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# Influence of oxygen deficiency on growth and function of plant roots

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## Introduction

An increasing amount of the fruit-vegetables tomato, cucumber and paprika and a number of ornamental flowers grown in glasshouses are cultivated on artificial substrates such as rockwool and peat. Water and nutrients are supplied continuously by dripping a nutrient solution at the base of each plant. To prevent water shortage and accumulation of salts in the rooting medium the supply of nutrient solution is as an average twice the daily water uptake. Because of the variation in plant size and rate of water supply this ratio is often exceeded. Under these wet conditions diffusion of oxygen to the root system is assumed to be the limiting factor for root respiration and hence for growth and development of plants. Different from field conditions where rain showers can periodically cause oxygen deficiency, plants grown on artificial substrates are permanently supplied with suboptimal amounts of oxygen.

There are, however, great differences in sensitivity between plant species to oxygen deficiency in the root medium (Yu *et al.*, 1969). Oxygen transport from shoot to root was substantiated by the observation of large gas-filled spaces, aerenchyma, in shoots and roots of wetland plants (Armstrong, 1979). In non-wetland plants aerenchyma is formed in adventitious roots growing under anaerobic conditions, by degeneration of cortical cells (Trought and Drew, 1980). The tolerance of plants to oxygen deficiency is improved by mineral nutrition, especially nitrate (Guyot and Prioul, 1985). Trought and Drew (1981), explain this as an alleviation of the negative effect of oxygen deficiency on the mineral nutrition of the plant. Another explanation is that nitrate serves as an alternative electron acceptor to free oxygen in roots (Garcia-Novo and Crawford, 1973), thus enabling root respiration and root functions to continue. In line with this conception nitrate reductase activity is found to be enhanced in roots under anaerobic conditions (Lambers *et al.*, 1978). The aim of the experiments was to determine the change in root activity in relation to a reduction of root respiration of three species, maize, cucumber and tomato which are known to be differently sensitive to oxygen stress and to reveal the possible cause of these differences.

## Material and methods

### *Measuring system*

The measuring system for root growth and root activity is derived from a system described before (Veen, 1977). Only one plant is grown on the system with the roots situated in the root vessel, a transparent Perspex box measuring 20 × 15 × 15 cm. The stem base is fixed water tight with a closed-cell rubber stopper into an opening in the top of the root vessel (Fig. 1). With a small rotary pump the nutrient solution is pumped at a speed of $1\,l\,min^{-1}$ through the measuring chamber in which electrodes can be mounted. The measuring chamber is situated at the highest level in the system and can be moved up and down to keep the pressure inside close to atmospheric pressure which is necessary for a good functioning of the reference electrode. After passing the measuring chamber the nutrient solution flows back to the root vessel. Because the root vessel and the measuring chamber are closed, the volume of the nutrient solution in the system is displayed in the aeration vessel (A)

