EFFECT OF VARIOUS OXYGEN AND CARBON DIOXIDE CONCENTRATIONS ON COTTON ROOT DEVELOPMENT[1]

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(WITH NINE FIGURES)

Introduction

Under field conditions, the concentrations of oxygen and carbon dioxide at the surface of cotton roots are thought to be extremely variable. Gross analyses made by **LEONARD** (**10**) show the percentage of oxygen in the soil air under cotton to vary from 0 to 21 for Houston clay, from 10 to 21 for Sarpy fine sandy loam, and from 18 to 21 per cent. for Ruston sandy loam. While these values only approximate effective concentrations at root-gas interfaces, they do indicate in a relative way the conditions of aeration within different soil types. Although the differences in behavior of cotton on these different types of soil may be attributed to differences of particle size, fertility, water movement, and numerous other variables, the rôle of aeration appears to be quite important. Unpublished data of the present writers indicate that aeration and mechanical composition of the soil may prove to be an important factor in the distribution of the Fusarium wilt disease of cotton. The importance of soil aeration as it applies to the culture of plants in general is well appreciated, although many of the details are yet to be investigated.

The purpose of the present study was to determine the effect of carbon dioxide and oxygen mixtures on the growth and development of cotton roots in nutrient solutions. The study seemed necessary in order to obtain data for the proper interpretation of the behavior of cotton on different soil types with and without the presence of the Fusarium wilt disease.

Methods and materials

The general plan of the apparatus employed for subjecting the roots of seedling cotton plants to stated concentrations of oxygen and carbon dioxide alone and in mixtures with nitrogen is illustrated in figure 1. By means of a siphon arrangement, the gas in container B was forced through a nutrient solution in which the plants were supported. Although only one culture tube and one plant is illustrated in figure 1, two tubes were used, each containing two plants. A battery of several such units was used, each provided with a different concentration of gas. The entire equipment was operated before a large laboratory window or in the greenhouse. Supplementary illumination was provided by a fluorescent lamp which was operated continuously. In some experiments (not reported) no supplementary illumination was used; in others, artificial light supplemented daylight sufficiently

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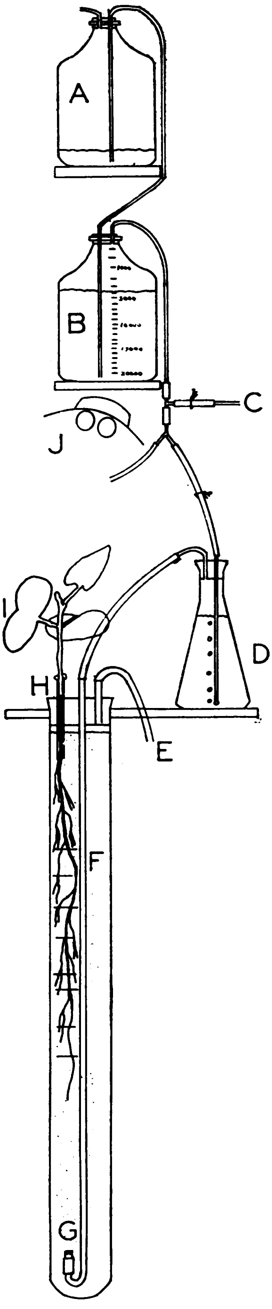

FIG. 1. A diagrammatic view of the apparatus used in aeration experiments. A, five-gallon bottle; B, five-gallon bottle calibrated to hold 20 liters of air at atmospheric pressure; C, air inlet for making gas mixtures; D, Erlenmeyer flask holding reserve nutrient solution; E, gas escape tube; F, culture tube; G, gas outlet; H, glass tube in which cotton plant was inserted; I, cotton plant; J, fluorescent light.

to make a photoperiod of 15 hours. The relationship between the percentage of oxygen and carbon dioxide and cotton root growth was similar to that obtained with continuous illumination. The culture tubes were placed in a darkened air- or water-bath in which the temperature was maintained at approximately 30° C. in some experiments and 28° C. in others. Air temperatures about the shoots were not controlled.

Compressed cylinder gases were either used directly or further purified by appropriate methods. They were introduced into the system through opening C and were utilized from container B (fig. 1). Approximately 40 liters of gas was passed through each pair of culture tubes in 24 hours. To prepare a given concentration of mixed gases, distilled water was forced from calibrated container B. The composition of the mixtures were determined at frequent intervals by a portable gas analysis apparatus. Oxygen concentrations were easily prepared accurately, although carbon dioxide concentrations frequently varied over a range not exceeding 10 per cent. of the required concentration. Dissolved oxygen in the culture solution was determined by the Winkler method (**1**).

A modified Knop's solution of approximately the same composition shown by LOOMIS and SHULL (**12**) was used in the culture tubes. In tests in which nitrogen was supplied in the ammonium form, ammonium chloride and calcium chloride replaced an equivalent quantity of calcium nitrate. The initial pH of the nutrient solution was adjusted at 5.4 to 5.6 using a weak solution of potassium hydroxide, the exact pH varying somewhat with different experiments. The nutrient solutions containing nitrates usually shifted to a pH of about 6.0 and the solutions containing ammonia to a pH of about 4.8 before being changed. If the solutions containing ammonia become too acid (pH of 4.0 or less) root growth ceased. The pH of solutions receiving high concentrations of carbon dioxide were stabilized by using a buffer composed of potassium acid phosphate and potassium hydroxide. The solutions were prepared from distilled water and most of them were changed at intervals of not more than three or four days. Solutions saturated with pure gases were not changed during the course of an experiment.

Cotton seeds of the Stoneville 2B variety were acid delinted, dusted with a mercurial disinfectant, and placed between moist paper towels for germination. If no seed disinfectant was used, a low yield of healthy seedlings resulted, even though all glassware, solutions, and apparatus were boiled or autoclaved before use. After the seeds germinated, the seed coats were removed with sterile forceps and the seedlings supported in tap water on a paraffin-impregnated cloth. After the cotyledons were expanded, healthy seedlings were selected, and the radicals were inserted through short glass tubes held in no. 7 rubber stoppers. A paraffin-petroleum-cotton-lint sealing compound held the seedlings in place and prevented exchange of gases through the openings after the stoppers were placed in the culture tubes. Air was passed through the culture solution for two or three days to permit the seedlings to become established. The air or gas was released through a sintered glass plate in the bottom of the 24-inch culture tubes so as to break up the gas bubbles.

