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The Use of Agar Nutrient Solution to Simulate Lack of Convection in Waterlogged Soils

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Agar at 0·1 % in nutrient solution ('stagnant solution') was used to prevent turbulence (convection), thus simulating the slow gas movements which occur in waterlogged soils. Wheat, aged between 6 and 16 d at the start of the treatment, was used to test plant growth and development in this stagnant solution for 8–15 d. K-MES buffer at 5 mol m^{-3} was used to retain the pH of the rhizosphere in the stagnant solution at pH 6·5.

The prevention of convection reduced dissolved oxygen concentrations in the bulk solution from 0·275 to below 0·05 mol m^{-3} after 1 d, while ethylene accumulated over 10 d to $6{\cdot}5 \times 10^{-6}$ m^3 m^{-3} (ppm).

Aerenchyma of nodal roots grown in stagnant solution comprised 22 % of the cross sectional area of the root 50 mm behind the root tip; this was similar to values recorded earlier for nodal roots of wheat in waterlogged soil and contrasts with 7·6 % for roots in non-flushed solution without agar (referred to in this paper as 'semi-stagnant solution') and 2·4 % in N_2-flushed solution.

Increases in dry weight and numbers of nodal roots with time were larger for stagnant and N_2-flushed, than for semi-stagnant or aerated solution. In contrast, seminal roots did not grow in stagnant solution, while seminal roots in N_2-flushed solution grew much less than in semi-stagnant or aerated solution.

In the stagnant solution, relatively high concentrations of N, K and P were required to avoid limitations in mineral uptake into the roots, due to the long diffusion pathway from the bulk solution imposed by the lack of convection. Nevertheless, our data show that the slow growth imposed by the lack of convection was due to factors other than low mineral nutrition. The most likely cause was the change in the dissolved gas composition of the root media, particularly of the rhizosphere.

In conclusion, in terms of anatomy and morphology the roots grown in the stagnant solution more closely resembled those from waterlogged soil than did those grown in either semi-stagnant or N_2-flushed solution.

Key words: *Triticum aestivum*, wheat, waterlogging, lack of convection, aerenchyma, root development, nutrient uptake.

Definitions: Anaerobiosis: induces severe tissue hypoxia i.e. used to describe conditions of very low O_2 concentrations; anoxia: absence of oxygen in plant tissues; hypoxia: low but not zero oxygen in plant tissues; stagnant solution: solution containing 0·1 % agar to prevent convection; semi-stagnant solution: non-flushed solution without agar.

INTRODUCTION

The diffusion of gases is 1×10^4-fold less in water than in air, so waterlogging in soil reduces O_2 and increases CO_2 and ethylene concentrations in the soil (Jackson and Drew, 1984). Only limited convection occurs in soil, i.e. diffusion dominates, resulting in different dissolved gas concentrations in the rhizosphere compared with the bulk medium (Armstrong, 1979).

Elucidation of changes in root characteristics associated with waterlogging in soil might be expedited if the changes in gas composition of the rhizosphere, due to limited convection and slow diffusion, could be simulated in solution culture. Current methods reduce O_2 concentration, but have the following disadvantages: (1) flushing with N_2 creates an excessive sink for O_2 around the roots and also removes CO_2 and ethylene; (2) semi-stagnant solutions, i.e. solutions not flushed with air or N_2, and without other forced turbulence, still give substantial convection. Convection prevents or mitigates establishment of any concentration gradients of O_2, CO_2 and ethylene between the rhizosphere and the bulk solution, while allowing substantial gas exchange between the solution and the atmosphere; and (3) flushing with mixtures of O_2, CO_2 and ethylene would still not simulate their concentration gradients in the rhizosphere of soil grown plants.

When convection in the root medium is slight or zero, dissolved O_2 may be lower in the rhizosphere than in the bulk soil in roots with no, or little, aerenchyma, while aerenchymatous roots will have radial oxygen loss, thereby generating higher concentrations of dissolved O_2 in the rhizosphere than in the bulk soil (Armstrong, 1979). Roots of 13–18 d old wheat grown in aerated solution have little, or no, aerenchyma (Thomson *et al.*, 1990), hence at the time of transfer to a stagnant, O_2-containing solution, O_2 in the rhizosphere would be lower than in the bulk solution. This

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pattern would be reversed with time, after the start of exposure of the wheat roots to stagnant solution, but only for the nodal roots in which aerenchyma is induced, not for the seminal roots which did not form aerenchyma, either at low O_2 or in semi-stagnant solution (Thomson *et al.*, 1990).

Gradients of dissolved CO_2 and ethylene between the bulk solution and the rhizosphere are harder to predict since they will depend on the relative rates of production and consumption of both these gases by soil microflora and plant roots.

This paper evaluates the potential of stagnant nutrient solution containing 0·1% agar ('stagnant solution') to prevent convection, in order to simulate the changes in composition of dissolved O_2, ethylene and CO_2 and their movement in the rhizosphere of roots in waterlogged soil. It should be emphasized that changes in composition of several other gases, which occur in waterlogged soil, are not simulated in the stagnant solution. Further advantages and disadvantages of the use of stagnant solution (0·1% agar) will be considered in the Conclusions.

