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Differentiation Between the pH Effect and the Bicarbonate Ion Effect in Causing Lime-Induced Chlorosis

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DIFFERENTIATION BETWEEN THE pH EFFECT AND THE BICARBONATE ION EFFECT IN CAUSING LIME-INDUCED CHLOROSIS

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INTRODUCTION

Lime-induced chlorosis has been recognized for many years as a problem where plants are grown on calcareous soils. There are many factors associated with and influencing this form of iron chlorosis and because of this it has been very difficult to determine the relationship between the factors and chlorosis.

There are high concentrations of bicarbonate in calcareous soils. Because of this high concentration, it was believed that the presence of the bicarbonate ion was causing chlorosis. It has been proposed that the pH of the growth medium was the causitive factor of lime-induced chlorsis. Most calcareous soils have a pH range around 8.0. At this pH the solubility of iron is very low, and it was believed that chlorosis was a result of iron being insoluble at a high pH. Iron chlorosis has been induced in plants by increasing the phosphorus concentration in the growth medium. Iron phosphates have a low solubility and it was believed that the available iron was precipitated by the phosphates. The interference of metals such as calcium, nickel, cobalt, zinc, and copper appears to be a factor affecting the absorption of iron by plants. It was thought that an interfering ion (Ni, Co, Ca, Zn) may interfere with the translocation of iron in the malic or malonic acid complexes (Tiffin and Brown, 1962).

Because of the complexity of the problem, nutrient

solutions have been used in studying iron chlorosis. They provided a means where most of the supposed factors could be held constant and one or two varied. Split-root and split-medium experiments have been used to separate the factors even more than in the conventional nutrient culture.

In a number of cases it has been observed that the iron content of chlorotic plants did not differ from that of non-chlorotic plants (Iljin, 1952). It was assumed that the iron was inactivated in the plant and thus unavailable for use. Attempts have been made to find those areas of inactivation by using radioautographs, and by sectioning the plants and running chemical analysis on the sections. It has been observed that the concentration of phosphorus tends to increase in the upper stems and leaves of chlorotic plants. De Kock (1955) proposed that the degree of chlorosis could be determined by calculating the ratios of phosphorus to iron. This ratio was higher in chlorotic plants than in non-chlorotic plants. In addition to this, the uptake of monovalent and divalent ions seems to be altered in chlorosis. The chlorotic plant seems to have an increase in monovalent ions and a decrease in divalent ions.

The concentration of the bicarbonate ion and the pH of the medium are two factors which have received considerable attention. Goss (1957) and Doney et al. (1960) found that when the bicarbonate ion was present in the solution culture there was an overall decrease in the nutrient ions taken up by the plant. Brown et al. (1960) separated the

bicarbonate ion from the other nutrients by allowing the plant roots to grow through some soil and into a bicarbonate solution below. They observed that the bicarbonate had no effect upon the uptake of other ions and concluded that the effect of bicarbonate, when present with the complete nutrients, was in maintaining a constant pH.

Many workers (Doney et al., 1960; Petersen, 1961; Porter and Thorne, 1955) have indicated that in addition to maintaining a constant pH, the bicarbonate ion has other effects which cause chlorosis. It has been very difficult to separate the effect of pH and bicarbonate. One reason has been that in the non-bicarbonate system the pH tends to decrease, and in some cases as much as one pH unit in four to six hours while the pH of the bicarbonate system remained constant (Miller and Russell, 1962).

This study was set up to investigate the following objectives: (a) To find a method for maintaining a constant pH of 7.8 in a hydroponic system without using the bicarbonate ion, (b) To differentiate between the pH effect and the bicarbonate effect in causing chlorosis in plants, and (c) To examine the various regions of the plants to see if there are areas of mineral accumulation that might explain the chlorotic symptoms.

REVIEW OF LITERATURE

The characteristics associated with lime-induced chlorosis are the same as those associated with iron deficiency chlorosis—interveinal yellowing of the leaves at the meristematic region combined with reduced plant vigor, increased monovalent and decreased divalent ion absorption.

Although no single factor has been found to adequately explain this physiological disease, many factors have been associated with it. Thorne, Wann, and Robinson (1950) observed that calcareous soils characterized by fine texture, high moisture content, poor aeration and cool temperatures intensify the development of chlorosis in plants.

It is well known that plants differ in their susceptibility to lime-induced chlorosis. Weiss (1943) found a recessive gene to be the contributing factor to the difference in chlorotic susceptible PI-54619-5-1 (PI) soybeans and non-susceptable hawkeye soybeans, Brown (1957) found a number of plants to be resistant to lime-induced chlorosis when grown on calcareous soil but developed copper deficiency when grown on organic soils. Other plants were observed to be just the opposite. Brown (1957) observed that plants containing a dominant iron terminal oxidase were more susceptible to chlorosis than plants containing a dominant copper terminal oxidase. It was suggested that the resistance

or susceptability to lime-induced chlorosis depends upon the terminal oxidase present in the plant.

Effects of Bicarbonate

Results indicate that with certain plant species increased carbon dioxide concentrations with the accompanying bicarbonate in the growth medium has a depressing effect on the growth (Stolwijk and Thimann, 1957), respiration (Miller and Thorne, 1956), mineral nutrient absorption (Jackson and Coleman, 1959), nutrient translocation within the plant (Rediske and Biddulph, 1955), and the rates of several enzymatic reactions (Miller and Evans, 1956).

In studying the inhibition of plant cytochrome oxidase systems by the bicarbonate ion, Miller and Evans (1956) found that the activity of cytochrome oxidase decreased as the bicarbonate concentration in the root medium increased. In an earlier experiment, Miller and Thorne (1956) indicate that the bicarbonate ion inhibited the respiration in the roots of plants containing a dominant iron terminal oxidase more than those containing a dominant copper terminal oxidase. Bonner (1950) and Bendall *et al*. (1958) observed that the succinic oxidase system was sensitive to the bicarbonate-carbon dioxide concentration. Baxter and Belcher (1955) suggest that accumulation of bicarbonate ion around roots unfavorably affects carbon dioxide excretion and internal pH and was the main factor in the metabolic disturbance leading to iron deficiency.

Another effect of the bicarbonate ion appears to be in decreasing the absorption of mineral nutrients by the roots. Wadleigh and Brown (1952) felt that bicarbonate ion induced chlorosis through its action on entry and activity of iron and that other arrangements in the chemical status of plants were largely concomitant with the effect of iron absorption and activity. Marcour (1952) indicated that the presence of bicarbonate in the nutrient solution almost completely prevented the uptake of radioactive iron. Goss (1957) found that bicarbonate significantly decreased the uptake and translocation of a number of mineral elements.

Doney (1959) found that increased bicarbonate levels tended to decrease the amount of phosphorus absorbed by bean plants, however, it seemed to increase the percentage of phosphorus in the stems and primary leaves over that of the control even though the total phosphorus in the plant was lower.

Walliham (1961) and Morcour (1952) observed that the iron concentration in the roots of sodium bicarbonate treated plants was lower than in sodium sulfate or sodium chloride treated plants. Marcour (1952) states that iron uptake seems to be slowed down by the presence of bicarbonate ions at the surface of the roots and the iron already present in the cell is more or less immobilized by organic acids or by bicarbonate and carbonate ions.

Heller et al. (1940) found that sodium bicarbonate treatments reduced the calcium content in tomato plants very

markedly. Olsen et al. (1949) states that the bicarbonate ion in calcareous soils appears to decrease the calcium content which, in turn, increases the solubility of phosphorus. The higher phosphorus and lower calcium could be responsible for the resultant chlorotic plant.

Effect of Phosphorus

Many workers feel that the effect of phosphorus in causing lime-induced chlorosis is in precipitating the iron in the root medium and in the conductive tissues of the plants. Brown et al. (1959) state that phosphorus can cause iron chlorosis in some plant species or varieties. They suggest that phosphorus can accumulate inside the plant in such proportions as to inactivate iron.

Chandler and Scarseth (1941) found that as the phosphorus content of the soil was increased there was an increase in chlorosis and a reduction in the iron content of peanut and alfalfa plants. In nutrient experiments using PI and hawkeye soubeans, Brown and Tiffin (1960) found that by increasing the phosphorus concentration in the nutrient solution the absorption of iron was greatly decreased and the phosphorus concentration in the exudate increased. Sideris et al. (1943) reported that an increased supply of phosphorus increased the amount of iron precipitated by the plant roots.

Doney et al. (1960) suggests that the total uptake of iron from the growth medium is influenced by the phosphorus

concentration. They found that plants grown in nutrient solutions with low phosphorus contained more iron than plants from the high phosphorus solution.

Aiyar (1946) found that increasing concentrations of phosphorus caused an increase in the phosphorus content of the roots, but a decrease in the nitrogen and iron content. Biddulph and Woodbridge (1952) observed that as the phosphorus content of the nutrient medium was increased, roots, stems, and cordate leaves continued to build up in phosphorus content even after trifoliate leaves were adequately supplied. They concluded that the excess phosphorus in the plant may be responsible for immobilizing iron and other ions.

De Kock (1955) noted that as the oxygen supply to the roots increased from 1 to 20 percent there was an increase in the phosphorus content of the leaves and the stems.

The pH of the root medium has an effect upon the absorption and form of phosphorus. Arnon et al. (1942) showed that the amount of phosphate absorbed by a plant varied both with the plant and the pH of the nutrient solution. Biddulph and Woodbridge (1952) state that the pH effects the permeability of the absorbing cell membrane. They found a characteristic uptake of phosphorus for each pH level of the nutrient medium. Their results indicated that movement of the phosphorus from stems and petioles to leaf blades was impaired at pH 7 and higher. The resultant accumulation of phosphorus in stems and petioles at this pH constitutes a medium rich in phosphorus through which other ions being

transported to the leaf blades must pass.

Ion Distribution

The ion distribution within the plant has been used as a measure of chlorosis. De Kock (1955) maintains that it is possible to distinguish between chlorotic and non-chlorotic plants by the phosphorus to iron ratio, with chlorotic plants having a larger ratio than non-chlorotic plants.

Baxter and Belcher (1955) and Warnock (1952) are of the opinion that immobilization of iron within the plant is not the direct cause of the observed chlorotic condition. Thorne et al. (1950) believe that the disturbance in the monovalent to divalent ion ratio is a result of chlorosis rather than a cause of it.

Oserkowsky (1932) states that in some plants it has been observed that chlorotic symptoms apparently attributable to iron deficiency were not always accompanied by a shortage of iron in affected tissues. De Kock (1955) noted that chlorotic plants had an accumulation of iron in the interveinal tissue and a very limited amount of iron in the veins. This is in agreement with the results of Biddulph (1951) which indicates that iron and phosphorus accumulate in the roots and conductive tissues of plants suffering from chlorosis.

