WATER STATUS AND GROWTH INITIATION IN *POPULUS*

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ABSTRACT

Water content and water potential in stools of three clones of **Populus** spp. were studied over the dormant period. To determine the site and magnitude of water loss, sealed and unsealed cuttings were held in a range of relative humidities and weighed periodically. Water status, growth initiation and early growth were determined using water culture techniques, and polyethylene glycol to produce differences in osmotic potential.

Water content was at a minimum at leaf-fall, and rose thereafter until bud-break. Gradients along the wand tended to become reversed, indicating upward water movement. Water potential rose slowly until mid-winter and then declined, at least until bud-break.

Overall water loss was proportional to relative humidity and duration of exposure. Cut ends were the major sites of loss. At low relative humidities, bud presence was associated with reduced water loss, with small but significant clonal differences.

Root primordia and buds showed a restricted development below –11 bar, while subsequent growth of shoots and roots were severely limited below –4 bar. Water content and water potential were highly correlated with the osmotic potentials applied. Small osmotic potentials often induced greater growth responses than zero potential treatments.

The significance of the experiment is discussed in relation to water status, viability of cuttings and to larger material used in plantings in the field.

INTRODUCTION

Soil conservation techniques in New Zealand hill country rely heavily on the use of *Populus* because of the ease of vegetative propagation of clonally pure stock and the rapid rates of growth which occur in favourable conditions. The planting of long poles allows foliage to be established beyond the reach of stock, and the root systems developed by most species are very effective in binding unstable soils. Recent disease problems may temporarily restrict the future use of the genus (van Kraayenoord *et al.,* 1974).

Losses of poles used for soil conservation purposes are high, however (Edwards 1968, 1969). While the reasons for these are obscure, water deficits in the planting material may be responsible. The relationship between a severe water stress and subsequent

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failure of poles has been recorded in at least one case, where poles grown in a nursery with sandy soil in an unusually dry season had a high mortality rate (van Kraayenoord, *pers comm.*).

The study reported here investigated three aspects of water status and growth initiation in *Populus*:

a. seasonal changes in water status of annually harvested stools,

b. sites and significance of water loss from cuttings,

c. establishment of levels of water stress which limit growth initiation in cuttings.

MATERIALS AND METHODS

Three clones were used: *Populus euramericana* (Dode) Guinier cv. I-78, *P. yunnanensis* (Dode) and *P. deltoides* spp. *angulata* Ait. cv. Carolinensis. In the text, these will be referred to as I-78, *P. yunnanensis* and *P. angulata* and I-78, Yun. and Ang. in the tables.

Plant material used in the study was derived from seven-year old stools and consisted of one season's growth, 1.5-2 m long and up to 2 cm in diameter. All three clones were used in each of the three sections of the study. Changes in water status during dormancy were traced by sampling at 3-week intervals from 14 April to 7 September. Five wands per clone of 0.9-1.3 cm diameter (outside) were further subdivided into three equal parts *viz:* the upper, middle and lower regions. Each region was further divided into five 1-cm units for water content determinations and 10-15 0.5 cm units for water potential determinations.

Water loss from cuttings was studied using saturated solutions of salts to maintain relative humidities of 100.0, 93.0, 80.5, 55.5 and 0.0% in closed containers (Winston and Bates, 1960). Cuttings were 2.5 cm long and had 1.0-1.3 cm outside diameter, half being sealed at both cut ends with paraffin wax and the rest left unsealed. Within each sealing treatment, half of the cuttings had a lateral bud at the distal end and half did not. Temperature was maintained at $20.0 \pm 1.0°C$. The water content of each cutting after six 24-hour periods was calculated.

A range of water stresses was imposed on cuttings 10 cm long with 1.0-1.3 cm outside diameter, harvested on 1 August some six weeks prior to budbreak and after an adequate cold treatment. A growthroom maintained at $20.0°C \pm 1.0°C$ and lit by a bank of fluorescent tubes giving 68 lux was used. A 12/12 hour light-dark sequence was applied. The cuttings, cut level with the tip of a lateral bud at the distal end, were sealed at both ends with wax.

Four holes were cut in the plastic tops of blackened, 150-ml paper cups and a sealed cutting was inserted in each hole so that 7.5-8.0 cm was immersed in the solution.

