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Action of plant roots in diminished oxygen supply.

Benjamin Isgur
University of Massachusetts Amherst

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I Introduction and Purpose

Adequate soil aeration has long been considered as essential to normal healthy development of most mesophytic plants. Soils which are impervious to air due to standing water must be artificially drained before any crop may be profitably grown upon them.

If, then, aeration is essential, the oxygen supply may become a limiting factor in plant growth.

In order to isolate a factor relating to plant metabolism for the purpose of investigating its influence on growth, it becomes necessary to vary it and it alone, leaving all other factors constant. The soil is usually too complex a body (no two soils being exactly alike) to make it suitable as media in which only one factor is varied. In fact, an experiment performed in soil, as a medium, cannot usually be repeated and have all the factors exactly alike. There are too many unknown quantities present to allow the investigator to draw definite conclusions Sachs (62), Knop (28), and Nobbe in the period from 1859 to 1865 developed the general technique used in water cultures today. Since that time, plant physiologists have used the water culture method for studying plant reactions to nutrients and to other essential factors--physical as well as chemical. Since the water culture is a synthetic medium, the advantages

for studying plant reactions are obvious The water cultures may be modified in practically any way desirable and known factors kept constant.

Recently Gericke (19) of California has grown vegetables, notably tomatoes, in water culture, and claims huge increases in yields over soil grown plants. Gericke's experiments have even led to commercial enterprises wherein a large number of greenhouse operators have gone over to the growing of tomatoes in water cultures. The tomatoes thus grown are considered to be equal or better in quality to those grown in soil. The Subcommittee on Technology of the National Resources Committee (58) reported in 1937 that tray agriculture, the growing of plants in water culture, was one of the thirteen new developments which may have a profound influence on our social set-up in the near future.

With water culture technique used as a means for plant investigations and the possibility that water cultures may at some time in the near future be used extensively in the production of vegetables by commercial growers (probably under greenhouse conditions) it becomes desirable to investigate the relationship of roots to oxygen supply--especially so since one type of practice keeps roots submerged.

The purpose of this work, then, is to obtain direct

evidence concerning the relationships of roots to oxygen when the roots are submerged in water.

II Review of Literature

A.Oxygen-Root Relationships

The existing data concerning the oxygen-root relationships are mainly of an indirect nature and although many research workers, notably Allison and Shive (2), Livingston and Free (17), Bergman, and others have shown that aeration appears to be beneficial to plant growth, there still is some doubt whether the beneficial effects observed are due directly to the oxygen supplied in the aeration process.

It has been shown that different species of plants vary widely in their response to the variations in the amount of oxygen in the soil. In 1925, Cannon (9), in an exhaustive series of experiments, found the following facts to be true for plants grown in soils:

(1) When oxygen was entirely removed from the soil growth ceased in all species (30), although most of these species were able to maintain a very slow growth-rate in as little as 0.5% oxygen--but only for a limited period of time. This means that if the soil contained 0.5% oxygen that plants could not continue to grow for more than a very short time.

(2) Although not conclusive, some evidence has been found to the effect that certain plants, notably cotton, may allow an inward diffusion of oxygen through the stem to the roots. From the data gathered in this work and the work previously cited, the general conclusions have been drawn that plants need a supply of oxygen for their roots in order to function properly. Textbooks are generally agreed that oxygen is necessary to proper root functioning.

It is to be noted that whereas in soil, plants cannot continue growth when the oxygen content falls below 0.5% or 5000 parts per million, that plants do well in water culture when the dissolved oxygen content lies between 7 and 9 parts per million--or .0007-.0009%, an oxygen content--one thousandth of the amount needed in soil. If, then, oxygen is essential to plant growth, why do plants when raised in water culture do so well? Why will plants which are rooted in water-logged soil do much more poorly than will plants which have their roots entirely submerged in water? These facts seem to lead us to the possibility that perhaps the effect of oxygen is not a direct one but an indirect one. It leads us to the possibility that there may be certain factors present in

soils which are not found in water cultures and that the beneficial effects of aeration are indirect rather than direct.

Also, Dr. L.H. Jones has noted that roots of some plants when grown in water culture differ in having fewer if any, root hairs. The difference in oxygen requirements may be tied up with this factor.

On the other hand, Sachs (62), as early as 1860, found that plants growing in aerated culture solutions apparently were benefited and showed increased dry weight. This was especially true for those plants whose roots completely filled the culture jars.

Allison and Shive in a study of the effect of aeration on the growth of Soybean plants in sand and water cultures, with and without continuous renewal of solution, obtained greater growth of both roots and tops with aeration than without in the cultures with continuous solution renewal; but with only periodic renewal of solution, the aeration benefited the root growth of the plants, but not appreciably the top growth.

Arker (4), in 1901,reported that the growth of lupine roots was greater in both soil and water cultures when a stream of air was allowed to pass through the culture medium.

Loehuring (31), in 1934, showed that it was possible to decrease the size and growth of plants by very rapid aeration, using moist air.

Most workers in this subject have considered the influence of oxygen to be of paramount importance. However, some investigators have even suggested that the carbon dioxide content of the culture solution or soil might also be involved in this relationship.Free(17) has shown that bubbling carbon dioxide through culture solutions containing buckwheat plants produced injury in a few hours and death in a few days. Using the technique of Free, Knight(27),in 1924, found that with corn plants he obtained a better correlation of root growth of plants with the carbon dioxide content of the solutions in an inverse ratio than with the oxygen content in a direct ratio. From these facts Knight concluded that carbon dioxide and not oxygen was the limiting factor in his experiments.

In all these experiments, as they were performed, it is obvious that the data does not necessarily indicate the necessity of oxygen for plant roots nor does it indicate that the plant roots use oxygen directly. The only way in which definite indications can be obtained is by means of direct measurements.

B. Nitrogen-Plant Relationships

Inasmuch as the oxygen in the soil is considered to have some bearing on the oxidation of ammonia, it is necessary to investigate any work which may have been done along these lines.

It is generally accepted that most plants absorb the necessary nitrogen as inorganic compounds, especially in the form of nitrates. In early investigations, workers found that although most plants preferred nitrates, there were some plants which apparently did equally well on ammonia nitrogen. It was even discovered that there was some disagreement among experimenters as to whether certain plants preferred nitrate or ammonia nitrogen. However, the apparent discrepancies were explained away by some as due to the fact that these experiments were not performed under sterile conditions and did not, therefore, preclude the possibility that the ammonia nitrogen might have been changed to nitrate nitrogen before assimilation by the plant. Since 1889, however, considerable work has been done under sterile conditions to determine the relative value of nitrate and ammonia salts in plant nutrition.

Among the experiments conducted for the purpose of determining the relative values of nitrate and am-

monia salts in plant nutrition were those of Boussingault (7) who found that in sand culture, nitrates were preferable to ammoniates.

Knop(28), Sachs(61), and others, found that not only were nitrates preferable for water cultures but that ammonia salts were toxic.

Up to the last decade of the nineteenth century, the view was generally held that ammonia must first be oxidized to nitric acid before the plant could take it up.

Muntz (42), in 1889, found that Zea mays, and Cannabis sativa absorbed and assimilated ammonia from salts. Pitsch(46) at the same time found that oats grew well on ammonia in sterile cultures, but grew better on nitrates. Pitsch overlooked the physiological acidity produced by ammonium chloride. By physiological acidity is meant the acidity resulting in a medium when the plant uses the basic ion of a salt in larger quantities than the acidic ion. In the case of ammonium chloride(NH_4Cl), the plant will utilize the ammonium ion in larger quantities than the chloride ion, thus causing the formation of hydrochloric acid in the medium.

Experiments were now instituted to control both physiological acidity and nitrification. Kossowitsch(29), in 1897, and Maze(34), in 1898, using sand cultures show-

ed ammonia nitrogen no worse and in some cases better than nitrate nitrogen. Kruger(30), and Treboux(74) obtained similar results.

However, experimenters are far from unanimous in their opinion of the value of the two sources of nitrogen. Frequently poorer growth was obtained on a mixture of sulfate of ammonia and calcium carbonate than on a mixture with nitrates. Later Hutchinson and Miller(22) obtained increased growth of peas by the addition of calcium carbonate to ammonium sulfate even though the growth was less than that produced by the use of nitrates. Thomson(71), in 1922, found that calcium carbonate added to a solution of ammonium sulfate gave increased growth at first but at the conclusion of the experiment did not give as good growth as did the plants which were grown on nitrates alone.

Prianischnikov(48), in 1934, used ammonium nitrate with the idea of maintaining physiological neutrality, but found that the ammonium ion was utilized faster than the nitrate ion so that in effect ammonium nitrate must be considered as a physiologically acid salt. It is of interest to note, especially in conjunction with the present work of the writer that although at first the ammonium ion may be more rapidly absorbed, that as the plant grows older, the nitrate form seems to be preferred.

More direct proof of this fact is the work of Schulow(68) in 1912, who grew Maize in sterile cultures containing ammonia and nitrate forms of nitrogen. His results were as follows:

Table I

The results of Schulow showing the relative absorption of nitrate and ammonia nitrogen at different ages

After	34 days	88.6 mg.	NH_3	and	35.2 mg.	NO_3
"	52 "	150.2 "	"		155.7 "	"
"	80 "	203.0 "	"		243.4 "	"

This work also indicates that young plants prefer ammonia to nitrate nitrogen but the reverse being true as they grow older.

Prianischnikov(57), in 1934, in an exhaustive investigation showed that the following four factors exert an important influence on the rate and form of nitrogen absorption:

(1) Amount of carbohydrates---the smaller the amount of carbohydrate present the greater the relative absorption of nitrates and the less ammonia ions absorbed.

(2) Reaction of media---the more acid the media the less able is the plant to take in nitrogen in the form of ammonia and the more nitrate is used.

(3) Age of the plant---as the plant grows older, the less able is it to use nitro-

(4) Concentration of the solution---the more concentrated the solution the less nitrogen as ammonia will be absorbed and the more nitrogen as nitrate will be used.

Here too is indicated that plants are better able to utilize ammonia in their younger stages.

Clark and Shive (11), in 1933, showed that under various pH conditions the rates of absorption of ammonia nitrogen per unit of plant tissue decreased, and the rates of absorption of nitrate nitrogen increased as the plants became older.