Stagnant solution (0·1% agar) has been used previously to study radial O_2 loss from roots of intact plants (Armstrong, 1969, 1971), effects on elongation related to reduced radial oxygen loss from roots caused by the lack of convection (Healy and Armstrong, 1972), development of aerenchyma and number of adventitious roots in *Rumex* species (Laan *et al.*, 1989; Visser *et al.*, 1996, respectively) and germination and growth of rice coleoptiles (Setter and Ella, 1994).

The experiments presented here evaluate the use of stagnant solution (0·1% agar) in terms of changes in dissolved gas composition and the consequent changes in root anatomy and morphology. We compare responses of intact wheat plants, grown with their roots in stagnant, N_2-flushed, semi-stagnant and aerated solutions. Measurements were made of aerenchyma, dry weights and lengths of main root axes and laterals. Mineral nutrient deficiencies may occur due to the long diffusion pathway from the bulk of the stagnant solution to the root epidermis, as a result of the lack of convection. Hence, nutrients in the rhizosphere may become depleted and we therefore tested internal nutrient concentrations and growth over a range of external nutrient concentrations.

MATERIALS AND METHODS

Preparation of agar in nutrient solution

Agar was routinely dissolved in nutrient solution at a concentration of 0·1% (w/v), but there were comparisons with 0·05 and 0·2% agar. To dissolve the agar all solutions were autoclaved at 120 °C for 15 min and then cooled overnight to room temperature. Magnetic stirring until the temperature of the solution fell below 80 °C prevented the formation of lumps.

Mineral nutrients (composition given under plant culture) were added before autoclaving. Nutrient solutions containing phosphate and Ca^{2+} were autoclaved separately and cooled to 45 °C before mixing, at which time micronutrients were also added.

Checking convection

A drop of 0·5% neutral red, made up in the same agar concentration as that being tested, was placed either 10 mm below the surface of the agar solution, or 10 mm above the base of a 25 cm^3 vial filled with agar solution. In other tests a dyed layer was placed either on the surface of the solution or at the base of the vial. The position of these drops and/or layers was observed over time.

Plant culture

Seeds of *Triticum aestivum* (cv. Gamenya) were germinated in the dark at 20 °C on plastic mesh over a solution of 0·5 mol m^{-3} $CaSO_4$. After 3 d the seedlings were transferred to 10% strength nutrient solution (composition of full strength nutrient given below).

On day 6, the plants were transferred to a phytotron under natural light. Light intensities varied between 1000 and 2000 μmol m^{-2} s^{-1} (PAR); day length was about 11 h. Temperature varied in 12 h cycles: 20 °C in the day and 15 °C during the night. At the time of transfer, the plants were also put in $3{\cdot}2 \times 10^{-3}$ m^3 pots, 4 plants per pot, in full strength nutrient solution aerated at 240–270 ml min^{-1}. The composition of the nutrient solution at full strength was (mol m^{-3}): NO_3^-, 4·5; SO_4^{2-}, 2·0; $[H_2PO_4^- + HPO_4^{2-}]$, 0·08; Ca^{2+}, 2·0; NH_4^+, 0·5; Mg^{2+}, 0·4 and K^+ either at 3·3, or at 6·1 when the solutions contained 5 mol m^{-3} MES. MES was used in all experiments except in the minus MES treatment during the test on rhizosphere pH (described later in this section). The initial pH was 6·5. Micronutrients (mol m^{-3}) were: B, 0·006; Cu, 0·0008; Zn, 0·0012; Mn, 0·001; Mo, 0·0001 and Fe sequestrene 0·015. Mild chlorosis in the expanding portion of the leaves indicated that iron deficiency often developed 10–12 d after germination. $FeSO_4$ at 0·06 mol m^{-3} was added daily to the solution, until the leaves became dark green again.

The stagnant treatment was started by immersing roots in 0·1% agar nutrient solution. At the start of this treatment the plants were usually 13–16 d old. The exception was the experiment to test the pH of the rhizosphere (see below). Transparent cylindrical vessels (16 × 16 cm, with a solution volume of $3{\cdot}2 \times 10^{-3}$ m^3) had lids made of PVC, and the plants were placed in holes with a diameter of 1·3 cm, sealed with tape over the cotton wool supporting the plants. The distance between the lid and the solution was 0·5–1·0 cm. Temperatures in the stagnant solution were 18·1 °C at 0900 h, 21·3 °C at 1200 h and 23·6 °C at 1600 h. Temperatures in the N_2-flushed and aerated solutions differed from these values by, at most, $\pm$0·4 °C.

Plant measurements

Plant growth was assessed by fresh weights and, after drying at 80 °C, by dry weights of shoots, nodal and seminal roots. Root morphology was evaluated from root numbers and lengths of main axes measured with a ruler; total length of seminal and nodal roots was measured using a Comair

root length scanner. Total length of root laterals was calculated from total root lengths minus total lengths of main axes.

