A PHYSIOLOGICAL INTERPRETATION OF NURSERY STOCK CONDITIONING THROUGH INTENSIVE ROOT WRENCHING

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Abstract: Conditioning studies conducted in South East Queensland nurseries with 1-0 Caribbean pine seedlings have demonstrated the need for intensive, in situ root wrenching schedules in obtaining successful outplanting performance under sub-tropical conditions.

Investigations into some physiological aspects of the conditioning phenomenon are reported for Caribbean pine seedlings raised under both nursery and glasshouse environments.

The conditioning process is interpreted as a composite alteration of metabolic pattern under strong hormonal control resulting from induced plant water deficits. Observed changes in hormonal activity, root growth potential, stomatal sensitivity and osmoregulatory capacity are viewed as adaptive-type responses to stock conditioning which provide plants with mechanisms for both avoidance of and tolerance to water stress.

Additional Keywords: P.caribaea var. hondurensis, abscisic acid, osmotic potential, turgor potential.

ROOT WRENCHING PRACTICE

The practice of root wrenching, defined as the partial severence and concomitant mild disturbance of nursery-stock root systems <u>in situ</u>, is not new (see Goudie 1935). New Zealand nurserymen, however, recently adapted the time-honoured practice of conservative, spade root wrenching, i.e. infrequent and deep root severence, to a stock conditioning or hardening regime <u>per se</u> (van Dorsser and Rook 1972). They introduced the technique of frequent, shallow, mechanised root wrenching as a means of conditioning 1-0 <u>P.radiata</u> D.Dor seedlings to withstand transplanting stress.

This cultural conditioning treatment was soon trialed elsewhere, including Queensland where intensive root wrenching was identified as an essential prequisite for the successful outplanting establishment of 1-0 <u>P.caribaea Morvar. hondurensis</u> Barr. et Golf. (Caribbean pine) seedlings (Bacon and Hawkins 1977).

Most studies of root wrenching practice have focused on the development of specific root wrenching schedules with documentation of gross morphological characteristics and outplanting performance of treated stock. Few studies in comparison have been concerned with gaining an understanding of the basic physiological processes and responses involved (Bacon 1980). Nevertheless there is currently a widespread acceptance of root wrenching as a stock conditioning tool (Boyer and South 1984, Duryea 1984).

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PHYSIOLOGICAL STUDIES

Experimental Stock

Caribbean pine seedlings were grown either in the nursery or glasshouse. Nursery stock were 10 months old at lifting and had received one of the following treatments during the four months prior to lifting;

N1: Untreated controls

N2: Root wrenched at monthly intervals

N3: Root wrenched at weekly intervals

Root wrenching was carried out using a taut wire drawn by a tractor. The measure of seedling water status during the nursery phase was relative water content (RWC), measured gravimetrically on detached fascicles. The first root wrenching lowered RWC significantly (91.2% to 76.3%), the last root wrenching lowered RWC significant only in the N2 (monthly) stock (89.4% to 79.1%). RWC recovered in all stocks after 2 to 4 days.

Glasshouse stock were raised singly in plastic tubes $(1\ 336\ cm^3)$ for 6 months and subjected to the following treatments during the final three months,

Gl: Watered daily and roots left intact

G2: Watered daily and roots cut once a week

G3: Watered once a fortnight and roots left intact

G4: Watered once a fortnight and roots cut once a week

Roots were cut with a sharp blade and left otherwise undisturbed. Leaf water potential (Ψ_1), measured as xylem pressure potential on cut shoots in a pressure chamber, assessed seedling water status during the glasshouse phase. The initial root severence lowered Ψ_1 from -0.49 to -1.12 MPa. Recovery to pre-stress levels was rapid. Thereafter the effect of root cutting on Ψ_1 decayed quickly, particularly in the well-watered treatments (Gl and G2). After the first two root severences only droughted stock (G3 and G4) recorded major reductions in Ψ_1 (minimum of -1.70 MPa).

Both nursery and glasshouse conditioned stock, i.e. those subjected to cyclic water stresses, were identified as small, sturdy plants with a reduced leaf area and an improved root/shoot ratio.

At the completion of the nursery - or glasshouse - conditioning phase seedlings were transferred to a range of controlled environments (benign to desiccating) in growth cabinets.

Selected Growth Processes

Bacon and Bachelard (1978) have reported on the measurement of transpiration, net photosynthesis, ^{14}C translocation to roots and root growth capacity of the nursery-grown stock after transplanting. The influence of stock pretreatment in the nursery on all these growth processes was significant and the results are summarised in Table 1. Weekly root wrenched stock (N3) were physiologically more active than monthly treated stock (N2) which in turn were more active than the untreated controls (N1).