C. Conclusions from Review and Statement of Problem

From the review of the literature, it may be seen that there is much controversy concerning the necessity of oxygen for the normal development of plant roots. The evidence is mainly indirect and the validity of the theories proposed has been questioned by many investigators. Furthermore there seems to be some difference in the amount of oxygen required by plants grown in soil and those grown in water culture media.

Although not conclusive, some evidence has been found to the effect that certain plants, notably cotton, may allow an inward diffusion of oxygen through the stem to the roots. If, this is so, then some investigation about the

relation of oxygen in water around roots is desirable and it would be necessary to carry on this work with all oxygen cut off from access to the roots.

In order to design an experiment which would offer direct evidence of the influence of oxygen on plant roots, it is necessary, then, to enclose the roots in an air tight container, and then to measure the oxygen present at the beginning and at the end of the experiment.

It was considered that if a supply of oxygen for the roots from the surrounding media is needed to carry on metabolic processes within the plant, then, with an oil seal the plant would soon succumb; for, the small supply of oxygen (7-8 p.p.m.) dissolved in water would be completely utilized in a very short time. It would also be necessary to have adequate checks, plants grown in culture solution on the surface of which no oil would be used in order to see how fast oxygen could be supplied to the water from the air. Another group of plants might be placed into water which had previously been boiled to remove all the air, and then an oil film added. Plants growing in these boiled cultures presumeably would have no access to oxygen,

Since there would be some transpiration in these plants, and since some of the nutrient solution would

have to be changed after several days, it would be necessary to devise some means of adding oxygen free nutrient solution as needed in order to keep the level of the culture media well above the root zone, and at the same time not increase the oxygen content of the original media. Figure I shows how this was done, and a description of the method will be found in the section on apparatus and methods.

By using the above set up, it will be possible to investigate what happens to the roots when they are deprived of oxygen, (2) to investigate the amount of oxygen needed by plant roots for carrying on the necessary metabolic processes, and (3) to investigate the interrelations of environmental factors when plants are deprived of oxygen.

III Research

A. Apparatus and Methods

1. Adding Oxygen Free Nutrient Solution

A large 2-liter erlenmeyer flask was filled about three-fourths full of the nutrient solution and allowed to boil for several minutes. As soon as the flame was removed a thick film of oil was introduced in order to prevent any oxygen from dissolving into the solution from the air. After the nutrient solution had been cooled sufficiently, a siphon was set up--one end being placed under the oil film in the Mason jar to which nutrient solution was to be added while the other end was placed under the oil film in the erlenmeyer flask from which the nutrient solution was to be added. A petcock was used to control the quantity of solution introduced. This simple piece of apparatus is illustrated in Fig.I.

2. Determination of Dissolved Oxygen

The determination of dissolved oxygen was made by the micro-Winkler method with modifications as needed. The sampling of the water for the oxygen determination was done by means of a modification of the apparatus

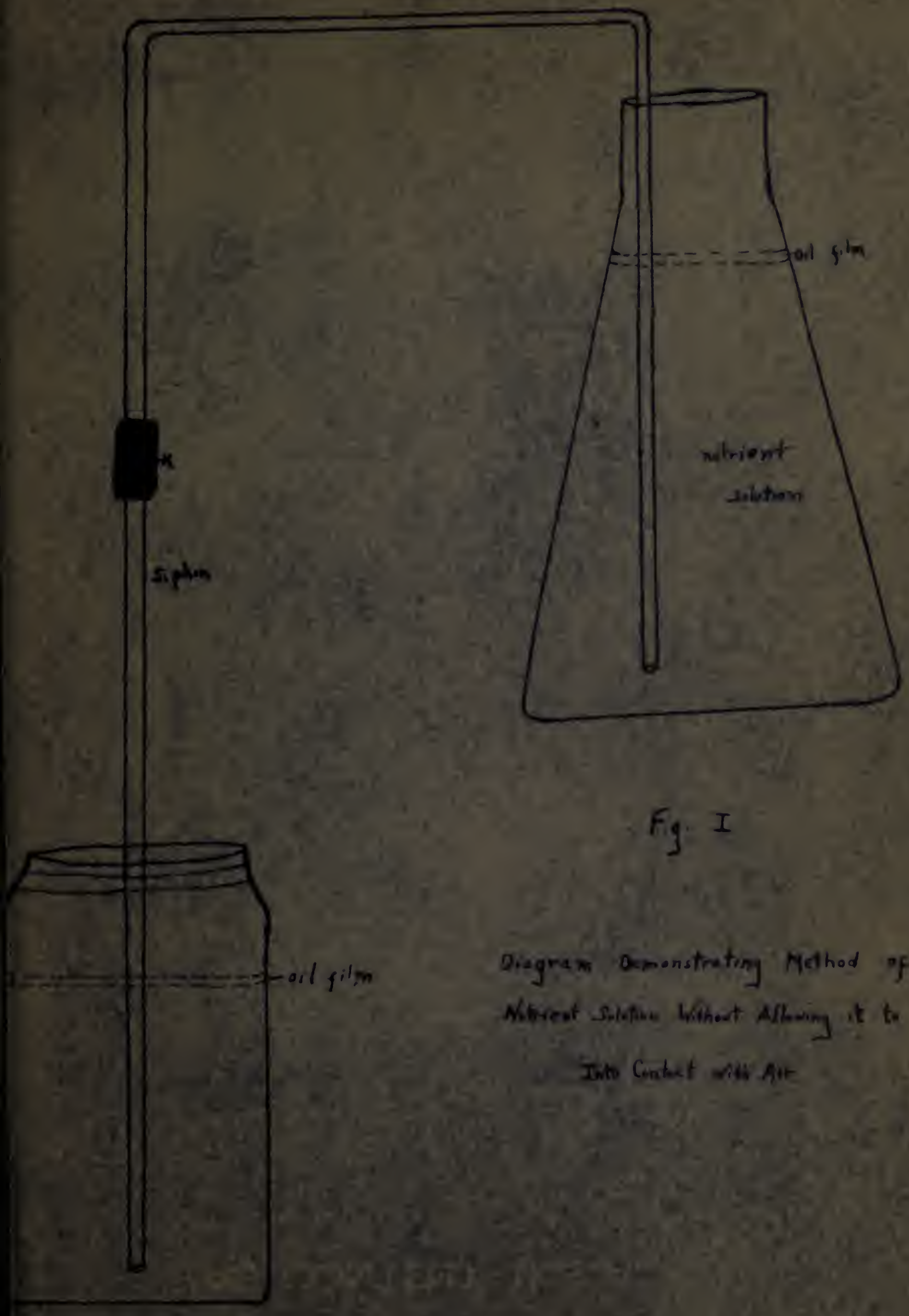


Fig. I

Diagram Demonstrating Method of Adding Nutrient Solution Without Allowing it to Come Into Contact with Air

used by Allison and Shive. This apparatus was modified to better suit the needs of this experiment.

Figure IIA and IIB are diagrams of the sampling apparatus. Using the apparatus as set up in Figure IIA, glass tube "a" detached at "b" is inserted beneath the oil of the culture media and then a few cc. of solution removed in order to free the sampling vial of any oil which might have been caught in the tube when it was inserted into the liquid. After a few ml. of the solution have been removed, tube "a" is connected at "b" to the rest of the apparatus and a sample of the solution taken up by supplying suction at tube "c". The media is allowed to fill the container "d" and empty itself several times in order to make certain that the solution to be tested has been kept from contact with the air.

When the solution in container "d" has been obtained free of air bubbles and free from any contact with the air, tube "a" is removed, at the same time making certain that clamp "b" is closed. In order to admit the chemicals for the oxygen determination, tube "c" must also be removed and the chemicals delivered by means of 1 cc. pipettes graduated to hundreths as shown in Figure IIB. Several precautions must be observed in order to insure success with this method. The author has found that it was neces-

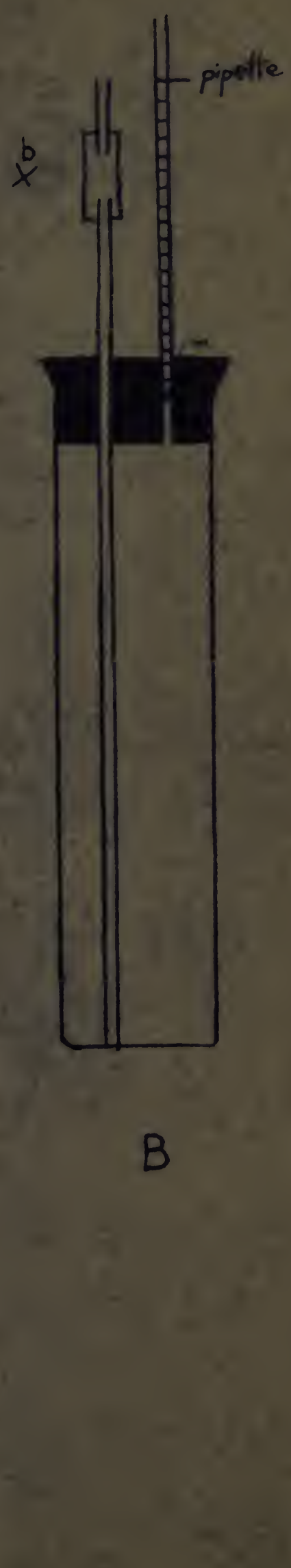


Fig II

Sampling Apparatus

sary to loosen screw-clamp "b" enough to allow some of the solution to just run over at "m" in order that the introduction of the pipette would not trap air bubble into the solution thereby vitiating the results. This must be done each time a pipette is used. After the pipette is introduced, the quantity of reagent added to the solution can be readily controlled by means of screw-clamp "b". It is essential that the sample be taken at the temperature at which the plant has been growing. The culture solution may not be removed from the greenhouse to the laboratory in order to obtain the sample; for if the solution is brought into a warmer room, bubbles of gas form on the walls of the container, and a truly representative sample, free of gas bubbles, is virtually impossible to obtain.

