EFFECT OF CARBON DIOXIDE ON ABSORPTION OF WATER AND NUTRIENTS BY ROOTS[1]

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H . T . C H A N G   A N D   W .   E .   L O O M I S

The importance of soil aeration to plant development has been recognized since the beginnings of agriculture. As our knowledge of the physiology of roots has grown, the beneficial effects of soil aeration have been assigned to a stimulation of root growth (4) and thus an increase of root area and penetration, and more recently to a direct stimulation of aerobic processes of salt accumulation. HOAGLAND and his associates particularly have shown that the rate of mineral accumulation in roots containing respirable carbohydrates may be positively correlated with the oxygen supply (8, 24).

Although many data upon the toxic effect of carbon dioxide on protoplasm are available, this phase of the soil aeration problem has been generally overlooked. FREE (7) and KRAMER (16) have shown a reduction of water absorption by roots with increased CO_2 concentrations. HOAGLAND and BROYER (9), however, thought the effect of CO_2 related to its displacement of oxygen, and ARRINGTON and SHIVE (3) concluded that reduced CO_2 concentrations were not a factor in the beneficial effects of the aeration of a modified Knop solution. Many other reports of the beneficial effects of the aeration of solutions and soils have stressed the importance of O_2 and ignored or minimized the rôle of CO_2.

The studies reported here were planned to test the toxicity of CO_2 accumulation as contrasted to an O_2 deficiency, and to study the effect of CO_2 upon the absorption of water and the more important nutrients.

Methods

Seedlings of wheat (*Triticum aestivum*, var. Thatcher), maize (*Zea mays indentata*, var. U.S. 35), and rice (*Oryza sativa*, var. Blue Rose) were started in quartz and grown in a four-salt solution, roughly that given by LOOMIS and SHULL (18) modified by reducing the phosphorus concentration by half. The plants were grown in quart jars, three plants per culture, until 5 to 7 weeks old, with solution changes at weekly intervals and additions of iron in ferric citrate as required. Enough plants were started that six cultures of well grown, uniform plants were available for each treatment, except in the rice experiment where lots of eight cultures per aeration treatment were divided between two pH levels.

Solutions were changed two days before an experimental run so that the plants would not be in a state of mineral starvation. Fresh solutions of accurately known composition were then weighed into the treatment jars, gas treatments started, and the plants transferred and left for two days and one night (36 hrs.). Air, CO_2, or N_2 was bubbled briskly through the

treated lots for ten minutes out of every hour throughout the period.   Nitrogen cylinders were not available for the experiments with maize, and a 5-mm. layer of paraffin oil was poured over one set of solutions after the plants were inserted to reduce both oxygen absorption and carbon dioxide loss by the solutions.   In the rice experiments half of the solutions in each treatment were brought to an initial pH of 4.0 with H_2SO_4 and held at this point with further additions of acid as required.   This treatment was intended to differentiate between the directly toxic effects of CO_2 and its effect on the pH of the solution.   Cultures treated with CO_2 alone remained near pH 4.0 throughout the period, even though the bottles were not tightly stoppered to prevent loss of CO_2 between treatments.

At the end of the test periods the jars were reweighed to obtain water losses, and samples were taken for determination of total solids, nitrogen, phosphorus, calcium, magnesium, and potassium.   Analyses were corrected for water losses and absorption calculated as milliliters of water or milligrams of element per culture.   Analytical methods for nitrogen, calcium, and magnesium were those given by LOOMIS and SHULL (18).   Potassium was determined by the ceric sulphate method and phosphorus by the stannous chloride, colorimetric method.

Five experimental runs were made: the first with wheat on November 13 and 14, 1942; the second with wheat on January 1 and 2, 1943; the third with maize on February 17 and 18, 1943; the fourth with maize on March 6 and 7, 1943, and the fifth with rice on August 24 and 25, 1943.   Evaporation conditions varied, being poorest for the third experiment with maize and best for the August experiments with rice.   The original solutions varied also, but were always made up in one lot sufficient for the experiment and analyzed in triplicate with the residual solutions to obtain their exact composition.   When absorption was low, as of phosphorus and calcium in some of the experiments, its determination by difference of the content of the solutions before and after absorption by the plants was not fully satisfactory because of the accumulation of experimental errors.   The physiological condition of the plants probably varied also in spite of uniform handling, for the variation in some of the series was wide and the responses of the three species differed in some respects.

