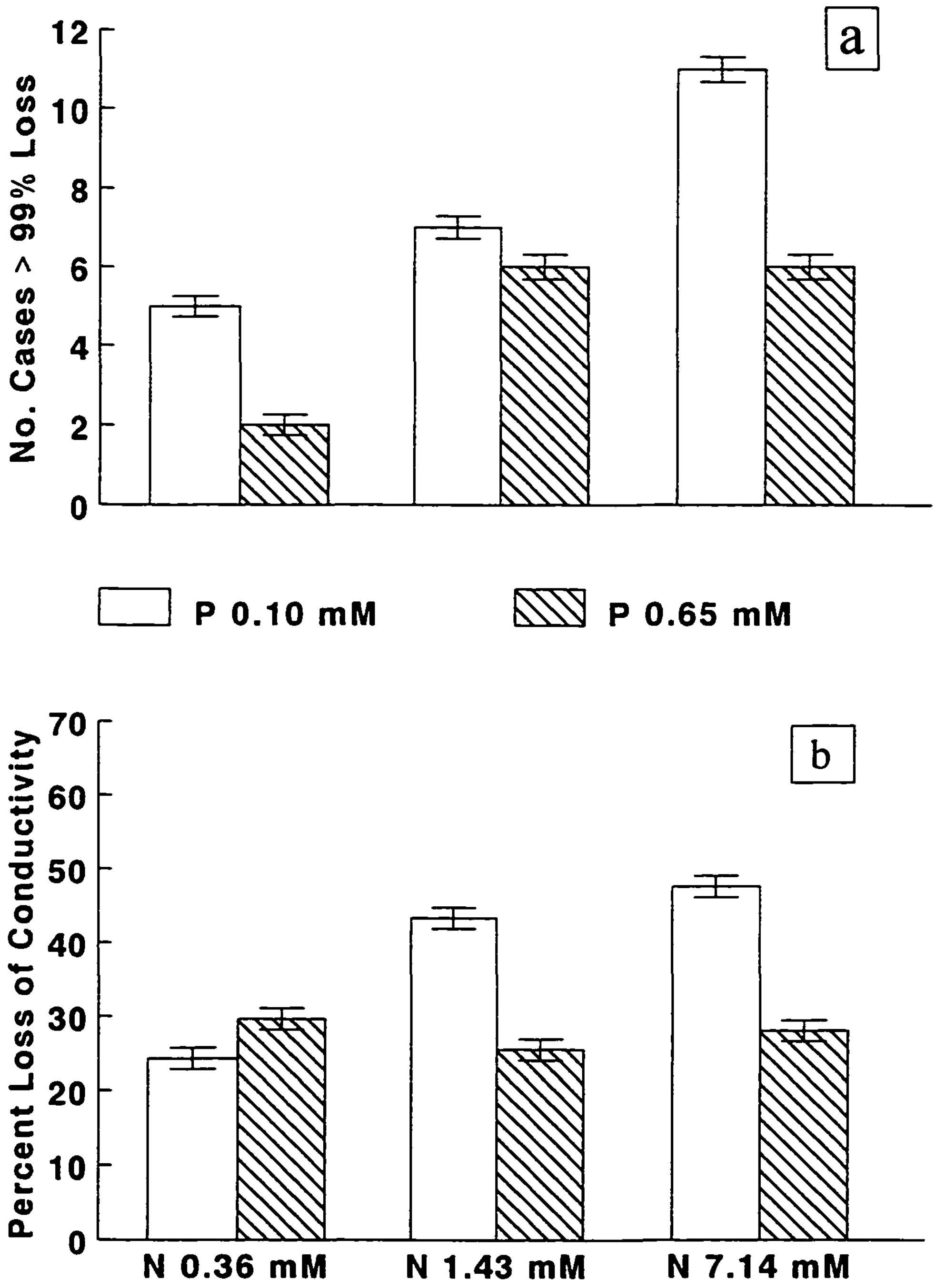

a
No. Cases > 99% Loss
0
2
4
6
8
10
12
P 0.10 mM
P 0.65 mM
b
Percent Loss of Conductivity
0
10
20
30
40
50
60
70
N 0.36 mM
N 1.43 mM
N 7.14 mM

Figure 2.4.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 not shown (n=96 per clone).

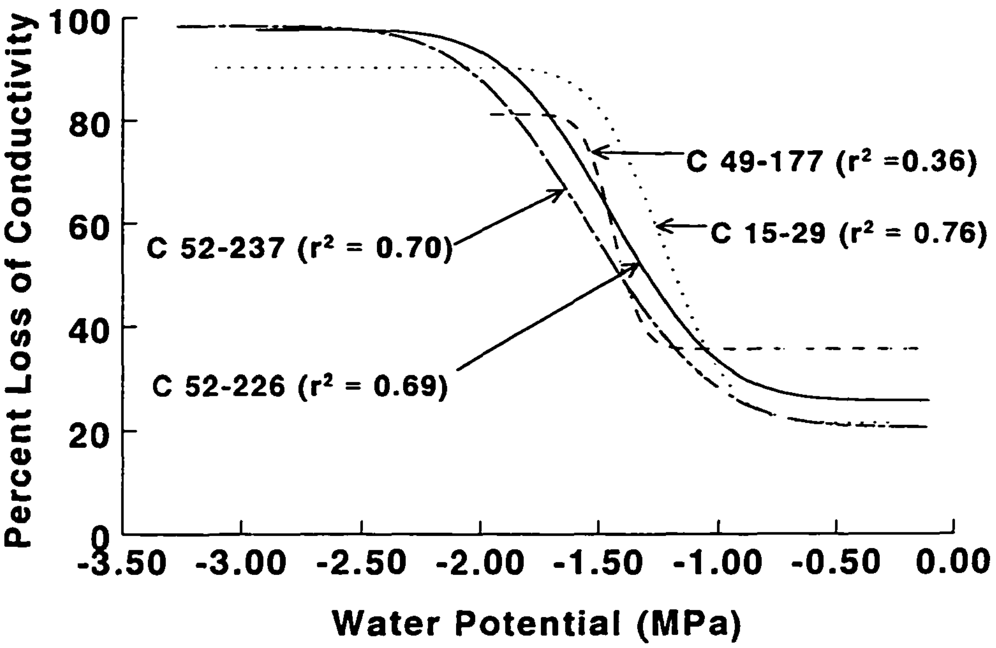

Percent Loss of Conductivity
100
80
60
40
20
0
-3.50
-3.00
-2.50
-2.00
-1.50
-1.00
-0.50
0.00
Water Potential (MPa)
C 49-177 (r^2 =0.36)
C 15-29 (r^2 = 0.76)
C 52-237 (r^2 = 0.70)
C 52-226 (r^2 = 0.69)

Figure 2.5. Scanning electron micrographs of bordered pit membranes of clone 52-237 at low N and low (a) and high (b) P. 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 thicker and shows no rips. For drought-sensitive clone 52-237 (d) the membrane appears fragile and is badly damaged.

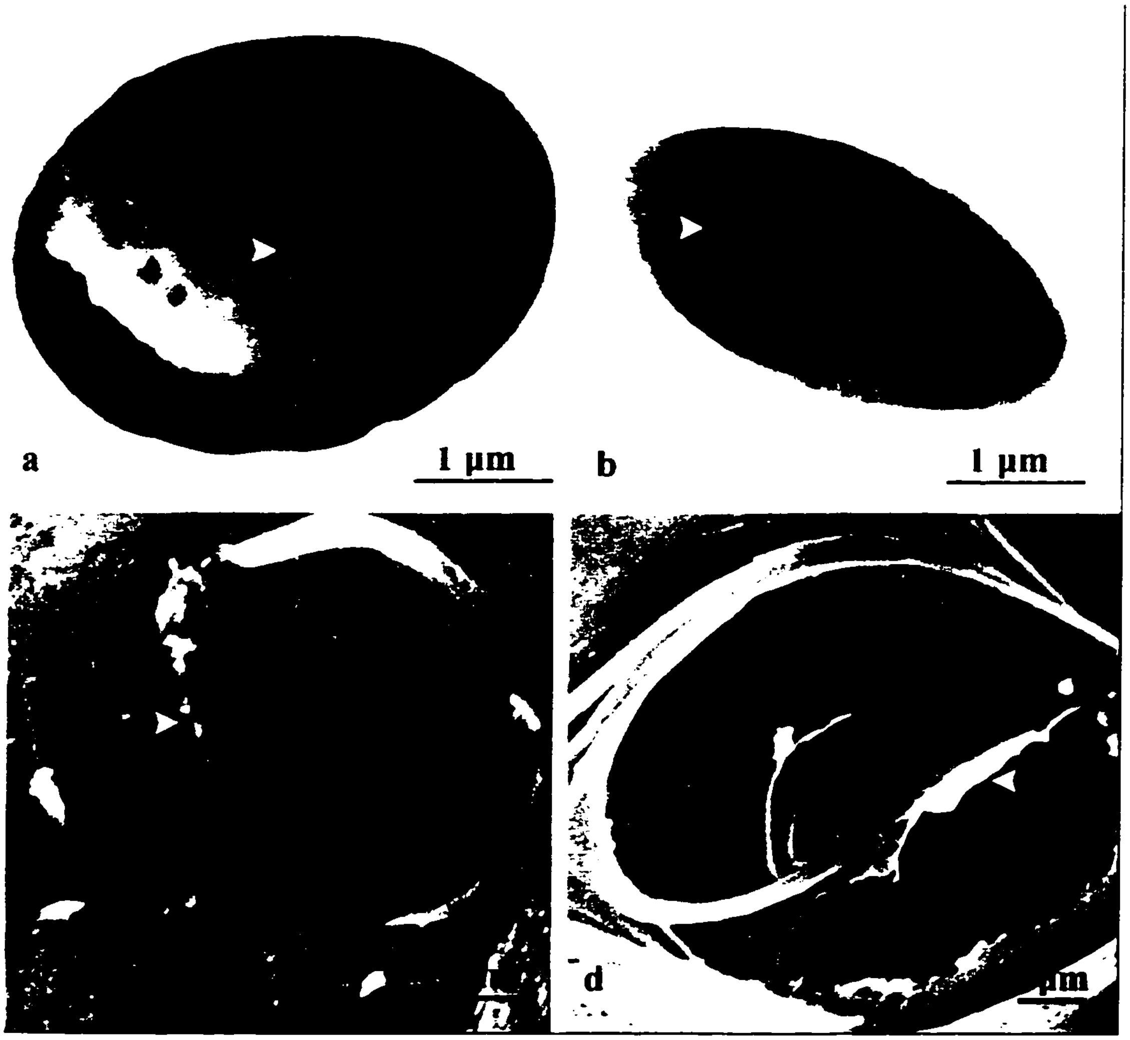
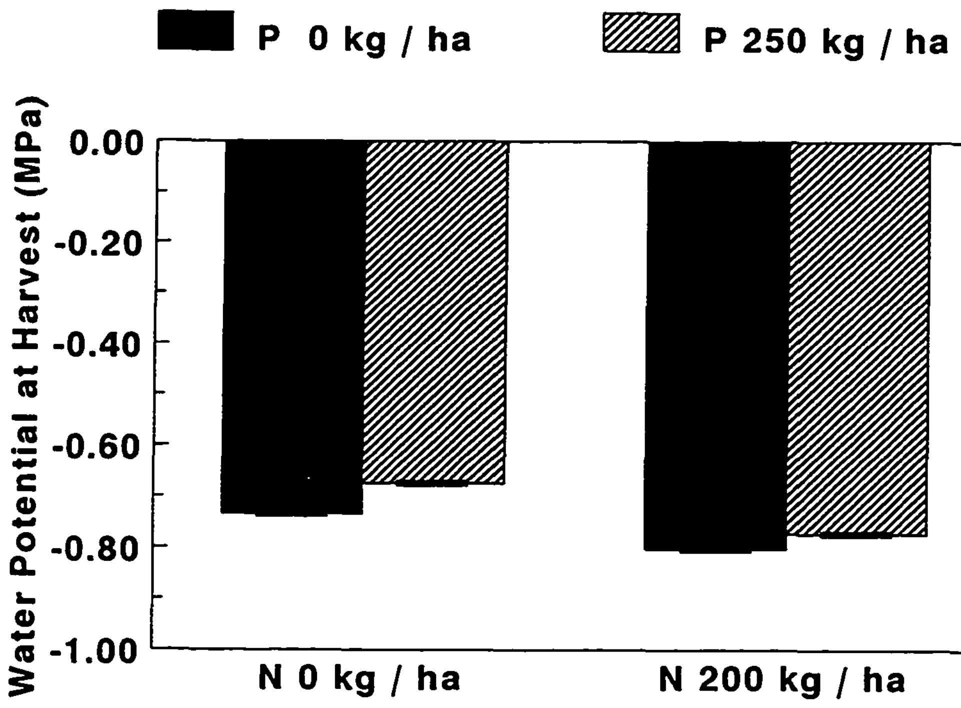

a
1 µm
b
1 µm
d
µm

Figure 2.6 Mid-day xylem water potentials measured on leaf petioles in the field, at Morningstar plantation, for clone 49-177 at two levels of N and two levels of P (n=12) (p=0.05).


P 0 kg / ha
P 250 kg / ha
Water Potential at Harvest (MPa)
0.00
-0.20
-0.40
-0.60
-0.80
-1.00
N 0 kg / ha
N 200 kg / ha

Chapter 3

MINERAL NUTRITION AND CAVITATION IN POPLAR II: EFFECTS OF PRECONDITIONING, NITROGEN AND POTASSIUM

3.1 INTRODUCTION

Nitrogen fertilization increased drought-induced cavitation in hybrid poplars, reducing potential productivity on dry sites by blocking the flow of water up the xylem vessels (Harvey and van den Driessche 1997). It was hypothesized that supplemental N increased transpirational water loss and xylem vessel diameters, making vessels more vulnerable to some types of cavitation. Increased P supply often counteracted the N effect, reducing cavitation at each N level (Harvey and van den Driessche 1997). Nitrogen and P are the nutrients most limiting to growth, followed by K (Clancy et al. 1995). In one study, poplars needed more K than *Platanus, Alnus, Betula* or *Fraxinus* species (sycamore, alder, river birch or ash), and *P. maximowiczii* grew more rapidly with added K (Wittwer and Immel 1980). If K fertilization is needed on a site, it is important to understand the implications for cavitation.

High levels of P increased leaf K concentrations within each clone (Chapter 2), an interaction which has been reported in the literature (Houman et al. 1991). This could be one way P decreases cavitation in severe drought. Potassium may affect conductivity. It serves as an enzyme cofactor, an osmoticum and an electrolyte at membranes, and is necessary for stomatal control (Taiz and Zeiger 1991). Potassium also affects wood anatomy, including vessel wall thickness and vessel length (Cutter and Murphey 1978).

High N supplies lowered leaf and stem K levels in hybrid poplars (Chapter 2) and spruce (van den Driessche and Ponsford 1995). High N also tended to increase cavitation. Therefore, since increased N decreased K and increased cavitation, and P increased K and decreased cavitation, possibly these effects are really due to changes in K and only incidentally to changes in N and P. Root K also decreased at high N (Harvey and van den Driessche 1997), which might reduce the water potential gradient between soil and roots, decreasing water uptake (Taiz and Zeiger 1991). Potassium fertilization increased drought resistance of *Triticum aestivum* L. (wheat) (Pier and Berkowitz 1987) and *Pinus sylvestris* L. (pine) (Christersson 1976). Further testing of K effects seemed warranted to determine if K fertilization might improve drought (Chapter 4) and cavitation resistance of poplars .

Some poplar clones become drought hardened (drought resistance improved), after a first episode of drying (Tschaplinski and Tuskan 1994, Gebre and Kuhns 1993, Liu and Dickmann 1993). Mechanisms included osmotic adjustment in the first two studies and increased stomatal control in the third. Potassium contributes to both osmotic adjustment and stomatal control, so should influence degree of drought hardening (Marschner 1986). It would be interesting to know whether this is true, and whether the adjustments leading to drought hardening also decrease vulnerability to cavitation.

Cavitation is reversible in a few species, perhaps due to positive root pressures or phloem-transported IAA (indole 3-acetic acid) increasing vessel solutes and thus osmotic potential (Salleo et al. 1996). The cyclic drying involved in drought preconditioning would provide an opportunity to measure cavitation before and after rewatering.

The objectives of this study were:

1. to further elucidate the mechanisms of N-induced increases in cavitation,
2. to understand the effects of K supply on cavitation and thus on drought productivity,
3. to discover whether the N and P effects on cavitation might be explained by their effects on K concentrations in the plant,
4. to study the influence of drought hardening or preconditioning on vulnerability to cavitation, and possible nutrient effects on preconditioning, and
5. to determine whether drought-induced cavitation is reversible in hybrid poplars.

3.2 MATERIALS AND METHODS

3.2.1 Plant Material

Six poplar clones were used: [1]

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[1] (TxD) were operational clones supplied by Cees van Oosten, MacMillan Bloedel, and clones 3 and 6 were from Peter McAuliffe, Scott Paper Ltd., New Westminster, B.C.

1) 49-177 *Populus trichocarpa* x *P. deltoides* (T x D) used in Chapter 2 and
2) 184-411 (TxD), both diploids which are productive on dry sites;
3) Robusta (*P. euramericana* (Dole) Guinier), well characterized, most drought resistant of five clones compared by Havaux et al. (1988);
4) 52-226 (TxTxD) used in Chapter 2 and
5) 17-50 (TxTxD), two drought sensitive triploids; and
6) *P. trichocarpa* (Torr. & Gray) (Blom), more drought sensitive than *P. deltoides*, many TxD hybrids (Pezeshki and Hinckley 1982) and Robusta (Havaux et al. 1988).

The clones are numbered in order of assumed decreasing drought resistance (and will be referred to by number hereafter). Rankings for the four operational MacMillan Bloedel clones are for productivity on dry plantation sites in the Pacific Northwest (Cees van Oosten, pers. comm.), and for clones three and six are estimated based on the literature. *Populus trichocarpa* is reported to have very little stomatal control (Braatne et al. 1992), and was chosen to help elucidate stomatal components of cavitation. Clones 1 and 4 exhibited contrasting strategies of cavitation avoidance (Chapter 2), so they were included again.

3.2.2 Plant Culture and Nutrition

Cuttings 23 cm long were planted April 4, 1996 in clean sand (Table 3.1 nutrient analysis), one tree per 8-litre plastic pot. They were in two polyethylene greenhouses so that water supplies could be manipulated, but otherwise were grown under ambient conditions. Inside each greenhouse, 360 trees were randomly arranged (including 36 extras for another experiment (Chapter 5)). Light averaged 80% of available PAR, and temperatures ranged from lows of 0.5°C in April to highs of 29.2 °C in July and 27.5 °C in August. Water was supplied as needed by a timed overhead watering system except when drying was scheduled.

Nutrient treatments, drought treatments and clones were completely randomized within each greenhouse (block). Trees were 30 cm apart, 30 rows of 12 trees in each greenhouse with 60 cm aisles for access around each group of 6X6=36 trees. One metre of clearance was left at each end of the greenhouses.

Most trees developed at least two main stems per cutting, except clone 5 often had only one. Any extra stems beyond the first two were removed as they appeared.

Two levels of N were fully crossed with three levels of K to give six levels of fertilization. Hereafter, nutrient treatments will be referred to as N1 (0.71 mM N supply as NH_4NO_3), N2 (7.14 mM N), K1 (no K added), K2 (0.26 mM K as K_2SO_4), and K3 (2.57 mM K). Other essential nutrients were supplied at the following concentrations: P, 0.33 mM; Ca, 1.00 mM; Mg, 0.82 mM; Cu, 0.47 μM, Mo, 0.03 μM; Zn, 0.47 μM; B 0.02 mM and Mn, 0.36 μM (van den Driessche 1990) (Table 3.1). Complete nutrient solutions were added by hand once a week after bud flush for four weeks, then twice weekly, 400 ml per pot, except during drought.

3.2.3 Growth

1. Cutting diameters were measured the day of planting. Growth in the first season is greater with larger cuttings (Cees van Oosten, pers. comm.), so cutting diameter was used as a covariate in statistical analyses of growth.
2. Heights and diameters of all trees were measured before drought.
3. Biomass allocations to root, shoot and leaf at different levels of nutrient supply and drought were compared by harvesting separately, drying at 80°C, then weighing (mg). All plant material (n=648) was eventually dried and weighed, whether harvested before drought, at the time of cavitation measurement (3.2.8) or at the final harvest.
4. Leaf area was measured on a sub-sample of leaves from all trees in greenhouse one with a Delta-T Leaf Area Meter, using leaves removed for ψ_{xp} tests. These leaves were weighed to obtain an area:weight conversion factor for final leaf area calculations from whole tree leaf weights.

3.2.4 Leaf Physiology

1. Leaf water potential of the petiole, representative of ψ_{xp}, was measured with a pressure chamber before testing stems for cavitation.

2. Gas exchange of the eighth fully-expanded leaf (leaf plastochron index 8) was measured at intervals and under drought and rewatering with the Li-Cor 6400 in greenhouse one (Chapter 4).

3. Stomatal closure points were calculated as the ψ_{xp} below which average stomatal conductance is less than 0.1 mol m^{-2} s^{-1}.

4. Two days into the final drought, leaf osmotic potential (mmol kg $^{-1}$) of the ninth fully expanded leaf was determined with a Johns Scientific Wescor 5500 Vapour Pressure Osmometer (n=648) (see 4.2.2).

3.2.5 Drought Treatments

There were three drought treatments, each involving one third of the trees:

1. Controls (well-watered). These were hand watered individually when water was withheld from other trees in the greenhouses.
2. One Severe Drought. Water was withheld once only, at the end of the experiment in August. After two days without water, four days of cavitation testing began (along with water potential and gas exchange measurements) (3.2.8).
3. Cyclical drought and rewatering. Water was withheld for 72 hours in July until the larger trees began wilting. After cavitation measurements (3.2.8), normal watering was resumed for 10 days. Finally, these trees were dried again synchronously with the severe drought treatment in August.

Two adjoining greenhouses were used to provide fixed time blocks. Drought treatments were separated by 10 days between the two greenhouses due to time constraints, so that greenhouse one was completely tested and harvested by August 10, and greenhouse two by August 20.

3.2.6 Cavitation

Cavitation was measured by the dye perfusion method (Chapter 2). This was done:

1. after a first cyclic drought of one third of the trees (hardening treatment),
2. after rewatering cyclically droughted trees, and

3. at the final harvest on controls, once-droughted, and redroughted hardened trees.
For cyclic drought, to ensure enough samples, only one stem per clone x nutrient combination was cut per greenhouse after the first drought (replicate two, randomly chosen). After rewatering for 10 days, the second stem of the same tree was cut and cavitation measured again to determine reversal of cavitation. That left two replications for the final harvest (Table 5.1 b to d.i).

Stem segments perfused with safranine dye (which would only travel through and stain uncavitated vessels) were frozen for future sliding microtome sectioning and microscopic examination. At this time, undyed (cavitated) and dyed (functioning) vessels were counted to calculate percent loss of conductivity (PLC). Vessel number was counted at the same time.

3.2.7 Plant Anatomy

1. Vessel diameters: 40 μm cross-sectional slices were cut from the centre of each stem segment used for cavitation measurement. These slices were permanently mounted on a microscope slide and examined under a Zeiss microscope with attached Cohu Camera. An image was transferred to a video monitor and analyzed with the Bioscan Optimus 3.14 program to compare vessel diameters between nutrient levels and clones. All vessels more than 20 μm in diameter were measured vertically and horizontally for two views per slide, choosing views randomly from the central area of the xylem (n=216).
2. Maximum vessel length was determined as longest stem segment through which air could be forced. This was done for two replications per clone x nutrient treatment (n=72).
3. Vessel length distribution was measured (Chapter 2) for clones two and four (one drought sensitive and one drought resistant T x D clone) for low and high N and K treatments, two replications (n=16).
4. Stomatal anatomy: clear nail polish was used to make leaf prints at least 1 cm in diameter (Ceulemans et al. 1978, 1995). The prints were taken from the seventh fully expanded leaf, near the midrib between the second and third veins, both abaxially and

adaxially. Stomatal length and density were ascertained from these prints (as in 1 above). The same trees were used as for maximum vessel length determination (n=72).

3.2.8 Nutrient Analysis

Leaf, stem and root samples were dried, weighed, ground and analyzed for macro- and micro-nutrient (Table 5.1). Samples of each plant part from three identically treated trees were combined into composite samples for economy.

Vector analysis was used to determine possible nutrient interactions (Timmer and Armstrong 1987, Haase and Rose 1995). Relative nutrient content was plotted against relative nutrient concentration, normalized to controls at 100.

3.2.9 Statistical Analysis

The experiment was set up as a randomized complete block factorial design (6 clones x 3 K x 2N x 3 drought levels) with three replications within each of two blocks. Since block, clone, treatment and drought level are all fixed effects, it was a fixed model with the error term for all main effects and interactions being experimental error (except for nutrient concentrations, Chapter 5.2). The ANOVA linear model and table are shown in Appendix 2 (Anderson and McLean 1974). SAS (Statistical Analysis System) general linear models procedure (proc glm) was used for most tests.

Linear and nonlinear regressions (proc reg and proc nlin in SAS) were used to determine effects of different parameters on cavitation. Curve fitting of data from recent experiments (Chapter 2) showed the relationship between ψ_{xp} and cavitation (percent loss of conductivity or PLC) was described best by the cumulative (sigmoid) equation from the Slidewrite program (and this equation fit stomatal conductance curves best as well). The destructive dye perfusion method (3.2.8) was used to determine PLC at a given ψ_{xp} Where more than one measurement was needed per tree (as in cyclically droughted trees before and after rewatering), different stems were used, and repeated measures statistics performed, followed by ANOVAs on the differences to pinpoint changes.

For other parameters measured, such as leaf area and vessel diameter, linear regressions were carried out versus PLC at a given ψ_{xp} to determine relationships. Multivariate analyses, including multiple regressions (Maxr and Rsquare in SAS proc reg) (Pimentel 1979), were used to develop hypotheses to explain nutrient and clonal responses to cavitation.

Means were compared with Duncan's Multiple Range Tests and Least Square Means. Planned contrasts were performed between drought resistant and drought sensitive clones, control versus once-droughted trees, and linear and quadratic K. Normality of the residuals of data sets was tested with proc univariate in SAS (Steel and Torrie 1980)

3.3 RESULTS

3.3.1 Cavitation

3.3.1.1 Cavitation at final harvest

Percent loss of conductivity due to cavitation (PLC) was greater at N2 than at N1 except in well-watered controls (p=0.0001) (Figure 3.1a). Hardened droughted trees had more PLC than once droughted trees (at N2), which in turn cavitated more than controls (p=0.0001) (Figure 3.1a), although hardening only significantly increased PLC in clones 1 and 6 (49-177 and *P. trichocarpa*) (p=0.0093) (not shown). Using ψ_{xp} as a covariate reduced the difference between the two N levels, but there was still significantly more cavitation at N2 (Figure 3.1b) (p=0.0001).

At each N level, there was more cavitation at a given ψ_{xp} at K2 than at K1 in unhardened trees (quadratic contrast, p=0.0235) (Figure 3.1b). When ψ_{xp} was not adjusted for, K2 led to more cavitation than K3 but not K1 (p=0.0238) (Figure 3.1c).

There were clonal differences in PLC alone and in cavitation at a given ψ_{xp} (p=0.0001) (Figure 3.2 a, b). Drought resistant clones (1-3) had less PLC than drought sensitive clones (4-6) (contrast p=0.0101) and in particular diploid T x D hybrids (clones 1 and 2) had less PLC than triploids (clones 4 and 5) (p=0.0003).

Midday xylem water potential was less negative at low than high N (p=0.0001), and at high N, ψ_{xp} was less negative after drought in hardened than unhardened trees (p=0.0001) (Figure 3.3a). Water potential was no longer well-related to PLC after hardening, increasing variability.

3.3.1.2 Cavitation Before and After a Drying Cycle

To test reversibility of cavitation, PLC was compared in 72 trees sampled after the first cyclic drought and again 10 days after rewatering, and for 72 other identically treated trees after a second drought (part of the final harvest). Water potentials were highest after rewatering at N2 (p=0.0477) (Figure 3.3b) except at K2 (p=0.0025) (Figure 3.3c) (NxK p=0.0005). There was more cavitation with greater N supply at the first drought, after rewatering, and in hardened trees (p=0.0054) (Figure 3.4a). PLC was not significantly decreased by rewatering within either N level (Figure 3.4a), although there was a marginal decrease at N2 when PLC was more than 50% (p=0.063) (not shown). At low N, there was greater PLC after rewatering, but when ψ_{xp} was used as a covariate this difference was not significant (Figure 3.4b). This covariate helped adjust for post sampling drought (up to three hours) before simultaneous rewatering. The clone x drought interaction was only marginally significant (p=0.063), but means tests showed clone one had significantly lower PLC after rewatering (Figure 3.5), especially at N2 (not shown).

3.3.2 Nutrient Analysis and Growth

The growing medium (sand) probably supplied adequate K, Ca and Fe without fertilization (Table 3.1). Foliar K concentrations appeared non-limiting even at K1, but N concentrations were only low to adequate (Marschner 1986) (Table 3.2). Greater N supply increased N in all plant parts; improved leaf and stem Cu, Mg, and Mn and root Ca and Mg; but decreased P and K in all plant parts. N also decreased leaf Ca, B, and S; stem B, Ca and Zn; and root Zn, Fe, Mg, Mn, and Al. Increased K supply increased K in all plant parts (especially the leaves), and increased stem Mn, and root Ca, but decreased leaf and stem Ca, Cu, Mg, S and Zn; leaf B and Mn; stem Al and root Mg and Al (all $p \leq 0.05$). More N often decreased the K effect on other nutrients. Stem N concentration was greater at K2 than at K1 or K3 (p=0.054) according to two means tests (Duncan's

Multiple Range and Least Square Means) (not shown).

