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OXYGEN CONCENTRATION IN PRIMARY ROOTS OF BROADBEAN, LUPIN AND PEA SEEDLINGS AS MEASURED WITH A MICROELECTRODE

W. Stępniewski[1,4], *J. Zausig*[2], *G. Przywara*[1], *R. Horn*[3]

[1] Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, P.O. Box 201, 20-290 Lublin 27, Poland
[2] Department for Soil Physics, University of Bayreuth, 8580 Bayreuth, P.O. Box 101251, Germany
[3] Institute for Plant Nutrition and Soil Science, University of Kiel, 2300 Kiel, Olshausenstr. 40, Germany
[4] Technical University of Lublin, Department of Engineering and Environment Protection, Nadbystrzycka 40 20-618 Lublin, Poland

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A b s t r a c t. The paper presents method of measuring the internal oxygen concentration of intact non-lignified plant roots with an oxygen sensitive Clark - type microelectrode, also allowing the measurement of oxygen partial pressure without interference from electrolytic properties of the root tissue. The use of a motor driven micromanipulator generated continuous profiles of oxygen pressure across the roots. The method was used for measuring the radial distribution of oxygen partial pressures within primary roots of 7-12 days' old broad bean, (*Vicia faba* L. ssp. minor), pea (*Pisum sativum*), and lupin (*Lupinus angustifolius* L.) seedlings. It has been stated that anoxic conditions can exist in the meristematic root zone even under atmospheric concentration of oxygen outside the root. The axial oxygen partial pressure within the roots of the flooded seedlings decreased with the distance from the root tip. Flooding the soil reduced scattering of the results and "smoothed" the dependence of axial oxygen pressure on the distance from the root tip. It also lowered the values of axial oxygen partial pressure in the case of lupin which showed the highest axial oxygen partial pressures as compared to the other plant species studied.

K e y w o r d s: Lupinus angustifolius L, Pisum sativum, Vicia faba, oxygen in roots, oxygen microelectrode.

INTRODUCTION

Interest in the ability of plants to colonise wetland habitats or survive soil waterlogging focus research attention on the processes of root aeration by the movement of oxygen from the shoots through the root tissues. This phenomenon and its adaptive significance has been documented in numerous publications (e.g., [1,8]). However, despite this interest, few researchers have investigated the internal aeration of plants with the help of oxygen-sensitive microelectrodes.

Fiscus and Kramer [5] used a rather large electrode consisting of a 254 μm diameter platinum wire to measure the radial movement of oxygen in excised roots of corn and jackbean. Vartapetian [13] showed that oxygen does not diffuse from the atmosphere to the root through the stem in 50 day-old plants of pumpkin, a dry land species. He used a small electrode of an unspecified diameter inside and outside the roots. Bowling [3] was probably the first to use a true microelectrode with a tip diameter of 1 μm to show that an oxygen gradient exists between the epidermis and the protoxylem of excised roots of *Helianthus annuus* L., growing in an air-saturated culture solution.

Tjepkema and Yocum [11,12] and Tjepkema [10] investigated internal oxygen partial pressures in nodules of soybean and within the actinorhizal root nodules of *Myrica Gale* L. using microelectrodes with diameter of 2 μm and 40 - 60 μm. Their measurements showed that the cortex of soybean nodules has an inner layer without intercellular gas spaces that form a barrier to oxygen diffusion into the central tissue.

To study the internal root aeration of intact plants, Armstrong [1] used a cylindrical platinum electrode to measure radial oxygen losses from plant roots and Armstrong *et al.* [2] used a series of equations to estimate internal root aeration following such measurements.

The first to examine the problem of internal aeration of intact wetland plants with *in situ* measurements were Hook and McKevlin [7] who estimated oxygen partial pressure in the rhizosphere and intact roots of seedlings of loblolly pine.