In order to determine the amount of dissolved oxygen in parts per million parts of water the following modifications of the micro-Winkler method were adopted: To the sampling tube which held approximately 30 ml. of solution was added o.1 ml. of a manganous sulfate (Mn. SO_4. $2H_2O$) solution made by adding enough water to 100 grams of manganous sulfate to make 250 cc. of solution. Three tenths (o.3) of a cc. of a potassium iodide(KI)-Sodium hydroxide (Na OH) solution(made by mixing 75 grams of potassium

iodide (KI) with 250 grams of sodium hydroxide (Na OH) and then made up to 500 cc. with water) was then added and the sampling container rotated to mix the contents. A precipitate forms which must be just dissolved by means of concentrated sulfuric acid (H_2SO_4). About 0.15-0.20 cc. of sulfuric acid are needed. Once it is acidified, the sample may come into full contact with the air and suffer no change.

B. Reagents, Materials, And Chemical Reactions

1. Oxygen Determinations

a. Reagents

0.1 cc.	----------	100 grams of $MnSO_4.2H_2O$ made up to 250 cc. with water
0.3 cc.	----------	75 grams KI, 250 grams NaOH, made up to 500 cc.
0.15-0.20 H_2SO_4	------	conc. H_2SO_4 Sp. g. 1.84
$Na_2S_2O_3.5H_2O$	--------	approximately N/40 solution
Starch	----------	0.5% solution

b. Reactions

The reactions which take place in this determination are as follows:

$$MnSO_4 - 2NaOH \qquad Mn(OH)_2 - Na_2SO_4$$

$$2Mn(OH)_2 - O_2 \qquad 2MnO(OH)_2$$

$$MnO(OH)_2 - 2H_2SO_4 - KI - MnSO_4$$
$$----- I_2 - 3H_2O - K_2SO_4$$
$$2Na_2S_2O_3 - I_2 ----- 2NaI - Na_2S_4O_6$$

These reactions show that the determination of oxygen is not a direct one but depends on the oxidation of the iodide ion (I^-) to iodine (I_2) which is measured by means of sodium thiosulfate ($Na_2S_2O_3$).

2. Nitrate Determination

A modification of the phenoldisulfonic acid method of Harper, was used for the determination of nitrates. A 25cc. portion of the solution to be tested was transferred by means of a pipette to 3 inch evaporating dishes, and evaporated to dryness. The evaporating dishes were allowed to cool and 2 cc. of phenoldisulfonic acid (made by dissolving 25 grams of pure white phenol in 150 cc. of concentrated H_2SO_4, and then adding 75 cc. of fuming H_2SO_4 --- the whole being heated at 100°C for two hours) was added. The dishes were rotated so as to bring the reagent into contact with all the residue. The reagent and residue were allowed to stand for 10 minutes and then 15 cc. of cold distilled water was added with continual stirring. Finally, the whole solution was made slightly alkaline by the addition of dilute ammonia (1-2),

diluted and compared with a standard solution containing 1 p.p.m. of nitrogen as nitrate nitrogen.

3. Solubility of Oxygen in Water

The solubility of oxygen in water at various temperatures is shown below in Table II. Like any other gas, the solubility of oxygen in water decreases with an increase in temperature.

Table II

Showing Relationship Between Oxygen
Dissolved and Temperature of
Water at Equilibrium

Temp. ^{o}C	Solubility p.p.m.
0	14.60
5	12.65
10	11.23
11	10.98
12	10.74
13	10.50
14	10.27
15	10.05
16	9.84
17	9.64
18	9.44
19	9.25
20	9.07
21	8.90
22	8.73
23	8.57
24	8.42
25	8.27
26	8.13
27	8.00
28	7.87
29	7.75
30	7.64

Averages from Winkler, Fox, and Roscoe
and Lunt.

4. Culture Solution

Calcium nitrate $Ca(NO_3)_2$ ----------.0084 M per liter
Primary potassium phosphate (KH_2PO_4) ------.0041 " " "
Magnesium sulfate ($Mg\ SO_4.7H_2O$) ----------.0225 " " "
Ferric potassium tartrate ---------- 1 mg." "
Traces of Boron and Manganese

The above is Shive's solution which the writer has used successfully for soybeans before.

5. Oil

The oil used was a pure, colorless mineral oil obtained from the Wentworth Pharmacy at Amherst, Massachusetts.

6. Soybeans

Soybeans, variety Manchu, were allowed to germinate in pure quartz sand and removed to culture solution when the cotyledons began to unfold. The culture solution was the modified Shive's nutrient solution .

C. Preliminary Experiments

It was deemed advisable to perform some preliminary experiments in order to determine the accuracy of the methods as well as their limitations.

1. Effect of Concentration on Dissolved Oxygen

To determine whether the presence of salts in the culture medium would have any influence upon the determination of dissolved oxygen, several beakers were filled with various concentrations of the culture media to be used, and determinations of the dissolved oxygen content made.

These determinations were made after the solutions had come to equilibrium with the air. The following table gives the results of these tests:

Table III

Showing the Effect of Concentration of Media on the Dissolved Oxygen

Conc. of Media	Dissolved O_2 (p.p.m.)
0.0	7.90
0.2	7.88
0.5	7.91
0.8	7.90
1.0 equals conc. of media used.	7.91

From the above table it may be seen that the concentration of the media makes no difference in the amount of oxygen dissolved in the nutrient solution. Therefore the removal of nutrients by the plant would have no effect on the ability of the media to hold dissolved oxygen.

2. Delicacy of Tests

The next experiment was designed to investigate the magnitude of the error which might be caused by differences in the amounts of reagents added. The following table presents the data obtained when (a) great care was taken to use the exact amount of reagents suggested, and (b) no great care--about twice the amounts of reagents added.

Table IV
Showing the Effect of Adding
Varying Quantities of Reagents
on the Accuracy of Tests
Temp.24.5°C

Treatment	Dissolved Oxygen p.p.m.
(a) great care taken with reagents	7.74
(b) No great care taken with reagents- about twice the amounts used as suggested in method.	7.76

It may be seen from Table IV that only moderate care need be taken with reagents used.

3. Effect of Oil On Dissolved Oxygen

Since oil was used to act as a seal in order to keep the oxygen out of the culture medium, a test was made to investigate the influence of oil on the dissolved oxygen content of the culture media. A culture solution which contained a surface film of oil was shaken in order that some of the oil would be distributed in the water. Samples of culture media were then removed, at the same time making certain that several bubbles of oil were caught in the samples. Dissolved oxygen was determined with the following results:

T able V
Showing Effect of Oil
on Oxygen Determinations
Temp. 24.5°C

Treatment	Dissolved Oxygen p.p.m.
Water(containing oil drops)	7.75
Water carefully removed from under the oil (no oil drops present)	7.76

Table V shows that little if any error can creep in through the inclusion of any small bubbles of oil which may be caught in the sample tube used for dissolved oxygen determination. Notwithstanding, it was deemed advisable to take oil free samples.

4. Accuracy of Determination of Small Quantities of Oxygen

In order to investigate whether minute amounts of dissolved oxygen could be determined by this method, water was boiled for fifteen minutes and the dissolved oxygen content determined. As there is some dissolved oxygen in the reagents, values below 0.05 p.p.m. are unreliable. The results obtained on a large number of samples ranged from 0.01-0.05 p.p.m.

5, Effectiveness of the Oil Seal

In order to investigate the effectiveness of the oil seal the following procedure was used. Plants were placed in quart Mason jars which contained the culture media and to which were added 30 ml. of oil to serve as a seal. The diameter of the jar at the region of the oil seal was 92 mm. When the plants were removed after 48 hours it was found that the plant roots had brought the dissolved oxygen content down to below 0.05 parts per million. With a pipette oil was removed to leave the desired number of ml. of oil behind. The following table gives the results of these tests.

Table VI
Showing Effectiveness of Oil Seal

Oil (ml)	Dissolved Oxygen (p.p.m.) (at end of)					
	24hrs	48hrs	72hrs	84hrs	7days	14days
10	2.01	5.08	7.99	7.89	8.03	7.85
15	1.05	4.22	5.40	5.97	7.94	7.92
20	0.09	1.44	1.84	2.08	3.11	4.14
25	0.33	0.53	0.78	0.72	1.90	2.69
30	0.05	0.05	0.05	0.07	1.98	1.97

From Table VI, it may be seen that an oil seal of 25-30 ml. would be an effective oxygen seal for all practical purposes. The above preliminary tests showed that it was possible to keep out oxygen for the period of the experiment and that the method used for oxygen determination was a suitable one.

Figure III

This photograph shows at the right the method of obtaining a sample of solution from the mason jar for oxygen determination. In the block which contains the vials one may see the pipette introduced into the vial after the sample is taken.

Figure IV

This photograph shows how the plants were held in the culture solution. The plants in "1" are older plants than those in "2". These plants were grown with their roots in culture solution whose surface was covered with a film of oil. These plants grew without anyapparent difficulty.

D. Main Work

1. Influence of Plant Growth on Dissolved Oxygen

The stem of the soybean plant was put through a one hole stopper (cork) and held in place by means of a ring of non-absorbent cotton batting. The roots were placed (see Fig. IV) in quart mason jars which held the culture media (thus inhibiting the growth of algae) the jars were enclosed in galvanized iron jackets which are not shown in photograph.

In this experiment ninety plants were used. The plants were placed in three major divisions designated as Divisions I, II, and III, These major divisions were each sub-divided into five minor divisions of six plants each, designated as A,B,C,D, and E. The treatments were as follows:

Division I -- culture solutions exposed to the air
Division II -- culture solutions covered with layer of oil
Division III-- culture solution first boiled, then covered with layer of oil.