Survival rates mirrored this treatment ranking (90.6%, 75.0% and 21.9% respectively). The greater capacity for root growth of the root wrenched plants, thereby re-establishing early contact with moisture reserves in the rooting medium, did help minimise the development of plant water deficits (maximum - 2.25 and-1.68 MPa in N1 and N3 stock).

Table 1: Influence of stock treatments on selected growth processes of Caribbean pine seedlings after transplanting from the nursery.

		Growth Processes $\frac{1}{-}$					
Stock Treatments		Transpiration	Photosynthesis	Translocation	Root Growth		
N1	Untreated	1.0	1.0	1.0	1.0		
N2	Wrenched Monthly	1.4	1.7	3.7	7.4		

3.0

6.3

9.5

2.1

Plant Growth Substances

N3 Wrenched

Weekly

Chromatographed extracts from both nursery - and glasshouse - grown seedlings were tested in appropriate bioassays for auxin, gibberellin, cytokinin and inhibitor activity (Bacon and Bachelard 1979). Stock conditioning either by root wrenching in the nursery or droughting in the glasshouse induced marked changes in the level of some growth substances (Table 2). Gibberellin and cytokinin concentrations were significantly reduced by pre-stressing treatments while inhibitor levels were significantly increased. Auxin levels remained relatively constant.

Table 2: Relative changes in endogenous concentrations of plant growth substances in Caribbean pine seedlings at time of transplanting.

Stock Treatment	Auxin	Gibberellin	Cytokinin	Inhibitor	
Nursery Grown					
Nl Untreated	1.0	1.0	1.0	1.0	
N3 Wrenched Weekly	1.1	0.4	0.4	1.6	
Glasshouse Grown					
Gl & G2 Watered	1.0	1.0	1.0	1.0	
G3 & G4 Droughted	0.9	0.3	0.4	2.2	

Class of Growth Substance

¹/ Rates expressed relative to untreated controls.

Subsequent work identified the major inhibitor present as abscisic acid (ABA). Using electron-capture, gas-liquid – chromatography for accurate ABA determinations the relationship between ABA content and leaf water potential (Ψ_1) was derived for glasshouse-grown stock (Figure 1). At a threshold Ψ_1 (c -1.0 MPa) ABA content increases dramatically in all seedlings irrespective of treatment regime.

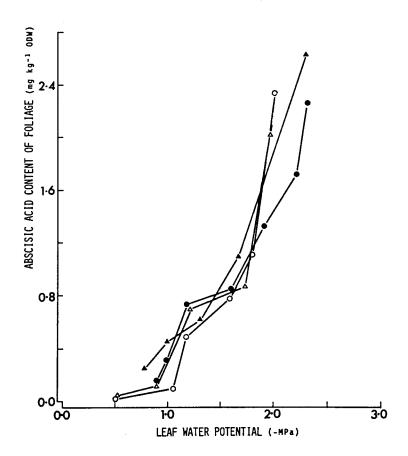


Figure 1: Relationships between foliage-abscisic acid content and leaf water potential of glasshouse-grown Caribbean pine seedlings. Glasshouse treatments included; Gl(O): watered daily and roots left intact, $G2(\Delta)$: watered daily and roots cut once a week, $G3(\bullet)$: watered once a fortnight and roots left intact, $G4(\Delta)$: watered once a fortnight and roots cut once a week.

Water Relations

Glasshouse-grown plants were transferred immediately after rewatering into a desiccating environment and the water status of plants monitored during the subsequent drying cycle. Measurements were made of relative water content (RWC), leaf water potential (Ψ_1), leaf osmotic potential (Ψ_o), measured psychrometrically on macerated needles, and leaf pressure potential (ψ_p) measured as the difference between ψ_1 and ψ_o .

The moisture characteristic curves depicted in Figure 2 (a plot of ψ_1 versus RWC) show that conditioned plants, those pre-stressed by cyclic droughts (treatments G3 and G4) lost only a small amount of tissue water for a relatively large decrease in ψ_1 . The opposite is true for plants raised under daily watering schedules (treatments G1 and G2).

