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Research Station for Floriculture and
Glasshouse Vegetables
Location Naaldwijk
Postbus 8, 2670 AA Naaldwijk
Tel. 0174-636700, fax 0174-636835

ROOT ARCHITECTURE, OXYGEN STRESS AND OXYGEN TRANSPORT

Literature and proposed experiments

Experiment 5.005

Chr. Blok
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INDEX

1. INTRODUCTION

This review is part of a project studyin g transport processes in substrates (Wever, 1999; Disco, 1999). The review focuses on the transport of oxygen. The transport properties of water (hydraulic conductivity and water retention characteristics) are debated elsewhere (Nowak, 2000).

A large number of gas transport models has been developed for use in soil science (Rolston, 1986). It is still difficult to evaluate the results of the models for substrates used in horticulture (Baas et al, 2000; Caron and Nkongolo, 1999). Especially substrates with low volumes of substrate particles e.g. peat and rockwool, are felt to behave different. Another problem connected with the use of models is the too simple description of actual root systems. For these reasons most models are believed to fail to deliver practical values.

It is hoped models can be developed which define critical conditions for oxygen stress in most common horticultural substrate. Such models could replace much of the difficult and costly direct measurements of oxygen transport and oxygen use. Models could effectively replace the repeated measurements for different substrate water contents and different plant sizes. The required input data would be confined to a maximum anticipated respiration rate, physical parameters like sample dimensions, substrate density and tortuosity and general physical constants like oxygen diffusion rate in air and water.

Oxygen stress is known to influence growth in many ways. It is therefore also measured in many ways (Gislerød and Kempton, 1983; Drew and Stolzy, 1996; Cherif et al, 1997; Rong et al, 1999). There is, however, not one accepted way to describe oxygen stress. A quantitative definition of oxygen stress is, however, a prerequisite to the evaluation of any model in terms of consequences for practical growing.

The aim of this review is:
- to define experiments to create and compare different root systems
- to define experiments to create and study oxygen stress in a controlled environment
- to define experiments to compare and improve transport models in cultivation experiments with substrates used in horticulture

Chapter two describes general root morphology and factors affecting the morphology. Chapter three describes the effects of root zone oxygen stress on the plant. The fourth chapter describes and discusses the models used. The models investigated were OXSI (Tariku, 1999) and SUBOX, a spreadsheet based on a formula of Millington (Bakker et al, 1987). In chapter five the discussion is concluded by proposing a strategy for the practical measurement of oxygen transport in substrates. In Appendix one experiments aimed at increasing the number of root tips are proposed. In Appendix two experiments are proposed to create and measure oxygen stress. In Appendix three the model SUBOX is described in detail.

2. ROOT MORPHOLOGY

A generalised dicot root system is shown in Figure 1. The root system consists of basal roots, lateral roots and root hairs. Adventitious roots are not considered here as their role in common horticultural growing is thought to be of little importance (Visser et al, 1996). It is believed the three root types shown are genetically different, as it is possible to breed tomatoes lacking any of the three types (Zobel, 1996). Still a mutually exclusive functional definition is difficult. Basal roots may be described as larger roots growing down and branching from the base of the hypocotyl. Lateral roots are vertical branch roots of a basal root, often regularly spaced along this base root. Root hairs are elongated epidermal cells, typically 0-7 mm long, 5-10 micrometer in diameter. Obvious shortcomings of this morphological definition include for example the status of a down-going branch of a base root.

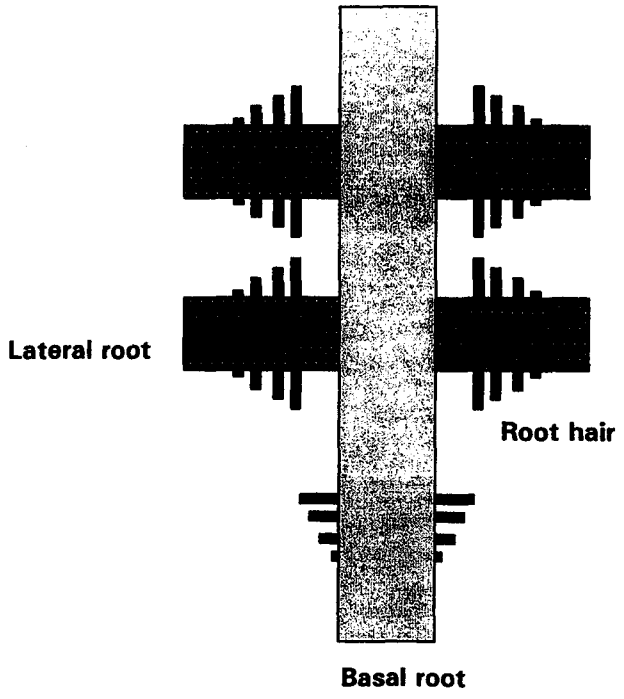

Figure 1- Schematic representation of three genetically different roots

The general function of the three root types as known today is stated here in brief (Marschner, 1995). The base roots grow towards favourable environments in terms of nutrients and water and at the same time avoid adverse circumstances. Lateral roots are the main way of exploiting water and local nutrient rich spots (McCully, 1995). Root hairs are thought to be the plants way of extending the depletion zone around roots (Tinker and Nye, 2000).

2.1 BASAL ROOTS

Enhanced basal root formation
The basal root formation is believed to be genetic in origin (Zobel, 1996). Branching of the basal root occurs only if growth is hindered by adverse circumstances. Such circumstances are thought to reduce the production of cytokinins. Cytokinins suppress branching of the basal root in lateral roots and possibly the branching into additional basal roots (Marschner, 1995). The uncommonly long unbranched roots in NFT cultures may thus be explained as cytokinin induced apical dominance.

Enhanced basal root elongation
Basal root elongation is stimulated by an increase in carbohydrate supply, calcium concentration in the substrate, and an optimal CO_2 level.

Roots are weak sinks depending on carbohydrates allotted to them by above ground processes (Nagel, 1998; Figure 2). When the total amount of carbohydrates produced by photosynthesis increases, the part transported to the roots and therefore root growth, increases in proportion (Scheible et al, 1997). Thus absolute root growth increases with light, which may explain the commonly observed poor root growth in winter propagation. An increase in shoot nitrate level, in plants with a high nitrate supply, reduces the amount of carbohydrates partitioned to the roots and therefore reduces root growth (Scheible et al, 1997).

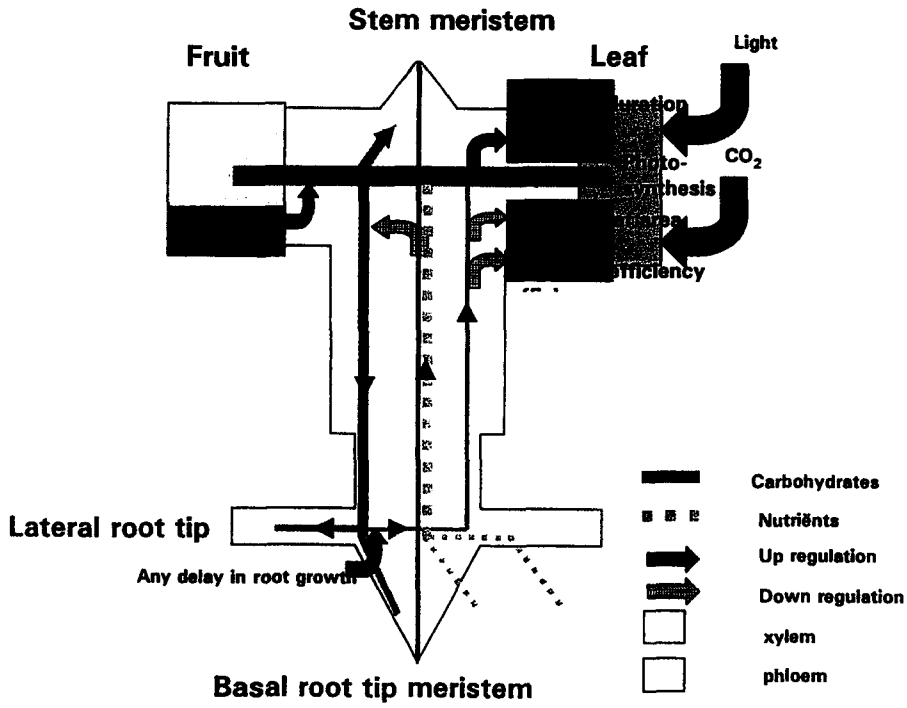

Figure 2 - Carbohydrate and nitrogen flow in relation to root growth

Root growth decreases when not enough calcium is bound to the plasma membrane of the root tip (Yermiyahu et al, 1997). Thus an increase in the concentration of another cation may reduce elongation by competition for binding sites. The competition for binding sites between calcium, potassium and magnesium is well known (Willumsen et al, 1997).