Vector analysis can summarize much of this information. Analysis of N, P and K for all six treatments (Figure 3.6a), with N1K1 as reference, showed a deficiency of N in the lower N treatments (Table 3.2), but a dilution of P and K. An examination of K treatment effects on all nutrients at low N (Figure 3.6b) reveals that K was at luxury levels at N1K2, but in excess (toxic) at N1K3, with growth decreasing as more K was supplied. Uptake of most other nutrients was depressed at N1K3, particularly B, Mg and Ca. At high N, using N2K1 as a reference (Figure 3.6c), growth increased as more K was supplied. There may have been a deficiency of K at N2K1, sufficient to a slight excess of K at N2K2, and luxury levels at N2K3. There was some secondary dilution, particularly of Mg, Ca, and Zn.

Growth parameters (all p=0.0001) were increased by greater N supply (Table 3.2): stem volume, leaf area (LA), size and number; or weight of the plant parts or whole tree (TW). Higher N supply decreased the root:shoot ratios and increased the leaf area ratios (LA/TW) (Table 4.1) (both p=0.0001). Potassium fertilization decreased leaf areas (linear contrast p=0.0485) and leaf area ratios (p=0.0006), but at N2, increased tree weight (p=0.012)(Table 3.2) and leaf thickness (p=0.0025) (not shown). At high N, K2 led to more (though smaller) leaves (p=0.002) (Table 3.2) but the smallest stem volume (p=0.046) (Table 3.2). Leaf loss after the first drought was greater at N2 (p=0.0001), and decreased at K3 (linear contrast p=0.0366) (Figure 3.7)

3.3.3 Anatomical Measurements

At higher N supply, there were increases in vessel diameters (p=0.0001) (Figure 3.8a) and lengths (44.2±2.2 (SE) vs 37.7±2.2 cm) (p=0.0468) (mainly clones two and five at N2) (p=0.0095). Vessel diameters were greatest at N2K3 and least at N1K3 (p=0.0009) (Figure 3.8a). Thus, N2 resulted in the greatest percentage of vessel area per stem cross-section at K3 (p=-0.0008) (Figure 3.8b). There were more stomata per unit area and per tree at N2 (p=0.0001), although stomata were also smaller (p=0.0001 for abaxial) (Chapter 4, Table 4.1). There were clonal differences in vessel length (p=0.0221) (Figure

3.9a), vessel diameters (p=0.0001) (Figure 3.9b) and stomatal length (p=0.0001) (Figure 3.9c). Triploid clones had wider and longer vessels and longer stomata ($p \leq 0.05$). Clone six had no adaxial stomata.

3.3.4 Gas Exchange and Osmotic Potential

Before drought, N2 increased transpiration for clone three only (p=0.0005), but K3 decreased it overall (p=0.0141) (Figure 3.10a). At the final harvest (post drought), N2 decreased transpiration (p=0.0001) as did K2 (adjusted for ψ_{xp} as a covariate) (p=0.0327) (Figure 3.10b) (N x K NSD). Increasing N supply increased osmotic potential (p=0.0001) and osmotic adjustment of hardened trees (p=0.0001) (Chapter 4) but decreased stomatal conductance of droughted trees (0.46±0.05 (SE) vs 0.16±0.05 mol $m^{-2}s^{-1}$) (p=0.003). Potassium did not affect mean post-drought stomatal conductance, but at N2, stomatal closure (determined mathematically) occurred first at K2 (-1.15 MPa), then at K1 (-1.75 MPa), but not until -2.67 MPa with K3 (Figure 3.11a). Stomatal closure points (0.1 mol $m^{-2} s^{-1}$ or less) of once-droughted trees were mathematically similar for T x D clones, (Figure 3.11b), (although clone five stomata appeared to close earlier), but clone three did not experience stomatal closure and clone six had overall lower conductances. After hardening, only the drought sensitive T x D clones (4 and 5) experienced stomatal closure (not shown).

3.3.5 Nutrient Relationships to Physiological Measurements

According to regressions of data for all trees at the final (post-drought) harvest, transpiration, ψ_{xp} and stomatal conductance were negatively correlated with PLC, and vessel diameters weakly positively related to PLC (Table 3.3). Water potential, transpiration and conductance were affected by leaf nutrient concentrations. Correlations often appear weak because water deficits are the main cause of changes in gas exchange and PLC, and drying time or ψ_{xp} explain most of the variances.

To illustrate which leaf, stem and root nutrients most affected PLC alone or at a given ψ_{xp}, further regression data is presented (Table 3.3). PLC was decreased by more leaf P and B (clone 5), stem Ca and B and root P. Cavitation was positively related to leaf Zn, stem S, N and Mn, and root B and N. Clones five and six were especially affected by nutrient content. Variation in PLC was best explained by ψ_{xp}, abaxial stomatal length, stem K and S, and root Ca and Mg, when transpiration was not included as a variable.

3.4 DISCUSSION

Fertilization with N or some levels of K may increase vulnerability to cavitation. Embolism formation was positively related to N supply whether N was increased from deficient to barely adequate levels, as here, or from barely adequate to high (Harvey and van den Driessche 1997), and whether trees were drought hardened or not. Potassium only affected cavitation of unhardened trees. High levels of K sometimes decreased cavitation, but, contrary to expectations, moderate supplies of K tended to increase cavitation at both N levels.

3.4.1 Potassium Effects on Cavitation

Potassium appeared to have conflicting effects on cavitation. High K supply tended to decrease tension on the xylem at the leaf level, and thus cavitation, by decreasing both transpiration per unit area and relative leaf area. On the other hand, K may have increased vulnerability of the xylem to cavitation, partly by increasing vessel diameter. The moderate K supply may have been sufficient to induce the cavitation-increasing effects on the xylem but not cavitation-decreasing effects on ψ_{xp}. Potassium tended to accumulate in the leaf at the highest K supply, decreasing transpiration. Moderate K supplies did not increase leaf K as much, nor decrease transpiration

Any unique effects at moderate K could provide clues to the increased vulnerability. Moderate K tended to increase stem N, which was positively related to cavitation. At high N supply, stem volume was least with moderate K, so there was growth inhibition. More and smaller leaves at moderate compared to low or high K would decrease the surface diffusive resistance, and increase the surface area for water loss (although leaf temperatures might be cooler, decreasing vapour pressure deficit (Kramer and Kozlowski, 1979)). There were signs of water stress at moderate (compared to low or high) K: lower transpiration rates after drought, more negative ψ_{xp} after rewatering, and earlier stomatal closure. Conventionally, one would assume that stomatal conductance and transpiration would be positively related to cavitation, with more water loss increasing water stress and thus cavitation. In this experiment, transpiration rates and stomatal conductance were negatively correlated with cavitation. Decreases in transpiration and conductance were symptomatic of cavitation, supporting the hypothesis that plants often operate on the brink of runaway cavitation (Tyree and Sperry 1989). This illustrates the difficulties in interpreting regressions.

Some characteristics were seen only at the highest or lowest levels of K. At high K, larger vessels may have increased water conductance, and smaller leaf area ratios would mean relatively less surface area for water loss. There was less transpiration at the highest K level before drought, and this might conserve water, leading to less negative ψ_{xp} and greater leaf retention. This may have compensated for any K-induced increase in xylem vulnerability. Both stem S and leaf Zn were decreased at the highest (but not at moderate) K supply, and stem S and leaf Zn were positively related to cavitation. At the same time, only high K supply increased root K concentrations, which might increase ψ gradient between soil and roots, increasing water uptake (Taiz and Zeiger 1991).

Zero K supply led to significantly less stem N and more stem P than moderate K. This could be important as N increases cavitation while P often decreases it, possibly by decreasing vessel pit membrane pore diameter, thus making it more difficult for embolisms to move from vessel to vessel (Chapter 2).

3.4.2 Nitrogen Effects on Cavitation

More light was shed on why N increases cavitation. Foliar N concentrations were limiting (treatment means 0.79 - 1.16%) in this experiment (Marschner 1986), so N fertilization effects were more pronounced than in our previous work (foliar N levels 1.17-3.21%) (Chapter 2). Nitrogen was shown to increase vessel diameter and length (the latter mainly in two clones) as well as cavitation. This is a further indication that vessel volume could have some influence on drought-induced cavitation within *Populus* or within each clone, even though this is not generally the case among species or even individuals (Tyree and Sperry 1989). *Populus balsamifera*, but not *Alnus*, displayed more vulnerability to cavitation in plant organs with the largest diameter vessels (Hacke and Sauter 1996b), and possibly *Populus* vessel diameter is more related to drought-induced cavitation than in other species because of an unusually narrow safety margin of ψ_{xp} before cavitation (due to their evolution in riparian habitats) (Hacke and Sauter 1996b). Within the species *Ceratonia silique* L. (carob), larger diameter vessels were also more likely to cavitate (Salleo and Lo-Gullo 1989).

As in an earlier experiment, greater N supply increased stomatal number (although decreasing stomatal size), and it also increased leaf area and size, all of which might increase water loss given a finite water supply in pots. Again, this would increase absolute PLC, although not PLC at a given ψ_{xp}, and the difference in cavitation between low and high N supply was less (but still significant) when cavitation was adjusted for ψ_{xp}. As in our previous work, increased N supply decreased P concentrations as well as K concentrations (except at high K), so these nutrient interactions could be important to the N effect on cavitation.

Greater N supply affected levels of other nutrients which could be related to cavitation resistance. It decreased stem Ca and B, both of which may contribute to membrane stability and integrity (Marschner 1986). Calcium increases lignification, which might slow hydrolysis at pit membrane sites.

3.4.3 Reversibility of Cavitation

Drought-induced cavitation in stem xylem vessels is sometimes considered irreversible in diffuse-porous trees, unless perhaps they can exert positive root pressures. However, Salleo et al. (1996) contended that there could be reversal in laurel, due to IAA-induced loading of solutes into the vessels, increasing their osmotic potential and water uptake. Tracheids of *Pinus sylvestris* may also reverse cavitation under negative pressures (in stem segments), possibly by diffusion of gases from the embolized conduits

(Edwards et al. 1994). On average, there was not a decrease in cavitation after rewatering, but there was some evidence of reversal at high N, at least for clone 49-177. Although poplars are not among the species known to develop positive root pressures during the growing season (Kramer and Kozlowski 1979; Hacke and Sauter 1996b), exudation of water from the leaf (guttation) is considered a symptom of positive root pressure (Kramer and Kozlowski 1979) and has been reported in clone 49-177 (C. van Oosten, R. van den Driessche, pers. comm.). Positive root pressures, gas diffusion or IAA accumulation may dissolve embolisms (Salleo et al. 1996) in this clone. Contrary to the findings of Salleo et al. (1996), this reversal was not less at high levels of cavitation for poplar.

3.4.4 Preconditioning and Cavitation

Even though preconditioning increased water potential, it did not decrease cavitation. This could be partly because the first cyclic drought introduced cavitation that was not reversible on average. When trees were rewatered for 10 days after drought, at low N there was a tendency toward increased PLC and a corresponding decrease in ψ_{xp}. At high N supply, PLC did not usually recover with rewatering, but ψ_{xp} nevertheless increased substantially from about -2 to -1 MPa. This may have been due to the drought-induced leaf loss, reducing leaf surface area for transpirational water loss to compensate for decreased water uptake through embolized vessels. At the end of the first drought period, transpiration was very low with high N supply. This may have conserved remaining water supplies and protected the xylem from further cavitation. Transpiration recovered substantially after rewatering, although not always to pre-drought levels.

In this experiment, the drought resistant clones on average experienced less cavitation than the drought susceptible, whereas the reverse was true in Chapter 2. This is because in the previous work, cavitation resistance of hardy trees was manifested only at critically low water potentials, whereas low potentials were the norms in the present experiment. Drought-resistant clones in the earlier experiment appeared to avoid high levels of cavitation because of stomatal closure just before the point of catastrophic cavitation.

Stomatal closure points did not appear different for drought hardy clones in this experiment after one drought, however. After hardening, the hardier clones (along with *P. trichocarpa*) no longer experienced stomatal closure, probably because they did not reach such negative water potentials as the susceptible clones. *Populus trichocarpa* is known to have poor stomatal control (Braatne et al. 1992) (Chapter 4) and thus is considered less drought resistant. It was not especially vulnerable to cavitation however, (as Sperry (1996) also noted), and had different coping mechanisms such as lack of adaxial stomata, and continuously low stomatal conductance and transpiration rates which were less affected by drought. The low transpiration rates, and consequent low cavitation, are probably due to the high leaf K concentrations maintained by this clone: when PLC was adjusted for K concentration, *P. trichocarpa* had a high vulnerability to cavitation. The triploid clones, which were also susceptible to cavitation, had longer, wider vessels, which might be more vulnerable to cavitation, and longer stomata which would increase the xylem tensions leading to cavitation.

Ability to continue transpiring (and photosynthesising) in drought was an indicator of cavitation resistance for the six clones used in this experiment.

3.5 CONCLUSIONS

This research helped clarify the effect of N fertilization on cavitation. Conduits were larger in volume with higher N supply, perhaps more vulnerable to drought-induced cavitation, and certainly more likely to cavitate with winter freezing (Hargrave et al. 1994). More N predisposed the trees to greater water loss and thus greater tensions on the xylem, with more stomata and greater leaf area per gram of tree weight. Finally, N fertilization greatly decreased the P needed to maintain small pores in the xylem vessel pit membrane, and the K necessary to prevent transpirational water loss.

Moderate K additions, probably at the sufficiency level, tended to increase cavitation and symptoms of cavitational stress such as stomatal closure. This could have been due to more and smaller leaves leading to more surface area for water loss, or to a tendency to increase plant N concentrations while decreasing P concentrations, both leading to more cavitation. It now seems likely that N and P effects on K concentrations play a secondary role in influencing cavitation resistance. Potassium at luxury levels may sometimes confer protection against cavitation relative to moderate K levels, perhaps by decreasing transpiration and leaf area ratios, and thus xylem tensions. However, vessel diameters were increased further at high N supply by more K, which would make the tree more vulnerable to freezing-, and possibly drought-induced, cavitation.

Drought hardening did not appear to reduce vulnerability to cavitation, even though it increased water potentials, because it introduced cavitation which was seldom reversible. Cavitation of leaf petioles led to leaf loss, which probably helped maintain water supplies.

Finally, trees which combined cavitation resistance with productivity on dry sites could maintain transpiration and photosynthesis in drought. Although high concentrations of leaf K may increase cavitation resistance by stomatal closure, this closure would also decrease photosynthesis and growth in drought. The T x D hybrids which are less successful on dry sites (especially 52-226) could not avoid low xylem water potentials even by early stomatal closure (possibly due to more permeable leaf cuticles). These findings lend weight to the theory that trees which are productive in drought operate on the brink of catastrophic cavitation to maintain better growth rates, but can avoid the most dangerously low water potentials (Tyree and Sperry 1989).

Table 3.1 Sand nutrient content in mg L^{-1} (n=7) with additional nutrients which were added as 400 ml in solution twice a week. Sand pH 6.56, nutrient solution pH 4.12 to 5.3.

Nutrient	mg L^{-1} added	mg L^{-1} sand	Nutrient	mg L^{-1} added	mg L^{-1} sand
Al	-	21.58±4.0	Mg	20	4.71±1.0
Al [1]	-	6.13±0.7	Mg [1]	20	67.33±2.4
B	0.2	nm [2]	Mn	0.02	0.59±0.16
Ca	40	4.57±0.8	N	10, 100	nm
Ca [1]	40	336.5±11	Na	-	8.64±0.6
Cu	0.03	0.07±0.01	P	10	0.54±0.1
Fe	4 once	20.22±3.4	S	28-67 [3]	0.17±0.1
K	0, 10, 100	3.00±0.8	Si	-	58.14±9
K [1]	0, 10,100	15.10±0.8	Zn	0.03	0.02±0.0

[1] Morgan's acid extraction results. Other extractions are with deionized distilled water (C. Dawson, pers. comm.).

[2] not measurable, quantities too small.

[3] 1.6 g S per pot added as slow-release gympsum to ensure optimum S for all treatments

Table 3.2. Growth and nutrient concentration (g kg^{-1} dry weight) responses to N and K fertilization, all clones, where N1 is 0.71mM N supply, N2 is 7.14mM, K1 is 0mM K supply, K2 is 0.26mM and K3 is 2.57mM.

Variable	N1			N 2			N x K
	K1	K2	K 3	K1	K2	K3	p≤
Growth							
Tree weight (g)	24.30 c[1]	24.15 c	23.18 c	87.70 b	86.85 b	91.03 a	0.012
Stem vol (cm^2)	9.90 c [2]	10.50 c	10.45 c	30.14 a	27.57 b	30.42 a	0.046
Root:shoot wt	0.413 a	0.463 a	0.468 a	0.253 b	0.246 b	0.293 b	0.001 [N]
Leaf area (m^2)	0.104 c	0.101 c	0.095 c	0.416 a	0.40 ab	0.397 b	0.001
Leaf size (cm^2)	26.21 d	24.81 d	29.44 d	64.37 b	59.57 c	68.53 a	0.002
Leaf number	47.45 d	49.33 d	40.17 d	72.55 b	82.46 a	65.42 c	0.002
Nutrients							
Leaf N July	8.38 c	8.38 c	8.66 c	12.00 a	11.84 a	10.84 b	0.001
Leaf N August	7.93 b	7.84 b	7.86 b	11.81 a	11.96 a	11.11 a	0.001 [N]
Stem N "	3.03 c	3.08 c	2.92 c	3.73 b	4.13 a	3.87 ab	0.001 [N]
Root N "	5.06 b	5.23 b	5.08 b	6.99 a	6.89 a	6.80 a	0.001 [N]
Leaf K "	14.45 c	16.40 b	20.27 a	6.44 f	7.46 e	13.01 d	0.001
Stem K "	7.56 c	8.41 b	10.08 a	4.06 e	4.78 d	7.43 c	0.001
Root K "	8.17 bc	8.56 b	10.04 a	5.27 d	5.61 d	7.92 c	0.001[N,K]
Leaf P "	1.96 a	2.01 a	2.03 a	1.33 bc	1.45 b	1.23 c	0.001 [N]
Stem P "	1.16 b	1.20 b	1.24 a	0.62 d	0.67 c	0.63 cd	0.027
Root P "	1.59 a	1.55 a	1.55 a	0.95 b	0.92 b	0.90 b	0.001 [N]
Stem S "	0.419bc	0.408bc	0.392 c	0.483 a	0.514 a	0.434 b	0.024
Leaf Zn "	0.049ab	0.045 b	0.046ab	0.051ab	0.052 a	0.037 c	0.008

[1] Means within a row followed by the same letter are NSD by Least Squares Means test

[2] Stem volume = ht/3 (πr^2) before any droughting

[N] Significantly different by N level instead of N x K ([N,K] differs by N and K, not NxK)

Table 3.3. Regressions and multiple regressions with associated signs and coefficients of determination (r^2) for which $p \leq 0.05$. Coefficients, where multiple, are in decending order of importance. Only coefficients which significantly affect the dependent variable ($p \leq 0.05$) are included, and the best combinations are presented (highest r^2). All values are from the final post-drought harvest.

Dependent Variable	Coefficients	Sign	r^2
ψ_{xp} [1]	K leaf + Mg leaf	++	0.11
Transpiration	S leaf +K leaf +Cu leaf	+ + -	0.10
Conductance	Cu leaf + P leaf	- +	0.07
PLC [2]	Transpiration	-	0.61
PLC	ψ_{xp}	-	0.41
PLC	Stomatal conductance	-	0.33
PLC clone 6	Transpiration, ψ_{xp} each	- -	< 0.3
PLC	Vessel diameter	+	0.06
PLC	P leaf	-	0.06
PLC	ψ_{xp} + Zn leaf + P leaf	- + -	0.47
PLC clone 6	Zn leaf	+	0.28
PLC clone 5	P leaf, B leaf each	- -	0.3, 0.2
PLC	Stem S + Mn + Ca + B	+ + - -	0.23
PLC clone 5	Stem N, S, Zn, Fe each	+ + + -	0.2-0.3
PLC	ψ_{xp} + S stem + N stem	- + +	0.47
PLC	Root P + Root B	- +	0.16
PLC	ψ_{xp} + Root N + root P	-+ -	0.47
PLC: Best with ψ_{xp}	ψ_{xp} + abaxial stomata length + K stem + S stem + Ca root + Mg root	- + + + - -	0.57

[1] Xylem water potential

[2] Percent loss of conductivity due to cavitation

Figure 3.1. Percent losses of conductivity (PLC) at low and high N supplies (N1 = 0.71 mM vs N2 = 7.14 mM) at the August harvest a) for well-watered controls, once droughted trees and hardened droughted trees (n≈72 per bar); and for unhardened trees at low, medium and high K supply (K1 = 0 mM, K2 = 0.26 mM, K3 = 2.57 mM) either b) adjusted for leaf water potential (ψ_{xp} used as a covariate in the analysis), or c) not adjusted (n≈48 for each bar) (error bars represent standard error).

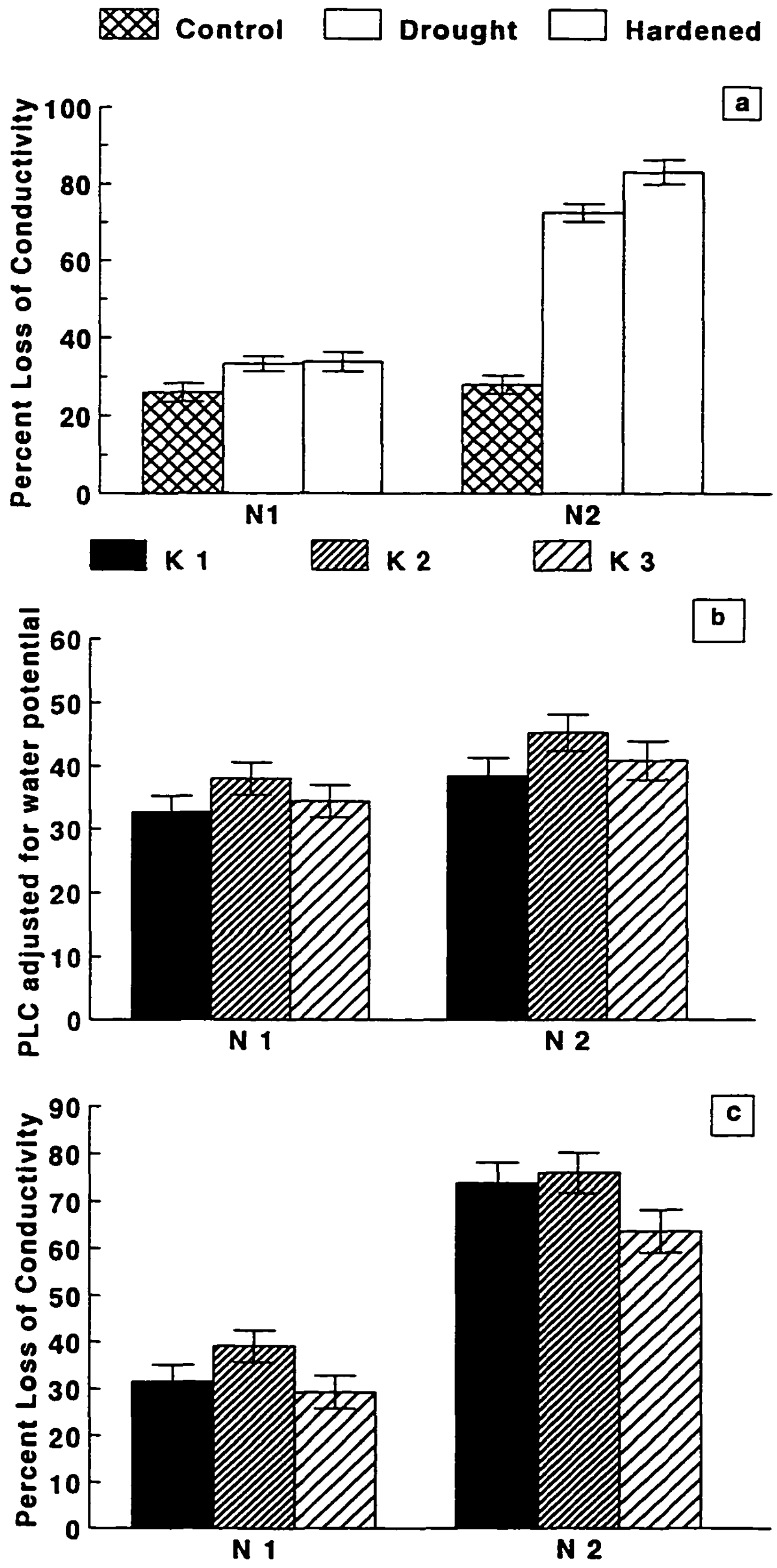

Control
Drought
Hardened
a
Percent Loss of Conductivity
0
20
40
60
80
100
N1
N2
K 1
K 2
K 3
b
PLC adjusted for water potential
0
10
20
30
40
50
60
N 1
N 2
c
Percent Loss of Conductivity
0
10
20
30
40
50
60
70
80
90
N 1
N 2

Figure 3.2. Percent losses of conductivity at the August harvest for poplar clones 1 (49-177), 2 (184-411), 3 (*P. euramericana* Robusta), 4 (52-226), 5 (17-50) and 6 (*P. trichocarpa* Blom), where clones 1-3 are drought resistant and clones 4-6 are drought sensitive. Percent losses are a) adjusted for xylem water potentials or b) plotted against leaf water potentials to produce clonal vulnerability curves (n≈72 per clone) (error bars represent standard errors).

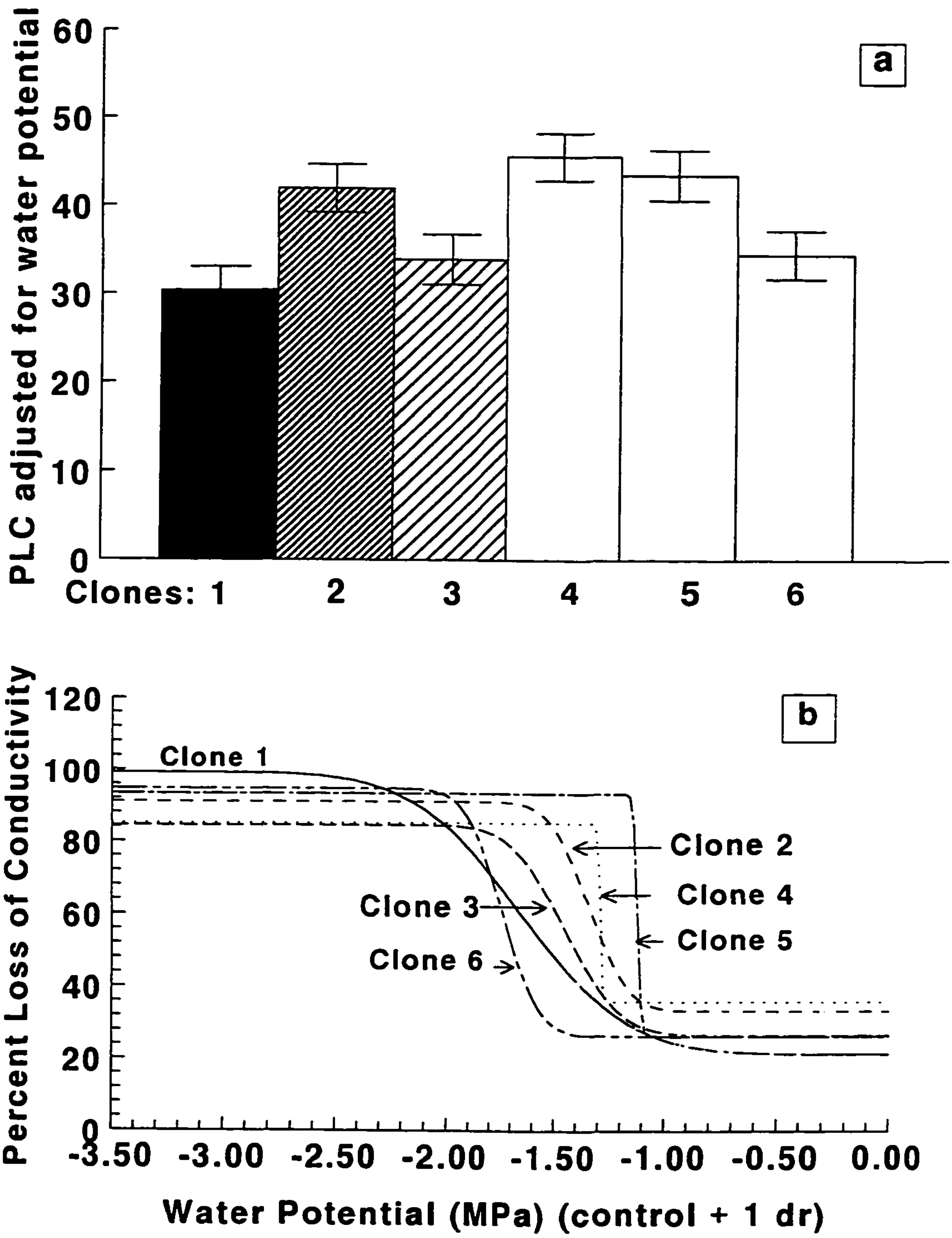

a
PLC adjusted for water potential
0
10
20
30
40
50
60
Clones: 1
2
3
4
5
6
b
Percent Loss of Conductivity
0
20
40
60
80
100
120
Clone 1
Clone 2
Clone 3
Clone 4
Clone 5
Clone 6
-3.50
-3.00
-2.50
-2.00
-1.50
-1.00
-0.50
0.00
Water Potential (MPa) (control + 1 dr)

Figure 3.3. Xylem water potentials measured in the leaf petioles of trees supplied with low and high N (N1 = 0.71 and N2 = 0.714 mM) for:

a) well-watered controls, once-droughted trees and hardened droughted trees at the final (August) harvest (n=72 per bar) and

b) cyclical drought trees after one drought in July, after ten days of rewatering (n=36), and after a final drought in August (n=72); and

c) trees supplied with low, medium and high K (K1 = 0 mM, K2 = 0.26 mM, K3 = 2.57 mM), averaged over both N levels, measured after one drought in July, after ten days of rewatering (n=24), and after the final drought in August (n=48) (error bars represent standard errors).

