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Plant, Cell and Environment (1989) **12**, 57–62

Discrepancies between water potential isotherm measurements on *Pinus ponderosa* seedling shoots: xylem hysteresis and apoplasmic osmotic potentials*

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Received 20 May 1988; accepted for publication 15 June 1988

Abstract. Sap expression, air drying and a combined technique were used to measure the water potential isotherm of *Pinus ponderosa* Laws. seedling shoots with the pressure chamber. Discrepancies between water relations parameters derived from these techniques can be partially explained by air entry into air drying tissues, hysteresis in the xylem water potential isotherm and dilution of apoplasmic solutes during sap expression.

Key-words *Pinus ponderosa*, pressure chamber, water potential isotherm, hysteresis, osmotic potential

Introduction

The relationship between water content and water potential in a system is called a water potential isotherm (Noy-Meir & Ginzburg, 1967). Tyree *et al.* (1978) list the water relations parameters that can be determined from a plant water potential isotherm. They are the symplasmic and apoplasmic water volumes, the weight-averaged osmotic potential of the symplast at full turgor and at any other hydration state, the weight-averaged turgor pressure of the symplast at any hydration state, and the bulk elastic modulus of the cell walls. A central assumption of water potential isotherm analysis is that the apoplasmic fraction of the tissue remains constant as the tissue is dehydrated (Tyree & Jarvis, 1982).

Scholander *et al.* (1964) introduced the pressure chamber technique for plant water potential isotherm determination, and there are now three variations that differ in method of tissue dehydration and water loss measurement. In the sap expression method, plant tissue is dehydrated in the pressure chamber and water loss is monitored by weighing expressed sap (Tyree & Hammel, 1972). In the air drying method, plant tissue is dehydrated outside of the pressure chamber by evaporation at atmospheric pressure. Changes in water content are measured by weighing the tissue either before or after measurement with the pressure chamber (Hinckley *et al.*, 1980). In the combined sap expression-air drying method, sap expression is used to dehydrate the tissue but water loss is measured by weighing the sample between pressure chamber measurements (Wilson *et al.*, 1979). In the combined method, water loss can be monitored by weighing the tissue either before or after the interval of sap expression.

Jones & Higgs (1979) and Ritchie & Roden (1985) showed discrepancies between water relations parameters derived from sap expression and air drying techniques. They attributed the discrepancies to disequilibria in tissue water status during the pressure chamber measurement but did not test this. It is the present author's hypothesis that the discrepancy between sap expression and air drying water potential isotherm curves is caused by a change in the xylem water content of air dried tissues and from dilution of apoplasmic solutes during sap expression. Gas entry and entrapment in the xylem of air dried tissues would lower the apoplasmic fraction and shift the air drying water potential isotherm to a region of lower water content. Scholander *et al.* (1964, 1965), Boyer (1967) and Jachetta, Appleby & Boersma (1986) found that tissue dehydration method has an effect on the concentration of solutes in the apoplast. As the pressure chamber measurement must be adjusted to correct for solutes in the apoplast (Boyer, 1969), dilution of solutes during sap expression may also contribute to the discrepancy between water potential isotherm curves.

In this experiment, sap expression and air drying water potential isotherm measurements were made on *Pinus ponderosa* Laws. seedlings to confirm for this species that the shape and placement of the water potential isotherm is dependent upon dehydration method (Jones & Higgs, 1979; Ritchie & Roden, 1985). The combined sap expression-air drying technique of Wilson *et al.* (1979) was added for comparison. To support my hypotheses, the water potential isotherm of *Pinus ponderosa* woody-xylem was monitored with a thermocouple psychrometer. Hydration and dehydration curves were generated to test for hysteresis in woody-xylem water holding capacity. Apoplasmic osmotic potential was also determined during sap expression and air drying water potential isotherm measurements.