Root growth is optimal at CO_2 levels of 0.5-1% (Zobel, 1995; Mathooko, 1996).

Reduced basal root elongation
Basal root elongation is reduced by many parameters, including resistance of the substrate, aeration and hormones like cytokinins and auxines. The role of ethylene and abscissic acid is related to oxygen stress and is discussed in chapter three.

An increase in resistance to penetration is related to a reduction in root elongation (Wraith and Wright, 1998). A corresponding reduction in above ground production is thought to be the result of a negative feedback function of carbohydrates not used for root growth on photosynthesis (Schaffer et al, 1999; Figure 2). The same explanation is given for the reduction in above ground growth which results from a restricted root volume (Van Gurp and De Bruin, 1990; Thomas and Strain, 1991; Van Iersel, 1997; Mugnai et al, 2000). Klapwijk and Wubben report the opposite, but their data show the same tendency and may only lack a sufficient number of repetitions (Klapwijk and Wubben, 1988). Root restriction in substrate compared to root restriction in water culture showed a more pronounced effect on above ground growth for substrate (Goto et al, 2000). One might speculate that the ease with which a root may turn away from an obstacle is of importance. Cytokinins might be involved in the down regulation of shoot growth as described above, but none of the authors cited mentioned cytokinins.

If the oxygen concentration in soil-air drops, the root elongation is stopped (Jackson, 1980; Soffer and Burger, 1988). A critical content of 10% oxygen or 35% air is mentioned (Marschner, 1995; Baas and Warmenhoven, 1995). These values are probably only valid for a specific crop-substrate system. In chapter three the limitations to the use of this type of critical value will be discussed. A drop in oxygen concentration around the roots also triggers the formation and transport of the hormones ethylene (and its precursor ACC) and abscissic acid (ABA).

Cytokinins are produced in growing root tips and reduce basal root elongation (Marschner, 1995; Figure 3). Cytokinins are transported to the above ground parts and improve the efficiency of photosynthesis. It not only increases the light use efficiency, but also increases the life span of a single leave, the leaf area duration (Drüge, 2000). The production of cytokinins is increased by nitrate.

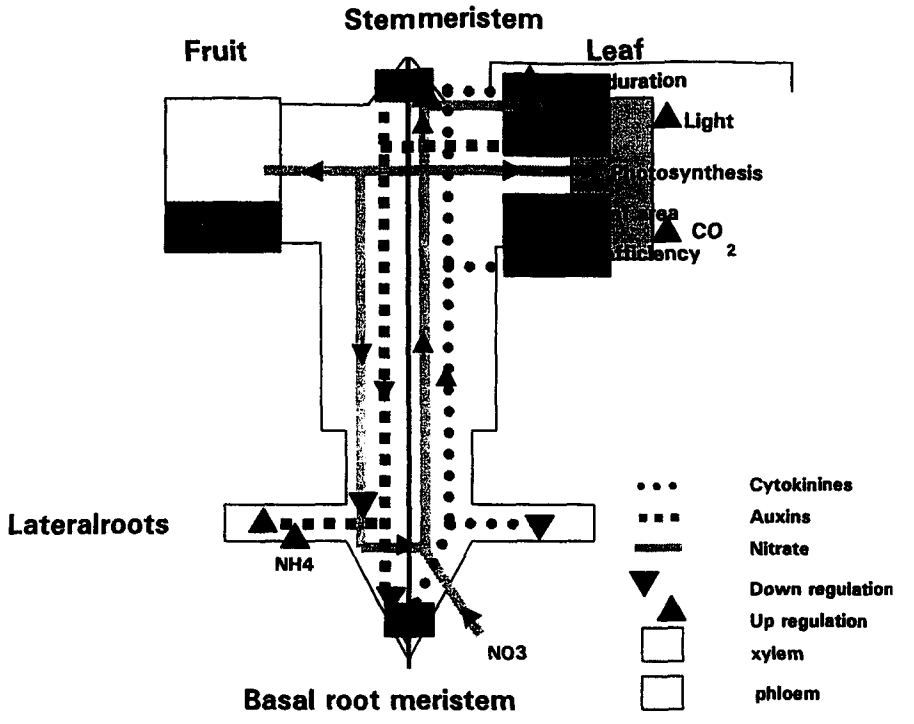

Figure 3 - cytokinins, nitrate and auxins flow in relation to root growth

Auxins like IAA are produced in shoot meristems (Figure 3). An increase in nitrate level in xylem sap promotes the formation of IAA (Zhang and Forde, 2000). IAA moves down in the plant and performs multiple functions. Free IAA promotes elongation of the stem as well as leaf elongation (Van Iersel, 1997). GA and cytokinins may compensate a lack in IAA for leaf elongation. IAA concentrations above a threshold level promote the formation of lateral roots (Morelli and Ruberti, 2000; Zhang and Forde 2000). External ammonium supply increases lateral root formation (Drüge, 2000). The effect of the ammonium probably is the increase in phloem unloading of IAA at the lateral root (Wiesler, 1997).

Among factors affecting root elongation not considered here are Aluminium concentration, soil acidity and gravity (Marschner, 1995).

2.2 LATERAL ROOTS

Patches of nutrients of which the uptake depends on diffusion, especially anions like phosphate and nitrate, enhance the phloem unloading of sugars and thus promote formation and growth of lateral roots (Wiesler, 1997; Figure 2). Anything that lowers the level of cytokinins, including cutting the basal root, increases lateral root formation (Glenn, 2000). IAA concentrations of over 10^{-7} mole/l also promote the formation of lateral roots (Salisburry and Ross, 1985; Moreli and Ruberti, 2000; Zhang and Forde 2000).

2.3 ROOT HAIRS

Root hair formation and root hair elongation are increased by low concentrations of P, N and K and therefore by relatively low water contents as lower water contents decrease diffusion (Marschner, 1995). Root hair formation in water vapour saturated air is extensive. Ethylene and auxines somehow stimulate root hair formation (Gilroy and Jones, 2000). The root hair tips need a substantial calcium influx and are able to grow positively towards a calcium gradient (Gilroy and Jones, 2000).

2.4 CONTROLLING MORPHOLOGY

The complex and often counterproductive rules described so far are partly scheduled in Figures 2 and 3. As culture measures like flooding induce multiple changes it soon is quite difficult to predict the overall outcome.

One way to promote above ground growth seems to increase the number of root tips formed. This will increase the level of cytokinins exported to the leaves and subsequently will increase the photosynthetic efficiency of the leaves. Based on Figures 2 and 3 experiments are proposed in which different root systems are created (Appendix 1). The mechanisms chosen are an increase in ammonium nutrition and root pruning.

