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Trans-Root Electrical Potential in Roots of *Plantago media* L. as Affected by Hydrostatic Pressure: The Induction of an O_2 Deficient Root Core

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Excised roots of *Plantago media* L. were subjected to small hydrostatic pressure gradients. Pressure applied with a displacement pump easily extracted gas present in cortical lacunae consequent on the gas saturation deficit of the bathing solution. The temporary pressure treatment resulted in an irreversible decrease in the O_2 diffusion rate through the root tissue and consequently in O_2 deficient root cores.

Therefore electrogenic pumps located at the symplast/xylem interface, i.e. in the centre of the root, were inhibited by pressure.

The longitudinal distribution of the xylem pumps could be computed: in *Plantago media* they are located in the basal parts of the root. The effect of pressure on the electrogenic xylem pumps demonstrated here should be taken into account when the process of ion transport from the root to the shoot is studied while the root is pressurized.

Key words: Electrogenic xylem pumps — Hydrostatic pressure — *Plantago media* — Respiration — Trans-root potential.

Roots of two *Plantago* species contain electrogenic ion pumps at the symplast/xylem interface (De Boer et al. 1983). The supply of oxygen was shown to be a crucial factor for the maintenance of xylem pump activity. At normal aeration of the bathing solution the activity of these pumps was often below its maximum capacity whereas a sudden pump inhibition was observed when the oxygen partial pressure of the bathing solution (pO_2) was reduced to 10%.

A rapid hyperpolarization of the trans-root potential (TRP), at the change of normoxia to anoxia, indicates inhibition of the electrogenic xylem pumps (see Fig. 1). On the other hand a depolarization of TRP, as a result of anaerobism or addition of uncouplers to the medium, means inhibition of electrogenic ion pumps in cortical cells. So, TRP is a summation of the membrane potential of cortical cells and xylem parenchyma cells.

Root cortical cells have to be almost anaerobic before the electrogenic component of the membrane potential disappears (Bravo and Uribe 1981, De Beer and De Boer unpublished results). The activity of the xylem pumps then is obviously a useful indicator for the occurrence of anaerobic root tissue.

This is of interest as the oxygen supply to the stelar root tissue has been a subject of discussion for a long time. In their now classical theory Crafts and Broyer (1938) postulated hypoxic conditions in the root core as being the important factor for centripetal ion transport. However, Bowling (1973) measured directly the radial oxygen gradient in sunflower roots and concluded

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Abbreviations: D_e, effective diffusion coefficient for oxygen; J_v, flow rate of xylem sap; pO_2, oxygen concentration, at air saturation it is 21%; TRP, trans-root electrical potential difference.

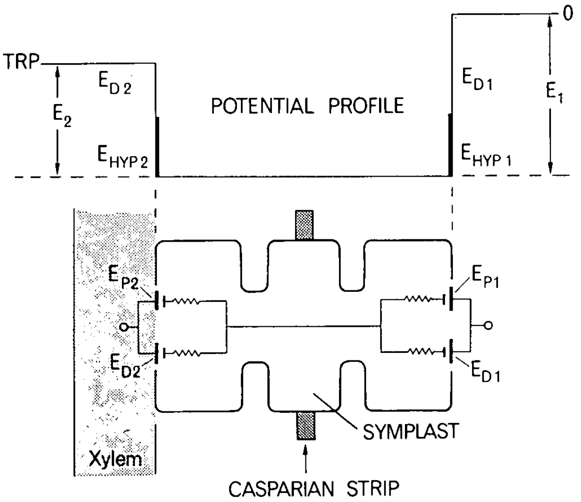

Fig. 1 Schematic illustration of the electrophysiological organization of the root. The membrane-potential of epidermal/cortical cells (E_1) and that of xylemparenchyma cells (E_2) results from the electromotive force (e. m. f.) of the passive channels (E_{D1}, E_{D2} respectively) and, in parallel with these channels the e. m. f. of the electrogenic pump (E_{P1}, E_{P2} respectively). The trans-root potential is the difference between the two membrane potentials: $TRP = E_1 - E_2 = (E_{HYP1} + E_{D1}) - (E_{HYP2} + E_{D2})$.

that stelar cells were certainly not under oxygen constraint. This conclusion is true for thin roots (Bowling used roots with a diameter of 0.4–0.5 mm) but does not hold for roots of larger diameter (Fiscus and Kramer 1970). Recently Van Noordwijk and De Willigen (1984) demonstrated that the conlusions reached in the two aforementioned articles differed only as a consequence of the diameter of the roots used. Van Noordwijk and De Willigen conclude that in roots of 1 mm diameter 20% of the root volume is not aerated adequately.

Starting from the conclusion of Van Noordwijk and De Willigen it is evident that any treatment reducing the effective diffusion coefficient of oxygen (D_e) in root tissue can affect the O_2 supply of stelar cells. One such treatment commonly used is pressurizing excised root systems (Jackson and Weatherley 1962, Kuiper and De Boer 1980, Lopushinsky 1964). It is general practice to raise the hydrostatic pressure gradient between bathing solution and xylem vessels to simulate transpiration in excised roots in order to study the effect of mass flow on ion transport across the root. Although not often mentioned it is well known, among others by own experience (Kuiper and De Boer 1980), that the appearance of the roots changes during such a pressure treatment; being white before, but translucent afterwards.

Meiri and Anderson (1970) showed that roots become translucent when air, normally present in cortical lacunae, is replaced by water. They investigated one consequence of this waterlogging, namely a decrease in the cortical resistance to water flow and suggested too the possibility that pressure results in oxygen deficiency in roots considering that the oxygen diffusion coefficient in air is 10,000 times greater than that in water.

In the present experiments the effect of pressure and low pO_2 on the development of anaerobic cores in roots were investigated. The activity of the electrogenic xylem pumps, measured by means of TRP, was used as an indicator for O_2 deficient stelar tissue. Moreover, the respiration rate of tap roots, both before and after a pressure treatment, was measured and the change in D_e upon application of pressure was calculated.