Materials and methods

Pinus ponderosa seedlings were grown in 5 × 25 cm polyethylene containers filled with a potting mix of

*Research conducted in partial fulfillment of the degree of doctor of philosophy, Department of Forestry and Resource Management, University of California, Berkeley, CA 94720, U S A

equal volumes of sand, peat moss and shredded redwood bark. Seed were germinated and initially grown in a greenhouse for 8 months, and moved outside for 16 months. Seedlings were fully hydrated at the start of each experiment. Roots were washed from soil and submerged in a covered, aerated water bath overnight

Sap expression

A shoot water potential isotherm was determined with the sap expression method (Tyree & Hammel, 1972) on seven seedlings in June, six in July and six in October 1986. An overpressure of 0.5 MPa for 12.5 min was used for each sap collection increment. 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 seedling to restrict evaporative water loss.

Air drying

The air drying method (Hinckley *et al.*, 1980) was used to measure the shoot water potential isotherm of six seedlings in June, six in July, and six in October 1986 for comparison with seedlings measured by the sap expression method. The air drying method was used on six additional seedlings in August 1986 for comparison with seedlings measured using the combined sap expression–air drying method.

Combined sap expression–air drying

A combined sap expression–air drying technique was used to measure the shoot water potential isotherm of six seedlings in August 1986 for comparison with shoots measured by the air drying method. Six shoots were also measured in October 1986 for comparison with both sap expression and air dried shoots. The combined procedure followed that of Wilson *et al.* (1979) with the following modifications. A stable pressure chamber end point and weight measurement were taken both before and after each sap expression interval. This produced two water potential isotherm curves for each seedling; one curve derived from end points determined immediately after the overpressure interval and one curve derived from end points determined after air drying but before the overpressure interval. Seedling weights were determined both after releasing pressure and before applying pressure.

Water potential isotherm analysis

Sap expression, air drying and combined sap expression–air drying shoots were dried at 65 °C for 48 h and weighed. Full turgor osmotic potentials and apoplasmic water contents were calculated from water potential isotherm extrapolations (Tyree & Hammel, 1972). A *t*-test was used to compare the means of parameter values for a given month.

Xylem hysteresis

Psychrometric water potential isotherms were measured on the woody-xylem of six seedlings to determine the magnitude of hysteresis in the wetting and drying of the xylem. Seedlings were immersed in a water bath and the shoots separated from roots at the cotyledon whorl. Shoots were cut into six 1.5-cm stem segments while still under water and the tissue exterior to the vascular cambium removed from the woody-xylem. Woody-xylem segments were blotted and weighed.

Woody-xylem wetting and drying curves were measured over the water content ranges of 0–15%, 0–25% and 0–45% weight loss relative to the fully hydrated weight. First, woody-xylem segments were incrementally dehydrated and water potential measured with a thermocouple psychrometer (SC-10, Decagon Devices Inc., Pullman, WA, U.S.A.)[1] calibrated with standard NaCl solutions (Lang, 1967). Woody-xylem segments were then incrementally rehydrated and water potential measured. Xylem segment weight was determined immediately after water potential measurement.

Secondly, woody-xylem segments were dehydrated immediately to 15, 25 or 45% weight loss relative to full hydration. Water was incrementally added until water potential approached full hydration. Samples were then incrementally dried to original dehydration levels. Water potential and sample weight were determined after each drying and rehydration increment. After the final psychrometric measurement woody-xylem segments were dried at 65 °C for 48 h and weighed.

Apoplasmic osmotic potential determination

Air drying. Six seedling shoots were excised and measured in the pressure chamber. The pressure was increased 0.1 MPa and expressed sap from the cut end blotted with a filter paper disc. The discs were sealed in a thermocouple psychrometer chamber (C-52, Wescor Inc., Logan, UT, U.S.A.)[1] calibrated with standard NaCl solutions (Lang, 1967). After sample equilibration, thermocouple microvolt output and chamber temperature were recorded. Chamber pressure was reduced to atmospheric and the shoot dehydrated on a laboratory bench. Shoots were remeasured as the tissue dried and sap samples collected for psychrometric evaluation.

