Gas-filled root porosity in response to temporary low oxygen supply in different growth stages

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Abstract

The development of gas-filled root porosity in response to temporary low oxygen supply was tested for a range of edible and ornamental crops: rice, maize, wheat, sugar beet, tomato, cucumber, sweet pepper, carnation, gerbera and rose. In a first experiment, the roots of tomato, maize and gerbera had a higher gas-filled root porosity, E_p (% v/v), when grown permanently in a non-aerated instead of aerated solution. The E_p of roots increased during two weeks when half the root system of a young plant was transferred to a non-aerated solution; in older plants this response was not seen. Carnation had a negligible gas-filled porosity in all treatments. In a second experiment, a comparison was made between high (20 kPa) and low (about 2 kPa) O_2 partial pressure in a recirculating nutrient solution. Half of the root system was transferred to low O_2 at various growth stages. In most species older plants did not increase E_p on exposure to low O_2. For tomato, sweet pepper and rose, E_p was normally in the range 3-8% (v/v). Young plants of cucumber, wheat and sugar beet also had an E_p in that range, but in older plants values ranged from 1 to 3%. Transverse root sections examined by light microscopy showed, on average, 60% more intercellular spaces in the root cortex than the measurements of gas-filled porosity, probably because some gaps and spaces in the cortex were not gas-filled. This effect was most pronounced in tomato. A negative pressure in the cortex may be needed for gaps to be gas-filled. An exodermis may increase the effectiveness of gas spaces in the cortex by closing the gas channels and, by offering some resistance to water uptake, allowing a negative pressure head in the cortex which keeps gaps gas-filled. A redox dye method was developed to study the length of root which is effectively supplied with oxygen, as a function of E_p. Results indicated that for every percent E_p the root can remain aerated over at least 1 cm in a non-aerated medium under the conditions of the test.

Introduction

Complete aeration of a root system in soil is only secured if the distance between any root cell and the nearest part of that root in contact with air-filled macropores or cracks is bridged by internal (longitudinal and radial) oxygen transport. Complete root-soil contact facilitates uptake of water and nutrients (Veen et al., 1993), but aeration of the root will be difficult if such complete contact exists along the total length of a root. Root sections with incomplete root-soil contact and thus in (partial) contact with macropores can be regarded as 'breathing sections' (Van Noordwijk et al., 1993). Cellular structure outside the cortex (epidermis and exodermis) may influence entry of oxygen into such a breathing section, but also influences loss of

oxygen from the root tissue in the remaining part of the root. Longitudinal transport of oxygen inside the root depends on the rate of oxygen consumption by root and rhizosphere, on the gas-filled porosity of the root, on root diameter and on the resistance for gas exchange between root and rhizosphere (Armstrong, 1972; De Willigen and Van Noordwijk, 1989; Luxmoore et al., 1970). Both the gas-filled porosity and the resistance for gas exchange differ widely among plant species.

In rice and other wetland plants, O_2 is transported, in gas-filled channels, from aerial tissues to the roots (Crawford, 1982; Van Raalte, 1944). The concentration gradient which drives the diffusion process is created by oxygen consumption in the root tissue, and outside the root if the outer cell wall is permeable and permits leakage. An anaerobic rhizosphere acts as an approximate zero-sink for oxygen. The *schizogenic* aerenchyma of wetland plants extends almost to the root tip, in contrast with the *lysigenic* type which develops secondarily in differentiated cortical tissue (Konings, 1983). Van Raalte (1944) and Armstrong (1971) measured O_2 leakage from rice roots with a platinum electrode. The root tip released most O_2. A few cm from the tip O_2 leakage decreased, but it increased again where branch roots emerged. In experiments with an 'artificial root' Armstrong (1972) investigated the relationship between gas-filled porosity of the root and the possible depth of penetration in an anaerobic medium. Mathematical models of longitudinal O_2 transport have normally assumed a constant resistance along the length of a root for gas exchange between aerenchyma and root environment.

Many non-wetland plants have a low gas-filled porosity when growing under adequate external aeration, but can make roots with more porosity when aeration is insufficient (Drew, 1990). The increased porosity is normally based on a lysis of cortical cells in young roots, at some distance from the root tip. Small changes in gas-filled root porosity in the range 0-10% (v/v) have a major effect on the distance a root can penetrate into a poorly aerated medium (De Willigen and Van Noordwijk, 1989).

Quantitative information on aerenchyma formation is scarce, even for the major crops (Drew, 1984; Justin and Armstrong, 1987). Most of the available data was obtained from young plants; little is known on the possible decrease with age in the ability of roots to adapt to poor aeration; sensitivity to waterlogging may depend on the stage of crop growth at which the stress occurs (Cannell and Belford, 1980). Practical interest in internal aeration has increased in horticulture by the introduction of soilless culture techniques, which normally have a wet root environment, with more or less stagnant nutrient solution (Jackson et al., 1984; Nieuwenhuizen, 1984). Compared to soil conditions, however, this root environment is relatively constant with time, so roots have the possibility to adapt. Transport distances between well and poorly aerated parts of the root medium are so short that a small amount of gas-filled pores can be sufficient for adequate aeration. For field grown crops, poor aeration is a risk which may occur during a few wet episodes in a growing season or not at all if rainfall is well distributed (Boone and Veen, 1993). Episodes of poor aeration may occur at any stage in the growing season. Little is known about the effect of early aeration stress on the ability of a crop to survive later episodes of poor aeration (Cannell and Belford, 1980).