Results

The general picture given by the data in tables I, II, and III, however, is clear cut in indicating that CO_2 was the dominant factor affecting nutrient absorption under the conditions of the experiments and the most important treatment factor affecting water absorption.   Standard errors are given for all of the averages in the tables so that the statistical significance of any pair of determinations can be estimated at a glance.   In general the addition of CO_2 significantly reduced the absorption of every substance tested.   Bubbling commercial nitrogen through wheat cultures (table I) had no effect. It is not assumed that these cultures were O_2 free, but only that O_2 should

TABLE I

THE ABSORPTION OF WATER AND MINERALS BY WHEAT PLANTS RECEIVING
DIFFERENT TREATMENTS

SUBSTANCE ABSORBED	CONTROL	BUBBLED WITH			COMPOSITION OF ORIGINAL SOLUTION
		AIR	NITROGEN	CO_2	
EXPERIMENT I. NOVEMBER, 1942					
Water (ml.)	62.4 ± 2.8	70.0 ± 1.5	....	40.6 ± 4.6	800.0
Total salts (mg.)	84.9 ± 15.8	117.3 ± 10.6	....	25.7 ± 11.1	828.8
N	11.1 ± 0.3	17.9 ± 0.3	....	2.1 ± 1.2	44.3
Ca	3.1 ± 0.9	1.4 ± 0.5	....	0.02 ± 0.4	78.5
K	7.4 ± 1.5	19.0 ± 2.2	....	− 2.2 ± 1.4	129.6
EXPERIMENT II. JANUARY, 1943					
Water (ml.)	53.5 ± 4.0	56.7 ± 2.3	60.4 ± 3.7	39.5 ± 2.9	800.0
Total salts (mg.)	184.5 ± 4.9	185.4 ± 4.1	183.9 ± 3.5	150.3 ± 3.9	1132.5
N	18.0 ± 0.3	17.6 ± 0.3	17.1 ± 0.7	15.1 ± 0.4	64.8
P	2.5 ± 0.2	2.6 ± 0.2	2.1 ± 0.2	1.6 ± 0.5	16.2
Ca	10.3 ± 0.6	9.8 ± 1.5	10.6 ± 0.3	8.2 ± 1.0	95.8
Mg	7.0 ± 0.6	7.4 ± 0.4	7.3 ± 0.6	3.9 ± 0.5	52.2
K	24.0 ± 1.4	27.8 ± 2.0	25.5 ± 0.9	8.1 ± 2.2	135.1

have been reduced to approximately the level of the jars bubbled with CO_2. The effects of CO_2 are thus shown to have been directly due to this gas and not to the exclusion of O_2.   Aeration generally increased the absorption of potassium and of phosphorus by maize, while covering maize culture solutions with paraffin oil to reduce O_2 absorption and CO_2 loss reduced the