Growth of the seedling roots was determined by measuring the length of the tap root at 24-hour intervals. The experiments were terminated 14 to 16 days after the gases were first introduced. Notes were made on the appearance of the seedlings, and the fresh and dry weights of the shoots and roots were recorded.

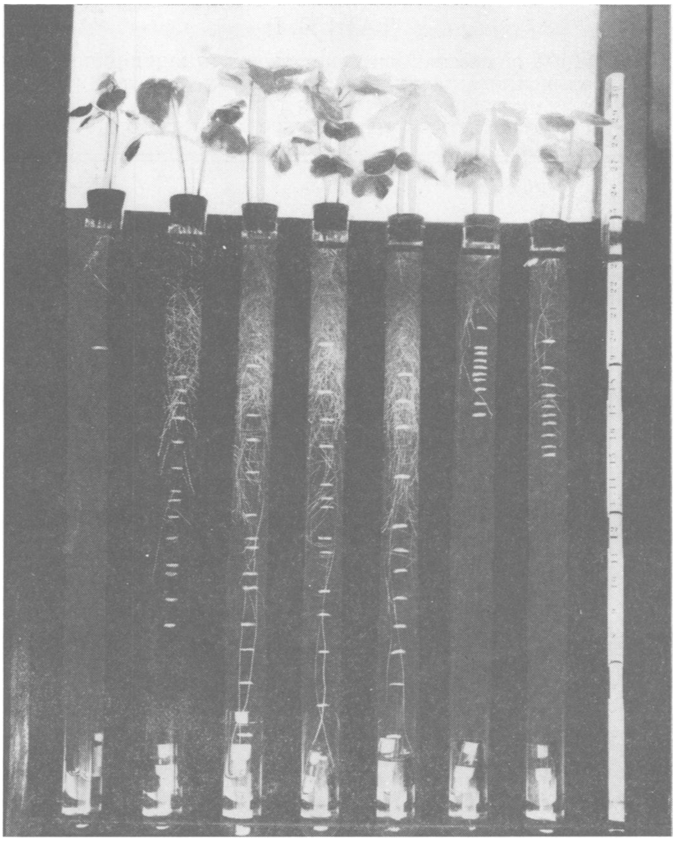

FIG. 2. The effect of various concentrations of oxygen on the daily elongation of cotton tap roots and the appearance of the tops (Experiment 13). Marks on the tubes represent tap-root elongation at 24-hour intervals. The gas mixtures from left to right were (percentages):

O_2	0	5	10	15	21	90	100
CO_2	10	10	10	10	10	10	0

A total of 20 separate experiments were conducted over a period of one and one-half years; although only seven of these experiments are actually referred to in the text, the results from the other experiments were similar to the ones reported.

Results

OXYGEN REQUIREMENTS

The optimum oxygen concentration for cotton root development was thought to depend upon a certain range of carbon dioxide concentration. To determine this range, oxygen, carbon dioxide, and nitrogen were combined in the proper proportion to form the desired mixtures. Oxygen was held constant at 21 per cent. while carbon dioxide and nitrogen were varied. Considerable root growth occurred up to 30 per cent. carbon dioxide while vegetative growth appeared to be excellent and unaffected over the same range. As a result of these tests, 10 per cent. carbon dioxide was selected as a constant with which to compare different concentrations of oxygen.

TABLE I

APPEARANCE OF 14-DAY-OLD COTTON SEEDLINGS GROWN IN LIQUID CULTURES SATURATED WITH CARBON DIOXIDE AND OXYGEN AT STATED CONCENTRATIONS AND WITH AMOUNT OF DISSOLVED OXYGEN IN THE CULTURES. RESULTS FROM SEVERAL EXPERIMENTS SUMMARIZED

GASES USED		DISSOLVED OXYGEN IN CULTURES*		APPEARANCE OF TOPS	APPEARANCE OF ROOTS
CO_2	O_2	WITH PLANTS	WITHOUT PLANTS		
%	%	*p.p.m.*	*p.p.m.*		
None	None	0.0	0.0	Little plants, small green leaves	Roots healthy, no growth. Branch roots developed if transferred to water saturated with air
10	None	0.0	0.0	Little plants, dark green leaves	Do.
10	0.5	Trace	0.1	Do.	Do.
10	1.0	0.3	0.4	Little plants, green leaves	Roots healthy, trace of growth
10	1.5	0.5	0.6	Medium plants, green leaves	Roots healthy, thick, some growth
10	2.5	0.8	1.0	Large plants, green leaves	Roots healthy, medium thick, good growth
10	5.0	1.8	2.0	Do.	Roots healthy, thin, very good growth
10	7.5	2.7	3.0	Do.	Do.
10	10.0	3.6	3.8	Do.	Do.
10	15.0	5.4	5.8	Do.	Do.
10	21.0	7.0	......	Do.	Do.
10	90.0	33.0	......	Medium plants, green leaves	Roots frequently unhealthy and parasitized, poor growth. Healthy roots produced if transferred to water saturated with air
None	100.0	37.0	......	Do.	Do.

* Temperature of 28° C.

The apparent optimum concentration of oxygen for seedling root elongation, with carbon dioxide held constant at 10 per cent. was found to range between 7.5 and 21 per cent. under the conditions described. Figure 2 illustrates a typical experiment in this series, and table I summarizes the results of all oxygen concentrations investigated. Figure 3 illustrates the variation occurring in three selected experiments.

Daily elongation of the tap roots is graphically illustrated in figure 3. During the first two weeks of seedling growth, tap-root elongation appeared to progress at a uniformly high rate. The greatest elongation for any one day was 67 mm.; it occurred at 21 per cent. oxygen and 10 per cent. carbon dioxide. Variation in root temperatures slightly above 30° C. sharply restricted the daily rate of tap-root elongation. Any prolonged reduction in intensity of illumination also limited root elongation, but the effect was not apparent for two or three days. Experiment 15 was conducted at light intensities somewhat lower than that of Experiment 17 (fig. 3). Although two plants were grown in the same tube and duplicate tubes used, the daily rate of elongation for each plant varied widely at times. No relation could be observed between stage of leaf development or leaf area and tap-root elongation.