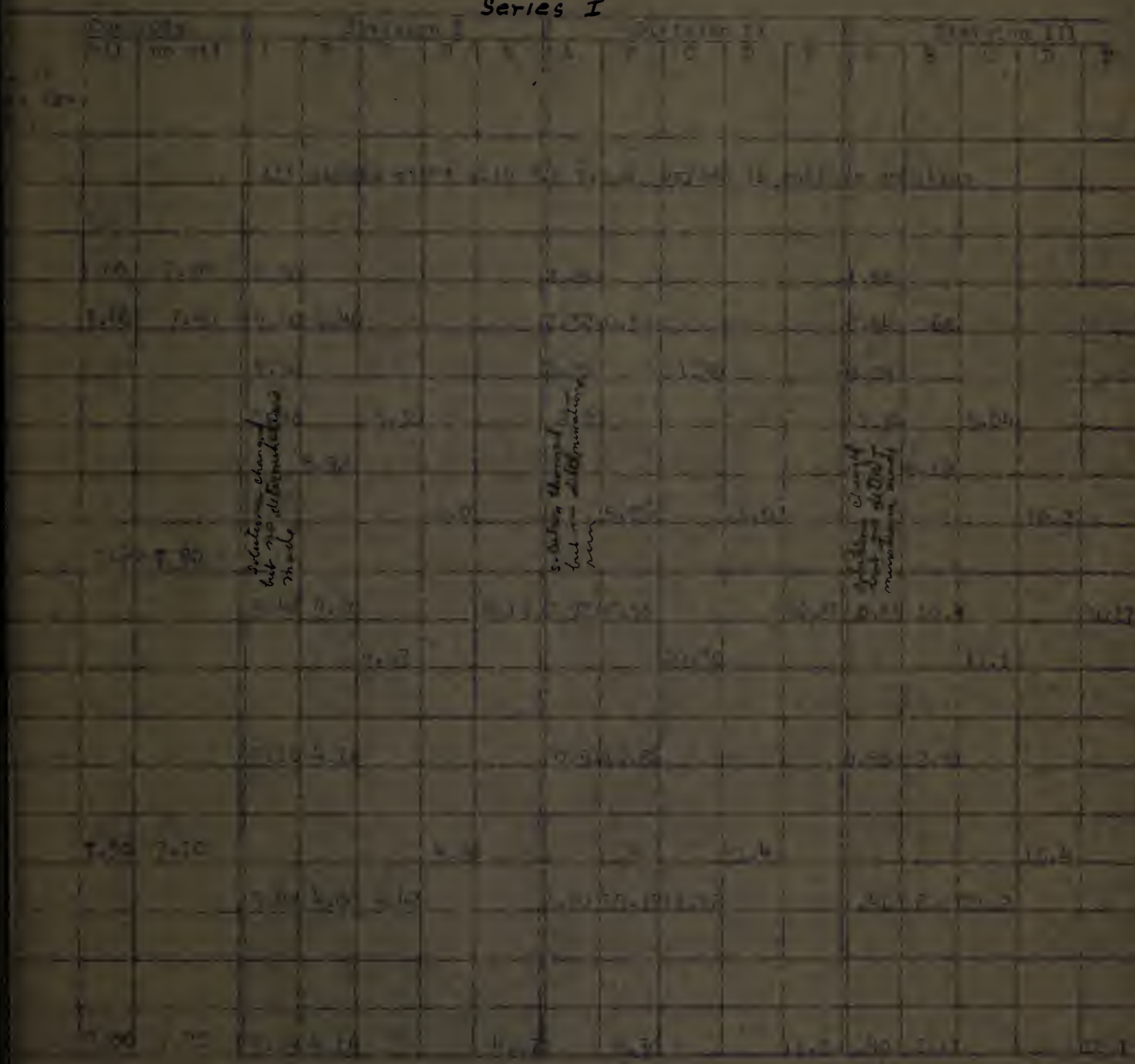
Sub-division A -- culture solution changed every day
" B -- " " " " 3 days
" C -- " " " " 5 days
" D -- " " " " 7 days
" E -- " " " " 9 days

It has already been shown that 25 cc. of oil proved an effective seal for the 1-9 days these experiments were run. At each change of the culture solution, determinations of the dissolved oxygen were made and average results taken. Table VII gives a resume of the results obtained from the first trial (Series I) while Table VIII shows the results of similar tests (Series II) made at a later date.

In Series I there was very little observable difference in appearance among the various groups of plants up to the last three days of the experiment. At that time, those plants which were under oil and subject to frequent changes of solution, began to turn yellow and appear wilted. It is to be emphasized that the more times a plant was changed during the experiment to new nutrient solution, the more wilted it appeared. This fact would seem to indicate that the plants wilted because in the transfer to new solution the roots would be drawn through an oil film and the more frequent the trans-

VII

Series I



fer, the more oil would coat the roots. Of course, as soon as the roots were submerged much of the oil would leave the surface of the roots and rise to the top--but nevertheless it was observed that some of the oil would remain clinging to the roots thereby coating the absorbing surface and reducing the amount of water and nutrients available to the plant.

From a study of Table VII, it may be observed that the plants of Division I (where no oil was used) never depleted the dissolved oxygen below 4.00 parts per million. The reasons for this fact are probably two-fold. As will be shown elsewhere in this paper, the rate at which oxygen is removed from the water by the plant decreases as the dissolved oxygen content decreases. Thus a plant which very easily removes oxygen from water when the dissolved oxygen content ranges between 7-8 parts per million finds it very difficult to remove oxygen when the dissolved oxygen content is merely 2-3 parts per million. The other reason is that, when the dissolved oxygen content falls to about 4.0 parts per million, the amount of oxygen being dissolved into the water from the atmosphere just equals the amount being used by the plant. In other words, an equilibrium is set up between the oxygen used by the plant and the amount of oxygen dissolved into the water. This

is true for plants of the size used in this experiment, but for plants either larger or smaller a different equilibrium point is reached. From Table VII, it is evident that as the plants grow larger the amount of oxygen removed before equilibrium is set up is ever greater.

Divisions II and III may be discussed together since after about twenty-four hours the oxygen content of Division II had been depleted to the point of that of Division III. Also, it was found practically impossible to obtain oxygen-free water by boiling and then pouring on an oil seal. Tests showed, in most cases, after the oil was poured on (even when the water had just been boiled) that the water still contained some oxygen. Hot oil is not as effective a seal as is cold oil.

In both Divisions II and III, the oxygen content after twenty-four hours falls to a low ebb. Here also, as the plants grow older they seem (in most cases) to be able to remove a greater amount of oxygen in a given time. It is also important to observe that after the plants have been under oil for a period of three or more days, abnormal values for dissolved oxygen are obtained. It is impossible for water at temperatures between 26^{O}-20^{O}C to contain more than 8.13-9.00 parts per million of dissolved elemental oxygen. Evidently, then,

instead of these high values being due to dissolved oxygen it may be due to some substance formed in the solution.

Since the checks---those cultures which contained a film of oil but no plants---showed either a normal quantity of dissolved oxygen or even a loss it may be assumed that the substance, whatever it may be, was produced by the plants themselves. This substance was capable of oxidizing the iodide of the potassium iodide (KI) to form free iodine (I_2).

2. Repetition of Tests Series II

The abve experiment was repeated in part in the following manner and with the following results:

(1) Six plants had their roots submerged (under an oil film) in Shive's nutrient solution. This nutrient solution was the same as the one previously used except that the elements manganese and boron were omitted.

(2) Six plants had their roots submerged under an oil film in distilled water. The roots of these plants were washed until there was evidence of neither nitrates nor nitrites in water that had been in contact with these roots for 12 hours.

Samples of these solutions were tested for oxygen-equivalent content at six, seven, and eight days after the roots had been submerged under oil. The results of these tests are collected in Table VIII.

It will be noted that tests for nitrites were made at each oxygen determination. The first distinct change in color was chosen as the end point of the titration, in those cases where the color tended to return. It should be noted that in the case of those plants grow-in distilled water none showed an indistinct or return-ing end point; while in only two cases out of seven were there no indistinct end-points in the nutrient cultures tested; namely, sample 1 of series A and sample 2 of ser-ies C. The explanation for this is unknown.

It is to be noted that in no case were nitrites pre-sent when only distilled water was used as a medium for the plant roots. When plants were growing in nutrient solution, nitrites were present in five cases out of seven---although when present the amounts were small. When this same test was run on series I, no nitrites were found produced in the nutrient solution except in a few cases and these were so few that they were not used in making up the results. The reason for the nitrites being produced this time may be due to some particular bacter-ial contamination which somehow was avoided in the previous

Table VIII

Series II - Dec.

Showing Oxygen - Equivalent Content of Solutions

Sample No.		Oxygen-Equivalent P.P.M.	Nitrites P.P.M.
Group A (2 Day Test) T=21.5°C			
Nutrient Solution	1	0.52	0.0
Distilled Water	1	0.22	0.0
	2	0.54	0.0
Group B (6 Day Test) T=22.0°C			
Nutrient Solution	1	18.4	8.0
	2	5.3	12.0
Distilled Water	1	4.2	6.0
	2	3.1	0.0
Group C (7 Day Test) T=21.5°C			
Nutrient Solution	1	11.7	19.0
	2	5.8	10.0
Distilled Water	1	4.0	0.0
	2	5.2	0.0
Group D (8 Day Test) T=22.0°C			
Nutrient Solution	1	8.1	24.0
	2	18.4	12.0
Distilled Water	1	10.4	0.0
	2	4.7	0.0

trials.

Also it is to be noted that in no case were the results of the determinations as high as those previously found. This led to a study of reasons for this difference, which may be summarized as follows:

(1) The plants were somewhat younger in the previous tests. It was there shown that younger plants were more proficient on this score(i.e. in the production of the substance which acted so as to give high values for dissolved oxygen).

(2) It will be noticed from Table VIII, page 41, that it required several changes to reach the maximum production of oxygen equivalent. For example, in Division IIIunder "B"and "C", where nutrient solutions were changed every three days and five days respectively, it was not untill after several changes that the maximum point was reached. This was true in almost every case.

(3) It is important to note that plant roots (when an oil film is used to prevent the reintroduction of oxygen from the air) brought the dissolved oxygen content down to less than 1 p.p.m. in 48 hours; in fact, the average was slightly less than 0.5 p.p.m. This fact was determined from

Group A which was run with plants of the same size as those used in Groups B, C, and D. Reference is here made to Table VIII. It is a safe assumption that the oxygen content in Groups B, C, and D would all have given oxy-determinations very close to those of Group A if the determinations had been run at the same time; namely, after 48 hours. Because of this fact, the results obtained with Groups B, C, and D, especially with those plants whose roots were submerged in distilled water (since in these no nitrites were present), become eminently significant. It should be noted that tests revealed an average of 5.2- p.p.m. or fully 10.4 times as much for the average for Group B, C, and D as for Group A. In all these determinations the end-point was distinct---and also, no nitrites were present as a possible interference with the test. Thus even though the results of this test were far below that previously made, there is still evidence of the production of some substance which acts as an oxident.