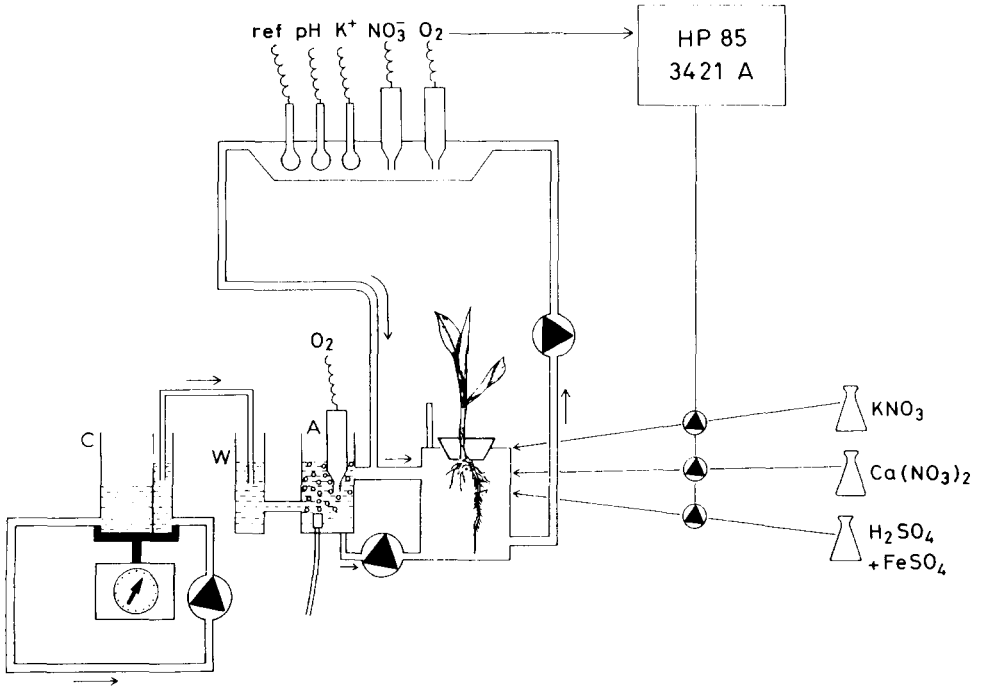



*Fig. 1.* Measuring system for simultaneous measurements of uptake of water, oxygen, potassium and nitrate, and the production of bicarbonate by a plant root system. For further details see text.

and vessel W, which have open connections with the main circulation stream.

Next to the system a perspex box (C) measuring 10 × 8 × 14 cm is placed on a digital balance. The box is separated into two unequal compartments by a wall with an opening 1 cm below the top. A small persistaltic pump circulates a nutrient solution without $NO_3^-$ and $K^+$ from the left to the right compartment, resulting in a constant level in the right compartment. With a syphon this constant level is transmitted to vessel W, so that in the measuring system the total volume of the solution is kept constant. The transpiration of the plant can be determined continuously by weighing the solution lost from box C. The nitrate concentration is measured using an Orion liquid ion-exchange membrane electrode 92-07, potassium using a Philips glass electrode G15-K and pH using an Electrofact 7G112 glass electrode. The reference is an Electrofact gel-filled electrode, type SR20. After amplification the electrode signals are permanently presented to a HP85 computer with a 3421A data acquisition/control unit. When the $K^+$ and $NO_3^-$ concentrations measured in the system are below a present level, known amounts of $KNO_3$ and $Ca(NO_3)_2$ are titrated to compensate for the uptake by the plant. Because the nitrate uptake usually exceeds the potassium uptake the potassium uptake is compensated with $KNO_3$ and the $NO_3^-$ uptake with an additional amount of $Ca(NO_3)_2$.

Because nitrate is the only nitrogen source, the pH usually raises due to $OH^-$ or $HCO_3^-$ production of the root system. This bicarbonate production is titrated with 0.25 mol sulfuric acid to achieve a constant pH of 4.5. At this pH all $HCO_3^-$ produced is liberated as $CO_2$. The $O_2$ electrodes are Electrofact Clark-type electrodes type Z152. One is positioned in the circulating nutrient solution, the second in the aeration vessel. Root respiration is measured and regulated by pumping a known amount of oxygen-rich nutrient solution from the aeration vessel to the root vessel using a CFF dosing pump type E 603. At the same time a same amount of the circulated nutrient solution flows back passively to the aeration vessel, keeping the water level constant. From the difference in oxygen concentration and the flow rate the net amount of oxygen pumped into the circulated system can be calculated. In a steady state situation the oxygen concentration in the root vessel is constant and this net amount of oxygen equals the amount taken up by the plant roots. In case of a non-stable oxygen

concentration root respiration is corrected for the change in concentration. Because only a limited amount of aerated nutrient solution is pumped into the root vessel the oxygen concentration in the circulated nutrient solution is always lower than in the aeration vessel.

The amount of oxygen supplied to the roots can be manipulated by changing the ratio of nitrogen and oxygen gas used for aeration. Nitrogen and oxygen was obtained from cylinders with compressed gas and mixed in the correct ratio using two adjustable mass flow controllers (Precision Flow Devices type 112). The reduction in root respiration however depends not only on the reduction in oxygen content of the gas mixture but also on the root respiration under optimal conditions, which is a function of the root size. In practice it is impossible to reduce the root respiration to a preset value. Therefore the reduction of root respiration and the effects on root activity of a number of comparable plants are averaged.

Every 15 minutes a series of 19 relevant data about the state of the system and the amounts titrated is registered on the HP85 tape cartridge. It is possible to register 15 successive days on one tape. The cumulative uptake of $NO_3^-$, $K^+$, $H^+$ and water were first plotted versus time and from periods of constant activity the uptake rates were calculated using regression analysis. In order to demonstrate the influence of oxygen stress on root activity independent of the root size, this influence is expressed as a percentage of the root activity under optimal conditions. Except for water uptake no significant differences in root activity have been found between the day and night period. Therefore the root activities under oxygen stress are calculated as the percentage of the activity averaged over the control period of about 24 h. Only the influence of oxygen stress on water uptake is calculated as the percentage of the water uptake of the corresponding period during the control day.