The experiments reported here provide quantitative information on the ability of a range of agricultural and horticultural crops to increase their gas-filled porosity in root tissue when exposed to poor external oxygen supply in various stages of their life cycle. Experiments were performed in a split-root system, where aeration was reduced for half the root system at a specified time, to test the ability of the roots to increase gas-filled porosity without too drastic effects on the shoot.

Not all gaps and intercellular space in the cortex are necessarily filled with gas. Van Noordwijk and Brouwer (1988) showed that, for maize, the gas-filled porosity measured by pycnometry was in agreement with measurements of intercellular spaces on microscopic sections, provided that all root diameter classes were equally represented in both samples. A comparison between microscopic observations and measurements of E_p for other species will be reported here.

Cortical gas spaces are normally continuous

within a single root axis, but barriers may exist between main axes and lateral roots. Anatomical evidence (Danilova et al., 1992) suggests that a lateral root, originating from the stele of a main root, normally has a fully developed epidermis inside the cortex of the main root and may even be covered by cell walls of collapsed cortical cells. Semi-quantitative evidence on the effects of such a barrier on oxygen transport can be obtained by using redox dyes (Coutts and Phillipson quoted by Crawford, 1989; Laan et al., 1989). Accordingly, a redox dye method was developed for visualizing oxygen leakage from roots, to study the effective transport distance for O_2, and to study possible barriers on the transition between main and lateral roots.

Materials and methods

Experiment 1

Plants were grown on solution culture with their root system split over two 8-L pots. The solution was either permanently aerated on both sides (+ control), permanently non-aerated (− control) or aeration was stopped in half of the root system at a certain growth stage, about two weeks before a measurement of gas-filled root porosity. Measurements of duplicate plants were made on three dates for tomato, maize, carnation and gerbera.

A nutrient solution of pH 5.8 was used containing 10.2 mM NO$_3^-$, 1.24 mM H$_2$PO$_4^-$, 2.90 mM SO$_4^{2-}$, 5.52 mM K$^+$, 3.48 mM Ca^{2+}, 2.89 mM Mg^{2+} and micronutrients. Nutrient solution in the pots was renewed once every two weeks for the first four weeks and once a week thereafter. Daily water use was replaced by demineralized water, half- or full-strength nutrient solution, depending on the electrical conductivity (EC) of the remaining solution in the pot.

Tomato (*Lycopersicon esculentum* (L.) Karsten cv. Moneymaker) was measured at first flowering, 22 DAP (Days After Planting), when the first truss ripened (62 DAP) and after 8 weeks of fruit production (118 DAP). Maize (*Zea mays* L. cv. Brutus) was measured when vegetative (30 DAP), at flowering (66 DAP) and

after fruits of the first truss were ripe (113 DAP). Carnation (*Dianthus caryophyllus* L. cv. Silvery Pink) was measured at 50, 114 and 163 DAP. Gerbera (*Gerbera jamesonii* Bolus cv. Fleur) was obtained from tissue culture (very small) and measured at vegetative (108 DAP), flowering (174 DAP) and an older growth stage (209 DAP).

The experiment started in spring in a cold greenhouse (average temperature 18 °C; minimum night temperature 12 °C). Tomato and maize grew very well. The temperature was rather cool for carnation early on in the experiment. The Gerbera experiment was performed in a heated greenhouse with an average day temperature of about 22 °C and a minimum night temperature of 12 °C.

Experiment 2

As a comparison of aerated and non-aerated solution involves a complex of factors, including the degree of stirring of the solution, in a second experiment in a recirculation system aerated nutrient solution was compared with nutrient solution with low O_2 pressure, obtained by bubbling with N$_2$ gas. The actual O_2 partial pressure varied between < 1 and 4 kPa during a day/night cycle. The oxygen concentration in the aerated system stayed close to 20 kPa. The response to a change from high to low O_2 supply of half of the root system was measured, without 'controls' where both half-root systems were grown at continuously high or low O_2 supply. Four recirculation systems were made, two for high and two for low O_2 solution, leading to two independent replicates. In total 48 plants, i.e. 96 split-root pots, were grown simultaneously. The nutrient solution had the same composition as in experiment 1.

A sequence of crops was grown on this system: cucumber (*Cucumis sativus* L. cv. Corona; measured at 30, 43, 57, 71, 99 and 126 DAP), sweet pepper (*Capsicum annuum* L.; measured at 26, 41, 53, 68, 82 and 110 DAP), tomato (*Lycopersicon esculentum* (L.) Karsten cv. Moneymaker; measured at 21, 35, 49, 65, 77 and 91 DAP), rose (*Rosa hybrida* L. cv. Sonia; measured at 42, 58, 71, 85, 114 and 143 DAP), carnation (*Dianthus caryophyllus* L. cv. Silvery Pink, measured

at 48, 62, 76, 91, 119 and 143 DAP), wheat (*Triticum aestivum* L. cv. Minaret; measured at 50, 66, 80, 102, 121 and 141 DAP), sugar beet (*Beta vulgaris* L. cv. Regina; measured in one experiment at 27, 32, 39, 46, 60 and 74 DAP; because of problems with tap root development, a second experiment, with a modified system for securing the plants, was measured at 70 and 84 DAP) and rice (*Oryza sativa* L. cv. Limas (an 'upland rice') and cv. Citandui (a 'lowland rice'); measured at 29, 38, 50 DAP).