One of the aims of the experiments was to get more insight into the longitudinal localization of the xylem pumps. This was intended as an experimental completion of the theoretical model made by Ginsburg (1972) of the localization of the TRP measurements in excised roots.

Materials and Methods

Plant material—Seeds of *P. media* L. were germinated in vermiculite. Fourteen day-old seedlings were transferred to trays containing 30 liter of a nutrient solution having the following

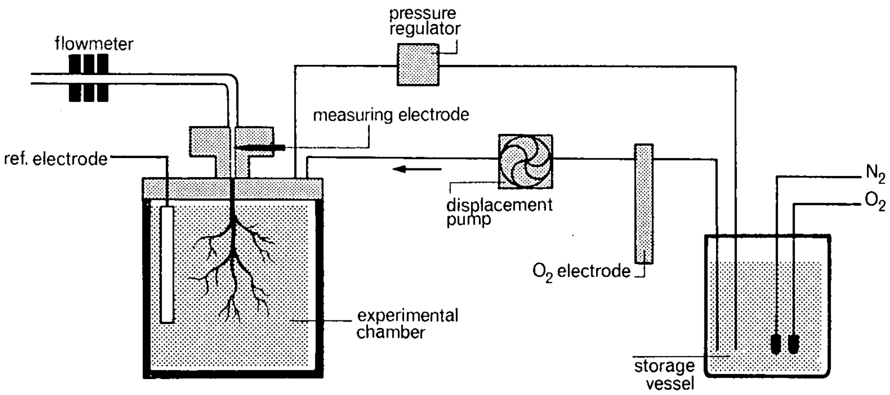

Fig. 2 Experimental setup for simultaneous measurement of trans-root potential (TRP) and the xylem sap flow rate (J_v).

composition: Major elements (mM), 1.25 KNO_3, 1.25 $Ca(NO_3)_2$, 0.5 $MgSO_4$, 0.25 KH_2PO_4; minor elements ($\mu g \cdot g^{-1}$), 5 Fe (as Fe-rexonol), 0.5 B, 0.5 Mn, 0.05 Zn and 0.05 Mo. The initial pH was increased to 6 with KOH (about 0.1 $mmol \cdot liter^{-1}$) and the solution renewed every 7 days. Plants were grown in a climate chamber at 20°C (day and night), with a 12 h photoperiod of 58 $W \cdot m^{-2}$ light intensity. The relative humidity of the air was 60%. The experiments were carried out on individual plants, grown for 30 to 40 days in the nutrient solution.

Experimental chamber—The experimental setup is shown schematically in Fig. 2. Since this was described in full detail before (De Boer et al. 1983), only a short description is included. Roots of *P. media* were excised and the neck of the root was sealed in a rubber stopper in the lid of the experimental chamber. TRP was measured with two Ag/AgCl reference electrodes filled with 3 M KCl. The reference electrode in the bathing solution was immersed completely in order to prevent the development of hydrostatic pressure gradients across the tip of the electrode when the bathing solution was pressurized. The flow rate of exudate (J_v) was measured automatically.

Pressure and aeration—The common way of increasing water flow across excised root systems is by pressurizing the bathing solution with compressed air at continuous aeration (Markhart III 1982). Using this method of applying pressure, in accordance with Henry's law, a continuous stream of tiny air bubbles appeared at the excised surface; a phenomenon comparable to the "caisson disease". Markhart III experienced similar problems (personal communication).

The bubbles so formed cause a technical problem since they disturb the measurement of TRP and J_v. Besides this, the effect of air bubbles in the xylem vessels inside the root on ion and water flow is not known. Therefore we used a displacement pump (Jabsco) to pressurize the root containing vessel (free of air), while aerating the experimental solution in a storage vessel under normal atmospheric conditions. In this way the amount of air dissolved per liter solution remained at 23.3 mg, independent of the pressure applied. This setup resulted in a completely bubble free exudate.

Root respiration and root gas volume—Respiration of tap roots was measured with a Yellow Springs Instruments oxygen meter (model 53). Tap roots, with a diameter ranging from 2 to 5 mm, were washed for 30 min in aerated culture solution after all lateral roots had been removed. These root segments were then placed in a cuvette (volume 95 ml) containing a solution similar to that used for growth. The cuvette was carefully sealed, excluding all air bubbles from the solution and the decrease in oxygen concentration of the solution was measured with an electrode mounted in the cuvette. The temperature was 20°C.

To determine the volume of gas filled lacunae, tap roots were blotted gently and weighed (W_1). Next, air was extracted from the roots by applying pressure for 30 min at 0.6 bar; air is extracted by the air saturation deficit of the bathing solution (Meiri and Anderson 1970). Roots were weighed again (W_2) and the gas volume was taken as $W_2 - W_1$ ml since water (density= 1 g·ml^{-1}) replaced gas. The gas volume was sometimes expressed as percentage of W_2 under the assumption that root density was close to one after air extraction.

Diffusion of oxygen within plant roots—Oxygen transport within root tissue is mainly by way of diffusion. Mass-flow with the transpiration stream can be neglected (Armstrong 1979). Assuming that a) the root is cylindrical in shape, b) all cells in a cross section have a uniform oxygen uptake rate per unit volume, c) transport characteristics with respect to oxygen diffusion are uniform in a cross section, d) longitudinal transport of oxygen in excised roots is negligible, then radial O_2 flow in a plane perpendicular to the root axis can be described as (1) (Armstrong 1979).

$$Ma^2\left\{\frac{a^2}{b^2}+2\log\left(\frac{a}{b}\right)-1\right\}=4D_e(C_e-C_i), \tag{1}$$

where C_e is the oxygen concentration in the bathing solution (g·cm^{-3}), and C_i is the oxygen concentration at distance a from the root centre; in our calculations C_i was taken as 2%, the concentration critical for respiration (Armstrong and Gaynard 1976) (g·cm^{-3}), M is the rate of oxygen uptake per unit volume of root when this volume is O_2 saturated (g·cm^{-3}·s^{-1}), D_e is the effective diffusion coefficient for oxygen (cm^2·s^{-1}), "b" is the root radius (cm), "a" is the radius of the inner root cylinder (cm) where the oxygen concentration is equal to C_i; in our calculations "a" is the radius of the inner cylinder where the O_2 concentration falls below the critical concentration of 2%.

