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XYLEM WATER HOLDING CAPACITY AS A SOURCE OF ERROR IN WATER POTENTIAL ESTIMATES MADE WITH THE PRESSURE CHAMBER AND THERMOCOUPLE PSYCHROMETER[1]

STUART P. HARDEGREE

USDA-ARS-AWMRU, 2000 East Allen Road, Tucson, Arizona 85719

ABSTRACT

The pressure chamber and the thermocouple psychrometer often provide different values when used to estimate plant water potential. One hypothesis to explain the discrepancy between instruments is that water movement between the xylem and symplast occurs during pressurization in the pressure chamber. Pressure chamber and thermocouple psychrometer measurements of *Pinus ponderosa* (Laws.) seedling shoots and mature *Quercus agrifolia* (Nee) shoots showed that the discrepancy is greater for *Quercus*. It was hypothesized that the xylem water content-water potential relationship of these species would explain the magnitude of the discrepancy between instruments. The xylem water holding capacity alone, however, does not explain the difference between species. The larger discrepancy in *Quercus* is likely due to a greater volume of water held in the xylem relative to the volume held in the symplast.

WATER POTENTIAL ESTIMATES made with the pressure chamber often do not correspond to those for the same tissue made with a thermocouple psychrometer (Ritchie and Hinckley, 1975). The graphical representation of the relationship between readings taken with the two instruments, however, follows a consistent pattern for most species. At high water potentials the psychrometer gives a more negative water potential estimate than the pressure chamber and at low water potentials the pressure chamber gives a more negative water potential estimate than the psychrometer (Ritchie and Hinckley, 1975). The pattern of the discrepancy between instruments is generally consistent but the relative magnitude at different water potentials is species and sometimes study specific. This suggests that a common mechanism causes the discrepancy but is manifest to different degrees in different species.

It has been suggested that the bulk of the discrepancy between pressure chamber and thermocouple psychrometer measurements is due to water movement between the symplast and the xylem during a pressure chamber reading (Boyer, 1967; Duniway, 1971; West and Gaff, 1971, 1976). At the end point of a pressure chamber reading, the xylem is relatively hydrated and the pressure component of water potential in the xylem is zero. When pressure is released, xylem tension increases and air may displace water, which moves into the symplast and raises the tissue water potential during the psychrometer measurement. Solutes in the apoplast offset the effect of water movement between the xylem and symplast (Boyer, 1969).

If the bulk of the discrepancy between instruments is caused by water movement between the xylem and symplast then the magnitude of the discrepancy should be related to the volume of air that can displace water in the xylem. In this study the water content-water potential relationship was measured for *Pinus ponderosa* (Laws.) and *Quercus agrifolia* (Nee) xylem to determine if water-holding characteristics can be used to explain the discrepancy between pressure chamber and thermocouple psychrometer measurements. The xylem sap osmotic potential was also measured to correct pressure chamber readings for apoplasmic solutes.

MATERIALS AND METHODS—*Pinus ponderosa* (pine) seedlings were grown in 5 × 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 8 mo and then moved outdoors for 16 mo. *Quercus agrifolia* (oak) shoot tissue was obtained from a mature

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tree grown on the University of California Oxford Tract in Berkeley, California.

Pressure chamber-thermocouple psychrometer relationship for pine—Seedlings were hydrated overnight by immersing their roots in an aerated water bath, and then dried to water potentials ranging from −0.2 to −4.0 MPa. The seedling shoot was severed from the roots 1 cm above the cotyledon whorl, bark stripped from the cut end, and a pressure chamber measurement recorded. The shoot was removed from the chamber and a 2 cm tissue sample immediately cut from the shoot 2 cm above the area from which the bark was stripped. A longitudinal cut was made and bark tissue exterior to the cambium removed. Bark tissue was sealed immediately in an SC-10[2] Decagon Devices thermocouple psychrometer chamber precalibrated with standard salt solutions (Lang, 1967). The thermocouple microvolt output was recorded until sample equilibration. Needles and leaves were not used for the psychrometric measurements because long equilibration times are required for tissues with a heavy cuticle. Less than 2 hr was required for vapor equilibration in the psychrometric samples.

