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Errors in the estimation of pre-excision plant water potential *

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Summary. Over the same water potential range, *Pinus ponderosa* (Laws.) seedling shoots lost less water when dried by sap expression than when air dried either before or after shoot excision (Fig. 1). It is hypothesized that this phenomenon was caused by air in the xylem elements of air-dried tissue and entrapment of the air during subsequent pressure chamber measurements. When shoots were dried by sap expression and pressure was released, the shoot water potential estimate became less negative unless pressure was reapplied immediately (Fig. 2). The pressure chamber reading of shoots dried intact, however, did not change after one hour of equilibration at atmospheric pressure (Fig. 2). It was concluded that there was air in the xylem of intact-dried shoots before excision but little or no air entry into xylem elements after excision. For the seedling shoots used in this study, therefore, it would be appropriate to calibrate the pressure chamber with thermocouple psychrometer measurements (Fig. 3).

Two types of experimental evidence bring into question the accuracy of plant water potential estimates made with the pressure chamber. Thermocouple psychrometer and pressure chamber estimates of water potential do not always agree (Ritchie and Hinckley 1975) and the magnitude of the pressure chamber end point has been shown to be dependent upon the method of tissue dehydration (Jones and Higgs 1979; Ritchie and Roden 1985; Hardegree 1989).

In theory, the pressure chamber should overestimate plant water potential by an amount equal to the osmotic potential of water in the apoplast (Boyer 1969). Water potential estimates from a pressure chamber, however, are frequently more negative than water potential estimates from a thermocouple psychrometer (Ritchie and Hinckley 1975). It has been hypothesized that this discrepancy is caused by the presence of air in the apoplast (Boyer 1967; Duniway 1971; West and Gaff 1971, 1976). For tissues at the same initial water potential, more pressure is required to

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reach a pressure chamber end point if there is air in xylem elements or intercellular spaces (Boyer 1967; West and Gaff 1971, 1976). The pressure chamber will, therefore, underestimate plant water potential if air is present in the xylem before sample excision. For tissues at the same initial water content, however, air entry into xylem elements will displace water into the symplast and raise the water potential of the sample (Scholander et al. 1965; Kaufmann 1968; Duniway 1971). Air entry into xylem elements after excision, therefore, will cause the thermocouple psychrometer to overestimate pre-excision water potential.

Hardegree (1989) showed evidence supporting the hypothesis that, once established, a xylem air phase would become trapped during subsequent pressure chamber measurements. Air entrapment in the xylem would explain why excised plant tissues dried by evaporation at atmospheric pressure retain less water at a given pressure chamber end point than excised tissues dried by sap expression (Hardegree 1989).

In this study the question was asked, which instrument, the pressure chamber or the thermocouple psychrometer, more accurately estimates pre-excision water potential in *Pinus ponderosa* (Laws.) seedling shoots? The assumption was made that discrepancies between pressure chamber and thermocouple psychrometer estimates of plant water potential can be attributed to the presence of air in the xylem (Boyer 1967; West and Gaff 1971, 1976). If this assumption is correct then the relative accuracy of each instrument would depend upon whether a xylem air phase was present before, or developed after excision. The assumption was also made that once air becomes established in the xylem, it will be trapped during subsequent pressure chamber measurements (Hardegree 1989). The presence of xylem air would, therefore, be indicated by a lower tissue water holding capacity than that of tissue with fully hydrated xylem.

Three experiments were undertaken to evaluate the accuracy of pressure chamber and thermocouple psychrometer estimates of pre-excision water potential in *P. ponderosa* seedling shoots. In the first experiment, the relationship between water content and water potential of shoots dried intact, before excision, was compared to that of shoots dried by post-excision sap expression and post-excision air drying. This was to determine if an air phase was indicated in the xylem of intact-dried shoots at the time of measurement in the pressure chamber. In the second experiment, shoots were dried by sap expression, allowed to equilibrate at atmospheric pressure for different lengths of time, and remeasured with the pressure chamber. This was to determine if there was a time dependence to the hypothesized phenomena of air entry and entrapment in the xylem of air-dried tissues. In the third experiment, thermocouple psychrometer measurements were made on tissue samples taken both before and after measurement with the pressure chamber. This was to confirm the discrepancy between pressure chamber and thermocouple psychrometer measurements in this species and to determine whether the relationship is affected by timing of the psychrometric measurement. The experimental results and underlying assumptions of the hypotheses are discussed.