Measurement of gas-filled porosity

Measurement of gas-filled root porosity was based on the specific volume (volume per unit weight) of roots before and after removing gas from the roots by evacuation; the method was modified from Jensen et al. (1969) and described by Van Noordwijk and Brouwer (1988). The gas-filled root volume was expressed as percentage of total root volume.

Division in three root classes

Root samples were subdivided into three classes for measurement. In the first experiment the root classes were A: root tips, B: thick roots, C: branch roots and older branched roots; if insufficient root material was available no subdivision was made. In experiment 2 root classes consisted of the first 1/5 (A; mainly root tips), the central 3/5 (B; mixed root age) and the basal 1/5 (C; mainly root bases) of the length of the root system as assessed, when spread out in water.

Comparison of E_p with total porosity on microscopic sections

For a range of crops root samples were collected and microscopic sections were observed. The diameter of the root and the cortex was measured in each section together with the percentage of cortical area which consisted of gaps or gas spaces. An average value, weighted for root diameter frequencies, of each sample was compared with E_p data of a duplicate sample. In some sections the diameter of the largest en-

scribed circle in individual gas spaces was also measured.

Redox dye system

A redox dye was used to visualize oxygen transport in roots, grown in a nutrient solution (Van Noordwijk et al., 1993). A plant was fixed to a plate and the root system spread out on a pin board under water; oxygen was removed from the root surface by applying nitrogen gas. A water vessel (aquarium) was prepared with a redox dye, cristal-violet $C_{25}H_{30}ClN_3$ (10 mg L^{-1}), applied from a concentrated stock solution prepared in ethanol. The dye will change from colourless to violet at a redox potential of about -0.5 mV, which is lower than the transition point for Indigo carmine (-0.15 mV) (Crawford, 1989) or methylene blue ($+0.02$ mV) (Trolldenier, 1988). The water was de-oxygenated and the dye brought into the colourless, reduced stage by adding sodiumdithionite ($Na_2S_2O_4$) and mixing the solution with an aquarium pump, without disturbing the surface. The plate with the root system was then placed into the solution; the amount of water in the vessel could be chosen such that either the shoot base was just under the water level ('high tide', oxygen in the roots has to come from the shoot) or that the basal part of the roots was exposed to air ('low tide'). The water surface was coated with floating plastic grains, to reduce oxygen enrichment of the water. The method was tested on rice, maize, tomato and pea grown in an aerated and a non-aerated nutrient solution. A blue colour developed on the root surface where oxygen was leaking out of the epidermis. The pattern of blue colouring was recorded on photographs and drawings. Maximum colour intensity was reached in about 2 h, but the extent of the blue zone was not time-dependent.

Results

Experiment 1

Gas-filled root porosity E_p for the continuously aerated (+), continuously non-aerated (−) and split-root treatments in experiment 1 are pre-

sented in Figure 1. In tomato, maize and gerbera the roots were able to increase E_p when growing continuously in non-aerated solution. The values for E_p were clearly different between the crops (N.B.: different scales are used on the Y-axis). In carnation, E_p was not significantly different from 0 and no response to the non-aerated treatment was found.

Tomato plants of about 20 and 50 days old were able to increase E_p in non-aerated roots to the level of continuously non-aerated roots in about two weeks (S−). The half of the root system where aeration (S+) was retained had an E_p above that of the aerated control (+), but below that of the non-aerated half. In the graph it is assumed that before the split-root treatment started E_p was the same as that for the aerated control roots (C+). When the split-root treat-

ment was repeated with plants of 118 days, after 8 weeks of fruit production, no response of E_p was found.

In maize, both sides of the split-root treatment increased E_p to values close to that of the permanently non-aerated whole root system. The E_p value for the permanently aerated control increased with time, possibly indicating that aeration in the pot which was full of roots was no longer sufficient. Gerbera was able to increase E_p in the split-root treatment only slightly in the two weeks after aeration to half of the roots was stopped.