In our opinion one of the major drawbacks to these measurements is the use of bare platinum electrodes linked to an external reference electrode. This arrangement requires a culture solution or cell sap to be used as an electrolytic junction. Measurements are in this case done under the assumption that a change of medium, for example from agar to cell sap, does not effect the electrode calibration. Recalibration for every new medium may be impossible, because a new calibration has to be obtained for different plant tissues. But this is only possible by *in situ* calibration in intact plant tissues, where it is difficult to ensure either the complete absence of oxygen or air-saturated conditions.

This paper presents a method for measuring internal oxygen partial pressures in intact plant tissues, where the oxygen-sensitive microelectrode as well as the reference one are situated in 1M KCl behind an oxygen-permeable membrane. Thus pO_2 can be measured in liquids and plant tissues as well as in the gas phase without interference from variation in electrolyte composition of cell sap. If such microelectrodes penetrate plant tissues with the help of a small electromotor attached to a micromanipulator, continuous radial profiles of nonlignified roots may be measured. The following describes the construction of this oxygen-sensitive microelectrode and the procedure of measurement.

The principal aim of the study was to test the hypothesis that oxygen partial pressure within the root tissue depends on the distance from the root tip as well as on the plant species and the conditions of the root growth prior to the measurements. Results of measurements in primary roots of lupin, broad bean, and pea grown under flooded and aerated conditions are presented.

MATERIALS AND METHODS

Preparation of seedlings

Seeds of broad bean, (*Vicia faba* L. ssp. minor), pea (*Pisum sativum*), and lupine (*Lupinus angustifolius* L.) were placed on paper tissue saturated with water containing a fungicide at a temperature 22 ± 2°C. The germinated seeds (with 10 - 20 mm radicles) were placed in test tubes (180 mm long and 17 mm internal diameter) filled with loosely packed (bulk density 1.2 Mg m^{-3}) soil material from Ap horizon of an Orthic Luvisol (Elizówka, near Lublin, Poland) sifted through a 5 mm sieve and placed in a growth chamber with visible light intensity 100 Wm^{-2} and 16 h day length. The day temperature was 22 ± 1°C, the night temperature was 20 ± 1°C, air humidity was 45 ± 5 % during the day and 70 ± 5 % during the night. The soil moisture tension was in the interval 10 - 50 kPa.

One day later (during emergence) the soil conditions were differentiated: half of the test tubes were flooded completely from the surface, while the second half were maintained under control conditions as during the preceding day. Thus the soil conditions will be referred to as "flooded" and "aerated". For each plant species 12 test tubes were prepared (one plant in each tube) and six of them were subjected to flooding. After 5 to 7 days from the onset of flooding the seedlings were washed out from the tubes and the soil particles were removed from the roots by gentle washing.

In that time the seedlings had main axial roots differentiated with respect to the species and the aeration conditions. The roots of the pea seedlings were up to 10 cm long and 0.6 - 2.5 mm in diameter in the flooded tubes and up to 17 cm long and 0.9 - 1.6 mm thick in the control ones. The length of the broadbean roots was up to 12 cm and 18 cm, while the thickness was 1.0 - 1.9 mm and 1.0 - 2.1 mm, for flooded and

aerated conditions, respectively. Lupin roots under flood conditions were very short and did not exceed 3 cm with the thickness 0.8 - 3.5 mm, while those under control conditions reached up to 17 cm in length and 1.5 - 4.0 mm in diameter.

Electrode construction

For the direct measurement of pO_2 in plant tissues we used an oxygen sensitive microelectrode similar to that described by Revsbech and Ward [9]. The tip diameter of our electrodes ranged from 80 to 120 μm. The platinum cathode and the reference electrode (Ag/AgCl) were placed in an aqueous electrolyte (1M KCl) in an outer glass casing. This was made from a soda-lime glass capillary (ϕ 5.0/7.0 mm) by shaping it with a pointed flame and using a microforge to form the extreme tip. The outer casing was sealed at the tip with a cold-curing silicone rubber membrane that is extremely permeable to oxygen (RTV 108, General Electric, Germany).