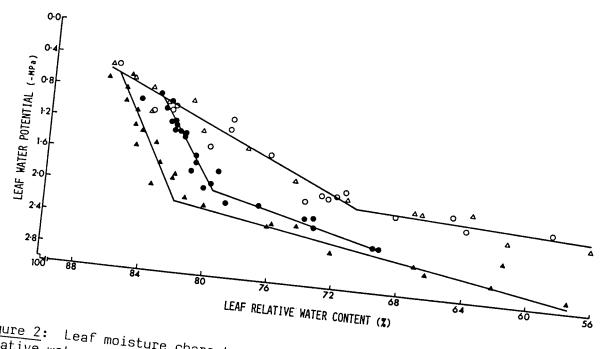


Figure 2: Leaf moisture characteristic curve (leaf water potential versus leaf relative water content) of glasshouse-grown Caribbean pine seedlings. Treatments

The plots of ψ_0 versus ψ_1 (Figure 3) reveal that conditioned plants possess significantly lower osmotic potentials (0.50 to 1.20 MPa) over the whole water potential range sampled (-0.50 to -3.00 MPa). The decrease in ψ_0 can be partially explained by a passive increase in solute concentration via tissue dehydration (c - 0.30 MPa maximum). The remainder is attributed to active solute build-up, termed osmoregulation or osmotic adjustment (Turner 1979).

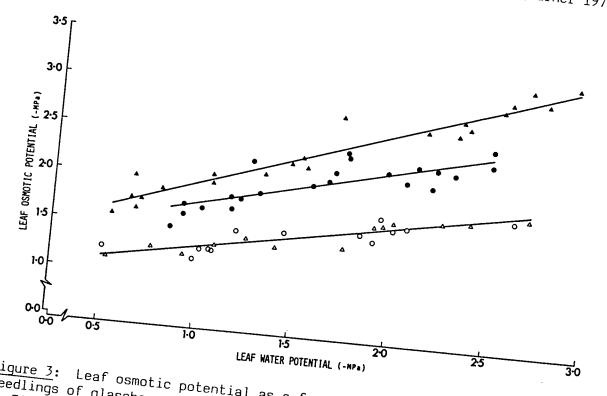
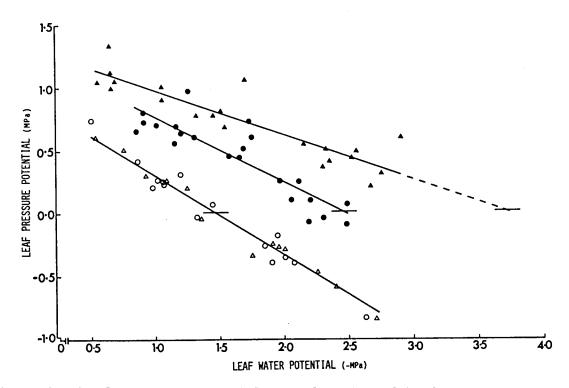


Figure 3: Leaf osmotic potential as a function of leaf water potential for seedlings of glasshouse-grown Caribbean pine. Treatments described in caption -346-

As a consequence of this osmotic adjustment, conditioned seedlings can maintain positive turgor over a wider water potential range than non-conditioned stock. The extent of this adaptation is shown in the ψ_p versus ψ_l plots provided in Figure 4. For instance, the ψ_l at which ψ_p approaches zero (the point of incipient plasmolysis) drops from -1.46 to -2.45 to -3.75 MPa in Gl plus G2 (watered), G3 and G4 (droughted) stock. Visual observations of seedling wilt support these analyses.



<u>Figure 4:</u> Leaf pressure potential as a function of leaf water potential for glasshouse-grown Caribbean pine seedlings. Treatments described in caption to Figure 1.

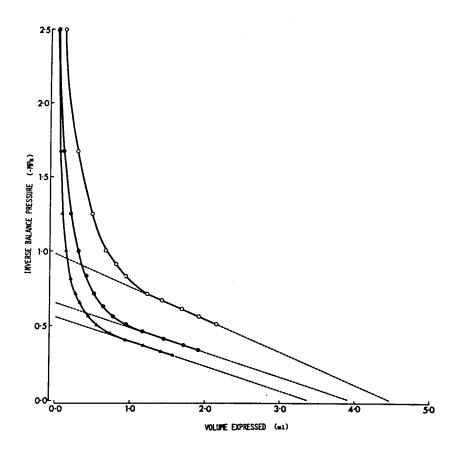
All pre-stressed seedlings (G3 and G4) survived 19 days of cabinet desiccation (ψ_1 down to -2.75 MPa) while none of the well-watered seedlings (G1 and G2) recovered from 15 days of desiccation (ψ_1 down to -2.60 MPa).