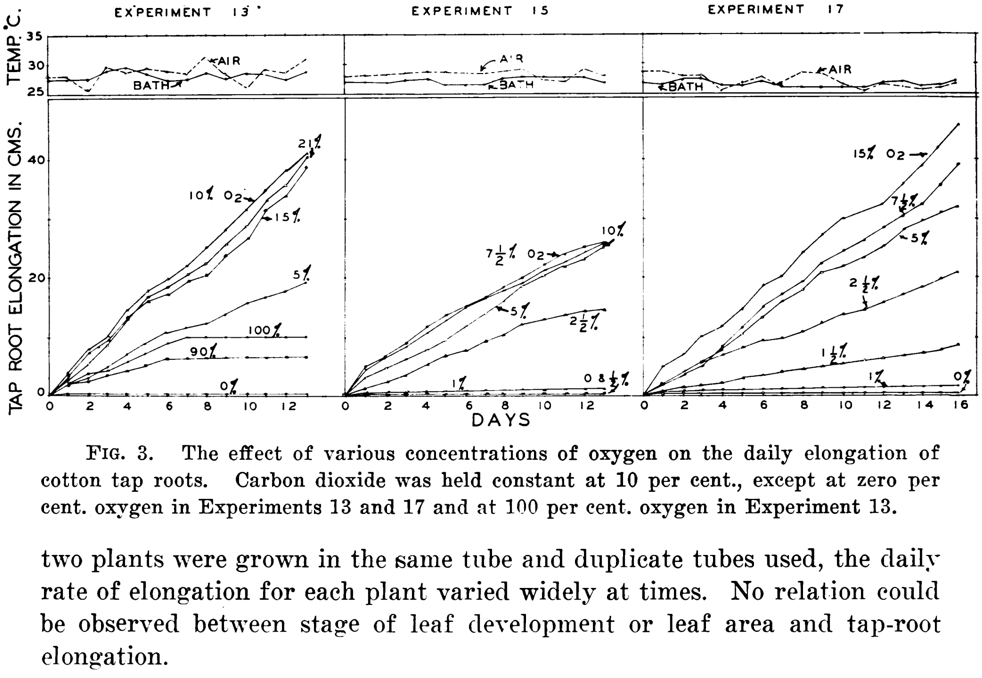

FIG. 3. The effect of various concentrations of oxygen on the daily elongation of cotton tap roots. Carbon dioxide was held constant at 10 per cent., except at zero per cent. oxygen in Experiments 13 and 17 and at 100 per cent. oxygen in Experiment 13.

The minimum concentration of oxygen tolerated by roots appeared to lie below one-half per cent. (figs. 2, 4) and zero per cent. (fig. 5). No root growth occurred under these conditions. The roots remained white and did not appear to be injured by anaerobic conditions. If air was substituted for the mixture containing zero oxygen, lateral roots were promptly initiated, and some of the old branch roots began to grow again. The tops of the plants were a darker green (when carbon dioxide was held at 10 per cent.) than those of plants receiving higher concentrations of oxygen.

At maximum concentrations of oxygen (90 and 100 per cent.), both root and top growth were restricted (fig. 2), but the color of the leaves was not affected as at low oxygen concentrations. If air replaced the gas mixtures of high oxygen concentrations, lateral root development was initiated, indicating that the pericycle was not permanently damaged. Microorganisms attacked the roots vigorously at high oxygen concentrations. Although care was exercised in preventing microbial contamination, it was difficult to prevent the roots from being decomposed at the high oxygen concentrations.

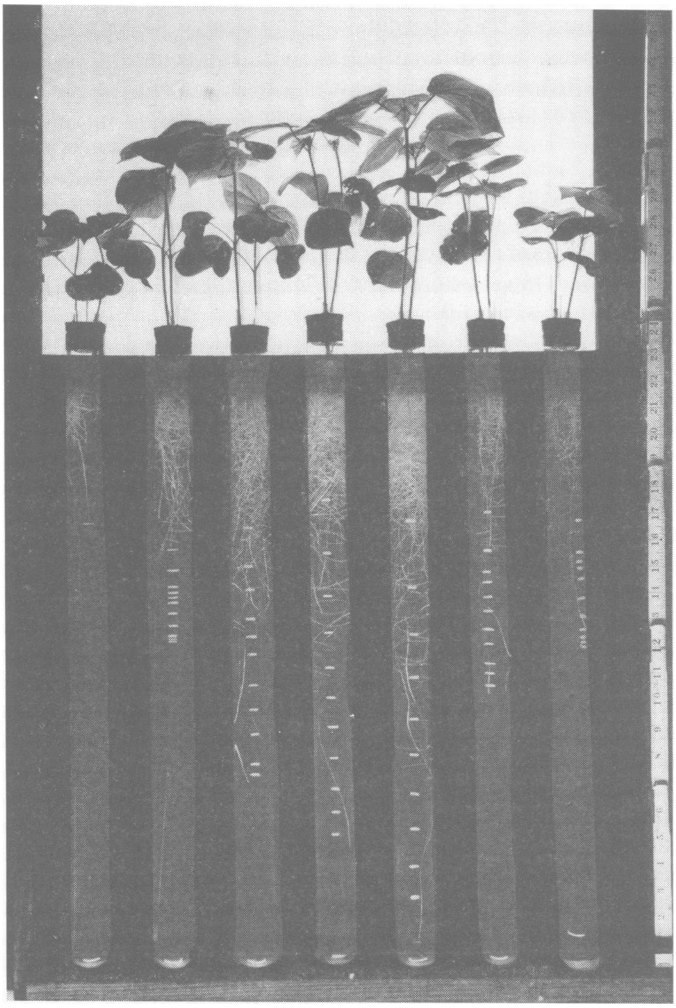

FIG. 4. The effect of various concentrations of oxygen on the daily elongation of cotton tap roots and the appearance of the tops (Experiment 5). Although the gas mixtures were the same as those used in figure 2, the bubbles were not broken up with a sintered glass plate, and the solutions were not kept saturated with oxygen. The "effective" percentages of oxygen were calculated from the determinations of dissolved oxygen. Marks on the tubes represent tap root elongation at 24-hour intervals. The calculated percentages of oxygen and carbon dioxide from left to right were:

O_2	0	2½	5	10	15	85	95
CO_2	10	10	10	10	10	10	0

If the concentration of oxygen was reduced or if air was substituted, decomposition ceased, and lateral roots developed. Species of Fusaria appeared to be dominant. The organisms present were non-parasitic at lower oxygen levels. This interesting observation deserves further study.