The cathode was made from a 5 cm long piece of platinum wire (ϕ 100 μm) etched in saturated KCN while 3V AC potential was applied. Then the Pt - wire was rinsed in concentrated HCl, water (millipore quality) and ethanol and then melted inside a thin glass capillary (Reedglas 8530 or 8533 Schott Glaswerke, Landshut, Germany). The tip of the cathode was exposed by grinding with a rotating abrasive disk (Type 462, Fa. Bachofer Laboratoriumsgeräte, Germany) and then etched in saturated KCN with 3 V AC to form a small recess. The cathode was pushed into the outer casing with a micromanipulator and fixed in the correct position with a fast-curing polyester (Pattex Stabilit express, Henkel KGaA, Dusseldorf, Germany). The electrode was filled with methanol and de-gassed in a desiccator under vacuum. The methanol was then replaced by 1M KCl. An AgCl covered silver wire was installed in the electrolyte as a reference electrode. Finally the top of the electrode was completely sealed with the polyester. Figure 1 shows the schematic drawing of the electrode.

Measuring equipment

The cathode was polarized at -700 mV with a constant voltage source. The current in the measuring circuit was determined with a nano-amperometer (N23, Fa. Knick, Berlin, Germany) and recorded with a chart recorder. The electrodes were calibrated in nitrogen-saturated water (pO_2 = 0.0 kPa) and air-saturated water (pO_2 = 20.5 kPa). Most electrodes gave signals of 2 to 7 nA in air - and 0.1 to 0.5 nA in nitrogen-saturated water. Calibration and measurements were done at room temperature (22 ± 2°C).

A small electromotor (PI Physik Instrumente, 7517 Waldbronn, Germany) attached to a micromanipulator (Fig.2) allowed the microelectrode to be driven with a constant speed of 0.1 mm min^{-1} through the plant tissue.

Measurement procedure

After washing the plants out of the soil they were then transferred to a plant holder made of 2 mm thick plexiglass with narrow channels for the roots (Fig. 2). Contact with water-saturated filter paper or careful wetting of the roots with water using a pipette, prevented the plants from wilting. After fixing the plant roots in the correct position, the plant holder was closed with a second 2 mm thick plexiglass plate. Predrilled 1 mm holes allowed the access of the electrode and radial penetration of the roots at various distances from the root tip.

A two-dimensional micromanipulator allowed the plant holder to be moved to the exact position required. A small motor drive attached to the micromanipulator of the electrode enabled the root to be penetrated at a constant rate of 0.1 mm min^{-1} to give a continuous profile of radial oxygen distribution. The root was rewetted from time to time to prevent drying.

All measurements were performed on plants grown in air. Each measurement lasted 20 to 40 min. After every measurement, the electrode signal was checked in air. Normally, the electrode response showed no drift but