The effective volume (V_e) in which the uptake rate of oxygen per unit volume equals M is:

$$V_e=\pi b^2 d-\pi a^2 d=\pi d(b^2-a^2) \tag{2}$$

where d is the thickness of a disk of unit volume, i.e. $d=\frac{1}{\pi b^2}$. The respiration rate per unit volume (=R) is then:

$$R=MV_e=M\left(\frac{b^2-a^2}{b^2}\right)\quad (\text{g·cm}^{-3}\text{·s}^{-1}) \tag{3}$$

Dividing (1) by the square of the root radius, b^2, and rearrangement gives:

$$M\left(\frac{b^2-a^2}{b^2}\right)=\frac{4D_e}{b^2}C_e+\frac{2Ma^2}{b^2}\log\left(\frac{b}{a}\right)-\frac{4D_eC_i}{b^2} \tag{4}$$

or:

$$R=pC_e+q\quad (\text{g·cm}^{-3}\text{·s}^{-1}) \tag{5}$$

the slope of this line being $p=\frac{4D_e}{b^2}$, whereas the intercept of this line is:

$$q=\frac{2Ma^2}{b^2}\log\left(\frac{b}{a}\right)-\frac{4D_eC_i}{b^2}.$$

In the present experiments the root radius b could only be approximated as the average root radius $\bar{b}$ since the shape of the tap roots is conical. The average root radius was calculated as: $\bar{b}=\sqrt{\frac{W_2}{3.14L}}$, where W_2 is the fresh weight of waterlogged roots (cm^3, assuming a root density of 1) and L is the total length of the root segments used (cm).

Oxygen uptake by roots is usually calculated from the decrease in O_2 concentration in the solution of a closed system. However, it is necessary to correct for changes in the cortical lacunae oxygen pool when it is significant relative to the oxygen pool in the bathing solution (Sand-

Jensen and Prahl 1982). The solubility of O_2 in air is 30 times higher than in water, 272.8 and 9.2×10^{-6} g·cm^{-3}, respectively, so 1 ml of air volume is equivalent to 30 ml of water.

The initial gas volume of the roots used (V_G) can be determined from the increase in wet weight when the roots are evacuated (Meiri and Anderson 1970). The total amount of N_2 in the cuvette, used for respiration measurements, remains constant during the experiment since measurements are performed in a closed system. When roots and solution are equilibrated with air, the total amount of N_2, M_{N_2}, is given by:

$$M_{N_2}=p_{N_2}(V_L s_{N_2}+V_G d_{N_2}) \tag{6},$$

where p_{N_2} is the partial N_2 pressure (0.765 bar at air saturation, 20°C), V_L is the water volume in the cuvette (ml), V_G is the gas volume of the roots (ml), s_{N_2} is the solubility of N_2 in water (1.901×10^{-2} g·liter^{-1} at 1 bar, 20°C), and d_{N_2} is the density of N_2 (1.1653 g·liter^{-1} at 1 bar, 20°C).

During O_2 uptake atmospheric pressure is maintained in the cuvette since part of the lid is elastic. Therefore:

$$p_{N_2}+p_{O_2}=\text{constant, or } p_{N_2}=0.977-p_{O_2} \tag{7}$$

p_{O_2} is known throughout the experiment as it is registered by the polarographic electrode: $p_{O_2}=\frac{s_{O_2}}{C_e}$, where C_e is the oxygen concentration in the solution (g·liter^{-1}) and s_{O_2} is the solubility of O_2 in water (4.339×10^{-2} g·liter^{-1} at 1 bar, 20°C).

Substituting equation (7) in (6) and rearrangement gives:

$$V_G=\frac{M_{N_2}-0.977\,V_L s_{N_2}+V_L s_{N_2} p_{O_2}}{0.977\,d_{N_2}-p_{O_2} d_{N_2}} \tag{8}$$

The total amount of O_2 in the system is given by:

$$M_{O_2}=p_{O_2}(V_L s_{O_2}+V_G d_{O_2}), \tag{9}$$

where d_{O_2} is the density of O_2 (1.3312 g·liter^{-1} at 1 bar, 20°C). Substituting equation (8) in (9) and rearrangement shows the relation between the partial O_2 pressure in the gas phase and the total amount of O_2 present in the system:

$$M_{O_2}=p_{O_2}\left(A+\frac{B+Dp_{O_2}}{C-d_{N_2}p_{O_2}}\right), \tag{10}$$

where $A=V_L s_{O_2}$, $B=M_{N_2}d_{O_2}-V_L s_{N_2} d_{O_2}\,0.977$, $C=0.977\,d_{N_2}$, and $D=V_L s_{N_2} d_{O_2}$.

In this way the decrease in oxygen concentration of the solution in t s can be corrected for the simultaneous decrease of the oxygen concentration in the lacunae. In the absence of any gas phase the amount of oxygen in the system can be calculated as $M_{O_2}=V_L\,C_e$.

