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THE EFFECT OF A LOW OXYGEN CONTENT OF THE MEDIUM ON THE ROOTS OF BARLEY SEEDLINGS

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ABSTRACT

Barley seedlings were cultivated on aerated nutrient solutions (air-plants), and on nutrient solutions through which nitrogen gas was passed (nitrogen-plants). Roots of nitrogen-plants died when the shoots were deprived of air. Roots of intact nitrogen-plants absorbed, from a solution which contained oxygen, less of this gas than roots of air-plants. In air, the respiration per mg dry-weight of roots of nitrogen-plants, was considerably higher than the respiration of roots of air-plants. It is concluded that in a solution deficient in oxygen, the roots of nitrogen-plants receive this gas from the shoots by diffusion along the intercellular spaces.

In short-term experiments, the uptake of chloride by nitrogen-plants, calculated per mg dry-weight from a solution deficient in oxygen, was about equal to the chloride uptake by air-plants from aerated solutions. The uptake of potassium by nitrogen-plants from a solution deficient in oxygen was higher than that by air-plants from an aerated solution. When nitrogen-plants were transferred to aerated solutions, the uptake of both chloride and potassium, was much higher than the uptake of these ions by air-plants.

The content of soluble sugars of the roots of nitrogen-plants was more than 100 % higher than that of the roots of air-plants; their protein-content was from 4 %–25 % higher.

1. INTRODUCTION

A well-developed system of large intercellular spaces is characteristic of the anatomy of most waterplants. In land plants the intercellular spaces are much smaller, and in many tissues, they are not more than narrow capillaries between the cells. When roots of land plants are growing in a medium which is deficient in oxygen, large irregular air-spaces may develop in the cortex, sometimes to such an extent that the cortex is reduced to a small number of radial cell plates (DUNN, 1921). These air spaces often contain oxygen even when this gas is not present in the surrounding medium. It has been demonstrated both in Cladium mariscus and in rice, that oxygen which enters the internal atmosphere of the leaf bases, diffuses from there into the roots (CONWAY, 1937, VAN RAALTE 1940). It seems logical to assume that the oxygen in the air spaces makes possible an aerobic metabolism in root cells.

WILLIAMS and BARBER (1961) recently pointed out that roots growing in an oxygen-deficient medium may have a much lower oxygen requirement than the tissues of normal land plants. These authors state: "Since we can hardly change the basic properties of living cells,

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this is equivalent to saying that the submerged portions of aquatic plants must contain the smallest possible amount of living tissue".

Actually the effect of submergence on the roots is to reduce the living tissue of the cortex. It is not, however, selfevident that this is the only way in which roots may adapt themselves to submerged conditions. Changes in the physiological processes of the root cells need not go so far as to change their "basic properties". It is, for instance, quite conceivable that the cells of submerged roots would make a more economic use of the available oxygen, than the cells of roots which are growing in a well-aerated soil.

The present work was intended to investigate the effect of growth in an oxygen-deficient medium on the physiology of roots. To this end, barley seedlings were grown either in an aerated nutrient solution or in a solution which contained no oxygen, for a period of six days. At the end of that period the effect on the roots of the two treatments was investigated.

In the following sections plants grown in aerated solution will be referred to as "*air-plants*", plants grown in oxygen-free solutions as "*nitrogen-plants*".

2. General methods

The barley variety, "Union Zomergerst", was used in most of the experiments. The seeds were germinated on wet filterpaper, in large petridishes which were kept in a dark room at a temperature of about 22° C for two days. The germinated seeds were transferred to a Hoagland nutrient solution of ¼ the normal concentration. After another two days the seedlings were mounted in the lids of cylindrical perspex vessels, the diameter of which was 6 cm and their height, 8 cm. They were provided with a vertical side tube for filling, and an outlet in the bottom to drain off the nutrient medium.

Two small side tubes, one near the bottom and one near the top, served as inlet and outlet for a stream of air or nitrogen. The lids had been perforated, and the plants were mounted in the lid by passing the coleoptile from below through one of the holes and holding them in position by means of cotton wool.

Sets of 20–40 seedlings were mounted in each lid. The lids were then sealed on the vessels and a layer consisting of a mixture of paraffin wax and bees-wax, was smeared on the lids around the plants to prevent any diffusion of oxygen from the air into the vessels. This had to be done very carefully, otherwise oxygen from the air diffused through the plugs of cotton wool in the holes of the lid into the vessels, even when the latter contained nitrogen at a pressure which was slightly higher than atmospheric. As a check on the air-tightness of the vessels, the gas that was passed through them was led into a small beaker containing a shallow layer of water. Gas bubbles ceased to come out when a leak occurred. These precautions are important, since only at low concentrations does oxygen limit the process of ion-uptake by the roots. Between the nutrient medium and the lid, a space of about

one centimeter remained in order to keep the grains free from the liquid in the vessels.

When the seedlings had been mounted in the lids they were allowed to grow for six days at a temperature of 21°–22° C. They were illuminated by means of Philips fluorescent tubes for 16 hours daily. The nutrient solution was a four times diluted Hoagland solution to which A–Z solution of the normal concentration was added. A stream either of air, or of commercial nitrogen, which had been purified by passing it through pyrogallol, or of nitrogen containing a certain percentage of oxygen, was passed continuously through the solution.