It was determined after measuring the water potential of pine samples that the psychrometric procedure could not be duplicated on the oak samples because bark tissue could not be separated cleanly from the xylem. The pressure chamber-thermocouple psychrometer measurement relationship was remeasured for a new group of pine seedlings planted one year later than the first. For this group of seedlings a bark segment was not used for the psychrometric measurement. After removal from the pressure chamber, a 1 cm segment was cut from the shoot 2 cm above the height to which the bark had been stripped. This segment was cut in half longitudinally and immediately sealed into the psychrometer sample chamber. Except for this difference the psychrometric measurement procedure followed that outlined previously.

Pressure chamber-thermocouple psychrometer relationship for oak—The oak samples were taken from a mature tree and could not be hydrated by root immersion in water. Oak branches were cut from the tree, recut under water, inserted into water-filled vials, placed in plastic bags and hydrated overnight. The branches were removed from the water and dehydrated by transpiration. Shoot segments cut from the branches were measured with the pressure chamber. A 1 cm segment was cut from the shoot, immediately after removal from the pressure chamber, and its water potential measured with the psychrometer.

Xylem sap osmotic potentials—The osmotic potentials of water expressed from pine and oak xylem were measured with a Wescor C51[2] thermocouple psychrometer precalibrated with standard salt solutions (Lang, 1967). Shoot tissue was subjected to an overpressure in the pressure chamber until sap flowed from the cut end. A filter paper disk was saturated with expressed xylem sap and immediately sealed into the psychrometer chamber for equilibration and water potential measurement.

Xylem water holding characteristics—Pine and oak shoot tissue was hydrated overnight in the same manner as outlined previously. Shoots were immersed in water and a razor blade was used to cut 1.5 cm long samples. The bark was stripped from the xylem while still under water. Xylem segments were placed in vials filled with distilled water and subjected to a pressure of 1.5 MPa with compressed nitrogen for 10 min to facilitate absorption of any gas emboli that might be present in the xylem. The samples were removed from the distilled water, blotted with a damp paper towel to remove excess surface water and sealed in the psychrometer chamber. Sample water potential was measured and sample weight, length and diameter recorded. The samples were dehydrated by evaporation, water potential was remeasured with the psychrometer and the sample was reweighed. This process was repeated until the sample water potential was below −4.0 MPa. The sample was then oven dried, at 65 C for 24 hr, and reweighed.

Thirty individual xylem segments were measured for each species. Fully hydrated pine and oak xylem segments were defined as having a 100% water content by volume. The water content as a percent volume (%V) was calculated from the equation:

$$\%V = (V_x - V_w)/V_x \qquad \text{(Eq. 1)}$$

where V_x was the xylem volume and V_w was water volume lost. V_w was calculated from the equation:

$$V_w = (W_o - W)/\rho_w \qquad \text{(Eq. 2)}$$

and represents the water volume of the initial hydrated sample displaced by air. W_o was the fully hydrated sample weight (g), W was the sample weight (g) and ρ_w was water density, assumed to be 1 g cm^{-3}.