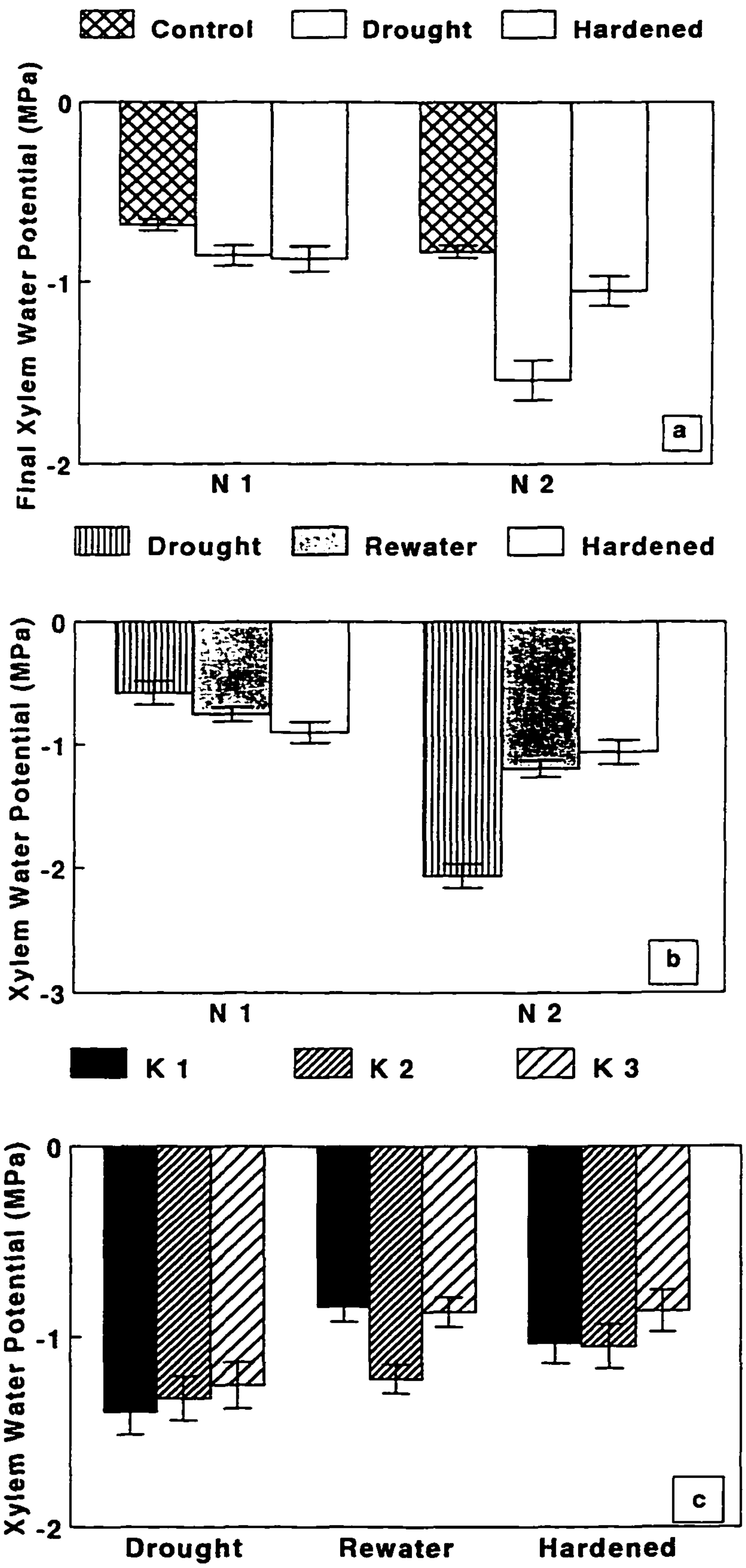

Control
Drought
Hardened
Final Xylem Water Potential (MPa)
0
-1
-2
N 1
N 2
a
Drought
Rewater
Hardened
Xylem Water Potential (MPa)
0
-1
-2
-3
N 1
N 2
b
K 1
K 2
K 3
Xylem Water Potential (MPa)
0
-1
-2
Drought
Rewater
Hardened
c

Figure 3.4. Percent losses of conductivity measured after one drought in July, after rewatering for 10 days (n=36 per bar), and after the final drought in August (hardened) (n=72 per bar), for trees supplied with N1 (0.71 mM) or N2 (7.14 mM N) (over all K levels) either a) unadjusted or b) adjusted for xylem water potential measured in leaf petioles (error bars represent standard error).

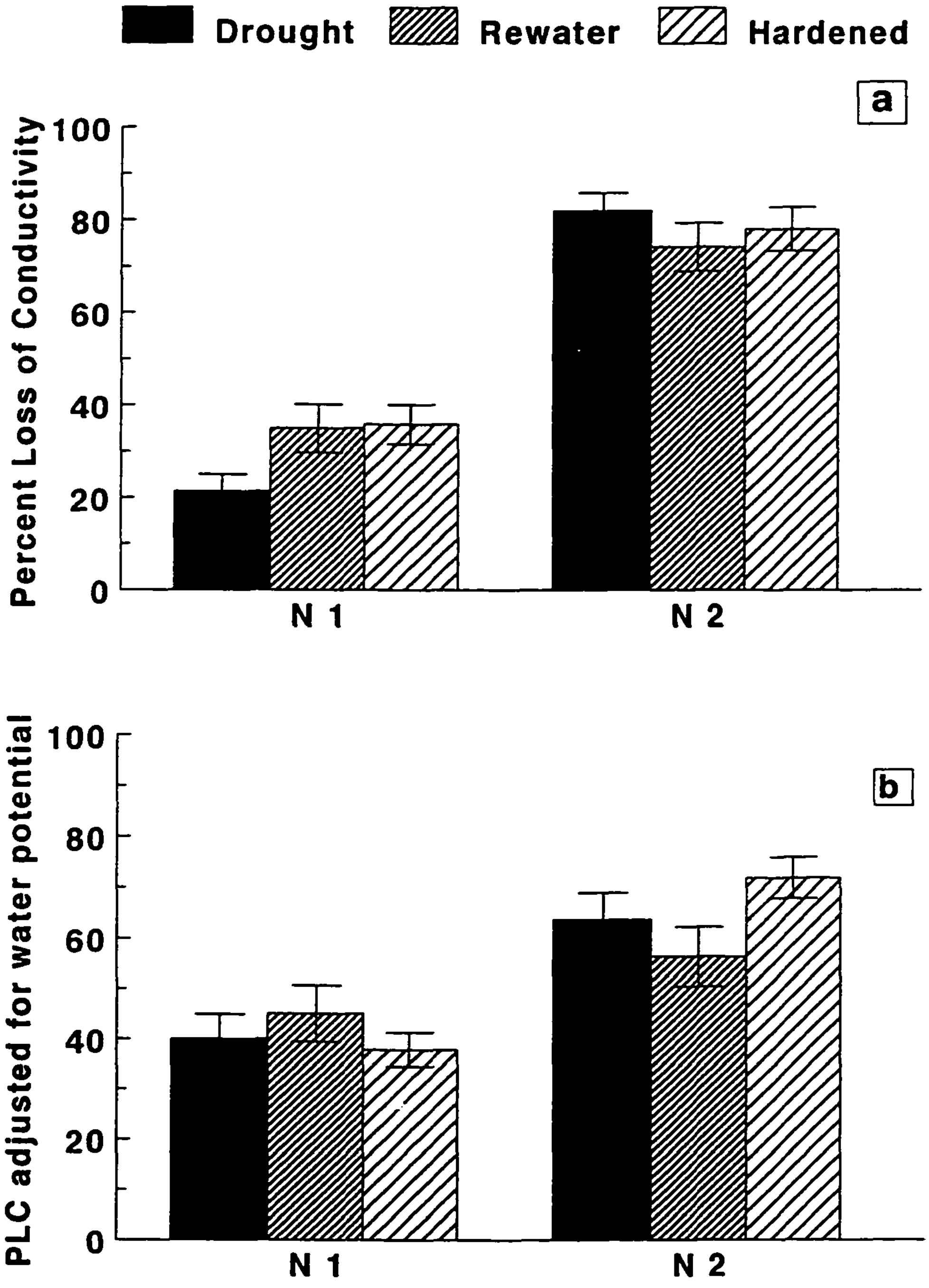

Drought
Rewater
Hardened
a
Percent Loss of Conductivity
0
20
40
60
80
100
N 1
N 2
b
PLC adjusted for water potential
0
20
40
60
80
100
N 1
N 2

Figure 3.5. Percent loss of conductivity over all nutrient levels for poplar clones 1 (49-177), 2 (184-411), 3 (*P. euramericana* Robusta), 4 (52-226), 5 (17-50) and 6 (*P. trichocarpa* Blom), where clones 1-3 are drought resistant and clones 4-6 are drought sensitive. Trees are droughted in July, rewatered for 10 days (n=12 per bar), and droughted again (hardy) (n=24) (error bars represent standard error).

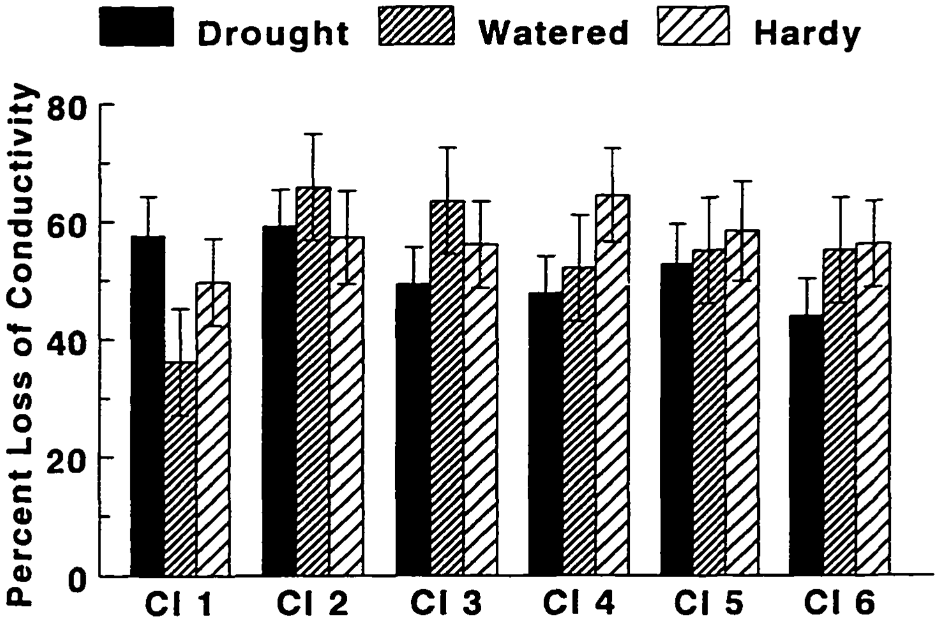

Drought
Watered
Hardy
Percent Loss of Conductivity
0
20
40
60
80
Cl 1
Cl 2
Cl 3
Cl 4
Cl 5
Cl 6

Figure 3.6. Vector diagrams showing relative foliar nutrient concentrations plotted against relative nutrient contents, and relative plant dry weights (all normalized to controls at 100) for a) N1 (0.71 mM) and N2 (7.14 mM) at K1 (0 mM), K2 (0.26 mM), and K3 (2.57 mM) where N1K1 is the reference; b) K1, K2 and K3 supply at N1 where N1K1 is the reference; and c) K1, K2 and K3 at N2 where N2K1 is the reference (n=36 composite samples per data point). Only nutrients referred to in the text are labelled.

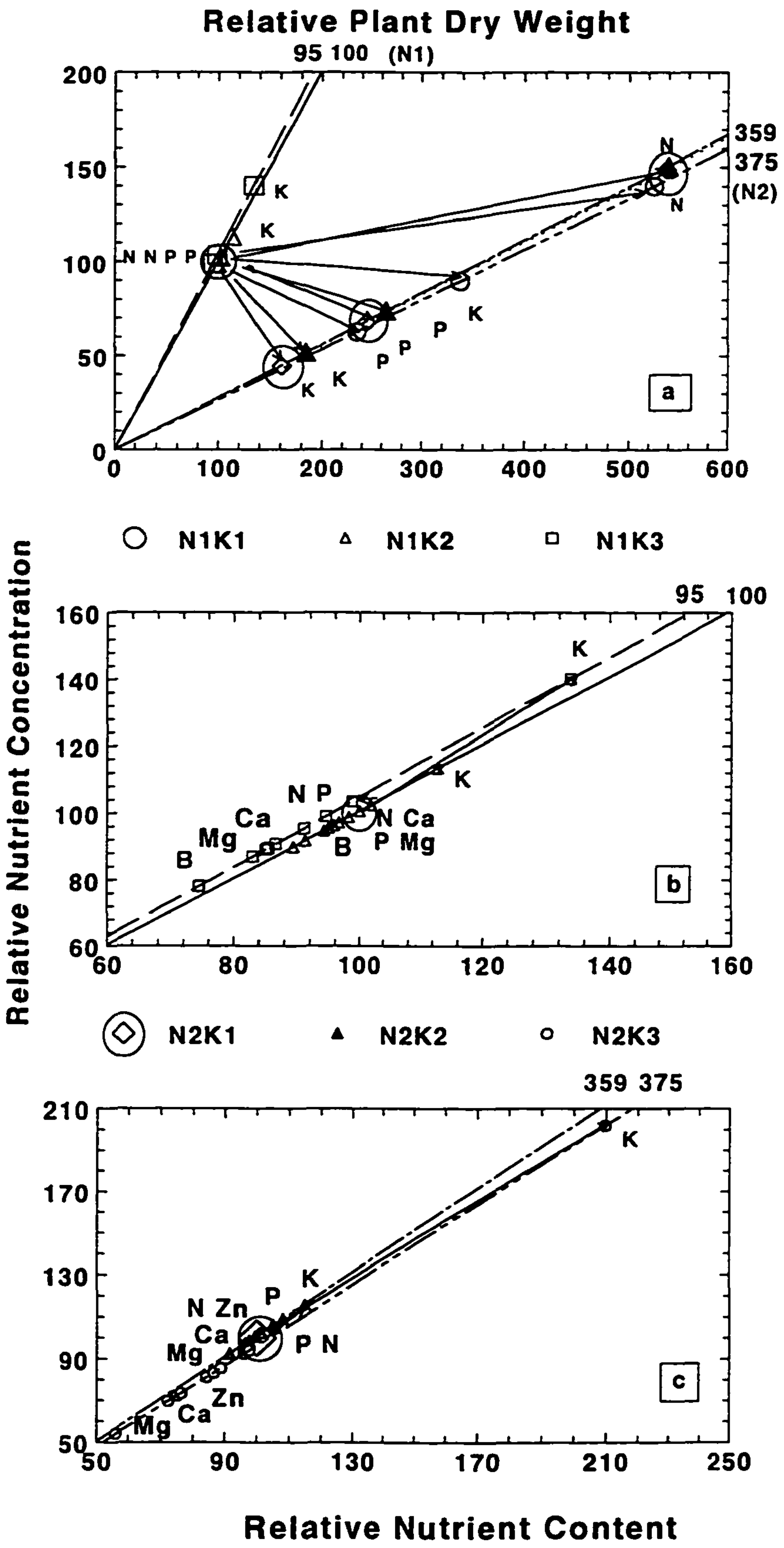

Relative Plant Dry Weight
95 100 (N1)
359
375
(N2)
200
150
100
50
0
0
100
200
300
400
500
600
N N P P
K
K
N
N
K
P
P
P
P
K
K
K
a
○ N1K1
△ N1K2
□ N1K3
95 100
160
140
120
100
80
60
60
80
100
120
140
160
K
K
N P
Ca
Mg
B
N Ca
B
P Mg
b
◇ N2K1
▲ N2K2
○ N2K3
359 375
210
170
130
90
50
50
90
130
170
210
250
K
K
P
N Zn
Ca
Mg
P N
Zn
Ca
Mg
c
Relative Nutrient Concentration
Relative Nutrient Content

Figure 3.7. Leaf loss in grams for trees droughted in July when nutrients are supplied as N1 (0.71 mM) and N2 (7.14 mM) at K1 (0 mM), K2 (0.26 mM), and K3 (2.57 mM) (n=36 trees per bar) (error bars represent standard error).

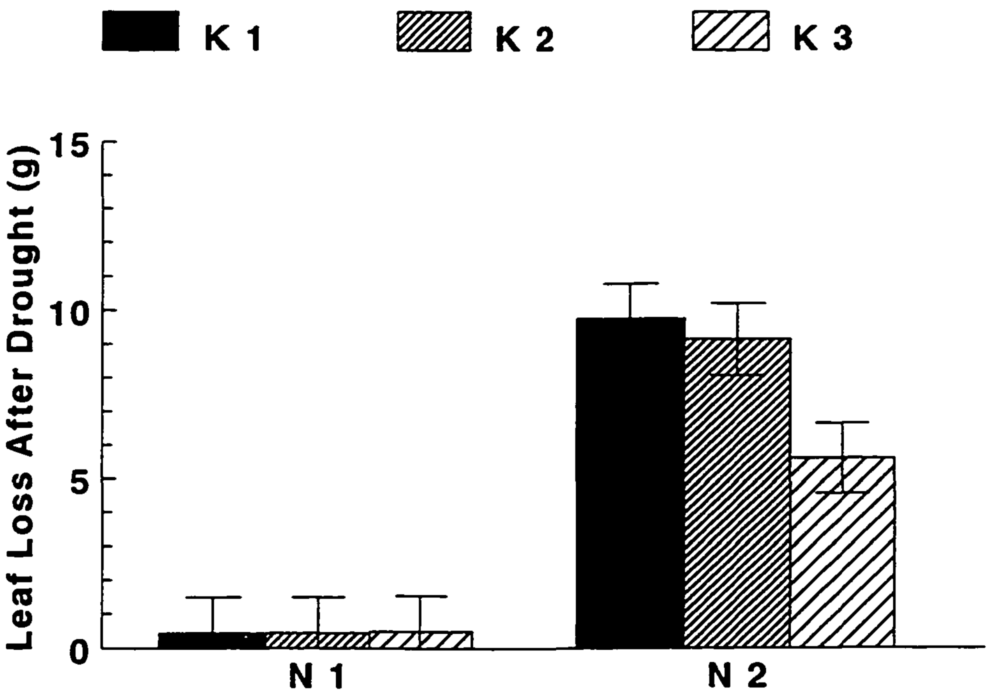

K 1
K 2
K 3
Leaf Loss After Drought (g)
15
10
5
0
N 1
N 2

Figure 3.8. Nutrients supplied at N1 (0.71 mM) and N2 (7.14 mM) at K1 (0 mM), K2 (0.26 mM), and K3 (2.57 mM) and a) vessel diameters were measured (n=36 per bar), which allowed calculation of b) percent of the stem cross-sectional areas which were vessel lumen.

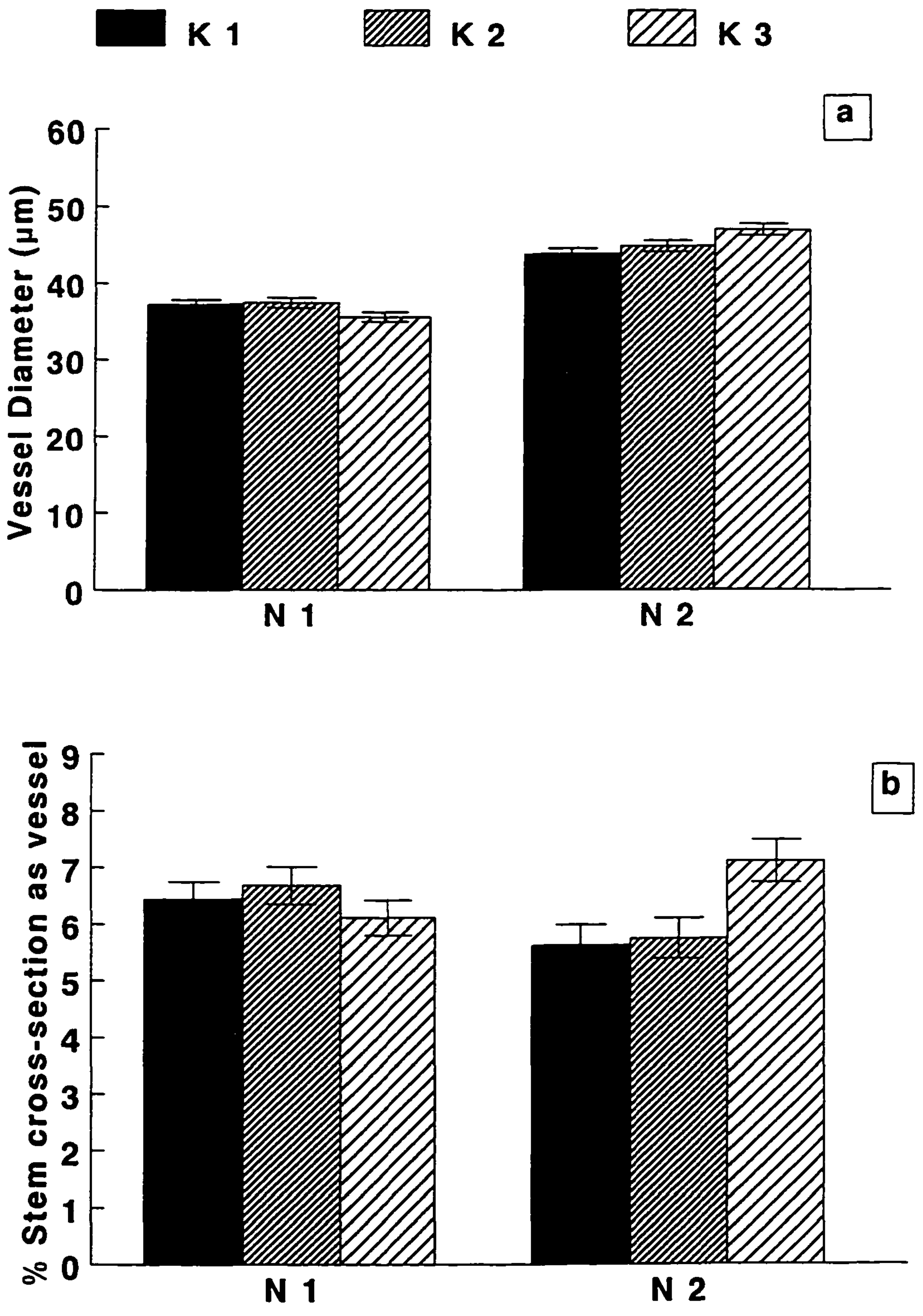

K 1
K 2
K 3
a
Vessel Diameter (µm)
0
10
20
30
40
50
60
N 1
N 2
b
% Stem cross-section as vessel
0
1
2
3
4
5
6
7
8
9
N 1
N 2

Figure 3.9. Anatomical characteristics which may affect cavitation of poplar clones 1 (49-177), 2 (184-411), 3 (*P. euramericana* Robusta), 4 (52-226), 5 (17-50) and 6 (*P. trichocarpa* Blom), where clones 1-3 are drought resistant and clones 4-6 are drought sensitive. Measurements included a) maximum vessel length (n=12 per clone), b) vessel diameter (n=36) and abaxial stomatal length (n=12) (error bars represent standard errors).

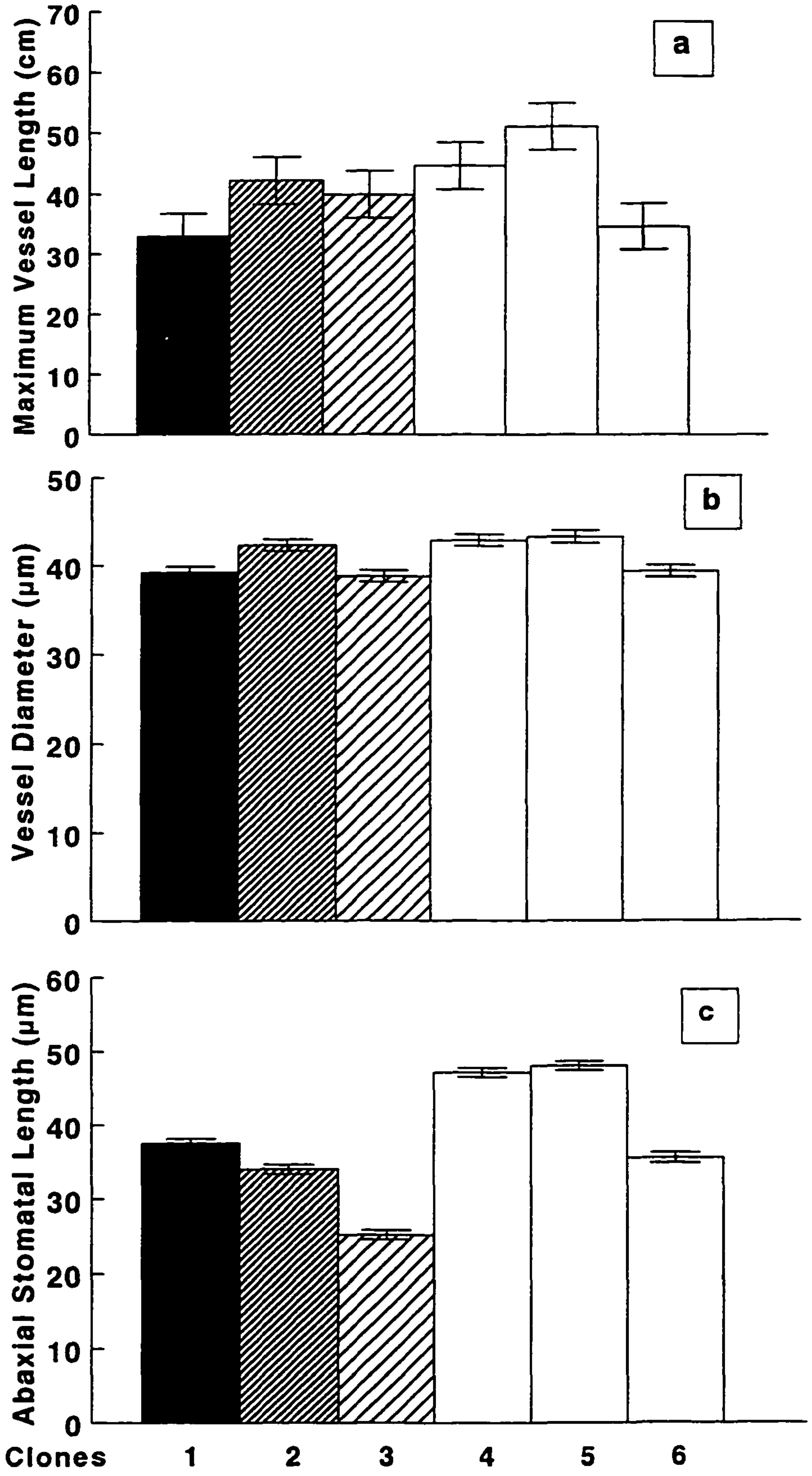

a
Maximum Vessel Length (cm)
0
10
20
30
40
50
60
70
b
Vessel Diameter (µm)
0
10
20
30
40
50
c
Abaxial Stomatal Length (µm)
0
10
20
30
40
50
60
Clones
1
2
3
4
5
6

Figure 3.10. Instantaneous transpiration rates of trees supplied with N1 (0.71 mM) and N2 (7.14 mM) at K1 (0 mM), K2 (0.26 mM), and K3 (2.57 mM), measured a) in July on well-watered trees (n=54 per bar) and b) in August after drought treatments (n=36) (error bars represent standard errors).

