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A REVIEW

MEASUREMENT OF ROOT POROSITY (VOLUME OF ROOT AIR SPACE)

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SOJKA R. E. *Measurement of root porosity (volume of root air space)*. ENVIRONMENTAL AND EXPERIMENTAL BOTANY, **28**, 275–280, 1988. —Root research can benefit under many circumstances from determination of the % fraction of root volume occupied by air (root porosity). Root porosity provides an indication of root reaction or adaptability to environments with insufficient oxygen availability. Three primary approaches have been used for root porosity determinations: cross-sectional ratios, pycnometry, and dynamic gas displacement. These three methods are explained and their relative advantages and disadvantages discussed.

INTRODUCTION

ROOT porosity (the fraction of root volume occupied by air) varies with many factors. These include: species,[11] root type,[16] distance from root apex,[11] bulk density of the surrounding soil or soil penetration resistance,[1] root temperature,[9] nutrient availability,[8] root oxygen availability,[3] root growth rate[4] and foliage light intensity.[9] Increased root porosity generally results from decreased availability of oxygen to roots, relative to metabolic demand, or from conditions promoting rapid root growth and elongation.[9] Root porosity data are valuable for the comparative evaluation of rhizosphere conditions, or of species or cultivar adaptation to oxygen-limiting environments, and for providing necessary inputs to root respiration models, which use porosity to partition internal diffusion between liquid and gas pathways in the root.[10,11]

Three approaches to measuring root porosity have been employed: visual determination of the ratio of air-space cross-sectional area to total root cross-sectional area, pycnometric volume displacement, and dynamic gas displacement. Each method has advantages and disadvantages; however, these aspects have not previously been critically reviewed. This review compares the reported methods and relative merits of each technique under various experimental constraints. The first two, visual determination and pycnometric volume displacement, are the most frequently used approaches. These techniques are described in detail. The third approach, dynamic gas displacement, is outlined more briefly, and the reader is referred to the original source material for more detailed development of theory and technique.

VISUAL CROSS-SECTIONAL RATIOS

Use of visual cross-sectional area ratios is perhaps the oldest, and initially the most widely used, root porosity determination method. The equipment needed includes a microscope, microscope slides, appropriate stains, microtome, and usually photographic or video equipment. A photo-

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graphic or video record of the samples is advisable. Most researchers utilize these images rather than direct observation to determine total root and root-pore cross-sectional areas.

There are numerous published, brief descriptions of the variations of this approach. As an example of the technique, the details of root preparation reported by DREW *et al.*[3] are described. In their method, root segments were dehydrated in a graded ethanol series, embedded in Fibrowax, and transverse sections 10 or 20 μm thick were cut on a microtome. Suberin was stained with Sudan IV dye, and lignin with safranin or (in unfixed, fresh sections) with phloroglucinol dyes. As a simple non-automated procedure, KONINGS and VERSCHUREN[8] made a series of transverse sections. These were magnified under a microscope and photographed. The images were then projected for enlargement and the root outlines and the air spaces were traced on paper. The cross-sectional surface areas of the root and air spaces were then determined with a planimeter. The areas of the air spaces were summed for each cross section (Aa) and the ratio of air space cross-sectional area to the overall root cross-sectional area (Ar) was determined for each selected position along the root. An average of the porosities along the root was taken to represent mean root porosity.

$$\% \text{ Porosity} = \frac{\Sigma Aa}{Ar} \times 100. \qquad (1)$$

JACKSON *et al.*[6] partially automated this technique by entering the outlines of the projected images into a computer file using a digitizing drawing board. The areas of the root and gas spaces were then computed automatically. Their method could conceivably be totally automated through use of a video image analysis system and video storage of the images.

The principal advantage of the visual cross-section technique is its simplicity. Most plant-oriented laboratories have sectioning equipment and microscopes. Furthermore little, if any, operator induced error is involved and the technique requires little, if any, training for consistency of results.

The greatest failings of the visual cross-section approach are: the occasional inability to positively distinguish between air spaces and other non-stained voids, the uncertainty of whether visually identified voids may have been flooded (if relevant—as for modeling of gas diffusion and root respiration) at the time of sectioning, and the need to assume that the porosity changes along the root are adequately represented by the number of cross sections made per unit length of root observed.

PYCNOMETRIC VOLUME DISPLACEMENT

Pycnometric volume displacement is currently the most commonly used root porosity determination method. The method is based on the weight increase which occurs when internal gas spaces of a root sample are flooded upon homogenization. It also relies on Archimedes' principle, which states that a submersed body is buoyed up (loses weight) by an amount equal to the weight of the fluid displaced. This technique's principle advantage lies in simplicity while still allowing an experienced technician to achieve good precision, repeatability and accuracy.

