DROUGHT EFFECTS ON LEAF ABSCISSION AND LEAF PRODUCTION

IN *POPULUS* CLONES

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Abstract: Leaf abscission and foliation responses to water stress were studied in potted plants of five *Populus* clones grown in a greenhouse. As predawn leaf water potential (Ψ_l) fell to -3 MPa, drought-induced leaf abscission increased progressively to 30% for data pooled across clones. As predawn Ψ_l fell below -3 MPa, drought-related abscission was about 50%. When combined with abscission rates in well-watered "phenological control" plants, abscission exceeded 80-90% in the most severely water-stressed plants. Clonal variation in water stress-induced abscission was statistically significant, but appeared to be confounded with clonal differences in leaf loss in control plants. Production of new leaf area ranged from 0 to 1,389 cm². Pooled across clones, maximum production of leaf area occurred in plants previously subjected to moderate water stress (-0.5 to -0.75 MPa). This stimulation of leaf development was associated with greater areas of individual leaves. At predawn Ψ_l < -2 MPa leaf area production was greatly decreased (<120 cm²). Generally, leaf area production during and after water stress did not vary systematically among clones. However, one clone of *Populus trichocarpa* appeared more capable than others of producing new leaves when severely stressed and allowed to recover. Results indicate substantial sensitivity of *Populus* clones to leaf abscission under water stress, a capacity for refoliation if water stress does not become severe, and some possibility of genetic variation among clones in these responses.

INTRODUCTION

Poplars are noted for rapid growth and biomass accumulation (Anderson and others 1983, Ranney and others 1987). As such they have received much attention from researchers interested in production of fiber and wood products. Physiologically and morphologically, members of the genus *Populus* are characterized by high transpiration and photosynthesis rates (Bugala 1973, Nelson 1984). They also exhibit prodigious production of leaf area that results from a predominantly free growth habit in which both preformed and neoformed shoots contribute to growth, especially in juvenile plants (Kozlowski and Pallardy 1997).

Many poplars are naturally found on riparian sites or in other situations in which the likelihood of significant seasonal water deficits is relatively small (Burns and Honkala 1990). When exposed to water stress poplars as a group show considerable inhibition of physiological function. Previous research has shown that leaf growth and photosynthesis is greatly reduced even under mild water stress (Braatne and others 1992, Dickmann and others 1992, Heilmann and Stettler 1985, Pezeshki and Hinckley 1982, Regehr and others 1975, Rhodenbaugh and Pallardy 1993). Another drought response of *Populus*, reported anecdotally in the literature, is premature leaf abscission (Dickmann and others 1992, Kozlowski and others 1991, Mazzoleni and Dickmann 1988).

Drought adaptation mechanisms of plants may be classified into broad groups based on responses of plants (Kramer and Boyer 1995). Drought tolerance, the capacity to endure at least modest meteorological droughts, is attained by combinations of physiological responses and morphological and anatomical features that: (1) reduce or postpone dehydration of plant tissues (=dehydration avoidance) or (2) confer the capacity to tolerate dehydration (=dehydration tolerance). Dehydration avoidance adaptations include those associated with acquisition of water resources (e.g., root system extent and patterns of exploration), with efficient internal water transport (e.g., vascular system adaptations), and with adaptations that reduce shoot water loss (e.g., stomatal closure, leaf abscission and high root-shoot ratios). Dehydration tolerance reflects the capacity of a plant to sustain physiological function and/or survive severe tissue water loss. By this classification scheme, poplars generally are considered dehydration-avoiding

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species, showing stomatal closure at high leaf water potential (Ψ_l) (Regehr and others 1975, Rhodenbaugh and Pallardy 1993), apparent predisposition to leaf abscission under water stress, and little leaf dehydration tolerance capacity (Braatne and others 1992). There are few data available, however, that have specifically related leaf loss to the level of water stress developed in leaves for any species (e.g., Pallardy and Rhoads 1993), and there are no data available for poplars. Additionally, there are few data available that document patterns of leaf production during and after drought in this taxon (Mazzoleni and Dickmann 1988).

Drought tolerance varies genetically within many species and genera of woody plants (see Pallardy 1981 for a review). Some investigations of genetic variation in water-relations-related traits of poplars have been conducted (e.g., Blake and others 1984; Braatne and others 1992; Ceulemans and others 1978; Gebre and Kuhns 1991, 1993; Pallardy and Kozlowski 1981; Rhodenbaugh and Pallardy 1993). However, there have been no comparative studies of leaf abscission responses among different poplar genotypes.