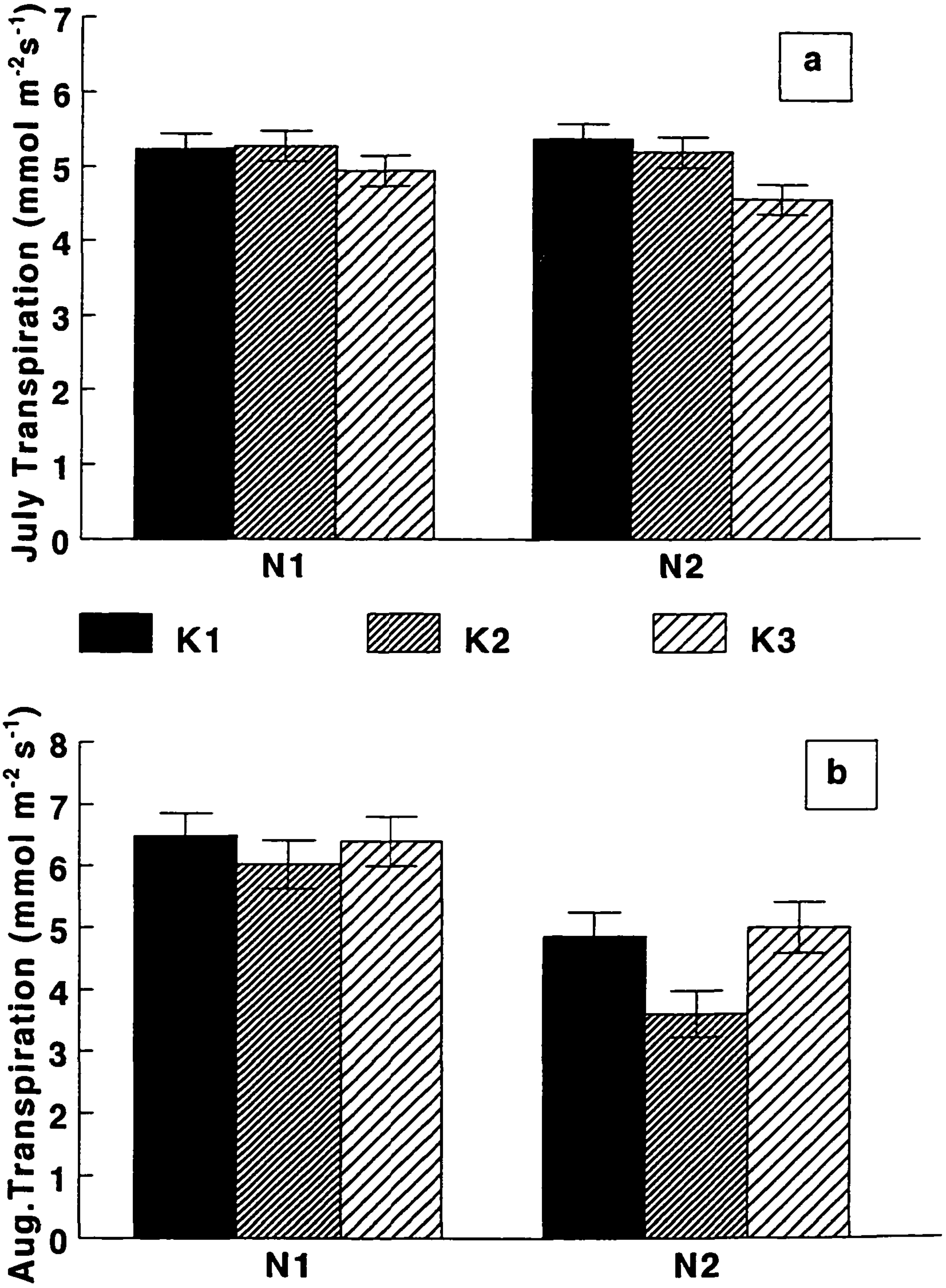
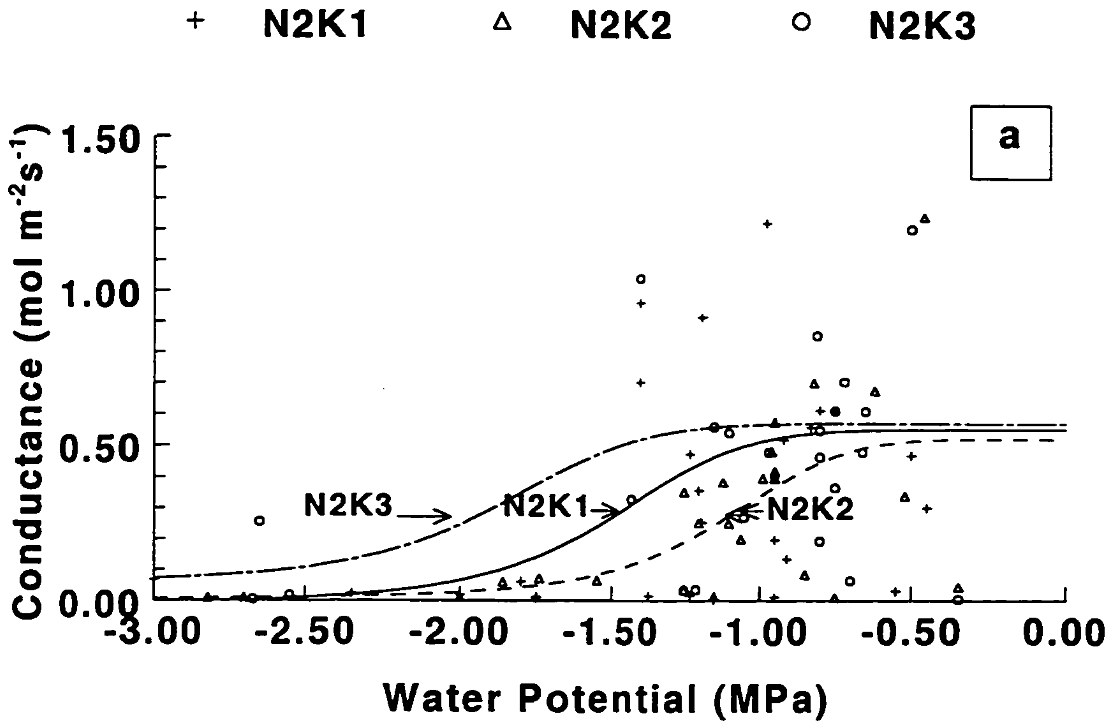
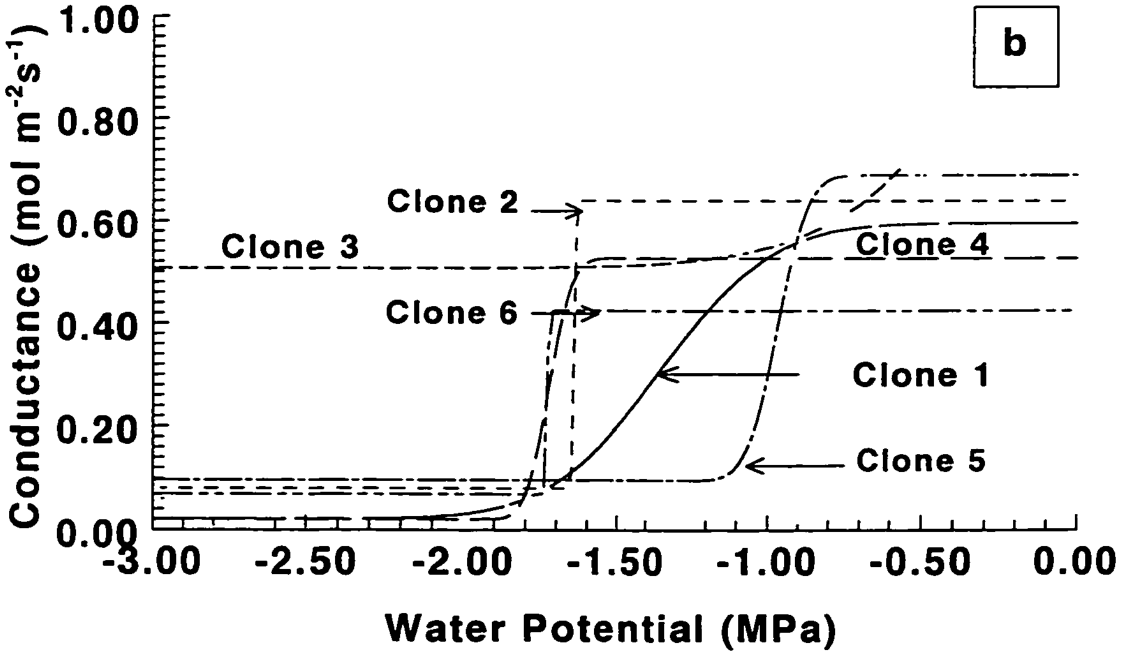

a
July Transpiration (mmol m-2s-1)
0
1
2
3
4
5
6
7
N1
N2
K1
K2
K3
b
Aug.Transpiration (mmol m-2 s-1)
0
1
2
3
4
5
6
7
8
N1
N2

Figure 3.11. Stomatal conductance plotted against xylem water potential measured in the leaf petioles. Cumulative (sigmoid) curves are fit to the data. Measurements were at the final harvest for a) trees supplied with N2 (7.14 mM) at K1 (0 mM), K2 (0.26 mM), and K3 (2.57 mM) (n=36 per bar) and b) unhardened poplar clones 1 (49-177), 2 (184-411), 3 (*P. euramericana* Robusta), 4 (52-226), 5 (17-50) and 6 (*P. trichocarpa* Blom) (n=24) (error bars represent standard errors).


+ N2K1
△ N2K2
○ N2K3
a
Conductance (mol m^{-2}s^{-1})
1.50
1.00
0.50
0.00
N2K3
N2K1
N2K2
-3.00
-2.50
-2.00
-1.50
-1.00
-0.50
0.00
Water Potential (MPa)


b
Conductance (mol m^{-2}s^{-1})
1.00
0.80
0.60
0.40
0.20
0.00
Clone 2
Clone 3
Clone 4
Clone 6
Clone 1
Clone 5
-3.00
-2.50
-2.00
-1.50
-1.00
-0.50
0.00
Water Potential (MPa)

Chapter 4

MINERAL NUTRITION AND DROUGHT RESISTANCE OF POPLAR: GAS EXCHANGE, OSMOTIC POTENTIAL AND PRECONDITIONING

4.1 INTRODUCTION

Fertilizing poplar plantation sites may affect drought resistance of *Populus*. For instance, van den Driessche (1984) concluded drought resistance is often decreased by high N levels and unchanged or increased by moderate N. Potassium is more often positively related to drought resistance (Christersson 1976).

Acidic soils are prone to K deficits (Clancy et al. 1995). Low K levels can decrease drought resistance of some trees like pine, although not spruce (Christersson 1976), and lead to loss of turgor and wilting with drought (Marschner 1986). Greater K supply may either increase or decrease transpiration (Christersson 1976). Christersson (1973) reported an inverse relationship between K and transpiration for drought-hardened but not for unhardened Scots pine seedings. Van den Driessche (1984) found both high and very low K decreased transpiration except in dormant seedlings, a quadratic effect which could explain some of the above contradictions.

Potassium did not change the water potential at which stomata closed in *Triticum aestivum* (wheat); instead it increased cytoplasmic desiccation tolerance (Pier and Berkowitz 1987). Potassium accumulated outside the chloroplast and could be exchanged for stromal H^+ with water stress, maintaining stromal pH and restoring photosynthesis. Potassium is one of the main osmotica in plants, leading also to higher levels of proline, and thus maintaining tissue water levels in drought (Marschner 1986). Osmotic adjustment maintains low osmotic potential by accumulating solutes to prevent tissue desiccation injury (Tschaplinski and Tuscan 1994). The adjustment is usually in the leaf in poplars, but one hybrid showed adjustment in the fine root.

In previous chapters, N fertilization led to decreased ψ_{xp} and increased cavitation in drought (Chapter 2, 3), while P improved the situation (Chapter 2). Increasing K supply had conflicting effects on ψ_{xp} and cavitation, depending on existing levels of the nutrient and on clones (Chapter 3). Here, the objective is to examine the effects of N and K fertilization, drought and preconditioning on photosynthesis, WUE and osmotic potential in potted clones of *Populus.*

Water use efficiency, or "ratio of dry matter produced to water used" (Kramer and Kozlowski 1979) is often chosen as a criterion for measuring resistance to drought. Nitrogen has been shown to increase WUE in conifers (Guehl et al. 1995, Brix and Mitchell 1986, Sheriff et al. 1986), decrease it in cotton (Radin and Parker 1979), or have no effect on well-watered Douglas-fir (Mitchell and Hinckley 1993). Excess N increased WUE of poplars under normal conditions but decreased it in moderate drought, although speeding recovery (Liu and Dickmann 1992b). High N increased ABA with drought for these clones, thus causing stomatal closure.

At full turgor, N did not affect osmotic potential of jack pine (*Pinus banksiana* Lamb.). Low N plants were more successful at maintaining turgor, mainly due to increased cell wall elasticity (Tan and Hogan 1995).

4.2 MATERIALS AND METHODS

4.2.1 Plant Material and Culture

Details of plant materials and their intensive culture are fully described in Chapter 3 (3.2.1-4). There were two blocks or greenhouses, six clones ranked in decreasing order of drought resistance, two levels of N (N1 or 0.71 mM and N2, 7.14 mM), three levels of K (0, 0.26 and 2.57 mM as K1, K2 and K3), three drought levels (controls, cyclically droughted, one severe drought), and three replications (n=648).

4.2.2 Gas Exchange and Osmotic Potential

Gas exchange measurements were made with a Li-Cor 6400 in July 1996 on all trees in greenhouse one (n=324). After that, the cyclical drought trees (n=108) were not watered for three days. Gas exchange was measured daily on 36 trees, and on the final day of drought, ψ_{xp} as well. Trees were rewatered for 10 days, and gas exchange and ψ_{xp} remeasured (n=36). Finally, they were droughted again along with the single-drought trees. After withholding water for three days, the final 4-day harvest began. Gas exchange and ψ_{xp} were measured on two out of three replications immediately before harvest (n=216).

Two days into the final drought, leaf osmotic potential of the ninth fully expanded leaf was determined with a Johns Scientific Wescor 5500 Vapour Pressure Osmometer. A leaf sample from each tree was placed individually in a marked plastic zip-lock bag in a cooler, and frozen within 15 minutes at -80°C to rupture cells and halt enzymatic changes. Osmotic potential of expressed leaf xylem solution was determined in mmol kg $^{-1}$.

4.2.3 Water Use Efficiency (WUE)

Instantaneous WUE was calculated from gas exchange measurements as net photosynthesis divided by stomatal conductance. Stomatal conductance, rather than transpiration, was chosen as the measure of water loss because it is not dependent on environmental variables such as vapour pressure deficit. Since the Li-Cor 6400 controls leaf temperature (set at 25°C to match average ambient conditions), some researchers believe that inherent differences in boundary layers and leaf temperatures (which would alter vapour pressure deficits) are not adequately accounted for by this method, in which case ^{13}C discrimination might be the method of choice for measuring WUE (Livingston, pers. comm.).

4.2.4 Nutrient Analysis

Leaf, stem and root nutrient content of the trees was determined at each drought level as described in Chapters 2 and 3.

4.3 RESULTS

Various levels of N and K supply affected pot-grown poplars in ways which might alter drought resistance. Morphological changes at N2 (Table 4.1) included increased tree weight, leaf area ratio, leaf loss, stomatal number per tree and per mm^2 (Chapter 3) and vessel diameters, and decreased stomatal length. Physical changes at K3 were only seen at high N supply, and included increased tree weights and vessel diameters, and decreased leaf area ratios ($p \leq 0.05$).

4.3.1 Water Potential and Osmotic Adjustment

Water potential and osmotic adjustment were altered by N and K supply (Table 4.2). Water potential was more negative at N2, but less so in hardened than unhardened droughted trees (Figure 3.3). Osmotic potential was more negative at N2 than N1, and osmotic adjustments (only seen in hardened trees) were greater at N2 (Table 4.2). Greater K supply did not affect osmotic potential overall. However, for some clones osmotic adjustment was greater at K3, while for others it was least at K3.

4.3.2 Water Use Efficiency (WUE)

Instantaneous WUE (determined by gas exchange) was influenced by nutrient supply (Table 4.2). Before drought, there was greater WUE (photosynthesis / conductance) at N2 than N1. This was because greater N supply increased net photosynthetic rates but not stomatal conductance (not shown) or transpiration. Although WUE was greater after the first drought ($1.3 \pm 0.0015 \times 10^{-4}$) than before drying ($2.1 \pm 0.000014 \times 10^{-5}$ vs) ($p=0.018$), hardening did not increase WUE significantly in successive droughts.

Nutrient treatment did not significantly affect WUE post drought, although it influenced gas exchange. Increasing N supply of once-droughted trees halved their net photosynthetic rates (Figure 4.1a) and transpiration rates (1.9 ± 0.4 mmol m^{-2} s^{-1} at N2 compared to 4.9 ± 0.4 mmol m^{-2} s^{-1} at N1) ($p \leq 0.001$).

Averaged over both N levels, WUE was greater at K3 than K2 or K1 (Table 4.2). The highest level of K decreased transpiration in well-watered trees, without decreasing photosynthesis. After drought, at K2, there was less photosynthesis (adjusted for water potential) and transpiration and conductance were reduced at N2. There was also less wilting at K3 ($p \leq 0.05$).

4.3.3. Preconditioning

Preconditioning, or a previous cycle of drought, altered several parameters of drought resistance. Net photosynthetic (Figure 4.1a) and transpiration rates during the final drought were higher in hardened than unhardened trees, water potentials were less negative, and there was less wilting (Chapter 3). There was osmotic adjustment, or an increase in osmolality, in hardened trees (Figure 4.1b). Leaf area ratios were greater (0.0046±0.00005 for hardened trees vs 0.0044±0.0005 for once-droughted and controls) but tree sizes smaller (49.3±0.7g vs 58.5±0.7 g for once droughted trees and 60.7±0.7 g controls). ($p \leq 0.05$) Hardened trees often developed small branches from the petiole scar, with many small leaves.

At the final harvest, drought hardened trees differed in nutrient concentrations from controls and once droughted trees. Hardened plants (at N2) had increased concentrations of leaf K, N, P, S, B, Cu, and Zn. At K3, leaf K did not increase with hardening. Also at N2, hardening increased stem K, N, P, S, and decreased stem Cu, and clones 1 and 5 had more stem Mn with hardening. Hardening increased root N and Ca at N2, and at N1, decreased root Al, Mg and Fe. There was less root S at the first drought but not after hardening ($p \leq 0.05$).

4.3.4 Late Season Changes

Net photosynthetic rates of well-watered trees increased from mid-July to mid-August, overall and at each N level (Figure 4.1c) ($p \leq 0.05$). Nutritional changes occurred during the same period (Table 4.3). Nutrient concentrations of K and P increased in well-watered trees, while N concentrations decreased (N supply was inversely related to K and P concentrations (Chapter 3)). Beginning July 30, fruiting bodies of the mychorrhizal fungus *Thelephora terrestris* (Shannon Berch, pers. comm., B.C. Ministry of Forests, Research Branch, Victoria, B.C.) began to appear, and were eventually in 8% of the pots of higher N supplied trees.

4.4 DISCUSSION

4.4.1 Nitrogen and Potassium Effects on Water Stress

Nitrogen generally increased water stress by decreasing water potential and increasing wilting, leading to stomatal closure. This was partially due to increased leaf area ratios. a common phenomenon in high N trees (Imo and Timmer 1992) which may lead to less water uptake per unit transpiring surface, and thus greater water stress (Oren and Sheriff 1995). More stomata per unit area and per tree might lead to more water loss per unit surface area as well. Increased N supply decreased P and K concentrations. Phosphorus could increase root conductivity (Skinner and Radin 1994, Andersen et al. 1989), so with more root P at low N there should be a better water supply per unit root surface area, coupled with relatively fewer leaves to supply. Larger vessels at high N might help adjust for relatively greater water needs from increased tree size.

Potassium generally decreased water stress. For some clones, it increased osmotic adjustment (which might have decreased wilting). Potassium increased ψ_{xp}, probably by decreasing transpirational water losses, as reported for Sitka spruce (Bradbury and Malcolm 1977) and drought hardened Scots pine (Christersson 1973).

4.4.2 Preconditioning (Hardening)

Drought preconditioning mainly occurred with the high N treatment, probably because this treatment was the most water stressed, so that hardening mechanisms were activated. Many leaf nutrients such as N and K became more concentrated with hardening at N2, which led to greater osmotic potentials and more osmotic adjustment. Theoretically, this should lower leaf water potentials while allowing turgor to be maintained, since ψ_{xp} equals osmotic potential plus turgor pressure plus gravitational pressure (Taiz and Zeiger 1991). However, hardened trees had less negative water potential. Stomata did not have to close, so transpiration and photosynthesis were maintained.

Greater concentrations of some nutrients with hardening might further increase photosynthesis: more N for Rubisco and Zn for activation of fructose-1,6-bisphosphatase for photosynthate partitioning (Marschner 1986). Thus, although higher N treatments led to more water stress during the first drought, the trees became hardened; in successive droughts they might continue to photosynthesize and grow, as for *Pinus resinosa* (Miller and Timmer 1994). However, in our work, leaf loss and stomatal closure in the previous drought period slowed growth so that in August the preconditioned trees were smaller. Earlier (Chapter 3), we found that cavitation which developed during the first drought was usually not reversed, so preconditioned droughted trees were at least as cavitated as once droughted trees (depending on severity of the previous drought). The cavitation would reduce the flow of water up the stem, but leaves lost during the first drought would also decrease the need for water. However, despite the loss of leaves during the first drought period, final leaf weight was decreased less than stem and root weight (greater leaf area ratios) with hardening. Increased leaf K and Cu might have decreased wilting in hardened trees (Marschner 1986), and less negative water potentials would also prevent wilting.

4.4.3. Osmotic Potential

Gebre and Kuhns (1991) speculated that hardening mechanisms of *P. deltoides* might include substances remaining in the leaves after drought (nutrients in our work), thicker cuticles, or greater water retention in cell walls. Hardened trees had greater foliar nutrient concentrations due to mobilization of nutrients during drought and rewatering (Chapter 5), and to decreased growth dilution. Cuticular thickness was not investigated in this study, but would probably be increased by low rather than high N supply (Kramer and Kozlowski 1979). Osmolality was positively related to N supply, and not to K, surprisingly (Marschner 1986). Perhaps compatible solutes like the amino acid proline (Taiz and Zeiger 1991) are more important to most poplar clones' osmotic adjustment. Gebre and Kuhns (1993) stated that inorganic ions were not important components of osmotic adjustment of two *Populus* clones, but Tschaplinski and Tuskan (1994) found N-based metabolites contributed little to osmotic adjustment of their clones.

4.4.4 Water Use Efficiency

High level of N supply increased WUE of undroughted, but not droughted, trees, as reported elsewhere for *Populus* clones in which increased ABA at high N supply accelerated stomatal closure (Liu and Dickmann 1992b). Since instantaneous WUE is photosynthesis divided by either transpiration or stomatal conductance, nutrient effects on WUE are due to their relative effects on these processes. Stomatal closure tends to decrease photosynthesis less than water loss and thus increase WUE in moderate drought, but with severe water stress, photosynthetic enzymes and chloroplasts are damaged (Taiz and Zeiger 1991). Before drought, increased N tended to increase photosynthesis without increasing stomatal conductance, thus increasing WUE. In drought, greater N supply decreased photosynthesis as much as conductance, indicating possible desiccation damage to the photosynthetic apparatus. Sometimes a productive *Populus* clone may resist this damage, increasing both stomatal closure and WUE in severe drought (Liu and Dickmann 1996).

Before drought, K increased WUE as for Sitka spruce (Bradbury and Malcolm 1977). After drought, photosynthesis was greatest at the highest levels of K and N. Perhaps K protected the mesophyll chloroplasts from drought-induced injury which may have occurred at high N supply, maintaining stromal pH (Pier and Berkowitz 1987). Moderate K supply decreased transpiration at high N at a given ψ_{xp}, but not enough to cause a statistically significant increase in WUE.

Pre-drought increases in WUE with either N or K fertilization might conserve soil water longer before water stress developed (but N may decrease drought resistance of trees once water supplies are depleted). Long-term productivity of a Douglas-fir stand was greatly increased by N fertilization, and soil water was only slightly depleted after seven years on an intermittently dry site (Brix and Mitchell 1986), so sometimes long-term as opposed to instantaneous WUE may be increased with greater N supply. However, many workers suggest limiting N fertilization on dry sites for conifers (Nilsen 1995, Raison and Myers 1992, Allen et al. 1990), since in severe drought N may increase water loss with little or no increase in growth (lower long-term WUE). Fertilization decisions may depend on the degree and frequency of drought: if short and not too severe, higher N might speed recovery (Liu and Dickmann 1992b) and allow rapid growth during the well-watered periods.

4.4.5 Late Season Changes

Photosynthetic rates of controls were greater in August than in July, despite lower concentrations of foliar N. Leaf K and P concentrations were higher in August, but K supply did not significantly affect photosynthesis in this experiment. However, relieving P and K deficiencies often increases photosynthesis (Marschner 1986), and even in August these nutrients were at less than critical levels for poplars (which are 1.8% for K and 0.24% for P) (McLennan 1996), especially at high N. Foliar P and K may have increased due to growth dilution of N, or because of late-season mycorrhizae. Deficiencies of P or K increase root exudates of sugars and amino acids which encourage mycorrhizal development (Marschner 1986). These increase P and K uptake by increasing root absorptive surface (Reid and Bowen 1979), thereby enhancing photosynthesis and growth (Marschner 1986). Although there were no fruiting bodies on the low N treated trees, the fungus was probably still present (Shannon Berch, pers. comm.) to increase photosynthesis at both N levels.

4.5 CONCLUSIONS

Drought hardening involved increased concentrations of many plant nutrients, which contributed to osmotic adjustment, and increased net photosynthesis (more leaf N and Zn). Increased nutrients in the stem and root might increase osmotic potential, and thus water potential gradients, increasing water uptake.

With ample water, both N and K increased WUE, but not in drought. Higher N supply increased water stress at the first experience of drought, perhaps due to increased leaf area ratios and more stomata, and perhaps due to decreased root P and thus less root conductivity. However, supplemental N increased hardening associated with osmotic adjustment. After hardening, N fertilization did not increase water stress as much, and K decreased water stress in drought, although it may not have affected hardening. Nitrogen fertilization levels should probably be tailored to site water supplies, and improving plant P and K status might increase water uptake and decrease transpirational water losses and mesophyll desiccation damage.

Table 4.1 Nutrient effects on physical parameters of droughted pot-grown poplars included tree weight (g), leaf area (LA) ratio ($m^2 g^{-1}$), leaf loss in drought (g) (lf loss), number of stomata per tree x 10^7 (stoma tr), and stomatal and vessel length (µm) (summarized from chapter 3). [1]

Variable	N1			N2			N
	K1	K2	K3	K1	K2	K3	p=
Tree wt	24.30 c	24.15 c	23.18 c	87.70 b	86.85 b	91.03 a	0.0120[2]
LA ratio	0.0044 b	0.0043 b	0.0042 b	0.0048 a	0.0047 a	0.0044 b	0.0001[3]
Lf loss	0.399 b	0.304 b	0.322 b	4.040 a	3.808 a	2.994 a	0.0001
stoma tr	1.870 b	1.841 b	1.888 b	9.277 a	10.131 a	10.568 a	0.0001
Stoma	39.77 a	38.59 ab	38.73 ab	36.27 c	36.74 c	37.28 bc	0.0001
Vessel	37.21 c	37.38 c	35.55 c	43.86 b	44.86 b	47.05 a	0.0009[2]

[1] Means within a row followed by the same letter are not significantly different according to the least squares means test.

[2] Probability for N x K interaction

[3] Probability of a statistically significant difference between levels of K $\leq$ 0.03.

Table 4.2. Nutrient effects on gas exchange parameters of pot-grown poplars included osmotic potential (ψ_π), osmotic adjustment ($\psi_{\pi\ ad}$), xylem water potential (ψ_{xp}), water use efficiency before droughting (WUE) (photosynthesis/conductance * 10^{-5}), photosynthesis (A) (µmol m^{-2} s^{-1}) adjusted for ψ_{xp}, change in A between first and final harvest averaged over all drought levels (A loss), initial transpiration (T) (mmol m^{-2} s^{-1}), final T adjusted for ψ_{xp}, and final stomatal conductance (g_s). [1]

Variable	N1			N2			N
	K1	K2	K3	K1	K2	K3	p=
ψ_π MPa	-1.437 b	-1.432 b	-1.432 b	-1.527 a	-1.520 a	-1.532 a	0.0001
$\psi_{\pi\ ad}$ "	0.027 b	0.039 b	0.023 b	0.163 a	0.080 b	0.172 a	0.0001
ψ_{xp}	-0.888 a	-0.814 a	-0.740 a	-1.207b	-1.130 b	-1.093 b	0.0001
WUE	1.791 c	1.722 c	1.825 c	2.662 ab	2.995 ab	3.514 a	0.0001[2]
A / ψ_{xp}	10.33 ab	8.04 c	8.76 bc	10.62 ab	8.52 bc	12.30 a	0.049 [2]
A loss	-1.56 b	0.51 b	-1.12 b	3.52 a	3.03 a	-1.05 b	0.014 [3]
T initial	5.237 a	5.272 a	4.942 ab	5.381a	5.203 a	4.568 ab	0.004 K
T /ψ_{xp}	6.48 a	6.02 a	6.40 a	4.86 b	3.60 c	5.00 ab	0.0001 [2]
g_s	0.577 a	0.395 ab	0.448 ab	0.223 b	0.032 c	0.240 b	0.002

[1] Means within a row followed by the same letter are not significantly different according to the least squares means test.

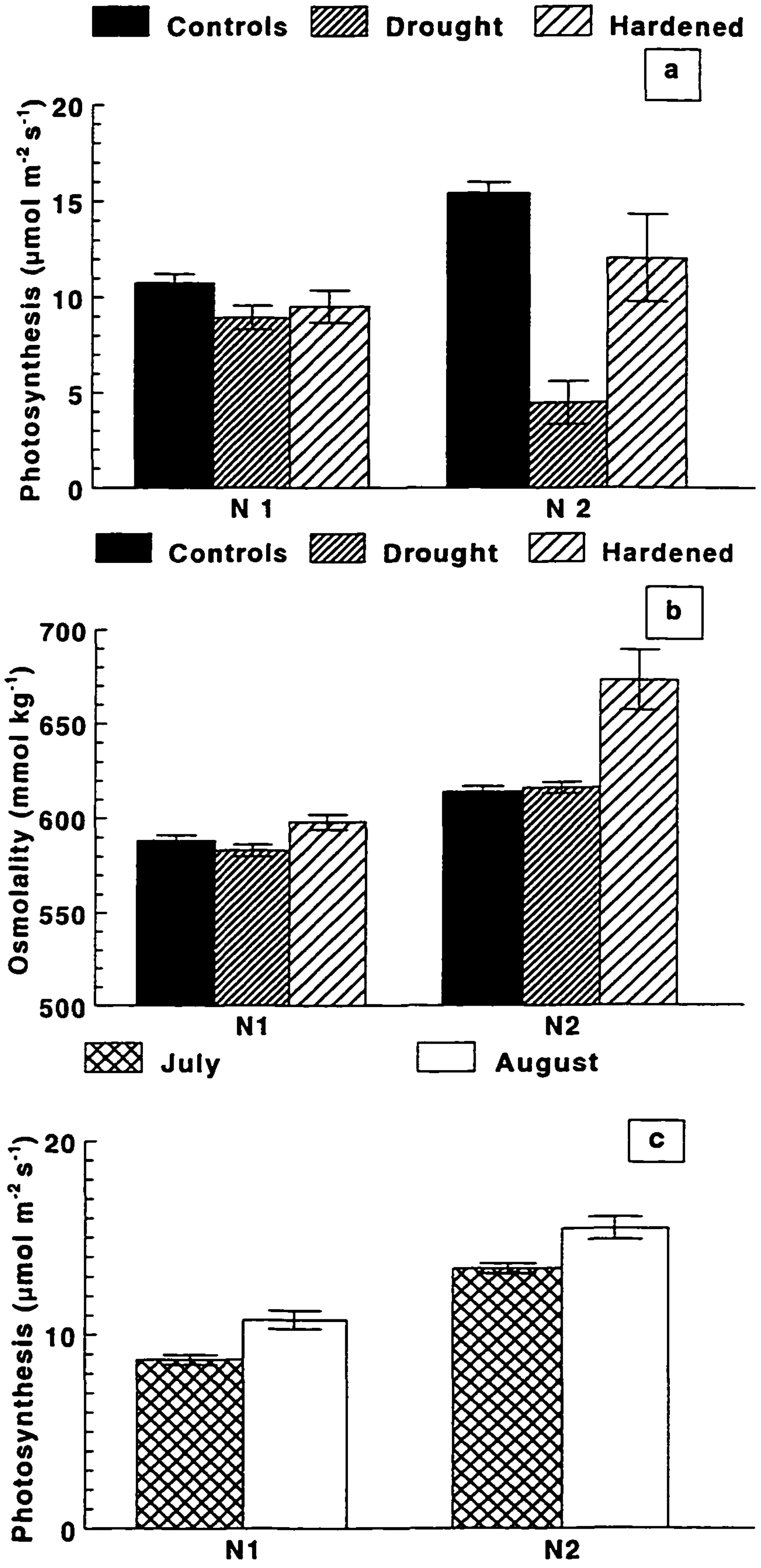
[2] Probability of a statistically significant difference between levels of K $\leq$ 0.03

[3] Probability for N x K interaction

Table 4.3. Foliar nutrient concentrations (g/100g) of well-watered controls, mid July and mid August 1996, at two levels of N supply (N1, 0.71 mM and N2, 7.14 mM).