Olsen (1935) and Biddulph (1951) both suggest that when iron is taken up from neutral or alkaline solutions

it can be precipitated as ferric phosphate in the vascular bundles along the veins of a leaf. Brown et al. (1959) noted that iron was inactivated internally in PI soybeans, principally by the combined efforts of phosphorus and calcium. In contrast, iron was absorbed and remained mobile in hawkeye soybeans under the same conditions of growth and element composition. They concluded that susceptibility to iron chlorosis appears to be relative in scope and depends on the capacity of a plant to absorb and hold iron in a soluble mobile form.

Lindner and Harley (1944) were able to show that in lime-induced chlorosis there existed a definite ratio between the calcium and potassium content of the leaves. Healthy green leaves have higher ratios while in chlorotic leaves the ratio was invariably low. They suggested that the high potassium level induced chlorosis by replacing the iron on the enzyme responsible for chlorophyll formation, thereby inactivating the enzyme. Wadleigh and Brown (1952) and De Kock (1955) observed that potassium content was higher in chlorotic leaves, both in the sap and in the dry tissue. However, they found no difference in the calcium content of chlorotic and green leaves.

pH EXPERIMENT

Introduction

In differentiating between the pH effect and the bicarbonate effect in causing iron chlorosis, it is important to have the same pH in the bicarbonate and non-bicarbonate treatment. Without this it would be very difficult to separate the two factors.

A number of investigators (Miller et al., 1962; Brown, 1959; Miller, 1960) have observed that the daily pH of a NaCl treatment or of a non-bicarbonate treatment constantly decreases. In working with nutrient solutions in which bicarbonate had not been added but in which the pH was maintained by the use of NaOH, Miller and Russell (1962) found that the pH decreased from the initial 7.8 value to as low as 6.8 within a period of from four to six hours. In contrast to this, the pH in the nutrient solutions containing bicarbonate remained constant for the duration of the experiment.

Hageman et al. (1961) used a carboxyl cation exchange resin as a means of controlling the pH of nutrient solutions in which they grew corn. They were able to maintain a pH in the range of 4.2 to 4.8 over a period of 10 to 14 days. From this initial idea of Hageman's, it was proposed that a buffered resin medium be established which would maintain a pH around 7.8.

The purpose of this experiment was to see if a non-bicarbonate system could be established which would maintain a pH of 7.8 that could be compared with a bicarbonate system.

Materials and Procedure

Dowex 50-W resin, a carboxyl cation exchange resin, was used as a means of maintaining a pH of 7.8 in a non-bicarbonate system. The resin was converted to the calcium form before adding it to the nutrient solutions. The calcium saturated resin was added at the rate of 25 g/l when used and in all cases the initial pH of the nutrient solution containing the resin was adjusted after the addition of the resin to 7.8 by using 0.1 N NaOH or HCl.

The resin could be reused after an experiment provided that it was once again converted to the calcium form. To do this the resin was first converted to the hydrogen form by washing in 5 percent (v/v) HCl. The calcium form was obtained by agitating the hydrogen form of the resin in a slurry of $Ca(OH)_2$ (7 percent w/v). The excess lime was removed by washing the resin with deionized water.

Three different experiments were designed to investigate the ability of the calcium saturated resin to buffer the nutrient solution and to test its effect on the plants growing in these solutions. Hoagland's No. 2 nutrient solution with iron supplied as ferric citrate was used in all of the experiments. In all of the bicarbonate treatments, sodium bicarbonate was added at the rate of 10 me/l.

These solutions were aerated with a 1 percent CO_2-air mixture. All other solutions were aerated with compressed air. The pH was measured with a Beckman zeromatic pH meter with a glass electrode.

The first experiment consisted of comparing the pH in resin-buffered and CO_2-HCO_3 buffered nutrient solutions in which no plants were grown. Gallon plastic freezer cartons were filled with the nutrient solutions and the solutions in half of the cartons were buffered with resin and the other half with bicarbonate. All of the solutions were adjusted to an initial pH value of 7.8. The pH of the solutions was checked daily but was not adjusted during the total 11 days of the experiment.

The second experiment was designed to see if the resin could buffer the pH of the nutrient solution as well as bicarbonate when plants were growing in the solutions and the solutions were at pH 7.8. Three treatments were set up: (a) resin, bicarbonate, and nutrient solution; (b) resin and nutrient solution; and (c) nutrient solution. Red kidney beans were grown. The pH of each container was checked twice daily and was adjusted to 7.8 with 0.1 N NaOH or HCl. The nutrient solution was changed every five days and the treatment lasted for fifteen days.

The third experiment was set up to see if the presence of calcium saturated resin in the nutrient solution had detrimental or beneficial effects on plants growing in the solution. Two treatments were used: (a) resin plus nutrient solution; and (b) nutrient solution. Red kidney beans

were used in this study. Two week old plants that had been germinated were selected for uniformity in size and were placed in the solutions. The treatments lasted two weeks after which time the plants were harvested. The pH of each container was checked twice daily and was adjusted to 7.8 with 0.1 N NaOH or HCl. The nutrient solution was changed every five days.

Results and Discussion

The results of experiment 1 are listed in Table 1. Both systems—resin buffered and bicarbonate buffered—received the same care. There was enough air being bubbled through the solutions to keep the resin slightly agitated. Over an eleven day period the resin was just as effective in maintaining a pH of 7.8 as the CO_2-HCO_3 system.

Table 1. Range of pH values in nutrient solutions as affected by method of pH control

Time	pH values under different methods	
	HCO_3-CO_2	Resin
Start	7.8	7.8
1 day	7.7	7.7
2 days	7.8	7.8
6 days	7.8	7.9
11 days	7.7	7.7

Table 2 contains the results of the second experiment. These data indicate that the resin was as effective in

controlling the pH as the bicarbonate when plants were growing in the solution. The table shows maximum deviation of the solution pH; although the solutions did not deviate this much in each twelve hour period. In the nutrient solution not buffered by resin or bicarbonate, the drift in pH was as much as one pH unit per twelve hour period.

Table 2. Maximum deviation of nutrient solution pH within a twelve hour period

	pH values under different methods		
	HCO_3-CO_2	Ca-resin	No buffer
Day time	7.7 — 7.9	7.65-7.85	6.8 — 7.8
Night time	7.7 — 7.9	7.65-7.85	6.8 — 7.8

The first two experiments indicate that both the resin and bicarbonate buffering systems are effective in maintaining the pH of the nutrient solution around 7.8 in the presence or absence of plants.

The effect that resin had on the concentration of nutrients in the solution is not known. It certainly had some effect on it because there was a high concentration of calcium added on the exchange sites of the resin. In the present experiment the concentration of nutrients in solution was not measured.

In order to determine the effect of resin on the plants experiment 3 was conducted. Table 3 contains the tabulated

data of this experiment. Each number represents the average fresh weight of four replications. Treatment 1 represents complete nutrient solution plus calcium saturated resin and treatment 2 only nutrient solution. There was a slight difference in the weight of the roots, upper stems, and leaves of the two treatments. The results indicated that the presence of resin stimulated larger growth in these three plant sections. A possible explanation for this difference not appearing in the primary leaves and lower stems is that these sections were partly developed before the treatment was applied. The other three sections had most of their growth during the treatment period.

Table 3. The effect of calcium saturated resin in the nutrient solution upon the fresh weight of red kidney beans

Treatment	Roots	Primary leaves	Lower stems	Upper stems	Leaves
			grams/pot		
1[a]	3.45[b]	2.45	2.44	4.03	11.06
2	3.18	2.54	2.58	3.38	9.48

[a]1=resin plus nutrient solution; 2=nutrient solution
[b]Each figure represents the average of four replications

In both treatments all of the plants were healthy and green. The plants in treatment 1 were slightly larger than those in treatment 2.

In a similar experiment performed by Hageman et al. (1961) resin was used to buffer the nutrient solution at

pH 4. They reported that the corn plants were bigger and appeared healthier than those grown in the solutions where pH was controlled by acid addition or was not adjusted. This response was possibly due to the increase in calcium made available to the plants from the exchange complex of the resin.

Summary

From this work it was concluded that: (a) The calcium saturated resin was as effective in maintaining a pH of 7.8 as the bicarbonate system. The resin allows a rather stable, high pH in a non-bicarbonate system. (b) Plants grown in solutions buffered with the resin were slightly larger than those grown in solutions where the pH was controlled by addition of NaOH and HCl.

COMPARISON OF PLANTS GROWN IN A MODIFIED SPLIT-ROOT SYSTEM WITH THOSE GROWN IN A CONVENTIONAL NUTRIENT SYSTEM

Introduction

The object of these experiments was to distinguish between the bicarbonate ion effect and the pH effect in inducing iron chlorosis at a pH of 7.8.

In the pH experiment a resin buffering system was devised which would maintain a constant pH of 7.8 in the absence of bicarbonate. This system was used to maintain a constant pH in the nutrient medium of the following experiments. The use of the resin buffer as a means of controlling pH in the non-bicarbonate solutions made it possible to separate the effect of bicarbonate from that of pH on inducing chlorosis.

Red kidney beans and hawkeye soybeans were used in the study to observe the response of two different plant varieties to bicarbonate. Distinguishing between the pH and the bicarbonate effect was done by comparing plants grown in nutrient solutions not containing bicarbonate with plant grown in identical solutions containing bicarbonate.

A split-root technique allowed the separation of phosphorus from iron. A factorial arrangement of treatments was set up using bicarbonate as the variable. This arrangement provided a means of separating the effect of bicarbonate

from that of pH when bicarbonate was with iron, or phosphorus, or with both. In addition, the conventional nutrient system allowed comparison of the bicarbonate effect when all of the nutrients were together. To measure the difference of the bicarbonate effect from that of pH, the visual appearance of the plants, the dry weights and chemical composition of the plant sections were compared for the various treatments.

Materials and Methods

This investigation consisted of two different experiments. One was a split-root experiment in which the roots of a plant were divided equally into two adjacent containers. In this experiment phosphorus and iron were in separate containers but the plant had access to both by means of the split-root system. Sodium bicarbonate was added as a treatment in a factorial arrangement.

The other experiment was a conventional nutrient culture. Treatments consisted of plants growing in complete nutrient solution with and without bicarbonate.

Two plant varieties were used—red kidney beans and hawkeye soybeans. They received similar treatment throughout the investigation as to the method of selecting the seeds, seed germination, treatment, harvesting and washing, separation into plant sections, and chemical analyses.

Solution culture

Deionized distilled water was used in the preparation

of the solution culture in all of the treatments. The nutrient solution was the same as Hoagland's No. 2 (Hoagland and Arnon, 1950) except for the iron which was supplied as ferric citrate.

Each container had the following common nutrients in me/l: Ca, 1.3 (plus that added by way of the calcium saturated resin); Mg, 1.3; NO_3, 2.0; K, 0.5; and S, 0.13. The minor elements were added to each container in the following concentrations in ppm: Mn, 0.7; B, 0.04; Zn, 0.02; Cu, 0.005; and Mo, 0.005. In the split-root experiment 1 me/l of phosphorus was added to one container and 5 ppm Fe was added to the other. In the conventional nutrient culture treatment 1 me/l of P and 5 ppm Fe were added with the other nutrients.

