5-1961

The Mineral Content of Various Sections of Some Plants as Influenced by Conditions Associated with Lime-Induced Chlorosis

Hyrum Del Var Petersen

Utah State University

THE MINERAL CONTENT OF VARIOUS SECTIONS OF SOME PLANTS AS INFLUENCED BY CONDITIONS ASSOCIATED WITH LIME-INDUCED CHLOROSIS

by

Hyrum Del Var Petersen

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Soil Chemistry

UTAH STATE UNIVERSITY
Logan, Utah

1961

ACKNOWLEDGMENT

The author expresses sincere appreciation to his wife and family for their help and encouragement while doing this work.

Sincere appreciation is expressed to Dr. R. L. Smith, Dr. Herman H. Wiebe, and Dr. Raymond W. Miller for their help and advice in conducting this research and in the writing and presentation of the results contained herein.

Thanks is due to Dr. Gene W. Miller for his help and suggestions in carrying out the research.

The author expresses gratitude to the Atomic Energy Commission for providing the funds necessary to make this research possible.

H. Del Var Petersen

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The so-called "lime-induced" chlorosis has been recognized for many years as a problem where plants are grown on calcareous soils. 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 vigor of the plant as a whole. Lime-induced chlorosis is unique in that the iron content of both the chlorotic plant and the soil do not always show a deficiency in iron when chemically analyzed. This leads to the theory that iron is inactivated in both the soil and plant.

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. In general increased chlorosis has also been noted under conditions of high pH. The pH and phosphorus effects appear to involve reduced iron solubility in the soil and within the plant while the exact effects of the bicarbonate ion on chlorosis have not been established.

DeKock (1955) found that the iron content of the plant tissues depends on the oxygen supply and the bicarbonate ion concentration. Whether the bicarbonate ion has its effect upon the adsorption of iron from the growth medium or within the plant is not known.

DeKock (1955) states that the ratio of phosphorus to iron in the plant tissues is a critical factor in lime-induced chlorosis. Hall and DeKock (1955) state that chlorotic and green plants can be distinguished from each other by their phosphorus to iron and calcium to potassium ratios.

Work done by Olsen (1935) indicates that leaves of chlorotic plants invariably contain more phosphorus than normal green plants. Olsen was of the opinion that iron in chlorotic plants was immobilized as insoluble ferric phosphate. Warnock (1952) also suggested that iron was immobilized within the plant.

In the light of these observations, it would seem that additional work could profitably be done to determine the regions of mineral inactivation, and the effect that certain chlorosis-causing factors have on inactivating some minerals with the plant. The object of this research was to study and compare the regions of mineral inactivation in plants grown on calcareous and non-calcareous soils and to observe the effects that various levels and combinations of bicarbonate, phosphate, and iron in the root medium have on the inactivation of minerals with the plant.

LITERATURE REVIEW

Soil Factors

There are many different soil factors which influence iron chlorosis or cause it to appear in plants. In some acid soils the amount of iron is too low and an actual iron deficiency exists. In other acid soils the elements manganese or copper may interfere with the plant's use of iron, even when enough iron is in the soil. Johnson (1917) found that in Hawaii, where soils are high in manganese, an iron chlorosis occurs that can be overcome by the addition of iron. He noted that the manganese to iron ratio, and not the total amount of either element present, was the important thing in causing chlorosis.

A common form of iron chlorosis occurs on calcareous soils where it is referred to as lime-induced chlorosis. Brown and Holmes (1953) state that lime-induced chlorosis in plants does not have a common causative factor. The problem is complex and the occurrence of chlorosis is dependent upon the iron requirement of the plant species and the available iron supply in the soil.

McGeorge and Breazeale (1956) found that lime-induced chlorosis exists in Arizona in the leaves of some fruit trees, field crops, and ornamentals growing in alkaline-calcareous soils which contain 3 to 5 per cent iron in various chemical combinations. They concluded that there is no deficiency of total iron in these soils and that lime-induced chlorosis is obviously due either to a low availability of iron in the soil or to iron immobilization in the plant.

Porter and Thorne (1955) found that high moisture, low temperature, high lime content, and high concentration of bicarbonate ion are soil factors which either contribute to or are associated with lime-induced chlorosis. DeKock (1955) found that the oxygen supply to the roots affects the translocation of iron to the leaves. Burtch et al. (1948) reports that high soil moisture together with low soil temperature are conditions most conducive to the development of lime-induced chlorosis.