By increasing the ammonium nutrition relative to the nitrates, the number of lateral roots should increase (Zhang and Forde, 2000). Previous research showed widely differing results such as effects on soil pH and induced calcium deficiency. Some experiments at least suggest extra root formation although the above ground production is not increased (Wiesler, 1997; Molitor and Fischler, 2000). Possible adverse effects are related to an ammonium-linked reduction in nitrate in the xylem sap flux. The reduction in nitrate reduces the level of auctions which reduces the leaf area formed (Van Iersel, 1997). The above ground production may be hindered by the extra oxygen consumption of the roots brought about by ammonium feeding (Molitor and Fischer, 2000). Ammonium feeding will also promote growth (and oxygen consumption) of micro-organisms in the root environment. In conclusion the effects of ammonium to nitrate ratio affect so many counteracting processes that the result on root formation in a specific experimental setting cannot be predicted but must me measured.

Root pruning is a common cultivation measure for some species of fruit trees (Glenn, 2000; Shackel et al, 2000; Davies et al, 2000). Root cutting in greenhouse cultures is less common but not unknown (Karni et al, 2000; Nijssen, 2000). It seems most effective to cut many root tips and little root mass by cutting off the thin outer layer of substrate (Appendix 1).

It should be mentioned that it is possible to change the rooting pattern genetically. A large project with beans proved it possible to select and engineer plants with an increased proportion of shallow roots (Lynch, 2001). This greatly improved the plants efficiency in phosphate uptake. One of the typical features of substrate growing in modern horticulture is the greatly reduced rooting depth. Up to now no efforts have been made to adapt the plants root morphology to this specific trait of horticultural practise.

3. OXYGEN STRESS

3.1 GENERAL

Root oxygen stress can be reflected in mild to severe reductions in oxygen consumption (Greg Cobb et al, 1995). Hypoxia and anoxia have been defined as O_2 levels at which ATP formation is respectively reduced and prevented (Morard and Sylvestre, 1996). Oxygen used by roots has to be transported through air and/or water in the soil and through water surrounding the root. The maximum amount of oxygen dissolved in water is reached when, at a given temperature, there is equilibrium with the oxygen content in air. Changes in air oxygen content then result in proportional changes in water oxygen content. The saturated oxygen content in water is also a function of temperature. When the temperature rises, the oxygen content drops (Table 1).

Table 1 - Maximum oxygen concentration in water

T	O_2	Unit
15	10.3	mg/l
20	9.3	mg/l
25	8.5	mg/l
30	7.8	mg/l

Creemers et al, 1984 with 21.0% O_2 in air

There are several reasons why anoxia risks in NFT-systems are higher in the summertime. First of all, the equilibrium oxygen content in water drops because of the temperature. Secondly, the oxygen use by the roots for maintenance rises with temperature. Thirdly the nutrient uptake and therefore the oxygen use for nutrient uptake is higher in the summer time. Oxygen problems in the summertime are reported for gully systems with nutrient flow technique (Gislerød and Kempton, 1983; Riviere et al, 1993). Surprisingly oxygen problems are not reported as common in substrate growing. This may be because the problem is not present or, in a mild form, not recognised (Baas et al, 1997). It may also be that the frequent irrigation with drip systems creates enough turbulence to ensure ample oxygen supply (Soffer and Burger, 1988; Riviere et al, 1993).

Table 2 - Critical oxygen concentration in water

T	O_2	Unit	Author	In µmole/l	mg/l
	3	mg/l	Gislerød and Kempton, 1983	100	3.0
24	60	%max	Jackson, 1980*	170	5.7
	3	mg/l	Riviere et al, 1993	100	3.0
	8	mg/l	Soffer and Burger, 1988**	250	8.3
	8	%max	Strojny et al , 1998	20	0.7
	0-4	ppm	Rong et al, 1998	125	3.8
	0.01	mmole/l	Yoshida et al, 1997	10	0.3
	1	ppm	Rong et al, 1999	30	1.0

* Damage is the reduction in root elongation

** Without stirring

In Table 2 critical levels found by various authors are shown. Comparison is difficult as techniques and damage criteria differ. Nutrient flow speed for example differs greatly

between authors. Examples of different criteria are: root elongation, root metabolite levels and above ground yield (respectively Jackson, 1980; Rong et al, 1999; Gislerød and Kempton, 1983). It seems that in substrate growing individual roots may stop growing long before the above ground production is affected.

Yield depressions of 10-30% were found for tomato (Gislerød and Kempton, 1983). Their critical values may be regarded as conservative for their method wasn't suitable to measure yield depressions between 0 and 5%.

For normal root functioning a certain amount of carbohydrates is required. Nagel, 1998, shows a figure in which the roots of a tomato plant use only 12% of the total amount of carbohydrates produced. 5% is used for growth, and 7% for respiration (respectively 40% and 60% of all carbohydrates imported in the roots). This corresponds roughly with data in Lambers and Scheurwater, 1996, who estimate 10-20% of the total assimilates are used in the roots, of which 50% is used for ion uptake, 40% for growth and only 10% for maintenance. This is close to practical tomato yield data of a dry weight yield of 3.0 kg tomato fruit mass, 2.5 kg stem and leaf mass and 0.1 kg root mass. It should be kept in mind that the dry mass produced by the root is 2-4 times the weight recovered at the end of the growing period because of roots dying and exudates lost (Berntson and Bazzaz, 1997; Toshiki et al, 1999).

The carbohydrate use by roots is thought to be closely related to oxygen use. One can either calculate or measure the quantity of oxygen a root system uses for growth, maintenance and ion uptake (Veen, 1981).

It is tempting to think that roots, when the nutrient supply is artificially stopped, will about halve their oxygen demand, as the nutrient uptake process no longer requires energy. This may, however, be obscured by the fact that the amount of oxygen necessary to produce a certain amount of ATP per unit carbohydrate respired is not necessarily constant. The plant has a choice between two pathways. Alongside the normal oxidative phosphorylation of ADP to ATP there is an "alternative pathway" which requires more oxygen per unit ATP produced (Lambers and Scheurwater, 1996). Depending on factors as ion uptake, more or less ATP is produced while the amount of carbohydrates respired fluctuates much less pronounced (Van der Werf, 1993).

3.2 SYMPTOMS

Uptake and growth
Root growth and uptake of most elements is active and requires energy. Growth and uptake stop when the oxygen level becomes low (Tachibana, 1991; Kitano et al, 1999; Morgan, 2000). This will only have consequences for the shoot if a very large part of the roots suffers from a lack of oxygen. Roots have the ability to upgrade the local uptake and thus to compensate for parts which are no longer active for whatever reason (Voogt and Van der Elzen, 1989; Glenn, 2000).

Ethanol
As described by Greg Cobb et al, 1995, the metabolic machinery depends on oxygen as electron acceptor. Without oxygen the production of ATP stops. Without ATP the efflux of protons from the root stops and the cytosol of root cells becomes acidic. These cells die within hours. When given time, the cell adapts to anoxia by starting ethanolic

fermentation and transport of lactate from the cells (Rong et al, 1999). Ethanolic fermentation produces ATP but much less efficient. To start this pathway genes for the production of enzymes necessary for the pathway have to be induced which takes some hours (Greg Cobb et al, 1995). Among these molecules are ADH (Alcohol DeHydrogenase) and lactate dehydrogenase. This implies that plants can adapt better to gradual changes in root oxygen supply than to sudden changes as instant flooding.

ACC and ethylene

A broad spectrum of stresses promotes the formation of ethylene from methionine via the formation of Ado-met and ACC. As the last step involves the oxidation of ACC to ethylene the reaction is dependent on the oxygen level. With some oxygen still available, ethylene will be formed in situ, but when no oxygen is available at all, ACC will accumulate (Mathooko, 1996). Water logging decreases the diffusion of ethylene away from the roots and thus promotes high root ethylene levels. High ethylene levels will cause aerenchyma formation (Blom-Zandstra et al, 1998). Ethylene will also cause leaf senescence. Interaction of ethylene and auxines is believed to increase adventitious root formation (Visser et al, 1996).

