CHANGES IN WATER POTENTIAL OF PINUS RADIATA FASCICLES DURING TEMPORARY STORAGE

BRIAN J. MYERS and IAN E. CRAIG
CSIRO Division of Forestry and Forest Products, P.O. Box 4008,
Queen Victoria Tce, A.C.T. 2600, Australia

(Received for publication 25 September 1985; revision 18 January 1988)

ABSTRACT

Excised **Pinus radiata** D. Don fascicles were stored in cold, humidified, glass vials for some hours prior to measurement of needle water potential using a pressure chamber. Tests of the technique with various types of fascicles suggested that the change in water potential during storage for up to 12 hours was within acceptable limits (<0.06 MPa) for fully expanded C+1 fascicles grown in the field, but not for current expanding fascicles nor for fascicles growing under mist irrigation in a shade house.

Keywords: water potential; needle storage; pressure chamber; **Pinus radiata**.

INTRODUCTION

The use of the Scholander pressure chamber (Scholander *et al.* 1964) for measuring the hydrostatic pressure of tissue apoplasm, which is assumed to closely approximate the water potential of the tissue symplasm (Passioura 1980), is widely accepted (Ritchie & Hinckley 1975). The main attractions of this technique over other methods of estimating tissue water deficits lie in its portability and simplicity of use in field applications.

In practice, however, several sources of error can influence the reliability of the readings, such as transpiration from the leaf between the time it is cut and the time it reaches the balancing pressure in the chamber. Baughn & Tanner (1976a) calculated that in rapidly transpiring leaves, the rate of change of water potential can be as high as 0.01 MPa/s and Turner & Long (1980) measured changes up to 0.7 MPa in the first 30 seconds after cutting. Precautions that can be taken to reduce this source of error include minimising the time interval between cutting and pressurising, humidifying the chamber during pressurisation, and wrapping the foliage in an impervious material such as plastic film, bag, or aluminium foil (Ritchie & Hinckley 1975; Baughn & Tanner 1976a; Turner & Long 1980; Leach *et al.* 1982).

In some situations (for example, when the number of measurements is large or the water potentials are very low) it is not feasible to measure all the leaves immediately and erroneous conclusions may be drawn because of changing potentials during a long sampling period. It is then desirable to reduce total sampling time by harvesting all

leaf samples rapidly and storing them for subsequent determination of water potential, provided that water potential does not change during storage.

There have been conflicting results reported on the storage of foliage using a variety of techniques (Baughn & Tanner 1976a; Hellkvist & Parsby 1976; Karlic & Richter 1979; Kaufmann & Thor 1982). Leaf water potential can change during storage because of a net transfer of water to or from the leaf or because of internal tissue changes such as cell expansion and respiration (Baughn & Tanner 1976b; Nonami & Boyer 1987). Water will move into or out of the leaf if there is a gradient of water potential between the leaf and its environment. The rate of water transfer depends on the magnitude of the gradient and on the resistance to flow through the stomata and cuticle. A zero gradient in water potential will be achieved only when the water potential of the leaf equals that of the water vapour in the air which, according to Slatyer (1967), is –1.29 MPa at 5°C and 99% RH and –2.59 MPa at 98% RH. Thus, a large potential gradient may exist even at very high humidities. The success of any storage technique in preventing significant changes in water potential will depend on its ability to maintain saturated air around the foliage, on the condition of the stomata and the cuticle, and on the degree to which cell enlargement and respiration can be reduced by low non-freezing temperatures.

Faced with an extensive monitoring programme of pre-dawn needle water potential in a large *Pinus radiata* field experiment, needle storage for some hours prior to measurement was desirable. This paper describes a technique for doing so and the effect of needle development, season, and initial water status on the stability of water potential during storage for up to 12 hours.

MATERIALS AND METHODS

Four tests were conducted on 11-year-old trees in a *P. radiata* plantation near Canberra, Australia. A major experiment had been established on the site with treatments including irrigation and fertiliser application. Sample fascicles were harvested from trees growing under contrasting soil moisture regimes that had developed different pre-dawn water potentials. A fifth test was conducted on 4-year-old clonal grafts of *P. radiata* growing under mist irrigation in a shade-house. Different pre-dawn water potentials were achieved by regulating soil water supply for several days prior to sampling.

The tests were conducted on single fascicles cut with a scalpel at the base of the fascicle sheath. Severed fascicles were placed into 25-mm-diameter glass test tubes, suspended by the needle tips from a slit in the rubber stopper. A wet rubber sponge in the base of the tube served to humidify the air. They were stored, surrounded by ice, in a styrofoam cooler box at 5°C. Between 10 and 15 seconds elapsed from excision to sealing in the tubes.

Each sample consisted of eight adjacent fascicles (three only in the shade-house test) which were stored in one tube. For each test, seven such samples were harvested from one or two adjacent twigs in quick succession. The water potential of each fascicle in one sample was measured immediately after cutting and every 2 hours thereafter for 12 hours.