Brown et al. (1959) found that lime-induced chlorosis has frequently occurred where green and barnyard manures have been plowed into the soils followed with irrigation and in areas of soil compaction and poor drainage. These conditions tend to increase bicarbonate, phosphorus, calcium, and manganese concentrations of the soil solution. They concluded that the development of chlorosis appears more related to the soluble phosphorus and calcium concentration in solution and the amount absorbed by the plant than to the bicarbonate ion concentration bathing the roots.

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 be conducive to iron chlorosis in some plant species or varieties. They found that phosphorus can accumulate inside the plant in such proportions as to inactivate iron.

Doney et al. (1960) found that increased pH in the external solution generally decreased the amount of iron absorbed by the plant.

Chandler and Scarseth (1941) found that as the phosphorus content of the soil was increased there resulted an increase in chlorosis and a reduction in the iron content of peanut and alfalfa plants. In nutrient experiments using Plant Industry-54619-5-1(PI) and Hawkeye (HA) soybeans, 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. He 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 Woodridge (1952) observed that as the phosphorus content of the nutrient medium is increased, roots, stems, and cordate leaves continue to build up in phosphorus content even after trifoliate leaves are being adequately supplied. They conclude that the excess phosphorus in the plant may be responsible for immobilizing iron and other ions.

DeKock (1955) noted that as the oxygen supply to the roots increased from one to 20 per cent 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 that there exists 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 is 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. Under conditions of high pH and with accumulation of phosphorus, some difficulty in the successful passage of ions to the leaf blades has been found.

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, 1953), and the rates of several enzymatic reactions (Miller and Evans, 1956).

In studying the inhibition of the plant cytochrome oxidase system 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 (1954) indicates 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 is the main factor in the metabolic disturbances 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.

Brown et al. (1959) found that more solution phosphorus appeared in the bicarbonate than in non-bicarbonate treatments, but the reverse was true in the plant material. This suggests a competitive effect of the bicarbonate ion on phosphate absorption or that phosphate may exist in solution in a form which is less available in the bicarbonate treatment.

Wallihan (1961) and Marcour (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, "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.

Brown and Wadleigh (1955) suggest that a possible sequence of events resulting from the presence of bicarbonate ion in the medium may be summarized as follows: The bicarbonate ion induces greater accumulation of monovalent ions in the leaves. This increase in the ratio of monovalent to divalent ions may affect the activity of iron, possibly through the precipitation of iron phosphate, which, in turn, reduces the chlorophyll concentration and thus brings about chlorosis. Finally, the degree of chlorosis is highly correlated with the depression in growth.

Ion Distribution

The ion distribution within the plant has been used as a measure of chlorosis. DeKock (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. In addition to the phosphorus to iron ratio, the ratio of monovalent ions to divalent ions in the plant tissue is used as a measuring device.

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. DeKock (1955) noted that chlorotic plants had an accumulation of iron along the veins and that normal healthy 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 indicate 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 HA soybeans under the same conditions of growth and element composition. They concluded that susceptibility to iron chlorosis thus 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 DeKock (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.

Summary

Lime-induced chlorosis is a common form of iron chlorosis found on calcareous soils. There are differences of opinion as to the exact cause of lime-induced chlorosis, however, there are some common characteristics associated with the plants suffering from the disease.

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) and non-susceptible (HA) soybeans. Miller (1954) found that plants containing a dominant iron terminal oxidase are more susceptible to chlorosis than plants containing a dominant copper terminal oxidase.

A number of soil conditions have been associated with lime-induced chlorosis. Some of these soil conditions are high moisture, poor aeration, low temperatures, high pH, large amounts of available phosphorus, an imbalance of macroelements and microelements, high lime, and high bicarbonate content of the soil. Chlorosis can apparently be caused by any one of these conditions but an interaction between any or all of them seems to be the case in most soils.

Some common characteristics of plants suffering from lime-induced chlorosis are a decrease in plant growth, an increase in monovalent and a decrease in divalent ion absorption.

SOIL EXPERIMENT

Methods and Procedure

Plants and Soils

Red Kidney beans (chlorosis susceptible), and tomatoes (Orsen Cannon Hybrid), and Hawkeye soybeans (soybeans and tomatoes being non-susceptible to chlorosis) were grown in a calcareous soil from Logan, Utah, and in a non-calcareous soil from Farmington, Utah.