As described by JENSEN *et al.*,[7] clean root samples are placed in a wide-mouth water-filled pycnometer vial (they suggested a Pyrex 25 ml capacity, Corning Model No. 1620*). It is better to insert the roots into a water-filled vial than to add water to a vial containing roots because fewer air bubbles are trapped among the roots. Any trapped air bubbles should be freed by manipulating the submerged roots in the pycnometer vial using a narrow powder spatula or similar tool. The temperature of the vial containing roots and water (T) is recorded. All subsequent pycnometer weight determinations must be made on materials returned to T to prevent errors from temperature-related density changes. The pycnometer, water, and intact fresh roots are then

* Names of equipment manufacturers and suppliers are provided for the benefit of the reader and do not imply endorsement by the Department of Agriculture.

weighed on an analytical balance (W_{fr+w}) to the nearest 0.0005 g. Next, the roots are removed from the pycnometer and blotted *gently* between absorbent paper towels or tissue until free water does not easily transfer to the blotting paper. One must be careful not to crush any roots, causing cytoplasmic fluids to be blotted away. The fresh roots are then quickly weighed (W_{fr}) in a tared covered Petri dish. If roots are suitable (not excessively wet) prior to submersion in the pycnometer, this weight can be determined at the outset and the need to pat the roots dry between towels eliminated.

The roots are then removed from the Petri dish and homogenized. This can be done by a variety of methods. A glass mortar and pestle or a small glass hand-held tissue grinder (e.g. Kimax No. 43950) work well. The use of a ball mill grinder and foam suppressant as suggested by JENSEN *et al.*[7] is not recommended because the homogenate heats up excessively, necessitating extensive and time consuming cooling in an ice bath to regain T. Also, the foam suppressant and unavoidable metal-flake contamination from the grinding process introduce excessive weighing errors. The entire homogenate is recanted into the pycnometer using the rinse water to return to the full pycnometer volume. The pycnometer and homogenate are adjusted to temperature, T, and weighed (W_h). Finally, the pycnometer filled only with water at temperature, T, is weighed (W_w).

Porosity can then be determined as follows:

$$\% \text{ Porosity} = (V_a/V_{fr})100 \qquad (2)$$

where V_a is the root air volume and V_{fr} is the fresh root volume. The term V_a is equal to the increase in water volume in the pycnometer due to destruction of the pore space by homogenization. Let ρ_w be the density of water at T. Then:

$$V_a = (W_h - W_{fr+w})/\rho_w \qquad (3)$$

and

$$W_h = W_{fr+w} + (V_a)(\rho_w). \qquad (4)$$

Because the submerged roots (by Archimedes' principle) decrease in weight equal to the weight of water displaced.

$$W_{fr+w} = (W_w + W_{fr}) - (V_{fr})(\rho_w). \qquad (5)$$

This allows calculation of V_{fr} simply as the volume of water in the pycnometer displaced by the roots:

$$V_{fr} = (W_w + W_{fr} - W_{fr+w})/\rho_w. \qquad (6)$$

With ρ_w canceling out of the numerator and denominator, and substituting relationships 3 and 6 into Equation (2), the equation becomes:

$$\% \text{ Porosity} = \frac{(W_h - W_{fr+w})}{(W_w + W_{fr} - W_{fr+w})}100. \qquad (7)$$

All steps for a given sample should use the same pycnometer vial at a single temperature. The temperature should remain constant to within a half degree centigrade to prevent weighing errors resulting from temperature dependency of water density. Ideally even the pycnometer lid and meniscus should be positioned exactly the same before each weighing for volume uniformity. Use of as large a root mass as possible in the pycnometer increases sensitivity. Root samples should be fresh and be covered and refrigerated during delays in the measurement process to reduce respiration. Predetermination of a temperature calibration curve for W_w saves time at the balance during actual measurements.

Variations of the above method have been used with varying degrees of success. Evacuation of the homogenized sample improves flooding of pore space and removal of air bubbles following homogenization.[12] Others have sought to simply eliminate the homogenization step by placing the submerged intact root system under vacuum in the pycnometer.[5,13] The latter technique employs the relationship:

$$\% \text{ Porosity} = \frac{100(V_a)}{V_{fr}}$$

$$= \frac{(WE_{fr+w} - W_{fr+w})/\rho_w}{(W_w + W_{fr} - W_{fr+w})/\rho_w}(100) \qquad (8)$$

which is similar to Equation (7), except that WE_{fr+w} (the weight of the pycnometer plus roots plus water after evacuation, filled to volume, and at temperature T) is substituted for W_h. EVANS and EBERT[5] reported difficulty using this technique with structurally weak roots such as those of rice.