Root growth was measured as increase in root volume using a method described before (Veen, 1977).

Nitrate reductase activity (NRA) was measured using the intact tissue assay of Jones and Sheard (1977). Samples of 2-g fresh-root tissue were taken in duplicate from root systems of stressed and control plants and vacuum infiltrated under $N_2$ in 25 ml of a medium containing 50 m*M* $KNO_3$, 1% n-propanol and 100 m*M* phosphate buffer at 27°C. Nitrite released into the medium after 30 and 60 minutes was determined with sulfanilamide and 0.02% N-1-naphtylethylene diamine. Optical density was measured at 540 nm.

*Growing conditions*

Maize (*Zea mays*, var. LC11), tomato (*Lycopersicum esculentum* L. var. Counter) and cucumber (*Cucumis sativus* L. var. Farbio) seeds were sown in moist sand in a climate room at 20°C, 80% relative humidity and a daylength of 16 hours. The light intensity was about 50 $\mu$E m$^{-2}$ sec$^{-1}$. After 7 days a number of uniform plants of each species were selected and grown on half strength Hoagland nutrient solution containing (m*M*): $KNO_3$, 2.5; $Ca(NO_3)_2$, 2.5; $KH_2PO_4$, 0.5; $MgSO_4$, 1.0 and trace element (mg l$^{-1}$) B, 0.1; Mn, 0.1; Zn, 0.01; Cu, 0.004; Fe, 7.5; Mo 0.01. Light intensity was about 150 $\mu$E m$^{-2}$ sec$^{-1}$ with a daylength of 16 h. The temperature was 22°C and the relative humidity 70%. After 3 to 4 weeks a plant was fixed in the measuring system and grown at the same environmental conditions. After an adaption period of about 3 days the respiration rate and other root activities were measured under optimal supply of oxygen and nutrients for one day. The day after this control day the respiration rate was reduced by choosing an appropriate ratio of $N_2$ and $O_2$ gas that was obtained from gas cylinders, and the effect on root growth, water and ion uptake and bicarbonate production was measured.

## Results

To compare the three plant species the root activities at optimal conditions on basis of unit root weight or unit leaf area are given in Table 1. Maize showed a relatively high root respiration rate, the potassium uptake per unit root weight of cucumber was significantly lower than that of tomato and maize. There was no significant difference in nitrate uptake between the three species, but the bicarbonate production of cucumber was significantly lower than that of tomato and maize. Tomato had the highest water uptake rate per unit leaf area and maize, being a $C_4$ plant, the lowest. The effect of a

*Table 1.* Oxygen uptake ($O_2$), potassium uptake ($K^+$), nitrate uptake ($NO_3^-$), bicarbonate production (Z) and water uptake rate ($H_2O$) of three plant species under climate room conditions at optimal oxygen supply. Values followed by the same letter are not significantly different ($P > 0.05$)

| Species | $O_2$ $mg\,g^{-1}\,h^{-1}$ | $K^+$ $\mu mol\,g^{-1}\,h^{-1}$ | $NO_3^-$ $\mu mol\,g^{-1}\,h^{-1}$ | Z $\mu mol\,g^{-1}\,h^{-1}$ | $H_2O$ $mg\,cm^{-2}\,h^{-1}$ |
|---|---|---|---|---|---|
| Cucumber | 0.13[a] | 2.3[c] | 5.3[e] | 1.5[f] | 8.0[h] |
| Tomato | 0.15[ab] | 3.8[d] | 7.1[e] | 2.8[g] | 10.9[i] |
| Maize | 0.20[b] | 3.9[d] | 6.8[e] | 2.9[g] | 6.2[h] |