After six days, the seedlings were used for the experiments. If ion-uptake was to be determined, the nutrient solution was replaced by 5×10^{-4} Mol. of potassium chloride. The absorption by the plants during a period of 5 hours was determined by measuring the concentration of the solution. Potassium was determined by flame photometry, and chloride was titrated with silver nitrate using a silver-electrode for establishing the end point. (PIPER, 1944) Each set of seedlings was dried in an oven; the dry weights of the shoots and the roots were determined separately.

3. EXPERIMENTS

3.1. *The effect of a low oxygen tension on ion-absorption*

The uptake of chloride by air-plants and by nitrogen-plants is shown in Table I. During the 5 hours in which the chloride uptake took place, the solutions of half of the sets of air-plants were aerated whereas the roots of the other half were in oxygen-free solutions. The nitrogen-plants were treated similarly, the chloride being taken up by the roots both from aerated solutions and from solutions in which oxygen was excluded. The results show that the absence of oxygen during uptake, decreases the chloride absorption. The effect of oxygen on nitrogen-plants is to increase their chloride absorption; these plants absorbed more chloride than did the plants which had been given an aerated culture solution during their total growth period.

Table II gives the results of a similar experiment. During the uptake of chloride, the roots of the seedlings were in a solution of the same oxygen content as that in which they had been cultivated, i.e. either in an aerated solution or in oxygen-free solutions. In this experiment the exclusion of oxygen from the nutrient solution had reduced the growth of the seedlings, root growth being affected more than the growth of the shoots. Apart from Experiment 6, the actual chloride uptake by air-plants was higher than that in nitrogen-plants; whereas chloride uptake per mg. root dry weight was lower in air-plants except for Expt. 1.

Table III shows the uptake of potassium by air-plants and by nitrogen-plants. As in the experiment of Table II, the absence of oxygen from the culture solution has decreased the growth of the roots more than the growth of the shoots, but this reduction in size of the root system has not affected the potassium uptake: the amount taken

TABLE I

The uptake of chloride by barley seedlings from aerated and from oxygen-free solutions. The seedlings were grown on diluted Hoagland solution; uptake was determined from 5×10^{-4} N potassium chloride Nitrogen gas was continuously bubbled through the oxygen free solutions, air was bubbled through the aerated solutions. Each figure gives the uptake and the dry weight, of a group of 20–24 seedlings.

Gas passed through the nutrient solution		uptake, μg Cl^-		dry weight, mg/set :	
during 6 days culture period	during 5 hours uptake expt.	total in 5 hours	per mg of root dry weight	roots	shoots
		1005	9.8	102.1	460.1
	1a air	1012	10.2	99.2	562.3
		1061	10.3	102.8	491.8
Expt. 1 air					
		632	6.6	95.6	475.1
	1b nitrogen	653	6.6	99.3	490.5
		572	5.9	96.7	483.9
		1207	12.0	100.6	547.2
	2a air	1264	13.2	95.7	555.3
		1274	13.1	97.2	635.7
Expt. 2 nitrogen					
		852	8.9	95.7	618.0
	2b nitrogen	692	7.6	91.4	694.2
		749	7.7	97.4	595.5
		845	9.1	92.4	469.0
	3a air	1040	10.3	100.9	522.1
		919	10.4	88.4	497.3
Expt. 3 air					
		706	7.7	91.7	503.1
	3b nitrogen	646	6.5	98.8	525.5
		540	5.6	95.9	513.8
		1278	12.9	99.0	479.5
	4a air	1230	12.1	102.3	504.6
		1218	13.0	93.7	510.3
Expt. 4 nitrogen					
		667	7.5	89.0	513.5
	4b nitrogen	781	8.2	95.7	480.2
		643	7.2	89.6	485.7

up by the small root system of the nitrogen-plants is about equal to the uptake by the larger root system of the air-plants. As the ratio uptake/shoot dry weight in air-plants is equal to that in nitrogen-plants, it may be argued that potassium uptake is determined by the size of the shoots. But the decreased uptake by air-plants when oxygen is excluded from the uptake solution, (sets 3 and 4) and the increase in uptake by nitrogen-plants when the uptake-solution is aerated (sets 7 and 8), show that it is the activity of the roots that chiefly determines the uptake, and not the size of the shoots.

The experiments described in this section have shown that seedlings of barley can be grown in oxygen-free solutions and that the uptake

TABLE II

Uptake of chloride from 5×10^{-4} *N KCl by barley seedlings from aerated and from oxygen-free solutions.*

"Air-plants" had been grown for 6 days on aerated solutions, "nitrogen-plants" on oxygen-free solutions. During the uptake experiment, the KCl solution of the "air-plants" was aerated, oxygen was excluded from the KCl solution of the "nitrogen-plants".

Each figure gives the uptake and the dry weight of a group of 20–24 seedlings.

Expt. no.		Uptake in 5 hours			total dry weight			
		μg Cl^-		μg/mg dry wght of roots	roots		shoots	
1.	air-plants	788	100 %	8.8	89.7	100 %	347.2	100 %
	nitrogen-plants	662	84 %	8.8	75.0	84 %	347.1	100 %
2.	air-plants	976	100 %	10.2	93.4	100 %	362.5	100 %
	nitrogen-plants	820	84 %	13.1	62.6	67 %	324.0	89 %
3.	air-plants	501	100 %	6.4	81.9	100 %	350.8	100 %
	nitrogen-plants	462	92 %	7.6	60.5	74 %	307.4	88 %
4.	air-plants	713	100 %	7.9	90.1	100 %	334.1	100 %
	nitrogen-plants	692	97 %	10.0	69.0	77 %	319.7	96 %
5.	air-plants	962	100 %	11.2	85.1	100 %	315.1	100 %
	nitrogen-plants	856	89 %	13.2	64.5	76 %	317.6	101 %
6.	air-plants	607	100 %	6.7	90.1	100 %	358.6	100 %
	nitrogen-plants	657	109 %	10.0	65.5	73 %	324.6	91 %

TABLE III

Uptake of potassium from aerated and from oxygen-free solutions of KCl *by "air-plants" and by "nitrogen-plants".*

Uptake from 5×10^{-4} N KCl during 5 hours.