Nutrient	N1			N2		
	July	August	p≤	July	August	p≤
N	0.845	0.791	0.0022	1.177	1.007	0.0001
P	0.162	0.202	0.0001	0.098	0.110	0.0549
K	1.224	1.694	0.0001	0.751	0.880	0.0060

Figure 4.1. Differences between well-watered controls, once-droughted trees, and droughted hardened trees in a) net photosynthetic rates in August at two levels of N, N1 (0.71 mM) and N2 (7.14 mM)) (n=36 per bar); and b) osmolality in August at N1 and N2 (n=108); and c) net photosynthetic rates of controls in July and August (n=36 per bar) (error bars represent standard errors) .


Controls
Drought
Hardened
a
Photosynthesis (µmol m-2 s-1)
0
5
10
15
20
N 1
N 2
Controls
Drought
Hardened
b
Osmolality (mmol kg-1)
500
550
600
650
700
N1
N2
July
August
c
Photosynthesis (µmol m-2 s-1)
0
10
20
N1
N2

Chapter 5

MINERAL NUTRITION AND DROUGHT RESISTANCE OF POPLAR: NUTRIENT RETRANSLOCATION

5.1 INTRODUCTION

Poplars tend to shed their leaves in drought as a cavitation-avoidance mechanism (Rood and Mahoney 1990). If nutrients were transferred before abscission from the leaf to the stem and root, efficiency of retranslocation might improve productivity (or at least reduce loss of productivity) under drought. Foliar N is retranslocated in drought from perennial C_4 grasses, so that photosynthetic rates after rewatering are lower than predrought (Heckathorn and DeLucia 1994). There must be a balance between photosynthetic losses and N conservation. Phosphorus and sometimes N retranslocation are less in drought than in fall in *Viburnum acerifolium* L. (maple-leaf viburnum) (Minoletti and Boerner 1994), and fall retranslocation is reduced by drought in *Hordeum vulgare* (barley) (Pugnaire and Chapin 1992), so early leaf loss due to drought may reduce internal nutrient recycling. If productive poplar clones do shed their leaves with drought, they may resorb more nutrients beforehand than unproductive clones.

Transfer of nutrients out of the leaves of deciduous trees before fall senescence may also increase long-term productivity (Killingbeck 1996), and increased retanslocation on nutrient-poor sites should confer a selective advantage. Sometimes this occurs (Pugnaire and Chapin 1992), but often there is greater retranslocation efficiency on fertilized than on unfertilized sites (Crane and Banks 1992). Nitrogen is indeed retranslocated in the fall for some poplar clones, more so in a more productive clone (Pregitzer et al. 1990). *Populus tremuloides* recovered about half of its leaf N and P, and 10% of leaf copper in fall (Killingbeck et al. 1990). Nitrogen, P or K supply, or clonal productivity on dry sites may be related to drought or fall retranslocation of essential nutrients in poplar.

Sometimes N, P and K accumulate during drought rather than being retranslocated, which has the advantage of increasing osmotic adjustment (Mattson and Haack 1987, Kemp and Moody 1984). Potassium may be a component of osmotic adjustment in poplars, part of the solute accumulation which lowers osmotic potential in drought and prevents tissue injury (Tschaplinski and Tuscan 1994). Thus, if not retranslocated, increased N and K supply might improve osmotic adjustment and drought preconditioning.

The objective of this portion of the study was to determine the extent of poplar nutrient retranslocation, both in drought and in fall, and its effect on drought resistance. Pot-grown *Populus* clones that retranslocate more efficiently may be more productive on dry sites, so the ability to conserve nutrients by resorption might provide another selection criterion. Nitrogen and K were varied, and drought resistant and drought sensitive poplar clones were subjected to three levels of drought, including cyclic drying. Nutrient concentrations were determined before and after each phase of drought and at abscission in fall to help resolve these questions.

5.2 MATERIALS AND METHODS

5.2.1 Plant Material and Culture

5.2.1.1 Experiment 1

Plant material and culture are described in Chapter 2 (2.2.1 to 2.2.2). Four clones of *Populus trichocarpa* x *P. deltoides* (T x D) (49-177, 15-29, 52-237 and 52-226) were greenhouse-grown in a sub-irrigation sand culture system. Nutrient treatments were three concentrations of N (0.36, 1.43 and 7.14 mM as NH_4NO_3) at two concentrations of P (0.10 and 0.65 mM as KH_2PO_4).

5.2.1.2 Experiment 2

Plant material and culture are described fully in Chapter 3 (3.2.1 to 3.2.4). Two greenhouses contained 720 potted poplar cuttings from six clones (numbered 1-6 in order of decreasing drought resistance) (49-177, 184-411, *P. euramericana*, 52-226, 17-50 and *P. trichocarpa)*. They were subjected to two N levels (N1 being 0.71 mM and N2, 7.14 mM), three K levels (K1 at 0, K2, 0.26 and K3, 2.57 mM), and three drought treatments, with three replications per nutrient treatment per clone randomly arranged in each greenhouse. .

Thirty-six trees per greenhouse (one extra replication per greenhouse per clone by nutrient treatment) were randomly distributed amongst the others. These trees received ample water and were in every way treated like the controls, but were not included in the cavitation experiments (Chapter 3). At the conclusion of the drought treatments in August 1996, these 72 trees had outgrown the greenhouses and were moved outdoors in their pots to a fenced compound with automatic sprinklers. Here they continued growing until leaf loss. Twice weekly fertilization was continued until the first leaves fell, then once weekly thereafter.

5.2.2 Nutrient sampling

5.2.2.1 Experiment 1

During their growing season, 192 trees were dried for 0, 4, 6 and 8 days to achieve a gradation of xylem water potentials (ψ_{xp}). Then leaves, stems and roots were harvested, dried at 80°C, weighed, ground, and submitted for nutrient analysis (2.2.2).

5.2.2.2 Experiment 2

Complete shoots were removed at five different sampling dates (Table 5.1). These shoots were separated into stem and leaf, dried at 80°C, weighed, combined for composite samples and ground in preparation for nutrient analysis. After the final harvests ("d" and "e," Table 5.1), the entire root system was washed, dried, weighed, ground and analysed for nutrient content. Chemical analyses of macro- and micronutrients were carried out (2.2.3) by technicians at the chemistry lab at the B.C. Ministry of Forests Glyn Road Research Station.

Thus, there were three drought treatments (Table 5.1):

1) controls, always well-watered,

2) one drought (trees droughted once at the end of the experiment, time "d" below) and

3) cyclical drought (trees droughted at time "b," rewatered at "c," redroughted at "d").

There were five sampling times (Table 5.1):

a) well-watered (n=108 leaf and stem samples per greenhouse, 36 per drought level),
b) post-cyclical-drought (n=36 leaf and stem samples per greenhouse)
c) rewatered (n=36 for each plant part per greenhouse)
d) final drought (n=108 leaf, stem and root samples per greenhouse, 36 per drought level)
e) fall (n=72 leaf, stem and root samples).

Drought treatments 1 and 2 were only sampled at times "a" and "d," always as 3-replicate composite samples. Drought treatment 3, cyclical drought, was sampled four times, and "a," "b" and "c" were single samples and "d" a 3-replicate composite sample.

Trees saved for fall nutrient determination "e" continued to grow until leaf abscission. Beginning in September, twice a week all yellow leaves were touched gently, and if they abscised, were collected, counted, dried and weighed. This ensured that leaves did not drop off unnoticed, and permitted recording of leaf loss dates. When the last remaining leaf fell November 11, all 72 trees were harvested. Leaves, stems and roots were dried, weighed, ground, and submitted for nutrient analysis (individual samples).

5.2.3. Leaf area

In Experiment 1, total leaf area of each tree was determined (Li-Cor Model 3100 leaf area meter).

In Experiment 2, leaves removed for ψ_{xp} determinations were measured for leaf area with the Delta-T Leaf Area Meter. They were then weighed, and a conversion factor calculated for each tree to estimate leaf area from total leaf weight.

5.2.4 Retranslocation Determination

In Experiment 1, nutrient concentrations and leaf nutrient pools at the four drought levels were compared.

In Experiment 2, nutrient mobilization was determined in two ways: at the final harvest, by comparing controls with once-droughted and cyclically droughted trees at the same date; and before and after each drought, comparing nutrient concentrations and nutrient pools for different shoots of the same tree (or an identically treated one).

Following Pugnaire and Chapin (1992), the following definitions were applied:

1) Retranslocation was the difference in nutrient pools in green versus senesced leaves.

2) Nutrient pools were grams of nutrient per square metre of leaf area.

3) Retranslocation efficiency was pool of resorbed nutrient (maximum minus minimum pools) divided by nutrient pool at maximum, converted to a percentage.

Retranslocation was also considered as difference in percent nutrient content at the various levels of drought (Heckathorn and DeLucia 1994). There was retranslocation if a nutrient was reduced in the foliage ($p \leq 0.05$) after either drought (drought retranslocation) or fall senescence (fall retranslocation).

4) Resorption proficiency, or terminal percentages, were considered most important by Killingbeck (1996). If N was < 0.7% and P < 0.05%, trees were considered proficient. The ultimate proficiency levels are thought to be at 0.3% for N and 0.01% for P.

5.2.5 Statistical Analysis

5.2.5.1 Experiment 1

Statistical methods are fully described in Chapter 2. As a randomized complete block split plot design, all main effects were tested against their interactions with block. SAS was used as below (5.2.5.2), with planned contrasts between drought resistant and drought sensitive clones, and for linear and quadratic N.

5.2.5.2 Experiment 2

The experiment was a randomized complete block factorial design (Appendix 2). Because composite samples were made of the three replications, there were zero degrees of freedom within each block for nutrient analysis, although there was replication due to the two greenhouses. Thus, a conservative test was used, whereby the largest interaction mean square was used as the error term instead of experimental error. This artificially underestimated the F value, so results were more significant than they appeared (Anderson and McLean 1974), which should be considered when ANOVA results are marginal ($p=0.05$ or slightly greater).

SAS (Statistical Analysis System) general linear models procedure (proc glm) was used for most tests. Means were compared with the Duncan Multiple Range test and Least Square Means. Planned contrasts were performed between drought resistant and drought sensitive clones, diploid versus triploid T x D hybrids, controls versus once-droughted trees, and linear and quadratic K. Repeated measures tests were used where consecutive tests were made on the same tree, with results further amplified by ANOVAs performed on the differences between the two measurements. In any case, each test was performed on a different stem and after different drought treatments.

5.3 RESULTS

5.3.1 Water Potential

Mean predawn ψ_{xp} in Experiment 1 decreased at each drought level (p=0.0410) (-0.15, -0.25, -0.45 and -0.72 MPa at 0, 4, 6 and 8 days of drought, with ψ_{xp} on days 0 and 4 significantly less negative than on days 6 and 8). Daytime ψ_{xp} was not significantly different at 0, 4 and 6 days (averaging -0.59 MPa) but was more negative after 8 days of drought (-0.92 MPa) (p=0.049).

Mean daytime ψ_{xp} in Experiment 2 varied by N treatment for droughted trees at each sampling date (p=0.0001) (Figure 3.3b), and by drought treatment at the final harvest (Figure 3.3a) (p=0.0001). Nitrogen fertilization decreased ψ_{xp} (-0.81 MPa at N1 v. -1.15 MPa at N2), and ψ_{xp} was higher in well-watered controls (-0.76 MPa) than in hardened droughted trees (-0.95 MPa), which were less stressed than once-droughted trees (-1.1 MPa) at the final harvest (p=0.0001).

5.3.2 Effects of Drought on Growth

In Experiment 1, control trees continued to grow (35.18 ± 1.06 g), but droughted trees weighed significantly less (averaging 28.31g with NSD at days 4, 6 and 8). Root weights, however, were NSD between droughted and control trees ($p \leq 0.05$).

In Experiment 2, leaf weight did not vary with drought level at N1. At N2, leaves of well-watered trees (37.79 ±0.42 g) were NSD in weight from droughted trees (36.63

±0.42 g), but leaves of hardened trees weighed significantly less (31.62 ±0.42 g) ($p=0.0001$). At both N levels, stems of well-watered trees (22.86±0.34 g) were NSD in weight from droughted trees (22.71±0.34 g), but again stems of hardened trees weighed significantly less (18.82±0.34 g) ($p=0.0001$). Roots of well-watered trees weighed more (14.21±0.23 g) than droughted roots (13.27±0.23 g), and both were heavier than roots of hardened trees (10.30±0.23 g) ($p \leq 0.0001$) (variances are standard errors).

5.3.3 Nutrient Mobilization

In Experiment 2, following changes in foliar nutrient pools in the same or similarly treated trees with rapid drought in July, increases of B, Cu, P, S, Zn and K occurred, and only K decreased again with rewatering (Figure 5.1). Foliar N and Mn did not increase during drought, but they did with rewatering ($p \leq 0.05$). Only foliar Mg and Ca may have decreased in drought, and that only marginally significantly in the first cyclic drought.

Nutrient concentrations were compared at the final August harvest for well-watered trees, those droughted rapidly once, and hardened droughted trees (Table 5.2). One drought increased foliar Cu concentrations, but hardened trees had higher levels of B, Cu, K, N, P, S and Zn compared to both well-watered trees at this harvest, and in July (Table 5.3). Stem nutrient concentrations were less affected by rapid drought, but K, P and S increased in the stem as well as the leaf (Tables 5.2). There were decreases in root Al and S, and, with preconditioning, in K and Mg (at low N supply). Only Ca concentrations increased with drought in the root ($p \leq 0.05$).

In Experiment 1, where drought was longer and slower, foliar N (Figure 5.2a) and P (Figure 5.2b) nutrient pools and concentrations (Table 5.3) all decreased with drought. There were concurrent increases in concentrations of stem and root N, no change in stem P, and marginal decreases in root P (Table 5.3). Leaf Cu did not change significantly with slow drought, but at high levels of N supply there were increases in concentrations of foliar Ca, Mg and Fe ($p \leq 0.05$). Changes in nutrient contents (concentration*weight) of stem and root were NSD before and after drought.

Nitrogen and Cu were resorbed from the leaves in fall in Experiment 2, and foliar K and P concentrations and nutrient pools also decreased at the higher N supply (Tables 5.2, 5.3). Leaf B, Ca, Mg, Mn and Zn increased in fall. A decrease in stem concentrations was seen for Al, Ca, Mg, Fe (at N1), P (at N1), Cu (at N2), S (at N2) and K, but total stem content of K, and of P in clone two, increased. Stem concentrations of Mn, N and Zn increased. Fall root concentrations were lower for Al, Ca, Fe, K (at K3 but not in clone 6), Mg, Mn, N (except at N2), S, and Cu, but total root Cu content increased. There were also higher root concentrations of B, and of P at higher N supply ($p \leq 0.05$).

5.3.4 Effects of Nutrition on Nutrient Mobilization in Drought

Nitrogen fertilization had much more effect on nutrient mobilization in drought than either K or P augmentation. Effects below are all significant statistically ($p \leq 0.05$). In Experiment 2 (not shown), a higher level of N supply caused:

1. drought-induced mobilization of B, Cu and S (nutrient pools) into the leaves;
2. leaf nutrient pools of N, P, Zn and Mg to accumulate in hardened droughted trees;
3. concentration increases of K, N, P and S in hardened droughted leaves;
4. greater increases in concentrations of N and S in the stems in droughted compared to undroughted trees;
5. greater decreases in concentrations of Cu in the stems of droughted trees compared to undroughted trees;
6. increased concentrations of stem P with drought in hardened trees;
7. root Mg concentration and root N content to increase with drought; and
8. higher concentrations of N in hardened compared to unhardened roots.

In Experiment 1, higher N supply caused foliar Mg, S and Fe concentrations to increase in droughted relative to undroughted trees (not shown).

K fertilization, like N fertilization, significantly affected absolute quantities of nutrients at every level of drought, but did not change nutrient movement in drought.

5.3.5 Effect of Clone on Drought Mobilization

There were no significant effects of clone on drought mobilization of nutrients in

Experiment 1. However, in Experiment 2 (Table 5.4), leaf Cu nutrient pool increased in all but clones 3 and 6. Leaf K increased in clone 2 with one drought and decreased in clones 3 and 4. After hardening and redroughting, clones 1, 2 and 4 had increased K but clones 5 and 6 were NSD and clone three lost K during hardening. Magnesium increased significantly after drought only in clones 2 and 5. Clone 3 retranslocated leaf P at the first drought, clone 2 increased P nutrient pool, and clones 1, 4, 5 and 6 only increased in P significantly after hardening. Leaf S was NSD for clone 6, increased after the first drought for clone 2, and increased after hardening only for clones 1, 4 and 5, while in clone 3 it actually decreased with hardening ($p \leq 0.05$).

5.3 6 Fall Retranslocation Efficiency

5.3.6.1 Retranslocation Efficiency (RE) and Fertilization

Four nutrients were resorbed from the leaves in fall (Cu, K, N, P), and fertilization affected the retranslocation efficiency of all four (Figure 5.3)

Leaf Cu was retranslocated most efficiently with the highest level of K fertilization (K3) ($p=0.021$), but N fertilization did not significantly affect it (Figure 5.3). Potassium was more efficiently retranslocated at N2 ($p=0.0001$), and in fact was not retranslocated at the lower N level. At high N supply, it was least efficiently resorbed at K3 ($p=0.0003$) ($p=0.002$). The higher level of N supply also increased RE of N ($p=0.002$). At both N levels, K3 led to the greatest RE for N (K $p=0.0004$) (NxK $p=0.014$). Finally, more N supply increased RE of leaf P ($p=0.0001$), and as was the case for K, there was no retranslocation at the lower N level. However, unlike for K resorption, leaf P was more efficiently retranslocated at K3 than at K1 (linear contrast $p=0.037$).

5.3.6.2 Fall Retranslocation Efficiency and Clone

The six clones in Experiment 2 varied in their ability to retranslocate nutrients in fall ($p \leq 0.05$) (Figure 5.4). Clone 6 (*P. trichocarpa*), the least productive clone (Chapter 3), was the most efficient for both Cu and N retranslocation, and Clone 3 (*P. euramericana*) was least for Cu, P and K, although not statistically different from some other clones. Drought resistant clones 1-3 were least efficient at retranslocating P ($p=0.002$), and comparing the diploid and triploid (more and less productive in drought respectively) T x D hybrids, the diploids retranslocated both P and K least efficiently ($p \leq 0.05$).

5.3.6.3 Resorption Proficiency

At all N and K fertilization levels, and for all clones, these poplar trees are proficient at resorption of N, approaching the ultimate levels, but not proficient at P resorption (Table 5.5). Increased N supply decreased resorption proficiency of N, P and Cu but increased K resorption proficiency. More K fertilization increased N and Cu resorption efficiency, decreased K efficiency and had no significant effect on P ($p \leq 0.05$).

Clones varied in their resorption proficiencies (Table 5.5). All clones were proficient at resorbing N but not P. Clones which grew well in drought were not more proficient, although Clone 1 ranked fairly highly, and Clone 6 fairly low.

5.3.7 Leaf Retention

5.3.7.1. Leaf Loss During Drought

It was earlier shown that N fertilization increased leaf loss during drought (partly because it increased drought stress), and K fertilization decreased leaf loss (Figure 3.7). The more drought-sensitive triploid T x D hybrids also lose more leaves than all other clones (Figure 5.5).

5.3.7.2 Leaf Loss in Fall

Fertilization with the highest level of K postponed final fall leaf loss ($p=0.02$) by 1.5 days on average, but initial leaf loss was earlier ($p=0.02$) by nine days (not shown). The most productive T x D hybrids on dry sites had the longest growing season until first leaf loss (contrast $p=0.026$) (Figure 5.6a), although they also had the shortest time to final leaf loss ($p=0.0001$) (Figure 5.6b). Clone 4 flushed a few days sooner in the spring than all other clones (not shown), which lengthened its total growing season, but it grew less between August and November than clones 5 and 6 (p=0.009) (Figure 5.6c). The growth of clone 5 in fall, probably due to longer leaf retention, made it not significantly smaller than the productive clones 1 and 2 by season's end.

5.4 DISCUSSION

5.4.1 Drought Intensity and Nutrient Mobilization

Drought-induced mobilization of foliar nutrients varied considerably between experiments: gradual application of drought resulted in retranslocation of N and P, but short sudden drought led to nutrient accumulation. Perhaps contradictory experimental results in the literature regarding concentration versus retranslocation of nutrients in drought may be due to variations in speed and severity of drying. Comparison of the two experiments is complicated by concentrations of N being lower in Experiment 2, despite similar levels of supply (7.14 mM high N treatments). Recirculating nutrient solutions in Experiment 1 greatly increased foliar concentrations compared to twice-weekly fertilizing in Experiment 2, with gradual leaching in between.

During slow drying in Experiment 1, nutrient movements more closely resembled those at fall leaf senescence, with N and P resorbed from the foliage. Nitrogen was probably retranslocated to the stem and roots, but the fate of P was less clear. Lower N or P concentrations in well-watered stems or roots could be partly due to growth dilution. As in fall, other nutrients became more concentrated in the leaf: Ca and Mg at higher N supply, and Fe, S and Zn. Unlike the situation in fall, K and Cu were not retranslocated from the leaves, and in fact K showed a marginal increase.

A rapid three-day drought in July caused no retranslocation out of the leaves except for a marginal decrease in Mg and Ca. Nitrogen concentrations remained stable during drought (while most other foliar nutrients increased), but increased with rewatering and were maintained at high levels until the second cycle of rapid drought in August. These high foliar N concentrations in hardened trees probably contributed to osmotic adjustment, and to enhanced photosynthesis and leaf water potentials during subsequent drought. Speculation on causes of nutrient movements in drought and fall (resorption of

Cu, K, N and P and concentration of most other nutrients), involves consideration of plant hormones.

5.4.2 Plant Hormones and Nutrient Mobilization

ABA is the hormone that initiates leaf senescence, and perhaps the series of events that lead to nutrient retranslocation (Thimann and Tan 1988). ABA probably stimulates ethylene production, which in turn decreases transport (and later increases the breakdown) of auxins. Normally auxins inhibit encoding of hydrolytic enzymes, so with a decrease in auxins, hydrolytic enzymes are produced which break down proteins, nucleic acids and chlorophyll (Taiz and Zeiger 1991). Rubisco, light harvesting protein and chlorophyll are broken down into their amino acid constituents, with arginine probably the main winter storage amino acid (Sagisaka 1974). Phosphorus is retranslocated in inorganic form as nucleic acids, ester P and lipid P (van den Driessche 1984). With short days in fall, arginine, other amino acids and soluble N may form bark storage proteins (BSPs) in poplars (Coleman et al. 1994, Langheinrich and Tischner 1991). Since bark storage proteins resemble the products of Win4 gene, perhaps Win4 products control accumulation and translocation of leaf N (Coleman et al. 1994).

Auxins (mainly IAA) from the leaf apical meristem at first inhibit, then later facilitate senescence (Taiz and Zeiger 1991). In the first stages of drought, when auxin transport is inhibited but breakdown has not yet begun, IAA may be controlling the nutrient concentration increases. Alternatively, cytokinins (particularly zeatin) are known to cause selective mobilization and accumulation of nutrients in various plant organs, and to inhibit senescence. Lower N supply decreased cytokinin-like substances in birch, leading to leaf senescence and retranslocation of foliar nutrients from older leaves to apical shoots (Darrall and Wareing 1981). In the present experiment, retranslocation and senescence were not inversely related to N supply, but conceivably greater N supply increased cytokinin concentrations. The ratio of cytokinin to auxin is considered significant: perhaps as auxins break down, a higher ratio leads to more nutrient accumulation (Taiz and Zeiger 1991) (as seen in the high N treatment in drought). Alternatively, low soil water potentials may signal zeatin synthesis.

5.4.3 Nutrient Mobilization in Drought

Leaf K increased in drought. This may increase survival, because K is an osmoticum. However, K is antagonistic to Ca and Mg (Diem and Godbold 1993) and greater K supply decreased Ca and Mg in the leaf and stem (3.3.2), probably to maintain the electrochemical balance (Diem and Godbold 1993). When K increased at first drought, Ca and Mg decreased marginally. With rewatering, K concentrations dropped as Mg and Ca increased.

Nitrogen concentrations did not increase in the first drought, but after rewatering, when K decreased, N increased: a possible N/K antagonism was seen in these clones (Chapter 3). Perhaps cationic polyamines like putrescine took the place of K in charge balancing, since elevated N supply increased osmotic adjustment while K did not (Chapter 4). The increased N might also result in more Rubisco or chlorophyll, because with hardening, there was more photosynthesis in drought at high N supply.

Nitrogen fertilization has been shown to increase poplar foliar ABA in drought (Liu and Dickmann 1992b), hastening stomatal closure, although mature leaves of *P. trichocarpa* (Schulte and Hinckley 1987) were insensitive to the ABA increase. Nitrogen-induced increases in both water stress and ABA probably caused the stomatal closure, inhibition of photosynthesis and leaf loss seen here in drought at high N. With hardening, stomata remained open in drought, indicating reduced ABA synthesis, lack of response to the hormone, or perhaps a blockage of ABA transport from the roots by xylem embolisms.

5.4.4 Nutrient Mobilization in Fall

All three nutrients most limiting to growth (N,P,K) (Clancy et al. 1995) were retranslocated in fall. Nitrogen fertilization had a profound effect on absolute nutrient concentrations, and also on movement of nutrients in drought (particularly into drought-hardened trees) and in fall. Potassium had a lesser, but still highly significant effect, on concentrations of most other nutrients, but in drought, increases in K treatment level only caused K retranslocation from the roots (perhaps to the leaves). Increased K supply had more effect on nutrient movement in fall than in drought, increasing retranslocation efficiency of Cu, N and P but not K. The latter may have evolved because there is less need for K at high K supply, but similar logic does not hold for N. More N is retranslocated at high N supply.

The trees in this experiment appeared proficient at N resorption, nearing Killingbeck's (1996) ultimate resorption levels. However, this is not particularly useful information, since mid-season concentrations approached proficient retranslocation levels at low N. Even though P was in short supply, retranslocation proficiency of P was not high. Poplars' growth response to P fertilization is greater than conifers' (Chapin et al. 1983), and the lower P retranslocation proficiency in hardwoods compared to conifers may be one reason for this (Killingbeck, 1996). High N supply decreased resorption proficiency (raised final concentrations) of N, P and Cu, but increased K resorption proficiency and K retranslocation efficiency (at low K supply). This could increase long-term productivity and drought resistance. Greater K supply increased N and Cu resorption, and final K concentrations, but did not affect fall P concentrations.

5.4.5 Clonal Variation in Nutrient Mobilization

There was significant clonal variation in both drought and fall nutrient mobilization. Clones which were more productive in drought did not follow a consistent pattern of nutrient mobilization. One of the two productive diploid T x D hybrids was most consistent in increasing foliar nutrient pools in drought, and the other did so after hardening. *Populus euramericana* was the only clone which resorbed foliar nutrients in drought, whether hardened or not. Its drought resistance ranking (3 of 6) was only estimated from the literature, and it probably should have a lower ranking according to productivity on dry sites (being less productive in general than all T x D's) (Chapter 3). *Populus trichocarpa*, possibly the least productive on dry sites and certainly the clone with the least stomatal response, seldom had changes in foliar nutrients with drought.

In this experiment, fall retranslocation efficiency and resorption efficiency did not correlate with clonal productivity on dry sites. Retention of leaves may be more vital to productivity, both during drought (when nutrients are resorbed less), and in the fall. For instance, triploid T x D hybrids, which are less productive in drought, lost the most leaves with water stress. One of these triploids had longer autumn leaf retention than the other: its greater fall growth increment caused the two clones to change productivity rankings. Diploid T x D hybrids, the most productive on dry sites, grew longest before first leaf loss.

5.5 CONCLUSIONS

In fall, N, P, K and Cu were retranslocated, P less efficiently than N. This may explain the strong growth response of poplars to P fertilization in the field. With gradual drought, N was relocated from the leaves to stems and roots, and P to the roots, but K and Cu remained in the leaves. Rapid severe drought did not allow time for retranslocation. Instead, foliar K concentrations increased (probably exchanged for Ca and Mg to maintain electrochemical balance), along with B, Cu and P. Nitrogen concentrations remained unchanged during a first drought, but increased with rewatering to contribute to drought preconditioning, as N supply was positively related to osmotic adjustment.

Increased K supply increased retranslocation efficiency of Cu, N, and P but not K. Resorption proficiency of K was increased with greater N supply, especially at lower K supply. This might increase productivity and drought resistance the following season. Neither retranslocation efficiency nor resorption proficiency correlated with clonal productivity on dry sites. Instead, leaf retention in fall and drought was more vital to productivity.

