## WATER RELATIONS OF WRENCHED AND UN-WRENCHED PINUS RADIATA SEEDLINGS ON BEING TRANSPLANTED INTO CONDITIONS OF WATER STRESS

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### SYNOPSIS

Internal water deficits and rates of transpiration of seedlings which had been unwrenched (C), root wrenched once (W/1), or wrenched at fortnightly intervals over the previous five months (W/F) were compared for seven days after being transplanted into conditions of water stress in a controlled environment cabinet. Relative turgidities of the needles of the seedling pretreatments were ranked in the order W/F > W/1 > C. Rates of transpiration of the W/F seedlings were significantly higher than those of the seedlings of the other two treatments. Differences in rates of transpiration between detached shoots of the three treatments were slight; those of the W/F seedlings had the highest rates. Seven days after the seedlings were transplanted the root systems of only the W/F stock showed many new roots. Results from this investigation support those from field trials which indicate that well-wrenched transplants survive dry conditions better than W/1 plants, and even more so than C plants, owing in part at least to the effect of repeated wrenching in producing a root system more capable of meeting the requirements of the plant after transplanting.

### INTRODUCTION

The normal test for quality of planting stock is to measure survival and initial growth rates of transplants in the field. The unpredictability of climate and site, however, make field trials unreliable and field experiments run the constant risk of being ruined by fungal or insect pests, weed growth or other interference. Even where satisfactory growth is made, large numbers of replicated plots are essential to eliminate chance variation in plant response. Controlled environment facilities which allow a specific set of weather conditions to be programmed at any time enable a seedling's performance to be gauged under known, controlled and reproducible conditions.

Over the last few years, a substantial part of the research effort on nursery and establishment practice at the Forest Research Institute has been to test the efficiency of several procedures for hardening *Pinus radiata* D.Don nursery stock (FRI 1964, 1965, 1966). Root wrenching has been included in

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these treatments and field trials have indicated the superiority of well-wrenched stock over unwrenched seedlings. Empirical research methods are, however, only slowly defining what physiological criteria of the planting stock are critical in obtaining a satisfactory field performance, and a more basic physiological approach to this problem might be expected to yield more informative results.

The present study is only part of a more general investigation into the relationship between the physiological condition of *P. radiata* and survival rate in the field. This investigation compares the internal water contents and rates of transpiration of wrenched and unwrenched *P. radiata* seedlings under conditions of water stress.

### MATERIALS AND METHODS

Fifteen-months-old, spring-sown *P. radiata* seedlings grown in the FRI nursery at Whakarewarewa were used in this study.

The seedlings had previously been given one of the following three degrees of root wrenching:

C — Unwrenched.

W/1 — Wrenched once only, 5 months previously.

W/F — Wrenched at fortnightly intervals over the previous 5 months.

Seedlings from these three treatments are illustrated in Fig. 1. Average root/shoot (oven-dried weight) ratios were C, 0.16; W/1 0.26; W/F, 0.44.

The W/F stock could be expected to have a high survival rate when planted out under adverse conditions, while the unwrenched seedlings would be virtually a complete failure. The W/1 and the W/F seedlings had such similar fibrous root systems that it was extremely difficult morphologically to distinguish between roots of these two treatments. However, while the W/1 stock had soft, green, actively growing shoots similar to the unwrenched stock, the shoots of the W/F plants were brown and lignified and terminal growth had ceased.

Five plants of each treatment were used in each of the three experiments in a randomized block design. Each seedling was carefully dug up, immediately planted in a bucket of soil, and watered to field capacity before being transferred to the artificially lit controlled environment cabinet (CSIRO Model LBH — described by Pescod, *et al.*, 1963). The unwrenched seedlings proved difficult to lift from the seedbeds without causing considerable damage to the deeply penetrating tap root.