Each figure gives the uptake and the dry weight of a set of 20–24 seedlings.

set no.	air-plants or nitrogen-plants	gas in solution during uptake experiment	total μg K^+ in 5 hrs	Uptake μg K^+ per mg dry wght of roots	μg K^+ per mg dry wght of shoots	Total dry weight roots mg	shoots mg
1.	air-plants	air	1147	13,7	3,5	84.0	331.6
2.	air-plants	air	1166	12.4	3.2	93.9	360.7
3.	air-plants	nitrogen	801	9.7	2.4	82.5	336.8
4.	air-plants	nitrogen	813	9.0	2.3	90.8	346.5
5.	nitrogen-plants	nitrogen	1014	16.2	3.4	62.6	297.9
6.	nitrogen-plants	nitrogen	1186	18.9	3.8	62.8	312.1
7.	nitrogen-plants	air	1195	19.5	4.1	61.2	292.4
8.	nitrogen-plants	air	1271	20.2	4.3	62.8	294.1

of chloride and potassium by these seedlings, calculated per mg root dry-weight, is often higher than the uptake by seedlings grown in aerated solutions.

3.2. *The effect of the oxygen in the atmosphere around the shoots*

VLAMIS and DAVIS (1944) have demonstrated that the capacity of barley plants to absorb potassium ions, is not impaired by a low oxygen content of the nutrient solution. They concluded that oxygen is translocated from the shoot to the roots.

BRYANT (1934), studying the effect of oxygen-deficiency on submerged barley roots, found that at low oxygen tensions large irregular air spaces develop in the root cortex. It is likely that these air spaces form a continuous system which is connected with the intercellular spaces of the leaves.

In order to investigate the effect on the roots of the atmosphere around the shoots, a perspex cylinder provided with two side tubes was placed over the leaves; it was screwed on top of the perspex vessels that contained the roots. By means of the side tubes in the upper cylinder the atmosphere around the shoots could be varied.

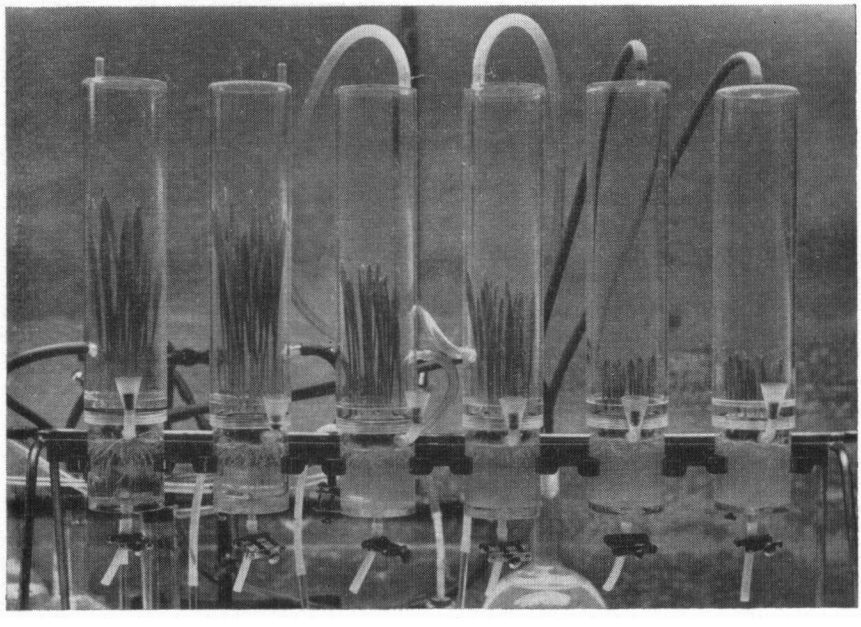

Photo 1.
Effect of the atmosphere around the leaves on the growth of the roots.
Roots in Hoagland solution through which a stream of purified nitrogen was led.
Leaves in: air (2 cylinders at the left)
nitrogen (2 cylinders at the right)
nitrogen + 1 % CO^2 (2 middle cylinders).
The roots are normal when the leaves are in air. Oxygen formed in photosynthesis is not sufficient for aeration of the rootsystem (middle cylinders). (Photo after 7 days).

In the experiment of photo 1, a stream of purified nitrogen gas was continuously blown through the solutions in all six vessels. The atmosphere around the leaves consisted of air in the two cylinders at the left, of pure nitrogen in the two cylinders at the right and of nitrogen containing 1 % carbon dioxide in the two middle cylinders. The plants were under these conditions for 7 days. The photograph shows the plants at the end of that period. The root systems belonging to the shoots which had been in air, had the normal appearance of nonaerated submerged barley roots. The roots in the other vessels had hardly grown; moreover, bacteria had developed abundantly in the nutrient solution, presumably on the substances which had diffused out of dead cells. These results show conclusively that root growth in an oxygenfree nutrient medium depends on the uptake of oxygen by the shoots. This confirms the results which SOLDATENKOV and CHAO (1961) obtained by a similar experimental arrangement with beans. The experiment also demonstrates the effect of photosynthesis.