The soils were treated with $NH_4H_2PO_4$ at 100 pounds of P_2O_5 per acre furrow slice. The soil was put in two-quart, plastic freezer cartons. The seeds were germinated and the plants were grown in these cartons.

The plants were grown in a plant growth cabinet with both temperature and light duration controlled. The plants were subjected to 18 hours of daylight with an average light intensity of 1600 foot candles and a temperature of 29° C. and 6 hours of darkness at 18° C.

Analytical Methods

Each bean and each soybean treatment consisted of four plants growing in a pot, and each tomato treatment consisted of two plants in a pot. Each pot was analyzed as a single unit. All treatments were replicated four times and the values averaged.

The plants were harvested after they had been in the treatment for three weeks. In harvesting, the plants were cut off just above the soil, dipped in distilled water and shaken, and separated into leaves, upper stems, and lower stems. The upper stems and lower stems were divided where the petiole of the first trifoliate leaf attaches to the stem.

The plant sections were placed in paper bags and dried for 24 hours in a forced air dryer at 70° C. After drying, the plant material was ground with a mortar and pestle. The plant material was analyzed by following the procedures suggested by Ulrich et al. (1959) and Wilde and Voigt (1955). Iron, phosphorus, calcium, and potassium were determined from the plant material on a dry weight basis using the orthophenanthroline method, the ammonium molybdate method, the E.D.T.A. method, and the flame photometer respectively.

Results and Discussion

The soybeans and kidney beans developed chlorosis when grown on the calcareous soil, but the tomatoes did not. On the average, the plants grown on the non-calcareous soil were larger than those plants grown on the calcareous soil; none of the plants grown on the non-calcareous soil showed signs of chlorosis.

Chemical analysis

The chemical analysis of the plants are recorded in table 1. Each value consists of an average of four replications.

The uptake of phosphorus was reduced in all plants grown on the calcareous soil. This is in agreement with some of the work of Arnon (1942) in which it was shown that the amount of phosphorus absorbed was dependent upon the plant and pH of the system.

With the exception of some very small differences, there was an increase in potassium absorption when the plants were grown on the calcareous soil. Lindner and Harley (1944) found the concentration of monovalent ions, potassium in particular, increased in plants suffering from lime-induced chlorosis. The results of Warnock (1952) and DeKock (1955) also show an increase in potassium in chlorotic plants.

Table 1. Chemical analysis of plants grown on a calcareous (C) and a non-calcareous (NC) soil.

Treatment[1]	Tomatoes			Hawkeye Soybeans			Red Kidney Beans		
	LS[2]	US[2]	L[2]	LS	US	L	LS	US	L
				mg P/g of dry plant tissue					
NC	3.03	3.60	5.41	4.46	4.92	5.53	4.60	4.34	4.87
C	2.29	3.37	3.76	3.01	3.97	4.29	3.39	3.47	3.41
NC/C	1.31	1.06	1.44	1.48	1.24	1.29	1.37	1.25	1.43
				µg Fe/g of dry plant tissue					
NC	56.9	31.0	130	41.6	56.5	108.2	39.2	55.8	98.1
C	45.6	26.9	114.5	30.8	36.3	63.0	41.9	41.9	109.1
NC/C	1.25	1.15	1.14	1.34	1.56	1.71	0.935	1.33	0.900
				per cent K of dry plant tissue					
NC	7.89	11.62	6.43	7.25	9.97	5.63	9.17	10.98	5.33
C	8.58	13.25	6.25	7.35	9.62	5.65	12.79	12.62	6.48
NC/C	0.920	0.879	1.029	0.986	1.03	0.996	0.717	0.870	0.822
				per cent Ca of dry plant tissue					
NC	2.36	1.72	3.20	1.92	1.97	1.93	2.30	1.26	3.35
C	2.77	2.12	3.96	2.30	2.16	2.23	2.08	1.36	3.03
NC/C	0.852	0.811	0.808	0.830	0.912	0.865	1.105	0.926	1.105

Table 1. Continued

Treatment	Tomatoes LS	Tomatoes US	Tomatoes L	Hawkeye Soybeans LS	Hawkeye Soybeans US	Hawkeye Soybeans L	Red Kidney Beans LS	Red Kidney Beans US	Red Kidney Beans L
				P/Fe Ratio					
NC/NC	52.8	116.1	41.6	107.2	81.0	51.2	117	77.7	49.6
C/C	50.2	125.2	32.8	97.7	109.3	68.0	80.9	82.8	31.3
				K/Ca Ratio					
NC/NC	3.34	6.76	2.01	3.78	5.06	2.92	3.99	8.71	1.59
C/C	3.10	6.24	1.58	3.15	4.45	2.53	6.14	9.20	2.14
				Ca/Fe Ratio					
NC/NC	415	555	246	462	349	178	587	226	341
C/C	607	788	346	752	595	354	496	325	278

[1] Average of four replications.