RESULTS—*Pressure chamber-thermocouple psychrometer discrepancy*—Oak exhibited a

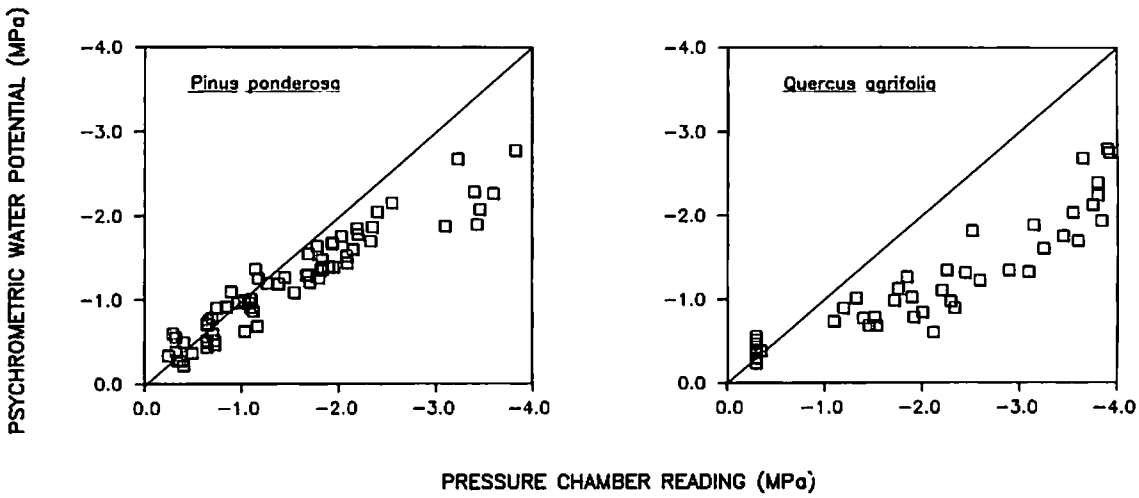

Fig. 1. Relationship between pressure chamber and thermocouple psychrometer measurements of *P. ponderosa* and *Q. agrifolia* shoots. Solid line represents the equipotential line. Pressure chamber measurements were corrected for solutes in the apoplast.

greater discrepancy between pressure chamber and thermocouple psychrometer measurements than did pine over the water potential range tested (Fig. 1). When a regression line was fitted to the data for each species, the curves could be distinguished at the 95% level of confidence. The coefficient of determination for these lines was 0.90 for pine and 0.84 for oak. The second set of data for pine, not shown here, was derived from shoot samples that included both xylem and bark. The slopes and intercepts of linear regression lines through the two pine curves were not significantly different at the 95% level of confidence.

The pressure chamber provides a water potential estimate that is bulk averaged over the whole tissue sample. It was assumed that an internal equilibrium existed in the shoot when the psychrometric measurement was taken and that the discrepancy between instruments was not due to a water potential gradient at the time of sampling. Hardegree (1987) found that the discrepancy between instruments for pine was the same whether the psychrometer measurement was taken before or after the pressure chamber reading. This indicated that an internal equilibrium is established quickly after release of pressure from the pressure chamber. Since the tissue was not transpiring rapidly, it was assumed that an internal equilibrium existed when the psychrometric sample was taken. It was also assumed that equilibrium was achieved during the pressure chamber measurement because end point values stabilized quickly.

The mean and standard deviation of xylem osmotic potential for 16 oak samples was -0.20 ± 0.043 MPa. For 10 pine samples, the mean and standard deviation of xylem osmotic potential was -0.15 ± 0.040 MPa. The correction factor for xylem osmotic potential was determined as the average of a separate sample and, thus, was a source of random error. The error was less than 0.03 MPa for both pine and oak at the 95% level of confidence. The data in Fig. 1 were corrected for the presence of solutes in the xylem by adding the mean xylem sap osmotic potential, for each species, to the individual pressure chamber water potential estimates.

Xylem water content-water potential relationship—The xylem of pine lost more water than that of oak, on a percent volume basis, over the water potential range studied (Fig. 2). The data shown in Fig. 2 were separated into two groups at -1.0 MPa where there was a change of slope of the water content-water potential relationship. Regression analysis showed that below -1.0 MPa the slopes and intercepts of the xylem water content-water potential relationship of pine and oak could be distinguished at the 95% level of confidence. Above -1.0 MPa the slopes but not the intercepts could be distinguished at the 95% level of confidence.

The amount of water held in the xylem relative to the amount held in the whole shoot was calculated for seven oak and six pine shoots. The oak xylem contained, on average, 28% of the water held in the shoot with a standard deviation of 9%. The pine xylem contained,