[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

Sap expression. Six seedling shoots were separated from roots and measured in the pressure chamber. Pressure was increased 0.1 MPa after equilibration and a sap sample collected for psychrometric analysis. The overpressure was increased to 0.5 MPa and exuded sap blotted. After 12.5 min pressure was reduced, a stable end point recorded and the process repeated. Apoplasmic osmotic potentials for both sap expression and air drying methods were plotted against pressure chamber end points.

Results and Discussion

Air drying and sap expression water potential isotherm curves measured with the pressure chamber show a consistent pattern. The air drying curve invariably lies in a region of lower relative water content (Fig. 1; Jones & Higgs, 1979; Ritchie & Roden, 1985). Plant water relations parameters are derived by linear extrapolation from these curves and are, therefore, affected by choice of dehydration method (Table 1; Jones & Higgs, 1979; Ritchie & Roden, 1985).

Xylem hysteresis

A change in the apoplasmic fraction of air dried tissues would contribute to the discrepancy between air drying and sap expression water potential isotherm measurements. The xylem of a shoot drying on the bench may lose water to the symplast through air entry or cavitation of a gas phase in the xylem cell lumina (West & Gaff, 1971, 1976; Duniway, 1971). It is hypothesized that this gas phase becomes trapped during the pressure chamber measurement, effectively reducing the amount of water in the apoplasmic fraction. This hypothesis is supported by data showing hysteresis in the woody-xylem water potential isotherm (Fig. 2). Hysteresis occurred over all water content ranges tested. Once a gas phase becomes established in the xylem it persists and further changes in tissue water content follow the lower loop of the hysteresis curve (data not shown). Hysteresis in the filling and draining of xylem elements would shift the air drying water potential isotherm curve to a lower absolute water content.

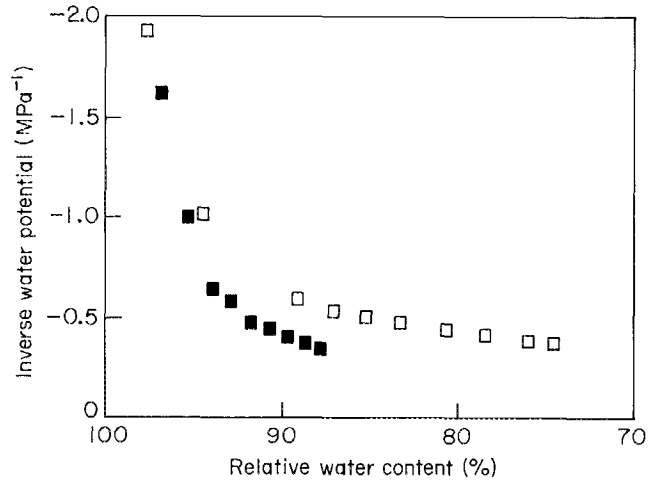

Figure 1. Comparison of air drying (□) and sap expression (■) water potential isotherms for seedlings of similar fresh weight and woody-xylem per cent dry weight

The initial composition and pressure of a xylem gas phase would be dependent upon the method by which the gas was propagated. Air entry into the xylem elements at the cut end of the sample would create a gas phase composed of air at atmospheric pressure. Cavitation of xylem water would result in a gas phase composed of water vapor at very low pressure. In time, diffusion of air into a water vapor pocket would raise the gas pressure to atmospheric (Oertli, 1971).

During a pressure chamber measurement, a xylem gas phase would become trapped because pores connecting xylem elements are of smaller diameter than xylem cell lumina. A small capillary will hold water against greater tension than a large capillary. As xylem tension is relieved during a pressure chamber measurement, the small pores connecting xylem elements will rehydrate first and prevent the escape of gas from the cut end.