Selection of seeds and plants

Uniform healthy seeds were selected and, after treating with seresan (Fungicide produced by duPont), were germinated by placing between layers of cheese cloth which were suspended over a rack in a large tray and saturated with tap water. Several days later healthy seedlings were selected for uniformity and transplanted into a water solution containing 1 me/l calcium as $Ca(NO_3)_2$. At this time the primary root was cut off leaving several of the secondary roots. These seedlings grew under normal-day conditions. When the secondary roots were about six inches long, uniform seedlings were selected and transplanted into treatment solutions.

Treatments

In the split-root experiments, the plants were suspended over two adjacent containers with the roots equally divided between them. The containers were gallon, plastic freezer cartons which had been painted on the outside with aluminum paint. Each carton contained complete nutrient solution, except for phosphorus and iron. In addition, each carton contained 25 g/l calcium saturated resin. (The preparation of the resin is explained in the pH experiment.) Phosphorus was added to one carton of each pair and iron to the other. This arrangement enabled the plants to absorb all required nutrients and eliminated direct contact in the solution between iron and phosphorus. Hereafter the solution containing phosphorus but no iron will be referred to as the phosphorus solution and the solution containing iron but no phosphorus will be called the iron solution.

In the split-root experiment, four different treatments were set up using bicarbonate as the treatment variable (Table 4).

Table 4. Treatments used in the split-root experiment

Treatment	Phosphorus solution	Iron solution
1	bicarbonate	bicarbonate
2	bicarbonate	no bicarbonate
3	no bicarbonate	bicarbonate
4	no bicarbonate	no bicarbonate

In the conventional nutrient culture experiment, the plants were placed in the painted freezer cartons. Complete nutrient solution and 25 g/l calcium saturated resin was added to each carton. In this experiment treatments consisted of complete nutrient solution with and without the addition of bicarbonate.

In all of the bicarbonate treatments, the bicarbonate was added as sodium bicarbonate at 10 me/l. To maintain the bicarbonate ion concentration a 1 percent CO_2-air mixture was bubbled through capillary tubes into the nutrient solution.

In the non-bicarbonate treatments the solutions were aerated by passing air from the compressed air line through capillary tubing into the solution. In both the bicarbonate and non-bicarbonate treatments there was enough air going through the solution to keep the resin agitated at all times.

The pH was maintained at 7.8. It was checked twice daily using a Beckman zeromatic pH meter with a glass electrode. The solutions were adjusted to the initial pH using 0.1 N NaOH or HCl. The nutrient solutions were changed every five days.

The plants were grown in a growth chamber where daylength, light intensity, and temperature were controlled. Plants were grown in a 16 hour light period at 78 F and an eight hour dark period at 65 F.

Harvesting

The plants were harvested fifteen days after the beginning of the treatment. Each treatment consisted of two plants which were analyzed as a unit. Each treatment was replicated four times.

In harvesting, the plants were separated into sections. In the split-root experiment the sections were the iron-root, the phosphorus-root, primary leaves, lower stems, upper stems, and remaining leaves. In the conventional nutrient culture experiment the sections were roots, root-stem, primary leaves, lower stem, upper stem, and remaining leaves. The root-stem was that section of the stem which was below the water level in the carton. The division between the lower stem and upper stem was immediately above the petiole-stem junction of the first trifoliate leaf.

After separation of the plant sections, the green weight was taken of each section. The sections were washed in 0.1 N HCl followed by two washings in distilled water. Each washing consisted of submerging the plant and gently agitating it for 15 to 20 seconds. After washing the plant sections were placed in paper bags and dried in a forced air dryer at 80 C.

Chemical analyses

The dry plant tissue samples were ground into a homogenous powder using a porcelain mortar. The mortar was used to eliminate possible contamination from a steel grinding mill.

The ground plant samples were weighed on a Mettler electric balance and transfered into 125 ml digestion flasks. Ten ml of concentrated nitric acid was added to each flask. The flasks were placed on a hot plate and heated at a low temperature until all of the organic matter had been digested. The flasks were then removed from the hot plate and allowed to cool. Five ml of perchloric acid were added to each flask and the contents digested until two ml of solution remained.

The digestion material was quantitatively transfered from the digestion flasks into 100 ml volumetric flasks and brought to volume. It was then filtered through a No. 1 Whatman filter paper and kept in storage bottles. The filter paper removed the silica from the digested solution.

Phosphorus was determined by using the nitric acid-molybdate-metavanadate method as described by Wilde and Voigt (1955). Iron was determined with ortho-phenanthroline following the method of Seywell and Cunningham (1937). Calcium and magnesium were determined by the ethylenediamine tetraacetic acid (EDTA) method as suggested and worked out by Patton and Reeder (1956) and Flaschka, Barnard, and Broad (1957). Potassium was determined by use of a Perkin-Elmer Model 52-c flame photometer with a propane burner. The method used was that suggested in the instruction manual for the flame photometer and in the Agricultural Handbook 60.

Results and Discussion

Plant-solution relations

Bicarbonate ion. From the Henderson-Hasselback equation

$$pH = pK + \log \frac{(HCO_3^-)}{(CO_2)} \qquad (1)$$

the approximate bicarbonate-carbon dioxide ratio at various pH values can be computed. This is done in Figure 1. It indicates that at pH 7.8 about 97 percent of the carbonic acid system exists as bicarbonate. Thus the advantage of maintaining a pH value of about 7.8 in the present investigation is evident.

In the presence of bicarbonate and carbon dioxide most of the sodium and potassium ions are associated with bicarbonate but some carbonate always exists. The question arises, is there enough carbonate present to make any differences? Using the proper dissociation constant (equation 2) and a 1 percent CO_2-air mixture with a bicarbonate ion concentration of 0.01 molar at 25 C, the concentration of carbonate was found to be 6.57×10^{-5} molar (equation 3). Umbreit (1949) concluded that this was such a small quantity

$$K = \frac{(HCO_3^-)^2}{(CO_2)\ (CO_3^{--})} = 5 \times 10^{+3} \qquad (2)$$

$$(CO_3^-) = \frac{(0.01)^2}{(3.05 \times 10^{-4})\ (5 \times 10^{+3})} = 6.57 \times 10^{-5} \qquad (3)$$

of carbonate that it could be neglected.

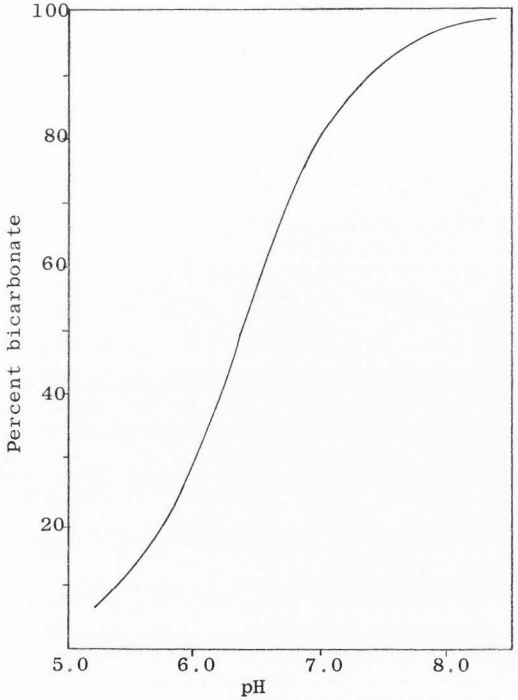


Figure 1. The percent bicarbonate in the carbonic acid system at various pH values

Precipitate. At the end of the experiment a white precipitate was observed in all of the solutions.

Pinevich (1927) found that when Knop's (Hoaglands No. 2) solution was made alkaline with NaOH or $CaCO_3$ a colloidal precipitate of calcium phosphate was formed which removed some of the iron.

Goss (1957), in a study on the effect of bicarbonate on the uptake of radioisotopes, observed a similar precipitate in his solutions. He determined the rate of formation of the precipitate in bicarbonate and non-bicarbonate solutions and observed a rapid rate of formation in both solutions. He examined the precipitate and found it to be largely amorphous tricalcium phosphate, with a small additional amount of magnesium carbonate present in the sample isolated from the bicarbonate culture. He concluded that the precipitate formed in the nutrient solution was not a major factor in explaining the effect of the bicarbonate ion on the uptake by plants of the isotopes used.

The precipitate formed in the present study may not be the same as that formed in Goss's or Pinevich's solutions; but, it seems probable that it could be in as much as the same nutrients were used in both studies. Olsen (1953) points out that calcium phosphates are complex, extremely variable, and little understood. Over a long period of time it might be expected that the precipitate formed would be different from those formed over a short time. Greenwald (1941, 1945) found that the solubility of calcium phosphate

was increased by the presence of bicarbonate, and he postulated the formation of calcium bicarbonate and calcium bicarbonate phosphate complexes. Brown et al. (1959) observed that the addition of phosphorus to the solution cultures containing $NaHCO_3$ increased the soluble calcium levels and that soluble phosphorus levels were constantly higher in $NaHCO_3$ treatments than in NaCl treatments.

Obviously, there is a possibility that some difference could exist in the nutrient concentration of the solutions with bicarbonate and non-bicarbonate treatments. However, this difference was not determined quantitatively.

Visual and weight relations

Visual appearance. The symptoms of iron chlorosis were associated with the treatments in which iron and bicarbonate were together. In the conventional nutrient culture experiment, iron, phosphorus, and bicarbonate were together in the nutrient medium. Under this arrangement the symptoms of iron chlorosis appeared to be more pronounced than in the split-root experiment where phosphorus was separated from iron and bicarbonate.

Plants differ in their susceptability to iron chlorosis, and for this reason red kidney beans and hawkeye soybeans were used. In the conventional nutrient culture experiment both red kidney beans and hawkeye soybeans developed chlorosis in the bicarbonate treatment but were green and normal looking in the non-bicarbonate treatment.

In the split-root experiment the differences in susceptability to iron chlorosis of the two plants was apparent. The red kidney beans did not show signs of iron chlorosis under any treatment, not even when bicarbonate was present with both phosphorus and iron. On the other hand, hawkeye soybeans developed chlorosis only when bicarbonate was with the iron. When bicarbonate was with the phosphorus and not with the iron, chlorosis did not appear.

One explanation why hawkeye soybeans developed chlorosis and red kidney beans did not develop chlorosis when bicarbonate was with the iron in the split-root experiment was the difference in the chelating ability of the two plants. Brown et al. (1960) found that chelating agents are numerous in plants and the kind and concentration are dependent upon the plant species. They found that roots differ just as chelating agents differ in their capacity to compete for iron, and that red kidney beans were able to draw more iron from solution than hawkeye soybeans.