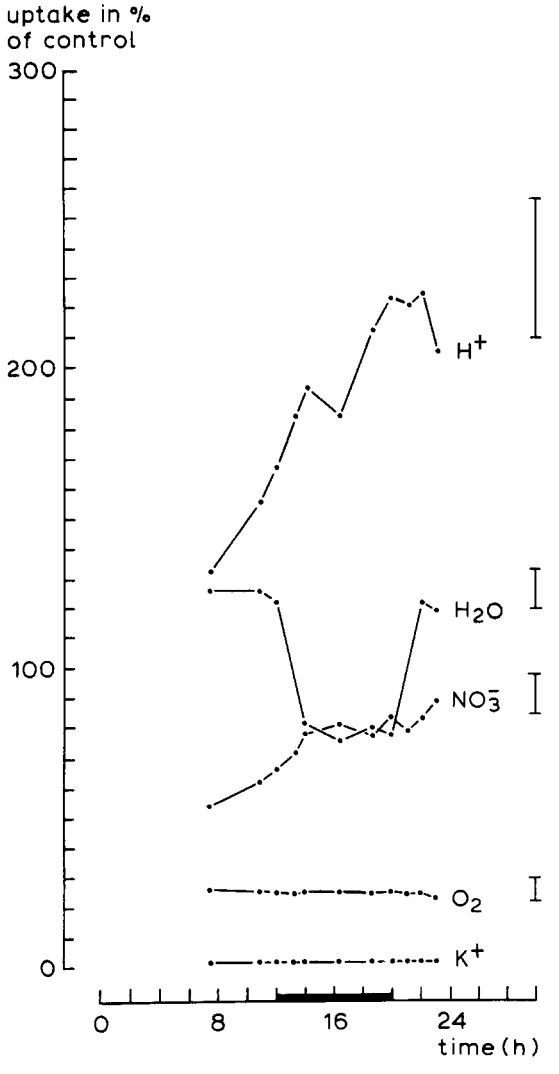



*Fig. 2.* Time course of bicarbonate production ($H^+$), uptake of water ($H_2O$), nitrate ($NO_3^-$), potassium ($K^+$) and oxygen ($O_2$) of a maize root system after reduction of oxygen supply. *Vertical bars* indicate standard deviation, the horizontal bar the dark period.

reduction in root respiration to 20% of optimal on a number of root activities of maize was measured (Fig. 2). $K^+$ uptake stopped immediately after reduction of oxygen supply, $K^+$ concentration in het root environment even raised due to leakage of $K^+$ out of the roots. Nitrate uptake was reduced to about 40% of the rate at optimal oxygen supply, but increased in time to about 90% of the level at optimal oxygen supply. The reduction of water uptake about 16 h after onset of oxygen stress occurred during the dark period. There was a 20% reduction of water uptake in the dark whereas there was no influence of oxygen stress in the light.

The most striking effect of reduction of oxygen supply to the roots was the increase in alkalinization of the nutrient solution by increased $HCO_3^-$ production of the roots. This is even more striking for cucumber (Fig. 3) where the rate of alkalinization of the nutrient solution after 24 h of oxygen stress was about 4 × the control rate. A reduction in oxygen supply to 20% of the maximal uptake rate induced an immediate reduction of the nitrate uptake to 40% of the control rate, but during the experimental period the nitrate uptake increased and after 24 h the uptake rate had recovered to 80%. Potassium uptake completely stopped at low-oxygen conditions and as with maize, $K^+$ concentration in the root medium increased due to leakage from the root system. Reduction of the oxygen supply to 20% of the maximal respiration rate caused death of the root tips of tomato, reduction of the water uptake rate and hence wilting of the plant. To study adaption of tomato plants to oxygen stress, oxygen supply was reduced to 40% of the maximal respiration rate. In spite of this lower oxygen stress, nitrate uptake was relatively more reduced. The recovery after 24 h treatment was only 50% of the control value (Fig. 4). Also water uptake showed a decrease towards the end of the stress period, and although the rate of bicar-

