A Split-Root Technique for Measuring Root Water Potential

KINGSLEY B. ADEOYE\(^1\) AND STEPHEN L. RAWLINS

Department of Soil and Environmental Sciences, University of California, Riverside, California 92521 and United States Salinity Laboratory, 4500 Glenwood Drive, Riverside, California 92501

ABSTRACT

Water encounters various resistances in moving along a path of decreasing potential energy from the soil through the plant to the atmosphere. The reported relative magnitudes of these pathway resistances vary widely and often these results are conflicting. One reason for such inconsistency is the difficulty in measuring the potential drop across various segments of the soil-plant-atmosphere continuum. The measurement of water potentials at the soil-root interface and in the root xylem of a transpiring plant remains a challenging problem.

In the divided root experiment reported here, the measured water potential of an enclosed, nonabsorbing branch of the root system of young corn (Zea mays) plants to infer the water potential of the remaining roots growing in soil was used. The selected root branch of the seedling was grown in a specially constructed Teflon test tube into which a screen-enclosed thermocouple psychrometer was inserted and sealed to monitor the root's water potential. The root and its surrounding atmosphere were assumed to be in vapor equilibrium.

While a plant transpires, water moves along a path of decreasing potential energy from the soil, through the roots, stem, and leaves into the surrounding atmosphere. Water movement through this soil-plant-atmosphere continuum (SPAC) is usually described by a modified form of the catenary equation van den Honert (15):

\[ Q = \frac{\psi_0 - \psi_a}{R_s} = \frac{\psi_0 - \psi_r}{R_r} = \frac{\psi_r - \psi_L}{R_L} \]

where \( Q \) is the rate of water transport through the system; \( \psi_0 \) is the average water potential in the bulk soil; \( \psi_a \) is the average water potential at the soil-root interface; \( \psi_r \) is the average water potential in the root xylem; and \( \psi_L \) is the average water potential of the leaves. \( R_s \), \( R_r \), and \( R_L \) are, respectively, average resistances to water movement from a point \( b \) in the soil to the root surface, from the root surface across the cortex into the root xylem vessels (radial resistance), and along the xylem vessels in the roots and stem to the evaporation sites in the leaves (longitudinal resistance). The units of the resistance depend on the units of \( Q \) and \( \psi \). When \( Q \) is in \( \text{cm}^3\cdot\text{s}^{-1} \), and \( \psi_0 \), \( \psi_a \), \( \psi_r \), and \( \psi_L \) are in bars, the units of \( R \) are \( \text{bar} \cdot \text{s} \cdot \text{cm}^{-2} \).

In equation 1, the relative magnitudes of soil resistance \( (R_s) \) and plant resistances \( (R_r + R_L) \) to water flow are uncertain, and have continued to be a major point of contention. Using a single-root model, Gardner (3) predicted that water potential gradients near a root would remain small except in dry soil, or when the root density was low and the transpiration rate was high. This implied a small \( R_s \). Results of greenhouse experiments by Gardner and Ehlig (4, 5) were interpreted to imply that resistance to water flow was greater in the soil than in the plant. Newman (10) later presented theoretical calculations, reviewed experimental evidence (11), and concluded that soil resistance would not become appreciable compared with plant resistances until the soil was near or below the permanent wilting point.

The divergent views on how to apportion the resistance to water flow in a soil-plant system are due largely to difficulties associated with experimentally measuring some of the crucial components in equation 1: the water potential at the root surface \( (\psi_0) \) and the water potential in the root \( (\psi_r) \). As a result, most calculations and estimates have been based on the soil hydraulic conductivity required to maintain assumed rates of water extraction at various soil water potentials (8, 16).

One logical approach to the problem is to measure separately the various quantities in equation 1. The rate of water transport, \( Q \), can be estimated in laboratory or greenhouse from successive weighings of potted plants, if surface evaporation is prevented. Water potential in the wet range of nonsaline soils can be determined by tensiometers. Soil psychrometers, while less accurate in the wet range, can be used to monitor the water potential or soil over the entire range of available water to plants. Leaf water potential can be measured \textit{in situ} with miniature intact leaf psychrometers (6) and dewpoint leaf hygrometers (12). With due precautions, leaf water potential can also be determined using excised tissue either by the psychrometer method or by the pressure chamber method. This leaves root water potential both at the root surface and in the xylem as the only unknown variables.

