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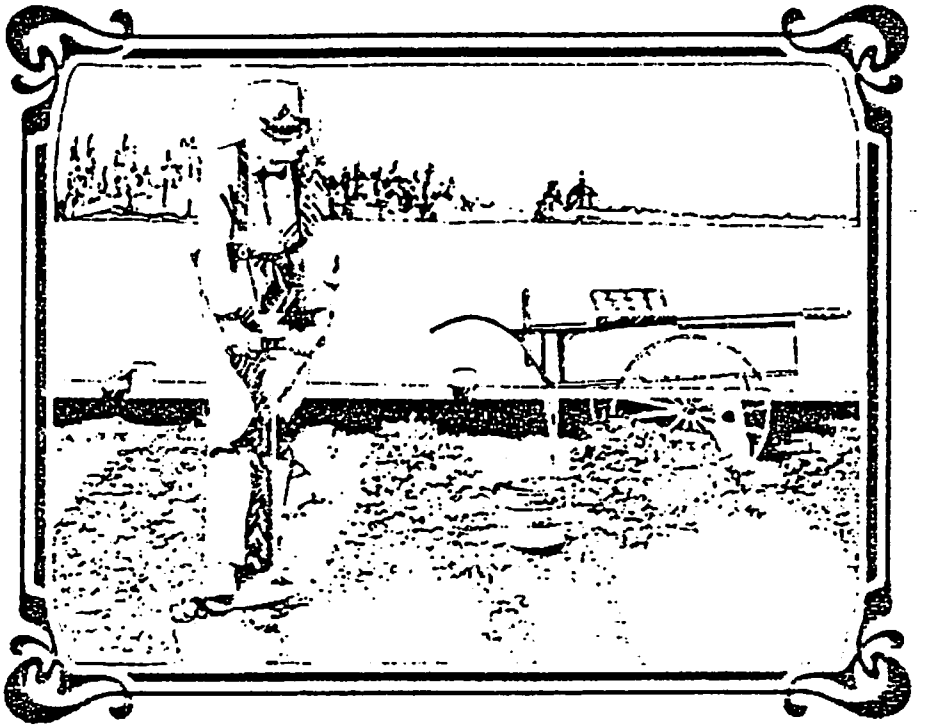

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ERRORS IN THE ESTIMATION OF PRE-EXCISION PLANT WATER POTENTIALS WITH THE PRESSURE CHAMBER

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ABSTRACT

In this experiment the pressure chamber and the thermocouple psychrometer were used to evaluate the assumption inherent in pressure chamber function that tissue water partitioning is the same at the end point as existed *in situ*. It was found for *Pinus ponderosa* seedling shoots that cavitation events in the xylem prior to excision, and partial filling of the gas filled cells under pressure, causes the pressure chamber to underestimate pre-excision plant water potential.

INTRODUCTION

The graphical representation of the relationship between a pressure chamber reading and a thermocouple psychrometer measurement on a plant tissue sample has been referred to as a pressure chamber calibration curve (Ritchie and Hinckley, 1975). Calibration curves have been used in the past to correct pressure chamber readings to more accurately reflect the water potential of the tissue sample. The use of a calibration curve for this purpose assumes that the thermocouple psychrometer, and not the pressure chamber, more accurately reflects the pre-excision water potential of the tissue.

A hypothesis has been put forth in the literature that water movement between the symplast and apoplast may contribute to the discrepancy between pressure chamber and thermocouple psychrometer measurements. An overpressure may have to be applied to rehydrate air filled pores before an end point can be achieved (Boyer, 1967; West and Gaff, 1971; 1976). Upon release of pressure, air entry into the xylem may displace water into the symplast, artificially elevating the psychrometric measurement (Dunlway, 1971). If water movement between the symplast and apoplast occurs during water potential measurement then the relative accuracy of the respective instruments depends upon: the degree of gas phase cavitation in the xylem prior to excision; the time dependence of air entry into the xylem after excision; and the magnitude of hysteresis effects in the filling and draining of xylem elements. Offsetting the error caused by changes in the partitioning of water is the effect of solutes in the apoplasmic fraction (Boyer, 1967).