The amount of dissolved oxygen in the nutrient solutions receiving the various percentages of oxygen-gas mixtures is given in table I as parts per million. The exact amount of dissolved oxygen varied with temperature. Estimations of dissolved oxygen enabled us to be certain that the rate of gas evolution through the nutrient solutions was sufficient to approximately saturate the solution. It also showed that solutions with no plants growing in them were consistently, though only slightly, higher in dissolved oxygen than solutions containing growing plants. When the rate of gas evolution through the solutions was reduced, the roots absorbed oxygen, and the actual concentration of oxygen in the cultural medium was less than the calculated amount introduced. Figure 4 illustrates the results of a typical experiment of this type. The actual concentrations of oxygen in the solutions was determined by analysis. When 10 per cent. carbon dioxide was used in the mixture, the limiting oxygen factor was well illustrated by reduced root and shoot growth at both low and high concentrations.

EFFECT OF FORM OF NITROGEN IN THE NUTRIENT SOLUTION ON OXYGEN REQUIREMENT

The effects of nitrate and ammonium nitrogen on root growth were compared at all oxygen concentrations from zero to 100 per cent. The chief interest was in the effect of nitrate and ammonium nitrogen on cotton root growth at low percentages of oxygen. The results from one such experiment are shown in figures 5 and 6 and summarized in figure 7.

Tap-root elongation was similar with both nitrate and ammonium nitrogen at all concentrations of oxygen. Roots subjected to zero per cent. oxygen remained white and appeared healthy with both nitrate and ammonium nitrogen. Shoot growth seemed greater with nitrate than with ammonium nitrogen, regardless of the oxygen concentration.

Up to 15 per cent. oxygen content tap-root elongation increased, while the weight of the root system was near or at its maximum at two and one-half per cent. oxygen. The lack of correlation between tap-root elongation and increase in weight of the whole root system was caused, at least in part, by the fact that the roots which developed at the lower oxygen concentrations were thick, while those produced at higher percentages of oxygen were slender; thus a given length of root at two and one-half per cent. oxygen weighed more than the same length of root produced at higher oxygen concentrations.

Cotton seedlings growing in solutions containing nitrates accumulated more nitrogen than did those growing in solutions containing ammonia. The extra nitrogen absorbed (in nitrate form) from the solutions containing nitrate over those containing ammonia seemed to accumulate as nitrate nitrogen within the plants, since the percentages of organic nitrogen in the whole plant was about the same in both the nitrate and ammonia series. The total absorption of nitrogen (in nitrate or ammonium form) increased with oxygen increase up to about two and one-half per cent. The differences in total uptake of nitrogen were largely caused by differences in plant size, rather than by oxygen concentrations. Nevertheless, the percentage of nitrate nitrogen in the plant increased with oxygen concentration and suggests better absorption of nitrate nitrogen at high than at low oxygen concentrations: The percentage composition is based on an analysis of the whole

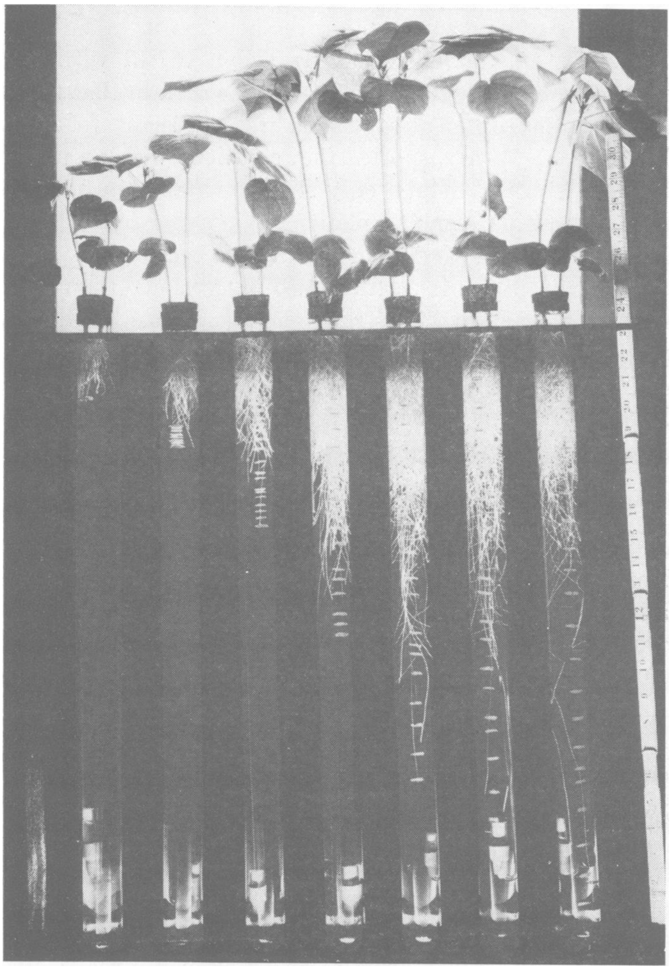

FIG. 5. The effect of nitrate nitrogen on the daily elongation of cotton tap roots and appearance of the shoots at various concentrations of oxygen (Experiment 17). Marks on the tubes represent tap-root elongation at intervals of 24 hours. The gas mixtures from left to right were (percentages):

O_2	0	1	1½	2½	5	7½	15
CO_2	0	10	10	10	10	10	10

plant, and the results may be affected by differences in the relative amounts of roots, stems, and leaves.