In the two cylinders at the right of Photo 1 photosynthesis must have been absent, or at least proceeding at a very low rate, since no carbon dioxide was supplied to the shoots. Accordingly, the shoots were short. In the two middle cylinders the nitrogen contained 1 % carbon dioxide and the shoots are rather well developed, though to a lesser degree than the shoots in the two cylinders at the left that had been growing in air.

Table IV gives the dry-weights of the shoots and of the roots in a similar experiment. The control plants had been growing on an aerated nutrient solution in a normal atmosphere. The dry-weights of the root systems in the nutrient solutions from which oxygen had been excluded were smaller than the dry-weights of the control roots. The reduction of the root dry-weight was greatest in the plants of which the shoots had been in pure nitrogen. When the shoots had been growing in nitrogen + carbon dioxide, the dry-weight of the roots

TABLE IV

Dry-weights of barley seedlings, the roots of which had been growing in oxygen-free nutrient solutions for 7days, the shoots being in air, in nitrogen + 1 % carbondioxide, or in pure nitrogen.

set no.	gas in nutrient solution	shoots in	dry-weight roots mg	average	dry-weight shoots mg	average
1 (control)	air	air	122.4	100 %	395.2	100 %
2 (control)	air	air	120.0		377.1	
3	nitrogen	air	77.4	63 %	407.6	105 %
4	nitrogen	air	74.4		404.2	
5	nitrogen	N^2+1 % CO^2	50.1	40 %	283.9	73 %
6	nitrogen	N^2+1 % CO^2	46.2		275.6	
7	nitrogen	N^2	31.0	26 %	121.4	34 %
8	nitrogen	N^2	32.6		138.4	

was higher due to photosynthesis by the leaves. However, the oxygen produced in photosynthesis did not prevent the eventual decay of the roots. In sets No. 3 and No. 4, the shoots of which had been growing in air, the exclusion of oxygen from the nutrient solution had caused a decrease in the dry-weight of the roots, but the roots were longer than those of the controls, and looked perfectly healthy.

3.3. *The transfer of oxygen along the roots*

The experiments of the preceding section have demonstrated that, for a normal development of barley roots in an oxygen-deficient nutrient solution, the shoots have to be in contact with atmospheric oxygen. It seems logical to conclude that oxygen is translocated from the shoots to the roots, but this assumption has to be proved experimentally. Translocation of oxygen may be demonstrated by analysis of the gas in the intercellular spaces of the roots. In the present experiments we investigated whether the roots gave off oxygen to the environment, since if this was the case, translocation along the roots must have taken place earlier.

The oxygen concentration in the medium was determined by a method after Tödt (1958) modified according Spruit [1]). The vessel that contained the plants was identical to the normal experimental vessels, except that it contained a gilded platinum electrode and was connected by means of a side tube to the mercury electrode (Fig. 1). The side tube contained saturated KNO_3 solution in agar. The surface of the mercury ($\pm$ 2 cm^2) was covered by a layer of calomel. The medium, a 5×10^{-4} molar solution of KCl, was vigorously stirred by means of a magnetic stirrer. When a potential difference of about 1 volt was applied to the electrodes the current passing through the solution was proportional to its oxygen content up to about 12 % of the saturation value. The current was measured by means of a Kipp A-70 galvanometer.

Two types of experiments were carried out. In the first, a lid containing a set of 24 seedlings was hermetically sealed on to the experimental vessel. The solution in the vessel was freed from oxygen by bubbling a stream of nitrogen through it, purified by means of pyrogallol solutions. When all the oxygen was removed, the nitrogen supply was shut off and the deflection of the galvanometer was recorded for a certain time.

Where the seedlings had been growing in an aerated solution during the previous six days, the oxygen content of the solution in the experimental vessel remained very low, but with seedlings which had been raised in an oxygen-free culture solution, the oxygen content, as indicated by the galvanometer, rose continuously. Evidently in these seedlings oxygen was translocated from the shoots to the roots. If the shoots were cut just above the lid the excretion of oxygen into the surrounding solution stopped, and this seemed to indicate that the

[1]) The authors are indebted to Dr. C. J. P. Spruit at Wageningen for many useful improvements of the original method.

presence of the leaves is necessary for the supply of oxygen to the roots. However, at close inspection of the stumps of the cut shoots, it was noted that small drops of liquid lay on the surface. If this liquid penetrated into the intercellular spaces, the diffusion path of oxygen from the atmosphere into the roots would be blocked. To avoid this