Polyethylene glycol (PEG), the molecular weight of which was cryoscopically determined to be 207.0, was used in ten concentrations to induce water stress, *viz.* 0, -1, -2, -3, -4, -6, -8, -11, -15 and -20 bar.

Solutions were renewed at 48-hour intervals after a first renewal at 24 hours. Twenty cuttings per clone per treatment were set up, and 5 of these were destructively sampled at 3 stages: (a) after 4 days, (b) when growth was first initiated in each clone and (c) 14 days after growth initiation. For each cutting sampled, water content was determined both above and below the water level, and Schardakov's dye technique (Knipling, 1967)

was used to determine water potential of the region of the cutting below the water level. In addition, observations of time of appearance, numbers and growth of shoots, root initials and roots were made at 2-day intervals. Measurements at termination also included shoot and root dry weights.

Growth and water status measurements were analysed as 2-factor factorial designs using measurements of outside diameter, pith diameter or the ratio of pith volume to total volume to obtain the most significant covariance analyses. Regression analysis was used to relate water content to water potential and growth to water status.

RESULTS

Seasonal Changes in Water Status

Changes in water potential and water content during the season are shown in Table 1. In general, water potential rose from leaf-fall to a mid-winter maximum and then fell, while the water content rose from a minimum at leaf-fall until budbreak.

Water potential gradients along the wands just reached significance on two occasions. In I-78, a water content gradient with high distal levels was present, diminishing over the dormant period. In *P. yunnanensis* a similar gradient became reversed. In both clones, the water potential of the proximal regions increased more rapidly than in the distal although in *P. angulata* a large gradient with high distal levels was maintained. Regression analysis between water potential and water content was just significant on only two occasions.

TABLE 1—Seasonal water status of 1-year wands grown on stools

(a)   Water content (%)

Clone	Position on Wand	14 Apl	5 May	26 May	16 Jun	7 Jly	28 Jly	18 Aug	8 Sep
I-78	Distal	51.5	51.1	53.5	53.4	55.5	52.5	54.7	55.1
	Middle	49.1	49.2	51.9	52.8	54.7	52.6	54.4	55.0
	Proximal	47.7	48.5	50.6	52.7	54.1	52.5	53.8	54.5
Yun.	Distal	56.4	51.1	50.3	50.7	55.8	58.4	55.8	58.2
	Middle	53.7	49.2	48.9	50.3	55.5	58.7	57.1	59.3
	Proximal	52.8	47.9	48.2	50.4	55.4	59.0	58.1	60.0
Ang.	Distal	60.1	57.6	54.1	57.7	58.6	57.7	57.2	58.5
	Middle	56.1	55.2	51.3	54.0	54.4	54.5	55.1	54.8
	Proximal	51.7	50.7	49.5	50.1	51.9	53.2	53.0	53.3
L.S.D. (5%)		1.3	1.4	0.9	1.0	0.8	0.7	0.7	0.7

(b)   Water potential (bar)

		14 Apl	5 May	26 May	16 Jun	7 Jly	28 Jly	18 Aug	8 Sep
I-78		—2.87	—1.87	—1.78	—1.25	—1.15	—1.50	—1.71	—2.68
Yun.		—2.81	—2.01	—2.17	—1.33	—0.76	—1.02	—1.28	—1.59
Ang.		—2.75	—2.64	—2.65	—1.73	—1.75	—2.07	—3.36	—5.17
L.S.D. (5%)		0.65	0.73	0.56	0.36	0.38	0.54	1.01	0.62

N.B.—Relation of water potential to position on wand did not reach significance
(5% level)

Effect of Relative Humidity on Water Loss

The effect of sequentially lower values of relative humidity was an increase in water loss which could be greatly reduced by sealing the ends. Small significant differences in clonal water content existed throughout the experiment, but overall, no significant clonal difference in the rate of water loss was found.