Results

Evacuation of roots easily extracts gas from the cortical lacunae (Meiri and Anderson 1970), as a result of the gas saturation deficit of the solution. The following experiment was performed to show that pressurizing the bathing solution with a displacement pump, as described in **Materials and Methods**, had a similar effect. Two roots were pressurized for 30 min at 0.5 bar. Root 1 was pressurized with the displacement pump, the gas saturation deficit being 11.7 mg·liter^{-1}. Root 2 was pressurized with air whilst air bubbled through the solution. Roots were fully immersed in the solution in the experimental chamber to prevent the development

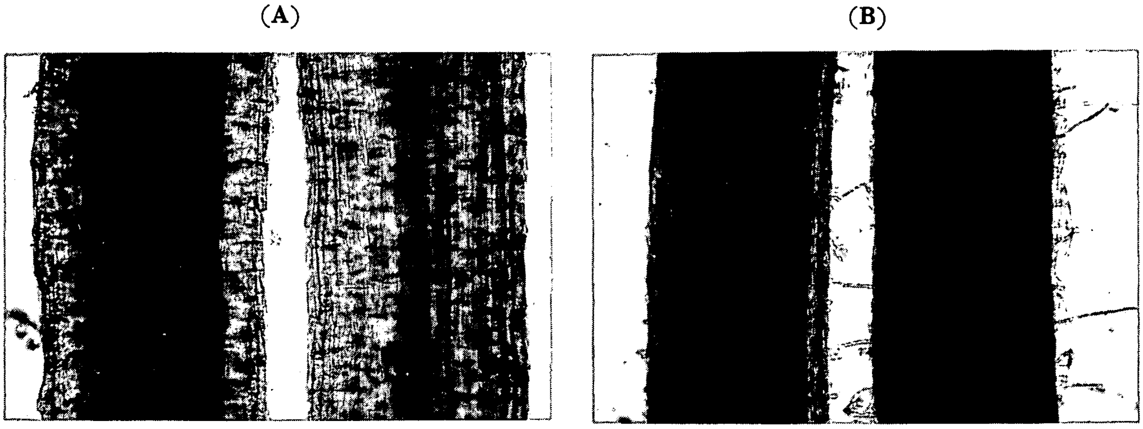

Fig. 3 Appearance of roots under the microscope before pressurizing (roots on the left hand side of Fig. 3A and Fig. 3B) and after pressurizing (roots on the right hand side of Fig. 3A and Fig. 3B) with the displacement pump (A) and with compressed air (B).

of a hydrostatic pressure gradient across the root. The appearance of the roots before pressure (left, Fig. 3A and 3B) when seen under the microscope was opaque, which is caused by the difference in refraction index of air and water. Root 1 became translucent (Fig. 3A) whereas the appearance of root 2 (Fig. 3B) was not changed by the pressure treatment, showing that a small air saturation deficit removes gas entrapped in cortical lacunae.

The importance of gas filled lacunae for the diffusion of oxygen was investigated by measuring respiration of tap roots before and after gas extraction. It was expected (Fiscus and Kramer 1970, Van Noordwijk and De Willigen 1984) in tap roots with an average radius ranging from 1.5 to 2 mm, that the total O_2 consumption would not be maximal at air saturation owing to the presence of an anaerobic core. Fig. 4 shows the rate of O_2 uptake from the bathing solution by a sample of tap roots in relation to the external oxygen concentration. The total gas volume of these roots was 0.61 ml (11.3% v/v), containing a considerable amount of O_2 compared with the amount of O_2 present in the 95 ml solution. Therefore, the apparent respiration rate before waterlogging was corrected for uptake of O_2 present in the gas phase, using equation (10). Without this correction the rate of respiration at atmospheric conditions is underestimated by ca 25% in the present experiment (Fig. 4).

Between 20 and 4% external oxygen (C_e) the corrected rate of respiration was directly proportional to C_e but at C_e values below 4% respiration rate declined sharply (Fig. 4). The effective diffusion coefficient of O_2 (D_e) could be calculated from the slope of the curve between 4 and 20%, using equation (5). After gas extraction the relation between rate of respiration

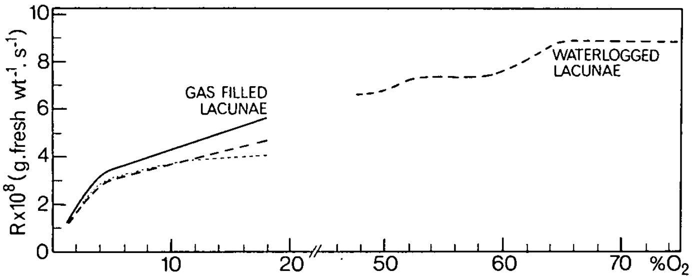

Fig. 4 Rate of oxygen consumption of tap roots of *P. media* in relation to the oxygen concentration of the bathing solution. ·······, Before pressure treatment, without correction for O_2 uptake from gas filled lacunae. ——, Before pressure treatment, after correction for O_2 uptake from gas filled lacunae. ----, After pressure treatment.