The major calibration curve assumption is that the pre-excision symplasmic and apoplasmic hydration states are more accurately reproduced during the psychrometric reading than at the end point of the pressure chamber measurement. In this paper a framework for evaluating this assumption will be presented.

MATERIALS AND METHODS

Water Potential Isotherm Analysis

Two-year-old *Pinus ponderosa* Laws. seedlings were rehydrated overnight by submerging their roots in aerated water bath. The bath and seedlings were enclosed in a large plastic bag.

Three types of water potential isotherm analyses were conducted on a shoot sample from these seedlings. First, a shoot sample was subjected to a standard pressure-volume analysis (Tyree and Hammel, 1972). Shoots were dehydrated by sap expression and the xylem exudate weighed. Second, a shoot sample was subjected to a bench drying water potential isotherm analysis (Neufeld and Teskey, 1986). Shoots were allowed to transpire on the laboratory bench between weight and pressure chamber end point determinations. Third, a seedling sample was dried intact to different water contents. The shoots were then excised, weighed, measured with the pressure chamber and reweighed.

After water potential isotherm analysis, the cut ends of the sap expression and bench drying shoots were inserted into a vial of water. The shoot and vial were then placed into a high humidity chamber. The shoot was weighed periodically as it rehydrated until its weight became constant. The tissue was then dried for 172.8 kiloseconds (ks) at 65°C and weighed.

After the pressure chamber measurement, the shoots from the intact drying water potential isotherm sample were allowed to equilibrate in the high humidity environment at atmospheric pressure. After 3.6 ks, these shoots were remeasured with the pressure chamber and reweighed. These shoots were then rehydrated and dried following the procedure outlined for the sap expression and bench drying shoots.

The Relative Water Content (RWC) of the seedlings at each pressure chamber measurement was calculated from the formula:

$$RWC = (W - DW)/(FW - DW)$$

where FW is the full turgor weight after rehydration, DW is the dry weight and W is the weight of the tissue at the end point measurement.

Water Potential Estimates and Pressurization History

Seedlings were rehydrated as before and the shoots were subjected to sap expression for different lengths of time to yield end point measurements of approximately 0.5, 1.2, 1.9 and 2.6 MPa. A stable pressure chamber end point was determined and pressure was released. Shoots were then allowed to equilibrate under high humidity conditions and at atmospheric pressure for either 0, 0.3, 0.9 or 1.8 ks. The shoots were then remeasured in the pressure chamber and reweighed. Two shoots each were measured for each combination of initial and point and equilibration time.

Calibration Curve Phenomena

Pine seedlings were rehydrated overnight as before. Shoots were excised at the cotyledon whorl and were allowed to dehydrate on the laboratory bench to different water contents. A razor blade was used to cut a shoot segment 1.0 cm long from the internode representing first year's growth. A longitudinal cut was then made to create a larger non-cuticular

air-tissue interface. The segment was sealed into a sample chamber of a Decagon Devices SC-10 Thermocouple Psychrometer Sample Changer. The shoot was then cut at the internode between the first and second year's growth and the bark was stripped from the basal 2 cm of the shoot. A pressure chamber end point determination was then made on the shoot. Immediately after the pressure was reduced, a 1.0 cm sample was cut from the shoot for psychrometric analysis. The microvolt output of the psychrometer was read with a Wescor HR-33T microvoltmeter connected to a strip chart recorder. The psychrometer was calibrated with standard salt solutions (Lang, 1967). Equilibration of the psychrometric samples was very rapid and the readings were taken within one hour of being sealed within the sample chamber.

RESULTS AND DISCUSSION

Figure 1a shows the sap expression and bench drying water potential isotherm data. Figure 1b shows the same sap expression data and the intact drying data. The data were separated at -1.7 MPa at an estimated break in the slope of the bench drying curve. An F-test was used to compare slopes and intercepts of the regression lines and showed the sap expression, bench drying and intact drying curves to be different at the 95% level of confidence.