Materials and methods

P. ponderosa seedlings were grown in 5 by 25 cm polyethylene containers filled with a potting mix of equal volumes of sand, peat moss and shredded redwood bark. The seedlings were grown

in the greenhouse for eight months and then moved outside for 16 months. All seedlings were hydrated before sampling by washing the soil material from their roots and submerging the roots in a container of aerated water overnight. The seedlings and container were enclosed in a large plastic bag to reduce evaporative demand while hydrating.

Experiment one

The relationship between shoot water content and pressure chamber end point was determined for seven shoots dried by sap expression in the pressure chamber (Tyree and Hammel 1972). An overpressure of 0.5 MPa for 12.5 min was used for each sap collection increment. After each overpressure interval, pressure was reduced until sap receded from the cut end, and then sequentially increased and decreased in increments of less than 0.1 MPa until a stable end point was achieved. A damp paper towel was placed in the bottom of a perforated plastic bag wrapped loosely around the shoot to restrict evaporative water loss.

The relationship between shoot water content and pressure chamber end point was also determined for six shoots air dried outside of the pressure chamber (Hinckley et al. 1980). After the initial end point measurement, pressure was slowly released, the shoot was removed from the pressure chamber and allowed to dry by evaporation at atmospheric pressure. The shoots were periodically weighed and remeasured with the pressure chamber as they dried out.

Shoots dried by sap expression and air drying were rehydrated by inserting the cut ends in water filled vials and storing them in a humidified plastic box. The shoots were then weighed repeatedly until water uptake stopped.

Forty-four additional shoots were air dried intact, without excising root from shoot. After drying to different water contents, the shoots were excised, weighed, measured once with the pressure chamber and reweighed. The intact-dried shoots were not rehydrated immediately after the initial measurement but were placed in a humidified plastic bag at atmospheric pressure for 1 h. The intact-dried shoots were then weighed and measured with the pressure chamber a second time for comparison to shoots dried by sap expression in Experiment two. After the second pressure chamber measurement, intact-dried shoots were rehydrated in the same manner as the shoots dried by sap expression and air drying.

After rehydration and weight measurement, all shoots were dried at 65 °C for 48 h and weighed. Relative Water Content (RWC) was calculated from the formula:

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

where W is the weight, DW is the dry weight and FW is the full turgor weight of the tissue after final rehydration. Shoot RWC was plotted against water potential estimates for the three dehydration treatments. The data for each dehydration treatment were separated into two linear regions at −1.7 MPa. A t-test was used to compare differences in slope and intercept between dehydration treatments.

Experiment two

In this experiment, pressure chamber measurements were made to determine if the relationship between shoot water content and water potential changes when a shoot initially dried by sap expression is then allowed to equilibrate at atmospheric pressure in a humidified plastic bag. Thirty-two shoots were excised, enclosed in the pressure chamber and pressure increased 0.5 MPa over that required to express sap from the cut end. Expressed sap was blotted with tissue paper. After 12.5 min, pressure was slowly lowered until sap receded from the cut end. Another 0.5 MPa overpressure was applied and the process repeated until the pressure chamber measurement approached a target end point of either 0.5, 1.2, 1.9, or 2.6 MPa. A stable end point was recorded, pressure slowly released, and the shoots weighed. The shoots were stored in a humidified plastic bag, at atmospheric pressure, for either 0, 5, 15, or 30 min, and one more pressure chamber reading and weight measurement taken. Two shoots were measured for each combination of initial pressure chamber end point and atmospheric equilibration time treatment. Pressure chamber measurements made before pressure release were plotted against measurements taken after atmospheric equilibration and regression lines calculated for the four equilibration time treatments. A t-test was used to test for differences in slope and intercept between treatments.

A regression line was also calculated for the data relating initial and post-equilibration pressure chamber measurements of the shoots dried intact in Experiment one. A t-test was used to compare slope and intercept of this line to those calculated for the four equilibration time treatments of shoots dried by sap expression. One of the 44 shoots dried intact in Experiment one lost a significant amount of water during atmospheric equilibration and was omitted from this analysis.