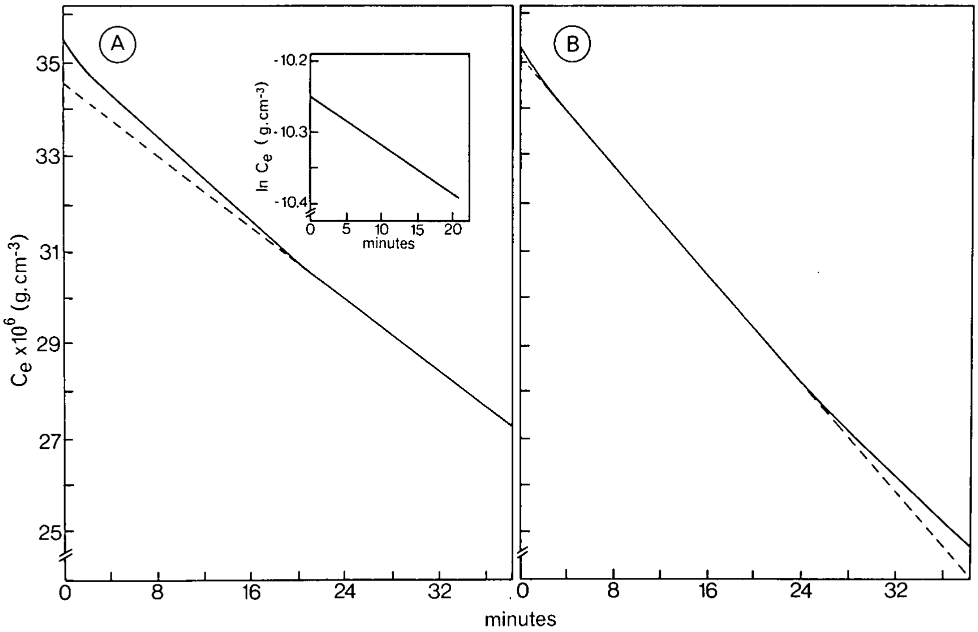

Fig. 5 Time course of oxygen concentration of the solution in the cuvette used for respiration measurements. Tap roots, equilibrated at 21% O_2, were at t=0 transferred into the cuvette, the solution of which had an O_2 concentration of 75%. A, roots with gas filled intercellular lacunae and B, waterlogged roots. The dotted line was drawn to indicate the deviation from linearity. The exponential part of the curve in Fig. A was plotted logarithmic in the insert; $r^2=0.9998$.

(=R) and C_e remained linear but the slope of the curve was smaller and thus apparently D_e. D_e in roots with gas filled lacunae was on the average 137% greater than D_e in waterlogged roots (Table 1).

The correction of R for O_2 uptake from the gas phase was based on the assumption that equilibrium exists between O_2 in the gas and water phase. The following experiment was carried out to investigate if indeed the O_2 exchange between these two phases proceeds at a considerable rate. Roots with gas filled lacunae were equilibrated in a solution containing 21% O_2 and then placed in the cuvette containing a solution with a partial O_2 pressure of 75% and the decrease of C_e was followed immediately (Fig. 5). Two processes determined the rate of O_2 uptake: 1) respiration, being constant at high values of C_e over the range 65 to 75% (see Fig. 4), and 2) diffusion of O_2 from the bathing solution at high oxygen concentration (75%), into the gas phase of the roots at low oxygen concentration (21%).

The oxygen concentration of the bathing solution as a function of time (t) can be mathematically described as a summation of a linear part (respiration) and an exponential part (diffusion into the gas phase):

$$C_e=a-bt+ce^{-dt}, \tag{11}$$

where C_e is the external O_2 concentration, t is the time and a, b, c, and d are constants. From d, the permeability constant of oxygen exchange across the root surface (k) can be calculated: namely $d=k\frac{V_i+V_e}{V_iV_e}$, where V_i is the volume of gas filled lacunae and V_e is the external water volume. The value of k as calculated from Fig. 5 is in the same range as k, calculated by Sand-Jensen and Prahl (1982) for roots of *Lobelia dortmana* L.: 0.020 and 0.022 cm·min^{-1} respectively. The surface area of the roots in our experiment was determined from the fresh weight of waterlogged roots and total root length.

The time delay of equilibrium of O_2 in the gas and water phase cannot be estimated on the basis of this k value since the surface area per unit volume of lacunae is not known. Fig. 5 shows however that the time delay of equilibrium during O_2 uptake measurements will be small as O_2 depletion is gradual. There was no exponentional decrease in C_e when the same experiment was performed with waterlogged roots (Fig. 5B). The minor initial deviation from linearity is probably the result of O_2 diffusion into the watery root tissue.

The experiments described so far point to an increased impedance for O_2 diffusion in the root tissue as a result of waterlogging by pressure. Therefore we compared the effects of anoxia and pressure upon TRP and the xylem sap flow rate (J_v). Fig. 6A shows the reaction of a so-called 'type A' root to anoxia. In type A roots two electrogenic ion pumps working in opposite directions, contribute to TRP (De Boer et al. 1983, see also Okamoto et al. 1979). The oscillations of TRP after reoxygenation are noteworthy and not uncommon when electrogenic ion pumps are subjected to sudden metabolic shifts (Gradmann and Slayman 1975, Okamoto et al. 1979). J_v decreased as soon as the solution was anaerobic. This may have been caused by a reduced cytoplasmic streaming and reduced conductivity of the plasmodesmata (Pitman et al. 1981).

Fig. 6B shows the effect of small hydrostatic pressures, applied to the same root system as used for the anoxia reaction. A pressure of 0.3 bar applied for 45 min had no effect upon TRP. When pressure was raised to 0.6 bar TRP hyperpolarized, very fast, after about 13 min. The hyperpolarization, indicating that the inner pump was inhibited, was followed by a depolarization, indicating the inhibition of the outer pump. The latter could be reversed by raising the O_2 concentration from 30 to 90%. The subsequent depolarization of TRP in response to