[2] LS means lower stem; US means upper stem; L means leaves.

When grown on the calcareous soil, the soybeans and tomatoes contained less iron and more calcium than when they were grown on the non-calcareous soil. The kidney beans had an increase of iron in the lower stems and leaves but a decrease of iron in the upper stems when grown on the calcareous soil. This suggests that iron might be inactivated in the lower stems of the chlorosis-susceptable beans (Doney et al., 1960).

Differences in the accumulation of iron in the leaves of the plants grown on the two soils are not as large as the differences observed by other workers (Warnock, 1952; March and Shive, 1925; Brown and Holmes, 1955). A possible explanation for this is that in this experiment the primary leaves were analyzed along with the trifoliate leaves. The primary leaves were large and green in both chlorotic and non-chlorotic plants, and in the case of the chlorotic plants contained as much or more material than all the trifoliate leaves combined. In contrast, in the green plants the total amount of material from the trifoliate leaves was much larger than that from the primary leaves. This relationship between the size of the primary leaves and the trifoliate leaves undoubtedly had its influence upon the results.

Ratios of various elements

There was no obvious correlation between the phosphorus to iron ratio and chlorosis. A possible reason for this could be that the primary and trifoliate leaves were analyzed together which masked the low iron of the chlorotic leaves. However, not all plants with iron deficiency have a shortage of iron in the affected tissues (Oserkowsky, 1932). DeKock (1955) and DeKock and Hall (1955) found the ratio of phosphorus to iron to be higher in plants showing chlorosis, and the ratio was postulated as a means of distinguishing between green and chlorotic plants. Warnock (1952) grew a number of chlorosis susceptable and non-susceptable plants in the field from which the soils used in this experiment were obtained. His ratios of phosphorus to iron were larger for chlorotic than non-chlorotic plants but his differences between the ratio of chlorotic and non-chlorotic plants were not as great as what DeKock (1955) suggests.

The increased ratio of potassium to calcium in the leaves has been found by some to be correlated with chlorosis (Lindner and Hardey, 1944; Wadleigh and Brown, 1952; DeKock, 1955). This difference is believed to be due to a change in the potassium content rather than in the calcium content (Wadleigh and Brown, 1952). Whether this is a cause of chlorosis or a result of chlorosis is not definite; however, Thorne et al. (1955) are of the opinion that this ratio is a result of chlorosis. Brown et al. (1955) showed that chlorosis was not correlated with either potassium or calcium or with their ratio but rather with monovalent cations or the ratio of sodium plus potassium to calcium plus magnesium.

In the present soil experiment the potassium to calcium ratio was lower in soybeans and tomatoes and higher in kidney beans when grown on the calcareous soil. This increase was due to an increase in potassium when the plants were grown on the calcareous soil.

Brown et al. (1959), using solution culture experiments, found that the potassium to calcium ratio was not related to chlorosis, but that the calcium to iron and the phosphorus to iron ratios were higher in chlorotic than non-chlorotic plants. The calcium to iron ratio was calculated for this experiment. For the tomatoes and soybeans this ratio was higher when the plants were grown on the calcareous soil. This is in agreement with the results of Brown *et al.* (1959). However, for the kidney beans this ratio was higher when the plants were grown on the non-calcareous soil. This is not in agreement with the results of Brown *et al.* (1959).

SPLIT-ROOT EXPERIMENT

Methods and Procedures

Solution culture and plants

Split-root experiments (Brown, 1956) were designed to determine the effects of the bicarbonate and phosphorus in causing chlorosis in the Red Kidney bean. The phosphorus and iron were always separated and the bicarbonate treatments were either (a) none, (b) added with the phosphorus, or (c) added with the iron.