Reviews of productivity in biomass plantations have emphasized the interception of radiation as a primary determinant of dry matter accumulation (Cannell 1985, Kozlowski and others 1991, Landsberg 1986). Water stress may decrease forest stand productivity because light interception by the canopy, which is a function of leaf area index and duration, is reduced. Dehydration avoidance attributes, such as extensive rooting, stomatal closure and leaf abscission are often effective mechanisms of drought tolerance, but they reduce above-ground productivity (Ludlow 1989). On the other hand, dehydration tolerance characteristics would appear to offer the potential for increases in drought tolerance with less inherent reduction in above-ground growth (Kramer and Boyer 1995, Ludlow 1989).

We report in this paper an experiment designed to quantitatively assess leaf abscission and production responses of clones of different genotypes of three species of *Populus*.

METHODS

The experiment was conducted in a greenhouse at the Forestry Sciences Laboratory at Rhinelander, WI. Eighty 10- to 15-cm long hardwood cuttings of each of two clones of *Populus trichocarpa* Torr. & Gray (T06, T09) and *P. balsamifera* L. (B03, B05) and one clone of *P. deltoides* Bartr. ex Marsh. (D12) were planted in plastic pots holding about 4 L of a potting mix. Slight modification of the basic methods of Parker and Pallardy (1985) and Pallardy and Rhoads (1993) were employed. Briefly, after a period of growth under optimal soil moisture conditions in a greenhouse, plants were allowed to dry slowly by withholding water. As predawn leaf Ψ [as measured with a pressure chamber (Ritchie and Hinckley 1975)] declined, plants were randomly selected and rewatered (Parker and Pallardy 1985). An additional five plants per clone were maintained in a well-watered condition as phenological controls. Leaf abscission was assessed by calculating, at the point rewatering began, the percent abscission of leaves present at the time water was withheld. After rewatering, plants were kept in moist soil for 6 weeks and new leaf area developed since the beginning of stress imposition was measured at this time using a LI-3000 Leaf Area Meter fitted with a belt conveyor (LI-COR, Inc., Lincoln, NE).

Considerable abscission was observed in phenological controls over the course of the experiment, possibly because of the shortening day length during the course of the experiment (August to November) (Addicott 1982). Hence, "drought-related leaf abscission" had to be calculated as the amount of leaf loss that exceeded that of the well-watered phenological controls. Unfortunately, leaf abscission was not assessed in the controls until late September, reducing the number of samples that could be validly analyzed for leaf abscission under drought influence, particularly in mildly-water-stressed plants. There was neither significant leaf abscission nor leaf growth in phenological control plants during the post-drought recovery period for water-stressed plants, allowing full use of samples for new leaf area analysis as a function of water stress.

Data were analyzed by Analysis of Variance (ANOV) using the General Linear Models Procedure of SAS (SAS Institute 1982). Drought-related leaf abscission and new leaf area were analyzed as dependent variables with clone, level of water stress and their interaction as independent variables. Percent abscission was arcsine (arcsin $\sqrt{\ }$) transformed before analysis to normalize the data (Zar 1984). Additionally, t-tests were conducted on arcsine transformed data to determine if drought-related abscission was significantly different from zero for data pooled

across clones. Leaf abscission of phenological control plants was analyzed using ANOV, but with only clone as an independent variable. Water stress levels were quantified into ranges of predawn Ψ_l, with a greater number of stress categories in the low- to moderate-water-stress range in analysis of new leaf area development. The latter categorization was associated with a greater number of samples available in this range for the new leaf area variable.

RESULTS

Pooled Data of All Clones

At all levels of water stress imposed, drought-related leaf abscission was significantly greater than zero (P<0.01) as determined by t-tests of individual stress level means. Drought-related leaf abscission over all clones increased significantly as predawn Ψ_l declined (Figure 1, Table 1). Leaf loss was progressively greater between 0 and -3 MPa and then increased abruptly below -3 MPa, reflecting nearly total leaf abscission under severe water stress. It is also worth noting that while we did not specifically assess mortality, the proportion of plants showing total leaf kill and complete suppression of new leaf development rose dramatically around -2 MPa (Table 2).