TABLE II

THE ABSORPTION OF WATER AND MINERALS BY MAIZE PLANTS RECEIVING
DIFFERENT TREATMENTS

SUBSTANCE ABSORBED	CONTROL	COVERED WITH OIL	BUBBLED WITH		COMPOSITION OF ORIGINAL SOLUTION
			AIR	CO_2	
EXPERIMENT III. FEBRUARY, 1943					
Water (ml.)	33.1 ± 2.7	32.7 ± 2.5	38.9 ± 4.2	28.4 ± 2.6	800.0
Total salts (mg.) ...	68.3 ± 4.2	64.2 ± 9.4	120.2 ± 7.0	42.2 ± 4.5	952.3
N	10.2 ± 2.6	5.5 ± 2.6	9.6 ± 2.9	3.7 ± 2.4	44.1
P	1.5 ± 0.3	1.0 ± 0.2	4.1 ± 0.2	0.7 ± 0.2	29.8
Ca	8.9 ± 1.7	8.9 ± 0.9	10.7 ± 0.3	5.1 ± 0.2	95.0
Mg	4.1 ± 0.6	4.1 ± 0.7	6.3 ± 0.6	2.7 ± 0.9	49.7
K	33.1 ± 1.1	17.5 ± 1.7	42.8 ± 2.5	− 7.8 ± 0.9	138.4
EXPERIMENT IV. MARCH, 1943					
Water (ml.)	89.2 ± 5.3	88.5 ± 4.3	97.6 ± 4.4	71.2 ± 3.8	800.0
Total salts (mg.) ...	189.7 ± 7.6	174.0 ± 6.3	265.4 ± 6.7	97.8 ± 4.7	948.5
N	43.9 ± 1.6	41.3 ± 1.4	44.2 ± 1.6	16.1 ± 0.7	51.2
P	4.0 ± 0.4	4.6 ± 0.4	8.3 ± 0.4	0.9 ± 0.2	30.4
Ca	13.1 ± 1.2	15.6 ± 1.3	20.5 ± 1.6	9.7 ± 0.8	96.1
Mg	7.7 ± 0.4	6.7 ± 0.3	8.9 ± 0.5	5.4 ± 0.4	49.4
K	31.1 ± 1.6	15.8 ± 2.7	51.9 ± 2.0	0.5 ± 2.3	140.7

TABLE III

THE ABSORPTION OF WATER AND MINERALS BY RICE PLANTS RECEIVING
DIFFERENT TREATMENTS*

SUBSTANCE ABSORBED	CONTROL	BUBBLED WITH		COMPOSITION OF ORIGINAL SOLUTION
		AIR	CO_2	
NO ADDED ACID, PH OF CONTROLS 5.0–6.0				
Water (ml.)	139.4 ± 7.4	155.5 ± 14.2	70.0 ± 14.6	800.0
Total salts (mg.)	48.8 ± 3.6	72.6 ± 4.5	1.6 ± 13.8	899.6
Nitrogen	8.0 ± 0.3	11.5 ± 1.1	0.9 ± 0.8	52.7
ADDED H_2SO_4, PH OF CONTROLS 4.0				
Water (ml.)	133.9 ± 12.9	133.1 ± 4.8	73.4 ± 12.6	800.0
Total salts (mg.)	16.4 ± 3.8	19.5 ± 3.6	7.3 ± 10.7	899.6
Nitrogen	5.1 ± 0.6	5.5 ± 0.2	0.6 ± 0.7	52.7

*Experiment V.   August, 1943.

absorption of potassium.   Other differences were either not statistically significant or were erratic.   Adding sulphuric acid to bring the pH of rice cultures growing at high temperatures to 4.0 significantly reduced total salt and nitrogen absorption, but the effect of CO_2 was much greater than the effect of H_2SO_4.

To facilitate comparisons of the effects of the several treatments on the absorption of the different substances the data were calculated to show the treatments as a percentage of the controls and grouped by substances absorbed.

THE EFFECT OF CO_2 ON ABSORPTION OF WATER

The effects of aeration and CO_2 on the water relations of the plants were measured by weighing the culture solutions without the plants before and after the treatment period.   The data are thus direct measurements of water absorption.   Since, however, the plants were turgid at the beginning and at least nominally so at the end of the experiments, they are also measurements of transpiration.   KRAMER's data (15) on the resistance of the root

TABLE IV

THE ABSORPTION OF WATER BY CONTROL AND TREATED PLANTS.   CONTROL DATA IN
MILLILITERS, OTHERS IN PERCENTAGE OF CONTROL

EXPERIMENT	CONTROL	AERATED	NITROGEN	OILED	CO_2
	ml.	%	%	%	%
I—wheat	62.4	112.2			65.1
II—wheat	53.5	106.0	112.9		73.8
III—maize	33.1	117.5		98.8	85.8
IV—maize	89.2	109.4		99.2	79.8
Va—rice control	139.4	111.5			50.2
Vb—plus acid	133.9	99.4			54.8
Average (%)	100.0	109.3	112.9	99.0	68.3

system to water movement, and his later observations (**16**) that this friction was increased by CO_2, indicate that differences in absorption rather than transpiration were determining. This conclusion is supported by KRAMER's (**16**) experiments in which bleeding from decapitated plants under suction was reduced by the same or a greater percentage as was transpiration of intact plants. HOAGLAND and BROYER (**9**) also found that CO_2 reduced the yields of sap obtained from decapitated plants under suction.