Porosity was evaluated by measuring buoyancy with a balance using Archimides' principle (Raskin, 1983).The vacuum infiltration required in this technique was for three periods of 40 s (cf. Thomson *et al.*, 1992). Aerenchyma formation was assessed for 100–120 mm long roots, by taking hand sections 50 mm from the root apex. Prior to sectioning the roots were infiltrated with water under vacuum. This technique is superior to cutting without vacuum infiltration, because it gives less distortion of the aerenchyma of the wheat roots and clearer visualization; without vacuum infiltration gas bubbles in the aerenchyma will give black images due to refraction of light. The root sections were photographed and the cross sectional area of the root occupied by aerenchyma determined using a Flinders Imaging MD-20 image analysis system.

Chlorophyll of the oldest leaf was assessed on the intact plant using a Minolta ('Spad 501') chlorophyll meter. The readings of the Spad meter were converted to chlorophyll concentrations, using an unpublished calibration curve for wheat leaves.

Plant tissues were dried at 80 °C and then ground. For nitrogen, the samples were digested and analysed according to the Kjeldahl method (McKenzie and Wallace, 1953) using an autoanalyser. For potassium and phosphorus, samples were digested in a mixture of $HNO_3/HClO_4$. Potassium was measured using atomic spectrophotometry (Perkin-Elmer 403), and phosphorus by the molybdovanadophosphate method (Boltz and Lueck, 1958).

pH of the rhizosphere

In this experiment the plants were only 6 d old when transferred to stagnant solution, and cylindrical pots with a volume of $1{\cdot}3 \times 10^{-3}$ m^3 were used.

Nutrient solutions containing 0·1% agar and 0 or 5·0 mol m^{-3} MES buffer were made up to a final concentration of 0·06% bromocresol purple. This indicator is bright yellow at pH 4·0 and ranges to dark purple at pH 7·0. Six d old plants were then grown with their roots in this nutrient solution for a further 14 d.

Oxygen in stagnant solution

Roots of 14 d old intact wheat seedlings were transferred to stagnant solution. During the measurement of dissolved O_2 concentration, the roots were lifted out of the agar. The agar solution in each cylinder (with the lid on) was then mixed with a magnetic stirrer for 30 s before O_2 concentration was measured with a Syland O_2-electrode. In preliminary tests this length of stirring provided good mixing, without measurable entry of O_2 from the atmosphere (the accuracy of the O_2 electrode is greater than 0·003 mol m^{-3} dissolved O_2).

Ethylene in stagnant solution

After roots of intact wheat seedlings had grown in the solutions for 0, 1, 2, 7 and 10 d, the solutions were stirred as described above, and five samples were taken using 1 ml Hamilton 'gas tight' syringes; the needles were sealed immediately after sampling with rubber bungs. Ethylene concentrations in these solutions were then measured as follows: 5 ml of solution was transferred to a 15 ml vial, closed with a puncture seal ('Suba seal') and shaken for 1–2 min. (This period was sufficient as shown in tests with longer periods of shaking, which included ethylene standards containing 5 and 10×10^{-6} m^3 m^{-3}. Gas was then removed from the head space and assayed according to Jackson *et al.* (1987).

Treatments

Treatments usually started 16 d after germination. The treatments were: (*a*) aerated; (*b*) stagnant without agar ('semi-stagnant'); (*c*) stagnant with 0·1% agar ('stagnant solution'); and (*d*) flushing with industrial N_2 gas. The initial O_2 concentration in the stagnant solution ranged between 0·14 mol m^{-3} and 0·28 mol m^{-3}.

Length of treatment varied between 8 and 15 d. Recovery was tested by growing two plants from each treatment in freshly aerated nutrient solution without agar for a further 6 d. These data are usually not reported, since relative growth rates and characteristics such as nodal roots and ratios of main axis to laterals tended to become similar in all treatments.

RESULTS

Convection

There was no convection in 0·1 or 0·2% agar solution: drops and layers containing neutral red remained distinct for at least 5 d. In 0·05% agar solution, the dye was completely dispersed within 3–5 d, while in deionized water the dyed drops and layers dispersed immediately.

pH of the rhizosphere

Without MES buffer, the colour of the rhizosphere of roots in agar solution became bright yellow within 1 d, indicating a pH of 4·0–4·5. In contrast, the bulk solution first remained purple (about pH 6·5), subsequently becoming light purple and 14 d after the start of the experiment its pH was approx. 5·0. The rhizosphere of plants grown in stagnant solution with MES remained purple. There was no further change in the colour patterns in the solution containing MES for the next 10 d.

TABLE 1. *Effect of 5 mol m^{-3} MES, pH 6·5, in aerated solution on fresh weight (g) of shoots, nodal and seminal roots of wheat (cv. Gamenya)*

	Initial weight	−MES	+MES	s.e.
Shoots	0·48	5·7	5·3	0·25
Nodal roots	0·013	0·8	0·49	0·075
Seminal roots	0·32	2·81	2·56	0·11

The seedlings were 13 d old at the start of the experiment and treated for 12 d. (TD Colmer unpubl. res.).