Needle water potentials were measured using a pressure chamber (Model 1000, PMS Instrument Co., Corvallis, Oregon) which was modified by inclusion of a soft rubber seal in the lid to enable rapid insertion of a fascicle and external adjustment of the tension on the seal during pressurisation. A slow pressurisation rate of 0.02 MPa/s was used. End-point determination was made to 0.01 MPa accuracy using a binocular microscope at 30× magnification. The chamber was humidified by a lining of wet rubber sponge.

Three of the tests on the plantation trees were conducted on fully expanded C+1 fascicles, one harvested pre-dawn in mid-summer, one pre-dawn in midwinter, and one at noon in midwinter. The fourth test on the plantation trees was conducted on current expanding fascicles harvested pre-dawn in early summer. The stomatal conductances measured with a null balance porometer (LI 1600, LI-COR Ltd, Lincoln, Nebraska) prior to cutting were zero pre-dawn and between 0.4 and 0.5 cm/s at midday. The test on the shade-house grown material was conducted on fully expanded C+1 foliage harvested pre-dawn in late summer. All tests included samples with high and low initial potentials (>-0.8 and <-1.2 MPa respectively) while some included a third medium level.

For each test, the departure of the measured water potentials from the initial potential was calculated and a repeated measures analysis of variance was conducted on these data.

RESULTS

The mean departures of the samples from the initial potentials at 2-hourly intervals for the five tests are shown in Fig. 1. The fascicles which showed the least departure from initial potentials during 12 hours of storage were the C+1 fascicles harvested pre-dawn and at noon in midwinter (no significant difference at $p < 0.01$) and the C+1 fascicles harvested pre-dawn in midsummer. While the latter did vary significantly from the initial potential, the maximum departure was –0.06 MPa at one time only and all other departures were –0.04 MPA or less which is within the normal reading accuracy of the pressure chamber, which we consider an acceptable departure. The average departure of the current foliage harvested pre-dawn in early summer was not significant for the first 4 hours but it subsequently dropped to 0.15 MPa below the initial value. Similarly, the average departure of the shade-house grown foliage increased rapidly from –0.05 MPa after 2 hours (no significant difference) to between –0.09 and –0.12 for the duration of the test.

There was a tendency for the transpiring C+1 fascicles harvested at noon to show a positive departure from the initial potential indicating that they were taking up water from the tubes while C+1 fascicles harvested before dawn from the plantation-grown trees tended to show negative departures suggesting that they were losing water. Similiarly, in all tests, there was a tendency for the samples at high initial water potentials to show negative departures and those at low initial potentials to show positive departures.

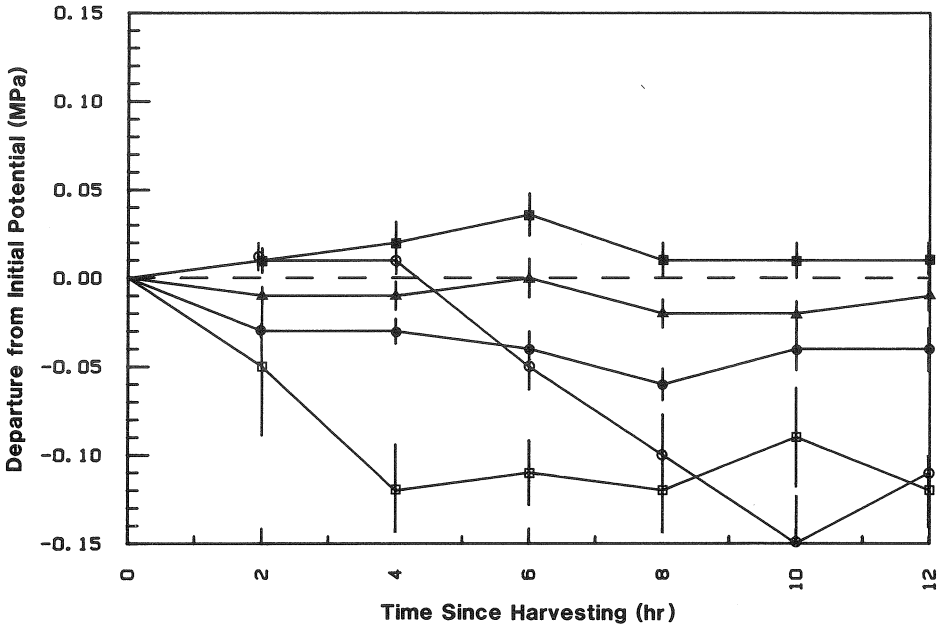

FIG. 1—Departure of **Pinus radiata** needle water potential from initial water potential during 12 hours' storage. Vertical bars represent one standard error.