TABLE 2—Water loss (as percentage of dry weight) from cuttings of all clones in a range of relative humidities

| | | | Relative Humidity | | | L.S.D. |
Day	100.0%	93.0%	80.5%	55.5%	0%	5%
(a) Sealed cuttings						
1	—0.0	—0.3	—0.9	—2.5	—3.5	—1.0
2	1.1	—0.5	—2.1	—4.7	—6.9	—1.5
3	—0.2	—1.2	—3.5	—7.6	—11.2	—1.8
4	—0.2	—1.5	—4.6	—10.0	—14.6	—2.0
5	—0.4	—2.0	—6.2	—12.9	—17.7	—2.3
6	—0.4	—2.4	—8.4	—15.6	—21.1	—2.5
(b) Unsealed cuttings						
1	—0.3	—1.4	—5.0	—10.8	—20.2	—1.0
2	—0.4	—2.1	—8.0	—17.7	—31.8	—1.5
3	—0.9	—3.5	—11.9	—25.3	—42.4	—1.8
4	—1.1	—4.2	—14.8	—30.5	—45.1	—2.0
5	—1.3	—5.1	—18.0	—35.1	—46.7	—2.3
6	—1.4	—5.9	—21.4	—38.4	—47.4	—2.5

At a relative humidity of 100%, all unsealed cuttings lost fresh weight, and all sealed cuttings gained fresh weight in the first 48 hours. *P. angulata* lost weight in the first 24 hours only. Subsequently, all clones lost fresh weight.

At relative humidities below 100%, both sealed and unsealed cuttings invariably lost weight. Those cuttings which had a bud lost water less rapidly than budless cuttings when in high relative humidities, but at low relative humidities bud presence was associated with a greater loss. The particular relative humidity at which the presence of a bud did not affect water loss varied with each clone. The effect of bud presence, although significant, was not large.

Effect of Water Stress on Growth

In general, water potentials of cuttings fell with decreasing osmotic potential of the growth medium. However cuttings of I-78, in solutions of —2 bar had higher water potentials than those in —1 bar solutions over all sampling times.

Cuttings of *P. yunnanensis* exhibited similar variations but not the well-defined trough in water potentials of I-78. *P. angulata* showed no such deviations. While these effects were seen in all seven 2-day observations, only the final observations (14-day) are presented for one clone (Table 4).

TABLE 3—Effect of bud presence on percent water loss. The results presented are the difference between cuttings with and without a bud. A positive value indicates cuttings with buds lost less water than those without buds.

	Day	% Relative Humidity (20°C)					L.S.D. (5%)
		100%	93.0%	80.5%	55.5%	0%	
I-78	1	0.23	0.01	−0.39	−0.27	−2.08	0.99
	2	0.19	0.24	0.61	1.44	−1.17	1.46
	3	0.17	0.69	1.39	2.16	0.24	1.78
	4	0.18	0.97	2.23	1.57	1.14	2.04
	5	0.13	1.42	3.17	1.35	1.17	2.26
	6	0.11	1.75	3.44	0.67	1.23	2.51
Yun.	1	0.02	−0.13	0.03	−1.33	−0.99	0.99
	2	−0.03	0.01	0.28	−1.01	−0.48	1.46
	3	−0.09	0.16	0.57	−1.66	−0.64	1.78
	4	−0.13	0.40	0.93	−1.55	−0.16	2.04
	5	−0.17	0.57	0.65	−2.17	−0.05	2.26
	6	−0.19	0.81	0.65	−2.34	0.18	2.51
Ang.	1	−0.05	−0.12	0.11	−0.87	−2.19	0.99
	2	−0.11	0.11	0.20	−0.71	−2.56	1.46
	3	−0.14	0.40	0.99	−1.17	−1.74	1.78
	4	−0.16	0.54	1.48	−1.41	−0.82	2.04
	5	−0.85	0.61	1.70	−1.95	−0.91	2.26
	6	−0.33	0.68	1.71	−2.46	−0.18	2.51

TABLE 4—Effect of osmotic potential of medium (in bar) on water status of cuttings: after 4 days (a); at growth initiation (b); and after 14 days growth (c). Initial water content for I-78 was 52.4%, and initial water potential for all clones was −1.53 bar. No significant interclonal differences were proved for water potential.

Medium	Water content (percent) Clone I-78			Water potential (bar) All clones		
	a	b	c	a	b	c
0	57.5	61.1	61.8	−1.9	−1.3	−2.8
−1	57.5	56.7	57.0	−2.0	−1.9	−4.1
−2	56.9	59.2	57.6	−1.8	−2.2	−5.5
−3	55.6	57.4	56.0	−2.3	−2.7	−6.3
−4	55.2	57.0	56.5	−2.5	−3.0	−8.5
−6	53.7	55.3	52.9	−2.8	−3.1	−9.7
−8	53.9	56.6	55.9	−3.2	−4.4	−12.6
−11	53.8	55.9	52.8	−3.3	−5.8	−12.2
−15	53.9	51.9	54.6	−3.9	−9.1	−16.6
−20	53.5	54.7	53.7	−4.5	−12.2	−19.8
L.S.D. (5%)	2.5	3.1	3.5	0.9	1.8	2.3

