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Quantification of air-filled root porosity: A comparison of two methods

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Introduction

Air-filled porosity of the root cortex is important for aeration of roots in situations where the external oxygen is insufficient. A quantitative theory predicting the depth a vertically growing root can penetrate into the soil is now available for simultaneous internal and external oxygen transport as a function of the air-filled porosities of soil and root (De Willigen and Van Noordwijk, 1987). Maximum depth of root penetration in the soil depends on root diameter, respiration rate, conductance of root epidermis-plus-exodermis for oxygen and air-filled porosity of both soil and root. Reliable methods for quantification of the air-filled porosity of roots or root segments are needed for practical applications of this theory. Two measurement techniques will be discussed here, direct measurements on microscopic sections and the pycnometer method as described by Jensen *et al.* (1969). To obtain root material with significant variation in porosity, maize plants were grown with and without aeration, including some factors stimulating or reducing the normal formation of air spaces via ethylene (Konings, 1983).

Materials and methods

Plant material

Maize plants were grown on a nutrient solution in a growth chamber (temperature 22°C, relative humidity about 40%) on a full-strength nutrient solution, replaced once a week; 3 plants were grown per 5-l pot from 2 weeks after sowing onwards. To obtain low root porosities, $AgNO_3$ was added to reduce ethylene formation; to obtain high porosities a low nitrogen supply was used in the presence of the ethylene precursor ACC (1-aminocyclopropane-1-carboxylic acid). Following Konings (1983), four treatments were used, each in two variants: AA. Aerated, with $AgNO_3$ (10^{-6} *M*), A. Aerated, N. Nonaerated, NN. Nonaerated, with ACC (10^{-5} *M*), low N-supply (3.4 instead of 10.2 me l^{-1}) during pretreatment and without N in the last week before harvest.

In variant 1 the pretreatments were implemented at the start of the solution culture period; in variant 2 treatments were only implemented in the last week before the harvest and the plants were aerated in a full-strength nutrient solution beforehand. Plants were harvested 11 weeks after sowing. All treatments were in duplicate.

Measurements on microscopic sections

The surface area of root pores in microscopic sections was calculated from visual estimates (calibrated with computer measurements) of the part of the cortex tissue occupied by air spaces and from measured diameters of stele, cortex and exodermis-plus-epidermis. Photographs of selected sections were compared by Dr H Konings with his quantitative results.

Pycnometer measurements

The pycnometer method is based on a comparison of the density of intact root tissue, including air-filled pores, and that of root homogenate without air. Intact root samples (1 to 3 g fresh weight) are carefully cut into pieces of 5 cm length directly before they are placed in a pycnometer

flask (50 ml). Root samples, pycnometer and water are allowed to come to room temperature beforehand. After it has been filled, the pycnometer is weighed. After removing the water adhering to the roots by centrifugation for 30 s in a household centrifuge, the fresh weight of the roots is determined. Air is removed from the roots by grinding them in a mortar, and the density of the root homogenate is measured in the pycnometer. Alternatively, air can be removed from intact roots in the pycnometer by applying vacuum (the two methods give equal results, the latter is more reproducible and easier). The temperature at the time of each pycnometer measurement is recorded.

Calculations for the pycnometer method

For calculations of air-filled root porosity the following quantities are used: ε_r = percentage air-filled porosity of the root (on a volume basis), F = (fresh) weight [g], V = volume [cm^3], X(T) = density of water at T°C [$g\,cm^{-3}$].

As subscripts are used: r = roots with air-filled pores, r* = roots without air-filled pores, a = air-filled pores, p = pycnometer filled with water, pr = pycnometer with roots and water.

As roots replace a volume of water equal to their own volume when put in the pycnometer, the following relation holds (at temperature T1):

$$F_{pr}(T1) = F_p(T1) - V_r \cdot X(T1) + F_r \qquad (1)$$

Rearranging gives:

$$V_r = \{F_p(T1) - F_{pr}(T1) + F_r\}/X(T1) \qquad (2)$$

After grinding we find for the root mass without air-filled porosity, at temperature T2:

$$F_{pr*}(T2) = F_{pr}(T2) + V_a X(T2) \qquad (3)$$

By rearranging we obtain:

$$V_a = \{F_{pr*}(T2) - F_{pr}(T2)\}/X(T2) \qquad (4)$$

From (2) and (4) we find for the air-filled root porosity, ε_r:

$$\varepsilon_r = \frac{100 \cdot V_a}{V_r}$$

$$= \frac{100\{F_{pr*}(T2) - F_{pr}(T2)\} \cdot X(T1)}{\{F_p(T1) - F_{pr}(T1) + F_r\} \cdot X(T2)} \qquad (5)$$

If T1 equals T2 the formula can be simplified to the form presented by Jensen *et al.* (1969). Values for X(T) can be found by interpolating between the following values for the density of water from Weast (1975): 0.99862, 0.99823 and 0.99707 $g\,cm^{-3}$ for 18, 20 and 25°C, respectively.