The fact that some substance which seemed to have oxidizing powers was produced led to an investigation of the literature to see whether other workers had ever reported the formation of oxidizing substances by plants. It was found that several reports had been made

concerning unidentified oxidants which apparently were produced by plant roots, but nowhere was it reported to have been produced in such quantity as was evident in those culture solutions which were covered with oil films, nor was it reported that anyone had limited the supply of oxygen to the roots.

Molisch (40), in 1885, appears to have been the first to demonstrate the oxidizing power of root secretions, and to show them as being capable of oxidizing various organic compounds. He found this oxidant capable of oxidizing various organic substances such as guaiacol, pyrogallol, and gallic acid.

Raciborski (59), in 1905, showed the oxidizing power of roots by using alpha-naphthalamine, benzidine, phenolphthalin, ferrous ammonium sulfate and leucomethylene blue.

Schreiner and Reed (66)(67), in 1909, considered that plant roots are able to carry on active extracellular oxidation chiefly by means of enzymes which they secrete. These investigations, using wheat plants, found that their roots were able to oxidize organic substances such as certain chromogens at a fairly rapid rate. The oxidizing power appeared to be most energetic in the region of the root where the root hairs are found and increasingly

less active as that portion of the root becomes older. These investigators also found that roots had greater oxidizing power in extracts of productive soils than in extracts of unproductive ones.

Borowski (6), in 1919, observed the oxidation of ammonium ferrosulphate by roots. He found that the oxidizing ability of the roots of different plants varies. He found that the roots of Sinapis to be weakest and Phoseolus strongest in oxidizing power.

3. Properties of the Oxidizing Substance

In order either to identify or to learn something about the nature of the oxidizing substance which was given off by the roots of these plants when put under oil, the following tests were made.

a. Boiling

The liquid of several jars which showed abnormal values for dissolved oxygen were removed from under the oil and vigorously boiled for five minutes. The results when tested for dissolved oxygen were as follows:

Table IX

Showing the Effect of Boiling on the Oxidizing Value*

Division	Oxidizing value* of culture solutions as shown by its ability to oxidize I^- to I_2	Oxidizing value* of culture solution after boiling for five minutes
II B	10.86 p.p.m.	10.54 p.p.m.
II B	14.00	13.98
II C	18.00	18.00
II D	41.40	41.35
II E	42.00	41.60
III B	12.80	12.02
III C	11.10	11.00
III D	15.40	15.33
III E	40.00	38.88

* Throughout this experiment the term oxidizing value will mean the equivalent amount of dissolved oxygen which would be needed to give the above values.

Table IX shows that the substance which causes the seemingly high oxygen values is not volatilized at boiling temperatures. It is improbable, therefore, that the substance is an enzyme, for enzymes are usually destroyed by boiling, or a gas because gases would be lost by volatilization.

b. Test for Nitrites

Nitrites in acid solution would be capable of oxidizing I^- to I_2. However, tests made by the Griess method which makes use of sulphanilic acid and naphthylamine hydrochloride showed that the solution contained no trace of nitrites. The nitrite test was made on all the solutions listed in Table IX.

c. Stability

Culture solutions showing abnormal values for dissolved oxygen were allowed to stand for given periods of time to investigate the stability of the oxidizing compound. Table X gives the results of this phase of the investigation. It shows that the substance produced deteriorates with standing and that it deteriorates more quickly when it stands exposed to the air than when kept under oil. It is probably for this reason that no abnormal values could be observed when there was no oil film covering the surface of the culture solutions. This was further substantiated in the experiment using aloin as an indicator. As may be seen the oxidizing power of the substance is lost rapidly when it is exposed to the air and thus any of the oxidant produced in the absence of the oil film (Series I-Division I) would be destroyed.

Table X

Showing the Effect of Exposure to Air
on the Oxidizing Equivalent*

No. of days left standing			12	14	16
Left tanding under oil film			7.90	7.94	8.20
Decrease in oxidizing power					
Left standing with oil-film removed			37	7.92	8.85
Decrease in oxidizing power	8.70	13.50	20.85	20.90	

* The solution used was a mixture of several culture solutions of abnormal oxygen values. The final mixture showed an oxidizing equivalent of 28.90 p.p.m.

There seems to be something present, greater in quantity than the oxygen dissolved in water and which is not discharged on heating. Hereafter, this substance will be called an oxidant.

4. pH of Solutions

The pH of the solutions of tests of Series I were taken at the end of the experiment by means of the quinhydrone electrode. These solutions were heavily buffered

with phosphates and therefore showed little change. Table XI shows the pH values of the culture solutions taken at the end of the designated number of days The pH of the original solution was 6.80.

Table XI

pH Values of Culture Solutions Taken at End of Designated No. of Days

Subdivision	Division I	Division II	Division III
A	6.90	6.71	6.82
B	6.88	6.87	6.40
C	6.70	6.80	6.80
D	6.70	6.70	6.80
E	6.70	6.70	6.80

5. Influence of Treatment on Dry Weight

At the conclusion of the experiment the plants of Series I were removed from the culture media and dried for 48 hours at a temperature of 105^{0}-110^{0}C. Table XII gives the values for the dry weights obtained. It may be seen from this table that the dry weights show no significant differences. The slightly lower results obtained for some of the groups that were under the oil may be attributed largely to the damage done to the roots by their frequent passage through the oil film.

Table XII

Dry Weights of Plants (grams)

Subdivision	Division I	Division II	Division III
A	1.85	1.60	1.50
B	1.70	1.50	1.59
C	1.40	1.37	1.58
D	1.38	1.45	1.50
E	1.51	1.78	1.59
Totals	7.84	7.70	7.76

There seems to be, then, no really significant difference in dry weight between those plants which have a greater quantity of oxygen supplied to them over those which have a lesser, or no oxygen supplied to them--at least for the time limit of this experiment.

6. Influence of Plant Debris on Oxygen Content

In order to determine whether some of the results obtained might not be due to some chemical reactions involved in the possible sloughing off of decayed plant material into the culture media, small quantities of root material were placed into culture solutions. Some of the culture solutions were given a surface coating of oil whereas the surface of some others were left exposed to the air. The following is a record of the results obtained after ten days.

Table XIII

Showing the Influence of Organic Matter on Dissolved Oxygen Content

Treatment	Dissolved Oxygen p.p.m. (avg. of six tests)
A. Organic matter in culture solution-coating of oil on surface	3.77
B. Organic matter in culture solution-no coating of oil	4.01
C. Check-no organic matter-coating of oil	7.81
D. Check-no organic matter-no coating of oil	7.94

The results in Table XIII make evident that the production of the oxident is not dependent upon the action of decaying organic matter.

7. The Influence of Age on Production of Oxidant

As is to be noted in Table VII, under anaerobic conditions an oxidant is produced, but it must also be noted that whereas the oxidant is produced in great quantities at first, there is a decrease in the quantity of the oxidant produced towards the end of the experiment. In order to determine whether the cause of the decrease in the production of the oxidant was due to the age of the plant

the following experiment was performed.

Soybean seeds were planted in pure sand and allowed to germinate. This time the plants were not removed until they had produced six leaves. Several days before these plants were removed another group of soybean seeds were placed in washed quartz sand and allowed to germinate. When the second set of soybean seed had just pushed their cotyledons above the surface of the sand both groups were removed and placed into nutrient solution in the same manner as before, i.e., with an oil film covering the surface of the nutrient solutions. Ten uniform seedlings were chosen from each group. The plants were kept in this solution for ten days; (more solution being added as needed in the manner already described) then removed and the nutrient solution analyzed for dissolved oxygen. The following is a tabulation of the results of this experiment.

Table XIV

Showing the Effect of Age of Plant on the Oxygen-Equivalent

	Oxygen-Equivalent p.p.m. (avg. of ten plants)
Plants which were just showing cotyledons	17.22
Plants showing six leaves	0.64

Table XIV shows that during the younger stages of the seedling the oxidant is produced whereas as the plant grows older there is little if any tendency for the production of the oxidant.

8. Effect of Number of Leaves on Oxygen Removal by Plants

In order to observe whether the leaves of the plant have any influence upon the absorption of oxygen by the roots, five series of six plants each were set up each with a different number of leaves removed, and allowed to remain in culture solution forty-eight hours. Oil was added after the plants were in position. Table XV shows the data collected on oxygen absorption. From this data it may be seen that the tops of plants exert an influence on the oxygen uptake of the roots. There is a direct relationship between the number of leaves on a plant and the oxygen uptake by the plant roots. As the number of leaves were reduced from twelve to none, the amount of oxygen absorbed was reduced from a point where practically all the dissolved oxygen was taken up to the point where practically none of the oxygen was removed.

Table XV

Showing the Effect of the Removal of Leaves on the Oxygen Absorbtion by Soybean Roots After 48 Hours
Temp. 22°C

Series		Avg. value for six plants p.p.m. of O_2
A	Check	1.05
B	3 lower leaves removed	4.00
C	6 lower leaves removed	4.20
D	9 lower leaves removed	6.45
E	Entire top removed	8.11

The plants chosen for this experiment were at an age where it would be expected that little of the oxydant would be produced, and also the time of the experiment (48 hours) was sufficiently small so that none or little could be produged as can be seen from Tables VII and VIII, pages 36 and 41 respectively.

9. Effect of Light on Oxygen Intake

In order to investigate further the affect of the tops of plants on the consumption of oxygen by roots, it was decided to investigate the effect of light, since photosynthesis is connected with the green coloring matter (chlorophyll) of the tops.

Accordingly, twelve uniform plants (bearing 14 leaves each) were chosen and all placed in nutrient sol-

ution and an oil film added. All the plants were removed to the greenhouse. Six of the twelve plants were placed under a cardboard box (precautions being taken for freedom of air movements) in order to exclude light. The other six plants were allowed to remain exposed to the light. After forty-eight hours, the plants were removed and dissolved oxygen determinations made with the following results.