Pressure-volume (P-V) curves were constructed (after Tyree and Hammel 1972) from measurements made on detached shoots sampled at the end of the glasshouse phase. Representative curves are detailed in Figure 5. In the examples shown, osmotic potential at maximum turgor (determined by extrapolation of the straight line portion of the P-V curve back to the ordinate) is estimated at -1.0, -1.5 and -1.8 MPa for watered (G1 plus G2), droughted (G3) and droughted plus root severence (G4) stocks. These data confirm via an independent methodology the existance of the osmoregulation response in conditioned seedlings.

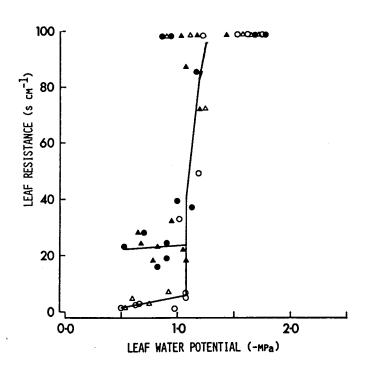
Stomatal Responses

The effect of water stress conditioning treatments on stomatal behaviour, measured as changes in leaf resistance with a ventilated diffusion porometer, was determined on seedlings transferred at the end of the glasshouse phase into a desiccating, cabinet environment. Figure 6 depicts the leaf resistance/leaf water potential relationship.

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 $\underline{\text{Figure 5}}$: Pressure-volume curves for Caribbean pine seedlings raised in the glasshouse. Treatments described in caption to Figure 1.



 $\overline{\text{Figure 6}}$: Relationship between leaf resistance and leaf water potential for Caribbean pine seedlings raised in the glasshouse. Treatments described in caption to Figure 1.

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Conditioned plants (G3 and G4) had markedly higher leaf resistance values (16 to 32 s cm⁻¹) than non-conditioned plants (G1 and G2) (2 to 7 s cm⁻¹) at high ψ_1 (> - 1.00 MPa). Partial stomatal closure in pre-stressed seedlings is likely but in addition stomatal pore area per unit leaf area was reduced. At a threshold ψ_1 of about - 1.10 MPa leaf resistance increased significantly indicating complete stomatal closure occurred. This response mirrors the abscisic acid build up at about - 1.00 MPa given earlier (Figure 1).

If the leaf resistance data is replotted on leaf pressure potential using the ψ_p/ψ_l regressions previously determined for the same experimental stock (Figure 4), threshold pressure potentials at which stomatal closure occurs at a ψ_p of 3.28 MPa in non-stressed plants and 0.95 MPa for pre-stressed plants (treatment G4). Stomata of conditional plants thus appear to be more sensitive to an approaching water stress.

THE CONDITIONING PROCESS

Caribbean pine seedlings can be conditioned to survive the shock of transplanting or the transfer to a desiccating environment by prior exposure to a number of repetitive plant water deficits. These plant water deficits can be imposed either through periodic root wrenching (common in nursery practice) and/or droughting (common in glasshouse practice). Root removal alone is not an effective conditioning treatment unless accompanied by an adequate reduction in leaf water potential.

The results presented here indicate that adequately pre-stressed Caribbean pine seedlings acquire resistance to water stress through a number of recognisable adaptations in plant morphology and physiology. These adaptive type responses to conditioning provide the plant with mechanisms for both avoidance of and tolerance to water stress (Bacon 1980).

Avoidance mechanisms: Two mechanisms of avoidance are identified; a reduction in water loss and a maintenance of water update. A reduction in water loss is achieved in three ways; high minimum leaf resistance, stomata sensitive to developing stress, and reduced foliage area. The maintenance of water update is achieved by an enhanced root system and root growth capacity.

Tolerance mechanism: Maintenance of turgor through a capacity to osmoregulate.

Since water stress is known to affect practically every aspect of plant growth and development (Hsiao 1973) it is reasonable to assume that periodic alteration of plant water status is responsible for initiating the adaptation or conditioning process. It is likely that the effects of turgor changes on plant hormone balances, particularly abscisic acid metabolism, are the basic stress-sensitive regulatory processes (Hanson and Hitz 1982).

CONCLUSION

The conditioning process is interpreted as a composite alteration of metabolic pattern under strong hormonal control resulting from induced plant water deficits. Observed changes in hormonal activity, root growth potential, stomatal sensitivity and osmoregulatory capacity are viewed as adaptive-type responses to stock conditioning which provide plants with mechanisms for both avoidance of and tolerance to water stress.

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