In order to study the absorption of nitrate and ammonium nitrogen at different concentrations of oxygen, the nutrient solutions were periodically analyzed. Results with the ammonia series suggested that more ammonium nitrogen was absorbed at high than at low concentrations of oxygen. The results with the nitrate series indicated the greatest loss of nitrate from nutrient solutions occurred at zero per cent. oxygen. Since a chemical analysis of the plant did not reveal any comparable accumulation of nitrogen

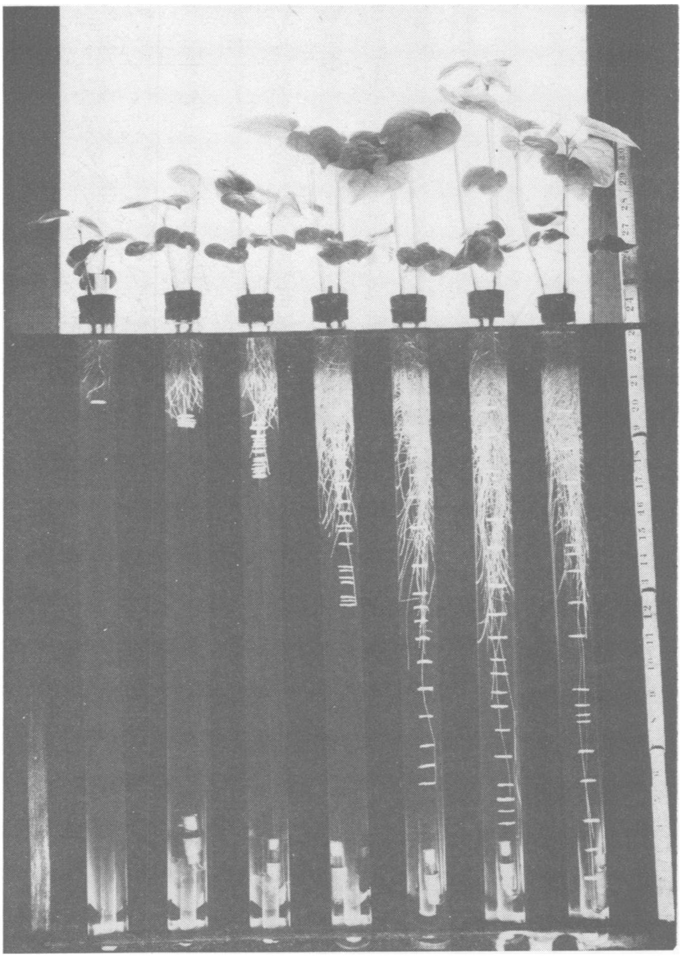

FIG. 6. The effect of ammonium nitrogen on the daily elongation of cotton tap roots and appearance of the shoots at various concentrations of oxygen (Experiment 17). Marks on the tubes represent tap-root elongation at intervals of 24 hours. The gas mixtures from left to right were (percentages):

O_2	0	1	1½	2½	5	7½	15
CO_2	0	10	10	10	10	10	10

within the plant, it was concluded that some nitrate nitrogen was lost anaerobically from the nutrient solution.

EFFECT OF CARBON DIOXIDE ON COTTON ROOT GROWTH

The influence of various concentrations of carbon dioxide on the daily elongation of the cotton tap root is shown in figures 8 and 9. Table II summarizes the results obtained from various experiments in which carbon dioxide was varied. The results of the three experiments shown in figure 8 indicate that a concentration of 60 per cent. carbon dioxide prevented all cotton root growth. Concentrations of carbon dioxide higher than 60 per cent. had the same influence on root growth and appearance of the tops as did 60 per cent. carbon dioxide. The higher concentrations of carbon

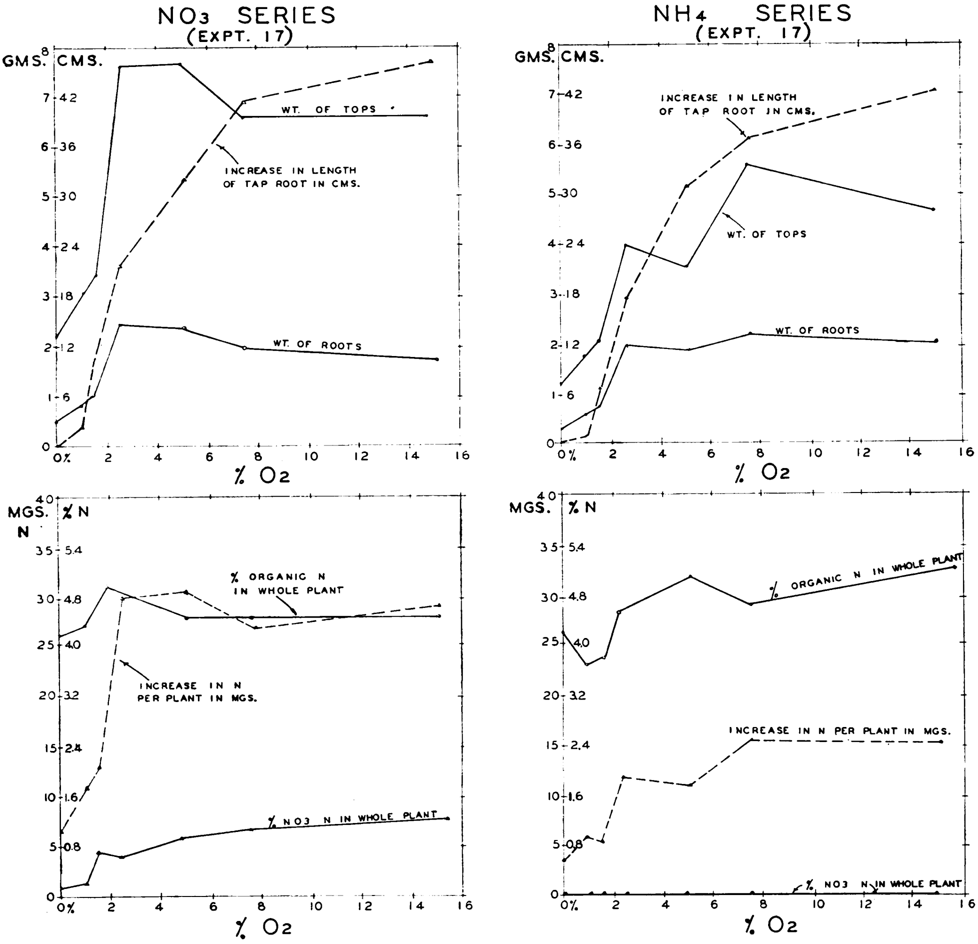

FIG. 7. The influence of various concentrations of oxygen and forms of nitrogen on the total elongation of the tap root, fresh weight of roots and tops, total absorption of nitrogen, and percentage of organic and nitrate nitrogen in seedling cotton plants.

dioxide resulted in wilting of the tops, especially when the cotton was in direct sunlight and when the air temperature was above 30° C. Wilting did not occur at the lower concentrations of carbon dioxide, with the exception of occasional wilting at 45 per cent. carbon dioxide.