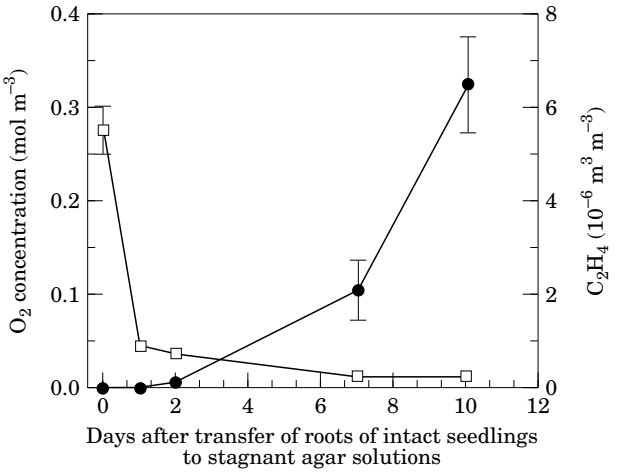

FIG. 1. Changes in O_2 and C_2H_4 in root media with time after transfer of roots of intact wheat seedlings to stagnant nutrient (0·1 % agar, no forced turbulence). O_2 is presented as the concentration of dissolved O_2, and ethylene as ppm (10^{-6} m^3 m^{-3}) in a notional gas phase that would be in equilibrium with the stagnant solution. The seedlings were 16 d old at the start of the treatment and there were three plants per 1 l pot. The values are means of four replicates ±s.e. □, O_2; ●, C_2H_4.

TABLE 2. *Porosity of seminal and nodal roots of wheat* (*cv. Gamenya*) *as a % of total root volume, and aerenchyma as a % of the cross sectional area of the root for nodal roots*

	% Root porosity		% Aerenchyma
	Seminal	Nodal	Nodal
Aerated	1·3	5·2	0·0
Semi-stagnant	4·2	9·3	7·6
Stagnant	3·0	14·8	22·3
N_2-flushed	2·4	3·8	2·4
l.s.d. (0·05)	2·0	2·4	3·2

Roots of intact plants were grown for 16 d in aerated nutrient solution and then treated for a further 15 d in aerated, semi-stagnant (no forced turbulence), stagnant (0·1 % agar, no turbulence), or N_2-flushed nutrient solution. Aerenchyma was evaluated at 50 mm from the tip and porosity for the entire root system. Means of four replicates with two plants per replicate.

Effects of MES on plant growth

MES at 10 mol m^{-3} did not affect growth of three medic species over 12 d (Ewing and Robson, 1991).

For wheat, MES at 5 mol m^{-3} and pH 6·5 in aerated solution, had no effect on fresh weight of seminal roots and shoots (Table 1). Fresh weight of nodal roots was reduced by 40 %. Whether these effects of MES would occur in stagnant solution is not known.

Changes in oxygen and ethylene concentrations in the stagnant solutions

Oxygen concentrations in the stagnant solution decreased rapidly from 0·275 to 0·045 mol m^{-3} within 24 h after transfer of roots of intact wheat seedlings to the solutions (Fig. 1). Over the next 6 d, there were further slow decreases to 0·011 mol O_2 m^{-3}. Ethylene concentrations increased from 0×10^{-6} m^3 m^{-3} at the start of the treatment to 2·1 and $6{\cdot}5 \times 10^{-6}$ m^3 m^{-3} in the notional gas phase in equilibrium with the stagnant solution after 7 and 10 d, respectively (Fig. 1). In the semi-stagnant solution O_2 concentration decreased from 0·095 to 0·03 mol m^{-3} between 1 and 10 d after starting the treatment (data not presented).

TABLE 3. *Dry weights* (*mg per plant*) *of shoots, seminal and nodal roots of wheat* (*cv. Gamenya*)

	Shoots	Seminal roots	Nodal roots	Shoot/root ratio
Initial weight	20	10	0	2·0
Aerated	160	30	12	3·8
Semi-stagnant	160	30	11	3·9
Stagnant	110	10	20	3·7
N_2-flushed	135	15	16	4·3
l.s.d. ($P = 0{\cdot}05$)	7	3	3	

Roots of intact plants were grown for 16 d in aerated nutrient solution and then for a further 15 d in either aerated, semi-stagnant (no forced turbulence), stagnant (0·1 % agar, no turbulence), or N_2-flushed nutrient solution. Means of four replicates with two plants per replicate.

Effects on roots and shoots

Porosity and aerenchyma. The porosity of nodal roots in the stagnant solution was 1·5 times greater than in the semi-stagnant solution, and three times greater than in the aerated and N_2-flushed solutions (Table 2).

Aerenchyma in the nodal roots grown in stagnant solution constituted 22 % of the cross sectional area of the root, which was three- and nine-fold higher than in the semi-stagnant and N_2-flushed solutions, respectively (Table 2). There was no aerenchyma in the roots of the aerated solution. The larger percentage cross sectional area for aerenchyma than for porosity in the roots in the stagnant solution (Table 2), is presumably due to different root tissues used for the evaluation of these characteristics; porosity measurements were on the roots as a whole, while aerenchyma was for a section 5 cm from the root tip. Porosity of the seminal roots never exceeded 5 %, and the only significant difference was a higher porosity for the roots grown in semi-stagnant, rather than in aerated, solution (Table 2).