Our results agree with those of KRAMER in showing that increased CO_2 concentration around the roots reduced water absorption while nitrogen gas had little effect. The data are summarized in table IV. They suggest that the oxygen concentrations obtained had no effect upon water absorption or loss under the conditions of these experiments. The gain of 9.3 per cent. in water loss with aeration is significant at the 5 per cent. level but is no greater than the gain from bubbling with nitrogen, and both could be assigned to a reduction in the average CO_2 content of the solutions. The oil treatment, which probably reduced oxygen absorption more than CO_2 loss, because of the differential solubilities of the gases in oil, had no effect on water loss. The reduced water loss from the jars receiving CO_2 was statistically significant in all but one test and highly significant as an average of all experiments.

The addition of sulphuric acid to bring the control solutions to the same pH as the CO_2 treated did not significantly reduce water absorption by rice. JACOBS (**11**), however, has shown that CO_2 may have a much greater effect on the internal pH of solutions or tissues protected by fatty membranes than other acids producing comparable external pH values. He assumed that CO_2 itself or undissociated H_2CO_3 is able to penetrate xylene, cottonseed oil, or protoplasmic membranes which are impermeable or only slowly permeable to non-fat-soluble acids. Once across the membrane the CO_2 dissociates to produce its equilibrium pH, which is near 4.0 in distilled water. Both JACOBS (**12**) and SEIFRIZ (**22**) have shown that CO_2 may increase the viscosity of protoplasm, and we may assume that the permeability of the root membranes to water is decreased by the changes set up.

The apparently greater sensitivity of rice to CO_2 than either wheat or maize is probably explainable on the basis of the higher greenhouse temperatures prevailing during the rice experiment, 30° to 35° C., in contrast to 20° to 30° C. for the other experiments. CANNON (**4**) has shown that CO_2 is more toxic at higher temperatures. A greater percentage decrease in water absorption might also have resulted from the very high transpiration rates during the rice experiment, even had the effect on root permeability been no greater.

THE EFFECT OF CO_2 ON ABSORPTION OF MINERALS

The treatments used and the analyses made varied somewhat among the five experimental runs. The data in table V are grouped around each analysis for convenient comparison. Absorption of the five elements tested was

affected in the order $K > N > P > Ca > Mg$. The absorption of all five was significantly reduced by CO_2, with the percentage reduction in the absorption of Ca and Mg paralleling that of water, the relative absorption of P and N being reduced to about half that of water and potassium being released from the roots in considerable quantities in two of four experi-

TABLE V

THE ABSORPTION OF NUTRIENT ELEMENTS BY CONTROL AND TREATED PLANTS.   CONTROL DATA
IN MILLIGRAMS PER CULTURE; OTHERS IN PERCENTAGE OF CONTROL

EXPERIMENT	CONTROL	AERATED	NITROGEN	OILED	CO₂
	mg.	%	%	%	%
ABSORPTION OF CALCIUM					
I—wheat	3.1	45.2			0.6
II—wheat	10.3	95.1	102.9		79.6
III—maize	8.9	120.2		100.0	57.3
IV—maize	13.1	156.4		119.1	74.0
Average (%)	100.0	104.2	102.9	109.5	52.9
ABSORPTION OF MAGNESIUM					
II—wheat	7.0	105.7	104.3		55.7
III—maize	4.1	153.6		100.0	65.8
IV—maize	7.7	115.6		87.0	70.1
Average (%)	100.0	125.0	104.3	93.5	63.9
ABSORPTION OF POTASSIUM					
I—wheat	7.4	256.7			− 29.7
II—wheat	24.0	115.8	106.3		33.7
III—maize	33.1	129.3		52.9	− 23.6
IV—maize	31.1	166.9		50.8	1.6
Average (%)	100.0	167.2	106.3	51.8	− 4.5
ABSORPTION OF PHOSPHORUS					
II—wheat	2.5	104.0	84.0		64.0
III—maize	1.5	278.3		66.7	46.7
IV—maize	4.0	207.5		115.0	22.5
Average (%)	100.0	196.6	84.0	90.8	44.4
ABSORPTION OF NITROGEN					
I—wheat	11.1	161.3			18.9
II—wheat	18.0	97.8	95.0		83.9
III—maize	10.2	94.1		53.9	36.3
IV—maize	43.9	100.7		94.1	36.7
Va—rice	8.0	143.7			11.2
Vb—plus acid	5.1	107.8			11.8
Average (%)	100.0	117.6	95.0	74.0	33.1