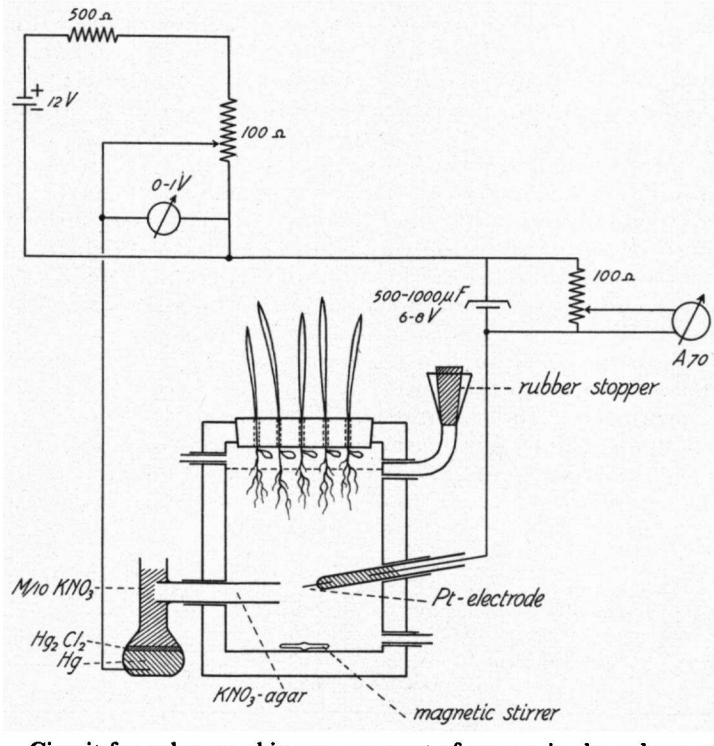

Fig. 1. Circuit for polarographic measurement of oxygen in the culture solution.

error, in the next experiments a stream of hot air from a hair dryer was directed on to the surface of the shoot stumps immediately after cutting the shoots. This caused any liquid which was oozing out of the vessels to evaporate so that no drops were formed. Under these conditions the excretion of oxygen by the roots continued after cutting the shoots. We may conclude from this experiment that in plants which have been raised in an oxygen-free solution, oxygen may diffuse by way of the large intercellular spaces into and along the roots and that for this process the presence of the leaves is not required.

The fact that the roots from plants in aerated solutions did not give off oxygen, does not necessarily imply that no oxygen is transferred along the roots. Excretion of oxygen into the environment requires that the rate of supply to the roots is higher than the rate at which the oxygen is used up. The excretion of oxygen by the roots of nitrogen-plants may be the result of a high rate of supply, or by their respiration

being less than that of the roots of oxygen-plants. In order to distinguish between these two possibilities, a comparison was made, —not of the oxygen excretion—but of the oxygen consumption by the roots of oxygen-plants and nitrogen-plants. To this end, the solution in the electrode vessel was saturated with air. From such a saturated solution the roots absorb oxygen. In Table V, columns 1 and 4 give the decrease of the oxygen content of the solution in galvanometer units after 45 minutes. Evidently, the roots of oxygen-plants absorb oxygen at a greater rate than the roots of nitrogen-plants. This agrees with the fact that, in an oxygen-free solution, the first do not give off oxygen.

After the roots had absorbed oxygen for 45 minutes, the shoots were cut, and as was stated above, the transport of oxygen to the roots is then blocked by the liquid which is excreted at the cut surface. The solution in the electrode vessel was again saturated with air by passing an air current through it for a few minutes. After that the decrease of the oxygen content that took place within 45 minutes was once more determined. Columns 2 and 5 of Table V show that now both types of roots absorb oxygen much faster than before the cutting of the shoots and, moreover, at equal rates.

This means that the respiration of both types of root systems is about the same. Consequently, the fact that the roots of intact nitrogen-plants excrete oxygen into an oxygen-free solution and in aerated solutions, absorb less oxygen than the roots of intact oxygen-plants,

TABLE V

Absorption of oxygen from a solution of 5×10^{-4} N KCl, *by roots of nitrogen-plants and by roots of air-plants.*

The figures, in galvanometer units, give the decrease in oxygen tension of the medium which took place with in 45 minutes.

nitrogen-plants			air-plants		
Decrease of oxygen content in 45 min.		dry-wt roots mg	Decrease of oxygen content in 45 min.		dry-wt roots mg
Plants intact	Top removed		Plants intact	Top removed	
18	30	36.3	16	20½	52.6
12½	28	38.0	14½	27	57.3
8	28	34.9	17½	27½	51.7
16	29	37.6	17	24	53.6
10½	24	38.5	17	24½	52.7
7½	19	41.0	13	19½	61.0
8	14	27.4	16½	17	54.3
10½	17½	32.3	14	17	44.2
10	23	30.8	13½	21½	40.9
7½	22	32.0	17	27	57.8
7	22	35.0	14	29	53.8
8½	28½	36.2	12	24½	55.1
average:					
10	23½	35.0	15	23	52.9

must be due to a greater rate of oxygen transfer along the roots of the nitrogen-plants.

3.4. *The respiration of roots of nitrogen-plants and of roots of air-plants*

The results of the last section have shown that severed root systems of nitrogen-plants and of air-plants use up the oxygen of the medium at equal rates (Table V). The total dry weight of the air-roots is, however, some 50 % more than that of the roots of nitrogen-plants. This implies that the respiration of the latter, calculated on a dry weight base, must be higher.

Respiration was determined by the Warburg method. The roots were cut into pieces of 2 cm length. The roots of each set of plants were distributed over two Warburg vessels. To one of these 3 ml of water were added, to the other 3 ml of 0,5 % glucose or, in a second experiment, 5×10^{-4} M KCl. The temperature during the experiment was 30° C. The results are given in Table VI. According to expecta-

TABLE VI

Respiration by nitrogen-plant roots and air-plant roots.

In Experiment 1 the roots in the Warburg vessel were suspended in water or in 0,5 % glucose; in Experiment 2 in water or in 5×10^{-4} N KCl.