Experiment three

The water potential of 32 shoots was estimated with both the pressure chamber and thermocouple psychrometer to determine if timing of the psychrometer measurement affected the discrepancy between instruments. Shoots were excised at the cotyledon whorl and air dried to water potentials ranging from 0 to −4.0 MPa as measured with the pressure chamber. A 1 cm long shoot segment was cut from the region of first-year growth 2 cm above the cotyledon whorl. A longitudinal cut was made to create a surface with a non-cuticular air-tissue interface and the water potential of the segment measured with an SC-10 thermocouple psychrometer (Decagon Devices, Pullman WA)[1] that had been calibrated with standard salt solutions (Lang 1967). The shoot was then cut at the internode of first- and second-year growth, and a pressure chamber measurement taken on the terminal segment. A stable end point was measured, pressure slowly released, and another 1 cm shoot segment cut for psychrometric analysis. Vapor equilibration of the psychrometric samples was usually achieved in less than 2 h. The relationship between pressure chamber and psychrometer measurements was plotted and regression lines calculated for the psychrometer data taken both before and after the pressure chamber measurement. A t-test was used to test for differences between slopes and intercepts of the regression lines.

Results

Although intact-dried and air-dried shoots did not have identical water release characteristics, they both lost more water than those dried by sap expression in the pressure chamber (Fig. 1). The regression lines for shoots dried by sap expression, air drying and intact drying were different at the 95% confidence level (Table 1).

The relationship between sap expression and air drying also held for tissues initially dried by sap expression and then allowed to equilibrate at atmospheric pressure without further water loss. The magnitude of the change in water potential estimate, however, was dependent upon the amount of time allowed for atmospheric equilibration (Fig. 2). When pressure was released and then reapplied immediately, the shoot water potential estimate changed very little. As the interval of equilibration at atmospheric pressure increased, the change in water potential estimate became pronounced. Pressure chamber readings of shoots dried intact, however, were seen to change very little after 1 h of equilibration at atmospheric pressure following the initial measurement (Fig. 2). All of the lines in Fig. 2 were significantly different from each other at the 95% confidence level with the exception of the 5 and 15 min equilibration lines (Table 1).

The relationship between water potential estimates made with the pressure chamber and thermocouple psychrometer was not affected by the timing of the psychrometric measurement (Fig. 3). There was higher variability in the post-pressurization psychrometric measurements but the regression lines in Fig. 3 could not be distinguished at the 95% confidence level (Table 1).

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[1] Mention of a trademark name or proprietary product does not constitute endorsement by the USDA and does not imply its approval to the exclusion of other products that may also be suitable

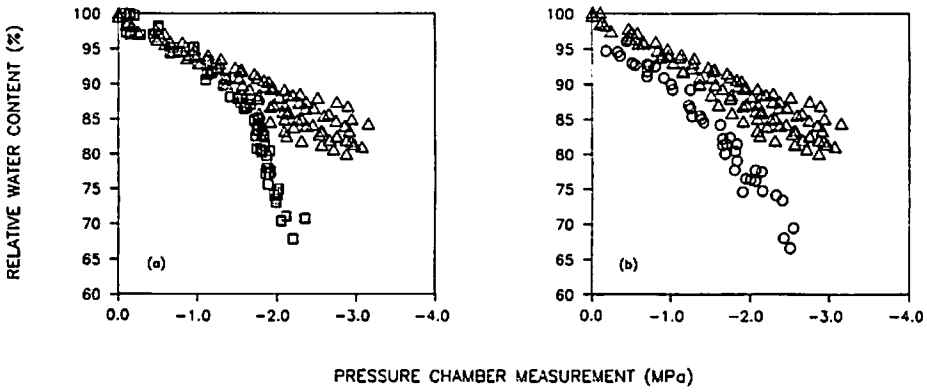

Fig. 1a, b. The relationship between pressure chamber water potential estimate and Relative Water Content for seedling shoots dried by (a) sap expression (△), and air drying (□), and (b) sap expression (△), and intact drying (○). Regression information is contained in Table 1

Table 1. Linear regression information for Figs. 1–3

(Fig. 1)					
Dehydration treatment	Water potential range (MPa)	Slope	Intercept	R^2	n
Sap expression	0 to −1.7	−6.218	99.7	0.9042	39
Air drying	0 to −1.7	−7.884	100.4	0.9219	31
Intact drying	0 to −1.7	−9.578	98.7	0.9318	26
Sap expression	−1.7 to −3.0	−4.991	97.2	0.4719	58
Air drying	−1.7 to −3.0	−30.472	136.0	0.8250	36
Intact drying	−1.7 to −3.0	−14.972	107.1	0.8159	18
(Fig. 2)					
Treatment	Atmospheric equilibration time between measurements	Slope	Intercept	R^2	n
Intact drying	1 h	0.884	0.127	0.9823	43
Sap expression	0 min	0.807	0.153	0.9923	8
Sap expression	5 min	0.745	0.100	0.9763	8[a]
Sap expression	15 min	0.663	0.132	0.9659	8[a]
Sap expression	30 min	0.638	0.004	0.9537	8
(Fig. 3)					
Timing of psychrometer measurement		Slope	Intercept	R^2	n
Before pressure chamber		0.650	0.156	0.9209	32[a]
After pressure chamber		0.638	0.206	0.8581	32[a]