Weights of roots and shoots. Growth of wheat was not affected by agar at 0·1 % (w/v) when the solutions were flushed with air (data not shown). In stagnant solution (0·1 % agar), there was no seminal root growth. Nodal roots were 1·7–1·8-fold heavier than aerated roots, while the increase in shoot dry weight for the plants grown with roots in stagnant solution was only 64 % of plants with aerated roots (Table 3). Plants with roots in semi-stagnant solution were similar in these aspects to plants with aerated roots. In contrast, intermediate values were found for plants grown with roots in N_2-flushed solution. Shoot/root dry weight ratios were somewhat higher in the N_2-flushed, than in all the other treatments (Table 3).

Root number and length. There were seven to eight nodal roots per plant in the stagnant and N_2-flushed solutions but

TABLE 4. *Development of nodal and seminal roots of intact wheat plants* (*cv. Gamenya*) *which were grown for 16 d in aerated nutrient solution and then treated for a further 15 d in either aerated, semi-stagnant* (*no forced turbulence*), *stagnant* (*0·1 % agar, no turbulence*) *or* N_2*-flushed nutrient solution*

Treatment	Number	Maximum length of main axis (mm)	Average length of main axis (mm)	Total length of laterals per main axis (mm)	Lateral/ main axis length ratio
Nodal roots					
Aerated	4	140	66	61	0·9
Semi-stagnant	5	201	98	103	1·0
Stagnant	7	138	76	103	1·3
N_2-flushed	8	102	62	84	1·4
l.s.d. ($P = 0·05$)	1·0	47	14	70	—
Seminal roots					
Aerated	5	305	215	772	3·6
Semi-stagnant	5	361	271	888	3·3
Stagnant	5	147	101	175	1·75
N_2-flushed	6	165	94	117	1·25
l.s.d. ($P = 0·05$)	0·96	39	22	80	—

The table shows average number, maximum length and mean length of individual main axes and the total length of lateral roots per main axis Means of four replicates with two plants per replicate.

TABLE 5. *Concentrations of N and K in shoots and chlorophyll concentration in the oldest leaf*

	N (mmol g^{-1} d. wt)	K (mmol g^{-1} d. wt)	Chlorophyll (mg g^{-1} d. wt)
Aerated	4·7	1·87	8·4
Semi-stagnant	4·3	1·78	8·8
Stagnant	2·9	1·25	5·8
N_2-flushed	3·7	1·39	7·6
l.s.d. (0·05)	0·2	0·09	0·5

Roots were grown for 16 d in aerated nutrient solution and then treated for a further 15 d in aerated, semi-stagnant (no forced turbulence), stagnant (0·1 % agar, no turbulence), or N_2-flushed nutrient solution. Means of four replicates with two plants per replicate for N and K^+ and four for chlorophyll.

only four to five in aerated and semi-stagnant solutions (Table 4). The maximum lengths of the main axes were: (1) for nodal roots: semi-stagnant > agar = air > N_2-flushed; and (2) for seminal roots: semi-stagnant > air > agar = N_2-flushed. The longer lengths of nodal roots in semi-stagnant and stagnant solutions than in air, was presumably associated with their earlier development. Nodal roots in stagnant and N_2-flushed solutions had 1·3–1·5-fold larger ratios of lateral to main axis length than roots in semi-stagnant and aerated solutions (Table 4). In contrast, in seminal roots the ratio was two to three-fold lower in stagnant and N_2-flushed solutions than in semi-stagnant and aerated solution (Table 4).

N, K and chlorophyll concentrations in the shoots. Table 5 shows that N and K concentrations in the shoots were in the order: air = semi-stagnant > N_2-flushed > agar. After reaeration, the shoots of plants with roots grown in stagnant solution were the only ones to show an increase in K concentration. The increase was about 10 % of the concentration at the start of recovery (data not shown).

Chlorophyll concentration of the oldest leaf was lowest for plants with roots grown in stagnant solution, intermediate when roots were in N_2-flushed solution, and highest in semi-stagnant and aerated solutions (Table 5).

Possible diffusion limitation on ion uptake in stagnant solution (*0·1% agar*) *and its effect on growth*

Checks were made to determine the concentration in the bulk solution at which diffusion started to limit uptake of nutrients by the roots in stagnant solution, and whether consequent lower nutrient concentrations in the plant tissues reduced growth. Concentrations of N, K, and P in the shoots were always substantially lower in the stagnant, than in the aerated, solutions (Table 6). N concentrations (dry weight basis) in the shoots of plants grown with roots in stagnant solutions decreased with external concentrations between 10 and 2·25 mol m^{-3} (Table 6). K concentrations in the shoots of plants were similar when grown in stagnant solution containing 1·6 and 3·2 mol m^{-3} K^+ and these concentrations were two-fold higher than at 0·8 mol m^{-3} K^+ (Table 6). In aerated solutions, increases in external concentrations of K^+ had no appreciable effect on the concentrations in the shoots, and increases in N were much smaller than in the stagnant solution (Table 6).