There were substantial and significant changes in water content between treatments and clones. Until growth initiation, water content of cuttings held at osmotic potentials of near zero rose, but fell in those of low potentials. The effect was more pronounced at the proximal region of the cuttings than in the distal which possessed an unsubmerged bud. After growth initiation, the water content rose in cuttings held under osmotic potential close to zero but remained unchanged in solutions of low osmotic potential. While changes in water content were similar for all clones, the water content of the proximal regions of *P. yunnanensis* rose considerably under near-zero osmotic potential.

Osmotic potentials below −6 bar reduced shoot growth to a low level, although more negative potentials allowed some growth even at −20 bar. In I-78 (Table 5) there was less shoot growth in −2 bar solutions than −1 bar solutions at all times, and a similar trough occurred in shoot growth of *P. yunnanensis* at −4 bar, but no trough was observed in *P. angulata*. Root initials were developed in all clones in solutions down to −8 bar, but below this level fewer were observed, only 33% of cuttings developing any initials at −15 bar.

The greatest number of root initials were developed at −3 bar in I-78, 0 bar in *P. yunnanensis* and −4 bar in *P. angulata*. Root growth from the initials was similarly inhibited, but at a much higher level—there was virtually no root growth below −4 bar in any clone. The time of appearance of root initials and subsequent root growth was increasingly delayed, as well as increasingly inhibited in solutions of more negative osmotic potentials.

Although these effects on root and shoot growth were seen in all clones, *P. angulata* appeared to be most sensitive.

There was a significant inverse relationship (not reported here) between water potential and water content at almost every sampling time, this being greater in the proximal region of cuttings than in the distal region, in all three clones. In addition, measurements of both water potential and water content were significantly related to concurrent measurements of shoot length, root number and root growth.

TABLE 5—Effect of osmotic potential of medium (in bar) on shoot and root growth. Results for clone I-78, 14 days after growth initiation.

Medium Potential	Shoot length (cm)	Shoot weight (mg)	Roots — length development initials	Roots — length development up to 1 cm	Roots — length development above 1 cm	Roots — totals per cutting length (cm)	Roots — totals per cutting dry weight (mg)
0	11.3	111.8	3.3	0.4	4.3	24.4	4.2
−1	6.4	70.6	8.4	0.6	3.9	12.5	1.6
−2	3.8	55.3	11.3	1.1	2.8	11.2	2.0
−3	2.0	31.6	13.7	0.5	2.6	8.8	1.9
−4	1.2	27.8	14.8	0.2	0.8	2.4	1.0
−6	0.7	16.6	12.3	0.0	0.0	0.0	0.0
−8	0.1	4.1	12.7	0.1	0.0	0.0	0.0
−11	0.0	0.0	6.2	0.0	0.0	0.0	0.0
−15	0.0	0.0	1.7	0.0	0.0	0.0	0.0
−20	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L.S.D. (5%)	1.2	—	3.2	0.8	1.3	5.7	—

DISCUSSION

The completion of leaf fall coincided with the minimum observed water contents, the distal leaves being last to fall. The soluble exports may have contributed to the higher water content observed distally through osmotic potential adjustments. The slow overall rise in water content at least until budbreak would suggest that slow cell-to-cell movement occurred in response to local gradients of water potential as suggested by Weatherley (1963). The source of water could be either from root reserves (which could be substantial in the light of the large root/shoot ratios found in old stools) or from a small root uptake in response to water potential gradients, or from absorption of precipitation by the wands, especially through the buds.

Most of the increase in water content had taken place by the time that water potential maxima were reached in mid-winter while vernalization requirements were also completed by this time. With bud activity slowly increasing due to rising temperature and an increasing day length, a consequent slow rise in solute concentration might follow inducing a decrease in water potential. The buds showed no visible changes during this period.

Water loss from cuttings at various relative humidities were shown to be primarily from the cut ends with total loss being influenced by cutting length. The presence of a bud appeared to effect a greater retention of water with a greater loss in unbudded cuttings at low relative humidities. The quantity of water involved was greater than the total water content of the bud itself, inferring that the bud influenced the water status of the tissue in its vicinity and only after vernalization had allowed the bud a slow increase in metabolic activity.