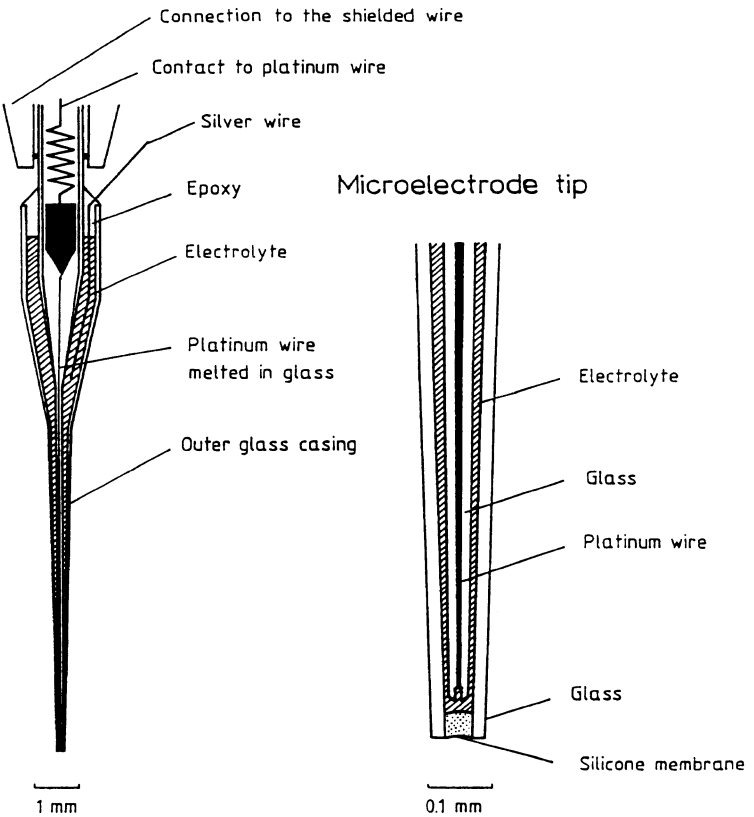

Fig. 1. Design of the oxygen-sensitive microelectrode.

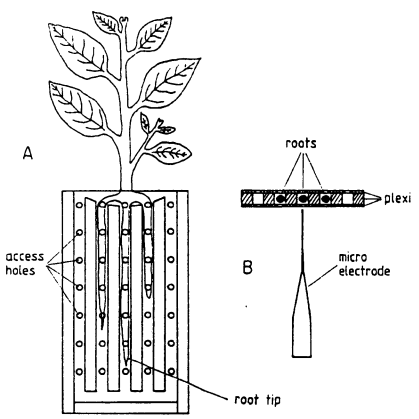

Fig. 2. Schematic drawing of the measurement of oxygen distribution within the roots: A - situation of the plant roots in the minirhizotron before the measurements; B - cross-section through the minirhizotron during the measurement of oxygen distribution.

sometimes a drift of up to 10 % occurred over several hours. Because calibration in air was done every 20 to 40 min it was possible to correct 8 measurements according to any shift in air values. If a drift of more than 10 % occurred the results were rejected.

To check if the response of the electrode was fast enough to follow the changes in oxygen partial pressure and thus to give correct readings, the motor drive was stopped in various positions and at different O_2 partial pressures. Readings stayed stable during at least 10 min after switching off the motor. Thus, oxygen consumption by the electrode itself did not induce a depletion of oxygen in the tissue and was fast enough to record internal pO_2 gradients over short distances.

Statistical procedures

Two types of statistical procedures were used. To evaluate the hypothesis concerning the existence of the dependence of the axial oxygen partial pressure on the distance of the measurement point from the root tip the corresponding correlation coefficients for linear ($y = a + bx$), multiplicative ($y = ax^b$), exponential ($y = \exp(a + bx)$), reciprocal ($1/y = a + bx$), and second degree polynomial ($y = a + bx + cx^2$) models were calculated using Statgraphics 5 programme. The best fit curve was drown in the figures in the case of the existence of a significant correlation between the two values of interest.

In order to test the hypothesis that axial oxygen partial pressure within the root depends on the plant species and the aeration conditions in the soil the analysis of variance was used and the Tukey's half intervals were calculated using the same programme.

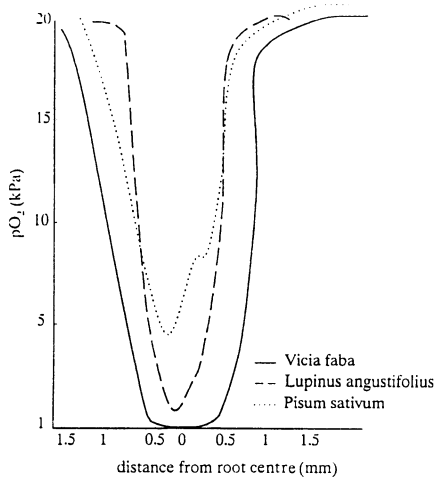

Fig. 3. Radial distribution of oxygen partial pressure in the roots of broadbean, pea, and lupin seedlings grown on wet tissue: solid line - broadbean, aerated conditions, 5 mm from the point of root/shoot divergence; dashed line - lupin seedling, flooded soil, 25 mm from the root tip; dropped line - pea, aerated soil, 78 mm from the root tip.