Various techniques have been used to measure root water potential, but all have been found wanting for one reason or another. With a special experimental arrangement, Kaufmann (7) allowed roots of pine seedlings to emerge from the bottom of a soil column into an atmosphere of humid air. From time to time, 40-mm-long root samples were taken and placed in the sample holder of a thermocouple psychrometer to determine the water potential. Measurements probably did not represent the true water potential of the roots growing in the soil, because the hanging roots were constantly maintained in an atmosphere of humid air. DeRoo (1) measured the water potential of tobacco roots by placing an entire root system together with any adhering soil in a pressure chamber. These measurements gave an effective average value of the water potential of the soil plus roots but not the true root water potential. Fiscus (2) made a good attempt at measuring root water potential of corn plants. In his experiments, specially constructed thermocouple psychrometers were attached \textit{in situ} to roots of corn seedlings growing in a container of soil. There was uncertainty, however, about which part of the root system was being measured. The tensiometer-potometer system of So \textit{et al.} (14) has a major limitation in that it can be used only above soil water potentials of ~0.85 bars. The work reported here is an

\(^1\) Former graduate student, Department of Soil and Environmental Sciences, University of California at Riverside, and Supervisory Soil Scientist, United States Salinity Laboratory, Riverside, respectively. Current address for senior author is: Department of Soil Science, Institute for Agricultural Research, Ahmadu Bello University, P.M.B. 1044, Samaru - Zaria, Nigeria.
attempts to develop a reliable technique to measure the root water potential of a transpiring plant growing in soil.

**MATERIALS AND METHODS**

**Measurement of Root Water Potential.** Root water potential of the test plant was measured based on the same premise that RAWLINS (13) used in measuring water potential within the stem of a plant; namely, that the potential at two points in a flow system would be equal if there were no flux between these points. In this case, rather than enclosing a leaf to eliminate flux, a root is enclosed. Consider a plant with its root system divided between two compartments, A and B. If the roots in B are not absorbing water, at steady state the potential at every point along this branch of the root system will equilibrate to the water potential at the base of the stem.

To make this measurement, we devised a divided root technique. A selected root of a corn seedling (*Zea mays* L., Bonanza) was grown in a modified half-strength Hoagland solution (9), contained in a small Teflon chamber, while the remainder of the root solution was grown in the soil. The osmotic potential of the nutrient solution was about -94 mbar. After adequate proliferation of the enclosed root, the nutrient solution surrounding it was siphoned off, the chamber was rinsed several times with deionized H₂O and mopped with tissue paper to remove droplets of water adhering to the roots and walls of the chamber. The strategy was to monitor the water potential of the roots enclosed in the chamber with a thermocouple psychrometer, so that the root tissue was in vapor equilibration with the air in the chamber.

The apparatus, shown schematically (Fig. 1), consisted of a root-thermocouple psychrometer chamber C, and stopper B, and a screen-enclosed thermocouple psychrometer A. The bottom section, C, was a section of a Teflon test tube with a 15-mm inside diameter. The middle section, B, was a removable Teflon stopper with two access holes drilled in it. Hole D, 9 mm in diameter, allowed the screen-cup thermocouple psychrometer, A, to be lowered into the chamber. The screen-enclosed psychrometer was made by replacing the ceramic cup protecting the thermocouple junction of a Wescor, a soil psychrometer with screen cups. These cups were about the same size as the ceramic cups, were made with stainless steel screens with openings of 28 to 30 μm. The selected root was directed into the bottom chamber through the access hole E, which was then sealed watertight with a soft wax. When the stopper and bottom sleeve were assembled, the root-thermocouple psychrometer chamber had an effective volume of about 2.65 ml. The whole unit was embedded in soil with about 5 mm of the stopper B protruding above the soil surface.

**Measurement of Soil and Leaf Water Potential.** Two ceramic cup soil thermocouple psychrometers (Wescor model PT51) were used to monitor the average water potential of the soil in each pot. Leaf water potential was determined on excised leaf tissues by using a pressure chamber apparatus and a sample chamber psychrometer. In the pressure chamber method, a thin strip of leaf was obtained by making a transverse cut with a razor blade from one edge of the leaf blade toward the midrib. The strip of leaf was inserted in the leaf holder and the pressure required to force sap out of the xylem through the cut end was determined immediately. In the psychrometer technique, leaf discs obtained by a 6.4-mm diameter paper punch were put in the sample holder of a Wescor Model C-52 sample chamber psychrometer. The discs were sealed in the chamber as rapidly as possible and allowed to reach temperature and vapor equilibration, which were usually achieved within 30 to 45 min. All psychrometers used in the experiments were precalibrated, using filter papers moistened with KCl solutions of known osmotic potential.

**Plant Environment.** Seeds were germinated in the dark on a thick pad of filter papers moistened with nutrient solution. The seedlings were removed when the coleoptiles were about 15 to 20 mm long and implanted in the Teflon chamber as described. The soil was Pachappa sandy loam contained in 500-ml Styrofoam cups. The plants were watered through pinholes at the bottom of the cup by placing the soil container in a 600-ml plastic beaker containing 75 ml of nutrient solution. The aerated nutrient solution in the root-thermocouple psychrometer chamber was renewed twice daily.