Figure 2 shows data on shoot and root dry weight for the C+ and C− control plants in experiment 1. For tomato and maize the above-ground dry matter production (Fig. 2A) of continuously non-aerated plants was about the
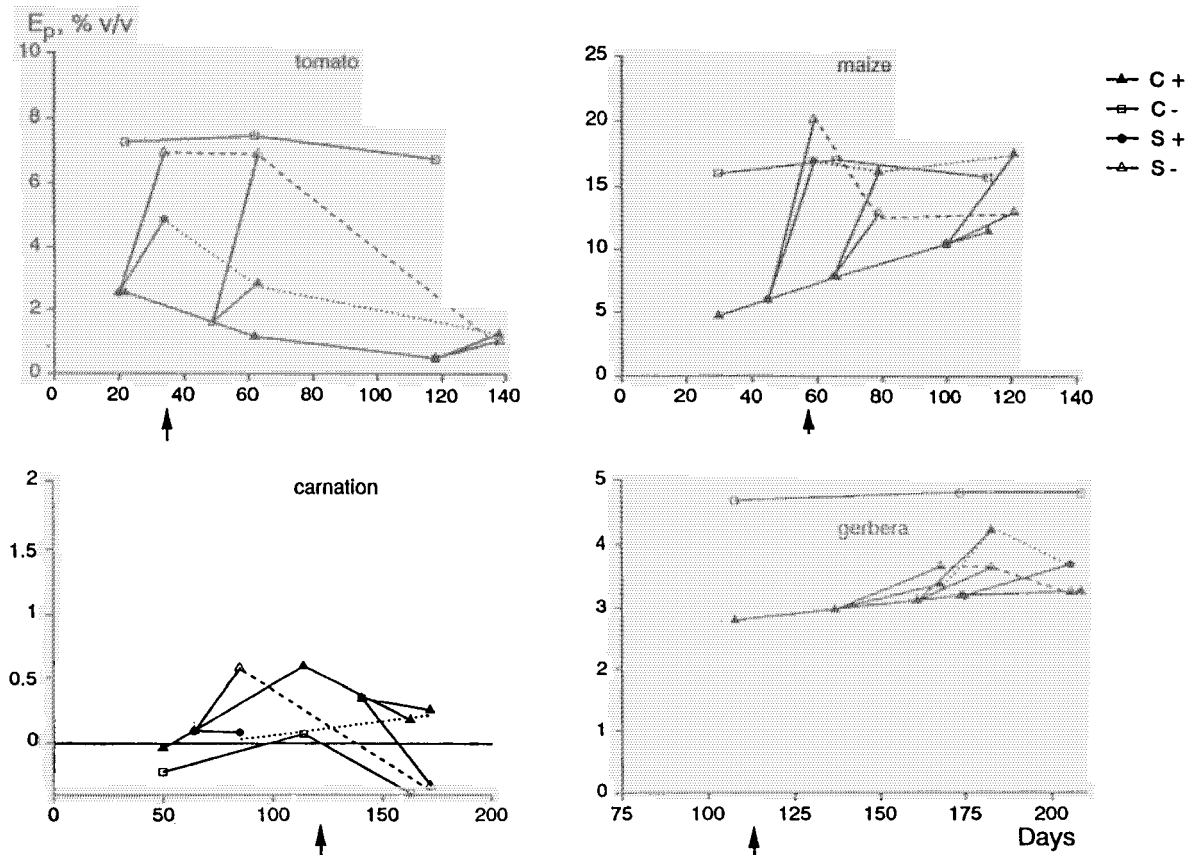

Fig. 1. Gas-filled root porosity in experiment 1 for three growth stages of tomato, maize, carnation and gerbera. Treatments were permanently aerated (C+), permanently non-aerated (C−) and split-root treatments where aeration was stopped in half the root system (S−) and continued in the other (S+), two weeks prior to measurement. Arrows indicate the date of flowering. The dashed line connects the S− points, the dotted line the S+ points.
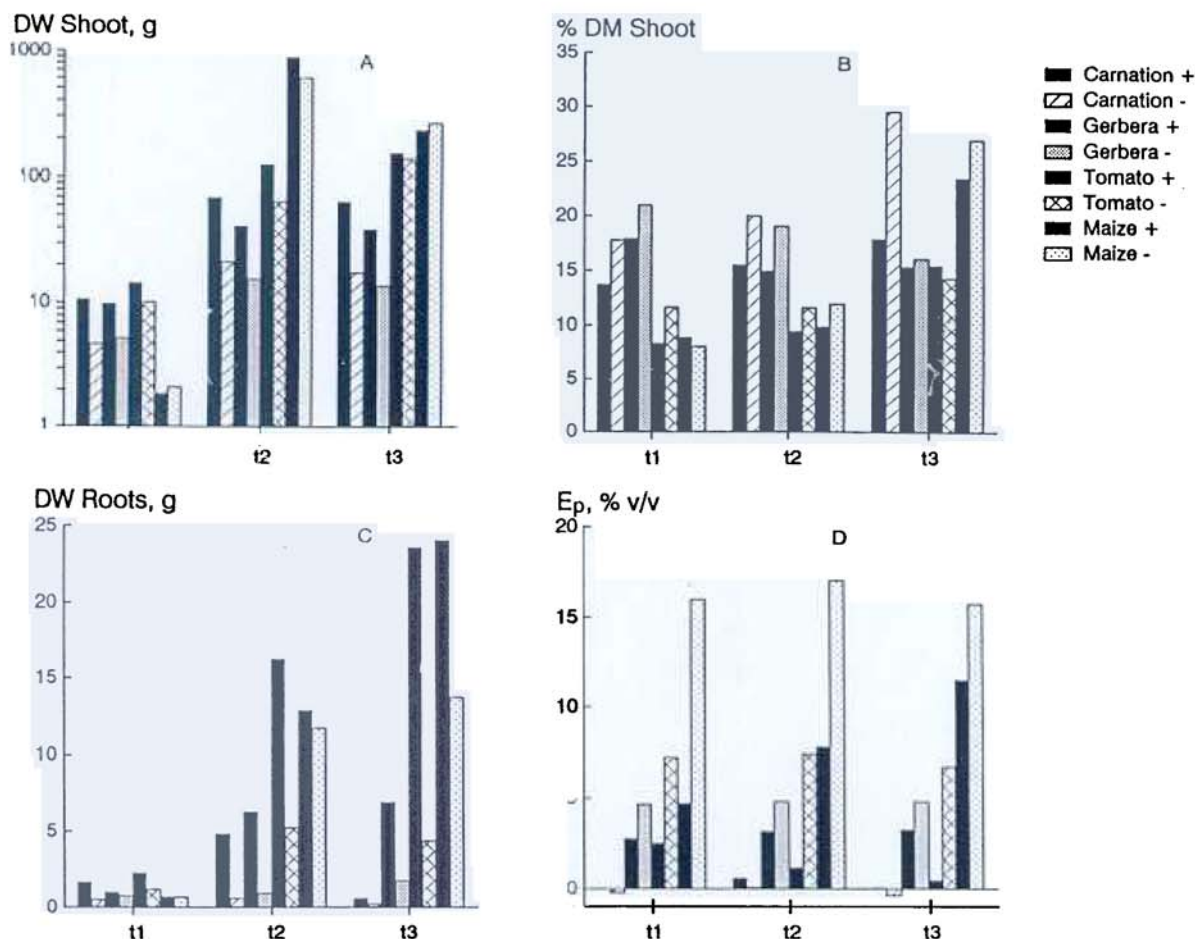
Fig. 2. Shoot and root dry weight (DW), dry matter percentage of the shoot and gas-filled root porosity of the root system for carnation, gerbera, tomato and maize, with (+) and without (−) aeration in experiment 1. The sampling times t1, t2 and t3 differed between crops (compare Fig. 1).