C+1 foliage, summer, pre-dawn ●
C+1 foliage, winter, pre-dawn ▲
C+1 foliage, winter, mid-day ■
Current foliage, summer, pre-dawn ○
C+1 foliage, shade house, pre-dawn □

DISCUSSION

The tests reported here support the published evidence that changes in water potential during temporary storage of excised leaves are highly variable and may be affected by a number of factors. Changes reported vary from 0.01 MPa over 24 hours for *Ginkgo biloba* L. (Karlic & Richter 1979), to 0.3 MPa within an hour for herbaceous species such as potato, capsicum, and soybean (Baughn & Tanner 1976a). The available evidence suggests that storage of broadleaved foliage may be less reliable than storage of conifer foliage (Karlic & Richter 1979) perhaps because of the greater resistance to water loss in the latter (Holmgren *et al.* 1965). Attempts to store mature leaves of several eucalypt species by a variety of techniques have been unsuccessful in maintaining water potential within acceptable limits (B. J. Myers unpubl. data).

One technique used to reduce water loss during storage, coating the leaves with vaseline, was successful for sclerophyllous evergreen species but not for deciduous broad-leaved species and not if the leaves were in contact with wet rubber sheets (Karlic &

Richter 1979). The inclusion of a source of humidity in the storage vials reduced the change in potential of *Pinus contorta* var. *latifolia* Engelm. needles during 4 hours' storage from about 0.4 MPa to less than 0.05 MPa, with the exception of needles under severe water stress, which changed by 0.12 MPa despite the humidification (Kaufmann & Thor 1982).

The present results show that, under certain circumstances, the change in water potential of *P. radiata* fascicles stored in the manner described will remain within acceptable limits for up to 12 hours. Field-grown fully expanded C+1 fascicles were successfully stored when harvested pre-dawn with their stomata closed, or at noon with their stomata open. They were also stored successfully when harvested pre-dawn in midwinter or in midsummer, although the departures were slightly larger in the summer. Presumably, a well-developed cuticle and efficient stomata contribute to the resistance to water flow into or out of these needles.

Under two of the test conditions, however, water potential changes did not remain within acceptable limits. Current-year fascicles which were still in the needle elongation phase dropped to 0.15 MPa below the initial potential during 12 hours' storage. This loss of water potential may have resulted both from the continued enlargement of cells during storage and from water loss through a poorly developed cuticle. Fascicles from the shade-house grown plants also stored poorly. While harvested pre-dawn, the stomatal conductance of these plants was not checked. It is possible that, as a result of growing under mist irrigation and in heavy shade, the stomata of these plants did not totally close at night or that the cuticle was poorly developed thus reducing the resistance to water loss during storage.

Our results indicate that detached *P. radiata* fascicles may be stored in cold humidified test tubes in the manner described for up to 12 hours without a change in water potential, but under some circumstances the potential may change beyond acceptable limits. Before using the technique for other than fully expanded C+1 fascicles grown in the field, we recommend that tests similar to those described here be conducted on the material to be stored.

ACKNOWLEDGMENTS

We are grateful to Dr R. Leuning of this Division for helpful discussions.

REFERENCES

BAUGHN, J. W.; TANNER, C. B. 1976a: Leaf water potential: comparison of pressure chamber and **in situ** hygrometer on five herbaceous species. **Crop Science 16:** 181–4.

——— 1976b: Excision effects on leaf water potential of five herbaceous species. **Crop Science 16:** 184–90.

HELLKVIST, J.; PARSBY, J. 1976: The water relations of **Pinus sylvestris**. III. Diurnal and seasonal patterns of water potential. **Phsyiologia Plantarum 38:** 61–8.

HOLMGREN, P.; JARVIS, P. G.; JARVIS, M. S. 1965: Resistance to carbon dioxide and water vapour transfer in leaves of different plant species. **Physiologia Plantarum 18:** 557–73.

KARLIC, H.; RICHTER, H. 1979: Storage of detached leaves and twigs without changes in water potential. **New Phytologist 83:** 379–84.

KAUFMANN, M. R.; THOR, G. L. 1982: Measurement of water stress in subalpine trees: effects of temporary tissue storage methods and needle age. **Canadian Journal of Forest Research 12:** 969–72.

LEACH, J. E.; WOODHEAD, T.; DAY, W. 1982: Bias in pressure chamber measurements of leaf water potential. **Agricultural Meteorology 27:** 257–63.

NONAMI, H.; BOYER, J. S. 1987: Origin of growth-induced water potential. **Plant Physiology 83:** 596–601.

PASSIOURA, J. B. 1980: The meaning of matric potential. **Journal of Experimental Botany 31:** 1161–9.

RITCHIE, G. A.; HINCKLEY, T. M. 1975: The pressure chamber as an instrument for ecological research. **Advances in Ecological Research 9:** 165–254.

SCHOLANDER, P. F.; HAMMEL, H. T.; HEMMINGSSEN, E. A.; BRADSTREET, E. D. 1964: Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. **Proceedings of the National Academy of Sciences, USA 52:** 119–25.

SLATYER, R. O. 1967: "Plant-water Relationships". Academic Press, London and New York, 366 p.

TURNER, N. C.; LONG, M. J. 1980: Errors arising from rapid water loss in the measurement of leaf water potential by the pressure chamber technique. **Australian Journal of Plant Physiology 7:** 527–37.