Leaf senescence

Leaf wilting because of senescence typically shows in the lower leaves, closest to the roots (Blom-Zandstra et al, 1998). The leaves hang down and soon become yellow from the margins to the peduncle. Leaf senescence is brought about by ethylene (or ACC) action. Normally ethylene is removed from the root system by diffusion into the soil air. When water logging occurs the production of ethylene or ACC is not only upgraded but also the diffusion is greatly reduced because roots become surrounded by water. The raised ethylene concentration causes aerenchyma formation in the roots. The ACC in the xylem sap, which is transported upward, is oxidised very soon after leaving the anoxic zone (English et al, 1995). Thus the lower leaves suffer the most.

Wilting and ABA

ABA is produced in roots and leaves in reaction to a broad array of stresses. A typical reaction to stress in the root environment, in which ABA is involved, is the closing of stomata. Before the stomata close the top of the plant loses turgor and the top leaves wilt. Possibly the photosynthetic efficiency is lowered too (Herde et al, 1999). Much research revealed that the stomata close in reaction to an increase in apoplastic ABA, i.e. not total leaf ABA. The plant is able to increase the apoplastic ABA concentration very fast, by releasing cell bound ABA (Drüge, 2000). The signal to start the release of cell bound ABA probably is a pH rise in the xylem sap (Mulholland et al, 1999; Wilkinson, 1999; Liang and Zhang, 1999; Netting, 2000). Such a change in xylem sap pH can be passed on as a change in xylem wall charge and is therefore not dependent on xylem sap flux. It may be passed on in minutes rather than hours (Koziolek et al, 2000; Netting, 2000). An increase in ABA in the leaf tissue will decrease leaf elongation rate, probably by reducing the cell wall extensibility (Mullholland et al, 1999; Incrocci et al, 2000).

Photosynthesis and leaf area duration

Another effect of anoxia is a decrease in leaf lifetime or as commonly described, a reduced leaf area duration (Yin et al, 2000). This affects all leaves and is usually not visible to an observer. Nitrogen deficiency or lack of cytokinins (Figure 3) can bring about a reduction in leaf area duration. Anoxia usually brings about both, less cytokinins and less nitrate transport (Drüge, 2000).

Cessation of shoot growth
Within days, as the dry weight percentage increases after uptake of elements ceases, dilution and internal redistribution of elements occur (Marschner, 1995).

Some aspects named by Marschner, 1995, but left out of this report are; nitrogen deficiency and sensitivity to exogenous phenolics.

3.3 CONTROLLED OXYGEN STRESS

In order to measure oxygen diffusion rates, either oxygen flux or oxygen content in various places in the substrate must be measured. Available techniques are measuring oxygen content in air chambers or air samples (Wever et al, 2001). A recent technique is the measurement of oxygen concentration with microfibres (Crisp, 1997). Theoretically it is also possible to measure oxygen as dissolved oxygen in water and to presume equilibrium with oxygen in the substrate air.

As Table 2 illustrates, little is to be expected from using any critical value for comparing different systems. Only oxygen levels below the very low concentration of 6 µmole/l hamper root cell metabolism (Greenwood, 1969; Jackson, 1980). Thus oxygen transport to the roots is limiting and not any "critical" level in the nutrient solution or substrate air. To compare systems, which differ in irrigation regime or substrate, the oxygen transport rate at a given consumption – the diffusion rate - is a much better parameter. With diffusion rate different substrates and water supply systems can be optimised to ensure proper oxygen refreshment at the root level.

If the oxygen requirement of part of a root system is not met, quite steep gradients must arise. The oxygen concentration will locally approach the aforementioned 6 µmole/l. The plant will, in reaction, increase uptake and root formation in those parts of the substrate, which are amply supplied. Above ground effects are absent or transient. When the total root oxygen requirement cannot be met by the supply, nutrient uptake must fall behind the amount needed for optimal growth. In reaction more carbon is allocated to the roots (Figure 2). Shoot morphological reactions must follow. One exception needs to be mentioned. When a root is deprived of oxygen suddenly, within minutes some shoot leaves may wilt. The reason is the root could not generate enough enzymes to prevent the build up of toxic reaction intermediates.

In appendix 2 various experiments are described which will render
- Root oxygen use of a healthy root system
- Prove the existence of a critical supply value
- Plant reaction to sub optimal oxygen supply
- Oxygen gradients for various substrate-plant systems

To prevent or at least reduce oxygen stress, several principles have been suggested. Water aeration as practised in Nutrient Flow Technique systems is of limited value. Oxygen consumption by a fairly small plant is in the order of 200 mg/plant/day or, in more general terms, 1 litre per m^2 /day (Baas et al, 2000). To supply such an amount of oxygen to a plant, at 20 degrees Celsius 125 litre of water (8 mg/l, Table 1) should pass the roots daily. Most substrate growing systems cannot meet this refreshment rate.

Increasing the amount of air in a substrate is an effective way in decreasing oxygen stress. This requires pores of a diameter that guarantees air entry even when the substrate is saturated. As drain is a requirement of most substrate growing systems and drain is only possible if some part of the substrate is saturated, saturation is a common state for parts of a horticultural substrate. Pores larger than 150 micrometer must be regarded as permanently air filled. An important prerequisite is that a large enough fraction of the pores must remain at least 150 micrometer. Roots and loose substrate particles can easily reduce pore diameters. Therefore only substrates as coarse perlite and clay granules meet these criteria. Once the critical transport parameters are known, substrates, which would benefit from an increase in the number of large pores, may be identified.

Another way to increase the air filled porosity is the use of a capillary connection to a lower water level. Thus water is removed according to the law of communicating vessels through a wick construction.

Flushing with irrigation water probably causes additional turbulence in the substrate air, thus refreshing the substrate air content by mass flow. The importance of mass flow for oxygen supply to roots in water culture is well known (Soffer and Burger, 1988). This might be the most important reason why oxygen stress in e.g. tomato growing, is not commonly reported. If so, this might also be the ratio behind an increase in yield when irrigation frequency (not quantity) is increased (Buwalda and Kim, 1994).

An even more drastically introduction of mass flow may be created with a research system called Active Drain System (ADS; Blok, 1999). Instead of creating drain by adding more irrigation water than the crop takes for transpiration, drain is sucked out of the substrate and replaced by fresh solution. The result is probably thorough refreshment of the air in the substrate. It may be difficult to separate oxygen effects from nutrient effects.

4. MODELS

4.1 GENERAL USE OF MODELS

Oxygen levels below 6 µmole/l hamper root cell metabolism (Greenwood, 1969; Jackson, 1980). If normal root functioning is possible at such very low oxygen concentrations, oxygen transport rate to the roots is limiting up to that point. This implies that any reported "critical" level in the nutrient solution or substrate air is only valid in the specific setting of that particular experiment. To compare systems, which differ in irrigation regime or substrate, the oxygen transport rate at a given consumption – the diffusion rate - is a much better parameter. With diffusion rate substrates and water supply systems can be optimised to ensure proper oxygen refreshment at the root level.

The oxygen diffusion rate is slowed by an increase in substrate depth and speeded by an increase in air filled pore space. Air filled pore space is, for a given substrate, dependent on water content and water content is a – not linear - function of depth (Van Genuchten et al, 1991). To take all these factors in account, various models exist (Tinker and Nye, 2000).

The basic models used
FLUX = Ds*d[c]/d[x]
In which FLUX is the amount of oxygen passing through a unit area per time unit, usually mg oxygen per square meter per second. The oxygen consumption by the roots creates a concentration difference, d[c], over a unit distance, d[x], in e.g. mg per m. The diffusivity in the substrate, Ds, is usually derived from the diffusivity of oxygen in air, Do, multiplied with a correction factor.