same as that for aerated controls gerbera and especially carnation were clearly affected aboveground. The percentage dry matter in the shoot (Fig. 2B) was dramatically increased in non-aerated carnation at the last sampling, but tended to increase also in other crops and at other sampling times. The dry weight of roots (Fig. 2C) was strongly reduced by poor aeration, even in maize and tomato where shoot dry weight was hardly affected. Figure 2D gives the gas-filled porosity for the four species on the same scale. Maize with the aboveground production suffering least from poor aeration, clearly had the highest E_p values, followed by tomato (small response aboveground) and gerbera (strong response). Split-root plants, where aera-

tion was stopped to half of the roots at a late growth stage, showed visible water stress. Some of the maize plants were close to collapse.

Experiment 2

In experiment 2 data were collected only from the two sides of split-root plants, one subjected to high and one to a low O_2 partial pressure (1-4 kPa). Two crops were grown in both systems. Carnation again developed almost no gas-filled root porosity. For tomato, the response to low oxygen pressure was less pronounced than that to lack of aeration (Fig. 1A).

Table 1 shows the results of an analysis of variance of experiment 2. For all crops, except

Table 1. Statistical significance of main effects (T = Time, F = Fraction, L = Low O_2) and their interactions in an analysis of variance of gas-filled root porosity E_p in experiment 2; the root system of rice was not fractionated. NS = no significant differences, *,** and *** = significant effect with $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

Treatment	Carnation	Cucumber	Tomato	Wheat	Sugar beet	Sweet pepper	Rose	Rice
T*L	**	NS	*	***	NS	NS	***	NS
T*F	NS	*	NS	***	NS	NS	***	–
L*F	NS	NS	NS	NS	NS	NS	NS	–
T*L*F	NS	NS	NS	NS	NS	NS	NS	

sweet pepper, the gas-filled root porosity changed significantly with time. Treatment of the roots for two weeks with a low O_2 concentration had a significant effect on gas-filled root porosity in tomato, wheat, rose and rice, but not in the other crops. Significant interactions of this treatment with time were found in carnation, tomato, wheat and rose. Division of the root system into three fractions (root tips, central and basal region) led to significantly different gas-filled root porosities in carnation, cucumber, tomato and rose; a significant interaction between this fractionation and time was found for cucumber, wheat and rose. No significant interactions were found between the low O_2 treatment and the fractionation of the root system. Second-order interactions were not significant.

As no significant interactions were found between the low O_2 treatment and the fractionation of the root system, Figure 3 presents treatment effects over time averaged over the three fractions for all crops. The data in Figure 3 are comparable with the interrupted and dotted line in Figure 1. In Table 2 the effect of fractionation is shown, averaged over low O_2 treatment and time.

Cucumber (Fig. 3B) had a higher E_p than tomato at early growth stages, but lower values later in their growth cycle. Sweet pepper (Fig. 3C) had a clear difference between the high and low O_2 sides of the root system early on and maintained E_p values of about 4% for low-O_2-treated roots. Rose (Fig. 3D) clearly responded to the treatment in a young stage, but at the time of the two last measurements its E_p value remained at or above 4%. For wheat (Fig. 3E) a

clear response in young plants was found, but a decline after flowering (Fig. 3E). Sugar beet showed no consistent difference between the two sides of the root system; average E_p declined from about 4 to 2% during the experiment. For rice (Fig. 3H) an increase in E_p with time was found, but the experiment was stopped before heading. No difference between the upland variety Limas and the lowland variety Cintandui was found, and only a small, but statistically significant, difference between the high and low O_2 sides of the root system.

Microscope sections

Cortical channels observed in root sections under a light microscope are usually termed 'aerenchyma' or 'air channels', although microscopic observations cannot assess whether they are gas-filled and whether the composition of this gas is similar to air. Here we indicate them with a neutral, morphological description 'intercellular channels'.