The pH was controlled by a potassium phosphate buffer and potassium hydroxide. The pH of even the 100 per cent. carbon dioxide solution usually did not shift much below 4.9. Evidently the pH was adequately controlled, even when pure carbon dioxide was bubbled through the nutrient solution. In preliminary tests when the phosphate buffer was not used, pure carbon dioxide produced a pH of 3.6 in the nutrient solution.

Concentrations of carbon dioxide up to 15 per cent. resulted in the production of long, slender roots. Concentrations of 30 to 45 per cent. carbon dioxide resulted in the formation of somewhat short, thick roots. Sixty per cent. carbon dioxide, and higher, prevented growth either in length or in thickness of roots.

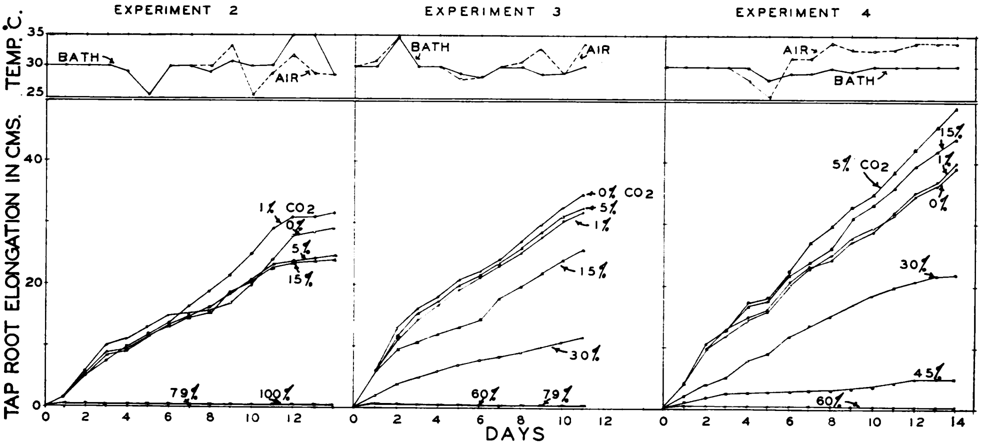

FIG. 8. The effect of various concentrations of carbon dioxide on the daily elongation of cotton tap roots. Oxygen was held constant at 21 per cent., except at 100 per cent. carbon dioxide.

When 60 per cent. carbon dioxide was replaced by air (21 per cent. oxygen and traces of carbon dioxide) branch roots developed. These results indicated that the pericycle was not destroyed by the high concentrations of carbon dioxide. The tap root was never observed to elongate further, after the introduction of air.

Low concentrations and complete absence of carbon dioxide resulted in no apparent inhibition of root growth. All traces of carbon dioxide were removed by bubbling the gas mixture through gas wash bottles containing potassium hydroxide.

When the temperature of the bath was 35° C. or higher for 24 hours (fig. 8, Experiment 2), root elongation almost ceased at all concentrations, although roots in five to 15 per cent. carbon dioxide were affected most promptly. The daily elongation of roots growing in the same environment varied for no apparent reason.

Discussion and conclusions

Cotton tap root elongation was entirely inhibited by gas mixtures containing one-half per cent. of oxygen or less. The roots remained white and apparently healthy under the above conditions for at least two weeks. If

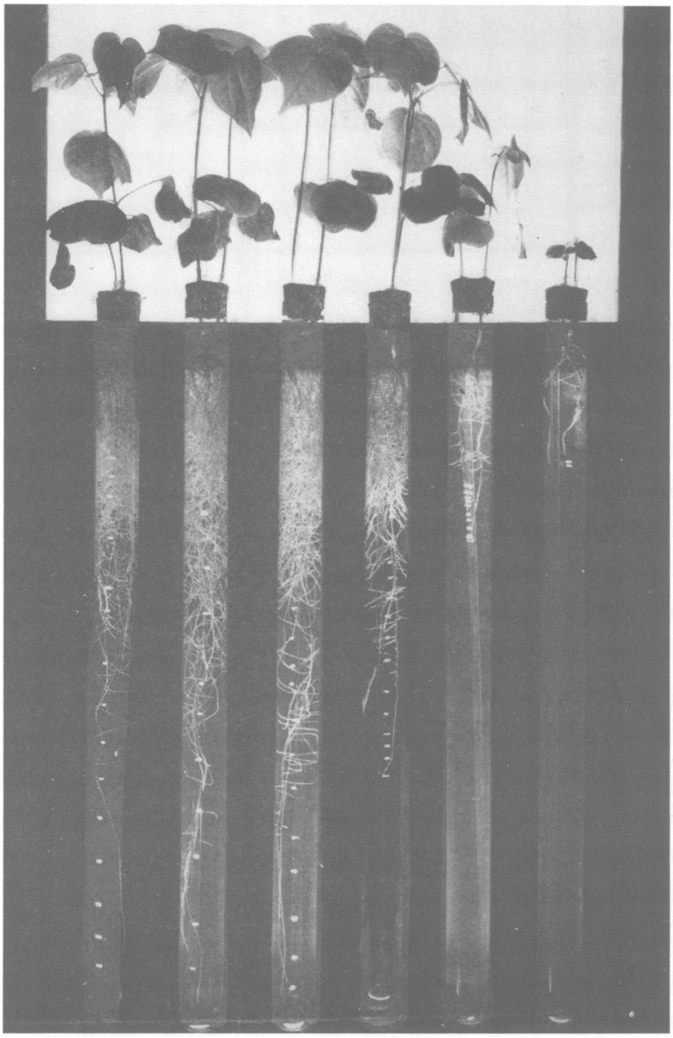

FIG. 9. The effect of various concentrations of carbon dioxide on the daily elongation of cotton tap roots and the appearance of the tops (Experiment 4). Marks on the tubes represent tap-root elongation at 24-hour intervals. The gas mixtures from left to right were (percentages):

CO_2	0	5	15	30	45	60
O_2	21	21	21	21	21	21

air containing 21 per cent. oxygen replaced the above gas mixtures, normal branch roots developed, and sometimes the branch roots began to grow again. Evidently, even very drastic reductions in oxygen around cotton roots, with either zero or 10 per cent. carbon dioxide, was not permanently harmful. The roots continued to absorb both nitrate and ammonium nitrogen, although not as well as cotton roots exposed to optimum concentrations of oxygen. The results suggest that the influence of extremely poor aeration of soil on the death of cotton roots is indirect. **Balls** (**2**) observed that cotton roots apparently became asphyxiated when the water table rose. **Leonard** (**10**) observed that when the soil was water-logged and very low in oxygen cotton tap roots were short and the tips dead. The influence of poor aeration on cotton roots might easily have been indirect by influencing the susceptibility of the roots to minor parasites.