P concentrations in the shoots of plants grown in stagnant solutions were two- and four-fold higher at 0·08 and 0·16 mol m^{-3}, respectively, than at 0·04 mol m^{-3} [$H_2PO_4^-$ and HPO_4^{2-}] (Table 6). Less pronounced increases were

TABLE 6. *K, P and N concentrations (mmol g^{-1} d. wt) in the shoots of wheat seedlings with roots grown in stagnant (0·1% agar, no turbulence) and aerated nutrient solutions containing different nutrient concentrations*

External nutrient (mol m^{-3})				N		K		P	
N	P	K stagnant	K Aerated	Stagnant	Aerated	Stagnant	Aerated	Stagnant	Aerated
10	0·16	6·0	3·2	2·31	4·51	0·37	0·79	0·14	0·36
5	0·08	4·4	1·6	1·97	4·23	0·35	0·83	0·08	0·30
2·25	0·04	3·6	0·8	1·48	3·83	0·18	0·88	0·04	0·19
s.e.				0·07	0·014	0·03	0·03	0·007	0·02

The seedlings were grown for 16 d in aerated solution and then for 8 d in stagnant or aerated nutrient solutions ($n = 3$, $\pm$s.e.). Dry weights of shoots, seminal and nodal roots were not affected by increase in external and internal nutrient concentrations.

TABLE. 7. *Dry weight (mg) and P concentrations (mmol g^{-1} d. wt) in the shoots of wheat seedlings that had grown for 16 d in aerated solution and then for another 9 d in stagnant solution (0·1% agar, no turbulence) at different P concentrations*

	P in tissues (mmol g^{-1} d. wt)		D. wt of organs (mg)		
External P (mol m^{-3})	Shoots	Roots	Shoots	Nodal roots	Seminal roots
0·16	0·13 ± 0·02	0·18 ± 0·005	133 ± 30	15 ± 1	26 ± 3
0·32	0·14 ± 0·01	0·20 ± 0·007	147 ± 25	17 ± 2	25 ± 2
0·64	0·15 ± 0·01	0·18 ± 0·004	127 ± 20	16 ± 1	27 ± 4
1·28	0·20 ± 0·01	0·25 ± 0·009	127 ± 22	15 ± 2	26 ± 3

($n = 3$, $\pm$s.e.).

found for shoots with roots in aerated solution (Table 6). Further increases in external P concentration resulted in only small increases in tissue P concentration between 0·16 and 0·64 mol m^{-3}, but at 1·28 mol m^{-3} external P, the P concentration in the shoots was 33% higher than at 0·64 mol m^{-3} [$H_2PO_4^-$ and HPO_4^{2-}] (Table 7). Similar trends were found for the roots (Table 7).

In plants with roots in stagnant solution, the observed increases in internal N, K and P, with increases in external concentrations, did not improve growth of shoots or seminal and nodal roots (Tables 6 and 7).

DISCUSSION

The use of 0·1% agar in nutrient solution to simulate the low convection in waterlogged soils was successful. There was a rapid decrease of O_2 during the first day in stagnant solution, while ethylene increased over 10 d; such changes are similar to those described for waterlogged soil by Trought and Drew (1980*a*).

The main features of the plant response to their roots being exposed to stagnant solutions were a higher % of aerenchyma and longer nodal roots than in conventional N_2-flushed solutions. Furthermore, the dispersion of the dye in the 0·05% agar solution over 3–5 d is consistent with the hypothesis of Healy and Armstrong (1972) that, at this agar concentration, convection is still substantial enough to prevent build up of high O_2 concentrations in the rhizosphere due to radial O_2 loss from the roots. In turn this loss of O_2 from the rhizosphere reduced root elongation relative to 3% agar (Healy and Armstrong 1972).

Changes in root characteristics in waterlogged soils and in the present stagnant solution (0·1% agar) (summarized in Table 8) could be caused by: (1) oxygen deficiency; (2) high ethylene or CO_2 concentrations (we have no information to speculate on the possible effects of CO_2); (3) interaction between high ethylene or CO_2 concentrations and oxygen deficiency; or (4) indirect changes due to growth reductions associated with changes in gaseous composition in the rhizosphere. For example, a relative increase in the length of lateral roots may be associated with cessation, or reduction, of growth of the main axes.

Aerenchyma

Aerenchyma will be discussed first because of its importance for roots in waterlogged soil and the substantial understanding about mechanisms of its formation. Aerenchyma in the stagnant solution (0·1% agar) was about 20% of the root sectional area, which was similar to values for wheat roots in waterlogged soil (Thomson *et al.*, 1992). In contrast, the percentages of aerenchyma were 7·6 and 2·4% for semi-stagnant and N_2-flushed solutions (Table 2). These results are therefore a prime example of the success of the use of agar to prevent convection in nutrient solution. Aerenchyma formation in maize roots is induced by ethylene (Drew, Jackson and Giffard, 1979; Jackson and Drew, 1984). At present there is no experimentation

TABLE 8. *Schematic presentation of root characteristics in the three hypoxic treatments and in the aerated solution*