Table 1. Results of Analysis of Variance with models of leaf abscission and new leaf area as dependent variables, and with clone, water stress level and their interaction as independent variables.

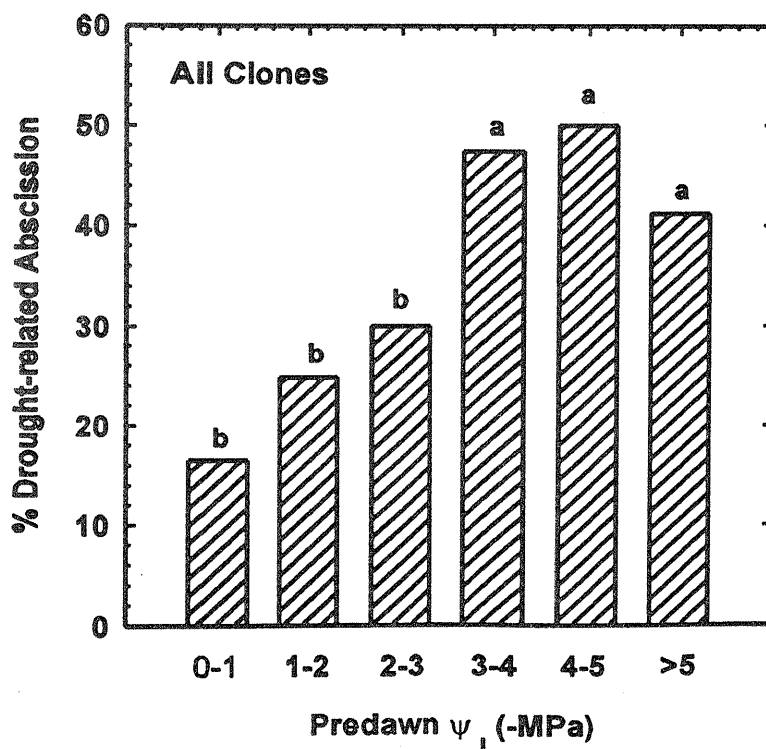| Effect	Drought-related Abscission	New Leaf Area
Clone	P<0.01	P<0.54
Stress Level	P<0.01	P<0.01
Clone x Stress Level	P<0.04	P<0.42

Figure 1. Mean percent drought-related leaf abscission of five *Populus* clones at several levels of predawn Ψ_l. Bars not capped by the same letter differ significantly (P<0.05).

Table 2. Percent of plants previously dried to several levels of predawn Ψ_l with no living leaves after 6 weeks at high soil moisture as a function of water stress levels. Data are pooled over five *Populus* clones.

Water Stress Level (MPa)	% Plants Lacking Living Leaves
0 to -1	3.2
-1 to -2	13.0
-2 to -3	56.7
-3 to -4	78.6
-4 to -5	100.0
<-5	87.0

Production of new leaf area after the stress period and a 6-week recovery period at high soil moisture peaked at intermediate levels of predawn Ψ_l, declining above and below this point (Figure 2). The production of new leaf area under mild water stress was not significantly different from that of the phenological control plants, although it was numerically lower. The production of new leaf area in plants water-stressed to predawn Ψ_l between -0.5 to -0.75 MPa exceeded that in the phenological controls by over 100 cm²; this difference approached the 0.05 level of statistical significance (P=0.09). New leaf area production between plants in the 0 to -0.25 and -0.5 to -0.75 MPa classes was significantly different (P<0.05, Figure 2). Predawn Ψ_l values less that -2 MPa greatly reduced production of new leaf area below that of the control- and mild- to moderately-water stressed plants, and these differences were highly significant (P<0.01, Table 1, Figure 2).