Transpiration was measured by determining the weight loss of seedlings growing in sealed buckets which had initially been watered to field capacity. Where detached shoots were used, the volume of water transpired was measured. Rates of transpiration were expressed per unit of leaf volume per 24 hr.

Plant water balance was measured as leaf water deficit and calculated as relative turgidity (Weatherley, 1950; Clausen



FIG. 1: Experimental radiata pine seedlings: left, wrenched once only, 5 months earlier; centre, unwrenched; right, wrenched at fortnightly intervals over previous 5 months.

and Kozlowski, 1965) — *i.e.*, field water content was expressed as a percentage of the water content of the needles at full turgidity.

### **EXPERIMENTS**

This study was carried out as three separate experiments, the first two of which investigated the internal water balance and rates of transpiration of entire, wrenched and unwrenched seedlings, while the third experiment employed detached shoots of wrenched and unwrenched stock.

#### Experiment No. 1

After being transplanted, the seedlings were given a day/ night temperature regime of  $20^{\circ}/15^{\circ}$ C with a uniform relative humidity of 95% for 24 hr to allow them to overcome the initial shock of being transplanted. Thereafter day/night temperatures of  $35^{\circ}/31^{\circ}$ C and relative humidities of 43/60% were provided.



FIG. 2: Changes of relative turgidity against time, for wrenched and unwrenched seedlings subjected to 35/31° C and 43/60% relative humidity.

Figure 2 shows the average relative turgidity values for the C, W/1 and W/F seedlings during the course of this experiment. (All times refer to when the seedlings were provided with temperatures over 30°C and low humidities.) Within seven hours the relative turgidities of the C seedlings were significantly lower than those of the W/F seedlings, and by 100 hr the relative turgidities of the C seedlings were significantly lower than those of the W/1 seedlings. (The 5% probability level was taken as indicative of any statistical significance.)

The *C* seedlings became severely wilted with dead needles on some plants during the latter half of the experiment and this showed as a drastic fall in foliage water content. Although the relative turgidities of the W/1 plants were consistently less than those of the W/F plants, the differences were not statistically significant at the 5% level.

Presumably the relative turgidities of the plants of all treatments fell markedly when the environmental conditions were changed from the cool, moist conditions of the 24 hr following transplanting to the hot, dry climate programmed for the experiment. This condition is suggested by the low relative turgidity values recorded at 3 hr. The sharp increases in the relative turgidities of the W/F and W/1 seedlings from 3 to 7 hr may have been due, at least in part, to the change from day to night conditions — *i.e.*, decrease in temperature and increase in humidity. The less severe desiccating conditions for the two hours before the 7 hr samples were collected probably enabled the W/F and W/1 plants to recover part of their water deficits. These data suggest that, whereas the seedlings of the two wrenched treatments were able to adjust rapidly to the dry conditions set in this experiment, the C plants were never able to recover.

When the plants were harvested at the end of the experiment, the roots of only the W/F seedlings showed many white growing tips.

Each of the potted seedlings was weighed immediately before the water stress conditions were programmed, then reweighed two and six days later. The average rates of transpiration expressed as "grams of water transpired per millilitre of foliage per 24 hr" were calculated over the first 48 hr, then from 48 to 141 hr for each of the seedling treatments. The rates of transpiration of the seedlings over the first and second part of this experiment are presented in Table 1. From 0 to 48 hr and from 48 to 141 hr in Experiment 1 the W/F

# TABLE 1: RATES OF TRANSPIRATION, EXPRESSED AS GRAMWATER LOSS PER ML OF FOLIAGE VOLUME PER 24 HR, OF C,<br/>W/1 AND W/F SEEDLINGS

	Expt. 1		Expt. 2		Expt. 3
	A	B	A	B	A
	0-48 hr	48-141 hr	0-68 hr	68-160 hr	0-70 hr
С	0.47	0.27	0.52	0.26	1.45
	(37)	(17)	(20)	(18)	(77)
W/1	0.93	0.74	0.72	0.40	1.56
	(60)	(52)	(29)	(29)	(83)
W/F	1.56	1.46	2.55	1.38	1.89
	(100)	(100)	(100)	(100)	(100)