TABLE II

APPEARANCE OF 14-DAY-OLD COTTON SEEDLINGS GROWN IN LIQUID CULTURES SATURATED WITH OXYGEN AND CARBON DIOXIDE AT STATED CONCENTRATIONS. RESULTS FROM SEVERAL EXPERIMENTS SUMMARIZED

GASES USED		APPEARANCE OF TOPS	APPEARANCE OF ROOTS
O_2	CO_2		
%	%		
21	None	Leaves green, top growth good	Roots thin, very good growth
21	1.0	Do.	Do.
21	5.0	Do.	Do.
21	15.0	Do.	Do.
21	30.0	Do.	Root growth reduced, somewhat thick
21	45.0	Leaves green, reduced top growth	Root growth greatly reduced, thick
21	60.0	Leaves dark green, very little top growth, tending to wilt in sunshine	No root growth. Branch roots developed if transferred to nutrient solution saturated with air
21	79.0	Do.	Do.
None	100.0	Do.	Do.

The studies of **Vlamis** and **Davis** (**16**) indicate that the oxygen requirement of excised roots of tomato, barley, and rice were similar, and yet the three plants reacted differently to anaerobic conditions. The evidence suggests that the reaction of the above plants to anaerobic conditions was related to their capacity to translocate oxygen from the stems to the roots. The capacity of cotton roots to remain alive and function well under anaerobic conditions is also probably related to its capacity to translocate oxygen from the shoots to the roots.

Conway (**6**), using *Cladium mariscus,* found a high oxygen content in intact roots but low oxygen in excised roots under similar conditions. The results suggest that oxygen is translocated to the roots. Differences in the ability of plants to move oxygen to the roots may in part determine their behavior under anaerobic conditions.

Under anaerobic conditions, short branch roots were produced near the base of the hypocotyl. The tissue was bulky and contained some chlorophyll. Probably sufficient oxygen was present within the tissue of the hypocotyl, either from translocation or from photosynthesis, to enable root initiation.

The minimum concentration of oxygen for cotton tap root elongation was not influenced by the form in which nitrogen was supplied (that is, as nitrate or ammonium nitrogen). The results of VLAMIS and DAVIS (**16**) likewise indicate that nitrate nitrogen is not superior to ammonium nitrogen for either growth or potassium absorption. SHIVE (**15**), working with soybeans, found that the greatest absorption of nitrate nitrogen occurred from nutrient solutions containing no oxygen, and absorption of nitrate decreased as oxygen in the nutrient solutions increased. The absorption of ammonia, on the other hand, was the reverse of the absorption of nitrate. The results were explained as indicating that nitrates were supplying oxygen to the roots under anaerobic conditions.

Nitrate nitrogen tended to accumulate in cotton seedlings as the percentage of oxygen in the gas mixtures was increased. In view of the observation that the percentages of organic nitrogen in the whole plants were about the same with different percentages of oxygen, the accumulation of nitrate may signify differences in rates of nitrate absorption. Appreciable accumulation of nitrogen, however, took place even under anaerobic conditions.

PEPKOWITZ and SHIVE (**14**), working on tomatoes and soybeans, found that the absorption of calcium, phosphorous, and potassium was least at zero parts per million oxygen and rose to an optimum at higher oxygen concentrations; nevertheless, appreciable absorption still occurred, even with the zero oxygen treatment.

VLAMIS and DAVIS (**16**) found that the accumulation curves for bromide and potassium with different percentages of oxygen were about the same for excised roots of tomato, barley, and rice. A loss of potassium occurred in the absence of oxygen. The accumulation curve for intact roots was quite different from the above. Intact rice roots (shoots attached) in the absence of oxygen accumulated appreciable quantities of bromide; those of barley less; and those of tomatoes almost none.

The lower limit of oxygen necessary for root growth varies somewhat with temperature. According to CANNON and FREE (**4**) cotton root growth would occur at concentrations of oxygen of slightly less than one per cent., if the temperature was 17° C., but a concentration of over one per cent. would be necessary if the temperature was 30° C. A small amount of cotton root growth occurred at 28° C. with one per cent. oxygen.

In the present investigation the optimum concentration of oxygen for cotton tap root elongation, as well as cotton root growth, was between seven and one-half and 21 per cent. oxygen, although five per cent. oxygen was only slightly inferior. GILBERT and SHIVE (**7**) reported that the optimum concentration of oxygen necessary for the production of green matter in soybeans was about six p.p.m.; in tomatoes, 16 p.p.m.; and in oats, four p.p.m.; or approximately equivalent to solutions in equilibrium with 15, 44, and 10 per cent. oxygen, respectively. BOYNTON and COMPTON (**3**) reported that oxygen concentrations of 15 per cent. or lower resulted in markedly poorer root and top growth of peach, apple, and prune trees growing in nutrient solutions than did 21 per cent. oxygen.

Excessive concentrations of oxygen resulted in reduced cotton root growth. Concentrations of either 90 or 100 per cent. oxygen gave similar results. Besides reducing the daily root growth, high concentrations of oxygen resulted in the roots becoming parasitized by what appeared to be chytrids in some cases and by species of Fusaria in others.

Root and shoot growth were reduced at oxygen concentrations above the optimum for root elongation. The leaves, however, appeared normal at the high oxygen concentrations, whether nitrate or ammonium nitrogen was used. GILBERT and SHIVE (**7**), working with soybeans, found that oxygen values of eight and 16 p.p.m. (equivalent to solutions in equilibrium with 22 and 44 per cent. oxygen) resulted in chlorotic leaves if nitrate nitrogen was present in the nutrient solution, but not if ammonium nitrogen was used. They referred to the chlorotic condition as one of "oxygen toxicity."