The volume of gas trapped in an individual pine tracheid at the end point of a pressure chamber

Table 1. Average full turgor osmotic potential and apoplasmic fraction estimates extrapolated from sap expression, air drying and combined air drying–sap expression water potential isotherm curves

	June	July	August	October
Weight averaged osmotic potential at full turgor (MPa)				
Sap expression	−1.09 ± 0 15	−1 20 ± 0 09	—	−1 45 ± 0.19b
Air drying	−1 33 ± 0 05	−1 72 ± 0.18	−1 49 ± 0 17a[1]	−1 45 ± 0.15b
Combined pre-pressure	—	—	−1 44 ± 0.25a	−1 48 ± 0 12b
Combined post-pressure	—	—	−1.62 ± 0.25a	−1.58 ± 0 13b
Weight averaged apoplasmic fraction (Relative water content)				
Sap expression	74 1 ± 3 6	78 2 ± 3 1	—	65 5 ± 8 8
Air drying	24.3 ± 3.2	25.8 ± 18 1	45 0 ± 13 5c	48.2 ± 9.7d
Combined pre-pressure	—	—	47 7 ± 16 4c	51 2 ± 7 8d
Combined post-pressure	—	—	44.3 ± 16 7c	49 2 ± 7 4d

[1]Means within columns with similar subscripts are not significantly different ($P \leq 0\ 05$)

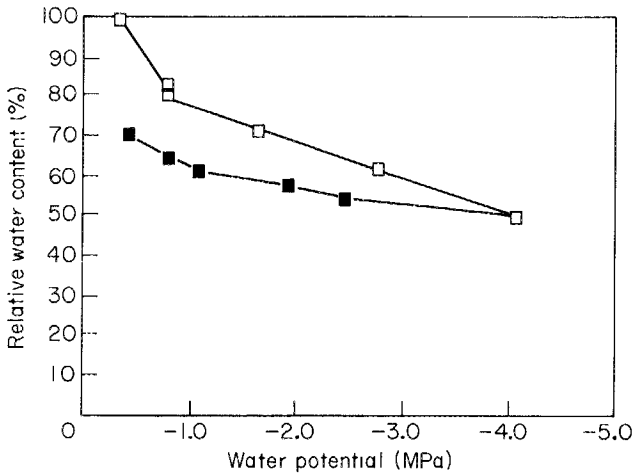

Figure 2. The woody-xylem water potential isotherm showing hysteresis between drying (□) and wetting (■) loops

measurement would be a function of the initial gas pressure and volume, and the air entry pressure associated with cell lumen diameter. The term 'air entry pressure' refers to the critical pressure drop across an air water interface required to either fill or drain a capillary. Water will be drawn into the gas filled cells by capillary forces as soon as tension is reduced below the air entry pressure of the tracheid lumina. As water moves into the tracheid, the trapped gas volume is reduced and the pressure increased. At the end point of a pressure chamber measurement, the absolute pressure of water in the xylem will be approximately 0.1 MPa. The pressure in the trapped gas phase will be greater than 0.1 MPa by an amount equal to the air entry pressure associated with the cell lumen diameter. The volume of trapped gas at this pressure would be governed by Boyle's law which states that for a fixed amount of gas, at a constant temperature, the product of pressure and volume is a constant.

The average tracheid inner diameter of seedlings used in this experiment was approximately 12 μm. The air entry pressure of a cylindrical capillary of this size is approximately 0.025 MPa. If the initial pressure of the trapped gas was 0.1 MPa, then the gas pressure after rehydration would be 0.125 MPa and the gas volume approximately 80% of the previous volume. If the trapped gas phase was composed of water vapor, the initial gas pressure would be approximately 0 003 MPa. Upon rehydration the equilibrium pressure of a water vapor phase would be 0.125 MPa with a corresponding final volume of about 2.4% of the initial volume. If the discrepancy between dehydration methods is caused by gas entrapment, therefore, the initial gas would most likely be air at atmospheric pressure.

