Radial Movement of Oxygen in Plant Roots

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ABSTRACT
The radial movement of oxygen in excised corn and jack bean roots was measured with a platinum wire electrode embedded in the root tissue. Measurements were made with the roots exposed to air and with the roots immersed in nutrient solution in the presence and absence of millimolar sodium azide. Effective rates of oxygen diffusion in the root tissue were also measured from 5 to 30°C and compared to the respiration rates of similar root segments over the same temperature range. Under conditions which allow the roots to exude freely, the interior of the root operates under an oxygen deficit. Inhibition of respiratory oxygen uptake by low temperature or azide treatment increased the flux of oxygen to the root interior.

This paper describes measurements of the rate of oxygen diffusion to the stele of roots. Although there has been interest in the oxygen supply to the stele of roots for many years, no actual measurements have been published. One reason for the interest is the Crafts-Broyer (1) theory of root pressure exudation, which assumes the existence of an oxygen deficiency in the stele. According to this theory, salt is accumulated by the well aerated cortical cells, moves into the stele through the symplast, and leaks out of the poorly aerated cells of the stele into the xylem. Back leakage of salt and inward diffusion of oxygen presumably are hindered by the endodermis. Attempts, therefore, were made to measure the rate of oxygen diffusion into the stele and to estimate the oxygen supply to the cells of the stele.

METHODS AND MATERIALS
The method used was similar to that described by Lemon and Erickson (5) and used by several other investigators for measuring the ODR in soil. The measuring system consisted of a length of 0.0254 cm diameter platinum wire sealed into the end of a glass tube and connected to a voltage source supplying 0.7 V, which is near the center of the applied voltage output plate. When the platinum cathode is placed in a solution and made several tenths of a volt negative to a saturated calomel electrode, oxygen is reduced at the platinum surface. After several minutes, a nearly steady state condition is obtained where the rate of oxygen reduction is controlled by the rate at which it can diffuse to the electrode surface. The current through the system can be measured and is related to the steady state oxygen flux at the electrode surface by

\[ i (10^{-4}) = \frac{nFA}{s_{\text{cm}}.t} \]

where \( i \) is the current in amperes at time \( t \), \( n \) is gram equivalents of oxygen per mole, \( F \) is the Faraday, \( A \) is the surface area of the electrode in cm\(^2\), and \( f \) is the oxygen flux at the electrode surface in moles of oxygen cm\(^{-2}\) sec\(^{-1}\) (5). The ODR generally reported in the literature is identical to the flux. If the current is expressed in microamperes, time in minutes, and the quantity of gas in grams, then the terms can be rearranged, and, according to Poel (8), the ODR can be calculated as

\[ \text{ODR} = \frac{i \times 60 \times 32 \times 100}{nFA} \text{expressed as g (10}^-6\) cm\(^2\) min\(^{-1}\). \]

Since the ODR values obtained with electrodes of different radii cannot be compared directly (6), and since the electrodes used in this study were much smaller than those generally used for soils, the ODR values reported here are expressed as percentages of ODR levels obtained with the same electrode in air-equilibrated nutrient solution. Calibration measurements were made by inserting the electrode into the mesh of loosely stacked cheesecloth squares which were flooded with nutrient solution, closing the circuit, and measuring the current at the end of a 5-min period when a nearly steady state diffusion rate was reached. Root ODR measurements were made by inserting the wire longitudinally into the center of freshly excised root segments, placing the root with the electrode inserted back into the nutrient solution, closing the circuit, and measuring the current after 5 min. Since the wire projected inside the root a distance of 4 to 5 mm, the values obtained were averages over this distance. Fresh sections of the root were made immediately after each measurement to verify the placement of the electrode. The diameters of the roots also were measured.

Measurements were made on excised roots of corn (Zea mays L.), Funk's Pioneer hybrid 3048MF and jack bean (Canavalia ensiformis L. [DC]) seedlings. The seeds were soaked overnight and germinated for 3 days on moist paper towels. The seedlings were then placed on styrofoam floats with their roots immersed in one-fifth strength Hoagland's solution number 1 (3). Roots were removed for measurement 3 days after transfer to the nutrient solution.