SOJKA *et al.*[15] had mixed success by substituting a lower density fluid (e.g. ethanol), determining a

density for the solid phase of roots and employing vacuum extraction to eliminate homogenization. For this method a value for root solids density (ρ_r) must first be established and the fluid density (ρ_L) must be known. Root solids density is calculated as:

$$\rho_r = \text{weight of dry roots} \div \text{volume of dry roots} \quad (9)$$

or

$$\rho_r = W_{dr} \div (W_L - [W_{dr+L} - W_{dr}])/\rho_L \quad (10)$$

where W_{dr} is the weight of oven dry (50°C) roots, W_{dr+L} is the weight of the pycnometer plus dry roots plus liquid at T and W_L is the weight of the liquid-filled pycnometer at T.

Then

$$\%\ \text{Porosity} = \frac{(V_{fr}) - (V_{rs} + V_{rw})}{V_{fr}} = 1 - \frac{V_{rs} + V_{rw}}{V_{fr}} \cdot 100 \quad (11)$$

where V_{fr} is the volume of fresh roots [Equation (6)], V_{rs} is the volume of root solids (W_{dr}/ρ_r), and V_{rw} is the volume of root water [$(W_{fr} - W_{dr})/\rho_w$].

Operationally, this is expressed in the relationship:

$$\%\ \text{Porosity} = \left(\frac{\dfrac{W_w + W_{fr} - W_{fr+w}}{\rho_w} - \dfrac{W_{dr}}{\rho_r} + \dfrac{W_{fr} - W_{dr}}{\rho_w}}{\dfrac{W_w + W_{fr} - W_{fr+w}}{\rho_w}} \right) 100 \quad (12)$$

or more simply:

$$\%\ \text{Porosity} = 1 - \left(\frac{W_{dr}(\rho_w - \rho_r) + \rho_r W_{fr}}{\rho_r (W_w + W_{fr} - W_{fr+w})} \right) 100. \quad (13)$$

The greatest disadvantages of the pycnometer technique are: the need for consistent sample handling through all procedural steps and for all samples, slowness (four–five samples per hour), numerous sources of error if good technique is not employed, and the inability to determine accurate porosity of hard (lignified) samples and excessively soft (crushable upon towel drying) samples.

DYNAMIC GAS DISPLACEMENT

Porosity determinations using dynamic gas displacement rely on the properties of gases in the root-pore spaces to measure pore space volume. These methods depend either on the diffusion of a marker gas[14] or the assumed behavior of an ideal gas under pressure.[2] These techniques have the potential advantages of speed, and the ability to determine pore space of difficult-to-grind materials, such as lignified tissue.

Saglio and Belgrand[14] weighed fresh root samples (W_{fr}), inserted them in a 16-ml test tube and sealed the test tube with a septum. Roots in the test tube were separated by a screen from 2 ml of 1 mM deoxycholate solution. The tube was evacuated by syringe and restored to atmospheric pressure with He; the sealed roots were then allowed to equilibrate at 20°C for 20 min. Following this period a 0.2-ml gas sample was removed for gas chromatographic (GC) response for He (R_1). The tube was then inverted, immersing the roots in the deoxycholate solution and trapping the diffused He. Care was taken not to trap bubbles between root segments. Roots were then quickly (<5 sec) transferred to a He-free tube with absorbent filter paper and again sealed using a septum. The weight of the tube and filter paper (W_t) and with roots later added (W_{t+r}) was determined. After 20 min of equilibration at 20°C a 0.5-ml gas sample was removed for GC response to He (R_2). At the end of each equilibration period, He partial pressures were assumed to be the same in the root air spaces and in the test tube. Finally, the weight of the tube and filter paper without roots, filled to volume with water, was determined (W_{t+w}). Since the mass of He in root air spaces resulting from enrichment in the first tube is the only source of He upon transfer to the second tube, root porosity can be determined by solving for V_a and V_{fr} as in Equation (2) from the following equations:

$$R_1 V_a + \alpha R_1 (V_{fr} - V_a) = R_2 (V_t - V_{fr} + V_a) + \alpha R_2 (V_{fr} - V_a) \quad (14)$$

where V_t is the volume of the second test tube and

α is a partition coefficient between the aqueous and gaseous phases for He. SAGLIO and BELGRAND[14] showed that in most instances negligible error results from assuming that tissue density (ρ_r) is nearly equal to 1 and that the solubility of He is small enough to ignore ($\alpha = 0$), thereby reducing Equation (14) to:

$$V_a = (R_2/R_1)(V_t - V_{fr} + V_a). \qquad (15)$$

Because ρ_w is equal to 1 gcm^{-3}, Equation (15) can be solved for V_a by substituting $W_{t+w} - W_{t+r}$, giving:

$$V_a = (R_2/R_1)(W_{t+w} - W_{t+r}) \div \rho_w \qquad (16)$$

and

$$V_t = (W_{t+w} - W_t) \div \rho_w. \qquad (17)$$

The value of V_{fr} must be obtained geometrically or pycnometrically as described in previous sections. If the solubility of He can be neglected but the tissue density is significantly different from 1, then

$$V_a = (R_2/R_1 - R_2)(V_t - V_{fr}). \qquad (18)$$

For low porosities (the authors suggest below 5%), the solubility of He can be accounted for under both tissue density assumptions. If ρ_r can be assumed equal to 1, then:

$$V_a = (R_2/R_1)(V_t - V_{fr} + V_a) - \alpha\left(1 - \frac{R_2}{R_1}\right)(V_{fr} - V_a) \qquad (19)$$

where

$$V_{fr} - V_a = W_{t+r} - W_t. \qquad (20)$$

If ρ_r differs significantly from 1, then:

$$V_a = (R_2/R_1 - R_2)(V_t - V_{fr}) - \alpha V_{fr}. \qquad (21)$$

Equation (21) provides a near correction for the He dissolved in the fluids of the root segments (it neglects the small fraction of V_{fr} occupied by V_a and V_{rs}). Agreement of SAGLIO and BELGRAND's[14] method with the pycnometric method[5] was nearly 1:1.

To the author's knowledge, use of the technique has not been reported beyond the initial publication. The major disadvantages of this technique would be lack of access to and skill with gas chromatography equipment. Given the time required for equilibrations and GC analysis, this method is probably not much faster than the pycnometric technique. It is also possible that not all gas spaces are measured. However, the method determines the porosity that is most effective in gaseous transport.

The technique of CARSTENSEN *et al.*[2] is, again, one that has appeared only in the original publication, to the best of the author's knowledge. In this technique, roots are floated in a series of isodensity Ficoll solutions in transparent sealed vessels. Pressure is monitored in the vessels and is increased by the introduction of nitrogen gas until the floating roots lose buoyancy and sink by the Cartesian Diver effect. This occurs through the compression of root gas volume caused by the pressure increase, leading to an increase in the mean density of the root segments. The pressure at which the root density matches the density of each suspending liquid results in sinking of the roots. The technique also accounts for the increased gas solubility with increased pressure. The change in volume of the root section under pressure results entirely from changes in the pore space volume, since it alone is compressible (these pressures occur typically in the range of 3 MPa). This provides a measure of the fractional volume occupied by gas within the root tissue. Derivation of theory and related equations[2] is somewhat lengthy; however, the operational relationship can be represented as follows:

$$\frac{\Delta\rho_r}{\rho_{ra}} = (C_s - C_F)(P_d - P_a) + X_a$$

where $\Delta\rho_r$ is the change in mean root density as the tissue is pressurized from atmospheric pressure (P_a) to the diving pressure (P_d) of the specific isodensity solution in which it is suspended, and where ρ_{ra} is the mean root density at atmospheric pressure. The compressibility of the solid–liquid phase of the root tissue is represented by C_s, and the compressibility of the specific isodensity Ficoll solution is C_F. The term X_a is the volume fraction of gas in the root samples at atmospheric pressure. CARSTENSEN *et al.*[2] solved for % porosity (100 X_a) using a graphic technique (Fig. 1). Plotting $\Delta\rho_r/\rho_{ra}$ against P_d for each isodensity series produces a curve which becomes linear at the pres-

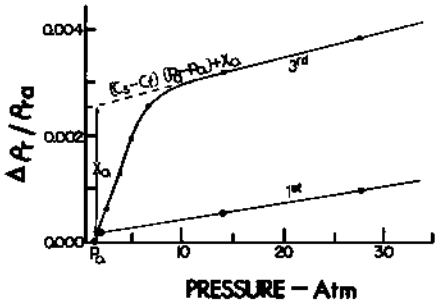

FIG. 1. Illustrative data for relative densities of pea root sections used to determine % root porosity (100 X_a). Data are presented for first and third millimeter sections of roots. Each point is the average of five freshly cut sections.[2]

sure at which root gas space collapses. Extrapolation of the linear portion of this plot to P_a gives the volume fraction of root air space (X_a) at atmospheric pressure. Although a graphical solution was employed by the authors, presumably regression analysis of the linear portion of the ($\Delta\rho_r/\rho_{ra}$) vs P curve could be used to automate the analysis, solving the function for $\Delta\rho/\rho_{ra}$ at P_a.

This technique appears to have the advantages of speed and simplicity and may hold promise for future use. However, caution may be in order, because porosity values reported for pea root segments by CARSTENSEN *et al.*[2] are in the low range of previously reported values for legume roots. This may be due to the use of apical or near apical root segments, but also may indicate that not all pore spaces are compressed in the method, or that refinement of theory or technique is still needed. The technique was not calibrated against visual or pycnometric methods.

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