ments.   The differences shown fit with the general concept of Ca and Mg as elements whose accumulation is least dependent upon metabolic effects, and of potassium as the element whose accumulation is most dependent upon a delicate balance of protoplasmic forces and reactions.   Calcium and magnesium could have been excluded by the same reduced permeability assumed to have acted upon water absorption.   The potassium excretion is not so

easily explained except as we assume that the accumulation of this element is dependent upon protoplasmic structure and/or negative charge, both of which were probably modified by the treatment. HOAGLAND and BROYER (9) found that CO_2 reduced Br accumulation, and VLAMIS and DAVIS (26) found that both K and Br were excreted from excised roots held with very low oxygen tensions or bubbled with CO_2.

Of the other treatments, aeration significantly increased potassium absorption in maize and wheat and phosphorus absorption in maize. No other effects were significant. Under certain conditions such absorption increases as are shown for K and P in the aerated cultures could be expected to increase plant growth (3). The results of KNIGHT (14), however, and particularly those of ARNON (1) suggest that such increases are the results of an improperly balanced solution rather than poor aeration, and that equally good results could be obtained by changing the salts or their concentrations. Bubbling the solutions with cylinder nitrogen for ten minutes of each hour, to give an O_2 reduction comparable to that obtained with CO_2, did not affect any of the reactions. Covering the solutions with paraffin oil reduced potassium absorption by maize to 50 per cent. of the control. No other effect was statistically significant.

Discussion

The evidence for a directly toxic effect of CO_2 on plant as well as animal protoplasm seems to be conclusive. JACOBS (10) used some plant materials in his research. Fox (6) worked with Nitella. VLAMIS and DAVIS (26) report, ''CO_2 was immediately lethal to all plants. . . . Other experiments with barley have indicated that a 20 to 30 per cent. partial pressure of CO_2 is toxic even if the entire residual pressure of 70 to 80 per cent. consists of oxygen.'' Although they do not stress the point, the data of HOAGLAND and BROYER (9) suggest that the roots of tomato plants were quickly killed by bubbling CO_2 through the solutions in which they were immersed. Other points are not yet established, however. We do not know the mode of action of CO_2 on protoplasm, the concentrations necessary for toxicity under varying conditions, or the practical importance of CO_2 toxicity in the field.

THE TOXIC ACTION OF CO_2

Fox (5) has reviewed the earlier work, most of it with animals including man, on the narcotic effects of CO_2, and has shown (6) that sea water saturated with the gas increases the viscosity of the protoplasm of *Nitella clavata*. Cells treated for one hour recovered with aeration but those left in CO_2 for two or three hours were killed. SEIFRIZ (22) observed almost identical reactions when *Physarum polycephalum* plasmodia were exposed to 50 per cent. CO_2 in oxygen. Anesthesia, as shown by the cessation of protoplasmic streaming, was complete within 60 seconds. Concentrations as low as 10 per cent. CO_2 gave symptoms of anesthesia. Carbon dioxide was the best narcotic for slime mold protoplasm of the substances tested, while ether was ineffective.

Distilled water saturated with CO_2 has a pH of about 4.0. ARNON, FRATZKE, and JOHNSON (2) and others have shown that hydrogen ion becomes directly and markedly toxic only at pH's toward 3.0 in the external medium. The rapid penetration of CO_2 through protoplasmic membranes, however, may bring about toxic internal changes in pH that are not reached with other acids. JACOBS (11) found that carbon dioxide dissolved in $NaHCO_3$ buffer at pH 6.9 was as toxic as in water at pH 3.9 and as quickly increased the internal acidity of immersed flower petals. Such changes in cellular acidity would tend first to decrease and then to increase the dispersion of the average protoplasmic colloid as the pH passed down through the isoelectric point or points. The effect of CO_2 is, however, to increase the viscosity. Such a change could be due to reduced hydration or to changes in molecular arrangement.