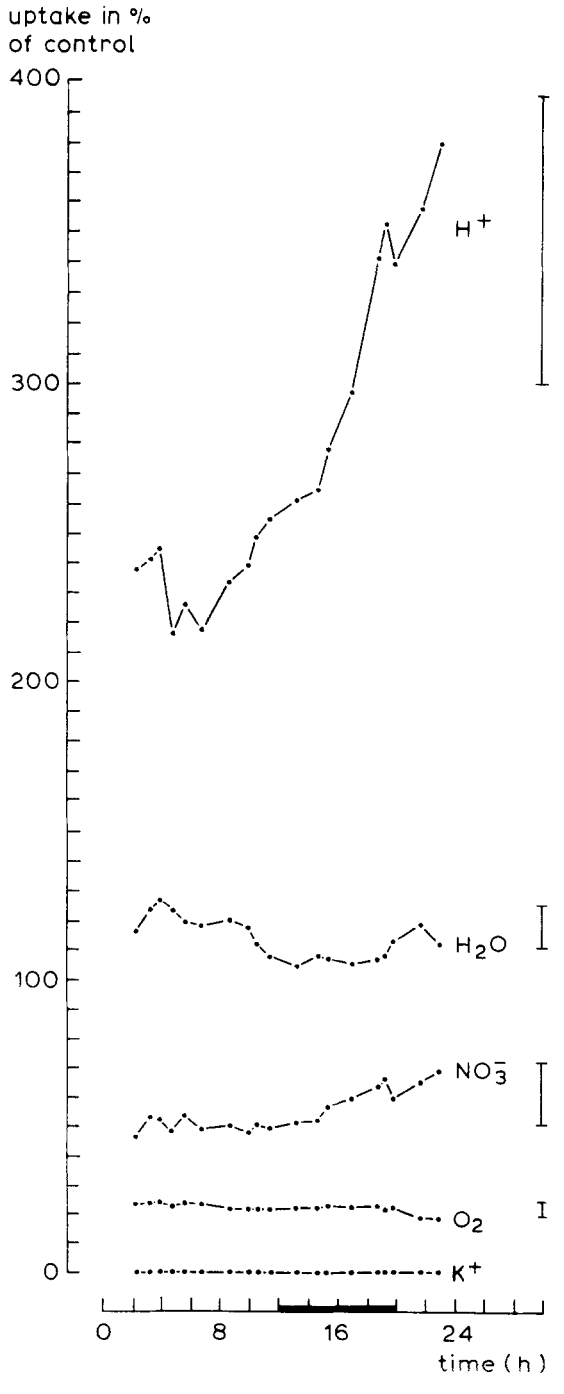



*Fig. 3.* Time course of bicarbonate production ($H^+$), uptake of water ($H_2O$), nitrate ($NO_3^-$), potassium ($K^+$) and oxygen ($O_2$) of a cucumber root system after reduction of oxygen supply. *Vertical bars* indicate standard deviation, the *horizontal bar* the dark period.

bonate production increased, this increase was clearly less than for maize and cucumber. As for cucumber and maize potassium uptake stopped immediately after reduction of oxygen supply and $K^+$ leaked into the root medium.

Bicarbonate production of the root system is supposed to be a reflection of the nitrate reduction rate of the root system. Therefore, nitrate reductase activity (NRA) of roots of the three species under control conditions and after 24 h of oxygen stress was measured (Table 2).

Cucumber had a significantly lower NRA than tomato and maize. The relative increase in NRA due to oxygen stress was also highest in cucumber, but in absolute sense the NRA in tomato roots increased more. The NRA in maize roots showed no significant increase at oxygen stress and the activity was between that of cucumber and tomato.

Figure 5 shows the effect of a reduced respiration rate on nitrate uptake a few hours after start of the

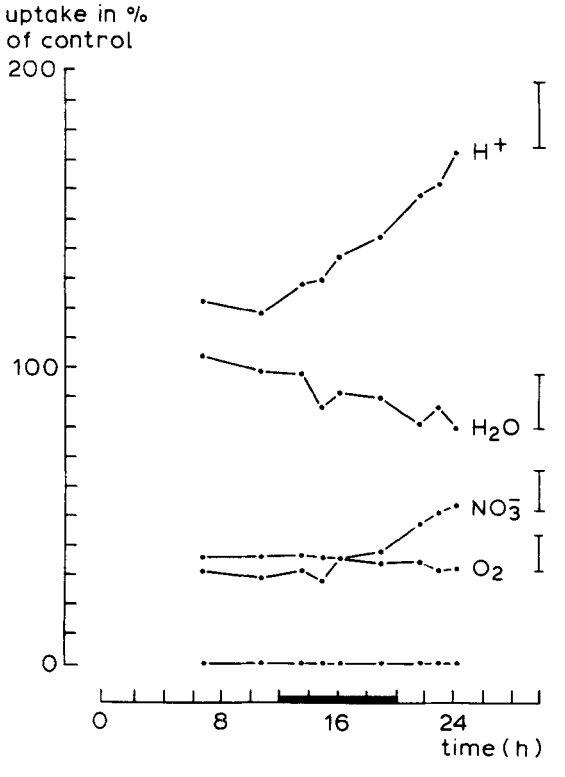



*Fig. 4.* Time course of bicarbonate production ($H^+$), uptake of water ($H_2O$), nitrate ($NO_3^-$), potassium ($K^+$) and oxygen ($O_2$) of a tomato root system after reduction of oxygen supply. *Vertical bars* indicate standard deviation, the *horizontal bar* the dark period.