It is interesting to note again that the effect of bicarbonate in causing iron chlorosis was more pronounced when phosphorus was separated from iron and bicarbonate. Brown et al. (1959) found that bicarbonate affected respiration of PI soybean roots more in conventional nutrient solutions when bicarbonate, phosphorus, calcium, and iron (pH 7.8) all bathed the roots together, than when iron was separated from bicarbonate, phosphorus, and calcium. It

is not the object to point out the relationship between respiration of the root and iron chlorosis in the leaves, but it is very interesting to note that there was a greater effect when bicarbonate, iron and phosphorus are together than when iron was separated from the other two.

In considering only the visual appearance of the plants it was evident that bicarbonate had an effect in causing iron chlorosis which was different from that of pH. The bicarbonate ion was not the only cause of chlorosis, in fact it had to be associated with the iron in order for chlorosis to appear. It did not appear when bicarbonate was only with the phosphorus solution. The effect of bicarbonate in causing iron chlorosis was even greater when bicarbonate, iron and phosphorus were together.

Weight relations. Tables 5 and 6 contain the average weights of the different plant sections of hawkeye soybeans and red kidney beans grown in the split-root system. Tables 7 and 8 contain the average weights of the different plant sections of hawkeye soybeans and red kidney beans grown in the conventional nutrient culture system.

The weight of the plants was correlated with the appearance of chlorosis. Plants which were the most chlorotic were the smallest in weight. The weight of the plant sections was also correlated with the presence of bicarbonate. In general, when bicarbonate was with the iron the weight was less than when bicarbonate was not with the iron. This relationship did not hold when bicarbonate was only with

Table 5. The weight of plant sections of hawkeye soybeans grown in the split-root experiment

P status	Root segment Fe	Root segment P	Primary leaves	Lower stem	Upper stem	Leaves
			grams			
			Iron with HCO_3^-			
P with HCO_3^-	0.170[a]	0.148	0.492	0.521	0.221	0.747
P without HCO_3^-	0.198	0.093	0.439	0.413	0.220	0.700
Mean	0.184	0.121	0.465	0.467	0.221	0.723
			Iron without HCO_3^-			
P with HCO_3^-	0.200	0.110	0.388	0.461	0.225	0.902
P without HCO_3^-	0.324	0.079	0.470	0.436	0.226	0.949
Mean	0.262	0.094	0.429	0.448	0.225	0.925

For comparison between:	L.S.D. .05
Any plant sections in any treatment Means	0.046
	0.033

[a]Each figure represents the average of four replications

Table 6. The weight of plant sections of red kidney beans grown in the split-root experiment

P status	Root segment Fe	Root segment P	Primary leaves	Lower stem	Upper stem	Leaves
			grams			
			Iron with HCO_3^-			
P with HCO_3^-	0.378	0.389	0.613	0.889	0.583	2.353
P without HCO_3^-	0.268	0.565	0.582	0.749	0.616	2.302
Mean	0.323	0.477	0.598	0.819	0.600	2.328
			Iron without HCO_3^-			
P with HCO_3^-	0.676	0.235	0.522	0.721	0.591	2.278
P without HCO_3^-	0.341	0.612	0.668	0.799	0.614	2.379
Mean	0.509	0.423	0.596	0.760	0.602	2.329
For comparison between:			L.S.D. .05			
Any plant sections in in any treatment				1.05		
Means				1.15		

[a]Each figure represents the average of four replications

Table 7. The weight of plant sections of hawkeye soybeans grown in the conventional nutrient culture experiment

HCO_3^- status	Root	Root stem	Primary leaves	Lower stem	Upper stem	Leaves
			grams			
with HCO_3^-	0.223[a]	0.151	0.383	0.250	0.128	0.353
without HCO_3^-	0.325	0.175	0.330	0.285	0.315	0.949
For comparison between:			L.S.D.	.05		
Plant sections				0.018		

[a]Each figure represents the average of four replications

Table 8. The weight of plant sections of red kidney beans grown in the conventional nutrient culture experiment

HCO_3^- status	Root	Root stem	Primary leaves	Lower stem	Upper stem	Leaves
			grams			
with HCO_3^-	0.262[a]	0.252	0.395	0.354	0.230	0.859
without HCO_3^-	0.410	0.209	0.311	0.370	0.438	1.524
For comparison between:			L.S.D.	.05		
Plant sections				0.046		

[a]Each figure represents the average of four replications

the phosphorus. Under these treatments in the split-root experiments, the various plant sections did not differ in weight.

The differences in weight of the plant sections appeared mostly in the roots, upper stems, and leaves. These sections were doing maximum amount of growth during the treatment period whereas the lower stems and primary leaves had done maximum amount of growth during the pre-treatment period.

Hawkeye soybeans in the split-root experiment had a decrease in the weight of the iron-root and in the leaves when bicarbonate was with the iron (Table 5). The other sections did not differ significantly from each other. However, there was a trend for more weight per plant section when bicarbonate was not present at all. In the conventional nutrient culture experiment, there was more weight in the roots, lower stems, upper stems, and leaves when bicarbonate was not present (Table 7). The differences in weight due to bicarbonate in the conventional nutrient system were greater than those in the split-root system. The weight differences have a positive correlation with the degree of chlorosis in hawkeye soybeans.

Red kidney beans when grown in the split-root system did not show weight differences in plant sections due to bicarbonate treatment (Table 6). The plants in this system did not show visual differences or weight differences. On the other hand, when the red kidney beans were grown in the

conventional nutrient culture system, chlorosis appeared and significant decreases in weight was observed in the roots, upper stems, and leaves (Table 8).

The differences in plant section weights are expressed graphically in Figure 2. This figure illustrates the tendency for a decrease in plant weight when bicarbonate was present.

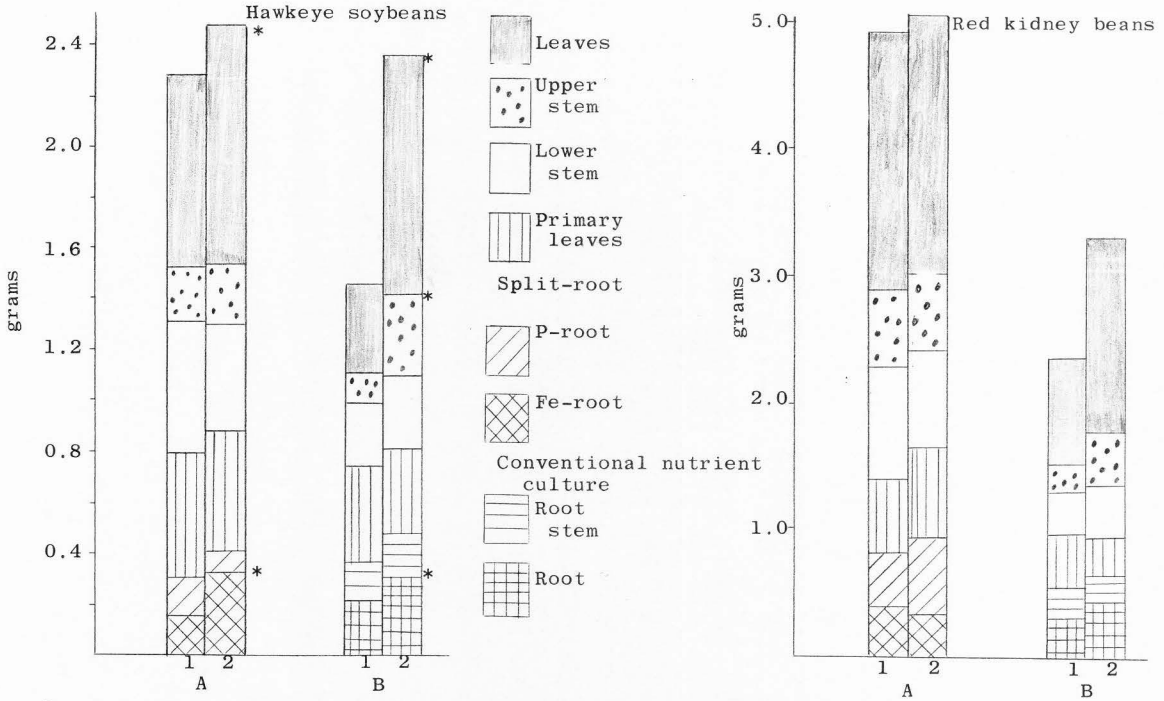
Phosphorus uptake and distribution

The values in Tables 9 and 10 represent the average concentration of phosphorus found in the different plant sections of hawkeye soybeans and red kidney beans when the plants were grown in the split-root experiment. Tables 11 and 12 give the average concentration of phosphorus in the plant sections of these plants when they were grown in the conventional nutrient culture experiment.

Hawkeye soybean, in the split-root experiment, had an increase in the mean phosphorus concentration in the upper stems and leaves when bicarbonate was with the iron (Table 9). There was also a trend for greater concentrations of phosphorus in these two sections when bicarbonate was with the phosphorus than when it was not but these values were not significant. The differences between the bicarbonate and non-bicarbonate treatments with soybeans were more pronounced in the conventional nutrient culture experiment (Table 11). The concentration of phosphorus was greater only in the upper stems and leaves with the bicarbonate treatment.

The phosphorus accumulation in hawkeye soybeans correlates with the appearance of chlorosis and with the differences in weight of the plant sections. Treatments in which

Figure 2. Weight of the plant sections of hawkeye soybeans and red kidney beans



1=Fe and P with HCO_3^-; 2=Fe and P without HCO_3^-; A=Split-root experiment; B=Conventional nutrient culture experiment; *=Sections which differ at or above the 0.9 probability level.