Two compounds of carbonic acid with protein have been reported; the formation of carbamic acid on the amino groups (23) and the formation of hydrogen-bond salts on the same groups (20). Carbamic acid compounds of protein derivatives are known only from their salts, especially of the earth metals. Amino acid carbamates form calcium salts by displacement of the hydrogens of both the amino and carbamic carboxyls. In proteins such calcium salts could presumably be formed between adjoining molecules, resulting in an increase in viscosity.

PAULI and STENZINGER (20) object to the carbamate theory of CO_2 toxicity. They found that various dialyzed animal proteins became positively charged upon treating with CO_2, and postulated the formation of weakly ionized, hydrogen-bond compounds. Both the carbamates, if produced, and the hydrogen-bond compounds would tend to form on the more basic amino groups above the alpha carbon, or possibly on cyclic nitrogen atoms. The residual bonds of the alpha amino nitrogen are too weak, especially after the formation of peptid linkages in protein synthesis, to expect them to form hydrogen-bond compounds. Compounding of these extra nitrogen atoms, on the other hand, would be little if at all affected by the polymerization of the amino acids into proteins. The dissociation of hydrogen-bond compounds into RH^+ and $OCOOH^-$ ions would increase the positive charge of the proteins, as observed by PAULI, and decrease their dispersion. The formation of such compounds might also affect the folding of the protein chains and the structure of the protoplasm while cross linkages between adjoining protein molecules reacting with the two hydrogens of the same carbonic acid molecule could rapidly increase the viscosity of the protoplasm, as observed by Fox (6) and SEIFRIZ (22).

A combination of rapid penetration of protoplasmic membranes, increases of cellular acidity, and the formation of specific, but weakly bonded, protein compounds seems to be the best available explanation of CO_2 toxicity. These reactions would fit the picture of a compound universally present in living cells becoming increasingly toxic above certain threshold concentrations. The formation of hydrogen-bond linkages on non-alpha amino nitro-

gen allows not only for reversibility and mass effects but for specific differ-
ences in CO_2 toxicity.   Those plants whose protoplasm contained the fewest
diamino and cyclic nitrogen groups would be most resistant to the action
of CO_2.

THE PRACTICAL IMPORTANCE OF CO_2 TOXICITY

Atmospheric oxygen is depended upon as an ultimate hydrogen acceptor
in the respiration of higher plants. We must assume therefore that soil or
culture aeration is beneficial at minimal oxygen levels because it replaces
this element. At the same time possible toxic accumulations of CO_2 are
swept away. The problem, therefore, is one of minimum oxygen levels,
maximum CO_2 tolerance, and expected gas concentrations around the roots
of plants growing in various environments.

Minimum oxygen levels have been estimated in various ways by many
investigators. STEWART and BERRY (24) found 20 per cent. oxygen in the
gas above the liquids used to be maximum for accumulation of K and Br by
storage tissue slices. At 3.8 per cent. oxygen, K accumulation was 70 per
cent. and Br 66 per cent. of maximum. At 1.4 per cent. oxygen, K was lost
to the solution. VLAMIS and DAVIS (26) found that excised roots lost K and
Br to solutions under partial oxygen pressures of less than 1 per cent., but
showed nearly maximum accumulation with 2.7 per cent. O_2. CANNON (4)
found that roots of most plant species grew with as little as 0.5 per cent. O_2,
and that maximum growth was obtained with from 2 to 8 per cent. O_2,
depending upon the species and conditions. TAYLOR (25) reports that
germination of wheat was reduced significantly by O_2 pressures of 5 per cent.
and was 50 per cent. of normal at 1 per cent. O_2. Rice germination was 90
per cent. of normal in purified N_2, with more than one-half the normal rate.
We may conclude from the data available that plant roots will develop and
function normally with 5 to 10 per cent. O_2 maintained in the soil gases and
slowly with 1 to 2 per cent. O_2.

Data on the effects of CO_2 are meager. CANNON (4) found that growth
of many roots was checked promptly by 25 per cent. but willow roots grew
in 45 per cent. CO_2 with O_2 normal or higher. SEIFRIZ (22) reports that
10 per cent. CO_2 was minimum for narcosis and 25 per cent. gave a rapid
reaction. Twenty to 30 per cent. of CO_2, even with 70 to 80 per cent. O_2,
was rapidly toxic to barley in experiments of VLAMIS and DAVIS (26).
KNIGHT (14) found that maize was killed in soil sealed with paraffin until
CO_2 accumulations reached 11.4 per cent.