*Table 2. In vivo* nitrate reductase activity ($\mu$mol.h$^{-1}$ on a fresh weight basis) in the roots of three plant species, grown at optimal oxygen supply and after 24 h of oxygen stress. Values followed by the same letter are not significantly different ($P > 0.05$)

| Species | Treatment | |
|---|---|---|
| | Control | $O_2$-stress |
| Cucumber | $0.64^a \pm 0.16$ | $1.22^b \pm 0.29$ |
| Tomato | $2.50^b \pm 0.73$ | $3.86^c \pm 0.36$ |
| Maize | $1.74^b \pm 0.37$ | $1.88^b \pm 0.53$ |

treatment. The oxygen uptake of maize roots could be reduced to 50% of the control rate, without influencing nitrate uptake. Below that point nitrate uptake decreased nearly linearly with oxygen uptake. In cucumber roots a reduction of oxygen uptake to 80% of the control rate had no influence on nitrate uptake; below that point nitrate uptake decreased linearly with oxygen consumption. Tomato was the most susceptible to oxygen stress and although measurements in the range between 70 and 100% oxygen uptake are missing, the data are situated around the 45° line, suggesting an equivalent reduction of respiration and nitrate uptake.

## Discussion

Root respiration is measured as the rate of oxygen uptake by roots from a circulating nutrient solution. It is assumed that the root medium is the only oxygen source for root respiration and that no oxygen is supplied to the roots by internal gas transport. A functional shoot to root internal gas transport was demonstrated in anaerobically grown pea roots (Armstrong, 1979). Several plant species, when grown under anaerobic condition, develop aerenchyma in the root cortex (Trought and Drew, 1980; Yu *et al.*, 1969), large gaseous spaces which provide low resistance channels for gas diffusion. Roots grown under aerated conditions contain also intercellular gas spaces. Considerable differences in root porosity exist between species which can be expressed as differences in specific weight of their roots. In our case the specific weights of the roots of maize, tomato and cucumber were 0.87, 0.96 and 0.97 g cm$^{-3}$ respectively. The roots of our plants are grown under aerobic conditions and only one day of oxygen stress is a too short period to induce aerenchyma. Not only the percentage porosity but also the continuity of the pores is an important factor determining the influence of gas filled pores on longitudinal oxygen transport. The functional importance of root porosity on oxygen transport from shoot to root under our experimental conditions was examined by cutting the shoot at the end of the experimental period and sealing the cut stem surface with a 1:4 mixture of bees wax and vaseline. No influence on the rate of oxygen uptake by the root system for more than one hour after this interference could be observed. So under our experimental conditions no oxygen transport from shoot to root occurred and the rate of oxygen uptake from the nutrient solution can be considered as equal to the root respiration rate. This conclusion is in accordance with results of Prioul and Guyot (1985) who found no influence of the oxygen concentration in the shoot environment on the rate of oxygen uptake from a nutrient solution of aerobic wheat roots. Wheat roots, grown for one week under anaerobic conditions, however, showed considerable internal oxygen transport, depending on the oxygen diffusion gradient. With all our plants the respiration could be reduced to a certain extent without influence on water uptake. Even reduction of the root respiration to 20% of the control rate had no influence on water uptake of cucumber and maize. Also Trought and Drew (1980) found no negative influence of anoxia on water uptake of young

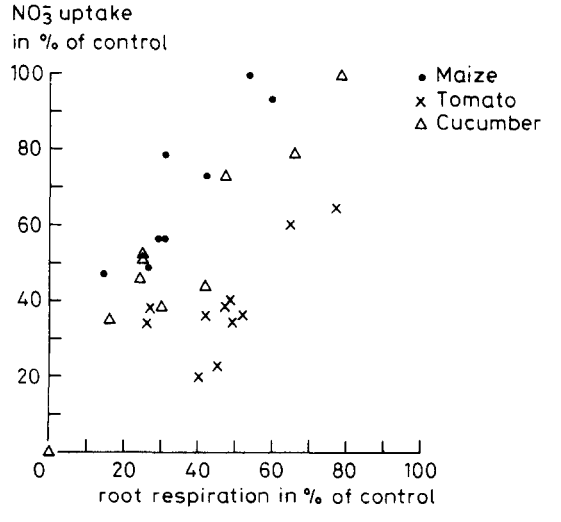



*Fig. 5.* Influence of root respiration on nitrate uptake of maize (●), tomato (×) and cucumber (Δ), about two hours after induction of oxygen stress.