Table 9. Phosphorus uptake and distribution in hawkeye soybeans grown in the split-root experiment

P status	Root segment Fe	Root segment P	Primary leaves	Lower stem	Upper stem	Leaves
			mg/g			
			Iron with HCO_3^-			
P with HCO_3^-	3.10[a]	3.42	1.82	2.00	2.81	3.80
P without HCO_3^-	3.20	3.58	1.66	1.78	2.37	3.36
Mean	3.15	3.51	1.74	1.89	2.59	3.58
			Iron without HCO_3^-			
P with HCO_3^-	3.11	3.55	1.70	1.57	2.11	3.04
P without HCO_3^-	2.90	3.96	1.53	1.98	1.84	2.56
Mean	3.01	3.75	1.62	1.77	1.98	2.80
For comparison between:			L.S.D.	.05		
Any plant sections in any treatment				1.14		
Means				0.58		

[a]Each figure represents the average of four replications

Table 10. Phosphorus uptake and distribution in red kidney beans grown in the split-root experiment

P status	Root segment Fe	Root segment P	Primary leaves	Lower stem	Upper stem	Leaves
			mg/g			
			Iron with HCO_3^-			
P with HCO_3^-	3.87[a]	4.84	4.19	2.24	3.77	4.48
P without HCO_3^-	3.75	5.82	5.51	2.12	3.44	4.37
Mean	3.81	5.33	4.85	2.18	3.61	4.42
			Iron without HCO_3^-			
P with HCO_3^-	3.92	5.27	3.36	1.74	2.97	3.52
P without HCO_3^-	3.92	4.95	5.27	2.03	2.97	4.19
Mean	3.92	5.11	4.31	1.88	2.97	3.85
For comparison between:			L.S.D.	.05		
Any plant sections in any treatment				1.71		
Means				0.70		

[a]Each figure represents the average of four replications

Table 11. Phosphorus uptake and distribution in hawkeye soybeans grown in the conventional nutrient culture experiment

HCO_3^- status	Root	Root stem	Primary leaves	Lower stem	Upper stem	Leaves
			mg/g			
with HCO_3^-	5.09[a]	2.76	3.09	3.97	5.53	5.40
without HCO_3^-	4.64	4.52	4.01	3.72	4.41	5.08
For comparison between:			L.S.D.	.05		
Plant sections				0.28		

[a]Each figure represents the average of four replications

Table 12. Phosphorus uptake and distribution in red kidney beans grown in the conventional nutrient culture experiment

HCO_3^- status	Root	Root stem	Primary leaves	Lower stem	Upper stem	Leaves
			mg/g			
with HCO_3^-	7.53[a]	2.59	5.66	2.91	4.92	7.77
without HCO_3^-	8.65	2.53	6.40	3.38	4.38	6.77
For comparison between:			L.S.D.	.05		
Plant sections				0.19		

[a]Each figure represents the average of four replications

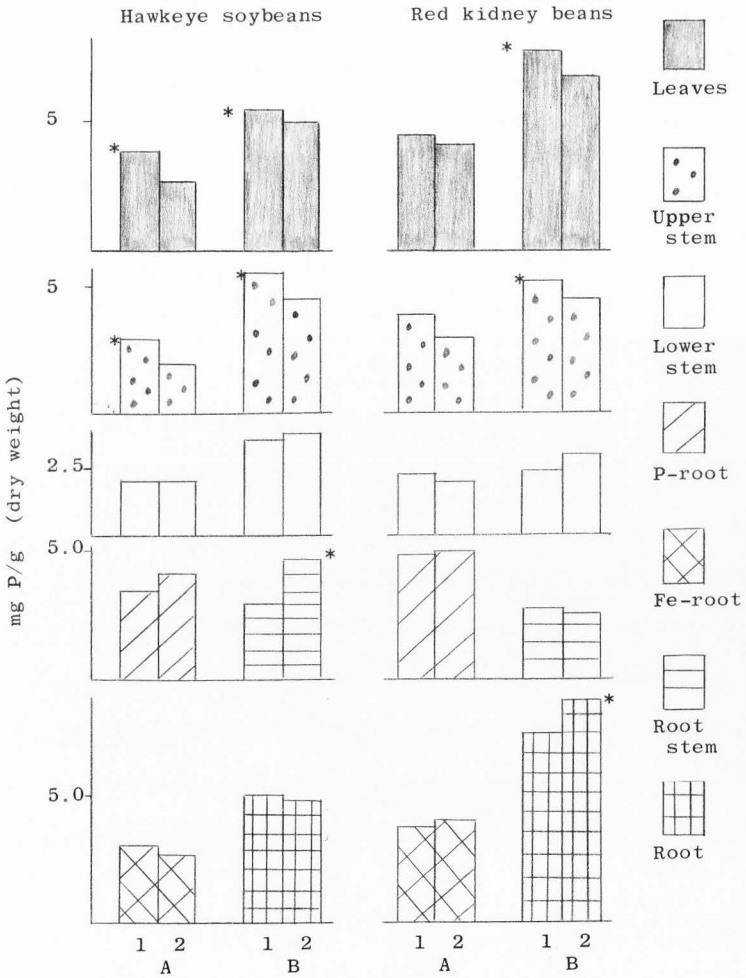
iron and bicarbonate were together caused chlorosis to develop. The weight of the upper stems and leaves was decreased but the concentration of phosphorus in these sections increased.

The sections of the red kidney beans grown in the split-root experiment did not show differences in phosphorus concentration due to bicarbonate treatments (Table 10). There was a trend among the mean values for a higher concentration of phosphorus in the upper stems and leaves when iron and bicarbonate were together.

Red kidney beans exhibited significant differences in phosphorus concentrations in the plant sections when grown in the conventional nutrient culture experiment (Table 12). The phosphorus concentration was higher in the upper stems and leaves but lower in the other sections with the bicarbonate treatment. This follows the same trend as the hawkeye soybeans and correlates with the appearance of chlorosis and decrease in weight of the plant sections.

The concentration of phosphorus in the various plant sections is expressed graphically in Figure 3. In the split-root experiments the values were selected from the treatments where bicarbonate was with both phosphorus and iron and where it was not present with either.

Table 13 lists the differences in phosphorus concentration in plant sections. A plus value indicates that there was a higher concentration of phosphorus when the plant was in the bicarbonate treatment. This table helps



1=Fe and P with HCO_3^-; 2=Fe and P without HCO_3^-; A=Split-root experiment; B=Conventional nutrient culture experiment; *=Sections which differ at or above the 0.9 probability level.

Figure 3. Concentration of phosphorus in the various plant sections of hawkeye soybeans and red kidney beans

Table 13. The difference in amount of phosphorus found in plant sections when plants were grown in solutions with bicarbonate and without bicarbonate[a]

Hawkeye soybeans			Red kidney beans	
Conventional nutrient culture experiment	Split-root experiment[b]	Plant section	Conventional nutrient culture experiment	Split-root experiment
+0.45	+0.20	Fe-root (root)	-1.12	-0.05[a]
-1.76	-0.47	P-root (root stem)	+0.06	-0.11
-0.92	-0.29	Primary leaves	-0.74	-1.08
+0.25	+0.02	Lower stems	-0.47	+0.21
+1.12	+0.97	Upper stems	+0.54	+0.80
+0.32	+1.24	Leaves	+1.00	+0.29

[a]Plus value means that there was more phosphorus in the bicarbonate treatment

[b]Values selected from those treatments where bicarbonate was with both phosphorus and iron and where it was not present at all

to illustrate the fact that phosphorus increased in the upper stems and leaves when bicarbonate was present.

An increase in phosphorus concentration of the upper stems and leaves in chlorotic plants has been observed by a number of workers and appears to be a common characteristic of iron chlorosis (Brown et al., 1959; Biddulph and Woodbridge, 1952). Brown et al. (1960) observed an increase in phosphorus concentration of the stems and leaves of red kidney beans when chlorosis had been induced by an excess of chelating agent.

A very important observation in the present experiment is that even though the concentration of phosphorus in the upper stems and leaves was greater in the bicarbonate treatment, the over all concentration of phosphorus in the plant was not increased. It was more pronounced in the conventional nutrient culture experiment (Table 12 and Figure 3). Doney et al. (1960) reported an increase in phosphorus concentration in the upper stems and leaves but a decrease in phosphorus concentration for the total plant when bicarbonate was present. Goss (1957), Brown (1959), and Miller et al. (1960) also reported a decrease in phosphorus concentration in the total plant as a result of bicarbonate treatments in conventional nutrient cultures.

Iron uptake and distribution

Tables 14 and 15 give the iron concentration in the plant sections of hawkeye soybeans and red kidney beans from the split-root experiment. Tables 16 and 17 give the

Table 14. Iron uptake and distribution in hawkeye soybeans grown in the split-root experiment

P status	Root segment Fe	Root segment P	Primary leaves	Lower stem	Upper stem	Leaves
			ug/g			
			Iron with HCO_3^-			
P with HCO_3^-	926.4[a]	104.5	39.6	31.9	36.1	29.9
P without HCO_3^-	716.0	238.8	44.6	34.6	47.2	30.7
Mean	821.2	171.7	42.1	33.2	41.7	30.3
			Iron without HCO_3^-			
P with HCO_3^-	1875.1	267.1	155.8	34.3	34.4	29.3
P without HCO_3^-	1717.7	218.9	142.4	43.9	48.6	34.9
Mean	1796.4	243.0	149.1	39.1	41.5	32.1
For comparison between:			L.S.D.	.05		
Any plant sections in any treatment Means				64.4 75.0		

[a]Each figure represents the average of four replications

Table 15. Iron uptake and distribution in red kidney beans grown in the split-root experiment

P status	Root segment Fe	Root segment P	Primary leaves	Lower stem	Upper stem	Leaves
			ug/g			
			Iron with HCO_3^-			
P with HCO_3^-	953.7[a]	71.2	53.5	32.6	31.2	35.7
P without HCO_3^-	1317.1	75.4	49.0	24.5	27.4	37.8
Mean	1135.4	73.3	51.2	28.5	29.3	36.8
			Iron without HCO_3^-			
P with HCO_3^-	1376.1	118.7	59.0	33.7	28.7	32.9
P without HCO_3^-	1343.7	105.5	46.9	26.7	30.3	31.0
Mean	1359.9	112.1	52.9	30.2	29.5	32.0
For comparison between:			L.S.D.	.05		
Any plant sections in any treatment Means				53.0 49.0		

[a]Each figure represents the average of four replications

Table 16. Iron uptake and distribution in hawkeye soybeans grown in the conventional nutrient culture experiment

HCO_3^- status	Root	Root stem	Primary leaves	Lower stem	Upper stem	Leaves
			ug/g			
with HCO_3^-	249.0[a]	85.0	44.2	40.0	55.9	52.0
without HCO_3^-	252.1	85.7	71.0	33.7	30.8	35.7
For comparison between:			L.S.D.	.05		
Plant sections				11.1		

[a]Each figure represents the average of four replications

Table 17. Iron uptake and distribution in red kidney beans grown in the conventional nutrient culture experiment

HCO_3^- status	Root	Root stem	Primary leaves	Lower stem	Upper stem	Leaves
			ug/g			
with HCO_3^-	1201.4[a]	129.1	48.9	14.7	13.1	34.4
without HCO_3^-	1249.1	99.2	62.7	30.5	19.6	46.2
For comparison between:			L.S.D.	.05		
Plant sections				30.0		

[a]Each figure represents the average of four replications

concentration of iron in the plant sections when the soybeans and kidney beans were grown in the conventional nutrient culture experiment.

The uptake of iron by hawkeye soybeans in the split-root experiment differed only in the iron segment of the root (Table 14). This difference was in the mean values of the bicarbonate with and without iron treatments. The concentration of iron in this plant section when bicarbonate was not in the iron solution was twice that found when bicarbonate was in the iron solution. The other plant sections did not show significant differences in iron concentration with any of the bicarbonate treatments.

For the hawkeye soybeans grown in the conventional nutrient culture experiment, the iron concentration was higher in the upper stems and leaves in the bicarbonate treatment than in the non-bicarbonate treatment (Table 16). The increase in the iron concentration of these two sections corresponds with the increase in phosphorus concentration with the bicarbonate treatment. These results suggest that iron may be precipitated by the phosphorus in these sections of the hawkeye soybean.