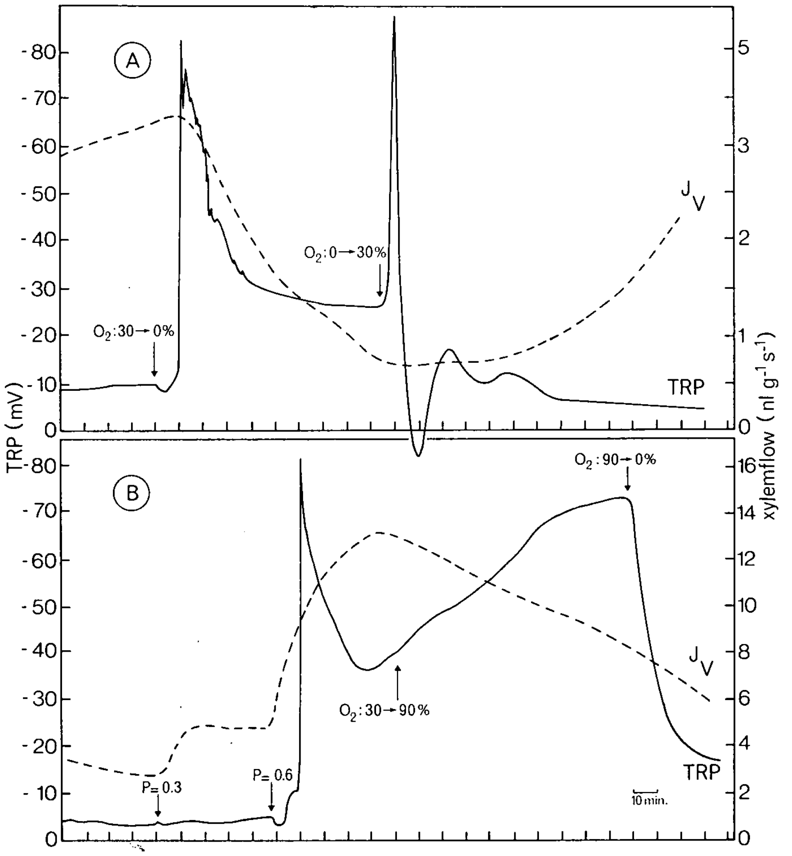

Fig. 6 A, response of trans-root potential (TRP) and xylem sap flow rate (J_v in $nl{\cdot}g^{-1}{\cdot}s^{-1}$) of type A root to anoxia and B, the response of the same parameters to hydrostatic pressure and changes in pO_2 of the bathing solution.

anoxia showed that the electrogenic ion pump at the plasmalemma of cortical cells was still functioning, even though pressure of 0.6 bar was maintained.

The increase of J_v between 0 and 0.3 bar was smaller than that between 0.3 and 0.6 bar (Fig. 6B). This nonlinearity of J_v versus pressure, in the pressure range of 0 to 1 bar, has been observed before (Lopushinsky 1964) and was explained by Meiri and Anderson (1970) as a decrease in cortical resistance to water flow as a result of advancing gas extraction in this pressure range.

To get insight into the effect of pressure as such, a 'type B' root was used. In type B roots only the outer electrogenic pump is active and therefore TRP is highly negative (De Boer et al. 1983). Any response of TRP in these roots thus reflects the activity of the outer pump only, the activity of which is shown by the anoxia respons of TRP (Fig. 7A). J_v decreased as soon as TRP depolarized and J_v recovered completely upon reoxygenation.

Fig. 7B shows the subsequent pressure effects. A pressure of 0.2 bar had little effect on TRP but TRP depolarized 23 mV when pressure was raised from 0.2 to 0.4 bar. A further pressure increase of 0.2 bar and a sudden pressure release hardly affected TRP. Since TRP repolarized when pO_2 was raised from 30 to 90%, the depolarization upon application of pressure was due to the inhibition of the outer pump by lack of oxygen. Keeping pO_2 at this high level, a sudden pressure switch from 0 to 0.6 bar had no effect on TRP; showing that pressure as such did not

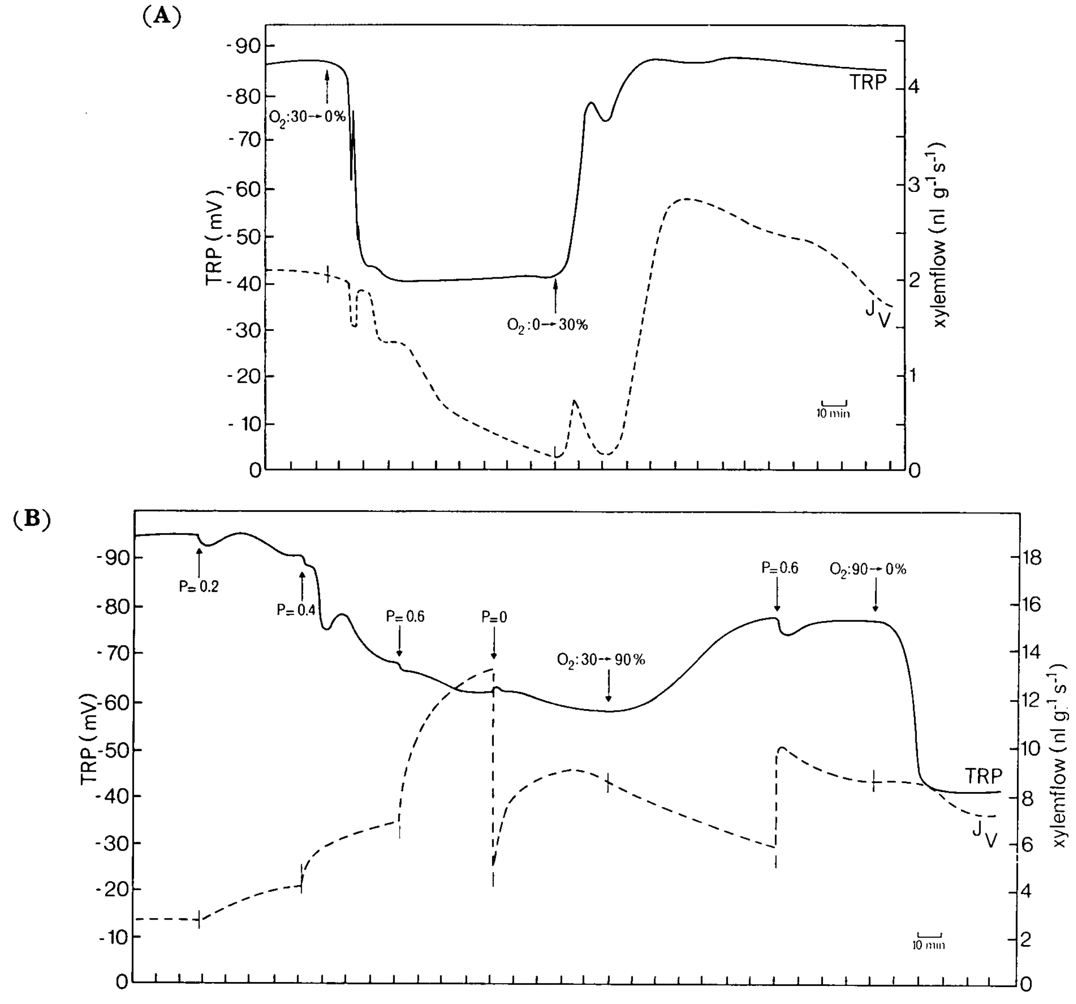

Fig. 7 A, response of trans-root potential (TRP) and xylem sap flow rate (J_v in $nl \cdot g^{-1} \cdot s^{-1}$) of type B root to anoxia and B, the response of the same parameters to hydrostatic pressure and changes in pO_2 of the bathing solution.