Expt. no.	roots from	roots in	respiration µl/mg dry-wt/hr
1	air-plants	water	5,8
	air-plants	water	6,1
	air-plants	glucose	6,7
	air-plants	glucose	4,4
	nitrogen-plants	water	10,1
	nitrogen-plants	water	9,7
	nitrogen-plants	glucose	8,0
	nitrogen-plants	glucose	10,9
2	air-plants	water	6,7
	air-plants	water	7,4
	air-plants	KCl	7,9
	air-plants	KCl	7,1
	nitrogen-plants	water	12,3
	nitrogen-plants	water	12,8
	nitrogen-plants	KCl	13,5
	nitrogen-plants	KCl	12,7

tion, the respiration of the roots of nitrogen-plants was higher than that of the roots of air-plants. Within the limits of the experiment glucose had no effect and it certainly did not raise the respiration level of air-roots to that of the nitrogen-roots. Lack of respiratory

substrate does not seem to be the cause of the lower respiration of the roots of air-plants.

Potassium chloride was added in the second experiment of Table VI to investigate whether respiration was increased by the uptake of ions. The result does not show such an effect. This need not imply that ion-uptake does not increase respiration, because it is not known if, under the conditions of the experiment, the excised root fragments absorbed any ions at all.

3.5. *The content of soluble sugars in nitrogen-plants and in air-plants*

HOAGLAND and BROYER's (1936) classical experiments on uptake have shown that low salt plants, which accumulate ions at a higher rate than high salt plants, have a higher sugar content. It may be asked whether, in our experiments, the higher rate of ion uptake of the nitrogen-plants was also correlated with a higher content of sugar.

For each experiment six sets of air-plants and six sets of nitrogen-plants were used. The chloride absorption during 5 hours in four sets of each type was determined. During the uptake the solutions were aerated. At the beginning of the absorption period 0,01 M glucose was added to the solution of 2 of the air-plant sets and of 2 of the nitrogen-plant sets. The roots of the remaining two sets of each type were used for the determination of the sugar content. The roots were dried in an oven at 65° C. The dry roots were weighed and cut into small pieces. 100 ml of 75 % ethanol were added. The roots were homogenized in a Waring Blendor. The homogenate was filtered through filterpaper in a Buchner funnel, and the residue was washed with 90 ml of 75 % ethanol. The filtrate was transferred to a 200 ml flask and was made up to volume with ethanol. An aliquot of 10 ml was pipetted into a tube containing 10 ml of anthrone reagent (200 mg anthrone, 8 ml ethanol, 30 ml water, 100 ml conc. sulphuric acid). The tube containing the reaction mixture was placed in a boiling water bath for 7 minutes and then rapidly cooled. The extinction at 620 mμ was determined and compared with a calibration curve made by adding anthrone reagent to known quantities of glucose.

Table VII shows the results of seven experiments. The roots of the nitrogen-plants contain, on a dry-weight base, more than twice as much soluble sugars as the roots of the air-plants. Glucose increased the chloride absorption by the roots of the air-plants in all seven experiments. The increase varied between 8 %–23 %. The effect of glucose on the absorption of chloride by the nitrogen-plants was less significant (increase in six out of seven experiments), and the increase was smaller, viz. 3 %–14 %.

These experiments lead to the conclusion that, at least in air-plants,

TABLE VII

Soluble sugars and chloride uptake by roots of barley seedlings.

Chloride was absorbed from aerated 5×10^{-4} M solutions of KCl.
0.01 M glucose was added to some of the KCl-solutions.
Each figure gives the average of 2 sets of 20–24 plants.

TABLE VII

Expt. no.	air-plants or nitrogen-plants	glucose added	chloride uptake from aerated KCl-solutions during 5 hours µg/mg dry-wt	%	soluble sugars µg/mg dry-wt	%
1	air-plants	—	7,8	100	—	
	air-plants	+	9,4	121	—	
	air-plants	—	—	—	68	100
	nitrogen-plants	—	10,3	132	—	
	nitrogen-plants	+	10,0	124	—	
	nitrogen-plants	—	—	—	154	226
2	air-plants	—	8,3	100	—	
	air-plants	+	9,6	104	—	
	air-plants	—	—	—	61	100
	nitrogen-plants	—	10,5	126	—	
	nitrogen-plants	+	11,3	136	—	
	nitrogen-plants	—	—	—	132	216
3	air-plants	—	7,1	100	—	
	air-plants	+	8,7	123	—	
	air-plants	—	—	—	68	100
	nitrogen-plants	—	10,4	147	—	
	nitrogen-plants	+	10,9	154	—	
	nitrogen-plants	—	—	—	198	290
4	air-plants	—	7,3	100	—	
	air-plants	+	7,9	108	—	
	air-plants	—	—	—	56	100
	nitrogen-plants	—	10,1	140	—	
	nitrogen-plants	+	10,4	144	—	
	nitrogen-plants	—	—	—	130	231
5	air-plants	—	7,2	100	—	
	air-plants	+	7,8	108	—	
	air-plants	—	—	—	67	100
	nitrogen-plants	—	9,3	130	—	
	nitrogen-plants	+	10,1	141	—	
	nitrogen-plants	—	—	—	153	228
6	air-plants	—	6,7	100	—	
	air-plants	+	7,9	118	—	
	air-plants	—	—	—	64	100
	nitrogen-plants	—	10,1	151	—	
	nitrogen-plants	+	11,3	169	—	
	nitrogen-plants	—	—	—	125	190
7	air-plants	—	6,8	100	—	
	air-plants	+	8,3	122	—	
	air-plants	—	—	—	66	100
	nitrogen-plants	—	10,3	151	—	
	nitrogen-plants	+	11,7	173	—	
	nitrogen-plants	—	—	—	144	218

the addition of glucose to the nutrient solution resulted in a slight increase of the chloride uptake. However, by this increase the rate of the chloride uptake by air-plants did not attain that of the nitrogen-plants. Consequently, although the higher sugar content of the nitrogen-plants may be one of the factors which cause their higher uptake capacity, it is not the only one.