When red kidney beans were grown in the split-root experiment there were no differences due to bicarbonate treatments (Table 15), even though the trend was for more iron to be taken up when bicarbonate was not in the iron solution. However, chlorosis did not develop in the red kidney beans in this experiment, therefore, differences in

iron concentration as a result of bicarbonate treatments would not be expected.

In the conventional nutrient culture experiment the red kidney beans developed chlorosis in the bicarbonate treatment. The concentration of iron in the root was lower in the bicarbonate treatment (Table 17). The concentration of iron in the other sections did not differ with the bicarbonate treatment.

The concentration of iron in the primary leaves, lower stems, upper stems, and leaves of hawkeye soybeans was about the same for the split-root experiment and the conventional nutrient culture experiment (Table 14 and 16). The red kidney beans exhibited the same trend (Tables 15 and 17). The reasons for this are not obvious but some possibilities will be discussed here and others later in the general discussion.

Iljin (1953) states that it was not an uncommon occurance for chlorotic plants to have the same concentration of iron as green plants. He found that plants suffering from iron chlorosis could have more, equal, or less iron than green plants growing in the same vicinity.

Rhoads et al. (1960) proposed that iron taken up by roots could be precipitated with phosphorus by products of CO_2 fixation. If this hypothesis could be proven it would provide an explanation for the results of this experiment as to why the concentration of iron did not show a greater decrease in chlorotic plants.

Calcium uptake and distribution

The calcium content of hawkeye soybeans and red kidney beans grown in the split-root experiment has been tabulated in Tables 18 and 19. Tables 20 and 21 give the calcium content of these plants when they were grown in the conventional nutrient culture experiment.

The uptake of calcium by hawkeye soybeans in the split-root experiment was decreased in all plant sections except for the iron segment of the root when bicarbonate was present (Table 18). There was a greater decrease in calcium uptake when bicarbonate was in the iron solution than when it was in the phosphorus solution. The calcium content increased in the iron segment of the root when bicarbonate was present and the greatest increase was when bicarbonate was with both the iron and phosphorus.

The concentration of calcium in the red kidney beans grown in the split-root experiment (Table 19) was the same as that of the soybeans. There was a decrease in calcium concentration in all sections except the iron segment of the root when bicarbonate was present. The calcium concentration in the root section increased when bicarbonate was present.

In the conventional nutrient culture experiment (Tables 20 and 21) both hawkeye soybeans and red kidney beans show a decrease in calcium concentration when bicarbonate was present except in the root which shows an increase in calcium.

Table 18. Calcium uptake and distribution in hawkeye soybeans grown in the split-root experiment

P status	Root segment Fe	Root segment P	Primary leaves	Lower stem	Upper stem	Leaves
			meq/g			
			Iron with HCO_3^-			
P with HCO_3^-	0.44[a]	0.38	0.94	0.45	1.16	0.69
P without HCO_3^-	0.29	0.59	1.04	0.59	1.28	0.76
Mean	0.36	0.48	0.99	0.52	1.22	0.73
			Iron without HCO_3^-			
P with HCO_3^-	0.41	0.51	1.14	0.60	1.49	0.90
P without HCO_3^-	0.28	0.46	1.29	0.72	1.52	0.85
Mean	0.34	0.48	1.21	0.66	1.50	0.87
For comparison between:			L.S.D.	.05		
Any plant sections in any treatment				0.06		
Means				0.05		

[a]Each figure represents the average of four replications

Table 19. Calcium uptake and distribution in red kidney beans grown in the split-root experiment

P status	Root segment Fe	Root segment P	Primary leaves	Lower stem	Upper stem	Leaves
			meq/g			
			Iron with HCO_3^-			
P with HCO_3^-	0.78[a]	0.48	2.20	0.46	0.72	1.17
P without HCO_3^-	0.73	0.63	2.44	0.51	0.71	1.29
Mean	0.75	0.56	2.32	0.49	0.73	1.23
			Iron without HCO_3^-			
P with HCO_3^-	0.74	0.58	2.56	0.61	0.91	1.47
P without HCO_3^-	0.67	0.53	2.73	0.69	0.94	1.61
Mean	0.71	0.55	2.64	0.65	0.92	1.54
For comparison between:			L.S.D.	.05		
Any plant sections in any treatment				0.05		
Means				0.05		

[a]Each figure represents the average of four replications

Table 20. Calcium uptake and distribution in hawkeye soybeans grown in the conventional nutrient culture experiment

HCO_3^- status	Root	Root stem	Primary leaves	Lower stem	Upper stem	Leaves
			meq/g			
with HCO_3^-	0.25[a]	0.39	0.76	0.62	1.15	0.70
without HCO_3^-	0.19	0.55	1.32	0.78	1.19	0.82
For comparison between:			L.S.D.	.05		
Plant sections				0.06		

[a]Each figure represents the average of four replications

Table 21. Calcium uptake and distribution in red kidney beans grown in the conventional nutrient culture experiment

HCO_3^- status	Root	Root stem	Primary leaves	Lower stem	Upper stem	Leaves
			meq/g			
with HCO_3^-	0.63[a]	0.32	1.98	0.52	0.49	1.02
without HCO_3^-	0.60	0.40	2.29	0.56	0.41	1.17
For comparison between:			L.S.D.	.05		
Plant sections				0.03		

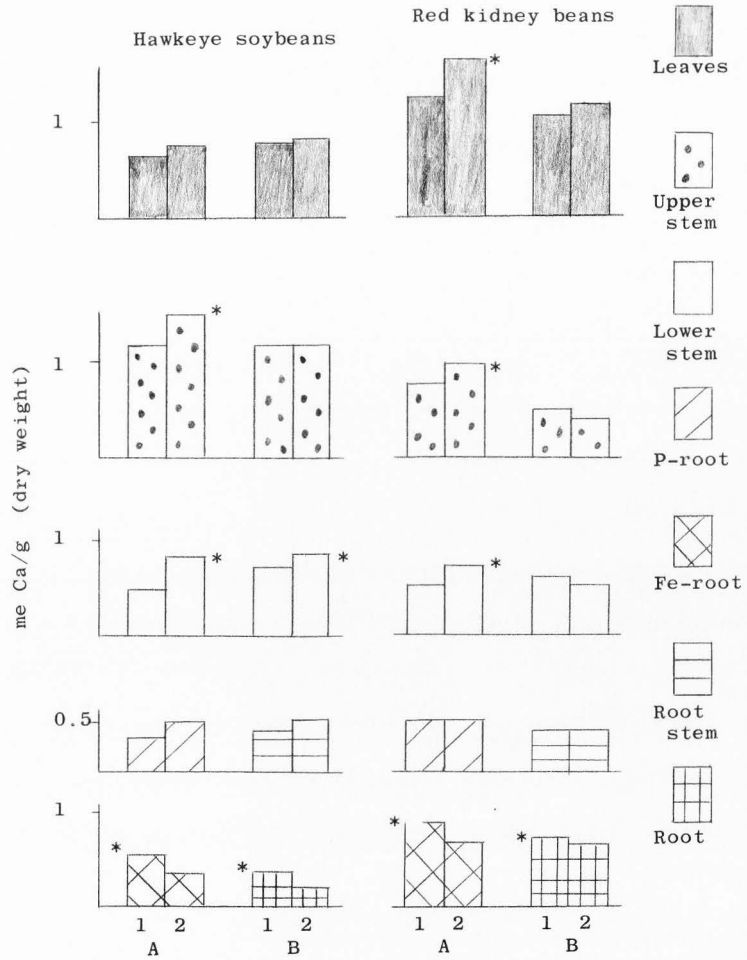
[a]Each figure represents the average of four replications

The concentration of calcium in the various plant sections is expressed graphically in Figure 4. In the split-root experiments the values were selected from the treatments where bicarbonate was with both phosphorus and iron and where it was not present with either. The figure points out the decrease in calcium concentration when bicarbonate was present.

A number of investigators have reported a decrease in the total calcium content of plants which were grown in the presence of high concentrations of bicarbonate (Heller et al., 1940; Gauch and Wadleigh, 1951; Wadleigh and Brown, 1952; De Kock, 1955).

Rhoads and Wallace (1960) proposed that the decrease in calcium to the stems and leaves and the increase in calcium in the roots is due to CO_2 fixation by the plant with a Ca-oxalate precipitate forming in the roots. They point out that in beans suffering from lime chlorosis, almost all water-soluble, non-volatile organic acids, and oxalates were increased. Bonner (1950) indicates that Ca-oxalate precipitates are known to occur widely in plants. If the above conditions are common among lime-intolerant plants, it might be expected that Ca precipitates would be increased in roots if oxalate was increased there.

The suggestion that Ca-oxalate precipitates in roots and decreases calcium in the leaves is in keeping with the results obtained by Dorsdoff et al. (1955), who attributed a decrease in calcium of leaves to the production of oxalate



1=Fe and P with HCO_3^-; 2=Fe and P without HCO_3^-; A-Split-root experiment; B=Conventional nutrient culture experiment; *=Sections which differ at or above the 0.9 probability level.

Figure 4. The concentration of calcium in the various plant sections of hawkeye soybeans and red kidney beans

from the nitrate fertilization in roots of tung trees.

The data of the present experiments indicates that in the presence of bicarbonate there was an increase in calcium content of the iron-root but not in the phosphorus-root. The reason for this is not obvious; however, it points out the fact that the effect of bicarbonate was more pronounced when it was with the iron than when it was with the phosphorus.

Uptake and distribution of magnesium and potassium

Magnesium. Tables 22 and 23 give the magnesium content for the different plant sections of hawkeye soybeans and red kidney beans grown in the split-root experiment. Tables 24 and 25 give the magnesium content of these plants when they were grown in the conventional nutrient culture experiment. All plants that developed chlorosis had a higher concentration of magnesium in the iron segment of the root than when they did not develop chlorosis. Chlorosis developed in all plants except the red kidney beans in the split-root experiment when bicarbonate and iron were together. In this respect the distribution of magnesium was like that of calcium. The distribution and concentration of magnesium in the upper sections of the plant—lower stems, upper stems, and leaves—was not consistant with any of the treatments. The reason for this is not known.

In the literature there are reports of both increases and decreases in magnesium content of chlorotic leaves.