In rice, intercellular channels extend across almost the full radius of the cortex, from exodermis to endodermis. In maize cortical cells collapse to form intercellular channels, bordered by cell remains. Several cortical cell layers remain intact between epidermis and the intercellular channels. In rose, the shape of intercellular channels was similar to that in maize. In tomato, secondary gas channels formed by the collapse of cortical cells, but a schizogenous type of aerenchyma was also present. This led to convoluted, spiral gas channels in the mature cortex.
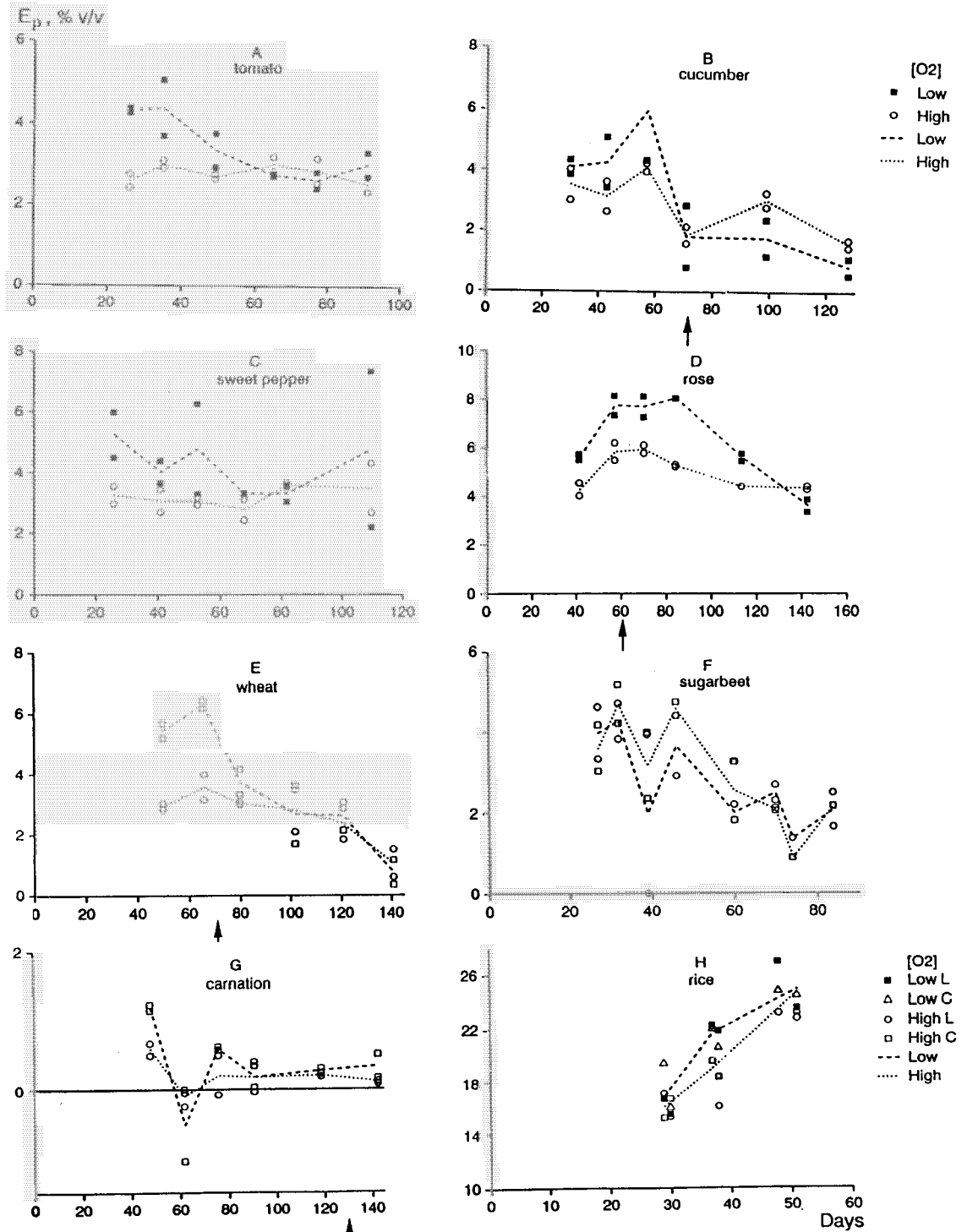
Fig. 3. Gas-filled root porosity E_p of roots (averaged over three fractions) with a low (dashed line) or high (dotted line) oxygen concentration in the nutrient solution, ten days prior to measurement (compare dotted lines in Fig. 1). The dashed and dotted line can be compared with those in Figure 1. Two varieties of rice were used (C = Citandui, L = Limas). N.B.: different scales are used for the Y axis; data are presented for duplicate plants, the line connects treatment averages.

Table 2. Gas-filled porosity of three root fractions, averaged over time and treatment. Differences between values followed by the same letter (in a single row) are not statistically significant

	Fraction		
	Central	Base	Average
Carnation			
Cucumber			
Tomato			
Wheat			
Sugar beet			
Sweet pepper			
Rose			

In Figure 4, gas-filled porosity (% v/v) is compared with the intercellular space (% a/a) in transverse sections of duplicate samples. Microscopical analysis showed, on average, 60% more intercellular spaces in the root cortex than the measurements of gas-filled porosity. Thus not all gaps and spaces in the cortex are gas-filled. This effect was most pronounced in tomato.