The beans were germinated between layers of water-saturated cheese cloth on a steel screen placed in a tray. After several days the primary root tip was removed, leaving several lateral roots. The plant roots were suspended in a tap water solution containing 1.3 me/1 calcium as $Ca(NO_3)_2$. The plants grew in this solution until the roots were 2 to 3 inches long and were then transferred to the treatment solutions. Each plant was suspended over two containers with the roots equally divided between the pair of containers. All containers had certain common nutrients. Phosphorus was added to one container of each pair and iron and other minor elements were added to the other container. This arrangement enabled the plants to absorb all required nutrients and eliminated direct contact in the solution between iron and phosphorus. Each container had the following common nutrients in me/1: Ca, 1.3; Mg, 1.3; NO_3, 2.0; K, 0.5; and S, 0.13. Minor elements were added to one of the containers of each pair in the following concentrations in ppm: Fe, 4.0 (as $FeCl_3$); Mn, 0.7; B, 0.04; Zn, 0.02; Cu, 0.005; and Mo, 0.005. Phosphorus was added to the other container, which contained no minor elements, at two levels, 0.06 and 10.0 ppm. The pots containing sodium bicarbonate (10 me/1) were maintained at pH 7.8 by aeration with a mixture of 1 per cent CO_2 in air. The pots containing sodium chloride (10 me/1) and those pots in which the sodium bicarbonate and the sodium chloride were absent were adjusted daily to pH 7.8 using 0.1 N NaOH. These we aerated at the same rate as the bicarbonate treatments.

The experiments were conducted in a growth chamber where temperature and light duration were controlled. The plants were subjected to 18 hours of daylight with an average of 1600 foot candles of light and a temperature of 29° C. and 6 hours of darkness at 18° C.

Analytical methods

The nutrient solutions were changed every week and the plants were harvested after being in the treatment for two weeks. Each treatment consisted of two plants. These plants were analyzed as one unit. Treatments were replicated three times and the values averaged.

In harvesting the plants, the roots and tops were separated and the tops were dipped in distilled water and shaken. The tops were separated into upper stems, lower stems, and leaves; dried at 70° C.; and analyzed for phosphorus, iron, calcium, and potassium as described in the Soil Experiment.

Results and Discussion

Before the plants were harvested, the following visual observations were taken on the plants; (a) the plants grown in the high phosphorus solution (10 ppm) were larger in vegetative growth than the plants grown in the low phosphorus solution (0.06 ppm), (b) the plants grown in the solutions containing bicarbonate were smaller than the plants grown in equivalent nutrient solutions without bicarbonate, (c) there was no observable difference in the size of the plants grown in solutions containing sodium chloride (10 me/1) and the plants grown in equivalent nutrient solutions without the sodium chloride, (d) a precipitate formed only in those pots containing bicarbonate with the iron. These plants were the only chlorotic ones.

The chemical analyses of the plants from the experiment are recorded in tables 2 and 3.

Chemical analyses

Phosphorus.--There was always more phosphorus taken up by the plants when the higher level of phosphorus was present (Fig. 1). Brown and Tiffin (1960) grew soybeans in nutrient solutions and found that as the phosphorus concentration of the nutrient solution increased the phosphorus concentration in the exudate also increased.

In the higher phosphorus treatment, there was always less phosphorus taken up by the plant when the bicarbonate was with the phosphorus. The bicarbonate tends to decrease the uptake of phosphorus when they are together (Doney, 1959; Goss, 1957; Brown et al., 1959). When the bicarbonate was with the iron (high phosphorus) a larger amount of phosphorus was taken up than when sodium chloride was with the iron. The reason for this is not obvious.

In the low phosphorus treatment when the bicarbonate was with the phosphorus, less phosphorus was found in the leaves and lower stems but more in the upper stems than when chloride was with the phosphorus. These results differ slightly from the results obtained when phosphorus was present at the higher level; however, they are in agreement with Doney's (1959) results in which he found that increased bicarbonate tended to increase the amount of phosphorus in the stems and primary leaves even though the total amount of phosphorus in the plant was lower.