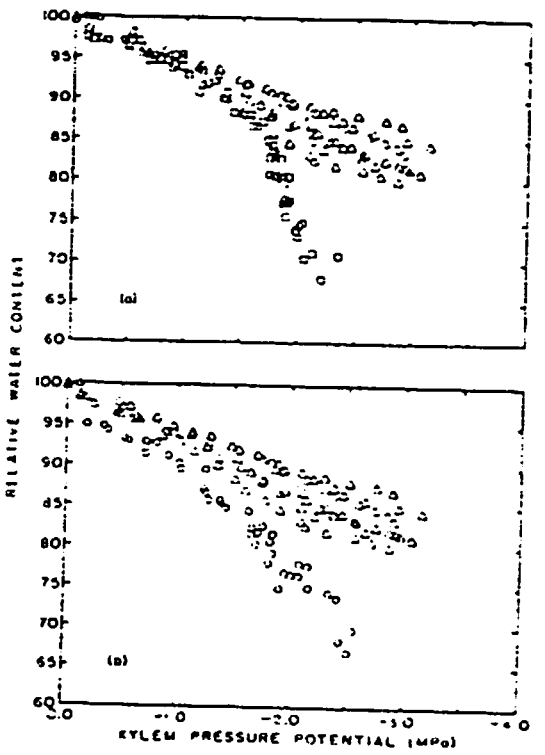

Figure 1. (a) Sap expression (Δ) and bench drying (○) and (b) sap expression (Δ) and intact drying (○) water potential isotherm data with relative water content based on rehydration of the tissues after the pressure chamber reading.

The data in figure 1 can be explained by cavitation and air entry events in the xylem and by hysteresis in the xylem water potential isotherm. The sap expression water potential isotherm represents the condition where the xylem is fully hydrated. With bench drying, air entry into the cut stem may occur and a gas phase will displace water in the xylem which will flow into the symplast. When pressure is applied to the stem during a pressure chamber reading, water will move from the symplast back into the xylem. The first structures to rehydrate in the xylem will be the smallest connective pores. The trapped gas phase will shrink but not be eliminated as the pressure is increased.

The volume of trapped gas at the end point of the pressure chamber reading will be a function of the pressure and volume of the trapped gas before pressurization and the air entry pressure associated with the internal diameter of the tracheids. As the xylem is rehydrated, water will enter the tracheid and the gas phase will be compressed. At the pressure chamber end point, the absolute pressure of the water in the apoplast will be 0.1 MPa and the pressure drop across the air-water interface of the the trapped gas phase will be equal to the air entry pressure associated with the tracheid diameter. Microscopic analysis of the woody-xylem of these seedlings yielded an approximate average tracheid inner diameter of 12 μm. The air entry pressure of a cylindrical hydrophyllic capillary with an inner diameter of 12 μm is approximately 0.025 MPa. The initial gas pressure inside the tracheids will be approximately 0.1 MPa, therefore, the gas pressure inside the tracheid at the end point will be approximately 0.125 MPa. This change in pressure will be accompanied by a volume change of only 20%.

Figure 1b shows that the intact drying seedlings exhibited a water potential isotherm most similar to the bench drying curve. This indicates that a gas phase was present in the xylem of the intact dried seedlings at the instant of end point determination. This gas phase could have been caused by either cavitation before excision or by air entry after excision.

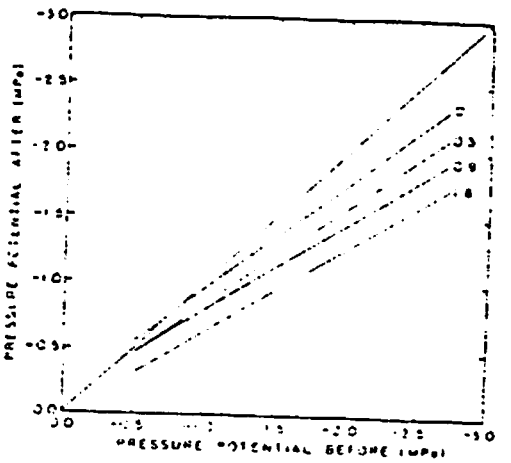

Figure 2. Regression curves for change in pressure chamber reading with time after sap expression "before" readings were taken with the pressure chamber using the sap expression method. "After" readings were taken with the pressure chamber after allowing the shoots to sit at atmospheric pressure in a high humidity chamber for 0, 0.1, 1.0 and 1.8 hrs. Each curve represents 6 measurements.