The difference in new leaf area exhibited between mildly stressed and moderately stressed *Populus* plants (Figure 2) was at least partially attributable to the greater number of new leaves produced during the longer drying period necessary to deplete soil moisture in the latter plants (Table 3). However, total new leaf production in both phenological control plants and plants subjected to stress-rewatering cycles was quite similar, differing significantly (P<0.05) only in the most severely water-stressed plants. *Populus* plants water-stressed to -0.5 to -0.75 MPa showed substantially and significantly greater (P<0.05) areas per leaf compared with plants that were more mildly stressed both during and after stress periods (Table 3). Moderately-stressed plants also had significantly greater areas per leaf than phenological control plants.
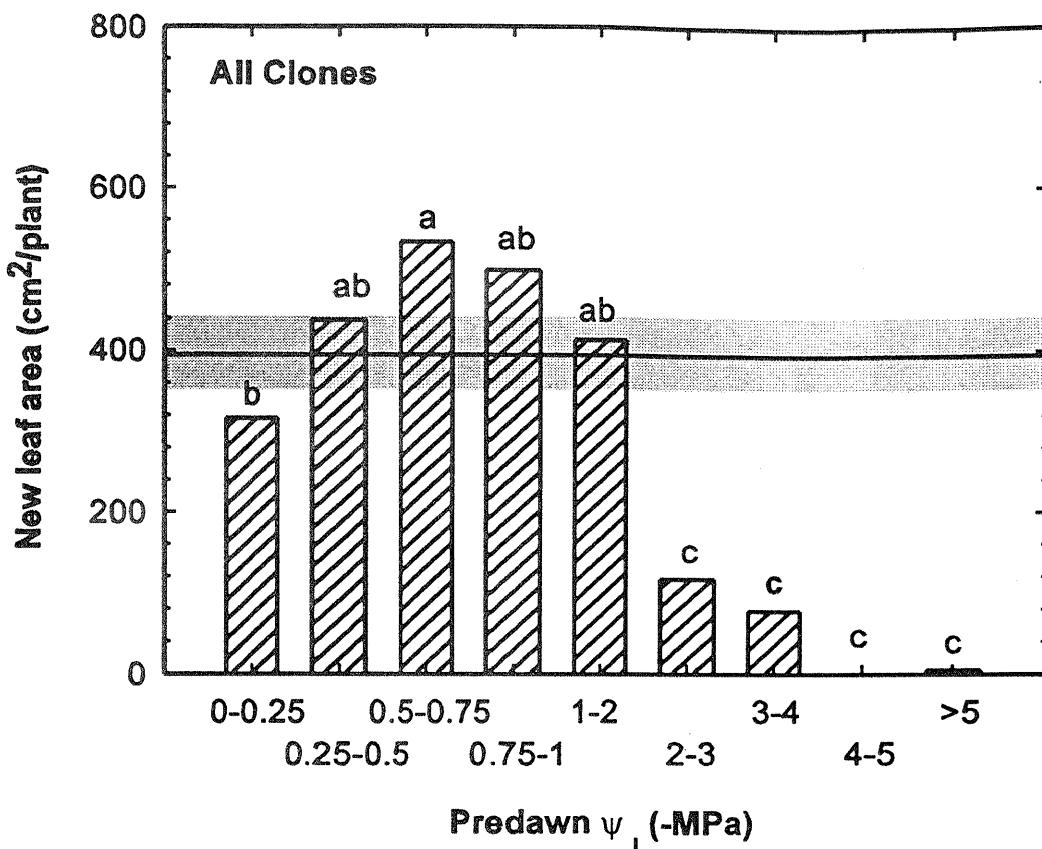
Figure 2. Mean new leaf area production following rewatering of five *Populus* clones previously dried to several levels of predawn Ψ_1. Horizontal line indicates mean new leaf area production of phenological control plants pooled across clones. Stippled area indicates ±1 SE of this value.

Table 3. Least squares means of leaf number and area per leaf for plants of *Populus* clones subjected to water stress and subsequently kept well-watered for 6 weeks. No plants fell within the -4 to -5 MPa stress level. Within a columns least square means not followed by the same letter are significantly different (P<0.05).

Stress Level Imposed	Number of leaves			Area per leaf (cm²)		
	During water stress	After water stress	Total	During water stress	After water stress	Total
0 to -0.25 MPa	2.63b	9.38a	12.00a	13.78b	32.90abc*	32.00ab
-0.25 to -0.5 MPa	2.41b	8.82a	11.24a	15.50b	35.60bc	30.31b
-0.5 to -0.75 MPa	5.63a	6.75ab	12.38a	33.44a	50.32a	41.94a
-0.75 to -1 MPa	7.22a	6.89ab	14.11a	25.11ab	39.06ab	29.62ab
-1 to -2 MPa	5.85a	7.12ab	12.96a	24.55ab	36.85bc	32.00ab
-2 to -3 MPa	5.55a	5.09bc	10.64a	18.42b	23.37bc	21.30bc
-3 to -4 MPa	6.00a	6.67abc	12.67a	18.06b	34.43abc	27.44bc
< -5 MPa	4.67ab	0.33c	3.84b	8.10b	8.5bc	6.15c
Phenological control	-	-	12.20a	-	-	29.54b

* Difference between 0 to -0.25 MPa and -0.5 to -0.75 MPa levels significant at P=0.06.