(Figures in parentheses indicate the relative rates of transpiration of the seedlings for that period of the experiment.)

plants showed significantly higher (0.5% level) rates of transpiration than the W/1 and C seedlings. Over the first two days the W/1 and C seedlings transpired at rates of 60 and 37% of those, respectively, of the W/F seedlings, and during the latter four-day period these rates dropped to 52 and 17%, respectively. The rates of transpiration of the W/F seedlings in absolute units were similar throughout the six-day experimental period.

It may be argued that expressing rates of transpiration on a unit leaf volume basis may have biased the results in favour of the W/F plants which were considerably smaller than the W/1 and especially smaller than the C plants. Average ovendried weights of the seedlings were W/F, 40.7; W/1, 56.8; C, 86.3 g. Even if transpiration is expressed on a per plant basis, the W/F and W/1 plants had 66 and 46% higher rates of transpiration than the C seedlings over the six-day experimental period.

### Experiment No. 2

The first experiment clearly showed that the responses of the W/F, W/1 and C seedlings to water stress were very different; the present study was designed to determine how the behaviour of the seedlings changed with an even greater water stress. Following transplanting, the seedlings were given more severe conditions of desiccation than in the first experiment — *i.e.*,  $36^{\circ}/33^{\circ}$ C and 27/45% day/night temperatures and humidities, respectively — and this environment was provided immediately without any initial recovery period.

The environmental conditions programmed in this experiment provided similar internal water deficits in the seedlings of the three treatments as did the conditions given in the first experiment, but there were significant differences in rates of transpiration between these two experiments.

Rates of transpiration of the W/1 and C seedlings were 29 and 20%, respectively, of the rates of the W/F seedlings (Table 1); these differences were significant at the 0.1% level, although the differences between the W/1 and C seedlings were not significant even at the 5% level. The reduced rates of transpiration of all seedlings during the second part of the experiment were presumably due to the soil in the immediate vicinity of the roots having dried out.

If transpiration is expressed on a per plant basis, the rates of the W/F and W/1 seedlings were 97 and 20% greater, respectively, than the average rate of the C seedlings.

The drier conditions programmed for the second experiment compared with the first considerably increased rates of transpiration of the W/F seedlings over the first two days *i.e.*, 2.55 g water per ml of foliage in Experiment 2 compared with 1.56 g/ml in Experiment 1 (Table 1). Surprisingly, rates of transpiration of the W/1 stock in Part A of the latter experiment were 78% less than the rates observed from 0 to 68 hr in the first experiment, whereas the C seedlings had similar rates of transpiration in the two experiments.

### Experiment No. 3

Having found in the first two experiments that the W/F seedlings transpired more rapidly, and also maintained higher internal leaf water contents than the W/1 and especially the C seedlings, this experiment with detached shoots attempted to define whether these differences in response were due to differences present in the shoots or in the root systems.

The shoots were cut off under the water, their cut ends placed in a measured volume of water and transferred to the controlled environment cabinet running at 35°/31°C day/night temperatures and 43/60% relative humidity (same conditions as for Experiment 1).

The rates of transpiration of detached shoots of the C and W/1 seedlings were 77 and 83%, respectively, of those of the W/F detached shoots (Table 1). The rates for detached shoots may be compared with those of Experiment 1 for entire seedlings as the environmental conditions were similar; from 0 to 48 hr in Experiment 1, the C and W/1 seedlings had rates of transpiration 37 and 60%, respectively, of those of the W/F plants. The detached shoots of each treatment had higher rates of transpiration than the entire seedlings of the same treatment, thus entire seedlings of the W/F, W/1 and C treatments had rates 82, 65 and 36%, respectively, of those of the detached shoots of the equivalent treatments.