3.6. *Protein content and potassium uptake*

The differences between air- and nitrogen-plants, reported in the previous sections, concern physiological processes. They may be caused by qualitative differences of enzyme systems, but it seems more likely that only the quantities of the enzymes per unit root weight of air- and nitrogen-plants differ. As the dry weight of the roots is chiefly determined by the quantity of cell wall material and starch, it is an inadequate measure for the enzymes, which form a part of the protein content of the protoplasm. The quantity of protoplasm cannot be determined, but the insoluble nitrogen present in the roots may be regarded as a measure of it. In this section experiments are described in which the uptake of potassium ions is related to the quantity of insoluble nitrogen in the roots.

The potassium taken up during 5 hours, by sets of 20 air-plants or

TABLE VIII

Uptake of potassium by air-plants and by nitrogen-plants and the insoluble nitrogen of the root systems.

The figures give the average uptake, and the average nitrogen content, of a set of 20–24 plants. The number of sets measured is given in column 3.

Expt. no.	air-plants or nitrogen-plants	number of sets invest.	potassium uptake µg/m dry-wt	%	insoluble nitrogen µg/mg dry-wt	%	K-uptake / N-content
1	air-plants	6	13,3	100	18,6	100	0,72
	nitrogen-plants	5	17,0	128	21,8	117	0,78
2	air-plants	6	11,8	100	17,9	100	0,66
	nitrogen-plants	6	18,8	160	22,4	125	0,84
3	air-plants	3	13,7	100	21,2	100	0,63
	nitrogen-plants	3	17,1	132	24,5	115	0,70
4	air-plants	4	14,2	100	18,9	100	0,76
	nitrogen-plants	6	15,9	112	23,2	123	0,69
5	air-plants	6	14,3	100	19,4	100	0,74
	nitrogen-plants	6	17,0	119	22,6	117	0,75
6	air-plants	6	13,3	100	21,0	100	0,62
	nitrogen-plants	6	17,0	127	24,0	114	0,71
7	air-plants	6	10,6	100	24,3	100	0,44
	nitrogen-plants	6	14,6	138	25,3	104	0,58

nitrogen-plants from aerated KCl solutions, was measured. After the uptake experiment the roots were cut, and their dry weight was determined. The dried roots were extracted with 3 portions of 25 ml distilled water on a shaking machine for 1½ hours. After that the nitrogen content of the roots was determined by means of the micro-Kjeldahl method.

In Table VIII the results of 7 experiments are shown. The potassium uptake by the roots of the nitrogen-plants is consistently higher than that of the air-plants and also their nitrogen content is higher. But a constant ratio between the insoluble nitrogen-content of the roots and their potassium uptake was not found, and the experiments therefore do not substantiate the fact that the potassium uptake of the nitrogen-roots is higher than that of the air-roots because the first contain more protoplasm per unit dry weight.

The fact that nitrogen-roots contain more insoluble nitrogen (i.e. protein) than air-roots, implies that they contain less of other insoluble compounds, especially cell-wall material and starch, This is not caused by a lack of carbohydrates, since in the previous section it has been found that nitrogen roots contain more soluble sugars. It seems probable that the limited air-supply to the roots causes a partial inhibition of the synthesis of polysaccharides.

4. Discussion

The present investigations have shown that the formation of air channels is not the only effect of anaerobiosis on roots, although it is by far the most conspicuous one. Other effects are: increased sugar content, a relative increase in cytoplasm, increased respiratory capacity and an increased uptake-capacity for chloride and for potassium. Some of these effects have been found by other workers. Bryant (1934) reported that the concentration of reducing sugars and total sugar of barley roots grown in an aerated culture solution, is less than that of roots grown in a non-aerated solution.

It may be asked what is the causal relationship between these effects. One of the underlying causes is a decreased polysaccharide content of the roots. The presence of large air spaces in the cortex of roots grown under conditions of anaerobiosis, will result in a smaller dry-weight per unit root length and also, as a consequence of this, in a relative increase of the living matter present in the roots. Unfortunately, although an increase of the living matter, determined as mg insoluble nitrogen per mg dry-weight, was found (Table VIII), a fixed quantitative relationship between this increase and the increase in potassium uptake was absent. In some of the experiments, the percentage increase of potassium uptake over the aerated control was higher than the percentage increase of insoluble nitrogen; in other experiments the increase of nitrogen was higher. The relative increase of the living matter of the nitrogen-roots cannot, therefore, be the sole cause of their increased capacity for ion-uptake and respiration.