These data indicate that CO_2 concentrations below 10 per cent. in the
soil gases are probably not highly toxic, but that concentrations above 15 or
20 per cent. may be lethal to some plants. Analyses of soil gases at depths
of 6 to 12 inches by LEATHER (17) and RUSSELL and APPLEYARD (21) agree
in showing typical CO_2 contents of 1.0 per cent. or less with O_2 concentra-
tions of about 19 to 20 per cent. LEATHER states, "The diffusion of gases
through soils at a depth of 12–15 inches is so efficient as to warrant the con-

clusion that cultivation of the surface soil is unnecessary for purposes of aeration.'' LEATHER, however, found CO_2 high around the roots of plants. RUSSELL and APPLEYARD recorded concentrations as high as 9.1 per cent. under rapidly developing *Festuca ovina,* and KNIGHT (14) observed 30.6 per cent. CO_2 in a garden soil sealed with paraffin. KIDD (13) found CO_2 concentrations at a depth of 12 inches as high as 20 per cent. when 3 inches of green grass was buried in an 18-inch pit. Control values were 1.0 per cent. Seeds of *Brassica alba* did not germinate over the decaying grass.

The available data indicate: (a) that plant roots vary in their sensitivity to CO_2; (b) that typical soils are low in CO_2 and high in O_2 so that a high resistance to CO_2 need not be a part of the normal picture of root development; and (c) that a high concentration of CO_2 in the soil air depends upon its rapid production by decay or root respiration as well as upon a low rate of gas exchange. The observation that CO_2 accumulation parallels O_2 depletion in upland soils is significant and indicates that slightly toxic to toxic CO_2 concentrations of 10 to 20 per cent. are probably more common than limiting O_2 concentrations of 1 to 2 per cent. Marsh soils, however, may be low in both O_2 and CO_2. Gases from flooded rice fields in India were nearly pure methane (17), and VLAMIS and DAVIS (26) were able to grow better rice bubbled with methane than with air. In spite of this resistance to low O_2 levels, rice was killed by CO_2 as quickly as tomato or barley.

Finally, CO_2 concentrations which reduce water and mineral absorption by one species in a plant community, even though not strongly toxic, may markedly affect competition. McCOMB and LOOMIS (19) have suggested for example that upland forest species are less tolerant of CO_2 than are grasses and prairie species. Decomposition of the high subsoil organic matter of prairie soils and respiration by grass roots would thus create conditions unfavorable to the invasion of grasslands by forest, particularly on heavy, poorly aerated soils. Treatments such as erosion, grazing, or cultivation which reduce the soil organic matter and/or the development of grass roots increase forest invasion.

Summary

Absorption of water by the roots of wheat, maize, and rice growing in water cultures was reduced 14 to 50 per cent. by bubbling CO_2 through the solutions for ten minutes out of each hour. Bubbling air through cultures at the same intervals increased water absorption 9 per cent.; in one experiment, bubbling with nitrogen increased absorption by 13 per cent. Adding H_2SO_4 to bring the solutions to the pH reached with CO_2 had no effect upon water absorption. None of the plants was visibly injured by treatments lasting 36 hours.

Carbon dioxide treatments reduced the absorption of five nutrient elements in the order $K > N > P > Ca > Mg$. Calcium and magnesium absorption was reduced about as much as water absorption. Potassium was excreted from the roots of many of the plants treated with CO_2. Nitrogen and phosphorus absorption were intermediately affected.

It is suggested that the toxic effect of CO_2 on plant protoplasm is associated with its specific ability to change the internal pH of cells and to form hydrogen-bond compounds with proteins. Reported maximum carbon dioxide concentrations in field soils rise above toxic levels, and CO_2 toxicity should be included with oxygen deficiency as a factor affecting plant development in poorly aerated soils. Because of the importance of roots in raising soil CO_2, this gas may be a factor in root competition, particularly in the deeper layers of heavy soils.

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