[a] Regression lines followed by the same letter could not be distinguished ($p \leq 0.05$)

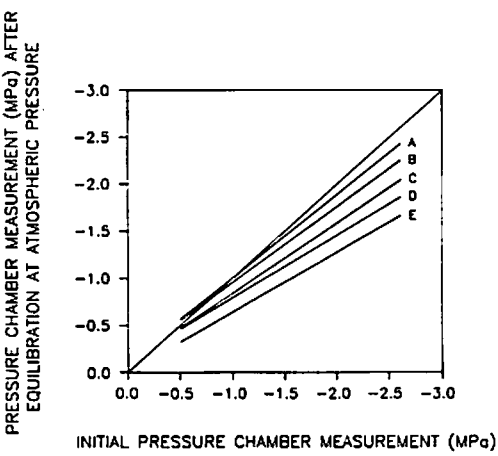

Fig. 2. The relationship between pressure chamber measurements made before and after an interval of equilibration at atmospheric pressure. Shoots dried intact and equilibrated at atmospheric pressure for one hour after the initial reading (A). Shoots dried by sap expression and equilibrated at atmospheric pressure for 0 (B), 5 (C), 15 (D) and 30 (E) minutes. Regression information is contained in Table 1

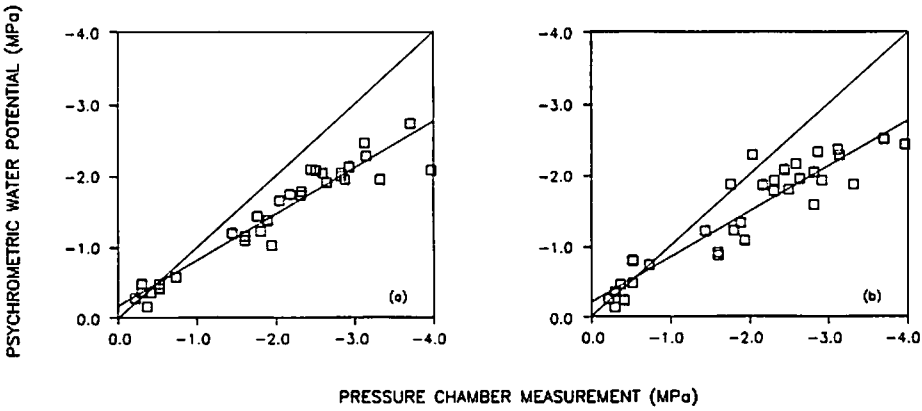

Fig. 3. a, b. Thermocouple psychrometer and pressure chamber measurements for *Pinus ponderosa* seedling shoots with the psychrometric determination made before (a) and after (b) the pressure chamber reading. Regression information is contained in Table 1

Discussion

A gas phase can form in the xylem of plant tissue by either cavitation of water (Milburn 1973) or air entry into cut xylem elements (Scholander et al. 1965; Kaufmann 1968; Duniway 1971). If gas entered cut xylem elements, it would be composed of air at atmospheric pressure but air would also diffuse into a water vapor pocket created by cavitation (Oertli 1971). The presence of air in the xylem can affect plant water potential estimation in two ways. If air is present before sample excision, the pressure chamber will underestimate water potential (Boyer 1967; West and Gaff 1971, 1976). If air enters cut xylem elements after excision, the thermocouple psychrometer will overestimate plant water potential (Scholander et al. 1965; Kaufmann 1968; Duniway 1971). Measurement errors associated with the discrepancy between in-

struments, therefore, cannot be assigned automatically to the pressure chamber unless the hydration status of the xylem is determined during the measurement sequence.