Clonal Differences

Statistical differences among clones in drought-related leaf abscission responses were evident (Figure 3, Table 1), but interpretation of these data was complicated by substantial clonal variation in abscission of the phenological control plants (Figure 4). Although analysis of variance of these latter data indicated no significant differences (P>0.05) among clones in leaf abscission among the control plants, clones with smaller amounts of leaf abscission (especially clones B03 and T09) tended to exhibit significantly greater levels of drought-related abscission (cf. Figures 3 and 4). Hence, clones B03 and T09 might be considered more susceptible to water stress-induced leaf abscission, but this pattern must be interpreted cautiously.
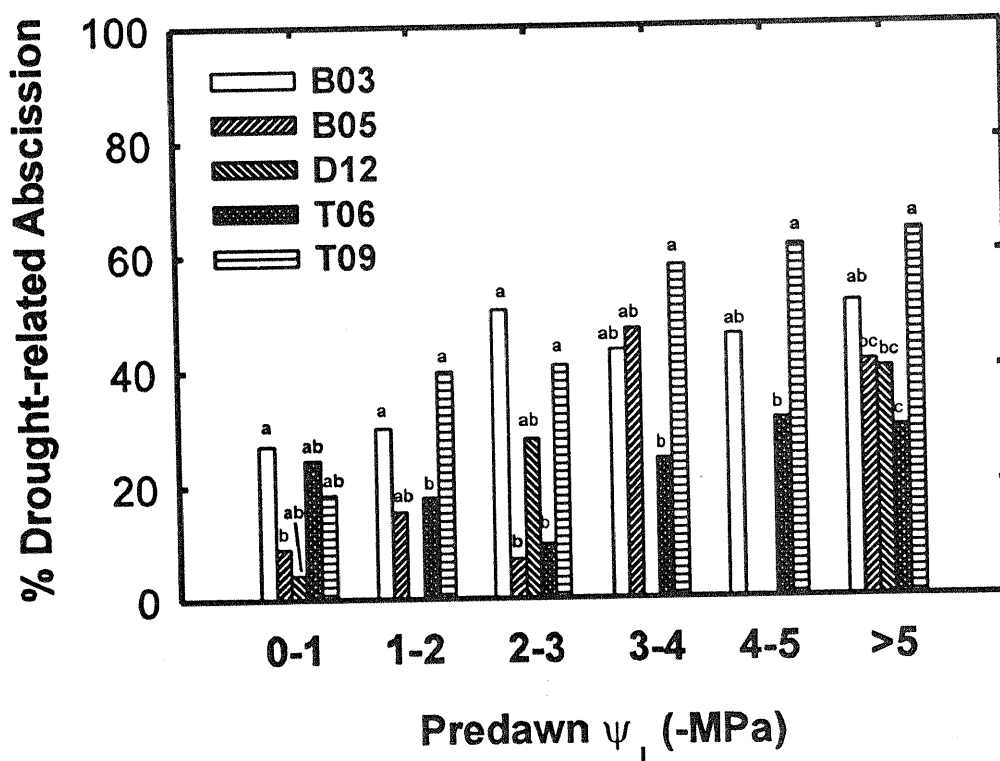