Table XVI

Showing the Effect of Light on the Oxygen Intake of Plant Roots

	Dissolved Oxygen p.p.m. (Avg. 6 plants)
Plants Kept in Darkness	6.71
Plants Kept in Light	2.24

T able XVI shows either that oxygen is not removed by plants in the dark or that the oxidant is made in the dark.

10. Effect of Oxygen on Dry Weight

It was thought advisable to investigate the influence of a deficiency of oxygen on the growth of older seedlings and to determine if possible, whether the production of

the oxidant might not have been the reason for the good growth of the young seedlings of the first experiment. Accordingly twenty plants (each bearing six to eight leaves) were removed from the quartz sand. Ten plants were placed in nutrient solutions under an oil film, whereas the other ten plants were placed in nutrient solution which did not have an oil film. The plants were allowed to grow for a period of 21 days. Nutrient solution was added to those plants which were under oil in the manner described heretofore, in order not to increase the oxygen content of the original nutrient solution. Nutrient solution was added to those plants which were not under an oil film merely by pouring the nutrient into the container without any thought to the addition of more oxygen.

It was found that although the roots (in the culture solution containing an oil film) were in perfectly healthy condition, that the bottom leaves began to appear wilted and some of the top leaves took on a spotted appearance on about the eighteenth day. A critical examination of the plant disclosed the reason for the sudden change in the tops. It was found that the region of the stem that was surrounded by oil was beginning to rot. This fact no doubt explained the observed difficulty. At this point it

was decided to harvest the plants. Table XVII shows the dry weights obtained.

Table XVII

Showing the Effect of Lack of Oxygen on Dry Weight

	Plants with oil film	Plants without oil film
Weight plants)	23.64	23.08
solved O_2 er 21 days)	0.06	3.63

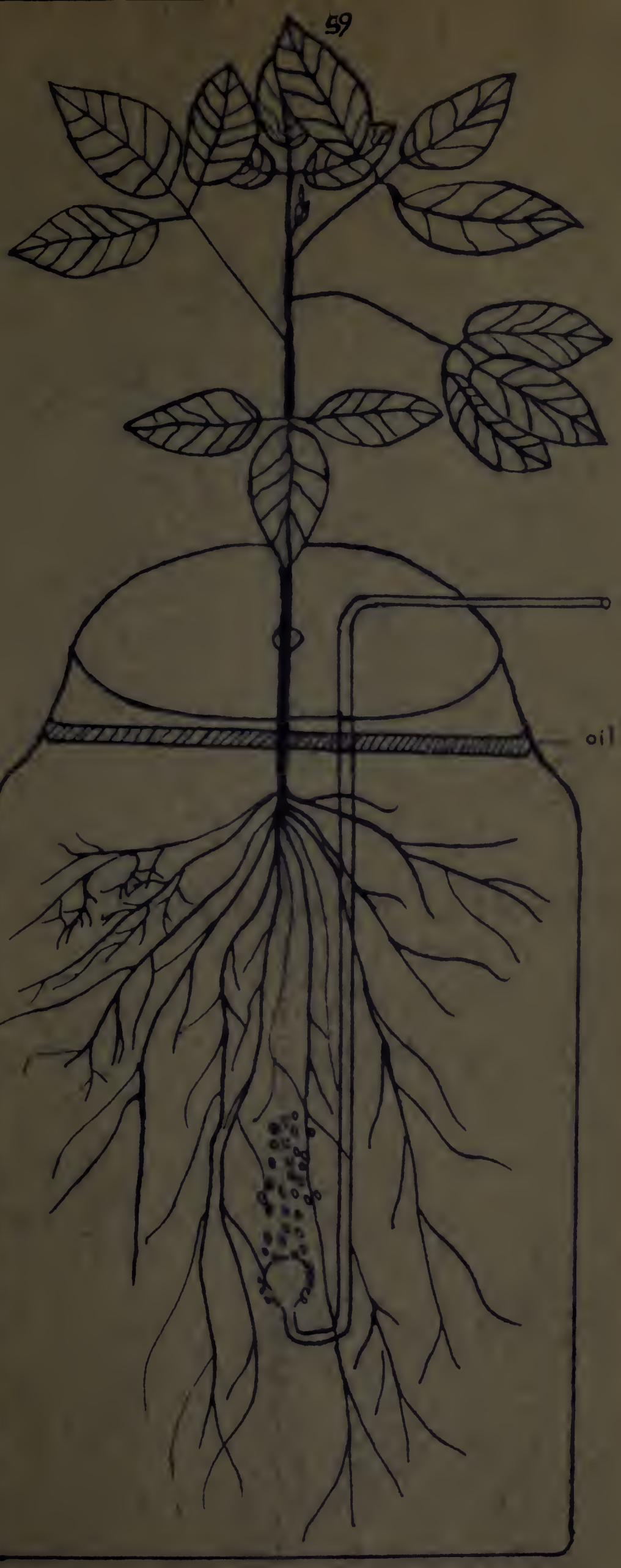
From the data in the above experiment we may see that the amount of dissolved oxygen in the nutrient solution which had no oil film was always greater than the amount of dissolved oxygen in the nutrient solution covered by an oil film. Notwithstanding this fact, the latter plants had a dry weight content slightly higher (although not significantly higher) than the former. Evidently the oxygen content of the solution does not exert so much of an influence on roots as has been thought in the past.

11. Influence of Oil Film on Production of Oxident

Since, as has been heretofore stated, after a period of time the contact of the oil made conditions such that

the stem began to rot at the point of contact, it was considered advisable to investigate the possibility that the oxidant might have its origen in that region, or that possibly certain reactions might be set up in that region where decay was progressing to account for the oxidant produced.

Accordingly, the following experiment was set up: six plants, Series B, (cotyledons just unfolding) were placed in culture solution. A film of oil (25 cc.) was introduced in order to coat the surface of the solution and to prevent air from penetrating the solution. Another six, Series C, were set up in exactly the same way but for the fact that the portion of the stem which was to come into contact with the oil film was coated with a collodion solution preparation. In this manner the stem in this region was prevented from coming into contact with oil film. Still another six plants, Series D, were set up in exactly the same way as were the first set with the exception that a glass tube penetrated the oil and through this tube a continuous stream of oxygen was introduced. Six plants, Series A, without an oil film was used as a check. The plants were all very young ones and were of an age which had always yielded large quantities of the oxidant.



Diagramatic Figure Showing Method of Introducing Oxygen into Culture Media

After ten days the experiment was discontinued and oxygen determinations made of all the culture media with the following results.

Table XVIII

Showing the Effect of a Continuous Stream of Oxygen on the Oxygen Content

	Oxygen Equivalent p.p.m. (avg. 6 plants)
A. Plants	3.6
B. Plants and oil	32.0
C. Plants (collodion on stem) and oil	34.3
D. Plants (continuous O_2 stream) and oil	4.8

This experiment shows that neither the oil nor the decay of the stem has anything to do with the production of the oxidant. It will be seen here as has been elsewhere noted that where there is present any oxyygen no oxidant tends to accumulate. This is particularly borne out in the case of the D series of plants where despite the oil film, no large accumulation of oxidant was found because of the presence of free oxygen. At the same time relatively large quantities of oxidant were produced in series B and C.

12. Oxidation of Organic Compounds

Since there was every indication that some oxidant was being formed by the plant roots, it was decided to investigate its ability to oxidize several compounds.

a. Benzidine

Benzidine oxidizes to a black substance. Enough benzidine was added to distilled water to make up a 5 part per million solution. Two series of six plants each were introduced into the solution---one series through an oil film. After twenty-four hours, the roots were all covered with a black dye.

The experiment with benzidine was repeated along more extensive lines as follows: A saturated solution of benzidine was made by the simple expedient of heating distilled water in contact with an excess of benzidine, then allowing the solution to cool. One portion of the solution was diluted ten times, another portion was diluted three times, while a third portion was used saturated. In this third portion, later, there precipitated out crystals of benzidine showing that it was saturated. These three solutions will be designated as, dilute, moderately concentrated, and saturated, in the order named.

An interesting observation made was that even when the saturated solution (which was left in contact with the crystals) of benzidine was exposed to the air (no oil film used) the solution remained colorless nor did the crystals at the bottom of the flask change from their colorless form-showing that oxidation could not occur under these conditions from the oxygen of the air. This test was run for a period of seven days.

The roots of all the plants which were put in a solution of benzidine in distilled water, whether the solution was dilute or saturated, were coated with the black insoluble substance. This transformation occurred in little more than twelve hours, for the plant roots were submerged in the solution in the late afternoon and were found coated with the black substance early the next morning.

The black substance was seen in the veins of the leaves and stems. Evidently, the benzidine had moved up the stem and even into the veins of the leaves of the plant whose roots had been submerged in the most concentrated solution of benzidine. The evidence of this fact was that the benzidine had been oxidized.in these regions to the extent that the stem and the major portions of the veins and veinlets were colored black, thus showing that oxidation was going on in these regions also. Combine this fact with

fact (as will be shown later) that
the ~~other~~, ~~and~~ the roots of this plant will not oxidize aloin when the tops are removed, and it seems probable that the oxidant may be found in the tops. If such products are in the tops they may be passed down to the roots. When the tops are removed the process would naturally stop.

It should be noted that it was fully three days after these trials were begun that the blackening of the stem and veins were first noted. Also at this time this same plant was becoming wilted and showed every evidence of dying. Because of this it was suggested by Dr. L.H. Jones that the benzidine was depriving the plant of oxygen thus causing its death.

The same general observations were made in the case of the moderately concentrated solution of benzidine, except that the evidence was not so marked, nor was the plant in such dire straits.

The plant whose roots were submerged in the dilute solution of benzidine seemed healthy even after a week of the trial, and even tho the roots were fully coated with the black substance. In this case there was no evidence that the benzidine had penetrated into the top of the plant.

The above experiment with benzidine brings out the following conclusions:

(1) That the oxygen dissolved in water, even when the water is in contact with the air, is insufficient to oxidize benzidine.

(2) That in considerably less than 24 hours the roots of the soybean plant could oxidize soluble colorless benzidine to an insoluble, black substance which coated the entire root.