Excessive concentrations of carbon dioxide, as well as low concentrations of oxygen, are considered to influence the growth of plant roots in poorly aerated soils. Concentrations of 60 per cent. carbon dioxide, and above, inhibited all cotton root growth even though oxygen was held at an optimum concentration. The pH, likewise, was held constant in these experiments. Evidently, the influence of high concentrations of carbon dioxide on root growth was not caused by a deficiency of oxygen nor by an excessively acid condition of the nutrient solution. If air containing 21 per cent. oxygen replaced the inhibitory concentrations of carbon dioxide, normal branch roots were formed. Evidently, high concentrations of carbon dioxide did not kill the cells of the pericycle. Top growth was very poor when high concentrations of carbon dioxide were used. When the plants were in direct sunlight and the air temperature was 30° C., or higher, the tops wilted.

NOYES (**13**) passed carbon dioxide through soil in which corn and tomato plants were growing, causing the plants to wilt and growth to stop. CANNON and FREE (**4**) studied the influence of different concentrations of carbon dioxide on the root growth of a number of plants. They found that high carbon dioxide concentrations inhibited the growth of all plants if the periods of exposure were sufficiently long, but that the sensitivity of different plants to different concentrations of carbon dioxide was not the same. Root growth of *Krameria canescens* ceased if carbon dioxide was 25 per cent. and if the temperature was 30° C.; growth did not cease if the temperature was 20° C. and root growth continued even at 75 per cent. carbon dioxide. Although 25 to 75 per cent. carbon dioxide prevented root growth of *Covillea tridentata*, growth could again occur if the above mixture were replaced by air.

Vlamis and **Davis** (**16**) have shown that pure carbon dioxide prevented the accumulation of bromide by both excised and intact roots of barley, tomato, and rice; it also resulted in wilting and cessation of growth. Similar observations on the effect of carbon dioxide on the wilting of plants have been made by other investigators. **Chang** and **Loomis** (**5**) found that carbon dioxide reduced the absorption of water and nutrients by wheat, maize, and rice when compared with aerated cultures of the same plants.

Concentrations of carbon dioxide between zero and 15 per cent. produced no appreciable influence on cotton tap-root elongation. Concentrations of 15 per cent. carbon dioxide occur very rarely in the soil air, as determined by gas analysis. In a previous paper (**10**) it was pointed out that concentrations of carbon dioxide may be very high adjacent to the surfaces of roots under some conditions, although the occurrence of such concentrations might not be revealed by a gross analysis of the soil air. It is possible that concentrations of even 60 per cent. carbon dioxide are sometimes attained. The concentrations of carbon dioxide at root surfaces under various soil conditions must be determined before the influence of carbon dioxide on root growth in a soil can be evaluated.

Concentrations of carbon dioxide from zero to 100 per cent. were studied in order to determine the influence of all possible concentrations which might possibly influence root growth in the soil. Normal root growth occurred in the absence of carbon dioxide. It does not appear possible, therefore, that minimum concentrations of carbon dioxide can be too low to seriously influence seedling cotton root growth. Further work should be done on the minimum requirements of plant roots for carbon dioxide.

Carbon dioxide is necessary for the growth of many microorganisms. **Jahn** (**9**) observed that the growth of the protozoan, Chilomonas, was reduced to less than one-fifth in carbon dioxide-free air when compared to ordinary air. **Longsworth** and **MacInnes** (**11**) found that carbon dioxide was necessary for the growth of *Lactobacillus acidophilus*. **Gladstone**, **Fildes**, and **Richardson** (**8**) observed that the passage of carbon-dioxide-free air through cultures of various bacteria stopped growth in some cultures, but not in others. In cultures not inhibited, carbon dioxide was probably formed within the cells themselves. Perhaps the reason cotton root growth was not influenced by the absence of carbon dioxide in the gas mixtures was because all cells of the roots were in contact with adjacent respiring cells; thus no cells were completely subjected to an atmosphere containing zero carbon dioxide, regardless of the composition of the gas mixture around the roots.

Roots in the same environment did not elongate at the same rate from day to day. **Cannon** and **Free** (**4**) likewise reported similar variations in what appeared to be identical plants. As far as could be ascertained, there was no relationship between root growth, leaf initiation, or leaf area.

Summary

A study was made of the oxygen and carbon dioxide requirements of cotton seedling roots in nutrient solutions.

Optimum oxygen concentrations for seedling root elongation of cotton appeared to lie between seven and one-half and 21 per cent. oxygen when carbon dioxide was constant at 10 per cent. The greatest rate of root elongation for any 24-hour period was 67 mm. in 21 per cent. oxygen and 10 per cent. carbon dioxide with culture temperature at 30° C.

The minimum oxygen requirement for cotton root elongation at 28° C. appeared to lie between one-half and one per cent. oxygen. Absence of oxygen around roots did not appear to be very harmful under the conditions studied. When air containing 21 per cent. oxygen replaced nitrogen gas (zero per cent. oxygen), new branch roots were initiated, and some of the old branch roots commenced to grow again.

Ninety or 100 per cent. oxygen resulted in reduced root elongation. Roots produced under such conditions were readily parasitized by chytrids and species of Fusaria.

Tap-root elongation at each concentration of oxygen was similar whether nitrate or ammonium nitrogen was used in the culture solution. Vegetative growth appeared improved with the nitrate nitrogen. More nitrate nitrogen than ammonium nitrogen was taken up by the plants.

The optimum concentration of carbon dioxide appeared to range between zero and 15 per cent. when the oxygen concentration was maintained at 21 per cent. The absence of carbon dioxide did not appear to affect root elongation. Concentrations of 60 per cent. carbon dioxide and above prevented all root growth. Root growth was reduced to 30 and 45 per cent. carbon dioxide, and the roots were rather thick.

Shoot growth appeared to be uniform in both height and fresh weight from zero to 30 per cent. carbon dioxide, reduced at 45 per cent. carbon dioxide, and greatly reduced at 60 per cent. carbon dioxide and above. The shoots frequently wilted at 60 per cent. carbon dioxide and above, especially when the plants were in direct sunlight and the temperature was over 30° C.

The writers conclude that the cotton plant is able to withstand anaerobic soil conditions and that this ability is, perhaps, related to the translocation of oxygen from the tops to the roots.

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