RESULTS AND DISCUSSION

Typical oxygen distribution curves for the roots of broadbean, pea and lupin are presented in Fig. 3. The patterns of radial distribution of oxygen within the root tissue were similar for the seedlings of all the three plant species. Oxygen partial pressure decreased from ambient 20 kPa towards the root centre. At the root axis the concentration reached a minimum value and then symmetrically increased when the electrode tip started to move towards the opposite root wall. This minimum oxygen partial pressure in the root centre will be referred to throughout the text as the axial oxygen partial pressure.

There remains the question whether our readings are genuine reflections of oxygen partial pressure in plant tissues or if they are artificially low because the cell damage by the electrode increases respiration sufficiently to deplete oxygen. We cannot be sure on this point but our step readings when the electrode was stopped for 10 min without affecting the reading confirmed that the oxygen distribution curves obtained by us are genuine.

Axial oxygen partial pressures versus the distance from the root tip for the three plant species are presented in Figs 4 -6. In all three cases the results obtained for the flooded roots are characterised by a smaller scatter as compared with the aerated ones and a decrease of axial oxygen pressure with the distance from the root cap (and thus with ageing of the tissue) can be observed.

For broadbean (Fig. 4.) the axial pressures of oxygen under flood conditions decreased curvlinearly from 12 - 15 kPa at a distance 0.5 - 6 cm to about 2 - 8 kPa at a distance 9 - 11 cm from the root tip. The best fit was provided by a second degree polynomial model and the correlation coefficient was 0.98. In the aerated treatment the results were very scattered covering a range from 0 to 16 kPa but no significant correlation with the distance was found. It is interesting that zero concentrations of oxygen in the root centre were observed for the measurements taken 3 to 11 cm from the tip. It should be noted that upper values of the axial oxygen concentration can be, in some cases,

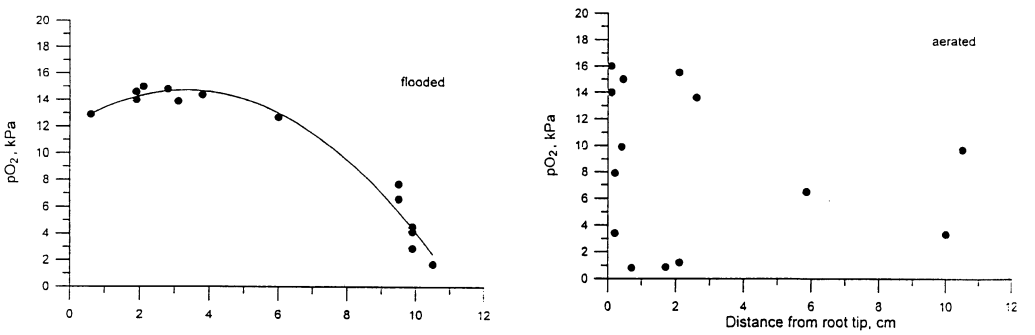

Fig. 4. Axial oxygen partial pressure in the roots of broad bean seedlings as a function of the distance from the root tip for flood and aerated conditions. For flood conditions $y = 12.06 + 1.60x - 0.24x^2$, $r = 0.98^{***}$, for aerated conditions - lack of significant correlation.