Table 2. Chemical analysis[1] of Red Kidney beans grown in a split-root system with phosphorus at 10 ppm

$NaHCO_3$ with Fe			NaCl with Fe			No salt		
LS[2]	US[2]	L[2]	LS	US	L	LS	US	L
mg P/g of dry plant tissue								
4.70	5.74	5.24	4.17	4.86	4.84	3.74	4.06	4.83
µg Fe/g of dry plant tissue								
21	64	63	36	72	115	38	45	123
percent K/g of dry plant tissue								
4.42	6.33	2.84	5.70	9.99	1.91	4.31	7.76	2.00
percent Ca/g of dry plant tissue								
1.48	2.10	2.08	1.95	2.22	3.53	2.11	1.92	3.20
P/Fe Ratio								
22.3	89.7	83.2	115.8	67.5	42.1	98.4	90.2	39.3
K/Ca Ratio								
2.98	3.01	1.37	2.92	4.5	.54	2.04	4.04	.63
Ca/Fe Ratio								
705	328	330	542	308	307	555	427	260

Table 2. Continued

NaHCO$_3$ with P			NaCl with P			No salt		
LS	US	L	LS	US	L	LS	US	L
mg P/g dry plant tissue								
2.44	3.23	3.43	3.53	4.74	5.97	3.74	4.06	4.83
μg Fe/g of dry plant tissue								
23	26	117	41	39	92	38	45	123
per cent K/g of dry plant tissue								
4.09	7.21	2.14	4.99	9.70	2.52	4.31	7.76	2.00
per cent Ca/g of dry plant tissue								
1.68	2.47	2.69	1.70	2.04	3.02	2.11	1.92	3.20
P/Fe Ratio								
106.1	124.2	29.3	86.1	121.5	64.9	98.4	90.2	39.3
K/Ca Ratio								
2.43	2.92	.80	2.94	4.75	.83	2.04	4.04	.63
Ca/Fe Ratio								
730	950	230	415	523	328	555	427	260

[1] Average of three replications.

[2] LS means lower stem; US means upper stem; L means leaves.

Table 3. Chemical analysis[1] of Red Kidney beans grown in a split-root system with phosphorus at 0.06 ppm

$NaHCO_3$ with Fe			NaCl with Fe			No salt		
LS[2]	US[2]	L[2]	LS	US	L	LS	US	L
mg P/g of dry plant tissue								
1.08	2.07	1.71	1.28	1.85	1.95	1.14	1.09	1.59
µg Fe/g of dry plant tissue								
37	99	81	38	50	101	42	41	125
per cent K/g of dry plant tissue								
4.40	6.70	2.44	4.95	8.37	1.76	4.46	7.80	2.01
per cent Ca/g of dry plant tissue								
1.40	1.71	2.36	1.89	2.22	3.11	2.01	2.27	2.87
P/Fe Ratio								
29.2	20.9	21.1	33.7	37.0	19.3	27.1	26.6	12.7
K/Ca Ratio								
3.14	3.92	1.03	2.62	3.77	.57	2.22	3.44	.70
Ca/Fe Ratio								
378	173	291	497	444	308	479	544	181

Table 3. Continued

$NaHCO_3$ with P			NaCl with P			No salt		
LS	US	L	LS	US	L	LS	US	L
mg P/g dry plant tissue								
1.04	1.48	1.75	1.08	1.09	2.00	1.14	1.09	1.59
µg Fe/g of dry plant tissue								
33	30	104	35	47	95	42	41	125
per cent K/g of dry plant tissue								
5.57	8.25	2.35	6.17	9.28	2.82	4.46	7.80	2.01
per cent Ca/g of dry plant tissue								
1.86	3.14	2.67	1.81	2.69	3.07	2.01	2.27	2.87
P/Fe Ratio								
31.5	49.3	16.8	30.9	23.2	21.1	27.1	26.6	12.7
K/Ca Ratio								
2.37	2.13	.91	2.73	3.11	.57	2.22	3.44	.7
Ca/Fe Ratio								
564	1047	257	517	572	323	479	554	181

[1] Average of three replications.

[2] LS means lower stem; US means upper stem; L means leaves.