(3) The oxidation mentioned in "2" can take place in a solution of benzidine in distilled water.

(4) In very highly concentrated solutions, the benzidine will pass up thru the plant and be oxidized to the insoluble compound.

(5) Those plants in which the benzidine had penetrated to the tops showed signs of dying. This might possibly be attributed to oxygen deprivation.

(6) It is possible that the seat of the oxidant may be found in the tops.

b. Aloin

Aloin is a yellow powder soluble in water. When aloin is oxidized it changes from a straw-colored solution to one that is deep red, depending on the proportion of the material oxidized.

Plants were removed from a nutrient culture and placed in an aloin solution made up to contain 200 mg. of aloin per liter of water. After twenty-four hours the plants were removed and samples taken for color. No attempt was made to register a quantitative measure of the color produced. Six plants were used in each treatment and the color changes recorded.

Table XIX

Showing Effect of Age of Plants
on Oxidation of Aloin

A_1--young plants (roots under oil film)-- Deep red (strong oxidation)
A_2-- " " (no oil film) -- " " " "
B_1-- plants 21 days old (under oil film)-- Pinkish (weak oxidation)
B_2-- " " " " " (no " ")-- " " "
C_1-- " 42 " " (under " ")-- No color change
C_2-- " " " " (no " ")-- " " "
Checks---no plants (film of oil) -- " " "

The results of this experiment are striking. They demonstrate clearly that the oxidant is produced in the younger stages of the plant's life but as the plant grows older, there is a definite loss of its ability to produce the oxidant.

Furthermore, it should be noticed that there is as

much oxidation of the aloin in the culture medium free of an oil film as there is of the one which has the oil film present. It seems, then, that the reason for a lack of evidence of the oxidant's presence in the previous experiment was due to the fact that for some reason or other the oxidant would disappear in that nutrient media where the oxidant could come into contact with the air. In other words, the substance would be destroyed in the air. If, however, the oxidant could be utilized at once to oxidize the aloin, then the oxidant could be measured in terms of oxidizing power.

The tests with aloin were repeated with much the same results as before. The nutrient solution used was similar in every respect to that reported in the body of the dissertation except for the fact that both Manganese and Boron were eliminated by request of Dr. Eisenmenger. The fact that the solution was deficient in these elements may possibly explain the appearance of the lower leaves on some of the plants as well as some differences which may appear between these results and the ones obtained previously. The plant roots were first placed in nutrient solution and were considered fully acclimated after they had produced a considerable number of new rootlets.

On the evening of December 14, 1939, the following

three sets of experiments were set up; -

1. Four plants were placed in a solution composed of 200 mg. of aloin per liter of distilled water. The surface of the liquid was covered with a film of oil.
2. Two plants were placed in a solution composed of 200 mg. of aloin per liter of the nutrient solution. An oil film was used to cover the surface of the solution after the roots were introduced.
3. Two-quart Mason jars containing a solution of aloin in distilled water in the same proportions as above were instituted as checks. The surfaces of the solutions were covered by oil films but no plants were introduced. These were to provide a means for observing the effect of air oxygen.

All plants were washed for three days by continually changing the water until no test for nitrates or nitrites could be detected in the water after the plant was submerged for several hours. As a further check a similarly washed plant was left in distilled water for the duration of the experiment and then tested for any nitrates or nitrites which might have exosmosed from the tissues of the plant roots. No sign of either could be found.

On the next morning, not more than seventeen hours

after the test was begun, all the solutions, with the exception of the checks, had turned varying shades of red showing that the aloin had been oxidized. Since some of the solutions contained nothing but water and aloin, the plants would seem to be the responsible agents.

It was suggested that nitrites might be the cause of the oxidation. Accordingly, check solutions for nitrites were made up. In one was placed a large pinch of sodium nitrite (more than one gram) and in the other about 1/5 as much. It was felt that for the purposes of this test greater accuracy was unnecessary. Over the surface of each solution was placed an oil film. No change of color developed in the three days following, showing that the nitrites could not be responsible for any change in color of the aloin even in the case of the nutrient solution above.

After the three days, tobacco plants were introduced into the aloin solutions to which the sodium nitrite had been added. There was evidently too much nitrite present for its toxic action was clearly evident. The plant in that aloin solution containing the high concentration of sodium nitrite died overnight and the solution of aloin did not change color. The plant in the aloin solution

containing the lower concentration showed signs of minor distress. In this case, however, the plant had turned the aloin slightly pinkish, showing that some oxidation had taken place. Another aloin solution containing but a trace of sodium nitrite was also used. Here the plant appeared very healthy and the solution had turned a deeper pink than in the second case. Tabulated the data appear as follows:

Table XX

Showing the Effect of Plants in Nitrite Solution of Aloin

Treatment	Appearance of plant after 24 hours.	Color of aloin Solution
1. plant in highest conc. of $NaNO_2$	Wilted and dying	Yellow
2. plant in medium conc. of $NaNO_2$	Wilted and dying but not in so bad a state as "1"	Pinkish
3. plant in low conc. of $NaNO_2$	turgid and healthy	Deeper pink than "2"

Since the pH of the solution might be a factor, especially in conjunction with the $NaNO_2$ in the oxidation of aloin, the pH of the following solutions were taken electromerically.

Table XXI

Showing pH of Aloin Solutions

	pH
Distilled water	5.7
Aloin solution - oil film - plant (plant had been in this solution 3 days, and aloin had changed color)	5.6
Aloin solution - oil film - plant (high conc. of $NaNO_2$)	5.7
Aloin solution - oil film - plant (low conc. of $NaNO_2$)	5.7

Table XXI shows that the pH of the solution did not change in any of the solutions so we may assume it was no factor in the aloin experiment.

In another experiment, two healthy plants were placed in a solution composed of 200 mg. of aloin in distilled water. An oil film was poured over the surface of the solution after the roots were introduced. The tops were cut off at a point below the cotyledons and a very thick coat of vaseline applied to the cut end. At the end of 48 hours there was still no signs of any oxidation having taken place.

The above experiment, when studied in the light of the experiment outlined on pages 53 and 54 , throws further information on this subject. The work on pages 53

and 54 showed that the tops influence the uptake of oxygen by plant roots. A plant with top removed cannot take up oxygen, neither can it oxidize aloin in 48 hours.

Since there is evidently oxidation going on in the stem and leaves of plants, evidence of which was shown under the benzidine tests, it is possible that some substance is passed down into the roots from the tops.

One other fact should be mentioned here; namely, that after a period of 6 days the reddish color in the distilled water began to dissapear. The reason for this is unknown although it may be possible that after this period of time the metabolic processes within the plant were interfered with because of the lack of minerals. This seems plausible since the aloin remained redder in the nutrient solutions.

The above experiment with aloin brings out the following facts:

1. That the oxygen normally dissolved in water (about 7-9 p.p.m. under normal conditions) is not sufficient to oxidize the aloin.
2. Nitrites alone did not oxidize aloin, at least within the time limit of this experiment.
3. The plant was able to oxidize aloin in distilled water after tests showed that neither nitrates nor nitrites were present.

4. 4. This oxidation will take place in considerably less than 24 hours.
   5. The fact that the plants in apparent distress because of the toxicity of the nitrites were either wholly incapable of oxidizing the aloin or capable of oxidizing it to a limited extent only tends to indicate that plant decay was not the factor. In any case decay of healthy tissue under water would be only limited in the few hours necessary to show this effect.
   6. The pH of the solution did not change during the aloin test.
   7. It appears that the plant itself is the responsible agent for the oxidation of the aloin.
   8. The tops of the plants seem to be necessary for the oxidation of the aloin.
   9. There was a fading of the reddish color of the oxidized aloin in distilled water after six days.

13. Oxidation of Ammonia

Starting with the knowledge that young plants can use ammonia nitrogen better than can older plants and also from the above experiments of the writer that an oxidant is produced much more prolifically by younger plants than

by older ones, it was considered as a possibility that there might exist a relationship between these two facts.

In order to test this possibility a preliminary experiment was set up in which the nutrient solution containing twelve young plants (whose cotyledons had just unfolded) was tested every twelve hours by means of diphenylamine to determine if any nitrates were formed. It was discovered that in the early mornings and in the evenings of sunless days that there would be distinct evidences of nitrate formation, whereas in the evening of sunny days there would be at the very utmost only a trace of nitrates. Evidently any nitrates formed were utilized almost immediately under conditions of strong sunlight.

In order to obtain quantitative results for nitrification, 24 uniform young plants (whose cotyledons had just unfolded) and 24 uniform older plants (bearing 8--10 leaves) were chosen and placed in distilled water. These plants were kept in distilled water with frequent changes for several days. The frequent changes of water was for the purpose of washing off any nitrates which might possibly be found on the surface of the roots or which might possibly diffuse into the water from within the plant tissue itself. To make certain that all the nitrates were washed

out, the water was tested by means of the diphenylamine method.

When it was certain that the water no longer showed any reaction to the diphenylamine the plants were divided into four series of twelve plants each. Each series being composed of six young and six old plants.

A stock solution was made up to contain 5 cc. of molar ammonium sulfate per liter of water. This solution was used for each series of plants, the pH of the solution being adjusted by the use of sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH) as required. Half of each series was placed in solution whose surface was covered by an oil film composed of 25 cc. of mineral oil. For the purpose of adequate checks series D, E, F, and G were installed. Series D, E, and F were similar in every respect to series A, B, and C with the exception that they contained no plants. Series G contained plants but had no ammonium sulfate, $(NH_4)_2SO_4$, added to the water.

Since, as was previously discovered, nitrate nitrogen would accumulate only in the absence of strong sunlight, the tests for nitrates were made in the early morning.

Table XXII gives results of these tests. As may be gathered from this table, young soybean plants appear to be better able to oxidize ammonia to nitrates than are

the older plants. As may be seen, this is true in every case. Also it is worthy of note, that the change to nitrates takes place much more rapidly at neutral reactions than at either alkaline or acid reactions. However, it must be emphasized that there must be an uptake of nitratesby the plant roots and it has been shown that more nitrates are absorbed under conditions of high sunlight than otherwise, It is therefore impossible at present to know definitely whether less nitrate was found in the solutions which held the older plants because less was formed or because more was absorbed by the plant roots. However, if it is assumed that there is an oxidant formed as postulated, and more by young plants than old, then less nitrates might actually be formed in those solutions which contain the older plants. This fact, in some measure may serve to explain why most workers, as shown above, have found that young plants are better able to assimilate ammonia nitrogen than are the older plants.