Table 5.1 Harvest and drought treatments, Experiment 2. All trees harvested at a given time (a-d) within a clone x nutrient x drought treatment form a single sample, so at "a" and "d" samples are 3-shoot composites. Diagrams a - d show the situation after the harvest described in column 1.

Harvest Protocol	Drought Treatment	1 2 3 (reps)
Each greenhouse contains 3, 2-shoot trees of each clone x nutrient x drought treatment.	1. Control	√ √ √ [1]
	2. One Drought	√ √ √
	3. Cyclic Drought	√ √ √
a) Harvest shorter shoots before first drought, except only take rep 1 of drought 3. Withhold water from cyclic drought trees after harvest.	1. Control	/ / /
	2. One Drought	/ / /
	3. Cyclic Drought	/ √ √
b) After 3 days drying cyclic droughted trees only, cut large shoots of drought 3, replication (rep) 2 (July 24 [3]). Rewater all.	1. Control	/ / / [2]
	2. One Drought	/ / /
	3. Cyclic Drought	/ \ √
c) After rewatering for 10 days, cut second shoots of drought 3 rep 2 (now larger). After harvest, dry drought treatments 2 & 3 together.	1. Control	/ / /
	2. One Drought	/ / /
	3. Cyclic Drought	/ √
d i) Withhold water 2 days from treatments 2 & 3 (August 6 [3]), then randomly harvest reps 1 & 3 of all drought treatments over 3 days.	1. Control	/
	2. One Drought	/
	3. Cyclic Drought	\
d ii) On the 6th day of drought, cut the final shoot per drought treatment. This ensures one very droughted shoot per composite sample.	1. Control	
	2. One Drought	
	3. Cyclic Drought	
e) An extra set of controls was grown for November harvest. Shoots were singled at time "a" and moved outside at time "d ii."	1. Control	/

[1] Each tree was pruned to 2 main stems (√). If no second stem, a branch was taken at "a."

[2] Xylem water potential of shoots from "b" to "d i" was measured before cutting.

[3] Date for Greenhouse 1. Greenhouse 2 was 10 days later at each step.

Table 5.2 Nutrient concentrations (g kg^{-1}), for (1) August controls, once-droughted, and hardened redroughted trees, and (2) August controls versus fall (well-watered until November) harvest (Experiment 2).

Nutrient	Control [1]	One [1] drought	Hardened, [1] redroughted	p value #1	Fall	p value #2
Al leaf	0.042	0.033	0.044	0.211	0.055	0.1000
Al stem	0.012a [2]	0.011a	0.011a	0.538	0.005b	0.0001
Al root	3.145a	2.93ab	2.683b	0.020	0.912c	0.0001
B leaf	0.039a	0.039a	0.042b	0.0009	0.076c	0.0001
B stem	0.014	0.015	0.015	0.341	0.015	0.885
B root	0.012b	0.012b	0.012b	0.451	0.014a	0.0001
Ca leaf	9.647b	9.678b	9.945b	0.428	15.797a	0.0001
Ca stem	5.016a	4.985a	5.052a	0.700	4.537b	0.0001
Ca root	8.006a	8.230b	8.650c	0.0001	4.810c	0.0001
Cu leaf	0.0057c	0.0062b	0.0068a	0.0001	0.0038d	0.0001
Cu stem	0.0037a	0.0036a	0.0039a	0.304	0.0028b	0.0021
Cu root	0.041a	0.046a	0.048a	0.318	0.022b	0.0001
Fe leaf	0.060	0.140	0.081	0.578	0.077	0.099
Fe stem	0.023a	0.021a	0.102a	0.408	0.013b	0.001
Fe root	5.631a	5.177ab	4.640b	0.058	1.552c	0.0001
K leaf	12.978ab	12.779b	13.260a	0.050	10.895c	0.0001
K stem	6.856b	6.9779b	7.329a	0.0001	5.160c	0.0001
K root	7.896	7.294	7.595	0.131	7.653	0.279
Mg leaf	3.144b	3.154b	3.193b	0.680	4.783a	0.0001
Mg stem	1.291a	1.309a	1.270a	0.336	1.019b	0.0001
Mg root	3.114a	2.992ab	2.872b	0.046	1.766c	0.0001

Mn leaf	0.171b	0.170b	0.172b	0.902	0.335a	0.0001
Mn stem	0.051b	0.051b	0.051b	0.958	0.070a	0.0001
Mn root	0.312a	0.326a	0.298a	0.368	0.171b	0.0001
N leaf	8.982a	8.960a	11.311b	0.0001	3.697c	0.0001
N stem	3.315c	3.407c	3.653b	0.009	4.749a	0.0001
N root	5.754b	5.907b	6.397a	0.0006	4.714c	0.0001
P leaf	1.554b	1.544b	1.920a	0.0001	1.368c	0.0060
P stem	0.901a	0.921a	0.934a	0.151	0.851b	0.0069
P root	1.268	1.241	1.222	0.468	1.259	0.816
S leaf	1.961c	1.978c	2.223b	0.0001	2.376a	0.0001
S stem	0.416b	0.439ab	0.470a	0.0003	0.410b	0.341
S root	1.393a	1.268b	1.435a	0.0007	1.055c	0.0001
Zn leaf	0.042c	0.046bc	0.051b	0.0089	0.692a	0.0010
Zn stem	0.032b	0.033b	0.033b	0.637	0.043a	0.0001
Zn root	0.044	0.042	0.037	0.179	0.040	0.489

[1] August harvest

[2] Means within a row (August test 1) followed by the same letter (or no letter) are not significantly different according to Duncan's Multiple Range Test.

Table 5.3 Nutrient concentration changes in Experiment 2: July (3 day drought), August (2-6 day's drought); August hardened trees (2-6 days); Experiment 1 (4-8 days), and fall. Concentrations increased (+), decreased (-) or were unaffected (0). "N1" is low N (0.71 mM), "K3" is high K (2.57 mM), "high N" is 7.14 mM, and "Cl" is clone.

Nutrient	July, 3 day first drought	Aug., 4 day first drought	Aug., 4 day hardened	Experiment 1, 4-8 days	Fall at abscission
Al leaf	0	0	0	0	+ p=0.1
Al stem	0	0	0	0	-
Al root	0	- marginal	-	0	-
B leaf	+	0	+ high N	0	+
B stem	0	0	0	0	0
B root	0	0	0	0	+
Ca leaf	- marginal	0	0	+ high N	+
Ca stem	0	0	0	0	-
Ca root	0	+	+	0	-
Cu leaf	+	+ high N	+	0	- [1]
Cu stem	0	0	- high N	0	- high N
Cu root	0	0	0	0	- [2]
Fe leaf	-	0	0	+ high N	+
Fe stem	0	0	0	0	- N1
Fe root	0	0	0	0	-
K leaf	+	+ high N	+	+ marginal	- high N [1]
K stem	0	0	+	0	- [2]
K root	0	0.	0	0	- K3, Cl 6 [3]
Mg leaf	- marginal	0	0	+ high N	+
Mg stem	0	0	0	0	-
Mg root	0	0	- N1	0	-

Mn leaf	0	0	0	0	+
Mn stem	0	0	0	0	+
Mn root	0	0	0	0	-
N leaf	0	0	+	-	-
N stem	0	0	+	+	+ [2]
N root	0	0	+	+	- [3]
P leaf	+	0	+ high N	-	- high N [1]
P stem	+	0	0	0	- N1, Cl 2 [2]
P root	0	0	0	- marginal	0 [3]
S leaf	+	0	+ high N	+	+
S stem	+	0	+ high N	+ 8 days	- high N [1]
S root	-	-	0	0	-
Zn leaf	+	0	+	+ 8 days	+
Zn stem	0	0	0	0	+
Zn root	0	0	0	0	0

[1] Nutrient content also decreased at N2

[2] Nutrient content increased at N2

[3] Nutrient content increased

Table 5.4 Drought nutrient mobilization (nutrient pools) by clone: increased nutrient pool (+), decreased pool (-) or no significant effect of clone x drought (0). Changes are after one drought unless otherwise noted.

Clone	Cu	K	P	Mg	S
1	+	0 [1]	0 [1]	0	0 [1]
2	+	+	+	+	+
3	0	-	-	0	-
4	+	- [1]	0 [1]	0	0 [1]
5	+	0	0 [1]	+	0 [1]
6	0	0	0 [1]	0	0

[1] increased in hardened droughted trees

Table 5.5 Resorption proficiency, or foliar concentrations of nutrients at fall leaf drop.

Factor	N%	P%	K%	Cu ppm
N1	0.33	0.23	1.69	3.29
N2	0.41	0.39	0.48	4.24
Probability	0.0001	0.0001	0.0001	0.0001
K1	0.38 a [1]	0.14	0.81 b	4.10 a
K2	0.39 a	0.14	0.90 b	3.97 a
K3	0.34 b	0.13	1.56 a	3.22 b
Probability	0.0048	0.2309	0.0001	0.0001
Clone 1	0.32 c	0.11 bc	1.13 b	3.00 d
Clone 2	0.36 bc	0.20 a	0.79 c	3.13 cd
Clone 3	0.43 a	0.21 a	1.27 ab	3.74 b
Clone 4	0.38 ab	0.09 c	0.80 c	3.92 b
Clone 5	0.33 c	0.09 c	1.10 b	3.61 bc
Clone 6	0.40 ab	0.12 b	1.43 a	5.19 a
Probability	0.0001	0.0001	0.0001	0.0001

[1] Means followed by the same letter within N, K or clone are not significantly different at $p \leq 0.05$ according to Duncan's Multiple Range Test.

Figure 5.1 Foliar nutrient pools (g m^{-2}) of B, Cu, Ca, K, Mg, Mn, N, P, S and Zn from Experiment 2 in July (a) before the first drought, (b) after a three-day drought, (c) after rewatering for 10 days, and (d) at the August and (e) fall (senesced leaves) harvests. The August harvest consisted of redroughted hardened trees (d-H), once-droughted trees (d-D), and controls (d-C) (n=72 composite samples for each bar) (error bars represent standard errors).

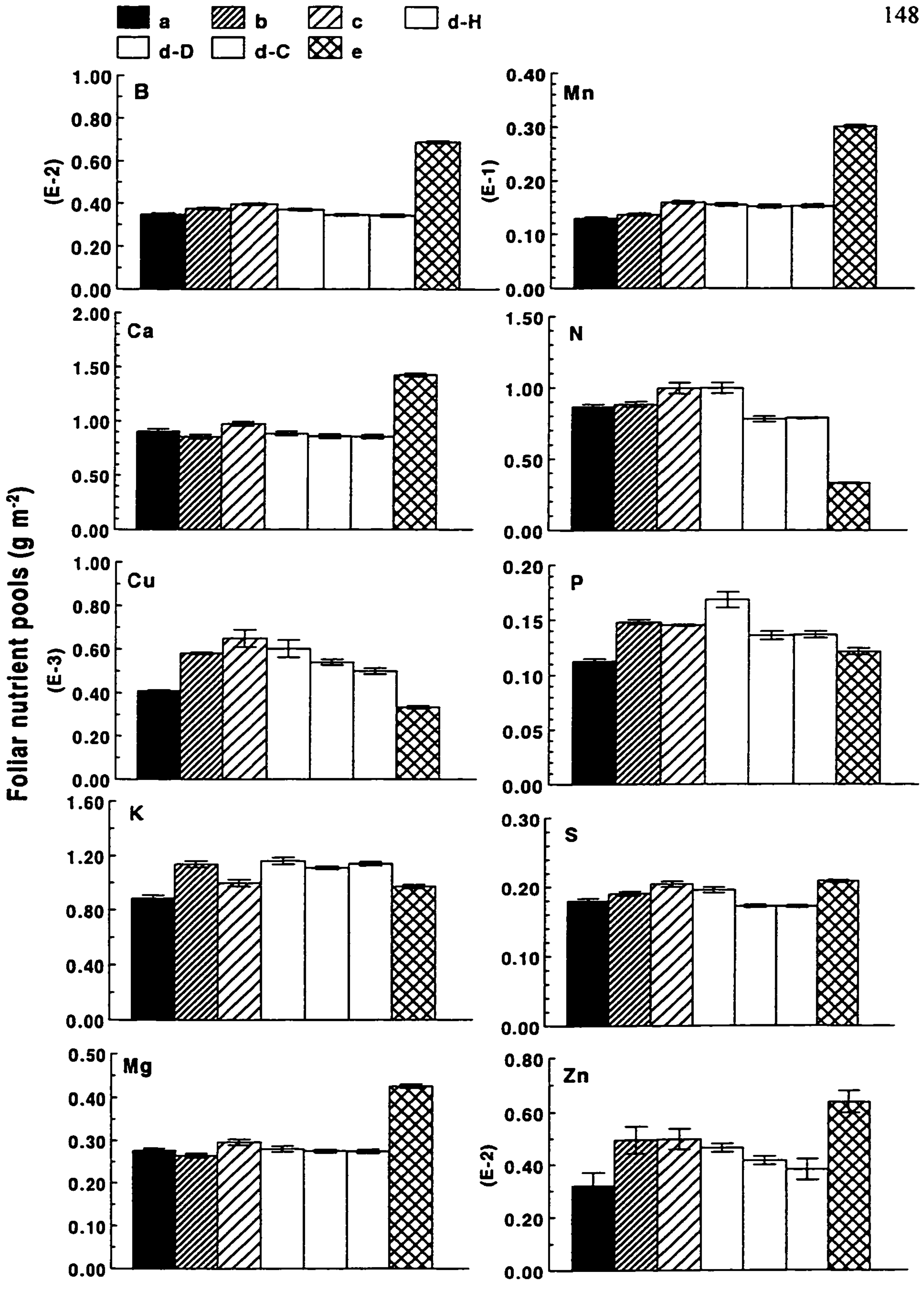
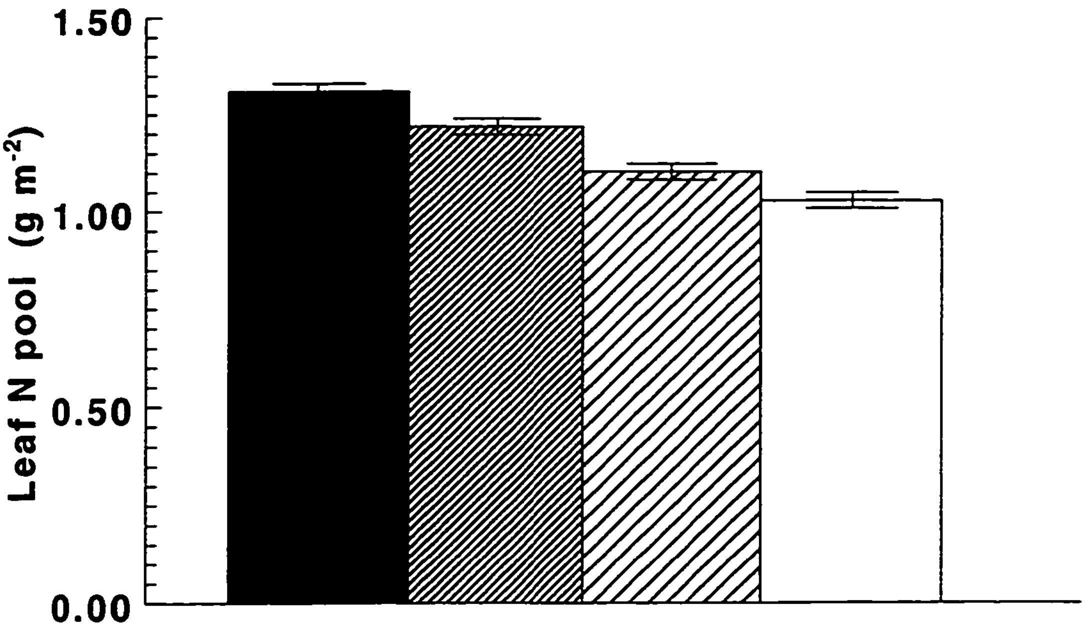

a
b
c
d-H
d-D
d-C
e
Foliar nutrient pools (g m-2)
B
(E-2)
1.00
0.80
0.60
0.40
0.20
0.00
Mn
(E-1)
0.40
0.30
0.20
0.10
0.00
Ca
2.00
1.50
1.00
0.50
0.00
N
1.50
1.00
0.50
0.00
Cu
(E-3)
1.00
0.80
0.60
0.40
0.20
0.00
P
0.20
0.15
0.10
0.05
0.00
K
1.60
1.20
0.80
0.40
0.00
S
0.30
0.20
0.10
0.00
Mg
0.50
0.40
0.30
0.20
0.10
0.00
Zn
(E-2)
0.80
0.60
0.40
0.20
0.00

Figure 5.2 Foliar N pools (g m $^{-2}$) from Experiment 1 after 0, 4, 6 and 8 days of drought for a) N and b) P (n=48 single-tree samples per bar) (error bars represent standard errors).


1.50
1.00
0.50
0.00
Leaf N pool (g m-2)

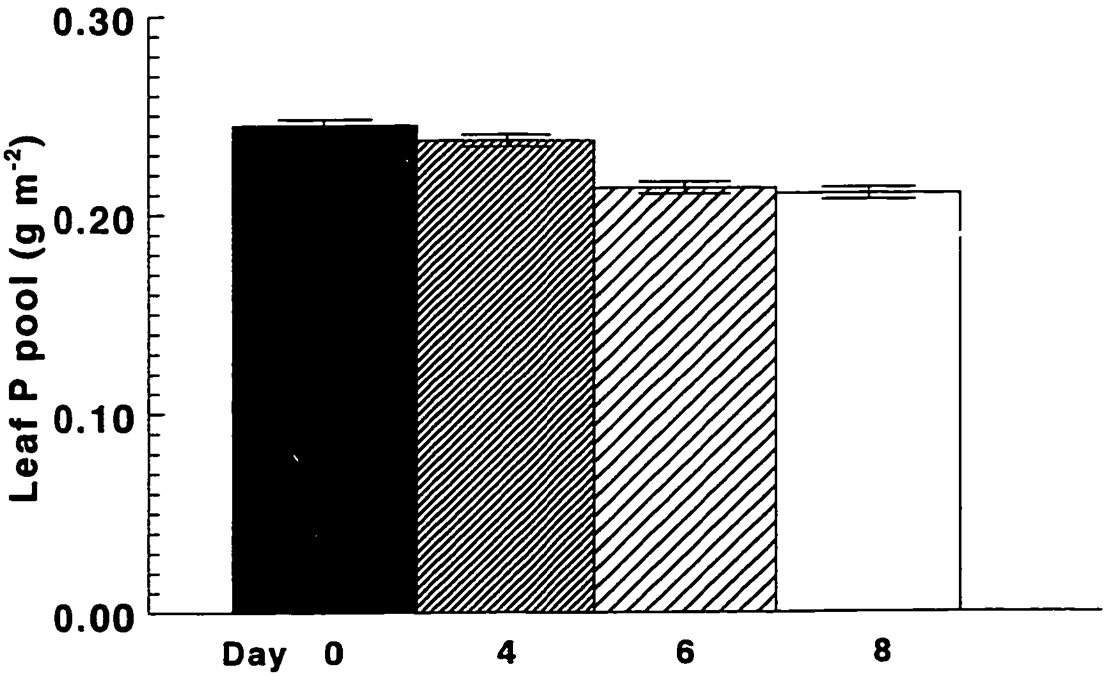

0.30
0.20
0.10
0.00
Leaf P pool (g m-2)
Day 0
4
6
8

Figure 5.3 Fall leaf nutrient retranslocation efficiency, in Experiment 2, at 3 levels of K (K1= 0mM; K2= 0.26 mM, K3= 2.57 mM) and 2 levels of N (N1= 0.71 mM and N2=7.14 mM) for Cu, K, N and P.

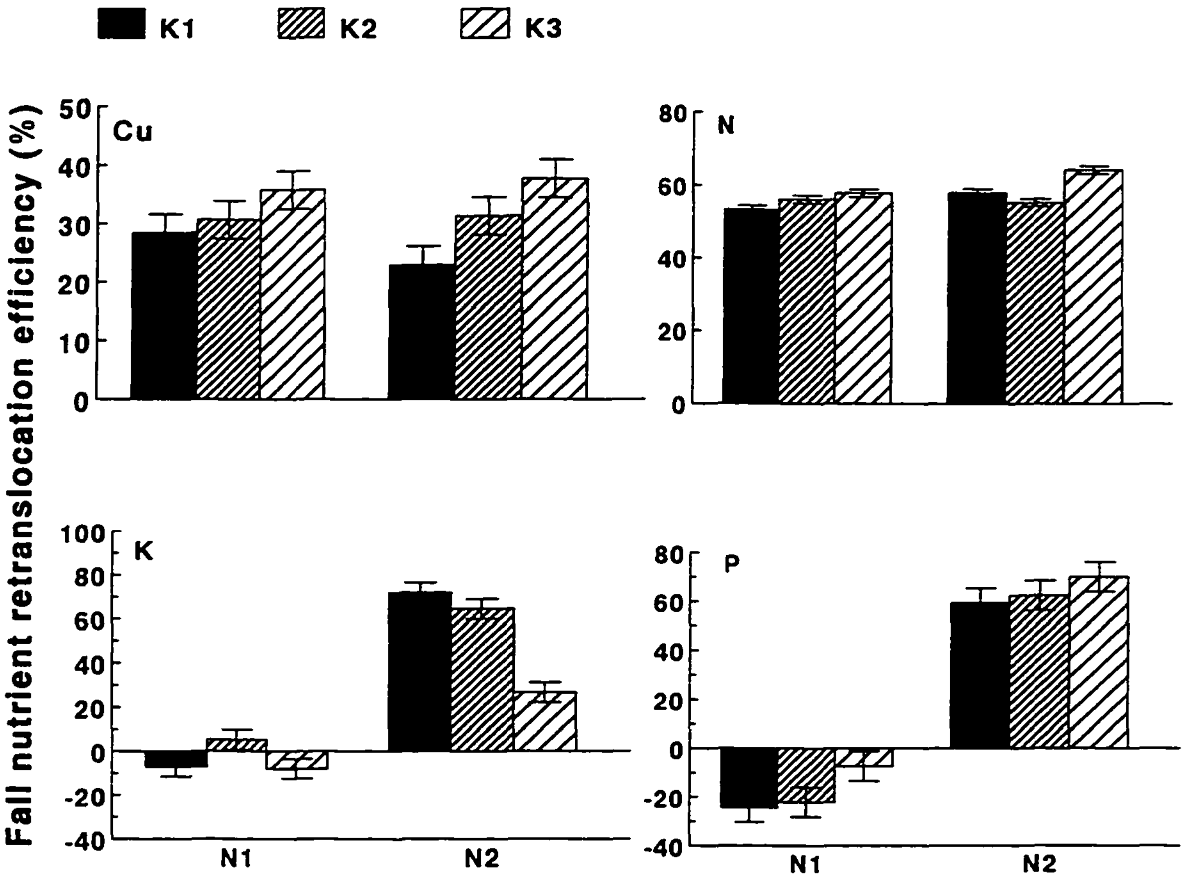

K1
K2
K3
Fall nutrient retranslocation efficiency (%)
Cu
0
10
20
30
40
50
N
0
20
40
60
80
K
-40
-20
0
20
40
60
80
100
P
-40
-20
0
20
40
60
80
N1
N2
N1
N2

Figure 5.4 Fall leaf nutrient retranslocation efficiency, Experiment 2, for 6 clones in descending order of productivity in drought: clones 1 (49-177), 2 (184-411), 3 (*P.euramericana*), 4 (52-226), 5 (17-50) and 6 (*P. trichocarpa*) for Cu, K, N and P (n=12 per bar) (error bars represent standard errors).

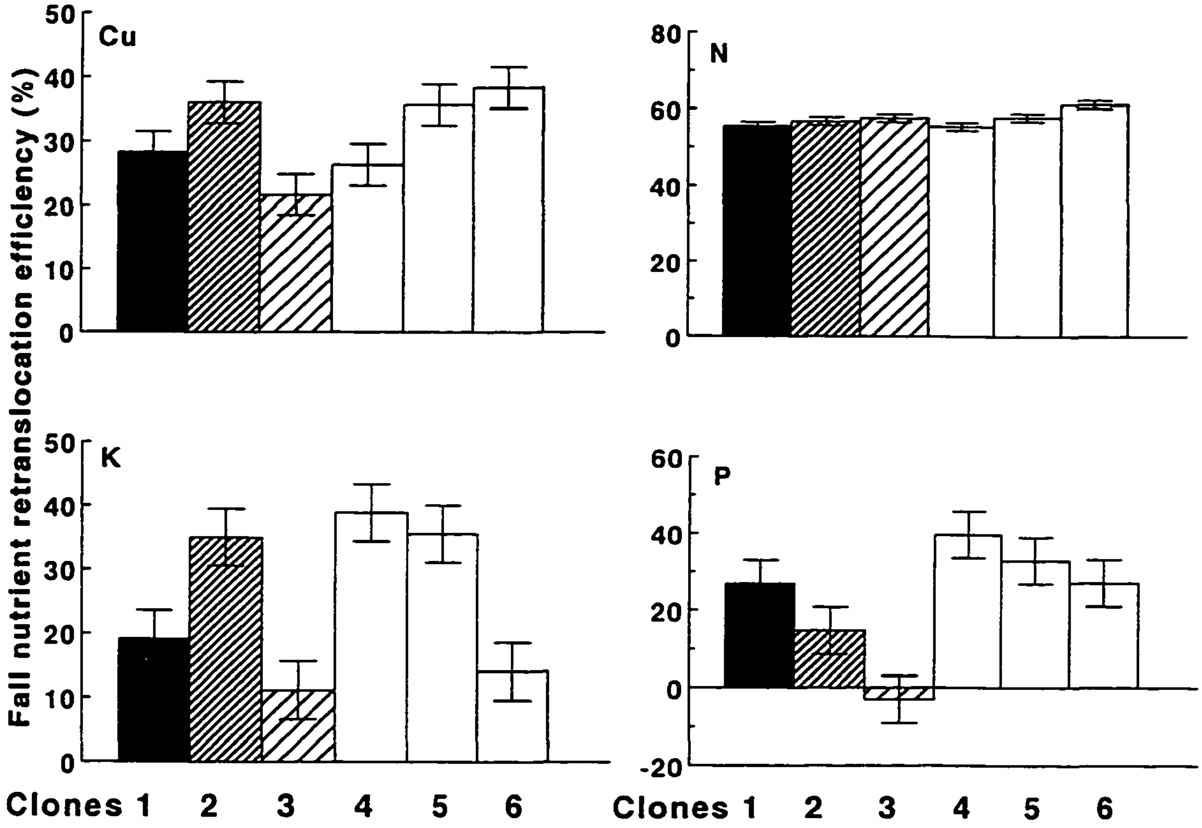

Fall nutrient retranslocation efficiency (%)
Cu
0
10
20
30
40
50
N
0
20
40
60
80
K
0
10
20
30
40
50
P
-20
0
20
40
60
Clones 1
2
3
4
5
6
Clones 1
2
3
4
5
6

Figure 5.5 Leaf loss in grams during drought, Experiment 2, for 6 clones in descending order of productivity in drought: clones 1 (49-177), 2 (184-411), 3 (*P.euramericana*), 4 (52-226), 5 (17-50) and 6 (*P. trichocarpa* Blom) (n=108 per clone) (error bars represent standard errors).