Hardegree (1989) showed evidence supporting the hypothesis that air in the xylem would be trapped during subsequent pressure chamber measurements. As pressure on the plant tissue increased, water would move from the symplast into the xylem, the small pores connecting xylem elements would rehydrate first and the gas become trapped. Gas entrapment in the xylem would explain why excised plant samples lose more water when dried by evaporation at atmospheric pressure than by sap expression in the pressure chamber (Fig. 1 a, Jones and Higgs 1979; Ritchie and Roden 1985; Hardegree 1989). Plant tissue dried intact also loses more water at any water potential than tissue dried by sap expression (Fig. 1 b, Jones and Higgs 1979). If this discrepancy is also caused by air in the xylem then the relative accuracy of the pressure chamber and thermocouple psychrometer will depend on whether the air phase was created before or after excision.

The water potential estimate of shoots dried by sap expression became less negative after pressure was released (Fig. 2) and it is hypothesized that this change in water status was also caused by air entry and entrapment in the xylem. The magnitude of the water potential change, however, was dependent upon the length of time before pressure was reapplied (Fig. 2). One might infer from this evidence that excision would only cause a small change in the water status of intact-dried tissues if the pressure chamber measurement was taken immediately. The pressure chamber readings of shoots dried intact, however, changed very little after 1 h of equilibration at atmospheric pressure (Fig. 2). This supports the hypothesis that an air phase was already present in the xylem of intact-dried tissues and that relatively little additional air entry occurred after excision.

An alternative explanation for the data in Figs. 1 and 2 is that the discrepancy between sap expression and air drying treatments was caused by a lack of equilibration during the pressure chamber measurement. Jones and Higgs (1979) and Ritchie and Roden (1985) attributed discrepancies between dehydration methods to disequilibria in tissue water status during the pressure chamber measurement but did not test this. The plant tissue dehydrated by sap expression, however, may not have come to equilibrium during the overpressure interval (Tyree and Dainty 1973) and if equilibrium was not obtained, water potential would have been underestimated in these tissues (Turner et al. 1984). The data in Fig. 2 would then suggest a time dependence of equilibrium and not of air entry into xylem elements. This explanation may not be valid in this case, however, because a stable end point was determined after each overpressure interval and subsequent overpressures were never in excess of 0.5 MPa (Tyree et al. 1978). It is more likely that greater disequilibria occurred during the measurement of air-dried shoots because they were subject to a greater pressure change for every reading than were the shoots dried by sap expression. Disequilibria in the air-dried shoots, however, would have generated a discrepancy in the opposite direction of that noted here (Turner et al. 1984).

It has been suggested that a lack of equilibration may also account for discrepancies between pressure chamber and thermocouple psychrometer measurements (Turner et al. 1984). Turner et al. (1984) attributed differences between pressure chamber and thermocouple psychrometer water potential estimates to water potential gradients across actively transpiring tissue. The transpiration rate of shoots used in

the present study, however, particularly below a water potential of −1.7 MPa, was relatively slow. The timing of the psychrometer measurement was also seen to have had a minor effect on the magnitude of the discrepancy between instruments, suggesting that equilibrium was established quickly after release of pressure from the chamber (Fig. 3).

It should be noted that although the intact-dried and air-dried shoots lost more water than those dried by sap expression, their water release characteristics were not identical (Fig. 1). One factor that may have contributed to the discrepancies between all treatments was the presence of apoplasmic solutes. Apoplasmic solutes tend to be flushed out during sap expression but remain and may be concentrated during post-excision air drying (Scholander et al. 1965; Boyer 1967; Hardegree 1989). Correction of the pressure chamber water potential estimate for apoplasmic solutes would, therefore, shift the air-dried shoot measurements closer to those of shoots dried by sap expression. It has been shown, however, that for *P. ponderosa* seedling shoots of this type the error is less than 0.15 MPa above a pressure chamber estimate of −2.0 MPa and does not exceed 0.3 MPa down to a pressure chamber estimate of −3.0 MPa (Hardegree 1989). It is not known how the apoplasmic osmotic potential of these shoots changes with intact drying.

Another factor that may have contributed to the discrepancy between intact-dried and air-dried curves in Fig. 1 would have been a small amount of air entry into intact-dried shoots after excision. Figure 2 indicates that a small amount of post-excision air entry may have occurred in the intact-dried shoots but this would account for less than 0.1 MPa of the discrepancy noted in Fig. 1.

Since air-dried and intact-dried shoots lost more water than shoots dried by sap expression (Fig. 1), and the water relations of intact-dried shoots changed very little after excision (Fig. 2), it was concluded that a gas phase was present in the xylem of shoots dried intact and that there was little additional air entry into xylem elements after excision. The data, therefore, indicate that the thermocouple psychrometer and not the pressure chamber gave a more accurate estimate of pre-excision plant water potential for the seedlings used in this study.

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