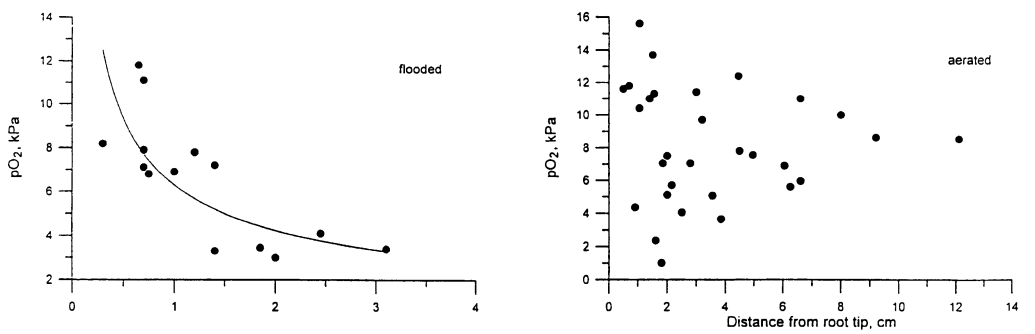

Fig. 5. Axial oxygen partial pressure in the roots of lupin seedlings as a function of the distance from the root tip for flood ($1/y = 0.072 + 0.087x$, $r = 0.79^{**}$), and for aerated conditions (lack of significant correlation).

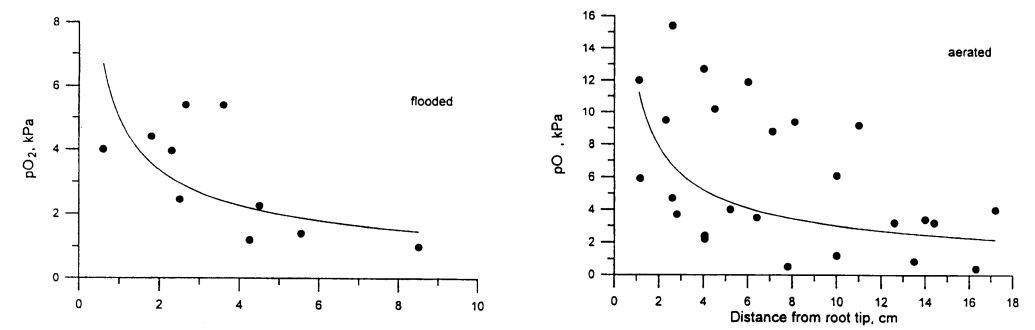

Fig. 6. Axial oxygen partial pressure in the roots of pea seedlings as a function of the distance from the root tip for flood ($1/y = 0.0473 + 0.11x$, $r = 0.84^{**}$) and aerated conditions ($y = 11.91x^{-0,598}$, $r = 0.47^{*}$).

uncertain as the position of the electrode tip with respect to the root axis was controlled only visually. Contrary to this the zero pressures are an unambiguous indication that anoxic conditions do exist in the meristematic root zone even at atmospheric oxygen concentration outside the root.

In lupin (Fig. 5.) a significant curvilinear decrease of axial oxygen partial pressure from more than 12 kPa to about 3 kPa was observed with the distance from the root tip (and thus with the root ageing). The correlation coefficient was 0.79 for the best fit reciprocal model. In the aerated treatment the values were very scattered from 1 to 16 kPa in the youngest parts of the roots (0.5 - 2 cm from the tip) and then they tended to approach 6 - 10 kPa. No significant correlation of the axial oxygen partial pressure with the position of the measurement point with respect to the root tip was found. It should be emphasised that in no case a zero oxygen pressure was observed both in flooded and in aerated roots.

In the pea roots significant negative correlations of the axial oxygen partial pressures with the distance from the root tip were observed both for flood as well as for aerated conditions, although in the latter case the correlation was weaker. For flooded roots the axial oxygen partial pressure decrease from 3 - 5 kPa in the youngest root part (0.6 - 3.5 cm from the tip) to about 1 - 2 kPa for the older section (4 - 9 cm). It should be emphasised that in the flooded pea roots the axial oxygen pressures were the lowest from all the treatments under consideration. In the aerated pea roots the values decreased with the root ageing from 4 - 16 kPa for the youngest section (1 - 3 cm) to 0.5 - 3 kPa for the older section (11 - 18 cm). Also a decrease of the scatter of the data occurred with increasing distance from the root tip.