Table 1 Data for four samples of tap roots, used for calculating the effective diffusion coefficient of oxygen (D_e) before and after gas extraction

No.	Radius (cm)	Gasvol. (% fr wt)	M[a] ($g \cdot cm^{-3} \cdot s^{-1}$)	D_e-before ($cm^2 \cdot s^{-1}$)	D_e-after ($cm^2 \cdot s^{-1}$)
1	0.163	8.0	6.0×10^{-8}	3.87×10^{-5}	1.63×10^{-5}
2	0.164	11.4	9.0×10^{-8}	2.51×10^{-5}	2.00×10^{-5}
3	0.142	9.2	7.3×10^{-8}	3.43×10^{-5}	1.34×10^{-5}
4	0.172	12.7	7.5×10^{-8}	5.26×10^{-5}	1.40×10^{-5}
x	0.160	10.3	7.45×10^{-8}	3.77×10^{-5}	1.59×10^{-5}
s.d.	0.013	2.1	1.23×10^{-8}	1.15×10^{-5}	0.30×10^{-5}

[a] M = the rate of oxygen uptake per unit volume of root when this volume is O_2 saturated.

influence TRP. In both type A roots and type B roots the relation between J_v and pressure was nonlinear.

Discussion

Oxygen diffusion in root tissue—The measurements on O_2 uptake by excised tap roots showed:

—that the amount of O_2 present in gas filled lacunae cannot be neglected when respiration is determined from the decrease of the O_2 concentration in the bathing solution,

—that the rate of oxygen diffusion in tap roots limits respiration, even under conditions of normal aeration,

—that gas filled lacunae facilitate O_2 diffusion into root tissue.

The effective diffusion coefficient of oxygen, D_e, apparently is the crucial factor for optimal O_2 supply. In thin onion roots (diameter 0.8 mm) O_2 uptake depended on pO_2 below 20% as D_e of these roots was small: $1.27 \times 10^{-5}\ cm^2 \cdot s^{-1}$ (Berry 1949). This D_e value is close to the D_e value calculated for pressurized tap roots: $1.59 \times 10^{-5}\ cm^2 \cdot s^{-1}$ (Table 1). Berry used the apex of onion roots and young root tissue usually is devoid of gas filled lacunae, just like older root tissue that has been subjected to pressure. The high D_e value of untreated tap roots (Table 1) shows that the presence of gas filled intercellular spaces facilitates O_2 diffusion to a large extent.

Fig. 8 shows the effect of D_e on the development of an oxygen deficient core as dependent

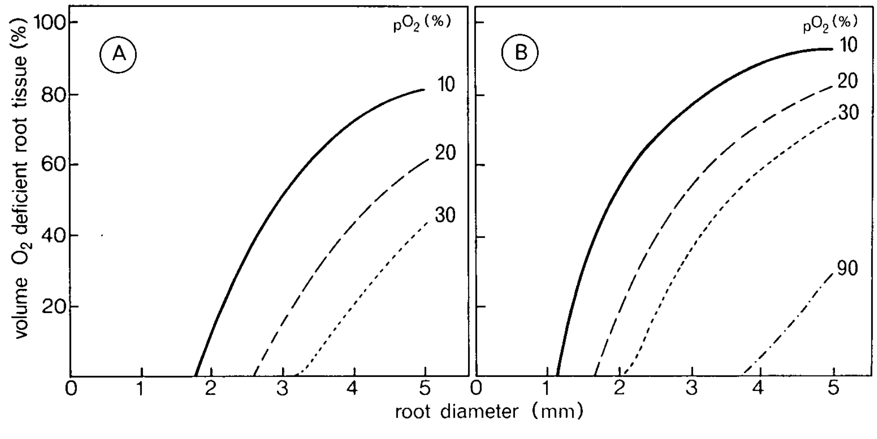

Fig. 8 Calculation of the development of an O_2 deficient root core as dependent on root diameter and the external O_2 concentration. Equation 1 was used to calculate the volume percentage of the root where pO_2 falls below 2% ($=C_i$). Average data from Table 1 were taken. A, before pressure treatment; $D_e = 3.77 \times 10^{-5}\ cm^2 \cdot s^{-1}$, and B, after pressure treatment; $D_e = 1.59 \times 10^{-5}\ cm^2 \cdot s^{-1}$.

on root diameter and pO_2; the data of Table 1 were used to calculate this figure using equation (1). An example may clarify the effect of pressure: root segments, 3 mm diameter, are fully aerated at 30% pO_2 (Fig. 8A) but pressure leads to O_2 deficiency in 35% of the root volume (Fig. 8B). It is not likely that in intact plants O_2 deficiency occurs so readily. In intact plants O_2 easily moves from the shoot to the root (Barber et al. 1962). It has been demonstrated for intact plants of *P. media* as well that there is O_2 transport from the shoot to the root (van Hartingsveld, unpublished results).

TRP and pressure—There are no examples showing the effect of pressure on TRP in the current literature as far as we are aware. Only Ansari and Bowling (1972) studied the relation between suction applied to the cut stump of sunflower roots and TRP. They found a slightly more negative TRP with increasing suction. A comparison between their results and ours is hard to make since the results of Ansari and Bowling gave no indication for the presence of electrogenic xylem pumps.