Measurements of ODR were made on roots immersed in one-fifth strength Hoagland's solution except for one series. In this series, the root segments were lifted up until only the tips were immersed, and the region being measured was exposed to air except for a thin film of nutrient solution adhering to the root surfaces.

Oxygen diffusion rates were also measured in roots treated with 1 mM sodium azide to inhibit respiration. For these measurements root systems of intact plants were transferred to nutrient solution containing the inhibitor. After 10 min the roots were excised and the ODR was measured while the roots remained in the same solution. The electrodes were recalibrated in the

1 This research was supported by Atomic Energy Commission Contract AT (40-1)-1827.
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3 Abbreviation: ODR: oxygen diffusion rate.
azide solution. Respiration measurements made on similar root segments indicated that oxygen uptake was reduced 50 to 80% by the sodium azide. The terminal 3 cm of the roots were cut at 0.5, 1, 2, and 3 cm from the root tip. Respiration was measured separately in each of the resulting four segments. These measurements were made polarographically after the method of MacDonald and Laties (7) in one-fifth strength Hoagland's solution.

Oxygen diffusion rates and respiration were measured over a temperature range from 5 to 30 °C. The roots were immersed in nutrient solution at the stated temperature for 15 min before measurements were made. The electrodes were calibrated at each temperature.

RESULTS AND DISCUSSION

A preliminary survey of the ODR over the terminal 10 cm of corn roots revealed very little change with distance more than 3 cm back from the tip; therefore, a more extensive study was carried out on the terminal 3 cm of corn and jack bean roots. Root ODR, respiration rate, and diameter of the various segments are shown in Figure 1. In general, the ODR values for jack bean are higher than for corn, even though the diameter of jack bean roots was greater for all segments measured. This may be accounted for partly because the jack bean roots have lower respiration rates and intercept less oxygen. In both plants, the

![Graph showing respiration rate, root diameter, and root ODR as related to the distance from the root tip of the two species. Each point represents the average of at least six measurements. The respiration rates are for the entire segment measured and the length of the line indicates the length of each segment. The ODR values are averages over the length of the electrode (4 to 5 mm) which was inserted into the basal portion of each segment. Here, the length of the line indicates the length of the electrode. Diameters were measured microscopically after the ODR measurements were made and represent the diameter near the basal end of each segment.](image)

![Graph showing the relationship between root respiration and root ODR over a 25°C range in temperature.](image)

![Table 1. Equivalent Respiration Rates, Calculated from Electrode Current and Expressed as Percentage of Measured Respiration Rate in Root Segments at Indicated Distances from the Tip.](image)
ODR increased with increasing distance from the tip up to 2 cm. This might be due to the lower rates of respiration encountered in going from tip to base, resulting in less oxygen being intercepted on its diffusional pathway to the center of the root. The increase in diameter which should lower the ODR is apparently compensated for over the first 2 cm by lower respiration rates and possibly by increases in the amount of intercellular gas space in the root tissue which would enhance the diffusion of oxygen.

It seemed possible from these data that the respiration rate of the root tissues is the main factor controlling the amount of oxygen reaching the center of the root. In order to test this hypothesis further, respiratory uptake of oxygen was decreased by the addition of azide to the roots in one series of experiments, and by lowering the temperature of the roots in another series.

Exposure of the roots to 1 nm azide caused large increases in effective ODR, as shown in Figure 2. Figure 3 shows that the same inverse relationship between root respiration and ODR exists when respiration is reduced by decreasing the temperature.