Distance of O_2 transport

Redox dye staining showed that, in most roots, a certain length of the root (epidermis) below the water surface was blue-stained. Rice formed the main exception, since the largest part of the root remained white, but the zone close to the root
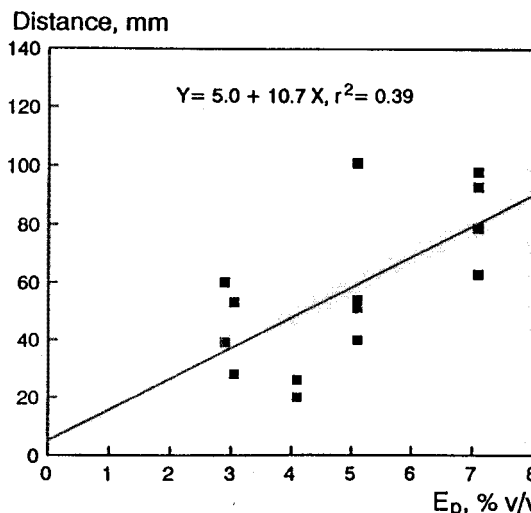

Fig. 5. Maximum distance from the water surface where the root epidermis was blue-stained related to gas-filled root porosity of duplicate samples. Data based on pea, tomato and maize.

tip was blue and root tips of emerging lateral roots were also blue.

Figure 5 shows the length of root which had a blue-stained epidermis as a function of E_p; rice roots were excluded from this graph. The regression line had a non-significant intercept and a significant ($p < 0.05$) slope. Results indicated that for every percent E_p the root can be aerated for about 1.1 cm while in a non-aerated medium.
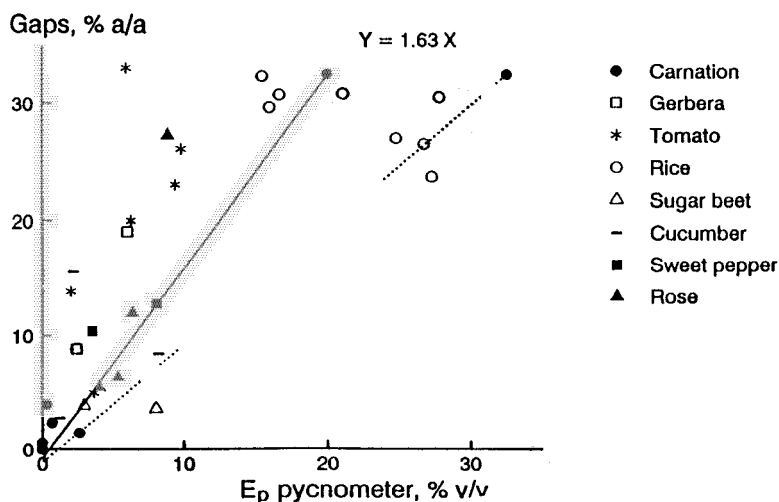

Fig. 4. Relation between gas-filled porosity E_p and the area of intercellular spaces and gas observed on microscopic sections (weighted average for diameter classes) on a duplicate sample. The dotted line indicates a 1:1 relationship, the solid line gives the average trend.

Branch roots close to the stem base also developed a blue surface, indicating no absolute barrier in the transfer of O_2 from a main axis to a branch root, although some evidence was obtained for an additional resistance Van Noordwijk et al., 1993).

Discussion

Aerenchyma formation

A continuously non-aerated control was included in experiment 1. In both tomato and maize, E_p of the root compartment which continued to be aerated was higher after two weeks than that in plants where the total root system was continuously aerated. The response in the non-aerated side was stronger than that in the aerated side, but apparently interactions at a whole plant level exist. This observation casts doubt on the normal interpretation of gas channel formation as a local process, based on perception of poor aeration in a root tip, hormonal (ethylene) signal transmission to and response by cortical cells (Drew et al., 1979). The data presented do not indicate whether increased porosity was due to newly formed roots or to changes in existing roots. The relatively small differences between root fractions are not conclusive in this respect, as no separation between young and old roots could be made. The majority of roots measured originated and grew before the change in aeration, especially on the low-O_2 treatment, as generally a shift of root growth to the aerated side was noticed (as described by Brouwer, 1981).

In experiment 2 no continuous aerated control was used. The results may therefore underestimate the ability of roots to form aerenchyma in response to poor O_2 supply, as this response would go unnoticed if the aerated side responds as well. Ethylene production from the precursor ACC requires oxygen and ACC can be transported rapidly inside the plant. Further experiments which include a permanently aerated control are needed and measurements of ethylene evolution on the aerated side of a split-root plane. The general decrease in response of E_p to poor aeration after flowering could be based on either an age effect in the root, as little new root growth occurs, or on changes in the hormonal balance in the plant.

Are all intercellular channels gas-filled?

Van Noordwijk and Brouwer (1988) showed, for maize, that gas-filled porosity measured by pycnometry agreed with measurements of intercellular spaces on microscopic sections, provided that all root diameter classes were equally represented in both samples. However, the present data indicate that a considerable fraction of intercellular spaces may not be gas-filled in other species, and that the term 'air channels' would not be correct. Whether intercellular spaces in the root are gas-filled or not depends on the size of individual spaces, on the (negative) pressure head and on surface characteristics. If virtually all resistance to water uptake is located in the endodermis, as is often assumed, gaps in roots growing in a nutrient solution would not be gas-filled, whatever their size, unless their lining is (strongly) water repellent. At least part of the resistance to water uptake must be located in an exodermis, external to the cortex for cortical gaps to be gas-filled. An interesting dilemma for the plant is that xylem vessels, at a low pressure, must be water-filled to function adequately as water conduits, while cortical spaces outside the main resistance for water uptake should be gas-filled. This is only possible if the cortical channels have a (considerably) larger diameter than xylem vessels, or have different wall properties. In younger xylem vessels, narrow inter-vessel pits may reduce the chance of embolism. In older vessels degradation of the pit membranes leads to embolism at less negative pressures than in younger vessels (Sperry et al., 1991).