Nodal roots				
% aerenchyma	stagnant	> semi-stagnant	> N_2-flushed	= air
Mean length main axes	semi-stagnant	> stagnant	> N_2-flushed	≈ air
Weight	stagnant	> N_2-flushed	> semi-stagnant	= air
Number of main axes	N_2-flushed	= stagnant	> semi-stagnant	= air
Ratio length laterals/ main axes	stagnant	≈ N_2-flushed	> semi-stagnant	= air
Seminal roots				
Mean length main axes	N_2-flushed	= stagnant	< air	< semi-stagnant
Weight	stagnant	< N_2-flushed	< semi-stagnant	≈ air
Ratio length laterals/ main axes	semi-stagnant	≈ air	> stagnant	> N_2-flushed

available to establish whether this conclusion also applies to wheat. Nevertheless, it is reasonable to assume that the large aerenchyma formation in nodal roots grown in stagnant solution is the result of the accumulation of ethylene within the tissues and the rhizosphere of the roots. Accumulation in the rhizosphere would not occur in either the semi-stagnant or the N_2-flushed solution because of convection and this in turn would deplete internal ethylene. Following the return from hypoxic to aerated solution, a decrease in the percentage of aerenchyma in the roots exposed previously to semi-stagnant and stagnant solutions, was presumably due to the rapid cessation of aerenchyma formation in newly formed root tissue in well aerated conditions (Watkin, Greenway and Thomson, unpubl. res.).

Growth in the stagnant solution

The dry weight and nutrient concentrations in the shoots of plants with roots in stagnant solution was the lowest of all the treatments (Tables 3 and 5, respectively). This was particularly surprising as there were longer main axes and laterals, and much more aerenchyma in the nodal roots when grown in stagnant than in N_2-flushed solutions. Furthermore, in stagnant solution the rhizosphere was likely to become oxygenated, at least around the nodal roots, due to the slow diffusion of O_2 lost from the roots. In contrast, in the N_2-flushed solution, O_2 outside the unstirred layers would be mixed rapidly with the bulk of the solution.

There are several possible explanations for the poor growth by plants with roots in stagnant solution: (1) roots in the N_2-flushed solution may receive 7–35% of their maximum O_2 requirement from the low level of O_2 in the nutrient (calculated by Kuiper, Walton and Greenway, 1994). This supply is presumably derived from impurities in the N_2 gas and from a small leakage from the air, and would be of particular advantage for the seminal roots since these have little or no aerenchyma and, hence, would suffer severe O_2 deficiencies in the stagnant solution; (2) high ethylene and/or high CO_2 adversely affected growth in the stagnant solution, while these high concentrations would not occur in the rhizosphere of the roots in the other solutions; (3) the leaves of the plants with roots in stagnant solution may have been nutrient deficient because their internal nutrient concentrations were lower than for plants with roots in the N_2-flushed solution and much less than in aerated solution (Table 5). However, there are no indications of N, K and P deficiencies, since the growth of shoots and of seminal and nodal roots did not improve even when higher external N, K and P concentrations increased their internal concentrations (Tables 6 and, for P, Table 7). An additional relevant observation was that despite this lack of growth response due to an increase in external P, the P concentration in leaves of plants with roots at 0·08 mol m^{-3} phosphate was 0·23% (on a dry weight basis; Table 6) higher than the deficiency level of 0·1% internal P (Mengel and Kirkby, 1982), but lower than the adequacy levels of 0·3–0·45% P on a dry weight basis (Mengel and Kirkby, 1982; Baker and Tucker, 1973, respectively). Thus, other adverse factors appeared to override a potential P deficiency. Of course, mineral nutrients other than N, K and P could be limiting. However, this seems unlikely since external P concentrations in the most comprehensive experiment (Table 2–4) were particularly low relative to P uptake by the roots. For example, the 16:4:1 ratio of uptake of N:K:P (data for wheat in a comparable growth stage by Buwalda *et al.*, 1988) was much narrower than the 63:76:1 ratio (N:K:P) for the concentration in the stagnant solution. Diffusion coefficients for most ions in aqueous media are roughly similar. Thus, if the diffusion limitations on P uptake do not lead to reduced growth, it is very unlikely that this would be so for other nutrients, since they are supplied in greater concentrations relative to their rate of uptake.

The suggestion that nutrient deficiency did not limit growth in stagnant solution is inconsistent with improvement of wheat growth in anaerobic media when supplied with higher concentrations of all nutrients (Trought and Drew, 1981; Huang *et al.*, 1994). Such discrepancies may be due to the history of the plants before transfer of roots to anaerobic media. Additionally, the duration of the anaerobic treatment is important since nutrients, accumulated during luxury consumption before anaerobiosis is imposed, may delay and/or mitigate deficiencies caused by the greatly reduced uptake during anaerobiosis (Trought and Drew, 1981). Furthermore, the duration of the experiment testing possible nutrient deficiencies was only 8 d (Tables 6 and 7). Hence, we cannot exclude the possibility that deficiencies

developed during the 15 d of treatment in the experiment in which we compared the response to stagnant and N_2-flushed nutrient solutions (Tables 3–5).