The high sugar content of the nitrogen-roots may be another cause of the increased uptake and respiratory capacity. In the experiments

of Table VII, the addition of glucose to the nutrient solution increased the potassium uptake by the air-plants by 8 %–22 %, but, as was pointed out in § 5, this increase was not sufficient to bring the uptake on a level with that of the nitrogen-plants. Here is a parallel with the high-salt and low-salt roots of HOAGLAND and BROYER (1936). These authors reported that when the nutrient solution was aerated, the sugar content of the roots decreased together with the uptake-capacity. The capacity for salt uptake could be restored "at least in part, by supplying sugar to the roots through the culture solution".

It seems logical to assume that the respiration of the roots of nitrogen-plants increases when the plants are transferred to aerated solutions, and that this increased respiration will be coupled with an increased uptake of ions. But Table III shows that even in non-aerated solutions, the capacity of nitrogen-roots to accumulate potassium-ions is higher than that of air-roots in aerated solutions. In this case, the high sugar content, the larger amount of living matter, (calculated per mg dry-weight,) and probably one or more factors which are still unknown, were more likely to promote the uptake, than was the restricted oxygen supply likely to decrease it. The way in which large air-spaces originated in roots which were transferred to non-aerated culture solutions, has been investigated by MCPHERSON (1939) with *Zea Mays*. After the transfer, so many cells of the cortex (which originally had the appearance of normal parenchyma) died and collapsed, that the volume occupied by intercellular spaces, eventually became greater than that occupied by living cells. Prior to the death of the cortex cells, the reaction of the cell wall to the cellulose reagent potaassium iodide + sulfuric acid changed. In the normal cells, the walls gave no cellulose reaction, whilst in the dying cells the cell-walls gave a blue colour with the reagent. MCPHERSON concluded that the cell-wall of normal cells contained protein that disappeared in dying cells, leaving cellulose. Evidently, chemical changes took place in the cell-walls, by which they were weakened. BRYANT (1934) found that the thickness of the cell-walls of roots of barley plants growing in non-aerated solutions, differed from that of the cell-walls of roots in aerated solutions.

The alterations, found by these authors in the cell-walls of the roots of nitrogen plants, suggest that decomposition of the polysaccharide of the cell-walls is a major factor in the formation of large intercellular spaces. This decomposition may be the result of a shifting to the left of the equilibrium in the complex reaction soluble sugars $\rightleftharpoons$ poly-saccharides. In other words, formation of new polysaccharide molecules from soluble sugars may also be inhibited.

According to this hypothesis, the formation of large intercellular spaces, the accumulation of soluble sugars and the relative increase of living matter in the roots of nitrogen-plants would be the results of one effect, viz. the inhibition of polysaccharide formation from sugars, by a low oxygen tension.

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THE EFFECT OF A LOW OXYGEN CONTENT OF THE MEDIUM ON THE ROOTS OF BARLEY SEEDLINGS

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ERRATUM

This photograph appeared in the previous issue on page 236. It is reproduced here because detail of the root-growth was lost in the previous printing.

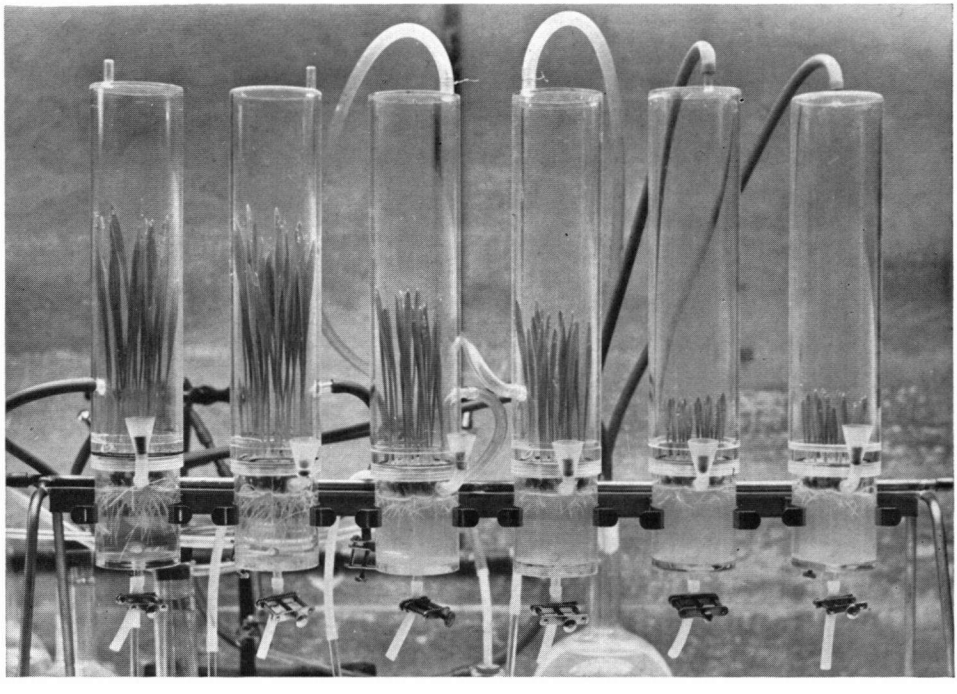

Photo 1.

Effect of the atmosphere around the leaves on the growth of the roots. Roots in Hoagland solution through which a stream of purified nitrogen was led. Leaves in:

air	(2 cylinders at the left)
nitrogen	(2 cylinders at the right)
nitrogen + 1 % CO^2	(2 middle cylinders)

The roots are normal when the leaves are in air. Oxygen formed in photosynthesis is not sufficient for aeration of the root-system (middle cylinders). (Photo after 7 days).