The plants were grown with a 12-h photoperiod provided by a bank of 16 1.2-m-long fluorescent lamps, supplemented by four 60-w incandescent bulbs. This combination of lamps produced a light intensity equivalent to about 308 μE m⁻² s⁻¹ measured with a Quantum Flux Meter (Lambda model LI-1905). The light bank was hung about one meter from the upper leaves of the potted plants, which were placed in 600-ml plastic beakers immersed in a constant temperature water bath maintained at 25 ± 0.01 C. The experiment was conducted in a temperature-controlled room at 25 ± 1 C.

**Root Air Supply.** Preliminary trials indicated that the roots inside the Teflon chamber deteriorated rapidly as a consequence of inadequate aeration after the screen thermocouple psychrometer was sealed in. This problem was resolved by providing a continuous stream of air through the chamber. The rate of air flow was adjusted to the estimated rate of O₂ consumption to prevent the roots from drying out. Daily values for O₂ consumption in soil range from 2.5 to 25 g day⁻¹ m⁻² root surface. As a guide, we assumed that an average daily quantity of 15 g m⁻² O₂ is used by microorganisms and plants in the top 250-mm depth of soil. On this basis, we estimated that the O₂ in the Teflon chamber would be depleted in less than 6 h, assuming the chamber was half-filled with roots.

**RESULTS AND DISCUSSION**

Several preliminary trials were made to develop and refine the technique for measuring root water potentials. These experiments were useful in identifying potential problems with the experimental setup. One immediate question was whether the roots inside

---

2 Trade names are given for the convenience of the reader and do not imply endorsement by the United States Department of Agriculture.
3 Wescor, Inc., 450 South Main Street, Logan, UT 84321.
4 Lambda Instrument Corp., P. O. Box 4425, Lincoln, NB 68504.
the Teflon chamber remained in good condition throughout each experiment. Each root chamber assembly was carefully dismantled to ascertain the conditions of the enclosed root. Measurements obtained with roots that were later found to be in poor condition, because of rotting or drying, were discarded.

Figure 2 shows a typical section through a Teflon chamber to display successfully grown roots in relation to the screen-enclosed thermocouple psychrometer. This plant had undergone two irrigation cycles spanning a period of approximately two weeks. The original single root branch had developed a massive network of fibrous roots completely enclosing the screen cup. As the chamber walls become lined with roots (Fig. 2), the measurement errors due to the walls being a vapor sink are minimized.

To make certain the roots were physiologically active, we determined their response to changing leaf water potential imposed by increased transpirational demand during a photoperiod. Psychrometer readings were taken every 40 min (Fig. 3). Water potential of the bulk soil roots, and leaves was -1.0, -2.0 and -4.0 bars, respectively, before the lights were switched on. Apparently, the water potential of the plant tissue did not increase sufficiently to come to equilibrium with the soil. This probably implies appreciable resistance in the stems and leaves. During the 6 h of this test, the root water potential declined steadily to a low of -6 bars. At this time the average leaf water potential was about -9.6 bars. The transpirational flux had caused the difference in potential between the bulk soil and the root xylem to increase from -1 to -5 bars, whereas that between the roots and the leaves increased from -2 to -3.6 bars. Within 40 min after the lights were switched off, the root water potential started to rise in response to decreasing transpiration. The observed effects of light on the water potential of roots were not unexpected, inasmuch as light controls the opening and closing of the stomates through which water is lost to the atmosphere.

Water potential measurements for a single plant during two extraction cycles following irrigations are shown in Figure 4. The water potential of the soil, roots, and leaves declined steadily as water was depleted from the soil. On the 6th day, water was applied and the water potentials of the soil, roots, and leaves rose accordingly. These observations indicate that the enclosed root could respond to changing water potential of any segment of the system.

CONCLUSIONS

The split-root technique described here seems to measure accurately the root water potential at the point at which the enclosed root joins the main root system. The assumptions that no flux existed within the enclosed root mass and that vapor equilibrium existed between these roots and the psychrometer chamber appear to be justified. Roots, unlike leaves, are not covered by a waxy cuticle that would impede movement of the small quantity of water necessary for equilibrium. Also, the chamber was nearly filled with a fibrous mass of roots and was closed for a long time before a measurement was taken. The fact that water potential within the chamber kept pace with changes in water potential of
the soil and leaves is evidence that these two assumptions were met.

Extrapolation from this point measurement to the water potential of the entire root system requires the additional assumption of negligible longitudinal resistance along roots. Although this is generally assumed to be true, this new technique offers the possibility of experimentally testing it by enclosing branch roots along a main root in different psychrometer chambers.

By using a psychrometer with this technique, one can measure water potential over the entire range expected in roots. The fact that the technique is nondestructive and in situ enables following water potential changes continuously in a dynamic system, by approximating the changes by a succession of steady states.

LITERATURE CITED