Apoplasmic solutes

Apoplasmic solutes appear to be flushed out when sap expression is used, but remain and may be concen-

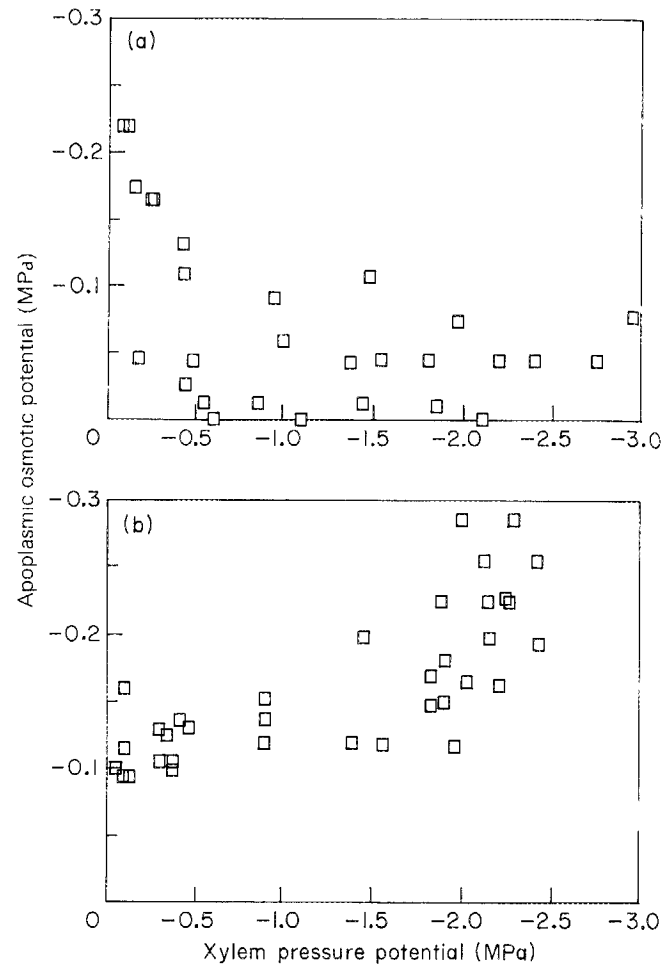

Figure 3. Apoplasmic osmotic potential as a function of xylem pressure potential (a) during sap expression and (b) air drying, water potential isotherm measurements

trated during air drying (Fig. 3; Scholander *et al.*, 1964, 1965; Boyer, 1967; Jachetta, Appleby & Boersma, 1986). Correcting the air drying pressure chamber measurements for apoplasmic solutes would shift the air drying isotherm closer to the sap expression isotherm.

The calibration curve for the psychrometer was non-linear at very high water potential. The microvolt output associated with a distilled water sample indicated a water potential of 0.05 MPa as calculated from the linear portion of the calibration curve. The apoplasmic osmotic potential estimates above −0.08 MPa in Fig. 3, therefore, are probably closer to zero.

Combined sap expression–air drying

Wilson *et al.* (1979) used the combined sap expression–air drying technique because tissue sample sizes were too small to recover significant quantities of sap. A combined sap expression–air drying technique can also be used to facilitate tissue dehydration after

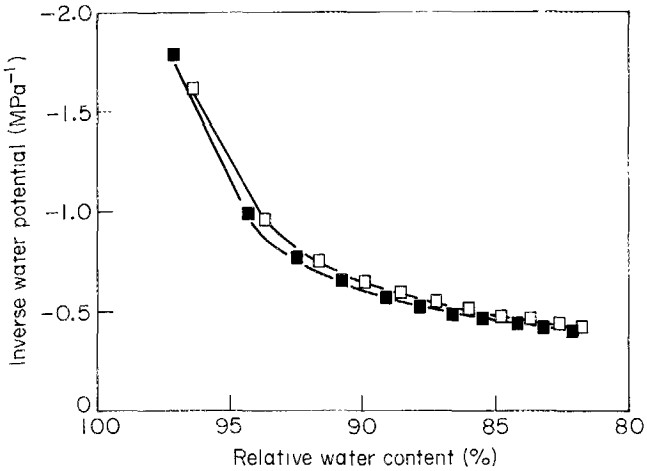

Figure 4. Combined sap expression-air drying water potential isotherm for one seedling Measurements for the upper curve (□) were taken immediately before an overpressure had been applied Measurements for the lower curve (■) were taken immediately after an overpressure had been applied.