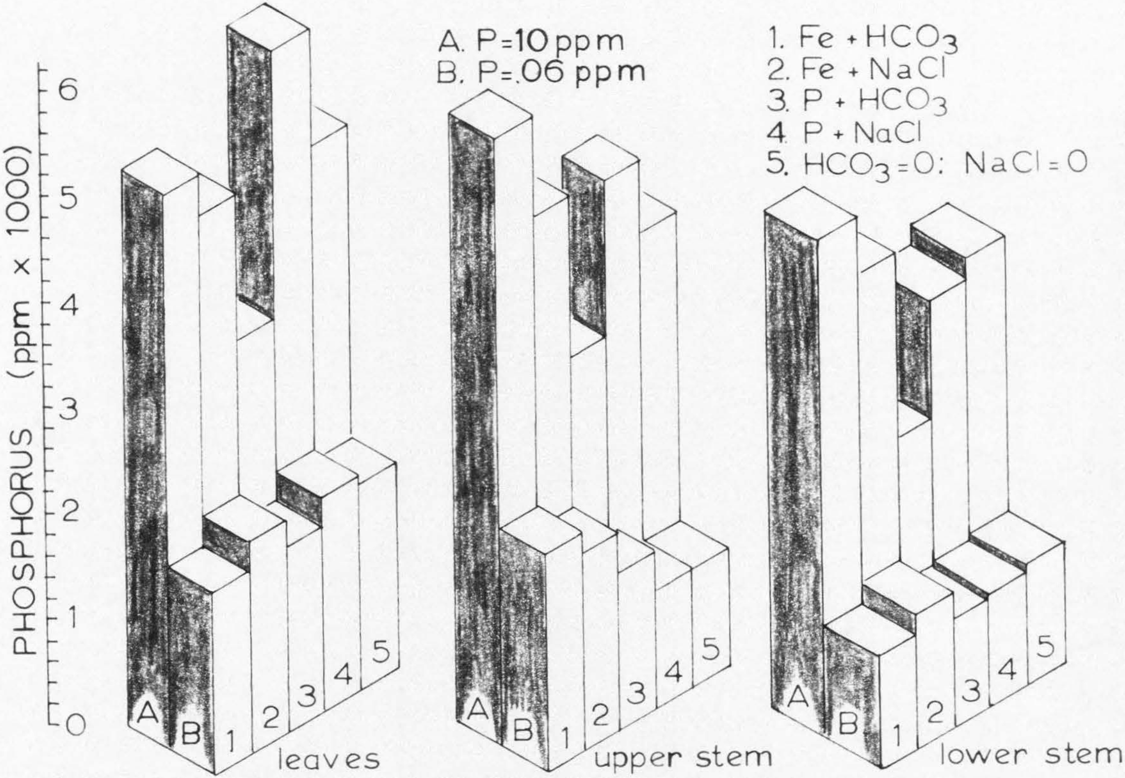

Figure 1. A comparison of the amount of phosphorus in the lower stem, upper stem, and leaves of Red Kidney beans when the plant was subjected to different treatments

Iron.--Less iron was taken up by the plants when the bicarbonate was with the iron than when sodium chloride was with the iron (Fig. 2). Marcour's (1952) results showed that the presence of bicarbonate in the complete nutrient solution almost entirely prevented the uptake of radioactive iron. Wallihan (1961) observed that the iron concentration in the roots of sodium bicarbonate treated plants was lower than in the sodium chloride treated plants.

In the upper and lower stem there seems to be slightly more iron taken up when sodium chloride rather than bicarbonate was with the phosphorus. This is parallel with the uptake of phosphorus in that more phosphorus was taken up when the sodium chloride was with the phosphorus. The difference in the amount of iron taken up from the low phosphorus solution and the high phosphorus solution are not large or consistent. These results differ from those of Aiyar (1946), Doney et al. (1960), and Brown and Tiffin (1960). Their results indicated that as the phosphorus concentration of the nutrient solution was increased, the uptake of iron decreased.

Potassium and calcium.--The uptake of potassium (Fig. 3) was usually reduced when bicarbonate was present, either with the phosphorus or the iron, the exception being that more potassium was found in the leaves when the bicarbonate was with the iron. These were the plants with chlorotic leaves. These results are in agreement with those of DeKock (1955) and Wadleigh and Brown (1952) where they found that the potassium content was higher in chlorotic leaves.

There was less calcium taken up when the bicarbonate was with the iron than in any other treatment (Fig. 4). The plants in this treatment were the only ones in which chlorotic symptoms appeared. When the bicarbonate was with the phosphorus there was an increase of