Figure 3. Clonal mean percent drought-related leaf abscission at several levels of predawn Ψ_1. Bars not capped by the same letter are significantly different (P<0.05).
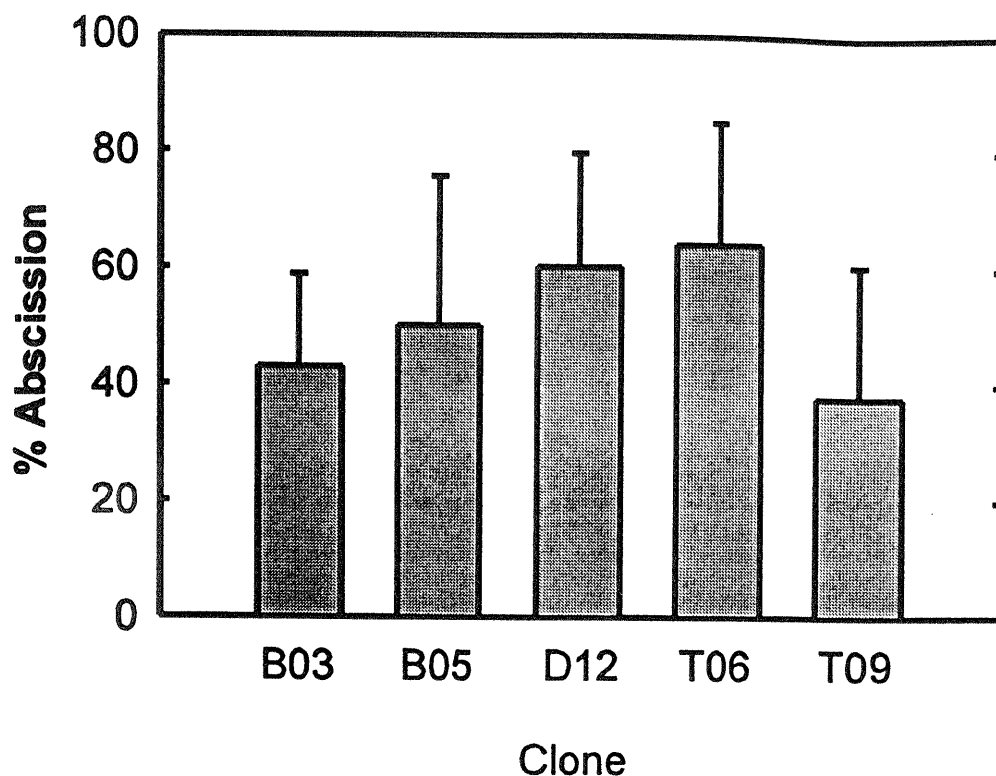
Figure 4. Mean percent abscission of phenological control plants of five *Populus* clones. Bars = 1 SD.

For the most part, new leaf area production during after stress did not differ among clones in any sustained fashion (Table 1). All clones showed capacity to produce substantial new leaf area to predawn Ψ_l values of -2 MPa (which would be characteristic of a moderate to severe drought). When predawn Ψ_l declined further, leaf area production showed a similar decline. There was one possible exception to this trend—Clone T06 appeared to possess the capacity to produce substantial amounts of new leaf area even when stressed severely (Figure 5). However, this pattern was not statistically significant (Table 1). There were no significant differences among clones nor was there a significant water-stress x clone interaction in the number of new leaves produced or in area per leaf (data not shown).

DISCUSSION

Populus clones exhibited enhanced leaf abscission under imposed drought. The levels of predawn Ψ_l shown to cause enhanced abscission in the present study can be expected during seasonal droughts in many areas where unirrigated *Populus* are grown, confirming field observations of the predisposition of this group to abscission (Dickmann and others 1992, Pezeshki and Hinckley 1982). The region of predawn Ψ_l around -2 MPa appears to be a critical threshold for *Populus*, as abscission accelerates with greater stress and production of new leaf area is greatly suppressed.
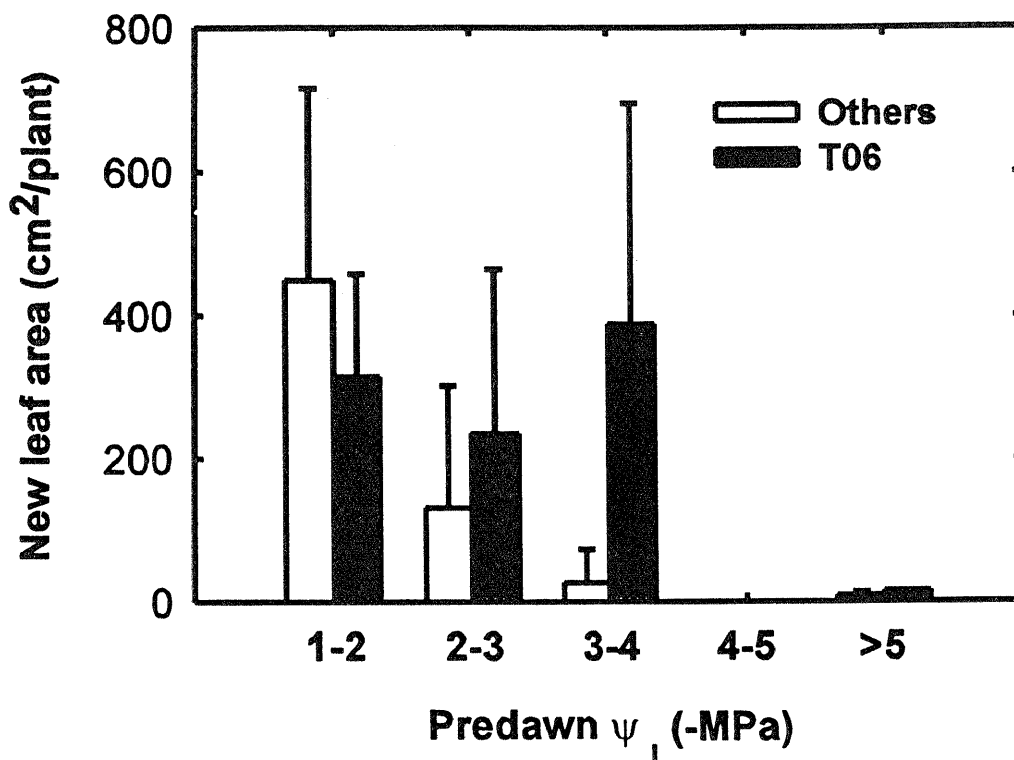
Figure 5. Mean new leaf area production rewatering of plants of clone T06 and all other clones previously dried to several levels of predawn $\Psi1$. Bars = 1 SD.