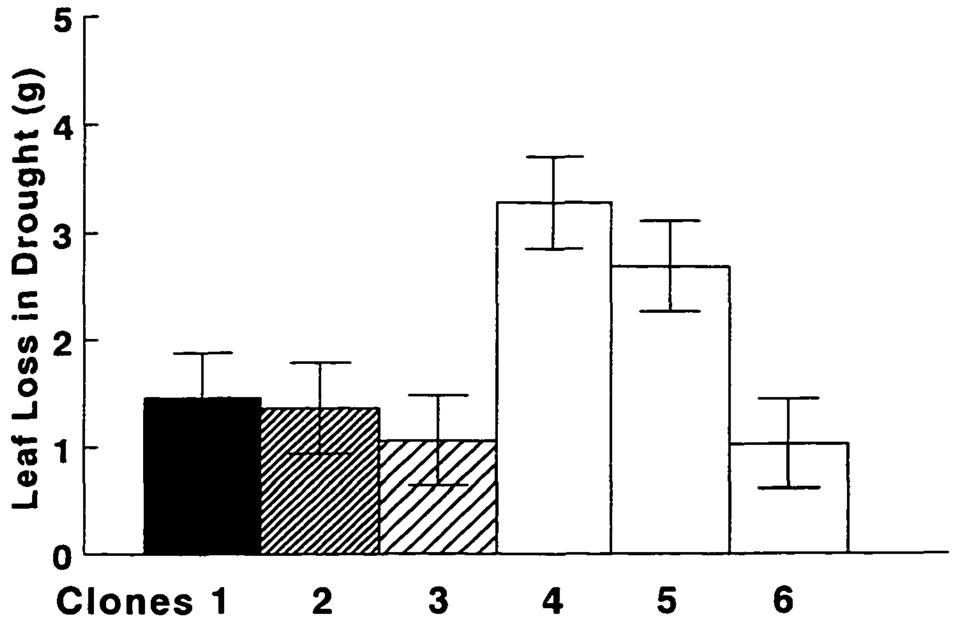
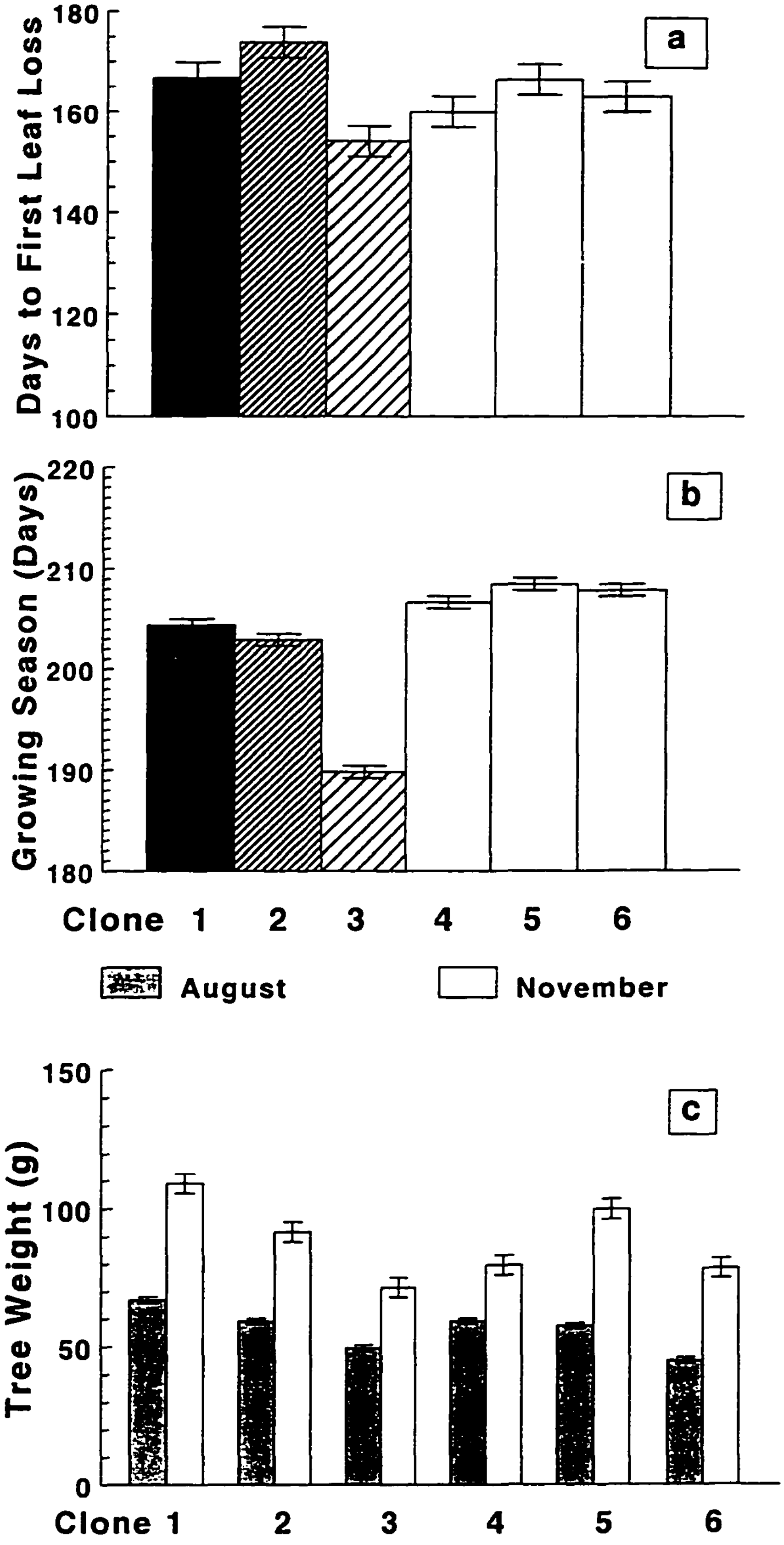

Leaf Loss in Drought (g)
0
1
2
3
4
5
Clones 1
2
3
4
5
6

Figure 5.6 Days from planting to a) first leaf loss in fall and b) last leaf loss in fall and c) tree weights in mid August and November for six clones in descending order of productivity in drought: clones 1 (49-177), 2 (184-411), 3 (*P.euramericana*), 4 (52-226), 5 (17-50) and 6 (*P. trichocarpa* Blom) (n=12 trees per bar) (error bars represent standard errors).


a
Days to First Leaf Loss
100
120
140
160
180
b
Growing Season (Days)
180
190
200
210
220
Clone 1
2
3
4
5
6
August
November
c
Tree Weight (g)
0
50
100
150
Clone 1
2
3
4
5
6

Chapter 6

DROUGHT RESISTANCE OF POPLAR: CHARACTERISTICS OF DROUGHT RESISTANT CLONES

6.1 INTRODUCTION

Selection criteria are needed for clones for the many plantation sites in the Pacific Northwest which are dry in summer. Productivity on dry sites is the prime consideration for many forest companies, and Cees van Oosten of MacMillan Bloedel has ranked growth performance in drought for various *Populus trichocarpa* x *P. deltoides* (T x D) hybrids which have grown well in the region. In growing and testing two sets of these clones, one objective was to look for characteristics of the best dry-site clones.

Behaviour of these clones was examined to see if the most productive clones were more cavitation resistant or more water use efficient, whether they exhibited greater photosynthetic rates or decreased transpiration rates and whether photosynthetic rates correlated with growth in potted trees.

6.2 MATERIALS AND METHODS

Plant material, culture and measurements are fully covered in previous chapters.

6.2.1 Experiment 1

For a sub-irrigation sand-culture system (Chapter 2), four T x D clones were chosen. The first two were drought resistant diploids, 49-177 and 14-29. Two clones were drought sensitive, a diploid (52-237) and a triploid (52-226). These were grown at three levels of N (N) (0.36 mM, 1.43 mM and 7.14 mM) and two of P (0.10 and 0.65 mM) with 4 drought treatments. Measurements were made of growth (height, weight, leaf area), nutrient composition, water potential (ψ_{xp}), cavitation, stomatal size and number, xylem vessel diameter and xylem vessel pit pore size.

6.2.2 Experiment 2

Poplar clones, numbered one to six in order of decreasing drought resistance, were chosen for a greenhouse pot experiment: (1) 49-177, (2)184-411, (3) Robusta, (4) 52-226, (5) 17-50, and (6) *P. trichocarpa* cv. Blom (estimated rankings for 3 and 6) (Chapter 3). These clones were grown at two levels of N (0.71 mM and 7.14 mM9) and three levels of K (0 mM, 0.26 mM and 2.57 mM) with three drought treatments: controls, one final drought, and hardened (pre-droughted before the final drought).

Growth, water potential, cavitation, stomatal and vessel size and number, gas exchange, osmotic potential, and nutrient concentrations were measured (Sections 3.2, 4.2, 5.2).

6.3. RESULTS

6.3.1 Experiment 1

Drought resistant clones had greater stem volume, height, vessel diameter, specific conductivity (Table 2.2), leaf areas and leaf area:root weight ratios (Section 2.2); decreased cavitation; less negative ψ_{xp} under severe water stress (Table 2.2); and fewer damaged xylem vessel pit membranes (Table 2.3). Concentrations of leaf B and Fe, leaf and stem Ca, Cu, Mg, Mn and Zn, leaf and root N, stem P and root S were lower and concentrations of stem K, root Mn and root P were higher in drought resistant compared to drought sensitive clones ($p \leq 0.05$).

6.3.2 Experiment 2

6.3.2.1 Osmotic Potential and Gas Exchange

Drought resistant clones were different from drought sensitive clones in gas exchange as well as in physical characteristics (Table 6.1). Resistant clones did not show more osmotic adjustment to drought or higher ψ_{xp}, whether considering all clones or just T x D hybrids. Resistant clones had lower instantaneous water use efficiency (WUE), maintained greater photosynthetic and transpiration rates (adjusted for ψ_{xp}) in drought when hardened (Figure 6.1a, b), and had greater initial (pre-drought) transpiration rates and stomatal conductance.

6.3.2.2 Physical Characteristics

Resistant T x D's experienced greater leaf retention during water stress than the drought sensitive TxD's, but *P. trichocarpa* had the fewest leaves lost (Table 6.1). Stomata were smaller in resistant clones and they had the most adaxial stomata (whereas clone 6 had none) (Figure 6.2), and total stomata per tree (Table 6.2). Resistant T x D clones had more abaxial stomata per unit area and per tree than the sensitive clones, but clone 6 had the most abaxial stomata and a moderate ranking in total stomata. Hardier trees had smaller diameter xylem vessels and less cavitation, but were larger at the August harvest with greater root:shoot ratios.

6.3.2.3 Response to Hardening

Resistant clones did not exhibit a consistent photosynthetic or transpirational response to drought and hardening (Figures 6.1 a, b) but there were significant clonal differences (p=0.0001): only Robusta and black cottonwood (3 and 6) did not significantly reduce their photosynthetic rates (and clone 6 transpiration rate) with drought, and clones 1 and 5 had the highest photosynthetic rates in redroughted hardened trees. Hardened resistant clones reached less negative ψ_{xp} over the course of the experiment: minimums for drought resistant clones were: -1.76 MPa (clone 1), -1.74 (2), and -1.65 (3); and for drought sensitive clones were -4.32 (clone 4), -2.12 (5) and -3.01 (6).

6.3.2.4 Response to Nitrogen

In response to N fertilization, the clones which were ranked more productive in drought increased in leaf area rather than photosynthetic rates (Table 6.3, 6.4). Leaf size increased more than leaf number (Figure 6.3a, b). Resistant T x D had fewer leaves than sensitive T x D at N2 (Figure 6.3b) but greater leaf areas (Table 6.3). Robusta, however, had many small leaves and branches.

Correlations between tree size (stem volume and tree weight) and net photosynthesis were statistically significant though weak (Table 6.5). There were stronger correlations between stem volume and total photosynthesis (net photosynthetic rate x leaf area) and tree weight and total photosynthesis, with an r^2 of 0.88 within clone 6. The strongest correlations of tree size were with leaf area, but leaf area and whole tree photosynthetic rates are not independent of tree size. Tree weight was more closely related to individual leaf area than to leaf number (r^2 of 0.51 vs 0.15) (not shown). Leaf areas per gram of tree weight, or leaf area ratios, were not related to stem volume, and only weakly negatively related to tree weight over all treatments. Within each N level, though, there were negative relationships (r^2 of 0.61 (N1) and 0.63 (N2)) for undroughted trees. There was also a negative relationship between specific leaf area (leaf area / leaf weight) and tree weight (Figure 6.4): the smaller clones (3 and 6) had thinner leaves ($p=0.0001$).

Drought resistant clones (and resistant T x D's) had lower leaf and stem K concentrations, and more root P and Ca. Resistant T x D's contained more leaf Ca, less leaf Cu, more root Al and Fe, and less root S than sensitive T x D's ($p \leq 0.05$).

6.4 DISCUSSION

6.4.1 Productivity in Drought

Clones ranked as more productive on dry sites were large clones under any conditions in my study, with high photosynthetic rates. These rates remained higher during drought, especially with preconditioning. *Populus trichocarpa* clones originating from drier sites in Washington also had higher net photosynthetic rates (Dunlap et al. 1993). Photosynthesis and growth of productive clones may have been maximized by their smaller, more numerous stomata in Experiment 2, as greater stomatal resistance decreases photosynthesis less than transpiration (Taiz and Zeiger 1991). Increased net photosynthetic rates, combined with reduced leaf loss in drought, increased whole tree photosynthesis after drought.

6.4.2 Response to Nitrogen Fertilization

Clonal response to N fertilization, whether mainly in leaf area or photosynthetic rates, could influence productivity differently before and after canopy closure. Clones Blom and Robusta had high photosynthetic rates at high N, yet were not especially productive.

Difficulties in relating instantaneous photosynthesis to growth may occur because long-term responses to drought and temperature change are not taken into account. As reported elsewhere, leaf area correlated best with growth (but was not independent of it), with productivity more related to leaf size than to leaf number (Ridge et al. 1986). Leaf area per gram of tree weight might be more meaningful, and was negatively correlated with growth, and lower in drought-productive clones. These had thicker leaves along with their large leaf areas, and relatively more root (in Experiment 2 where drought stress was greater). Thicker leaves may have increased photosynthesis per unit area (more chlorophyll and rubisco), and may also have smaller surface area:volume ratios, and thicker cuticles (to reduce nonstomatal water loss). At canopy closure, clones which respond to fertilizer mainly by increasing net photosynthetic rate (17-50 and Blom) may benefit more from increased N supply than the top two clones, 49-177 and 184-411, which respond by increasing leaf area.

6.4.3 Nutrient Concentrations in Drought Resistant Clones

Nutrient characteristics common to both sets of drought resistant clones were more root P and less leaf Cu and root S compared to sensitive clones. Phosphorus may increase root hydraulic conductivity (Skinner and Radin 1994), and for poplars, survival in drought may be more dependent on maintenance of hydraulic conductivity than on leaf dehydration tolerance (Braatne et al. 1992). Root P was also inversely related to cavitation (Table 3.3). Copper increases IAA accumulation (inhibiting root growth more than shoot growth), and Cu increases lignification (Marschner 1986). Perhaps stomata are more responsive if their guard cell walls are less lignified. Certainly leaf Cu was inversely related to transpiration and conductance (Table 3.3). Foliar S concentrations were low (less than 0.2% at high N and K (5.3) compared to optimums of 0.2-0.5 % (Marschner 1986)). Since sulfolipids are part of chloroplast thylakoid membranes (Marschner 1986), perhaps growth was increased by exporting S from root to leaf, resulting in lower root levels in the productive clones. Also, stem S was positively related to stem cavitation (Table 3.3). Possibly with lower root S there is not as much root cavitation, an important (though partially reversible) phenomenon in drought (Alder et al. 1996) which may limit gas exchange.

6.4.4 Other Drought Resistance Criteria

Robusta, one of the smallest clones in Experiment 2, should probably not be considered drought resistant according to our criterion of productivity on dry sites (although that remains to be tested), but might rank well if survival in drought were the criterion. It resembled the other drought resistant clones in many respects such as having less cavitation, more leaf retention in drought, and many small stomata.

Populus trichocarpa clone 93-968 was considered more drought sensitive than T x D hybrids (Braatne et al. 1992), but Blom had drought adaptations such as high leaf K concentrations (Chapter 3) which kept stomatal conductance and transpiration levels low, and absence of stomata on the top leaf surface (which is more exposed), a feature it shares with some other *P. trichocarpa* clones including Columbia River (Ceulemans et al. 1988). These factors decreased water stress despite poor stomatal control (Chapter 3), decreasing cavitation. Blom might grow unexpectedly well in prolonged moderate drought, since it maintained its admittedly low photosynthetic rate as it dried (possibly drought tolerant rather than drought resistant). However, *P. trichocarpa* may have greater cuticular conductance and fail to survive as long as hybrids after stomatal closure (Braatne et al. 1992).

6.4.5 The Role of Cavitation Resistance

Resistance to cavitation in severe drought may be an important aspect of productivity on dry sites. Since drought resistant clones were less vulnerable to cavitation, and cavitation avoidance may cause stomatal closure, they did not need to close their stomata as early in drought (especially after hardening), and thus could continue to photosynthesize and grow. Since stomatal closure increases instantaneous WUE (Taiz and Zeiger 1991), and they delayed stomatal closure, drought resistant clones appeared to have lower WUE. Dry-site clones of *P. trichocarpa* also had lower WUE under well-watered conditions than wet-site clones in a common garden study, perhaps because xeric clones were receiving more water than they were accustomed to, or they may transpire more to cool leaves (Dunlap et al. 1993). Since drought resistant clones were less severely cavitated at a given ψ_{xp}, xylem water flow was not blocked by embolisms: they could continue conducting water from root to leaf, replacing the water lost through their open stomata until supplies were exhausted.

In the field, water depletion would occur more slowly than in pots, especially with the greater proportion of roots in these resistant clones under dry conditions. In Experiment 1, resistant trees had greater leaf:root weight ratios, but in Experiment 2 they had smaller leaf area ratios and bigger root:shoot ratios. Drought tends to increase root growth under some circumstances (Ferrier and Alexander 1991), so perhaps root growth was favoured in the second experiment where mean ψ_{xp} was more negative. More root compared to leaf should make the internal water balance more positive, with more surface area for water uptake relative to water loss.

6.5 CONCLUSIONS

A variety of drought resistance mechanisms were seen, and clones would rank differently according to the selection criteria chosen. For commercial trees, continued productivity on dry sites is most desirable. Larger and thicker leaves correlated with increased growth. The clones which were more productive in drought had smaller, more numerous stomata, less leaf loss in drought, relatively more root, high photosynthetic and transpiration rates, especially when hardened, and more root P and less leaf Cu and root S. However, osmotic adjustment and WUE were not related to drought resistance for these clones. They responded to N fertilization by increasing leaf area more than photosynthetic rate, and in this respect might not benefit from additional N fertilizer after canopy closure. Resistant clones reached less negative water potentials and lower PLCs in severe, but not in moderate drought, and this may contribute to their productivity on dry sites.

Table 6.1. Clonal differences in Experiment 2: osmotic potentials (ψ_π) (MPa), osmotic adjustments ($\psi_{\pi\ \mathrm{ad}}$), water use efficiency (WUE) (A / g_s*10^{-5}), photosynthesis (A) adjusted for water potential (ψ_{xp}) (μmol m^{-2} s^{-1}), drop in A after drought (A loss), transpiration (T) (mmol m^{-2} s^{-1}), stomatal conductance (mol m^{-2} s^{-1}) (g_s), leaf area (LA) ratio, drought leaf loss (lf loss) (g), stomata per tree x 10^7 (stoma tr), stomatal length and vessel diameter (μm), cavitation (PLC), tree weight (g) and root:shoot ratios (Rt:shoot). Means followed by the same letter are NSD according to Duncan's Multiple Range Test.

Variable	49-177	184-411	Robusta	52-226	17-50	Blom	p=
ψ_π	1.42 c	1.50 b	1.58 a	1.51 b	1.44 c	1.43 c	0.0001
$\psi_{\pi\ \mathrm{ad}}$	0.066 b	0.141 a	0.048 b	0.070 b	0.071 b	0.057 b	0.0052
WUE	2.05 c	1.94 c	2.78 ab	2.36 bc	2.41 abc	2.97 a	0.0013[2]
A / ψ_{xp}	8.23 a	10.60 b	12.29	8.94 ab	8.21 a	10.29 ab	0.002[1H]
A loss	-0.312	1.053	-1.997	2.336	0.904	-0.417	0.030
T initial	5.97 ab	5.48 a	5.25 c	4.88 bc	4.81 c	4.21 d	0.0001[1]
T / ψ_{xp}	5.00 a	6.19 b	6.54 b	4.96 a	5.05 a	4.61 a	0.002[1H]
g_s	0.63 b	0.80 a	0.52 b	0.57 b	0.57 b	0.38 c	0.0001[1]
LA ratio	0.0043b	0.0040a	0.0054d	0.0039a	0.0043b	0.0049c	0.0001
Lf loss	1.46 a	1.35 a	1.06 a	3.28 b	2.6 b	1.03 a	0.000[2T]
Stoma tr	7.066 a	7.174 a	7.086 a	4.487 b	4.334 b	5.932 a	0.0001[1]
Stoma	37.53 b	34.00 c	25.17 d	47.10 a	48.03 a	35.39 c	0.0001[2]
Vessel	38.98 b	41.76 a	37.84 b	42.50 a	42.79 a	38.17 b	0.0001[2]
PLC	29.53 d	43.70 ab	37.35 bc	48.22 a	44.21 ab	31.95 cd	0.0001[2]
Tree wt	67.10 a	59.16 b	49.50 c	59.20 b	57.40 b	44.85 d	0.0001[1]
Rt:shoot	0.371 b	0.502 a	0.333 bc	0.415 b	0.286 c	0.230 c	0.0001[1]

[1] greater in drought resistant clones ($p \leq 0.05$)

[2] less in drought resistant clones ($p \leq 0.05$)

[H] hardened trees only

[T] less in drought resistant than in drought sensitive T x D clones only ($p \leq 0.05$)

Table 6.2 Stomatal characteristics by clone.

Clone	Length, μm adaxial	No per mm^2, adaxial	No per mm^2, abaxial	Total tree no x 10^7	Ratio: ad- to abaxial
49-177	37.5 b	72.5 c	145.8 bc	7.07 a	0.51 b
184-411	34.0 c	94.5 b	224.2 a	7.17 a	0.45 b
Robusta	25.2 d [1]	121.7 a	161.7 b	7.09 a	0.75 a [2]
52-226	47.1 a	68.3 c	130.4 c	4.49 b	0.52 b
17-50	48.0 a	40.4 d	125.4 c	4.33 b	0.33 c
Blom	35.4 c	0.00 e	236.5 a	5.93 a	0.00 d
p=	0.0001	0.0001	0.0001	0.0001	0.0001

[1] Stomatal length of Robusta was also 25 μm in a study by Cuelemans et al. (1995), smaller than Tx D clones.

[2] Stomatal ratio of Robusta was 0.61 elsewhere (Ceulemans et al. 1988), higher than all 6 other clones in the study.

Table 6.3. Clonal rankings at two levels of N supply (N1, 0.71 mM and N2, 7.14 mM) for net photosynthetic rate (A) (μmol $m^{-2}s^{-1}$), leaf area (LA) (m^2), whole tree photosynthesis (A*LA) and tree weight (TW) (g). Leaf area and tree weight are adjusted for cutting diameters ($p \leq 0.001$).

Clone	N	A	rank[1]	LA	rank	A*LA	rank	TW	rank
49-177	1	8.65	3 bc	0.104	3 ab	0.90	2 abc	26.33	2 a
184-411	1	10.61	1 a	0.085	5 b	0.90	2 abc	23.66	3 b
Robusta	1	9.63	2 ab	0.109	2 a	1.05	1 ab	20.95	5 b
52-226	1	7.93	4 bc	0.111	1 a	0.88	4 abc	29.94	1 a
17-50	1	7.77	5 cd	0.089	4 b	0.69	5 abc	21.36	4 b
Blom	1	7.64	6 cd	0.083	6 b	0.63	6 bc	16.36	6 c
49-177	2	10.84	6 g	0.481	1 c	5.21	3 ef	106.72	1 d
184-411	2	12.63	5 f	0.405	4 d	5.12	4 fg	92.78	2 e
Robusta	2	15.32	1 e	0.417	2 d	6.39	1 d	76.11	5 g
52-226	2	12.97	4 f	0.354	5 e	4.59	6 g	87.32	4 f
17-50	2	13.58	3 f	0.416	3 d	5.65	2 e	92.43	3 e
Blom	2	14.89	2 e	0.342	6 e	5.09	5 fg	72.20	6 h
p=		0.0001		0.0001		0.0009		0.0001	

[1] Rankings within a column followed by the same letter correspond to means which are not significantly different according to the least squares means test.

Table 6.4 Nitrogen response, percent increase by clone in net photosynthesis (net ps)(µmol m^{-2} s^{-1}), leaf area, whole tree photosynthesis (tree ps) (leaf area x net ps), and tree weight (g).

Clone	Net Ps	Leaf Area	Tree Ps	Tree Weight
49-177	125	463	579	405
84-411	119	476	569	392
Robusta	159	383	609	363
52-226	163	319	522	292
17-50	175	467	819	433
Blom	195	412	808	441

Table 6.5 Coefficients of determination (r^2) of stem volume and tree weight with net photosynthetic rates (μmol m^{-2} s^{-1}), total plant photosynthesis (leaf area * net photosynthesis), and leaf area ratios (LAR). All relationships are statistically significant ($p \leq 0.05$) except if 0.00.

Dependent Variable	Net Photo-synthesis	Total Plant Photosynthesis	Leaf Area (LA)	LAR [1]	LAR by N[2]
Stem Volume	0.097 0.05-0.35 [3]	0.37 0.28-0.54 [3]	0.48	0.00	0.00
Tree Weight	0.12 0.03-0.34 [3]	0.69 0.61-0.88 [3]	0.9	0.06 (-)	0.6 (-)

[1] Leaf area ratio=leaf area/tree weight and decreases in clones productive in drought

[2] Leaf area ratio is inversely related to growth within an N level in undroughted trees

[3] Range of r^2 for clones

Figure 6.1 Clones 1 (49-177), 2 (184-411), 3 (Robusta), 4 (52-226), 5 (17-50) and 6 (*P. trichocarpa* Blom), either undroughted (controls), droughted once, or hardened and then redroughted in August: a) net photosynthetic rates (n=12 per clone by drought level) and b) transpiration rates (n=12). Error bars represent standard errors.

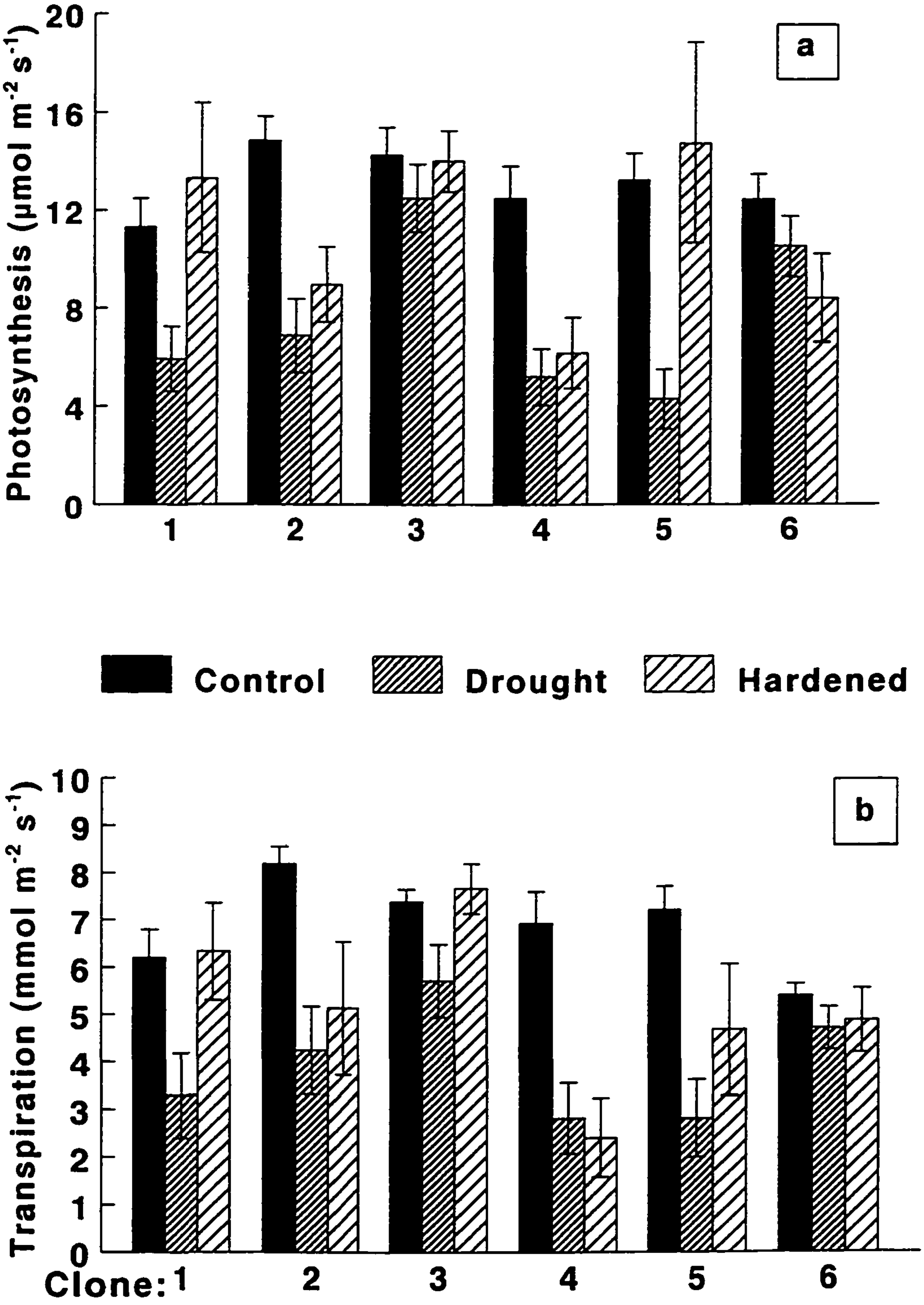

a
Photosynthesis (μmol m^{-2} s^{-1})
0
4
8
12
16
20
1
2
3
4
5
6
Control
Drought
Hardened
b
Transpiration (mmol m^{-2} s^{-1})
0
1
2
3
4
5
6
7
8
9
10
Clone: 1
2
3
4
5
6

Figure 6.2. Photomicrographs of adaxial leaf prints from the low N (0.71 mM), moderate K (0.26 mM) treatment showing clones a) 17-50 and b) Blom 6 (no stomata). Scale: 1"= 0.1 mm.

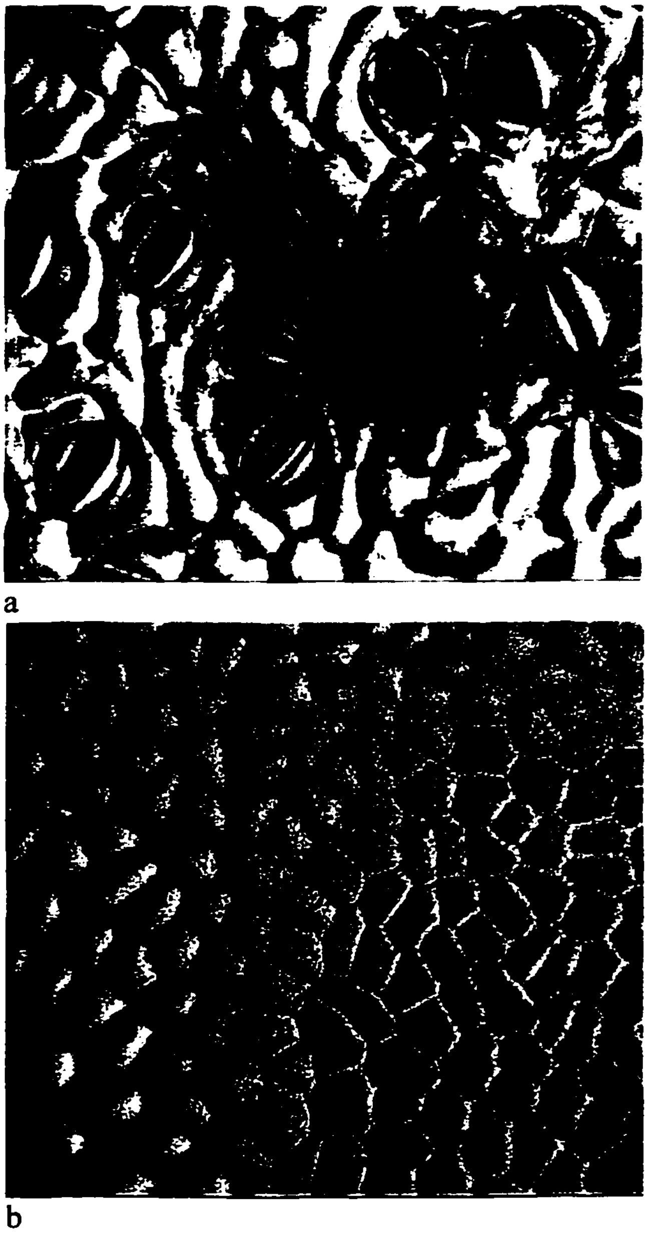

a
b

Figure 6.3. Clones 1 (49-177), 2 (184-411), 3 (Robusta), 4 (52-226), 5 (17-50) and 6 (Blom or *P. trichocarpa*) in August at low N (N1, 0.71 mM) and high N supply (N2, 7.14 mM) in Experiment 2: a) mean leaf size and b) mean leaf number (n=54 per clone by N level). Error bars represent standard errors.