Table 22. Magnesium uptake and distribution in hawkeye soybeans grown in the split-root experiment

P status	Root segment Fe	Root segment P	Primary leaves	Lower stem	Upper stem	Leaves
			meq/g			
			Iron with HCO_3^-			
P with HCO_3^-	0.43[a]	0.35	0.42	0.24	0.53	0.36
P without HCO_3^-	0.36	0.38	0.47	0.24	0.62	0.36
Mean	0.39	0.36	0.45	0.24	0.57	0.36
			Iron without HCO_3^-			
P with HCO_3^-	0.27	0.45	0.54	0.24	0.46	0.40
P without HCO_3^-	0.30	0.32	0.37	0.29	0.48	0.31
Mean	0.28	0.38	0.46	0.27	0.47	0.36
For comparison between:			L.S.D.	.05		
Any plant sections in any treatment				0.05		
Means				0.08		

[a]Each figure represents the average of four replications

Table 23. Magnesium uptake and distribution in red kidney beans grown in the split-root experiment

P status	Root segment Fe	Root segment P	Primary leaves	Lower stem	Upper stem	Leaves
			meq/g			
			Iron with HCO_3^-			
P with HCO_3^-	0.37[a]	0.39	0.47	0.19	0.24	0.32
P without HCO_3^-	0.43	0.35	0.62	0.24	0.31	0.34
Mean	0.40	0.37	0.54	0.22	0.28	0.33
			Iron without HCO_3^-			
P with HCO_3^-	0.43	0.63	0.50	0.22	0.26	0.44
P without HCO_3^-	0.53	0.44	0.58	0.28	0.37	0.34
Mean	0.48	0.54	0.54	0.24	0.31	0.39
For comparison between:			L.S.D. .05			
Any plant sections in any treatment				0.05		
Means				0.08		

[a]Each figure represents the average of four replications

Table 24. Magnesium uptake and distribution in hawkeye soybeans grown in the conventional nutrient culture experiment

HCO_3^- status	Root	Root stem	Primary leaves	Lower stem	Upper stem	Leaves
			meq/g			
with HCO_3^-	0.45[a]	0.21	0.30	0.25	0.56	0.35
without HCO_3^-	0.28	0.24	0.36	0.18	0.43	0.33
For comparison between:			L.S.D.	.05		
Plant sections				0.03		

[a]Each figure represents the average of four replications

Table 25. Magnesium uptake and distribution in red kidney beans grown in the conventional nutrient culture experiment

HCO_3^- status	Root	Root stem	Primary leaves	Lower stem	Upper stem	Leaves
			meq/g			
with HCO_3^-	0.51[a]	0.61	0.80	0.44	0.56	0.47
without HCO_3^-	0.36	0.73	0.79	0.44	0.34	0.67
For comparison between:			L.S.D.	.05		
Plant sections				0.06		

[a]Each figure represents the average of four replications

Olsen (1950) found that green leaves of sorghum had lower magnesium content than chlorotic ones. In the data published by De Kock (1955) on the amino acid content of several chlorotic and non-chlorotic plants, both increases and decreases in magnesium content are seen. However, the differences were not appreciable. In alkaline soils, sometimes, magnesium concentration is so high that the element is absorbed in quantities sufficient to cause toxicity. This might be one of the possible reasons for a higher concentration of magnesium in chlorotic leaves, as reported by Olsen (1950).

Potassium. The uptake and distribution of potassium in plant sections of hawkeye soybeans and red kidney beans grown in the split-root experiment has been tabulated in Tables 26 and 27. Tables 28 and 29 contain the concentration of potassium found in these plants when they were grown in the conventional nutrient culture experiment.

The trend in potassium uptake of the red kidney beans was different from that of the hawkeye soybeans. This was evident in both the split-root experiment and in the conventional nutrient culture experiment. When bicarbonate was with the iron, red kidney beans had more potassium in the primary leaves, lower stems, upper stems, and leaves and less in the roots than when bicarbonate and iron were not together. On the other hand, hawkeye soybeans had a decrease in potassium uptake in all plant sections when bicarbonate and iron were together. Table 30 helps to illustrate this trend.

Table 26. Potassium uptake and distribution in hawkeye soybeans grown in the split-root experiment

P status	Root segment Fe	Root segment P	Primary leaves	Lower stem	Upper stem	Leaves
			meq/g			
			Iron with HCO_3^-			
P with HCO_3^-	0.23[a]	0.23	0.80	0.76	1.29	0.94
P without HCO_3^-	0.25	0.20	0.86	0.92	1.29	0.96
Mean	0.24	0.21	0.83	0.84	1.27	0.95
			Iron without HCO_3^-			
P with HCO_3^-	0.25	0.19	0.78	0.91	1.53	1.04
P without HCO_3^-	0.33	0.16	0.80	0.94	1.55	0.94
Mean	0.29	0.17	0.79	0.93	1.54	0.99
For comparison between:			L.S.D.	.05		
Any plant sections in any treatment				0.05		
Means				0.08		

[a]Each figure represents the average of four replications

Table 27. Potassium uptake and distribution in red kidney beans grown in the split-root experiment

P status	Root segment Fe	Root segment P	Primary leaves	Lower stem	Upper stem	Leaves
			meq/g			
			Iron with HCO_3^-			
P with HCO_3^-	0.41[a]	0.48	1.18	1.35	2.23	1.27
P without HCO_3^-	0.43	0.64	1.07	1.30	2.15	1.14
Mean	0.42	0.56	1.12	1.32	2.19	1.20
			Iron without HCO_3^-			
P with HCO_3^-	0.63	0.50	0.97	1.32	2.13	1.14
P without HCO_3^-	0.79	0.74	0.94	1.17	2.13	1.10
Mean	0.71	0.62	0.96	1.24	2.13	1.12
For comparison between:			L.S.D.	.05		
Any plant sections in any treatment				0.08		
Means				0.08		

[a]Each figure represents the average of four replications

Table 28. Potassium uptake and distribution in hawkeye soybeans grown in the conventional nutrient culture experiment

HCO_3^- status	Root	Root stem	Primary leaves	Lower stem	Upper stem	Leaves
			meq/g			
with HCO_3^-	0.34[a]	0.65	0.72	0.60	0.97	0.95
without HCO_3^-	0.61	1.01	0.92	1.05	1.65	0.99
For comparison between:			L.S.D.	.05		
Plant sections				0.08		

[a]Each figure represents the average of four replications

Table 29. Potassium uptake and distribution in red kidney beans grown in the conventional nutrient culture experiment

HCO_3^- status	Root	Root stem	Primary leaves	Lower stem	Upper stem	Leaves
			meq/g			
with HCO_3^-	0.25[a]	0.87	0.91	1.40	2.18	1.52
without HCO_3^-	0.57	1.39	0.74	1.27	1.19	1.29
For comparison between:			L.S.D.	.05		
Plant sections				0.13		

[a]Each figure represents the average of four replications

Table 30. The difference in amount of potassium found in plant sections when plants were grown in solutions with bicarbonate and without bicarbonate[a]

Hawkeye soybeans			Red kidney beans	
Conventional nutrient culture experiment	Split-root experiment[b]	Plant section	Conventional nutrient culture experiment	Split-root experiment
-0.27	-0.10	Fe-root (root)	-0.32	-0.38
-0.36	-0.07	P-root (root stem)	-0.52	-0.28
-0.20	0.00	Primary leaves	+0.17	+0.24
-0.45	-0.18	Lower stem	+0.13	+0.18
-0.68	-0.26	Upper stem	+0.99	+0.10
-0.04	0.00	Leaves	+0.23	+0.17

[a]Plus value means that there was more potassium in the bicarbonate treatment

[b]Values selected from those treatments where bicarbonate was with both phosphorus and iron and where it was not present at all

It was expected to have an increase in potassium uptake in both plants when bicarbonate was present (Jackson et al., 1959; Russel, 1949; Ulrich, 1942). However, the conditions of the present experiment were different from the others. The close control of pH and the presence of the calcium saturated resin could possibly account for the results in the present experiment.

It is interesting to note that in the red kidney beans the distribution of potassium was just opposite to that of calcium, i.e., where calcium was low, potassium was high.

DISCUSSION

The pH of all the nutrient solutions was maintained at 7.8 $\pm$ 0.2 by using a calcium saturated carboxyl cation exchange resin. In addition to the resin, the bicarbonate treatments contained 10 me/l sodium bicarbonate. There was no difference in the pH of the bicarbonate and non-bicarbonate treatments. This arrangement made it possible to distinguish between the pH effect and the bicarbonate effect.

It seems logical that the presences of the resin in the nutrient solution would have an effect upon the concentration of the nutrients; however, the extent of this effect was not determined. Calcium from the resin was added to the solution. In preliminary experiments, plants grown in nutrient solutions containing the calcium saturated resin were slightly larger than those grown in identical solutions without the resin.

In the conventional nutrient culture experiment iron chlorosis appeared in both the red kidney beans and hawkeye soybeans with the bicarbonate treatment. It did not develop in the non-bicarbonate treatments. This has significance because both treatments were maintained at the same pH.

It has been very common for chlorosis to appear in conventional nutrient culture experiments with the bicarbonate treatment and for its absence in the non-bicarbonate treatments.

However, it has been difficult to maintain these systems at the same pH due to a drift in the pH of the non-bicarbonate treatment (Miller and Russell, 1962).

In the split-root experiment chlorosis developed in the hawkeye soybeans only when bicarbonate was with the iron. When bicarbonate was with the phosphorus chlorosis did not appear and the plants were normal and healthy. The red kidney beans did not develop chlorosis in the split-root experiment.

It has been established that plants differ in their susceptibility to iron chlorosis. This fact was emphasized again in these experiments. Brown et al. (1960) illustrated that red kidney beans had a greater ability to draw iron from solution than hawkeye soybeans. This could help explain why red kidney beans did not develop chlorosis in the split-root experiment when bicarbonate was with the iron.

The results of these experiments indicate that bicarbonate has an effect in causing iron chlorosis, and that it was a different effect than that of pH. However, the bicarbonate ion must be associated with the iron in order for it to induce iron chlorosis. The effect was greater when bicarbonate was with both iron and phosphorus. Brown et al. (1959) and Miller et al. (1960) report that the effect of chlorosis was, in part, the interrelationship between bicarbonate, iron, phosphorus, and calcium in the growth medium. However, in the present experiment the evidence shows that it was the interrelationship between bicarbonate

and iron, and that the effect was increased when phosphorus was with the bicarbonate and iron.

The way in which bicarbonate effects the plant in causing chlorosis is not known. There is reason and evidence to believe that bicarbonate works in a number of ways and in combination with a number of factors in causing iron chlorosis.

Bicarbonate is known to decrease the respiration in plant roots. Miller and Thorne (1956) found that bicarbonate ions markedly inhibited the respiration of excised roots from various plant species. Brown et al. (1959) observed that the bicarbonate ion inhibited the respiration of soybean roots to a greater extent when bicarbonate and iron were together than when they were separated.

Another way in which the bicarbonate ion could cause iron chlorosis is by competing with the root for the iron. The bicarbonate ion is a negative unidentate coordinating ligand (Kleinberg et al., 1960). As such it can form a chelate with the iron thus competing with the root for the iron. Brown et al. (1960) observed that roots and chelating agents competed for iron in the growth medium. As they increased the concentration of the chelating agent, iron was withheld from the plant and iron chlorosis developed.

Working with various iron chelates at pH 8.0 De Kock (1960) noted that chelates differ in their ability to supply iron to the plant. He also noted that when sodium bicarbonate was added to the medium, chlorosis was more severe than in the control which did not contain bicarbonate.