Figure 2 shows the time dependence of air entry into the xylem of the seedlings. Seedling shoots were first dehydrated by sap expression to yield the "before" reading. Pressure was released and then reapplied after different intervals of time. The seedlings were placed in a high humidity environment and lost almost no water between readings. When the pressure was reapplied immediately there was very little change in the pressure chamber reading. As more time elapsed, the difference between "before" and "after" readings increased. The regression lines shown in figure 2 were derived from eight samples each.

Figure 3 indicates the magnitude of post-excision air entry events in the xylem of intact dried seedlings. The time dependent nature of the air entry phenomenon makes it unlikely that the intact drying water potential isotherm curve shape is due to post-excision air entry. If the xylem gas phase in these seedlings was created through post-excision air entry then the curve in figure 3 would have shown more deviation from the isopotential line. Figures 2 and 3, therefore, support an hypothesis that the xylem gas phase in the intact dried seedlings was caused by pre-excision cavitation and not post-excision air entry.

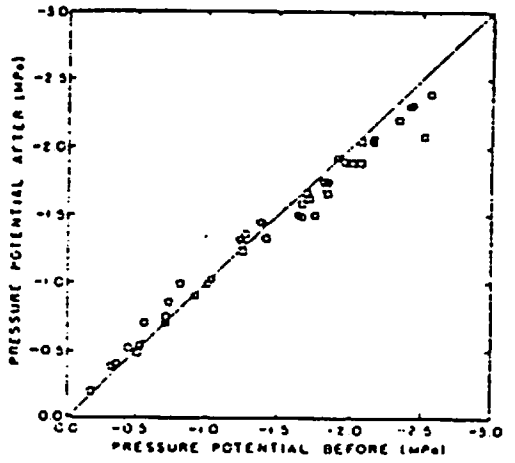

Figure 3. Change in pressure chamber reading after intact drying of seedlings. "Before" readings were taken with the pressure chamber from seedlings dried intact. "After" readings were taken with the pressure chamber after allowing the shoots to sit at atmospheric pressure in a high humidity chamber for 3.6 ks.

Figure 4 shows two pressure chamber calibration curves for these seedlings. A psychrometric measurement was taken both before and after the pressure chamber reading. The slopes and intercepts of the regression lines through the "before" and "after" calibration curves are almost identical but the "before" curve has less variability about the line. This is partially due to the greater degree of internal equilibrium of the shoot before the pressure chamber reading. The regression equations for the "before" and "after" calibration curves are $Y = 0.958\ X + 0.20$ and $Y = 0.639\ X + 0.05$, respectively. The R^2 values are 0.92 and 0.80, respectively. An F-test was used to test for equality of slopes and intercepts and it was found that the two curves cannot be distinguished at the 95% level of confidence.