Ds is a function of air free space, AFP (a fraction, no units), the space not occupied by substrate particles or water. As pores are not straight pipes but irregular in direction and width, Ds will decrease more than proportional with AFP. Thus most models define a tortuosity factor including AFP^a, in which a is between 2 and 4. This tortuosity factor is sometimes arrived at by fitting measured data (Bakker et al, 1987) and sometimes by theoretical analyses (Caron and Nkongolo, 1999).

Most models differ in their assumptions regarding the tortuosity factor, steady state, substrate homogeneity, and distribution of roots and homogeneity of oxygen uptake of all root positions.

Tortuosity
There is no single mathematical procedure fit for all soils and substrates to characterise the increase in way-length caused by the tortuosity of the pathway through the pores. There is a choice of calculated and experimentally fitted estimators of (the impedance by) tortuosity. The tortuosity factor is often estimated as $(TP)^{-2}*(AFP)^{3.4}$, in which TP stands for the total pore space fraction (Millingon in Bakker et al, 1987). For rockwool a tortuosity factor of $(AFP)^{3.0}$ was convenient for AFP values between 0.05 and 0.60 (Wever, unpublished based on a figure in Baas et al, 2000). It is, however, apparent that the tortuosity values with this formula are increasingly too low for AFP values from 0.60 to 0.96.

For AFP values below 0.10 the Millington estimate becomes sensitive to measurement errors. If, for example, the AFP is measured as 0.15 but is actually 0.10, the tortuosity factor is over-estimated 3 times. Another drawback of this estimator is the behaviour with substrates with a total pore space of over 0.50 (Table 3). It may be calculated that with an AFP of 0.20 this tortuosity factor for sand is 0.021, while it is 0.005 for rockwool. The difference is caused solely by the total pore spaces of respectively 0.45 and 0.96. It is hard to see a physical reason, which explains why the diffusion rate in rockwool should be 4 times lower than for sand when they both have an AFP of 0.20.

Table 3 - Comparison between the Millington formula for rockwool and soil

Rockwool			Soil		
TP	AFP	t	TP	AFP	t
96	10	0.00043	45	10	0.002
96	20	0.005	45	20	0.021
96	30	0.018	45	30	0.082
96	40	0.048	45	40	0.219
96	50	0.103	45	45	0.327
96	60	0.191			
96	70	0.323			
96	80	0.508			
96	90	0.758			

TP Total pore space l/l
AFP Air Free Pore space l/l
T tortuosity factor, TP^-2*AFP^3.3

Steady state
The formula FLUX = Ds*d[c]/d[x] is only valid when consumption by the roots and transport are in equilibrium. But it is likely that diurnal patterns in nutrient uptake result in diurnal patterns in root oxygen consumption (Kitano et al, 1999). Therefore models with oxygen consumption and gradients measured on a time scale of one day are to be preferred above shorter periods.

Substrate homogeneity
Substrates are usually far from homogeneous. Especially large and deep pores or pore-like structures improve the local oxygen supply.

Root distribution
Obviously roots concentrate at the bottom and the sides of substrates while most models assume a uniform distribution. Furthermore, if oxygen supply is continuously poor, roots will probably not enter such spots at all (Jackson, 1980).

Root oxygen consumption
Roots need oxygen to grow, for nutrient uptake and for maintenance. Root elongation ceases when the oxygen concentration is about half of that in air (Greenwood, 1969; Jackson, 1980). Nutrient uptake also stops when oxygen is depleted (Morard et al 2000). But maintenance respiration is another matter. First of all nitrogen can be used as an alternative source of oxygen/electron acceptor (Morard et al, 2000). Secondly roots have means of redistributing small amounts of oxygen through internal transport (Greenwood, 1969). Thirdly roots can down regulate their oxygen use smoothly with increasing hypoxia. It is therefore obvious that in a waterlogged profile patches of root

use less oxygen than anticipated and other patches use more, in compensation. Even on the root itself the oxygen consumption of the tip may be much higher than of a root part further away from the tip (Armstrong et al, 2000).

4.2 OXSI PROGRAM

The program is described in global terms by Tariku, 1998. The advantage of OXSI is its ability to take the effect local compaction into account.

4.3 SUBOX PROGRAM

The program is described in Appendix 3. An advantage of SUBOX is the better presentation of input data and the possibilities for dynamic graphs. An advantage, which is specific for substrates in horticulture, is the required input of pF data in cm layers. This is important when substrates are evaluated in a suction range from 1-30 cm. When pF data are actually averages of 5 cm samples, the relative error is considerable.

5 CONCLUSIONS AND PROPOSED STRATEGY

1. Gross root respiration must be measured as a basic input parameter for models
2. Actual gradients must be measured on a 2-4 cm scale
   2.1. For a well defined local oxygen consumption
   2.2. For a plant system in situ
3. Models must have an improved tortuosity factor
   3.1. Close to AFP for AFP > 0.30
   3.2. With an exponential decrease with decreasing AFP< 0.30

Ad 1. It is quite possible to alter the appearance and distribution of the root system (Dielman et al, 1998; Appendix 1). The actual root distribution is however, by no means a measure of the distribution of oxygen uptake. Roots can vary their local oxygen uptake from as jet unknown rates to almost zero. Oxygen may even be redistributed internally through roots. A decrease in nutrient uptake in one patch is actively compensated by an increase in nutrient uptake from other in patches with ample supply. Therefore the gross root respiration and the substrate oxygen transport properties offer the best way to evaluate possible oxygen distribution problems. Accurate measurement of the total root oxygen use is laborious but not very difficult. Appendix 2 shows a proven method (Wever et al, 2001).

Ad 2. The measurement of gradient in oxygen concentration on a 2-4 cm scale is difficult. It is believed that the measurements as proposed in Appendix 2 would be easier with a fibre optic spectrometer. This would allow for interval measurements (monitoring).

Ad 2.1. A local oxygen use of 0.1-10 mg/h will mimic the use of a root system. The resulting gradients would show possible risks of depletion. This method is easy to standardise but will probably over estimate the risks of depletion.

Ad 2.2. Roots are distributed fairly inhomogeneous. Roots also are able to compensate for patches with poor uptake. It is therefore likely gradients for a whole root system are not as steep as gradients for a single point artificial oxygen consumption of equal magnitude.

Ad 3. It is anticipated a simple but valid solution for the tortuosity factor will be formulated in 2001. Validation will be possible with the results of experiments described in Appendix 2. The tortuosity factor has to be close to AFP for AFP values above 0.3 as for AFP values above 0.3 the substrate diffusivity is not hindered by the pore geometry but is just the air diffusivity times the air filled volume fraction (Do * AFP). With decreasing AFP under 0.3 the tortuosity factor has to decrease more than proportional, as for infinitely small AFP's the substrate air diffusivity becomes very small but never zero. In fact, at some point the diffusivity in water will become larger than the diffusivity in air and will be rate determining for oxygen transport.

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APPENDIX 1. Experiments with root morphology

The strategy for research on root morphology changes is to focus on total root oxygen use and above ground dry mass production. This is done to avoid discussing different root morphologies with little or no consequences for the above ground plant. Such situations are thought common as roots have the capability to adapt the activity of local roots without changing the overall performance.

Airtight containers, e.g. cylinders or boxes, offer the opportunity to separate gasses in the root environment from the atmosphere. Thus many aspects of gas exchange may be studied. It is proposed to combine these airtight boxes with controlled substrate water content and controlled air supply (Figure 1). It is also proposed to use a previously used climate chamber set up in which cucumbers will be grown for about four weeks from the day of sowing.