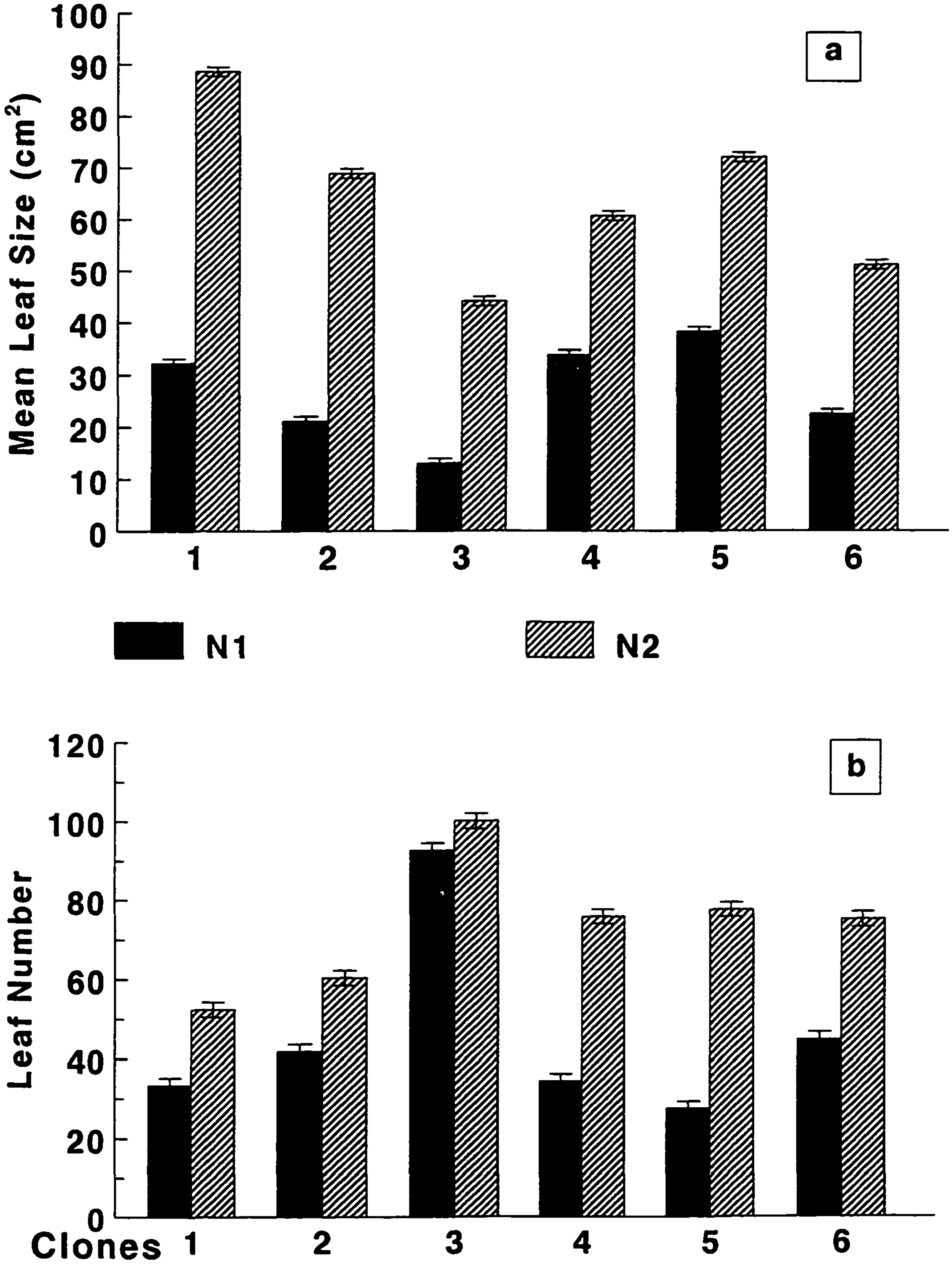
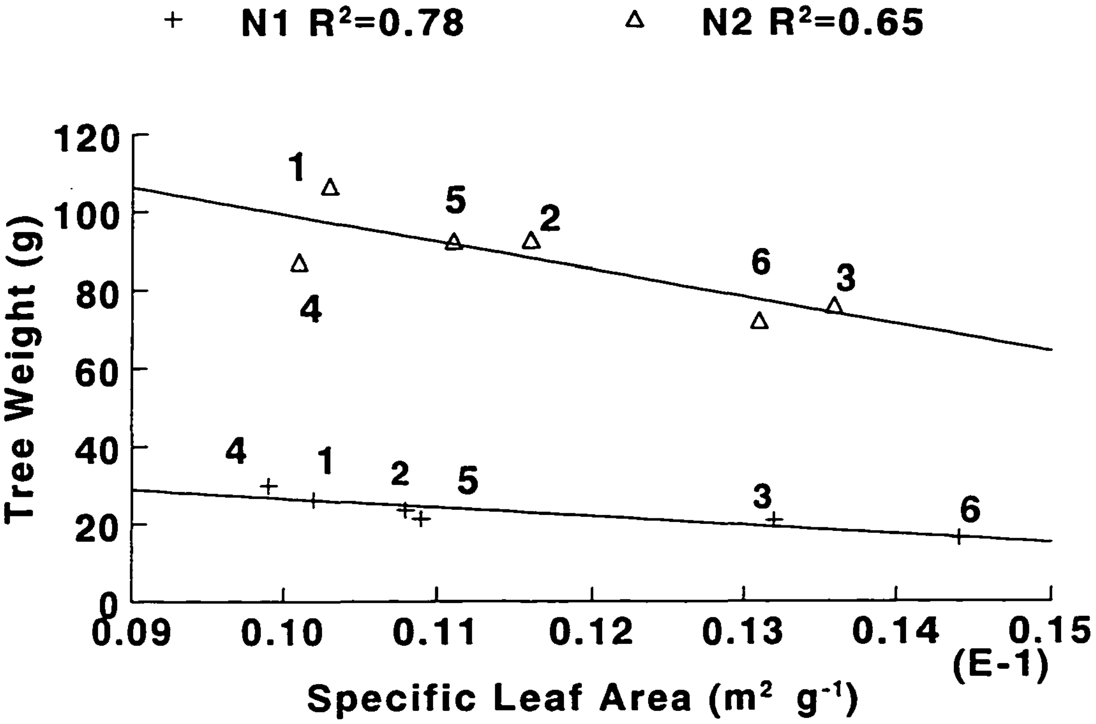

a
Mean Leaf Size (cm^2)
0
10
20
30
40
50
60
70
80
90
100
1
2
3
4
5
6
N1
N2
b
Leaf Number
0
20
40
60
80
100
120
Clones
1
2
3
4
5
6

Figure 6.4. Tree weight versus specific leaf area (leaf area/leaf weight) for 6 clones: 1 (49-177), 2 (184-411), 3 (Robusta), 4 (52-226), 5 (17-50) and 6 (Blom or *P. trichocarpa*) in August. Data points are means of 54 observations each.


+ N1 R^2=0.78
△ N2 R^2=0.65
Tree Weight (g)
120
100
80
60
40
20
0
0.09
0.10
0.11
0.12
0.13
0.14
0.15
(E-1)
Specific Leaf Area (m^2 g^{-1})
1
5
2
6
3
4
4
1
2
5
3
6

Chapter 7

CONCLUSIONS

Productivity in drought is determined by many aspects of poplar physiology, which in turn are influenced by nutrient supply, drought hardening, and clonal drought resistance in ways which have been investigated in this thesis (Appendix 3).

Nutrient effects on cavitation are of particular interest, because little information is available in this regard. In this study, increasing N supply from deficient to adequate levels, or from adequate to luxury levels, increased cavitation. Perhaps additional N made the xylem more vulnerable to cavitation by increasing conduit diameter, or creating more tension on the xylem. With greater N supply there was more water stress, possibly because of increased stomatal number, and more leaf compared to root. Phosphorus decreased cavitation at each N level, decreasing the size of xylem vessel pit membrane pores through which embolisms pass from vessel to vessel. Potassium had more complex effects on cavitation, and appeared to both increase vulnerability of the xylem vessels, and decrease tensions on the xylem at luxury (but not moderate) levels of supply.

This may also be the first direct study of the effects of preconditioning on susceptibility to cavitation. Hardened trees were at least as vulnerable to cavitation as unhardened, although embolisms may have played a role in the hardening process by decreasing water loss, for instance by leading to leaf loss. In looking at the effects of drought and rewatering on cavitation, it was found that drought-induced cavitation was seldom reversible in poplars, but some embolisms may have dissolved with high N supply in the most drought resistant clone.

A possible indicator of poplar cavitation resistance was retention of high transpiration rates during drought. Resistance to runaway cavitation was positively correlated with drought hardiness, but drought resistant clones in this study were vulnerable to moderate levels of cavitation.

This research also contributed to knowledge of nutritional effects on other aspects of drought resistance, such as instantaneous WUE and drought preconditioning. Nitrogen effects have been reported elsewhere, but are not always consistent, so it is useful to have another source stating that for one set of greenhouse-grown clones, greater N supply increased water stress, but also led to drought preconditioning and associated osmotic adjustment. Nitrogen fertilization increased WUE with ample water but not in drought. There are fewer studies on K effects on poplar drought resistance. Potassium tended to decrease water stress, especially at luxury K levels, under the conditions of this experiment. Like N, it increased WUE before but not after drought, but unlike N, did not contribute to preconditioning or osmotic adjustment.

The mechanisms of drought preconditioning and retranslocation in poplars are not fully understood, and this work augments that body of knowledge by following the details of nutrient movements during drought, rewatering, and subsequent drought. An example is that foliar N, which contributed to osmotic adjustment, only accumulated in the leaves after rewatering, not during an initial drought. Drought retranslocation results differed with speed and intensity of the drought process. Gradual drought resembled fall retranslocation, with resorption of N and P, but unlike the situation in fall, K and Cu remained in the leaves. With rapid drying over a few days, there was not time for retranslocation, and instead nutrients mainly became more concentrated. Leaf K was exchanged for Mg and Ca to maintain electrical charge balance, and contributed to stomatal closure.

Fall retranslocation was affected by nutrient supply. Increased K supply increased retranslocation efficiency of Cu, N and P but not of K itself. Increased N supply improved resorption proficiency of K at low levels of supply, which should have adaptive significance.

It was fortunate that operational clones ranked by productivity in drought were available for this study. Characteristics common to these drought-resistant clones could be useful indicators for future selections (Appendix 3). Observations on specific characteristics of the clones under study are also included for interested plantation managers (Appendix 4). Resistance to severe cavitation has already been mentioned as a trait common to hardy clones. Drought resistant (productive) clones also had more, but smaller stomata, less specific leaf area, less leaf loss, more root after drought, high rates of photosynthesis and transpiration (especially in drought after hardening), more root P and lower leaf Cu and root S concentrations. Surprisingly, they did not exhibit greater instantaneous WUE, or more osmotic adjustment, but despite delayed stomatal closure, they did not reach such negative water potentials in drought. The relatively larger root systems (containing more P to increase conductivity) may have increased water uptake; the less severely cavitated stem xylem ensured conduction of that water to the leaves; and the smaller stomata, thicker leaves, and slightly reduced leaf area ratios decreased leaf water loss despite stomata being open for continued photosynthesis and growth. Drought resistant clones responded to N augmentation more in leaf areas than in photosynthetic rates, and leaf area correlated more strongly with growth than did photosynthesis. This might not be true after canopy closure.

Future research could include pedigreed families of clones from the University of Washington, and more trials under field conditions. Based on our pot studies and some field work, N fertilization should not be overused on dry plantation sites in the Pacific Northwest, and some negative effects may be reduced by increasing tree P, and sometimes K, status. Productivity may be maximized by selecting clones which can maintain transpiration, photosynthesis and growth in drought, without being vulnerable to runaway cavitation.

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APPENDIX 1. ANOVA, EXPERIMENT 1

Linear Model

$$Y_{ijkl} = \mu + D_i + \delta_{(I)} + N_j + P_k + DXN_{ij} + DXP_{ik} + NXP_{jk} + DXNXP_{ijk} + R_{(ijk)} + C_l + CXD_{il} + CXN_{jl} + CXP_{lk} + CXDXN_{lij} + CXDXP_{lik} + CXNXP_{ljk} + CXDXNXP_{lijk} + E_{(ijkl)m}$$

where I=1,2; j=1,2,3; k=1,2; l=1,2,3,4; m=1,2,3,4 and

Y_{ijkl} = the variable to be analyzed

μ= overall mean

D_i = effect of the ith level of date effect, random

$\delta_{(I)}$ = restriction error

N_j = effect of the jth level of nitrogen effect, fixed

P_k = effect of the kth level of phosphorus effect, fixed

DXN_{ij} = effect of the interaction between day and nitrogen

DXP_{ik} = effect of the interaction between day and phosphorus

NXP_{jk} = effect of the interaction between nitrogen and phosphorus

$DXNXP_{ijk}$ = effect of the interactions among date, nitrogen and phosphorus

$R_{(ijk)}$ = restriction error

C_l = effect of the lth level of clone effect, fixed

CXD_{il} = effect of the interaction between clone and date

CXN_{jl} = effect of the interaction between clone and nitrogen

CXP_{lk} = effect of the interaction between clone and phosphorus

$CXDXN_{lij}$ = effect of the interactions among clone, date and nitrogen

$CXDXP_{lik}$ = effect of the interactions among clone, date and phosphorus

$CXNXP_{ljk}$ = effect of the interactions among clone, nitrogen and phosphorus

$CXDXNXP_{lijk}$ = effect of the interactions among clone, date, nitrogen and phosphorus

$E_{(ijkl)m}$ = experimental error

Appendix 1. ANOVA EMS

df	Source	EMS	Error
1	Date (D)	$\sigma^2 s + 4\sigma^2 e + 16\sigma^2 R + 96\sigma^2 \delta + 96\sigma^2 D$	-
0	δ(r.e.[1])	$\sigma^2 s + 4\sigma^2 e + 16\sigma^2 R + 96\sigma^2 \delta$	-
2	Nitrogen (N)	$\sigma^2 s + 4\sigma^2 e + 16\sigma^2 R + 32\sigma^2 DN + 64\phi N$	MS_{DXN}
1	Phosphorus (P)	$\sigma^2 s + 4\sigma^2 e + 16\sigma^2 R + 48\sigma^2 DP + 96\phi P$	MS_{DXP}
2	D x N	$\sigma^2 s + 4\sigma^2 e + 16\sigma^2 R + 32\sigma^2 DN$	-
1	D x P	$\sigma^2 s + 4\sigma^2 e + 16\sigma^2 R + 48\sigma^2 DP$	-
2	N x P	$\sigma^2 s + 4\sigma^2 e + 16\sigma^2 R + 16\sigma^2 DNP + 32\phi NP$	MS_{DXNXP}
2	D x N x P	$\sigma^2 s + 4\sigma^2 e + 16\sigma^2 R + 16\sigma^2 DNP$	-
0	R (r.e.[1])	$\sigma^2 s + 4\sigma^2 e + 16\sigma^2 R$	-
3	Clone (C)	$\sigma^2 s + 4\sigma^2 e + 24\sigma^2 CD + 48\phi C$	MS_{DXC}
3	C x D	$\sigma^2 s + 4\sigma^2 e + 24\sigma^2 CD$	-
6	C x N	$\sigma^2 s + 4\sigma^2 e + 8\sigma^2 CDN + 16\phi CN$	MS_{DXCXN}
3	C x P	$\sigma^2 s + 4\sigma^2 e + 12\sigma^2 CDP + 24\phi CP$	MS_{DXCXP}
6	C x D x N	$\sigma^2 s + 4\sigma^2 e + 8\sigma^2 CDN$	-
3	C x D x P	$\sigma^2 s + 4\sigma^2 e + 12\sigma^2 CDP$	-
6	C x N x P	$\sigma^2 s + 4\sigma^2 e + 4\sigma^2 CDNP + 8\phi CNP$	$MS_{DXCXNXP}$
6	C x D x N x P	$\sigma^2 s + 4\sigma^2 e + 4\sigma^2 CDNP$	-
0	E	$\sigma^2 s + 4\sigma^2 e$	-
144	S	$\sigma^2 s$	
191			

total df=(2X3X2X4X4)-1=191

[1] Restriction error

APPENDIX 2. ANOVA, EXPERIMENT 2

Linear Model:

$$Y_{ijklmn} = \mu + B_i + \delta_{(i)} + N_j + BXN_{ij} + K_k + BXK_{ik} + NXK_{jk} + BXNXK_{ijk} + C_l + BXC_{il} +$$
$$NXC_{jl} + KXC_{kl} + BXNXC_{ijl} + BXKXC_{ikl} + NXKXC_{jkl} + BXNXKXC_{ijkl} + D_m + BXD_{im} +$$
$$NXD_{jm} + KXD_{km} + CXD_{lm} + BXNXD_{ijm} + BXKXD_{ikm} + BXCXD_{ilm} + NXKXD_{jkm} +$$
$$NXCXD_{jlm} + KXCXD_{klm} + BXNXKXD_{ijkm} + BXNXCXD_{ijlm} + BXKXCXD_{iklm} +$$
$$NXKXCXD_{jklm} + BXNXKXCXD_{ijklm} + e_{(ijklm)n}$$

where $i = 1,2$; $j = 1,2$; $k = 1,2,3$; $l = 1,2,3,4,5,6$; $m = 1,2,3$; $n = 1,2,3$ and

Y_{ijklmn} = the variable to be analyzed

μ = overall mean

B_i = effect of the i^{th} level of block or greenhouse effect, fixed

$\delta_{(i)}$ = restriction error

N_j = effect of the j^{th} level of nitrogen effect, fixed

BXN_{ij} = effect of the interaction between block and nitrogen

K_k = effect of the k^{th} level of potassium effect, fixed

BXK_{ik} = effect of the interaction between block and potassium

NXK_{jk} = effect of the interaction between nitrogen and potassium

$BXNXK_{ijk}$ = effect of the interactions among block, nitrogen and potassium

C_l = effect of the l^{th} level of clone effect, fixed

BXC_{il} = effect of the interaction between block and clone

NXC_{jl} = effect of the interaction between nitrogen and clone

KXC_{kl} = effect of the interaction between potassium and clone

$BXNXC_{ijl}$ = effect of the interactions among block, nitrogen and clone

$BXKXC_{ikl}$ = effect of the interactions among block, potassium and clone

$NXKXC_{jkl}$ = effect of the interactions among nitrogen, potassium and clone

$BXNXKXC_{ijkl}$ = effect of the interactions among block, nitrogen, potassium and clone

D_m = effect of the m^{th} level of drought effect, fixed

BXD_{im} = effect of the interaction between block and drought

NXD_{jm} = effect of the interaction between nitrogen and drought

KXD_{km} = effect of the interaction between potassium and drought

CXD_{lm} = effect of the interaction between clone and drought

$BXNXD_{ijm}$ = effect of the interactions among block, nitrogen and drought

$BXKXD_{ikm}$ = effect of the interactions among block, potassium and drought

$BXCXD_{ilm}$ = effect of the interactions among block, clone and drought

$NXKXD_{jkm}$ = effect of the interactions among nitrogen, potassium and drought

$NXCXD_{jlm}$ = effect of the interactions among nitrogen, clone and drought

$KXCXD_{klm}$ = effect of the interactions among potassium, clone and drought

$BXNXKXD_{ijkm}$ = effect of the interactions among block, nitrogen, potassium and drought

$BXNXCXD_{ijlm}$ = effect of the interactions among block, nitrogen clone and drought

$BXKXCXD_{iklm}$ = effect of the interactions among block, potassium, clone and drought

$NXKXCXD_{jklm}$ = effect of the interactions among nitrogen, potassium, clone and drought

$BXNXKXCXD_{ijklm}$ = effect of the interactions among block, N, K, clone and drought

$e_{(ijklm)n}$ = experimental error

		2	2	3	6	3	3		
		F	F	F	F	F	R		
df	Source	i	j	k	l	m	n	EMS	Error
1	B_i	0	2	3	6	3	3	$\sigma^2 + 324\sigma^2\delta +$ $324\phi B$	-
0	$\delta(i)$	0	2	3	6	3	3	$\sigma^2 + 324\sigma^2\delta$	-
1	N_j	2	0	3	6	3	3	$\sigma^2 + 324\phi N$	MS error
1	BXN_{ij}	0	0	3	6	3	3	$\sigma^2 + 164\phi BN$	MS error
2	K_k	2	2	0	6	3	3	$\sigma^2 + 144\phi K$	MS error
2	BXK_{ik}	0	2	0	6	3	3	$\sigma^2 + 108\phi BK$	MS error
2	NXK_{jk}	2	0	0	6	3	3	$\sigma^2 + 108\phi NK$	MS error
2	$BXNXK_{ijk}$	0	0	0	6	3	3	$\sigma^2 + 54\phi BNK$	MS error
5	C_l	2	2	3	0	3	3	$\sigma^2 + 108\phi C$	MS error
5	BXC_{il}	0	2	3	0	3	3	$\sigma^2 + 54\phi BC$	MS error
5	NXC_{jl}	2	0	3	0	3	3	$\sigma^2 + 54\phi NC$	MS error
10	KXC_{kl}	2	2	0	0	3	3	$\sigma^2 + 36\phi KC$	MS error
5	$BXNXC_{ijl}$	0	0	3	0	3	3	$\sigma^2 + 27\phi BNC$	MS error
10	$BXKXC_{ikl}$	0	2	0	0	3	3	$\sigma^2 + 18\phi BKC$	MS error
10	$NXKXC_{jkl}$	2	0	0	0	3	3	$\sigma^2 + 18\phi NKC$	MS error
10	$BXNXKXC_{ijkl}$	0	0	0	0	3	3	$\sigma^2 + 9\phi BNKC$	MS error
2	D_m	2	2	3	6	0	3	$\sigma^2 + 216\phi D$	MS error
2	BXD_{im}	0	2	3	6	0	3	$\sigma^2 + 108\phi BD$	MS error
2	NXD_{jm}	2	0	3	6	0	3	$\sigma^2 + 108\phi ND$	MS error
4	KXD_{km}	2	2	0	6	0	3	$\sigma^2 + 72\phi KD$	MS error
10	CXD_{lm}	2	2	3	0	0	3	$\sigma^2 + 36\phi CD$	MS error
2	$BXNXD_{ijm}$	0	0	3	6	0	3	$\sigma^2 + 54\phi BND$	MS error
4	$BXKXD_{ikm}$	0	2	0	6	0	3	$\sigma^2 + 36\phi BKD$	MS error

10	$BXCXD_{ilm}$	0	2	3	0	0	3	$\sigma^2+18\phi BCD$	MS error
4	$NXKXD_{jkm}$	2	0	0	6	0	3	$\sigma^2+36\phi NKD$	MS error
10	$NXCXD_{jlm}$	2	0	3	0	0	3	$\sigma^2+18\phi NCD$	MS error
20	$KXCXD_{klm}$	2	2	0	0	0	3	$\sigma^2+12\phi KCD$	MS error
4	$BXNXKXD_{ijkm}$	0	0	0	6	0	3	$\sigma^2+18\phi BNKD$	MS error
10	$BXNXCXD_{ijlm}$	0	0	3	0	0	3	$\sigma^2+9\phi BNCD$	MS error
20	$BXKXCXD_{iklm}$	0	2	0	0	0	3	$\sigma^2+6\phi BKCD$	MS error
20	$NXKXCXD_{jklm}$	2	0	0	0	0	3	$\sigma^2+6\phi NKCD$	MS error
20	$BXNXKXCXD_{ijklm}$	0	0	0	0	0	3	$\sigma^2+3\phi BNKCD$	MS error
432	$e_{(ijklm)n}$	1	1	1	1	1	1	σ^2	-
647									

total df=(2X2X3X6X3X3)-1 = 647

Notes:

1. When nutrient contents of trees are compared, the three replicates within block are combined into a composite sample for economy. Then n=1, so there are zero degrees of freedom for experimental error. The interaction $BXNXKXCXD_{ijklm}$ mean square must be used as the error term for all F tests, providing conservative tests with only 20 degrees of freedom. If variances are small and equal for other interactions, and their F ratios are not significant at p=0.25, then interaction mean squares may be pooled to provide more degrees of freedom (Anderson and McLean, 1974).

2. When comparing drought retranslocation with fall retranslocation (using the 72 extra trees grown for that purpose), again replicates are combined so n=1 and the F ratio tests will be against the interaction, not the experimental error. Also, m (drought levels) = 4. In this case degrees of freedom for the interaction $BXNXKXCXD_{ijklm}$ would be 1X1X2X5X3 = 30.

APPENDIX 3. Effects of drought hardening, nutrition and clonal drought resistance (D.R.) on factors affecting productivity in drought ($p \leq 0.05$): positive (+), negative (-) or no (0) relationship. Variables are osmotic potential (ψ_π), osmotic adjustment ($\psi_{\pi\ ad}$), xylem water potential (ψ_{xp}), percent loss of conductivity (PLC), water use efficiency (WUE) ($A/g_s * 10^{-5}$), cases of wilting, photosynthesis (A) adjusted for ψ_{xp}, change in A between first and final harvest (A loss), transpiration (T), stomatal conductance (g_s), leaf area (LA) ratio, leaf loss in drought (lf loss), number of stomata per tree (stoma # tr), stomatal length, vessel diameter and tree weight (grams) (na=not applicable).

Variable	Hardening	Nitrogen	Potassium	Phosphorus	D.R.
ψ_π	+	+	0	na	0
$\psi_{\pi\ ad}$	+	+	+ or - [1]	na	- at K1
ψ_{xp}	+	- at hi N [4]	+ marginal	+ in field	+ expt 1 [4]
PLC	0 or + [1]	+ [4]	+ [2]	-	0 at lo ψ_{xp} [4] - at hi ψ_{xp} [4]
WUE	0	+	+	na	-
Wilting	-	+	- 1 drought	na	0
A / ψ_{xp}	+	+ or 1 [3]	- [2]	na	0
A loss in dr	-	+	+ [2]	na	0
T initial	na	0	-	na	+
T final	+	-	- [2]	na	+ [1]
g_s final	0	-	0	na	0
LA ratio	+	+	-	0	0
Leaf loss	na	+	0	0	-
Stoma # tr	0	+ [4]	0	+ or - [1]	+
Stoma µm	0	- [4]	0	0	- expt 2
Vessel µm	0	+ [4]	+ at hi N	0	0 triploid +
Tree weight	-	+ [4]	+ at hi N	0	+

[1] significantly different by clone

[2] at K2 of 3 K levels (0.26 mM supply)

[3] increased A for well-watered trees, decreased A for droughted trees

[4] seen in 2 experiments

APPENDIX 4. CLONAL CHARACTERISTICS

49-177 TxD diploid	This was the largest clone, with the least cavition in severe drought, and reached the least negative water potentials. Leaf loss in drought was low.
184-411 TxD diploid	Another large clone, less cavitation resistant than the other drought resistant clones, it had the most osmotic adjustment after hardening, and reduced leaf area ratios. This clone also resisted leaf loss in drought.
Robusta *Populus euramericana*	Robusta was probably not drought resistant according to the criterion of productivity on dry sites, but might rank well in drought survival: it had many characteristics of the other drought resistant clones (less cavitation, leaf retention in drought, and many small stomata). This clone had numerous small stems, branches and leaves; this may consume more photosynthate in respiration, and branchiness is usually considered undesirable commercially. Robusta (and Blom below) may allocate more carbon to short-lived fine roots or to root exudate, accounting for their small size.
52-226 TxD triploid	This was a fairly large clone, but lost leaves in drought, cavitated severely, and reached more negative ψ_{xp}. Photosynthetic rates were fairly low, and decreased quickly in drought. 52-226 responded less to hardening than other clones.
17-50 TxD triploid	Although 17-50 was mid-range in size in August, its longer growing season made it a top producer in Experiment 2 by November. Length of growing season has determined productivity rankings elsewhere (Dickmann et al. 1996), and on a mesic site this clone might be desirable, especially because it had fewer multiple stems. It may respond well to fertilization after canopy closure.
Populus trichocarpa "Blom"	Considered more drought sensitive than T x D hybrids (Braatne et al. 1992), it had more drought adaptations than expected: 1) high leaf K concentrations which kept stomatal conductance and transpiration levels low and 2) no stomata on the top leaf surface (which is more exposed), as for *P. trichocarpa* clone Columbia River (Cuelemans et al. 1987). These factors decreased water loss despite poor stomatal control, decreasing cavitation. Blom might grow better in drought than anticipated, since it maintained its admittedly low photosynthetic rate as it dried. However, it may have greater cuticular conductance and fail to survive as long as hybrids after stomatal closure (Braatne et al. 1992).

15-29 TxD diploid	This clone resisted cavitation in severe drought in Experiment 1 (although not as well as 49-177), and was productive, but was more vulnerable to leaf blotch, perhaps indicating susceptibility to nutritional disorders.
52-237 TxD diploid	52-237 was a small clone from Experiment 1, susceptible to drought and cavitation.