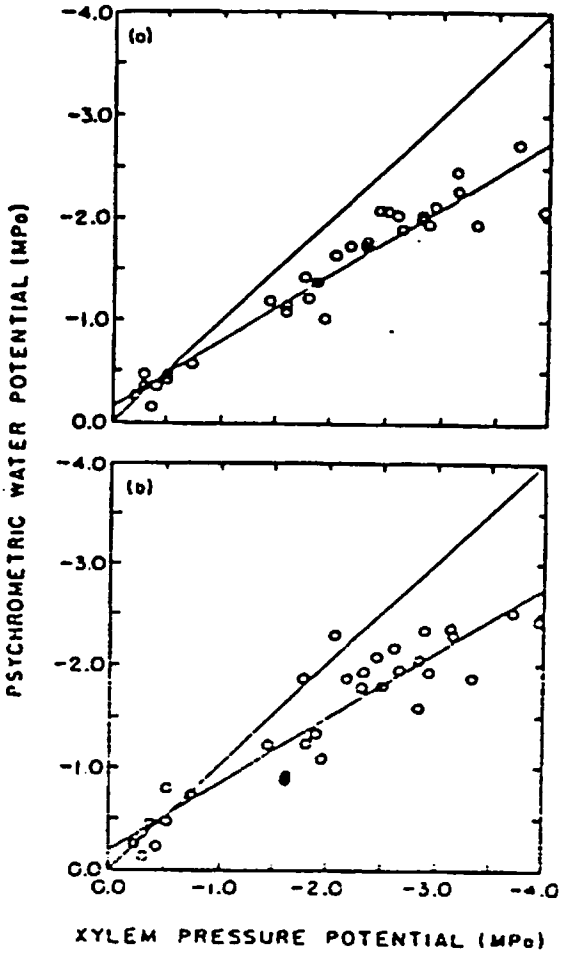

Figure 4. Calibration cures for P. ponderosa seedling shoots with the psychrometric determination made before (a) and after (b) the pressure chamber reading.

The hypothesized cause of the calibration effect illustrated in figure 4 is that a gas phase existed in the xylem prior to pressure chamber measurement. The pressure required to force water from the cut end was, therefore, greater in absolute magnitude than the tension in the apoplasmic water before measurement.

The results presented here are not applicable to other species and may not apply to P. ponderosa in general. The hypothesized calibration effect is sensitive to the xylem to symplast ratio and the seedlings used here were of relatively uniform morphology. Also, the dehydration of the intact dried seedlings was fairly rapid and the seedlings were well watered before starting the experiment. If the seedlings had been subjected to drought and then been rehydrated, gas emboli may have been present in the xylem from prior cavitation events. These emboli would have been able to expand under very low tensions and the calibration effect may have been greater at higher water potentials.

More work of this kind needs to be done with other species and under different drying conditions. Air entry would occur at lower water tension in vessel elements than in tracheids. Hysteresis in the water potential isotherm of vessel bearing xylem would be dependent upon the nature of the perforation plates. The large pores of simple perforation plates might not rehydrate until the gas phase in the rehydrating vessel was extruded from the cut end. If a gas phase is trapped in a vessel with a scalariform or reticulate perforation plate then the hysteresis effect would be maximized. A vessel with an inner diameter of 100 μm would have an air entry pressure of approximately 0.003 MPa. The rehydration of such a vessel with a trapped gas phase would be accompanied by a gas volume reduction of only 3%.

SUMMARY AND CONCLUSIONS

The pressure chamber and the thermocouple psychrometer are the two most widely used instruments for estimating the water potential of plant tissue. If sample excision is accompanied by air entry into the xylem then the psychrometer will overestimate the pre-excision water potential of the plant. If cavitation has occurred before excision then the pressure chamber, corrected for solutes in the xylem, will underestimate the pre-excision water potential of the plant.

For the pine seedling shoots studied here it was found that cavitation in the xylem likely preceded excision. There was also evidence that very little air entry into the xylem occurred after sample excision for intact dried seedlings. It can be concluded that for the pine seedlings used in this study, a calibration curve would be appropriate for correcting pressure chamber readings.

The pressure chamber is widely used for the measurement of a tissue water potential isotherm. Tyree et al.(1978) list the water relations parameters that can be calculated from this isotherm. It has been shown here and by others that the pressure chamber produces a different end point estimate for tissue at the same water content if the tissue is dehydrated by bench drying instead of by sap expression (Ritchie and Roden, 1985; Jones and Higgs, 1979; Hardegree, 1986). This discrepancy is most likely caused by air entry into the cut end of the bench drying sample and subsequent hysteresis in the rehydration of the xylem. A bench drying water potential isotherm, therefore, violates the assumption of a constant apoplasmic fraction. This in effect shifts the bench drying curve to a region of lower relative water content. As a result the extrapolated osmotic potentials may be erroneously high or low (depending upon the slope of the curve) and the extrapolated apoplasmic fraction will be erroneously low for bench dried tissues.

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