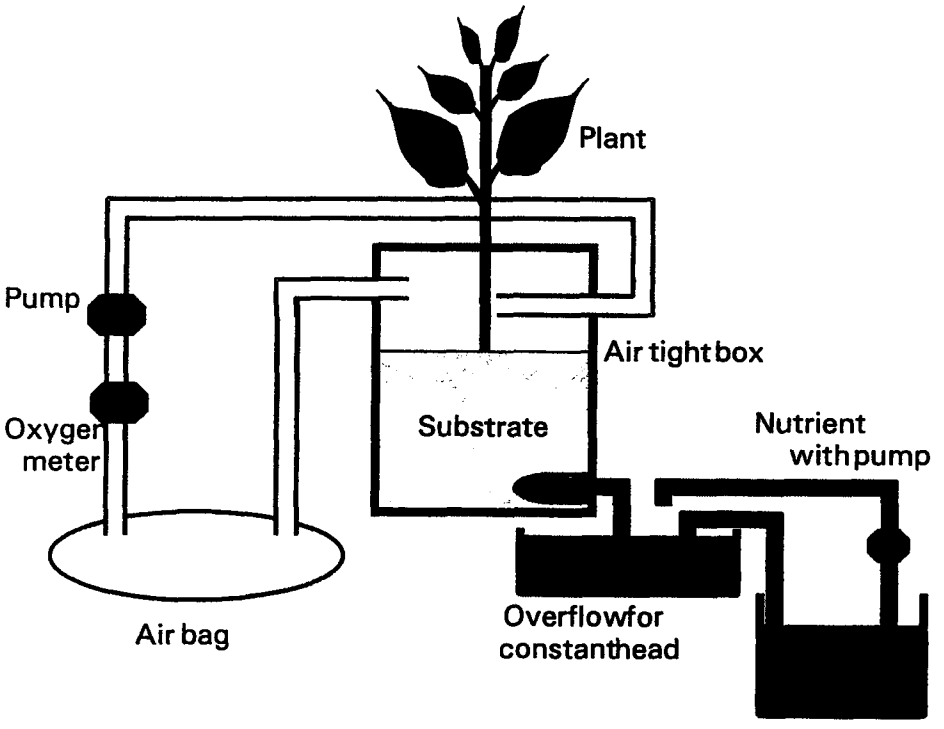

Figure 1 - System for controlled water and oxygen supply, showing airtight boxes with substrate and plant, an overflow, a nutrient solution supply and air bag

To create a constant water content in the airtight box, the airtight box is connected to an overflow. The water level in the overflow is held constant by a pump which re-supplies water from a nutrient solution container. This set-up ensures a constant suction height and therefore a constant water content in the airtight box. The supply of oxygen through water compared to the normal use is two orders of magnitude smaller (calculation Box 1). The pore size in the plug topping the connecting hose between airtight box and container is such that the connecting hose is always filled with water.

BOX 1 - Calculation on oxygen supply through water
The supply of oxygen to the roots through water uptake is considered to be insufficient. The daily oxygen consumption by the roots to be expected is at the most 100 mg (1 litre) of oxygen per day. The maximum anticipated water use is 0.5 litre water per day. The normal oxygen content of water is 9 mg/l when fully saturated. The oxygen supplied through the water is therefore only 5% of the total daily need.

As oxygen is used continuously, some form of supply is necessary to prolong the experiment. Therefore the airtight box top is connected with tubes to a bag with air. A pump and an oxygen/CO_2 meter are placed in the tube used to transport air from airtight box to air bag. As long as the transport of oxygen surpasses the anticipated use, oxygen consumption can be monitored online. The air bag size is chosen large enough to cover for the oxygen consumption of three days (calculation Box 2).

BOX 2 - Calculation on the minimal bag size
The maximum anticipated oxygen consumption is 100 mg/day, i.e. 3 mmole/day, i.e. 60 ml oxygen/day, i.e. 0.3 litre air/day. Therefore a 3.0 litre bag supplies enough oxygen for at least three days.

If the oxygen level in the supply system drops below a chosen limit, old air may be removed from the system and an equal amount of new air may be added. Simply changing the air bag for a fresh one does this. Small variations in air pressure in the system might create leaking. To prevent pressure differences the air bags are flexible but oxygen and carbon dioxide tight.

EXPERIMENT A Cucumber root growth as influenced by nitrogen source

Goal

- To measure the oxygen consumption rate and plant growth as influenced by the ammonium/nitrate ratio.

Hypothesis

Ammonium feeding increases lateral root formation. This should result in:

- More root mass
- More dry mass production as more root tips will produce more cytokinins which will improve the light use efficiency
- A higher root oxygen use as the uptake of ammonium and especially the uptake for extra growth require more oxygen

Treatments

The treatments are NH_4/NO_3 ratios of 1:10 (standard), 1:5 and 1:1. Other element are kept as stable as possible. KNO_3 is replaced by K_2SO_4 and KCl. The water content is set to 65% for all treatments and there is ample supply of oxygen. A treatment without plants is included to get an impression of the consumption by micro organisms. As micro organisms thrive on root exudates, this cannot but give a underestimation of the role of micro organisms.

EXPERIMENT B Cucumber root growth as influenced by root pruning

Goal

- To measure the oxygen consumption rate and plant growth as influenced by root pruning.

Hypothesis

Pruning the roots will stimulate the formation of more root tips. This should result in:

- More root mass
- More dry mass production as more root tips will produce more cytokinins which will improve the light use efficiency
- A higher root oxygen use as the uptake of ammonium and especially the uptake for extra growth require more oxygen

Treatments

The treatments are no pruning (standard), pruning the lowest 1 cm of the substrate away, pruning the lowest 1 cm and 1 cm of all four sides of the substrate away and pruning the lower half of the substrate away.

APPENDIX 2. Experiments with controlled oxygen supply

EXPERIMENT A Oxygen use of cucumber as influenced by water content

Goal
To measure oxygen use with different substrate water levels.

Hypothesis
At high water contents cucumbers grow slower. This is thought to reflect oxygen stress. Oxygen stress at high water content will be reflected by:
- lower oxygen consumption
- a steeper oxygen gradient declining with depth (transport will be limiting)
- a steeper decline in root number with depth
- higher CO_2 production as the result of an increase in fermentation.

Treatments
As reference; containers without plants. Water contents to set: 50%, 65%, and 90%. 3 repetitions thus 12 containers in total.

Table 1 - Measurements

Parameter	Meters	Frequency	Remarks
Oxygen use day	1	cont.	one container only
Oxygen use night	1	cont.	one container only
Carbon dioxide production day	1	cont.	one container only
Carbon dioxide production night	1	cont.	one container only
Oxygen use day	1	3x	after 20, 24 and 26 days
Oxygen use night	1	3x	after 20, 24 and 26 days
Carbon dioxide production day	1	3x	after 20, 24 and 26 days
Carbon dioxide production night	1	3x	after 20, 24 and 26 days
Water content	1	1/7d	
Water use day	3	5/wk	
Water use night	3	5/wk	
Leaf area	-	1/wk	
Fresh weight	-	once	
Dry weight	-	once	
Dry weight roots	-	once	
Oxygen gradient	-	1	3 samples in height per container
Carbon dioxide gradient	-	1	3 samples in height per container
Rooting pattern	-	once	

EXPERIMENT B Cucumber growth as influenced by oxygen supply

Goal

To measure the critical oxygen supply rate. Secondary goals are:

- to characterise growth affected by low oxygen availability
- to characterise rooting affected by low oxygen availability

Hypothesis

Sub optimal supply is a sure way of creating oxygen stress. This will reflect in:

- lower oxygen consumption
- a steep oxygen gradient declining with depth (transport will be limiting)
- a steep decline in root number with depth
- higher CO_2 production as the result of an increase in fermentation.
- a growth rate in equilibrium with oxygen supply

Treatments

The treatments are daily supply of 100%, 33% and 10% of the oxygen need per container i.e. 1000, 330 and 100 ml of oxygen respectively. To maintain equal volumes of total gas in the system, the air bags are filled with different amounts of air and nitrogen gas to a constant volume. The water content is set to 65% for all treatments. For measurements see Table 1. Figure 2 shows the probable pattern of oxygen stress reflected in the oxygen concentration in the system gasses. Note the periods of total depletion in the treatment with 10% of the anticipated oxygen needed.