Other physiological processes in poplars appear to be correspondingly sensitive to water stress. For example, photosynthesis in two of the clones studied here (B03 and T06) was greatly reduced as Ψ_l of leaves declined from -1 to -2 MPa (Rhodenbaugh and Pallardy 1993). In other more drought tolerant species, such as many *Quercus*, photosynthetic rates and leaf retention are sustained to substantially greater levels of water stress. In post and white oaks (*Quercus stellata* Wangenh., *Q. alba* L.) Ψ_l of -3 MPa or below was required to completely inhibit photosynthesis (Ni and Pallardy 1991, 1992). Even at such low Ψ_l values there was little tendency toward leaf abscission in these two species (Pallardy and Rhoads 1993). In contrast, in the same experiments a frequently riparian species (black walnut, *Juglans nigra* L.) showed patterns of photosynthetic inhibition and leaf abscission that were similar to *Populus* in the present experiment. These responses have obvious implications with regard to competitive ability on dry sites. The general restriction of these light-demanding plants to moist sites may be at least partly attributable to their poor prospects for adequate carbon balance in drought-prone habitats.

As might be expected, new leaf area production was greatly reduced if plants were severely water stressed. Interestingly, moderate water stress appeared to stimulate production of leaf area above that observed in well-watered phenological control plants, perhaps constituting a compensatory response to loss of leaf area via abscission. In the present study, the effect of moderate water stress appeared to be larger leaves, not an increase in number of leaves. Mazzoleni and Dickmann (1988) observed a similar pattern of stimulation of leaf growth in water-stressed *Populus* x *canadensis* 'Eugenei' plants after rewatering. There are obvious limits to this response, as new leaf area development in plants subjected to lower predawn Ψ_l values was greatly suppressed. We also observed a similar pattern of new leaf area development in response to water stress in *Juglans nigra*, but in this species leaf production was extended to -3 MPa (Pallardy and Rhoads 1993). Compensatory leaf area production might be advantageous under a regime of mild-to-moderate drought, as new, physiologically vigorous leaves could increase whole-plant photo-

synthesis. However, it should be remembered that this response attends the loss of existing leaf area that represents a significant loss of carbon and nutrient resources.

Clonal differences in leaf abscission and leaf growth responses were not well defined. Previous studies have shown genetic variation within *Populus* for a wide range of physiological responses (see Introduction). Additionally, genetic differences in autumnal leaf abscission patterns have been demonstrated (Barnes 1969). Although we detected statistical differences in clonal leaf abscission responses to water stress, the differences in abscission responses in control plants confounded meaningful comparisons of water stress responses among clones. More study is needed to confirm the putative differences displayed in the present experiment. There were no statistical differences among clones in production of new leaf area, but it is worth noting that one clone—T06—appeared to exhibit some capacity for sustained leaf production under severe water stress.

ACKNOWLEDGEMENTS

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