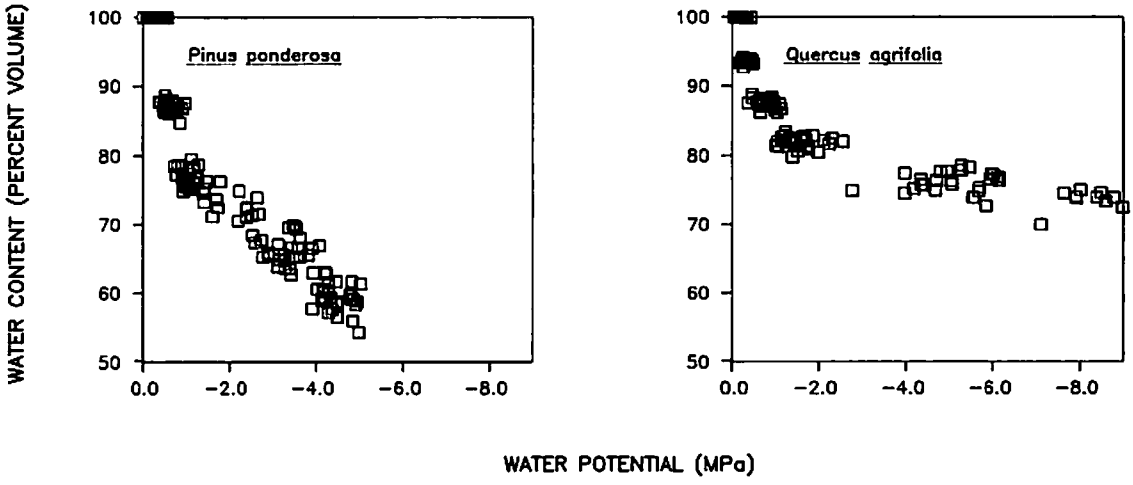

Fig. 2. Relationship between water content and water potential of *P. ponderosa* and *Q. agrifolia* xylem with water content expressed as a percent by volume.

on average, only 9% of the water held in the shoot with a standard deviation of 2%.

DISCUSSION—Discrepancies between pressure chamber and thermocouple psychrometer measurements in this experiment were similar in magnitude to those previously measured for pine and oak. Kaufmann (1968) measured a discrepancy as large as 1.6 MPa for *Quercus alba* and 0.5 MPa for *Pinus strobus* in the water potential range of 0 to −3 MPa, as estimated with the pressure chamber. In the same water potential range, Barker (1973) measured a discrepancy between the pressure chamber and a nonpsychrometric vapor equilibration technique of up to 1.0 MPa for *P. ponderosa*. In both of these studies pressure chamber readings were not corrected for the effect of solutes in the apoplast. If the pressure chamber measurements had been corrected for apoplasmic solutes, the discrepancy between instruments would have been greater.

The most convincing argument put forth to explain discrepancies between pressure chamber and thermocouple psychrometer measurements is that air-filled pores are present in the apoplast prior to, or are created after, samples are excised. Boyer (1967), Duniway (1971), and West and Gaff (1971, 1976) concluded that an air-filled pore would refill with water before an end point could be determined with the pressure chamber. Water under tension in the apoplast may move into the symplast when a shoot is excised or when pressure is released in the pressure chamber. The movement of water from the apoplast to the symplast raises the tissue water potential. Subsequent measurement of sample water potential with a thermocouple psychrometer reflects this increase (Boyer, 1967; Duniway, 1971; West and Gaff, 1971, 1976). The apoplasmic components most likely to lose water to the symplast are the xylem elements. Movement of water out of the xylem can occur following either cavitation of water or air entry.

If water movement out of the xylem contributes to the discrepancy between instruments, the magnitude of the discrepancy should reflect the anatomical characteristics of the sample tissue. These include the number, size and shape of xylem elements, size of the pores connecting xylem elements, and the xylem-volume to symplast-volume ratio. Also important would be the relationship between water content and water potential in the symplast. The characteristic pattern of xylem water loss alone does not explain why oak exhibits a greater discrepancy between instruments than pine. Pine xylem lost more water than oak, on a percent volume basis, over the entire water potential range studied. The most likely explanation of the larger discrepancy for oak is that this species has a higher proportion of water in xylem tissues than pine does. On a percent basis, there was three times as much water in the oak xylem than in pine xylem.

The thermocouple psychrometer has often been used to correct pressure chamber estimates of plant water potential (Ritchie and Hinckley, 1975). Calibration of the pressure chamber with the thermocouple psychrometer is a valid procedure only if the partitioning of water between the apoplast and the symplast is the same in the psychrometer tissue sample as occurred on the intact plant prior to excision. The partitioning of water between the symplast

and apoplast can change as a result of the cavitation of water in the xylem before or after sample excision, air entry into xylem elements, or from hysteresis effects in the filling and draining of the xylem during pressure chamber measurements (Hardegree, 1989). Unless these effects can be quantified there will be uncertainty about whether any instrument accurately estimates the water potential of the plant prior to sample excision.

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