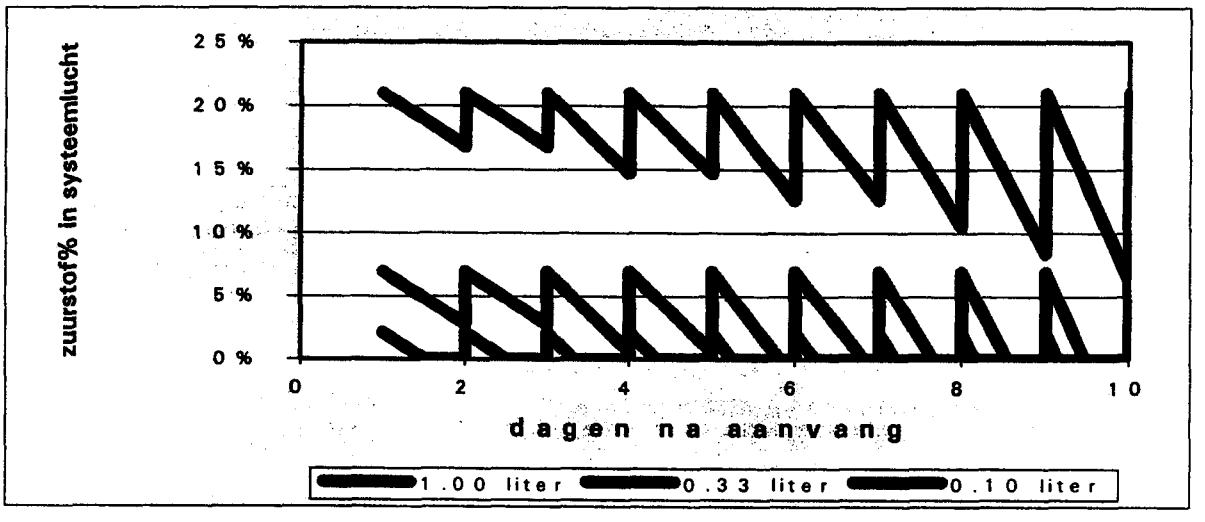

Figure 1 - Oxygen concentration in the system gasses in time

EXPERIMENT C Cucumber growth as influenced by air oxygen-concentration

Goal
To measure the influence of air oxygen-concentration on oxygen distribution in substrate

Hypothesis
Air oxygen concentration is proportional to the amount of oxygen dissolved in water. If oxygen transport through water plays a role of significance growth will be hindered by lower air oxygen concentrations. If oxygen is predominantly transported through air directly to the root surface, no differences in oxygen stress between treatments will be observed.

Treatments
Treatments are 21% oxygen, 7% oxygen and 2% oxygen in the air supplied to the airtight box. The air velocity in the system is set to ensure ample oxygen supply, even when the concentration is 1%. The air bags are filled with nitrogen gas and air to reach the right concentrations. To realise equal depletion time the size of the air bags will have to differ. With 21% oxygen a 5 l bag will do, with 7% of oxygen a 15 l bag is needed and with 2% of oxygen a 50 litre bag must be used. Figure 3 shows the probable pattern of oxygen concentration in the system gasses. Note that the average oxygen concentration in the 2% oxygen treatment is about 1%. The water content is set to 65% for all treatments. For measurements see Table 1.

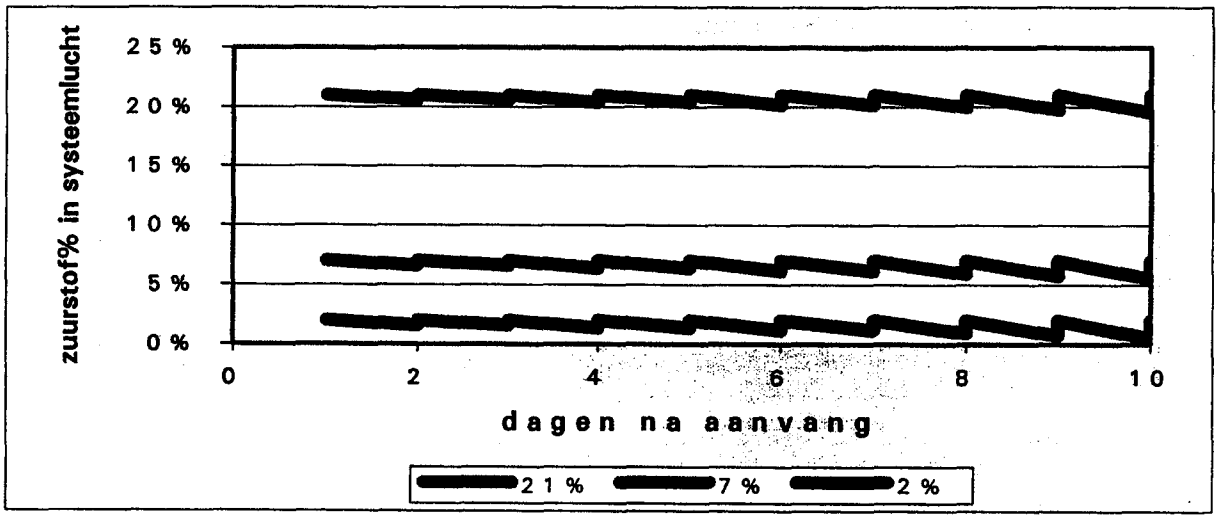

Figure 2 - Oxygen concentration in the system gasses in time

EXPERIMENT D Oxygen stress in cucumber as influenced by substrate

Goal
To establish differences in oxygen distribution within substrate.

Hypothesis
Different substrates at the same suction height will show a different internal oxygen distribution.

Treatments
Water content is set to −10 cm. There are three treatments with a different substrate. Perlite (fine), peat and rockwool. The oxygen supply is set to 90% of the anticipated need. For measurements see Table 1.

APPENDIX 3. Spreadsheet Oxygen gradient in Substrates SUBOX

SUBOX is presented as a set of 6 related tables.

Table 1 shows two physical constants and three physical relations, which underlay the Tables 2-6. The constants are the air oxygen content, 21%, and the diffusivity of oxygen in air. Both are expressed in consistent units to facilitate calculations in Table 2-6. The relations are Fick's First Law (the general diffusion formula), the water retention characteristic and a solution for diffusion in porous media.

Table 2 describes the substrate used. It gives name, pore fraction and water and gas filled fractions. All fractions are expressed as volumes as well. The volume filled by solid substrate parts is the difference between total substrate volume and the total pore volume.

Table three represents the measurement of the water retention characteristic and the subsequent mathematical formulation of the relation between water content and suction force. The first column shows the suction force applied to the sample expressed in column water suction at the bottom of the sample. It should be noted that this is slightly different from the procedure used for soil samples. In soil samples suction is defined as water column from the middle of the sample instead of the bottom. The procedure is adapted to make data independent of sample thickness and numbers of layers chosen in Tables 5 and 6. The second column, LWC, shows the measured values for water content in volume percentage. After this column the water retention curve should be either fitted with regression analysis or calculated according to theory. In this case the curve was fitted and the resulting formula can be found in Table 1. The third column, LWF, shows the water content expressed as a fraction as calculated with the formula in Table 1. The last column, WC, shows the total sample water content as a fraction. In this case a sample height of 12 cm is presumed (Table 4).

Table 4 is specific for situations with a closed airtight rooting compartment. It characterises the actual system in terms of container dimensions and substrate dimensions. It then goes on to calculate the total gas content of the system. The actual root mass is introduced as a measured input. In the next row a certain oxygen use per gram of root fresh mass is introduced, based on measurements or literature. This assumption is a simplification as an equal oxygen use over the root length or with height is rather unlikely. In the last rows of Table 4 the oxygen consumption of the system is calculated either per second or per day.