The effect of the distance from the root tip, which can be interpreted in terms of the physiological age of the root tissue, is for all the three plants similar in that the concentration decreases with the distance. This tendency was distinct for all the three plant species studied under flooded conditions, while under aerated treatment - it was observed only for pea roots.

The effect of flooding and of the plant species was tested statistically by analysis of variance using Statgraphics 5 and then the Tukey 95 % confidence halfintervals were calculated. The results of the statistical analysis are presented in Table 1. As it can be seen flooding caused a decrease of axial oxygen partial pressure only in the case of pea roots (from about 6 kPa to almost half of this). The decrease of the average concentration of oxygen due to flooding can be connected with either (or with the interaction) of the following causes: increase of the root diameter, increase of the

Table 1. The effect of flooding on the root diameters (mm) and axial oxygen partial pressure within the roots of particular plant species

Plant species	Soil moisture tension (kPa)	Root diameters (mm)	Oxygen partial pressure (kPa)	
			average for treatments	average for plants
Broad bean	0	1.42a	9.99a	6.12a
	15	1.48a	8.41a	
Lupin	0	1.92a	6.57a	6.67b
	15	2.04a	8.13a	
Pea	0	1.24a	3.15a	5.29c
	15	1.55a	5.93b	

Mean values in the first two columns for the same species and the general means in the last column for particular species followed by the same letter are not significantly different at P = 0.05 by analysis of variance.

radial root permeability to oxygen, and an increase of oxygen demand of the root tissue following a period of anoxia. The latter is the "respiration rebound" observed under such conditions [6]. The root diameters did not differ significantly for aerated and flooded treatments, as is presented in Table 1. Thus the factor of increased root diameter is eliminated. The phenomenon of increased respiration rate after a flooding period seems to be the beyond any doubt. The permeability of roots for gas diffusion was not studied so it is difficult to draw any conclusions on that.

The maximum values of the axial oxygen partial pressures were the smallest for pea roots under flood conditions, where they did not exceed 6 kPa while for broadbean and for lupin they were in the range 12 - 15 kPa.

The influence of flooding the roots caused the relationship of the axial oxygen partial pressure on the distance from the root tip to be more distinct and reduced scattering of the results. In the aerated roots the scatter of the results was from zero or almost zero to about 15 kPa so that the effect of the distance from the root tip is visible only for pea roots.

The effect of the plant species is presented in Table 1. It can be seen that the highest average value of the axial oxygen partial pressure was observed for lupine (6.67 kPa) where it differed significantly from the values for broadbean (6.12) and pea roots (5.29 kPa). It should be noted that lupine is the most flood sensitive of the plant species under consideration [6] and broadbean the most flood resistant. This was also confirmed by our observations in this experiment, where the lupin seedlings suffered apparently most under flooded conditions and their root growth rate was the smallest.

CONCLUSIONS

1. It has been shown that application of an oxygen sensitive Clark - type microelectrode and the use of a motor-driven micromanipulator allows for the continuous measurement of oxygen partial pressures across the nonlignified roots of plant seedlings.

2. It was stated that anoxic conditions can exist in the meristematic root zone even under atmospheric concentration of oxygen outside the root.

3. The axial oxygen partial pressure within the roots of the preflooded seedlings decreases with the distance from the root tip. This relationship was most distinct for broad bean.

4. Flooding the soil reduced scatter of the results and caused "smoothing" of the decreasing relationship of axial oxygen concentration versus the distance from the root tip as well as a decrease of axial oxygen partial pressure in the case of lupin.

5. The highest axial oxygen partial pressures were observed in the roots of lupin which differed significantly from those of the two other plant species.

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