With transfer from the low ambient field temperatures to laboratory temperatures of 20°C, there was a 24-hour lag before the retention effect at high relative humidities was observed. This implied a physiological change possibly associated with early changes in bud metabolism and the mobilization of reserve water. The sequence of events from other studies (Kozlowski 1971) has shown the involvement of auxin at an early stage in bud activity stimulating neighbouring tissue. Such changes would affect water potentials possibly via osmotic changes in the tissues concerned. Since several buds commenced budbreak within 6 days of treatment at high relative humidities, such changes may have occurred early in the experiment.

At the lower relative humidities, a very considerable overall loss occurred. Wolter and Kozlowski (1964) found that water was lost comparatively readily from buds of *Populus*, and the magnitude of loss at 0% relative humidity in this experiment suggested that the bud was not well protected. Burstrom (1948) showed that water loss from buds was influenced by bud size, water content, properties of bud scales and relative humidity, although Wolter and Kozlowski (*op. cit.*) did not find such intra-specific correlations. In this study, water loss was least in I-78 which also had the smallest bud dry weight and surface area. Further, the "crossover" point between the high and low relative humidity effects was also different between clones, I-78 being lower than *P. yunnanensis* or *P. angulata*. Thus I-78 appeared to be more resistant to water loss from the buds, probably because of bud size or budscale permeability.

The ability of cuttings to produce root initials even in solutions of low potential (i.e. when highly stressed) may reflect the comparatively isolated nature of the sites of

root initiation within the cutting, since commencement of growth was shown to be primarily a function of rising temperature. However when root elongation commenced, the root tissues were in intimate contact with the particular solution and root growth at this stage may have more truly reflected the effect of the imposed water stress. Similarly most bud growth was limited at potentials lower than −4 bar, although some growth was apparent in cuttings even at −15 bar.

Many of the growth and water potential measurements showed that there was slightly greater growth and lower water potential when cuttings were grown in −1 bar compared with zero or −2 bar. This trend was consistent in I-78 and frequent in *P. yunnanensis* but was not seen in *P. angulata*. Drew (1967a, b, c) similarly found that mineral-free water reduced growth in comparison to small osmotic potentials in growth media using minerals, and McWilliam *et al.* (1970) found a similar result using poly-ethylene glycol solutions to germinate grass and legume seeds. Milthorpe and Ivins (1966) further note that the most favourable water potential for early growth in fruit trees is nearer −1 than full hydration.

There is no obvious reason why growth should be increased by small osmotic potentials of the growth medium. Many workers equate maximum growth with maxi-mum hydration (Kramer, 1969), but there is some evidence to the contrary.

In I-78 and *P. angulata*, root development appeared to be from root primordia, formed during development of the wand. Larger numbers were observed on I-78 than *P. angulata*. In *P. yunnanensis* however, roots originated from proliferation of phellogen tissue about the lenticels and in longitudial cracks in the rhytidome. These observations are in line with the description by Frohlich and Dietze (1970) of different modes of rooting in the several sections of *Populus*.

Low available moisture levels in soil in late autumn and early winter are likely to limit those water reserves held in the stool, so lowering the water content even further at leaf-fall. The seasonal rise in water content and water potential noted here would then be restricted, unless absorption of rain or dew was sufficient to compensate.

Water potentials in the cutting material taken from stools before bud-break did not fall to levels which would limit subsequent growth, as indicated by the experiment using a range of osmotic concentrations. For these stools, recommencement of growth appeared to be a function of rising ambient temperature rather than limiting water stress. However it is clear that a small water stress can limit subsequent growth and that such a stress can develop rapidly in cuttings, particularly (a) those which have unsealed cuts, (b) those which are in an environment of even mild water stress and (c) probably those clones which have small buds in conditions of low relative humidity.

The overall magnitude of water loss from cuttings under constant relative humidity was shown to be comparatively large and in the field would lead to limiting levels of water stress within a period of days, even at high relative humidities. It appears likely that wands still attached to the parent stool may be receiving substantial replenishment by the redistribution of water reserves or possibly by root absorption even in dormancy. However, diurnal and other variations in relative humidity and temperature together with precipitation and dewfall may result in substantial reduction in the effect of prolonged low relative humidities noted here.

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