In Table five the general formulas of Table 1 are rewritten for use over a number of steps of the sample height. These formulas are the basis for Table 6 in which oxygen contents per layer are calculated. In the first row of Table five the suction force on the sample and the step size are defined. Now the number of calculation steps for Table 3 and Table 6 is known. To arrive at the diffusivity of oxygen per layer, DOL, the general formula for diffusivity from Table 1 is adapted. The term substrate gas content, GSC, is replaced by the gas content in the layer. The gas content in any layer is the substrate total pore space STP, minus the water content as fraction of that layer, LWF. Actual values for each layer are taken from Table 3. The oxygen flux through the top of the sample is of course equal to the total oxygen use divided by the sample top area. To arrive at a flux through any of the layers, it is simply assumed that all layers consume

an equal amount of oxygen. It may be noted that at this point it is not difficult to introduce a more complex pattern of oxygen with sample height. As literature does not suggest a credible pattern other than proportionality with depth, that proportionality is assumed. Once the flux per layer is known, the oxygen difference over the layer necessary to arrive at that flux is the mere result of putting the just derived values in Fick's First Law (Table 1). The oxygen concentration in any substrate layer may be estimated by subtracting the sum of all the differences over the above layers from the oxygen content in the air over the sample (21% in air).

Table 6 is already largely explained as the formulas of Table 5 are used to calculate the results for any layer of the sample. Note that Table 6 considers the twelve sample layers, not the theoretical step number of 18. The columns for suction force and water fraction per layer, UF and LWF, are derived from Table 3. The diffusivity of oxygen per layer decreases sharply with layer number as the tortuosity factor increases more than proportional with increasing water content. The oxygen flux, OFX, decreases with a constant value for each layer and reaches zero after the last layer as oxygen can hardly pass the sample bottom. The oxygen concentration difference over the layers increases although the amount of oxygen to be transported decreases steadily. The higher concentration difference is of course necessary to compensate for the increasingly impermeable layers. In terms of tortuosity one might say because of the more than proportional increase in way length. As a result, the oxygen content in the substrate drops more than proportional with depth.

CASE : 12 cm rockwool in cylindrical containers

Table 1 - Physical characteristics

Characteristic	Value	Units	Code	Calculation	Remarks
Oxygen content of the air	210	l/m^3	OA	-	
Diffusivity of oxygen in air	2.0E-05	m^2/s	ODA	-	
General diffusion formula (Fick's first law)				FLUX = - D*d[c]/dx = 1/A*dq/dt	basic textbooks
Water content from retention	Function of UF	l/l	WC	103.7-2.35*UF-9.8*$(UF)^2$	Fitted measured data
Diffusivity of oxygen in substrate (Millington formula)	Function of UF	m^2/s	DOS	ODA*$(STP)^{-2}$*$(SGC)^{3.4}$	Semi-empirical

D	diffusivity of a gas	D[c]	concentration difference	dx	distance
A	area perpendicular to the flux	dq	transported volume	dt	time
UF	suction force, in m	STP	substrate total pore fraction		
SGC	substrate actual gas content fraction				

Table 2 - Substrate characteristics

Characteristic	Value	Units	Code	Calculation
Substrate name	Rockwool			
Substrate total porosity	0.97	l/l	STP	-
Substrate water content	0.6	l/l	SWC	-
Substrate gas content	0.37	l/l	SGC	STP-SWC
Substrate volume	3	l	SV	-
Substrate total pore volume	2.91	l	TPV	STP*SV
Substrate dry volume	0.09	l	SDV	SV-TPV
Substrate water content	1.8	l	SWV	SWC*SV
Substrate gas volume	1.11	l	SGV	TPV-SWC
Suction force		m	UF	-

Table 3 - Water retention for a one centimetre layer

Suction force UF M	layer water content LWC V%	Fitted LWF l/l	Average over sample height WC l/l
0.00	97	1.04	
-0.01	97	1.01	
-0.02	97	0.99	
-0.03	96	0.96	
-0.04	95	0.93	
-0.05	90	0.90	
-0.06	90	0.86	
-0.07	85	0.82	
-0.08	80	0.79	
-0.09	75	0.75	
-0.10	70	0.70	
-0.11	65	0.66	
-0.12	60	0.61	
-0.13	55	0.57	0.81
-0.14	50	0.52	0.77
-0.15	45	0.46	0.73
-0.16	40	0.41	0.69
-0.17	35	0.35	0.65
-0.18	30	0.30	0.60
-0.19	25	0.24	0.55
-0.20	20	0.18	0.50
-0.21	12	0.11	0.45
-0.22	4	0.05	0.40
-0.23	0		0.37
-0.24	0		0.34
-0.25	0		0.32

Table 4 - System constants

Characteristic	Value	Units	Code	Calculation
Cylinder height	0.2	m	CH	-
Cylinder diameter	0.18	m	CD	-
Cylinder volume	5.1	l	CV	PI()*(CD/2)^2*CH*1000
Substrate height	0.12	m	SH	SV/CV*CH
Cylinder area	0.0254	m^2	CA	PI()*(CD/2)^2
System volume	15	l	YV	-
System gas volume	13.11	l	YGV	YV-SDV-SWV
System oxygen volume	2.75	l	YOV	OA*YGV
Root mass fresh weight	200	g	RFW	-
Oxygen consumption per gram root fresh weight	0.000059	ml/g/s	ROU	-
Oxygen consumption of the system	0.0000118	l/s	YOU	RFW*ROU/1000
Oxygen consumption per day	1.02	l/d	DYOU	YOU*3600*24

Table 5 - Preparation of layering

suction at cylinder bottom to reach SWC	0.06	m	SUC	table retention
total suction column	0.18	m	TSUC	SH + SUC
step size	0.01	m	dx	-
maximal step number	18	nr	STEPmax	TSUC/dx
diffusivity of oxygen per layer		m^2/s	DOL	ODA*(STP)^-2*(STP-LWF)^3.4
oxygen flux through top of the cylinder	0.00046371	$l/s/m^2$	TOFX	YOU/CA
Oxygen flux through any step of cylinder		$l/s/m^2$	OFX	YOU/CA*(1-(STEP/(SH/dx)))
oxygen concentration difference per layer		l/m^4	OCD	OFX/DOL*dx
oxygen concentration in substrate layer		l/m^3	OS	OA-(som(OCD))

Table 6 - Calculations per cm substrate, the yellow part is to be disregarded (surpasses the height of the actual example)

STEP nr -	UF m TSUC-(STEP-1)*dx	WC l/l see WC	DOL m^2/s see DOL	OFX $l/s/m^2$ see OFX	OCD $l/m^3/m$ see OCD	OS l/m^3	$[O_2]$ %	AFP Ratio air filled pores
1	0.18	0.30	5.5E-06	0.0004280	0.7719813	209.2	21%	0.67
2	0.17	0.35	4.1E-06	0.0003924	0.9600787	208.3	21%	0.62
3	0.16	0.41	3.0E-06	0.0003567	1.2058531	207.1	21%	0.56
4	0.15	0.46	2.1E-06	0.0003210	1.5309041	205.5	21%	0.51
5	0.14	0.52	1.5E-06	0.0002854	1.9663334	203.6	20%	0.45
6	0.13	0.57	9.8E-07	0.0002497	2.5572907	201.0	20%	0.40
7	0.12	0.61	6.4E-07	0.0002140	3.3693090	197.6	20%	0.36
8	0.11	0.66	4.0E-07	0.0001784	4.4954842	193.1	19%	0.31
9	0.10	0.70	2.4E-07	0.0001427	6.0573081	187.1	19%	0.27
10	0.09	0.75	1.3E-07	0.0001070	8.1636895	178.9	18%	0.22
11	0.08	0.79	6.7E-08	0.0000713	10.6589726	168.3	17%	0.18
12	0.07	0.82	3.0E-08	0.0000357	11.7728092	156.5	16%	0.15