Since we have determined the oxygen flux to the electrode surface, we can now convert this to a respiration rate for a volume of tissue equal to the volume of the electrode. And since the electrode reduces oxygen at a rate which is diffusion-controlled, we may imagine that this "equivalent respiration rate" represents the maximum possible rate of respiration for the equivalent amount of tissue. The relationship between electrode current and the equivalent respiration rate takes the form

\[
equiv \text{respiration rate} = \frac{32 \text{FW}}{nF} \text{g O}_2 \text{per sec per g tissue (3)}
\]

where \( W \) is the equivalent tissue weight in grams, \( i \) is in amperes, and the other terms are the same as equation 2. The volume of an electrode, such as those used, of 0.0254 cm diameter and 0.45 cm length, is 2.28 \( \times 10^{-4} \) cm\(^3\). Assuming a root density of 1 g cm\(^{-3}\), which is admittedly high, we get a tissue weight numerically equal to the volume of the electrode. Table I shows the results of such calculations made for various segments of corn and jack bean roots. The equivalent respiration rates were calculated and expressed as a percentage of the measured respiration rates for similar root segments. The values in Table I generally increase with distance from the tip because, while overall respiration decreases in this direction, the equivalent respiration rate, a function of electrode current, increases. Thus when the equivalent respiration rate for a small volume of interior tissue is expressed as a percentage of the measured respiration rate for the entire segment, the values tend to increase toward the base of the root. A comparison of the values for the two species in Table I reveals the large differences encountered between the two species used in this study. For example, the 1- to 2-cm segments of corn roots receive at the interior only enough oxygen to maintain a respiration rate of 35% of the over-all rate for the tissue, while in similar segments of jack bean this figure is 86%. These relatively large differences are probably due to differences in respiration rate, root diameter, and effects of organization of the tissues on oxygen diffusion. It should be pointed out that, if a considerable percentage of the tissue within the stele is nonliving, then treating the volume of tissue as being equal to the volume of the electrode may tend to overestimate the real oxygen requirement in the stele.

When 3-cm segments of corn roots were exposed to air, the root ODR rose to 75% of the calibration value in free nutrient solution. This represents an electrode current of 1.01 \( \mu A \) and an equivalent respiration rate of 3.65 \( \times 10^{-7} \) g O\(_2\) per sec per g tissue, which is approximately the respiration rate of similar segments measured in well stirred nutrient solution.

It is realized that insertion of the electrodes causes disruption of root tissue which might affect the rate of respiration. If respiration were materially increased, the equivalent respiration rate might be underestimated. However, there is no evidence that wound-induced increase of oxygen uptake occurs during the 3 to 5 min required for a measurement. The measurement of roots in nutrient solution and in air represents the two extreme situations to which the roots might be subjected. The actual condition in soil lies somewhere between these two extremes, and it therefore seems that the stele of ordinary roots operates with less than an optimum supply of oxygen. Furthermore, the percentage of oxygen is usually lower in the soil gas than in the air above; hence the oxygen deficit in the root interior may ordinarily be larger than indicated by our measurements. It seems probable that the oxygen supply in the soil is always suboptimal for maximum root respiration.

That an oxygen deficit exists within the stele when the root environment is at 21% oxygen is not surprising in view of the compactness of the endodermis and pericycle. It also is in accord with the hypothesis on which the Crafts and Broyer theory of root exudation is based. However, Yu and Kramer (10, 11) observed that the cells of the stele can accumulate ions as energetically as those of the cortex, both when freshly separated and when in intact roots. Furthermore, the oxygen uptake of separated stele was higher than that of the cortex. Thus, it appears that the stele of corn roots can accumulate ions even though it does not receive enough oxygen for its maximum rate of respiration.

Perhaps root functions are limited by an excess of carbon dioxide (9) or by inhibitors (4) rather than by the deficiency in oxygen. Possibly, roots are able to adapt so that they can function adequately with a low supply of oxygen because soil air usually contains less oxygen than the atmosphere above ground. It is reported that, although deficient aeration reduced root elongation, it does not prevent the development of branch roots which originate in the stele (2). This suggests that the internal tissues of roots are able to operate at a low level of oxygen.

In conclusion, it seems clear that radial diffusion of oxygen into the stele of corn and jack bean roots is too slow to provide enough oxygen to maintain the rate of respiration observed in well aerated tissue. However, more research is needed before the full significance of this observation can be evaluated.

**LITERATURE CITED**