wheat plants. Although only a limited number of data is available the results suggest that for maize the water uptake in the dark period is relatively reduced in case of oxygen stress (Fig. 2). This was not observed for cucumber and tomato. Tomato was much more sensitive to oxygen stress and even reduction of respiration to 40% caused a decrease in water uptake at the end of the experimental period.

Also Brouwer (1964) reduced respiration of bean roots with KCN and found no influence of the cyanide concentration on water uptake up to $10^{-5}$ *M*, at higher concentrations the water uptake rate decreased. Brouwer (1964) also reports the ion uptake to be more sensitive to KCN. In our experiments the nitrate and potassium uptake are influenced by oxygen stress although not to the same extent. Nitrate uptake 2 h after start of the treatment was for maize not influenced by reduction of the respiration to 50% of maximal (Fig. 5). At further decrease of respiratory activity also nitrate uptake rate decreased. For cucumber the nitrate uptake decreased when the respiration rate was reduced more than 20%, whereas for tomato there was no reduction of respiration without immediate effect on nitrate uptake.

In the control period the uptake of nitrate and potassium and the efflux of bicarbonate of maize and tomato was in electrical equilibrium: nitrate uptake nearly equalled the sum of potassium uptake and bicarbonate efflux (Table 1). In the stress period, however, nitrate uptake decreased and the bicarbonate efflux increased (Figs. 2, 3 and 4). When bicarbonate efflux exceeded nitrate uptake, potassium leaked out of the roots, giving the impression of a passive movement in a direction that leads to maintenance of electrical neutrality. This is in agreement with data of Jackson *et al.* (1976) who consider the nitrate uptake as the driving force for net potassium uptake. The rate of potassium efflux could not be determined because the relative change in concentration was too small in a volume of 5 l nutrient solution.

The nitrate uptake a few hours after decrease of the respiration rate for cucumber and maize decreased less than the respiration rate, but tomato in general showed a stronger decrease in nitrate uptake than in respiration rate (Fig. 5). The recovery of nitrate uptake during the 24 h stress period was also least for tomato. This recovery of nitrate uptake can be explained by the increase in nitrate reduction measured as $HCO_3^-$ efflux which reduces the internal nitrate level and stimulates the uptake mechanism (Deane-Drummond and Glass, 1983). This stimulation of NRA is least in tomato with consequently a minor stimulation of $NO_3^-$ uptake.

The nitrate reductase activity in the root system was determined in two different ways: as bicarbonate production of the root system and by an *in vivo* measurement using a representative sample of the root system and measuring the $NO_2^-$ efflux in the dark under anaerobic conditions. Although both methods indicate that the NRA is increased after a period of oxygen stress, the extent of the increment depends on the method used. The second method is more a reflection of the potential NRA, because the procedure includes an optimalization of the conditions. Therefore the bicarbonate output is used as a measurement of the NRA under our experimental conditions. Immediately after reduction of root respiration the bicarbonate efflux increased during the 24 h experimental period (Figs. 2, 3 and 4) and also nitrate reductase activity increased (Table 2). This increase in NRA is a common feature of plants under oxygen stress (Garcia-Novo and Crawford, 1973, Lambers *et al.*, 1978) but the extent of this increase differs between the species. The increase in bicarbonate production of oxygen-stressed cucumber plants, up to 4 times the control rate, is regarded as a mechanism of plant roots to eliminate excess NADH by insufficient oxygen availability and to overcome a period of oxygen stress. Maize, however, with only a limited increase in $HCO_3^-$ production was even more insensitive to oxygen stress than cucumber (Fig. 5). Its root porosity was, however, much greater than cucumber and tomato which for maize is regarded as the main mechanism to overcome a period of oxygen stress. In my opinion there is no internal transport of oxygen from shoot to root, but the porosity of the root system prevents radial oxygen gradients in the root by facilitating diffusive oxygen transport in the gas phase. In more compact roots of cucumber and tomato supply of oxygen to the central core and apical parts of the roots is more difficult, leading to a greater sensitivity at low oxygen conditions.

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