The similarity between the reaction of TRP upon anoxia (Fig. 6A) and pressure (Fig. 6B) indicated that the root core becomes O_2 deficient as a result of pressure. The respiration measurements (Table 1, Fig. 8) support this view and the pressure effect can be set out as follows: Pressure replaces the gas of the intercellular lacunae with water. Consequently D_e decreases and O_2 diffusion slows down. Gradually, in root segments from a certain diameter and greater, an O_2 deficient core develops and electrogenic xylem pumps located in this core are inhibited. The gradual nature of the process explains why the hyperpolarization of TRP (Fig. 6B) is not instantaneous, but only starts some 13 min after the onset of pressure.

It is possible that besides the stele inner cortical cells become O_2 deficient as well; the extent of which will depend on the size of the O_2 deficient core. All cortical cells contribute to TRP and depending on the number of cells that are inhibited TRP will depolarize to a greater or lesser extent. Such a reaction of TRP could be observed in type A and in type B roots (Fig. 6B and 7B respectively). At high pO_2 (90%) part of the inner cortical cells, O_2 deficient at 30% pO_2 after waterlogging, were reoxygenated as shown by the repolarization of TRP. Obviously 90% pO_2 was not high enough to supply stelar root tissue including the xylem pumps of type A roots with sufficient oxygen, as indicated by the anoxia respons of TRP (Fig. 6B).

The location of the xylem pumps—Fig. 8 shows that thin roots, diameter less than 1 mm, are sufficiently supplied with O_2. A pressure treatment does not affect the O_2 supply neither does a decrease in pO_2 to 10%. Since at pressure the activity of xylem pumps as measured by the present method, disappeared it is concluded that the TRP measurement does not include xylem pump activity in thin roots. On the basis of a theoretical model Ginsburg (1972) concluded that TRP reflects the electrical properties of the uppermost part of the root system. He represented the whole root by a two-dimensional analogue (Fig. 9). The potential difference measured between points A (exudate) and C (bathing solution) is that currently measured as TRP in excised roots. In this model the longitudinal extracellular conductivity has been omitted since this is very small (Ginsburg 1972). The sum of axial (i.e. along the xylem vessels) electrical resistances (R^x) in relation to the sum of the radial (i.e. across the symplast) resistances (R^s) determines which radial root segment contributes to TRP. So, if $\sum R_i^x \ll R_i^s$ TRP will be determined by radial segments far from the exuding end. The reverse is true when $\sum R_i^x \gg R_i^s$ what is the case in excised roots according to Ginsburg.

Our results provide some boundary conditions as to the localization of the TRP measurements in *P. media* roots. Fig. 8A shows that at pO_2 between 20 and 30% roots with a diameter of around 3 mm develop an O_2 deficient core. Recently we showed that in some roots (indicated with 'type C') the xylem pumps were only partially active at 20% pO_2 and were activated at 30% pO_2. This means that in these roots thick sections of the root (>3 mm) contribute to TRP. The present results show that the xylem pumps, after waterlogging, remained inhibited

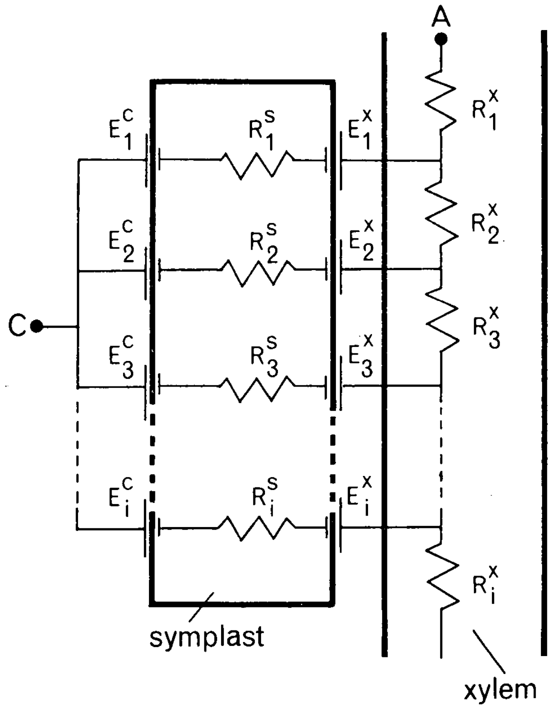

Fig. 9 Two dimensional electrical analogue of the plant root. E_i^c = the membrane potential of cortical cells of the ith length element, E_i^x = the membrane potential of xylem parenchyma cells of the ith length element, R_i^s = the radial, symplast resistance of the ith element and R_i^x = the longitudinal, xylem, resistance of the ith element. A=xylem exudate and C= bathing solution (after Ginsburg 1972).

even at 90% pO_2; this indicates that sections more than 3.7 mm thick contribute to TRP (Fig. 8B). The exact location of these xylem pumps as measured by the present method will be different for each individual root system, but generally it can be concluded that the TRP in *P. media* roots reflects the properties of the mature root segments.

On the basis of our measurements no judgement can be given about the presence of the electrogenic pumps in thin root segments. They may be operating there but may simply not be measured, since the ratio axial/radial electrical resistance is such that the TRP measurement includes only xylem pumps which are located closer to the excised surface. Direct measurement of the xylem potential in thin *Lolium* roots (Dunlop 1982) suggests that the xylem pumps are also operating in thin roots.

In studies dealing with the problem of salt resistance in plants much attention has been paid to the structure and function of xylem parenchyma cells (Kramer et al. 1977, Yeo et al. 1977, Läuchli 1976). The conclusion of these authors was that in mature root zones the xylem parenchyma cells reabsorp Na^+ from the xylem stream. This is of interest in view of the conclusion of the present work that the mature root zone of the glycophyte *P. media* contains electrogenic xylem pumps. The hypothesis that electrogenic xylem pumps provide the energy for reabsorption of Na^+ from the xylem stream in order to exclude Na^+ from the shoot of *P. media* will be the subject of further research.

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