### DISCUSSION

Although the ultimate test of tree seedling quality must remain its field performance, controlled environment facilities can allow a fuller understanding of the physiological mechanisms operative behind greater survival and more rapid growth following transplanting. Tranquillini (1965) used the artificial conditions of a wind tunnel to test the resistance of young *Pinus cembra* to severe drying conditions, observing that lignification of the shoot did not decisively increase drought resistance.

The relative turgidity technique was used to estimate the plant's internal water balance and to indicate plant viability. The relative turgidities of the seedlings of the two wrenched treatments were significantly greater than those of the unwrenched seedlings which showed a continued decrease with time; severe wilting was apparent at a relative turgidity of less than 70%. The relative turgidity measurements would suggest that the *C* seedlings would not survive planting out, and that the *W*/1 plants would succumb more quickly to adverse environmental conditions or mishandling than would the *W*/*F* stock. This result is in general agreement with data from field trials (FRI, 1965).

The observation that the W/F seedlings had significantly higher rates of transpiration per unit leaf volume than the W/1, and especially the C stock, was surprising as it had been assumed generally that the greater survival of wellwrenched stock was due to its greater control of transpiration water loss. In the latter part of Experiment 2 when the water supply to the roots of all three treatments was limited and its uptake decreased, the rates of transpiration of the three treatments fell proportionally, suggesting that there may be very little difference in control of water loss by wrenched and unwrenched stock, but this aspect was not studied in any detail. This investigation did show that the root systems of the repeatedly wrenched stock could support higher rates of transpiration and maintain higher internal water contents than could roots of the W/1 or C seedlings. Presumably the higher root/shoot ratios of their needles more readily than did the lower ratios of the other treatments.

The root/shoot ratios of 0.16, 0.26 and 0.44 for the C, W/1 and W/F seedlings, respectively, were calculated on an ovendried weight basis. Cameron (1967) has pointed out that the efficiency of a root system for absorbing water and nutrients will depend more on total surface area than on weight. The root systems of the W/F and W/1 seedlings were characterized by the presence of long, thin, fibrous roots with a large surface area compared with the few, thick roots in the seedlings of the C treatment (Fig. 1) which would enable the former plants to tap a larger volume of soil. This form of root system would place the wrenched seedlings at an even greater advantage than the root/shoot ratio values suggest.

Comparing the results from Experiments 1 and 3, the rates of transpiration of the detached shoots were higher than those for the entire seedlings of the corresponding treatment. Obviously each of the root systems, including those of the W/F treatment, was limiting transpiration to some extent.

Rates of transpiration *per se* are probably not of direct physiological importance to the survival of the seedling, but rather the internal water deficits largely determine whether or not the seedling survives. The highest rates of transpiration of th W/F seedlings, however, would suggest that they were able to carry on active photosynthesis soon after being transplanted. A higher level of metabolic activity was suggested also by the presence of many new root tips when the W/F plants were harvested at the end of the first two experiments. As Meyer et al. (1963) state: "the number of root tips borne by a root system is probably the most important index of its effectiveness in obtaining water and mineral salts from the soil". No evidence of any new root growth was observed in the W/1and C seedlings, although the W/1 root systems were outwardly very similar to those of the W/F seedlings. These differences in root regeneration potential between the W/F seedlings and the W/I and C plants must reflect differences in the physiological state of the plants at the time of lifting (Stone, 1967) owing presumably to repeated wrenching. The work of Stone and co-workers (e.g., Stone and Schubert, 1959; Stone et al., 1962; Krugman and Stone, 1966) has demonstrated the importance of a high root regeneration potential in obtaining satisfactory survival and growth of transplanted Pinus ponderosa and Pseudotsuga menziesii. It is interesting to

note that well-wrenched P. radiata stock appears to have a high root regeneration potential, which may well be linked with its high survival rate when transplanted under adverse conditions.

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