High external P concentrations lead to the risk of P toxicity; however, the P concentrations in the shoots at an external concentration of 1·28 mol m^{-3} P remained below the toxic level defined by Loneragan, Carroll and Snowball (1966). The sudden increase in P concentration in the plants grown in stagnant solution containing 0·64 and 1·28 mol m^{-3} P, respectively, may be due to an increase in a pathway for passive influx during anaerobiosis (Trought and Drew, 1980*b*), with the amounts of solutes being transported only becoming appreciable at high external concentrations.

Root development

Nodal root length. The most likely explanation for the longer main axes of the nodal roots in stagnant and semi-stagnant, than in N_2-flushed solution (Table 4), is the better internal oxygen supply to the root tips. Roots in both stagnant treatments have more aerenchyma than the roots from the N_2-flushed solution. Furthermore, in the stagnant solution, radial oxygen loss would be mitigated by oxygenation of the rhizosphere, generating a lower concentration gradient for radial O_2 loss; in contrast turbulence in the N_2-flushed solution would set the dissolved O_2 concentration at the outer boundary of the unstirred layer of the epidermis at the same low concentration as in the bulk solution. It would be of interest to evaluate these O_2 gradients, using micro O_2 platinum electrodes (cf. Armstrong *et al*., 1994).

Despite the relatively high O_2 supply to the roots in the stagnant solution, their growth was presumably still limited to some extent by restricted O_2 supply. This was suggested by the finding that elongation of 12 cm long roots was stimulated four-fold when the shoots were placed in gas containing 42 % O_2(Wiengweera, 1994).

Nodal root axes and total length of laterals. It is puzzling that there were more nodal roots and a higher ratio of length of laterals to length of main axes in N_2-flushed and stagnant solutions, than in semi-stagnant and aerated solutions. The effects cannot be readily attributed to ethylene, since concentrations of this phytohormone would almost certainly be higher in the two stagnant solutions than in N_2-flushed and aerated solutions (cf. Fig. 1). Perhaps, slow growth of the main nodal root axes in N_2-flushed and stagnant solutions induced more lateral root growth, i.e. apical dominance was weakened. This suggestion is not negated by the equal or shorter length of nodal roots in aerated than in stagnant nutrient solution, since a partial inhibition of elongation in the stagnant solution was conclusively demonstrated by increasing O_2 concentrations around the shoots (see above).

Seminal roots. For seminal roots, O_2 deficiency can probably account for the smaller weights and lengths of the main axes and the much smaller lengths of laterals in the stagnant and the N_2-flushed solutions than in the semi-stagnant and aerated solutions (Table 4), since the first two treatments are most likely to suffer O_2 deficiency. Major effects of ethylene are unlikely because of the different responses in the two treatments which are likely to be high in ethylene, i.e. semi-stagnant and stagnant solutions. The small proliferation of laterals of the roots in the stagnant and N_2-flushed solutions was despite their likely loss of apical dominance (cessation of elongation of main axes).

CONCLUSIONS

Before evaluating the advantages of the use of agar to prevent convection in nutrient solutions, we would first like to emphasize that several aspects of the agar system do not simulate waterlogged soils. These include: (*a*) the microflora in the stagnant solution (0·1 % agar) is presumably much smaller than in most soils, because the solutions were sterilized before use, and there would be much less substrate for the microflora in the stagnant solution than in soils. The microflora in soils is responsible for lowering redox potentials and associated increases in soluble heavy metals, and production of gases such an methane, N_2O and H_2S (Ponnamperuma, 1984) and of weak organic acids, which are end products of microbiological anaerobic catabolism (Drew 1990); (*b*) interaction between anoxia and infection by pathogens; (*c*) large consumption of radially lost O_2 from the roots by the microflora of the soil, creating a steeper O_2 gradient in the rhizosphere than would occur in the stagnant solution; (*d*) there may be more convection in some soils than in the stagnant solution; (*e*) the stagnant solution requires use of weak acids to buffer rhizosphere pH, and these weak acids may have side effects. Nevertheless, there are many advantages of the agar based root growth medium detailed in this paper, which balance these constraints. These are: (*a*) the stagnant solution simulates the changes in concentration of dissolved O_2, ethylene and CO_2 of waterlogged soil, and presumably also the concentrations and rates of movement of these dissolved gases in the rhizosphere. This simulation is not achieved or is achieved to a lesser extent, in other techniques, such as N_2-flushing and using nutrient solution without air flushing; (*b*) the composition of the stagnant solution is better defined than in soil, while the stagnant solution can be readily manipulated, for example to apply inhibitors of ethylene synthesis; (*c*) roots can be observed throughout the experiment using transparent vessels; (*d*) the stagnant solution (0·1 % agar) allows non-destructive evaluation, using short time intervals, of mineral nutrient uptake from the solution and of development of roots; for example their surface area (Sattelmacher, Klotz and Marschner, 1983) and volume (Sattelmacher, 1987); and (*e*) the possible straightforward transfer of roots from stagnant solution to fresh media allows the study of recovery.

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