If an error of 1°C is made in the correction for temperature X(T), the estimated root air-filled porosity ε_r may, for the values of F_r and F_p we use, deviate from the real value by about 1% porosity. In 25 measurements of F_p we found an average value of 49.9622 g and a standard deviation of 0.0057 g. Using this value we may expect for a porosity estimate ε_r of 5% that a 95% probability interval is 3.9–6.1%, under the conditions of our measurements. Duplicate samples usually differ by less than 0.5% in calculated ε_r.

Table 1. Air-filled root porosity (%) quantified by the pycnometer method and by direct observation; the first two columns indicate treatment; in the pycnometer method three categories of roots were measured, intact seminal, intact nodal roots and broken branch roots ('rest'); for the microscopic sections of intact nodal roots two diameter classes were used and a weighted average is given, based on the percentage of each class of roots in the root system as a whole; in the last two columns average root diameter (mm) and specific root length ($m\,g^{-1}$) are given

		Pycnometer method			Microscopic sections			Aver. Diam.	Spec. root length
		Seminal	Nodal	Rest	< 0.5 mm	> 0.5 mm	ave.	Diam.	Length
AA	1	-	3.3	-	0	0	0	0.67	123
	2	1.5	4.4	-	0.5	8.2	0.9	0.26	426
A	1	0.7	5.1	1.5	5.0	12.1	5.9	0.28	192
	2	1.7	4.5	2.8	3.4	5.4	3.5	0.30	504
N	1	5.1	15.0	7.5	15.4	23.5	15.6	0.32	387
	2	1.7	7.7	1.2	6.1	13.8	7.4	0.25	182
NN	1	6.8	18.8	11.0	15.3	21.6	15.9	0.33	305
	2	0	9.2	1.6	8.9	15.4	9.4	0.26	382

Results and discussion

Results are summarized in Table 1. The treatments of the maize roots induced the expected variation in air-filled root porosity. With both observation methods considerable differences were observed in air-filled root porosity between parts of a single root system. Comparison of the two methods therefore is only possible if representative samples are considered. In the pycnometer method, intact seminal roots always had a lower air-filled porosity than intact nodal roots. Broken branch roots, from both nodal and seminal roots, generally had an intermediate porosity.

For the microscope sections only intact nodal roots were considered. As porosity appeared to be related to root diameter, calculations were based on the average porosity of 10 to 20 measurements in two diameter classes (with an average standard error of the mean of 2.18% air-filled porosity) and on the percentage of roots in each diameter class found for the nodal root system as a whole.

When considered on this basis, the average porosities of nodal roots as estimated by the two methods agree well. The main exception is that in roots of treatment AA hardly any cavities in the cortex were observed, while the pycnometer method indicated an air-filled root porosity of 3 to 4%. As in the direct observations intercellular spaces in the intact root tissue are not measured, such a difference is not surprising. If 3.5% porosity is added to all microscopic measurements to account for such intercellular air cavities, the results are slightly higher than the pycnometer indicates, but within the range of experimental error.

Continuous presence of $AgNO_3$ in the solution had a pronounced effect on root morphology: branch roots were initiated, but hardly grew beyond the cortex of the main axes. This resulted in a high average root diameter (Table 1). Response of porosity to treatments was much more pronounced in variant 1, with continuous treatment differences, than in variant 2 where root conditions were only affected in the last week before harvest.

The results show agreement between the two methods of measuring air-filled root porosity. With the pycnometer a relatively rapid estimate of air-filled porosity of a well-defined part of the root system is possible. With the microscopic technique the relation between air cavities and root diameter can be studied in more detail, but more effort is needed to obtain a representative average value. In the study by Konings (1983) where the physiological mechanism of cavity formation was of prime interest, the microscopic technique, employed at a well defined distance from the root tip of nodal roots was appropriate. To obtain average values for calculations on root systems in the soil, the pycnometer method is preferable.

The difference in air cavities between main and branch roots is interesting as such. Thin roots have a better oxygen supply to all root cells than thick roots and hence need a lower air-filled root porosity provided that the external pathway through the soil yields oxygen. If, however, the root depends completely on internal oxygen transport, thin roots will lose more oxygen to the environment per unit volume of root tissue and hence need a higher air-filled root porosity to attain the same root length (De Willigen and Van Noordwijk, 1987). From our observations on microscopic sections and from the relevant literature, it seems that no continuity exists between air channels in the cortex of a main axis and air channels in the cortex of a branch root. At present we are investigating this matter experimentally. A diffusion barrier between air channels in main and branch roots may be advantageous to the plant if oxygen transport along the main root is insufficient to sustain all branch roots during a period of anaerobiosis in the soil. Priority of survival of the main axis may have ecological value for the plant, as it allows a rapid recolonization of the soil by new branch roots. Some observations on the response to flooding in the field agree with this view. Plumbing aspects of air channels and differences in air-filled porosity of different parts of a root system (Kozinka, 1979) have to be incorporated in models of oxygen transport to make them more realistic.

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