In a study of the bicarbonate and phosphorus effects on the uptake and distribution of chelated iron in soybeans, Hale et al. (1960) observed a competition for accumulation sites between the bicarbonate anion and FeEDDHA, and between $H_2PO_4^-$ and FeEDDHA.

There are other unidentate ligands such as OH^-, HCO_3^-, and $H_2PO_4^-$ which can compete with the ligands of the plant for iron (Wallace, 1962). If these ligands in the nutrient medium competed with the roots for the iron then the appearance of chlorosis could be explained on the basis of this competition.

Wallace (1962) suggests a competitive chelation theory as a proposed method of how the various ions could cause iron chlorosis. This theory could explain the appearance of chlorosis with high calcium solutions, high phosphorus solutions, and with high concentrations of metal ions (Lingle et al., 1963).

The competitive chelation theory aids in the interpretation of the present experiment. In the split-root experiment chlorosis did not appear in hawkeye soybeans in the non-bicarbonate solution; but, when the bicarbonate was added, the competition between roots and ligands was increased and the roots were unable to obtain sufficient iron. The red kidney beans were able to compete with the pH and bicarbonate for iron but when the phosphorus was added the competition for iron was so great that the red kidney beans developed chlorosis. Wallace (1962) points out that the

competition between ligand and plant for iron can be within the cell as well as in the root medium, and that competitive chelation does not exclude other factors such as ion toxicity.

The concentration of elements such as phosphorus and calcium was different in chlorotic plants and normal plants. It seems reasonable to assume that this difference in the concentration of the elements was due to the appearance of chlorosis rather than to the factor which caused chlorosis (Thorne et al., 1950). The concentration of phosphorus in this experiment was increased in the upper stems and leaves of chlorotic plants when chlorosis was caused by bicarbonate. Brown et al. (1960) reported the same distribution of phosphorus when chlorosis was caused by an increase in chelating agents.

SUMMARY

A calcium saturated carboxyl cation exchange resin was added to the nutrient medium as a buffering agent. The resin was found to be just as effective as bicarbonate and CO_2 in maintaining a constant pH of 7.8 $\pm$ 0.2.

By using the calcium saturated resin as a buffering agent to maintain a constant pH, it became possible to differentiate between the pH effect and the bicarbonate effect in causing iron chlorosis.

Red kidney beans and hawkeye soybeans were grown in nutrient solutions using both a split-root arrangement and the conventional nutrient culture arrangement. The split-root experiments allowed separation of iron and phosphorus with a factorial arrangement of the bicarbonate treatment. The nutrient solution was maintained at a pH of 7.8 $\pm$ 0.2 throughout the experiment.

Iron chlorosis developed in both the soybeans and kidney beans when bicarbonate was present in the conventional nutrient culture experiment. In the split-root experiment chlorosis developed in the soybeans only when bicarbonate was with the iron. It did not develop when the bicarbonate was only with the phosphorus. The kidney beans did not show signs of chlorosis with any treatment in the split-root experiment.

It was concluded that bicarbonate had an effect in

causing iron chlorosis above that of pH only when it was associated with iron. The effect was increased when bicarbonate, iron, and phosphorus were together. In solutions which were identical with the above except that bicarbonate was absent, chlorosis did not appear in the plants.

In addition to the appearance of chlorosis when bicarbonate was present with iron, there were differences in weight and in nutrient uptake and distribution. In the chlorotic plants—plants grown in solutions where bicarbonate and iron were together—there was a decrease in total plant weight. This decrease was due to the reduced growth in the roots, upper stems, and leaves. The bicarbonate, when associated with iron in the split-root experiment and with iron and phosphorus in the conventional nutrient culture experiment, had a direct or an indirect effect upon the plant which prevents metabolism and decreases plant growth.

The distribution and concentration of nutrients differed in the chlorotic plants and the non-chlorotic plants. In general there was a decrease in total uptake of every nutrient in the plant when grown in the bicarbonate with iron treatments; but, the concentration of the elements in various sections was sometimes higher in chlorotic plants than in non-chlorotic plants.

Phosphorus was more concentrated in the upper stems and leaves of chlorotic plants than in the normal plants. Calcium and magnesium were more concentrated in the iron-root when bicarbonate was with the iron.

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APPENDIX

METHODS OF ANALYSIS

Phosphorus Determination

Reagents

Nitric acid-molybdate-vanadate mixture

Solution 1: Dissolve 25 g of ammonium molybdate in 400 ml of warm water and cool.

Solution 2: Add slowly 1.25 g of ammonium metavanadate to 300 ml of boiling water, cool, and add 250 ml of concentrated nitric acid. Cool.

Pour solution 2 into a one liter volumetric flask and add solution 1. Mix well and dilute to volume with distilled water.

Phosphorus standard

Dissolve 0.3403 g of potassium dihydrogen phosphate in distilled water and dilute to one liter.

Procedure

1. Pipette 10 ml of the digest solution into a 50 ml volumetric flask.

2. Add 10 ml of the nitric acid-molybdate-vanadate mixture and dilute the solution to volume with distilled water.

3. The transmission of color is determined after ten minutes by means of a colorimeter with a 440 mu filter.

4. The amount of phosphorus in the sample is calculated from the size of the original tissue sample used in ashing and the dilution made in the determination.

5. This method gives a very stable color which can be determined even two weeks after development.

Iron Determination

Reagents

10 percent hydroxylamine hydrochloride

Place 10 g hydroxylamine hydrochloride in a 100 ml volumetric flask with 50 ml of distilled water. When solution is complete, dilute to volume with distilled water.

1.5 percent ortho-phenanthroline

Place 1.5 g of ortho-phenanthroline in a 100 ml volumetric flask with 75 ml of 95 percent ethyl alcohol. When solution is complete, dilute to volume with ethyl alcohol.

Potassium acid phthalate—sodium hydroxide buffer

Solution 1: Dissolve 20.4 g of potassium acid phthalate in 400 ml of water.

Solution 2: Dissolve 4.0 g of sodium hydroxide in 400 ml of distilled water.

Mix the two solutions together and dilute to one liter.

Standard solution

Dissolve 0.7022 g (reagent grade) ferrous ammonium sulphate, $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$, in about 100 ml of water and 10 ml of concentrated sulphuric acid, in a silica basin. Add 5 ml of concentrated nitric acid to oxidize the ferrous salt to ferric sulphate. Heat gently to expel oxides of nitrogen, transfer to a liter volumetric flask, and, when cold, dilute to mark. This solution is stable indefinitely and contains 100 micrograms of iron per ml. From this solution prepare a standard, containing 1 microgram of iron per ml, by diluting 10 ml to 1 liter and adding 15 ml of concentrated sulphuric acid before adjusting to volume.

Procedure

1. Pipette 5 ml of the digest solution into a 25 ml volumetric flask.

2. Add 5 ml of 0.1 M potassium acid phthalate-sodium hydroxide buffer.

3. Add 3 ml of the 10 percent hydroxylamine hydrochloride.

4. Add 1 ml of the 1.5 percent ortho-phenanthroline.

5. Dilute the solution and after 15 minutes the transmission of color is determined by means of a colorimeter with a 490 mu filter.

6. The amount of iron in the sample is calculated from the size of the original tissue sample and the dilutions made in the determination.

Determination of Calcium and Magnesium

Reagents

Sodium hydroxide-sodium cyanide solution (pH 12)

Dissolve 80 grams of NaOH (reagent grade) in one liter distilled water, cool and add 10 grams of sodium cyanide.

Ammonium chloride-ammonium hydroxide buffer (pH10)

Dissolve 67.5 grams of NH_4Cl in 570 ml of concentrated NH_4OH; add 10 grams sodium cyanide and dilute to one liter with distilled water.

Dye of Patton and Reeder (HHSNN Fisher catalogue)

Grind 1 gram of the dye with 200 grams of pure powdered K_2SO_4 or anhydrous Na_2SO_4. Grind in a porcelain mortar until a uniform color is obtained. Store in a brown container.

Eriochrome Black T indicator

Dissolve 0.5 grams of Eriochrome Black T, and 4.5 grams of hydroxylamine hydrochloride in 100 ml of 95 percent ethanol or methanol. Prepare fresh solutions at monthly intervals.

EDTA 0.01 N

Dissolve 3.723 grams of disodium dihydrogen ethylenediamine tetraacetate dihydrate in distilled water and dilute to exactly 2 liters. Standardize against the standard calcium solution.

Triethanolamine, 50 percent aqueous solution

Mix equal parts of triethanolamine (U.S.P. or N.F.) and distilled water.

Magnesium EDTA

Make up a saturated solution of MgEDTA. (Available from Hach Chemical Co., Ames, Iowa).

Standard calcium solution 0.01N

Dissolve 0.5000 grams pure dried calcium carbonate (Iceland spar) in 30 ml of approx. 1 N HCl and dilute to one liter. One ml of the solution contains 0.01 me or 0.2 mg. of calcium.

Equipment

Microburets

10 ml graduated at intervals of 0.02 ml.

Light source

Adjustable light source with tungsten bulb.

Procedure for calcium

1. Pipette 5 ml of the digest solution (containing from 0.4 to 2.0 mg of calcium) into a 125 ml Erlenmeyer flask and dilute with 25 ml of distilled water.

2. Add 2 ml of 50 percent triethanolamine.

3. Add 5 ml of the sodium hydroxide-sodium cyanide solution.

4. Add 25 mg of the Patton and Reeder dye.

5. Titrate with standard EDTA solution. The color change is from red through purple to blue. Because of the sharpness of the endpoint in the calcium determination it is necessary to have a tungsten light source so arranged that the light shines through the solution. The amount of calcium is determined by refering to a standard curve prepared by analysis of standard solutions.

Procedure for magnesium (calcium plus magnesium)

1. The amount of magnesium in the sample is determined by taking the difference between the amount of calcium and the amount of magnesium plus calcium in the sample.

2. Pipette 5 ml of the digest solution into a 125 ml Erlenmeyer flask and dilute with 25 ml of distilled water.

3. Add 2 ml of 50 percent triethanolamine.

4. Add 5 ml of the ammonium chloride-ammonium hydroxide buffer and 3 drops of Eriochrome Black T indicator.

5. Titrate with standard EDTA solution until the blue color persists.

6. The amount of magnesium is determined by subtracting the volume of EDTA used for calcium from that used for calcium plus magnesium and refering to a standard curve.

Potassium Determination

Reagents

Standard potassium chloride 0.02 N

Dissolve 1.491 g of dry potassium chloride in distilled water and dilute to exactly 1 liter.

Lithium chloride solution 0.05 N

Dissolve 2.12 g of dry lithium chloride in water and dilute to 1 liter.

Procedure

1. Pipette an aliquot of digested solution containing less than 0.1 meq of potassium into a 50 ml volumetric flask.

2. Add 25 ml of 0.05 N lithium chloride solution and dilute to volume with distilled water.

3. The potassium concentration is determined by reference to a standard curve.