Assuming a contact angle of $0°$ between water and the wall of a gas channel (so disregarding water repellent surface characteristics), the pressure P (in MPa) needed to displace a gas-water interface from a circular pore of diameter D (μm) can be calculated (Sperry and Tyree, 1990) as:

$$P = 4\frac{T}{D} \qquad (1)$$

where T is the surface tension of water (0.072 N m^{-1} at $20°C$).

For a pore of 2.8 μm diameter a pressure of 0.1 MPa is needed to make it gas-filled, for a pore diameter of 29 μm 0.01 MPa is needed. Measurements of the diameter of individual gaps showed most to be in the range 50-100 μm in rose roots and approximately 55 μm in relatively thick rice roots. In thin rice roots (minimum root diameter only 0.1 mm) gas channels, filling almost the whole cortex, were only about 20 μm in diameter. For many plants, 0.2 mm is a typical diameter for the finest roots; if half of the radius is occupied by cortical tissue, the maximum diameter of intercellular channels is about 50 μm. In thin roots, cortical spaces can only function as gas channels if an exodermis creates at least some resistance to water uptake, thus creating the suction required to fill intercellular spaces with gas. In tomato part of the aerenchyma appeared to be schizogenic; convolute strands of cortical cells remained, with plate-like gaps between them. In fine roots the distance between these plates may be insufficient to keep the gaps gas-filled. An interesting consequence of this interpretation is that day/night cycles in pressure head in the plant may induce changes in the degree to which cortical spaces are gas-filled. This hypothesis can be tested in further experiments. Measurements of the degree of water repellency of the linings of intercellular channels is needed to apply equation (1).

Differences between crops

The crops can be classified according to the E_p which developed under low oxygen supply (Table 3). From the horticultural crops tested, carnation was the only one without any ability to increase gas-filled porosity. The majority of carnation roots were very thin (diameter about

Table 3. Classification of crops by gas-filled porosity of the roots

Very high (>15% v/v)	Rice
High (8-15)	Maize
Intermediate (3-8)	Tomato, sweet pepper, rose, gerbera, cucumber[a], wheat[a], sugar beet[a]
Low (1-3)	Cucumber (old), wheat (old), sugar beet (old)
None (0-1)	Carnation

0.1 mm) and the cortex consists of only a few cortical cell layers. Thus, it appears that almost no space is available for intercellular pores of sufficient diameter. Practical experience in soilless culture showed that carnation is a difficult crop on wet soilless media. In the other crops a difference was found in the rate at which the crops could adapt to a poorly aerated medium. For gerbera, the response was slow, implying that the crop will probably be sensitive to relatively fast changes in the water content of the root environment in soilless culture. In a comparison of two gerbera cultivars (unpublished results) a difference in E_p was found, indicating that screening for higher E_p may help to select gerbera cultivars more suited for soilless culture. The redox dye test may be used in developing a relatively quick screening method. For most of the other crops a reasonable ability to increase E_p was found in young plants, but no ability to respond in older plants. This suggests that the sensitivity to anaerobiosis can be decreased if plants experience relatively poor aeration in a young growth stage. The difference between the two rice cultivars was less than expected; Zhang et al. (1990) found lowland rice varieties to have a thicker cortex and more aerenchyma than an upland variety tested.

Relevance under field conditions

On the basis of the current results we may expect that a root which can form an E_p of about 5% can be sufficiently aerated over a length of 5 cm in an anaerobic soil aggregate. Model calculations by De Willigen and Van Noordwijk (1989) predicted 4-7 cm penetration into anaerobic soil for 10% gas-filled root porosity. The difference between this model and the present results is that in the model the increased oxygen demand for a growing root tip was taken into account, and the experiment was based on existing roots. If the soil is not anaerobic, but has a limited O_2 supply the tip can extend further. While a root tip is penetrating into an aggregate, aeration is problematic because cortical gas channels do not extend to the tip where metabolic activity is highest and because oxygen has to diffuse across almost the complete diameter of an aggregate (Van Noordwijk et al., 1993). After the root has

entered a new macropore only the radius of the aggregate has to be bridged, from two sides. Additionally, oxygen demand per cm of root length for maintenance and uptake will be less than that required for root growth (Lambers, 1987). Once formed, a root may thus survive a certain reduction in oxygen supply when the soil becomes wetter. When the soil becomes water-logged, however, the oxygen stored in gas-filled pores may be depleted within a day (Boone and Veen, 1993). Physiological and biochemical mechanisms to survive temporary aeration stress may be essential (Drew, 1990; Wiedenroth, 1993), unless internal oxygen transport form aboveground tissues is possible. Even a low oxygen supply to the root tip (external or internal) may be important for survival of a root tip and for its ability to resume growth after return to aerobic conditions (Waters et al., 1991).

Acknowledgements

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