stomatal closure The shape of the water potential isotherm curve, however, is affected by the timing of weight and pressure chamber measurements (Fig. 4). It is hypothesized that the minor discrepancy noted between combined methods was caused by trapped gas going into solution during the overpressure interval. A sustained overpressure would enhance the rate at which trapped gas enters solution, hence, the volume of trapped gas present immediately after the sap expression interval, is reduced. The pre-overpressure water potential isotherm, therefore, occupies a position of lower water content for any given water potential Water relations parameters derived from the two types of combined curve, however, could not be distinguished from each other or from those derived from the air drying analysis (Table 1). The combined sap expression-air drying technique would be expected to have a very low apoplasmic solute error but this was not tested.

Water potential isotherm interpretation

Ritchie & Roden (1985) found that the average full turgor osmotic potential derived from the air drying technique is less negative than the same parameter derived from sap expression. In this experiment, full turgor osmotic potentials determined with the air drying technique were more negative or equivalent to the same average parameter derived from sap expression (Table 1). The direction of the difference between osmotic potential estimates will depend upon water potential isotherm slope and the magnitude of the discrepancy between dehydration techniques. Regardless of the effect of technique on the estimated osmotic potential, as long as the air drying curve lies above the sap expression curve (Fig. 1), the air drying apoplasmic fraction will be lower than that determined from sap expression (Table 1).

An error in the initial hydration of sap expression seedlings may have also contributed to the discrepancy between apoplasmic fraction estimates (Table 1). If there was a xylem gas phase present before the water potential isotherm determination, it may not have been eliminated during the initial rehydration. A continuous overpressure during sap expression would reduce the volume of trapped gas and contribute to the seemingly high apoplasmic fraction for the sap expression technique. Incomplete rehydration of the xylem would not preclude a high pressure chamber reading at the start of the analysis.

Hysteresis in the xylem water potential isotherm may also explain the findings of Neufeld & Teskey (1986). They measured the air drying water potential isotherms of shoots with varying amounts of foliage, and found that the estimated apoplasmic fraction of shoots with intact foliage was greater than that of shoots with only xylem and bark. Shoots with small amounts of attached foliage had negative apoplasmic fraction estimates. This is contrary to what one would expect since the apoplast to symplast ratio is higher in defoliated shoots. The hypothesized hysteresis effect in the air drying technique, however, is a function of the xylem to symplast ratio. A larger relative hysteresis effect in the defoliated shoots would have lowered the apoplasmic fraction estimates.

Xylem hysteresis and the concentration of apoplasmic solutes may contribute to the discrepancy between air drying and sap expression methods but the magnitude of this contribution was not specifically tested. The presented results apply only to one group of pine seedlings of relatively uniform morphology. The properties of plant tissue that may determine the magnitude of errors in water potential isotherm analysis are the apoplasmic osmotic potential, the relative volumes of apoplast and symplast, the morphology of the xylem and the nature of cavitation and air entry events. Such plant properties are highly variable between species. There are, however, many published studies that use sap expression, air drying and the combined technique to compare species and to infer ecological significance between water relations parameters and habitat preference (Abrams & Knapp, 1986; Bahari, Pallardy & Parker, 1985; Calkin & Pearcy, 1984, Jackson & Spomer, 1979; Jane & Green, 1983; Parker & Pallardy, 1982; Roberts & Knoerr, 1977; Roberts, Strain & Knoerr, 1980). The relationship between air drying and sap expression water potential isotherm measurements may need further analysis before valid interspecific comparisons of plant water relations parameters can be made.

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