Also, it is to be noted that compared to plants in neutral solutions there is a sharp decrease in nitrate formation both at high and at low pH values. This also seems, in part, to explain why other workers have found that plants in a neutral medium are better able to utilize nitrate nitrogen than ammonia nitrogen.

Table XXII

Showing Effect of Age and pH on Formation of Nitrates

Series	pH	Nitrates Formed (p.p.m.)			
		Oil Film		No Oil Film	
		Y.P.*	O.P.*	Y.P.*	O.P.*
A	4.0	2.6	0.8	2.3	1.2
B	7.0	5.5	2.7	6.0	3.1
C	8.5	0.8	0.1	0.7	0.0
D	4.0	0.0	0.0	0.0	0.0
E	7.0	0.0	0.0	0.0	0.0
F	8.5	0.0	0.0	0.0	0.0
G	6.8	0.0	0.0	0.0	0.0

* O.P. = old plants (8-10 leaves)
Y.P. = young plants (cotyledons just unfolded

The checks adequately show that in solutions having no plants no nitrates were formed. Also, it should be pointed out that those solutions to which no ammonium sulfate was added gave negative results for nitrates showing that no nitrates diffused out from the roots of the plants.

IV Conclusions

It is desirable to collect the data contained in the experiments performed and to integrate them, if possible. Also it is desirable to try to explain any apparent differences between the results obtained in this thesis with the results obtained by other workers.

It is noteworthy, then, that there is a wide difference between the minimum amount of oxygen which has been reported as necessary to plant roots when they are anchored in the soil, and the amount of dissolved oxygen available to plants when they are grown with their roots submerged in a nutrient solution. It must be noted that whereas in soil, plants cannot continue growth when the oxygen content falls much below 0.5% or 5000 parts per million, that plants do well in water culture when the dissolved oxygen content lies between 7 and 9 parts per million --- or .0007 - .0009%, an oxygen content, one thousandth of the amount needed in soil. The possibility is suggested, and seems to be borne out by experiment, that the effect of oxygen is not a direct one but an indirect one. The nitrogen of the soil is present, ordinarily, in organic form and must be changed by bacterial action (these bacteria needing oxygen) to the nitrate form be-

fore plants can utilize this nitrogen. However, in water culture, where the nitrogen is ordinarily supplied in either the nitrate of ammonium form, bacteria are not needed to convert organic to inorganic nitrogen.

It has been repeatedly observed that in water-logged soils, plants turn yellow, showing somewhat the same symptoms that would become apparent under a condition of nitrogen deficiency. In many cases when these same soils are aerated by artificial drainage, healthy plants may be grown in them. In the light of the experiments in water culture it is suggested that the yellowing of the plants may not be due so much to the lack of oxygen for the plant roots in the water-logged soils as to the lack of this oxygen for the bacteria which effect the change of organic nitrogen to the inorganic forms which thus become available to plants. It is suggested, then, that the need by plant roots for oxygen in the soil may be indirect rather than direct. To substantiate this point the writer has observed that supplying nitrates to grass in the very early spring when the ground is still very wet and bacterial action is low, will make the grass turn green sooner than on areas not so treated.

Again, under anaerobic conditions in the soil where there is usually an abundance of organic matter, it is

likely that anaerobic bacteria produce substances which are toxic to plants. Therefore later on in the summer when bacteria are very active, the additions of nitrates to water-logged soils may not have the same desired effect as additions made in the spring.

There is also the possibility that the roots of plants grown in water culture adapt themselves to these conditions by producing somewhat different roots. An observation made by Dr. L.H. Jones, that plants grown in water culture usually have roots devoid of hairs may bear this fact out, especially so since the writer has observed that as plants grown older it becomes increasingly more difficult to successfully transplant them to water culture.

It may well be that both the above factors; namely, the indirect effect of oxygen and the new type of rootlets developed under water culture conditions play an important part in explaining the difference that exists in the relationship of oxygen to roots in soil as compared to water culture conditions.

Again it is suggested that the increase in plant growth reported by some workers when culture solutions were aerated may have been caused not so much by any increase in the oxygen supply to the roots as by a stirring up of the media and therefore effecting a renewal

of the nutrient solution exposed to the surface of the feeding roots. In private conversation with Dr. Livingston of John Hopkins University, Dr. Livingston expressed the same view, and some work he is now supervising seems to bear this out.

As has been shown, when the nutrient solution is so controlled that no additional oxygen can enter, that plants will deplete the oxygen supply to a very low point, but that, at least during the time limit of this experiment, this low oxygen content had little if any effect upon the growth of plants over those plants grown in nutrient solution not so protected. Also, there is evidence that young plants at least produce a substance which the writer will call an oxidant because it was shown capable of oxidizing several substances.

That the substance is a true oxidant and not the result of the production of nitrites from nitrates by bacterial action is shown by the fact that both benzidine and aloin were oxidized in solutions of distilled water; as also by the fact that nitrites even in relatively large quantities when added to a solution of aloin could not oxidize it.

It seems probable that the oxidant is produced in the tops of plants and passed down thru the stem to the

roots. Evidence of this may be seen in the fact that benzidine was oxidized in the stem and leaves of the plant and that topless plants could not oxidize aloin solution.

The fact that the oxidant seems to be produced in largest quantities by young plants and that young plants appear to have the power of oxidizing ammonia nitrogen to nitrate nitrogen, seems to explain certain observations by many workers, notably Prianischnikov, that the younger the plant, the more able is it to use ammonia nitrogen; and also that as it grows older its ability to use ammonia nitrogen decreases so that increasingly it must get its nitrogen in the nitrate form. Added strength to this suggestion is gained when one realizes that more nitrate is oxidized from ammonia at pH 's which were also found by Prianischnikov to increase the ability of young plants to use ammonia nitrogen.

It is recognized that no care was taken to make the solutions sterile, but it is believed that adequate checks were maintained to insure recognition of microorganisms as a factor.

There is evidence that the tops influence the uptake of dissolved oxygen as well as the production of an oxidant, possibly thru a reduction in photosynthetic activity

as may be seen from the fact that little or no oxygen was removed from the solution both when the tops were removed and when the plant was removed from the light.

In conclusion, then, tops seem to be necessary in the production of the oxidant which is probably used by young plants to oxidize ammonia nitrogen and perhaps organic forms of nitrogen to nitrates. In the early spring this would be of great advantage to plants, since the soil is usually water-logged with little free oxygen present. The plants being young can take full advantage of oxidant production.

V Summary

1. A layer of oil approximately 0.45 cm. thick was found sufficient to prevent the entrance of appreciable amounts of oxygen into the culture solution. The oil provided a ready means for determining how much oxygen the plant actually used.

2. It was shown that the concentration of the culture media within the limits of this experiment would not influence the amount of dissolved oxygen which could be held in solution.

3. A method for adding oxygen-free nutrient solution to culture solutions was developed.

4. Sources of error which might arise during the oxygen determinations were investigated.

5. It was found that plants would deplete practically all the dissolved oxygen from the nutrient solution in a few hours.

6. If young plants are allowed to remain in an oxygen depleted solution, they apparently give off a substance which acts like an oxidant.

7. This substance could not be volatalized or destroyed by boiling but would be destroyed if allowed to stand in contact with the air for long periods of time.

8. The addition of plant debris to the culture solution had no observable effect on the production of the oxidant.

9. The age of the seedling had a profound influence upon the production of the oxidant, young plants producing the oxidant prolifically, whereas older plants either producing it in very minute quantities or not at all.

10. The amount of dissolved oxygen removed by the roots from the water culture is directly proportional to the number of leaves on the plant.

11. None or very little oxygen was removed by plants in the absence of light or when the tops were removed.

12. Within the limits of this experiment, the amount of oxygen in the solution had very little, if any, influence on the dry weight of the plants.

13. It was demonstrated that neither the oil nor any decay that might be set up in the region of the stem in contact with the oil, had anything to do with the production of the oxidant.

14. It was shown that plants do well in water culture when the dissolved oxygen content lies between 7-9 p.p.m. ----or .0007-.0009% ----an oxygen content one thousandth of the amount needed in soil.

15. Plant roots when growing in a solution of benzidine, were able to oxidize the benzidine to form a black insoluble substance.

16. The benzidine was absorbed by the plant roots and appeared in the stem and veins of the leaves where the benzidine had evidently been oxidized.

17. Those plants which had absorbed sufficient quantities of benzidine so that the stem and leaf-veins appeared black, began to wilt and died in a very few days.

18. The plants were able to oxidize yellow straw-colored aloin to its reddish form.

19. Plants would oxidize aloin even in distilled water. The same is true for benzidine.

20. It was demonstrated that nitrites could not oxidize aloin even when present in large quantities.

21. When the tops were removed, the roots lost their power to oxidize the aloin.

22. There is evidence to the effect that plants can oxidize ammonia to nitrates, and that the pH of the solution as well as the age of the plant seems to exert a profound influence on the intensity of the oxidation.

23. An explanation is offered for the apparent difference in oxygen requirement by the same plant in soil and water culture.

24. An explanation is offered for the fact that young plants are able to utilize ammoniacal nitrogen better than can older plants.

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We have examined Mr. Benjamin Isgur's dissertation both in the original and revised forms.

It is our opinion that Mr. Isgur has made a contribution to science and has stated his case in suitable form. We have personally watched Mr. Isgur at work, and consider his technique excellent. We believe him to be sincere, and found him particularly cooperative. The added work the committee desired was cheerfully performed and the results which appear in the final copy confirm the earlier work.

Mr. Isgur's thesis raises many questions which make it desirable that the work be continued.

We are glad to approve this thesis and recommend its submission to the Graduate Faculty.

C. A. Peters

Linus H. Jones

Special Committee Appointed by
Director Sievers

April 16, 1940

APPROVED BY:

H. W. Fessenden

Leon A. Bradley

Walter S. Eisenmenger

Committee on Thesis

DATE Dec 6 1938