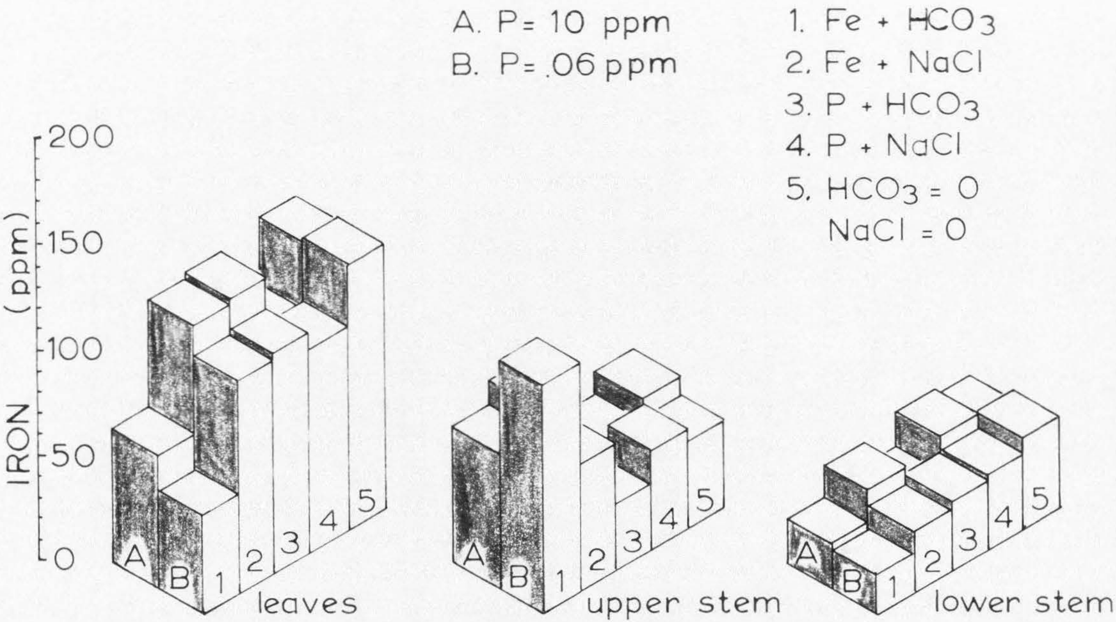

Figure 2. A comparison of the amount of iron in the lower stem, upper stem, and leaves of Red Kidney beans when the plant was subjected to different treatments

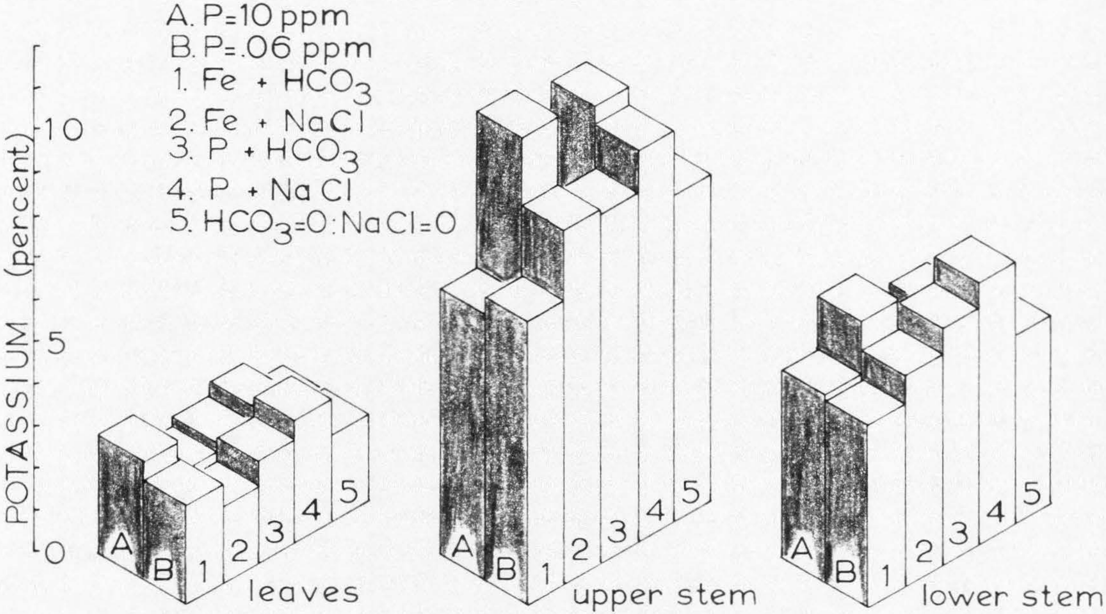

Figure 3. A comparison of the amount of potassium in the lower stem, upper stem, and leaves of Red Kidney beans when the plant was subjected to different treatments

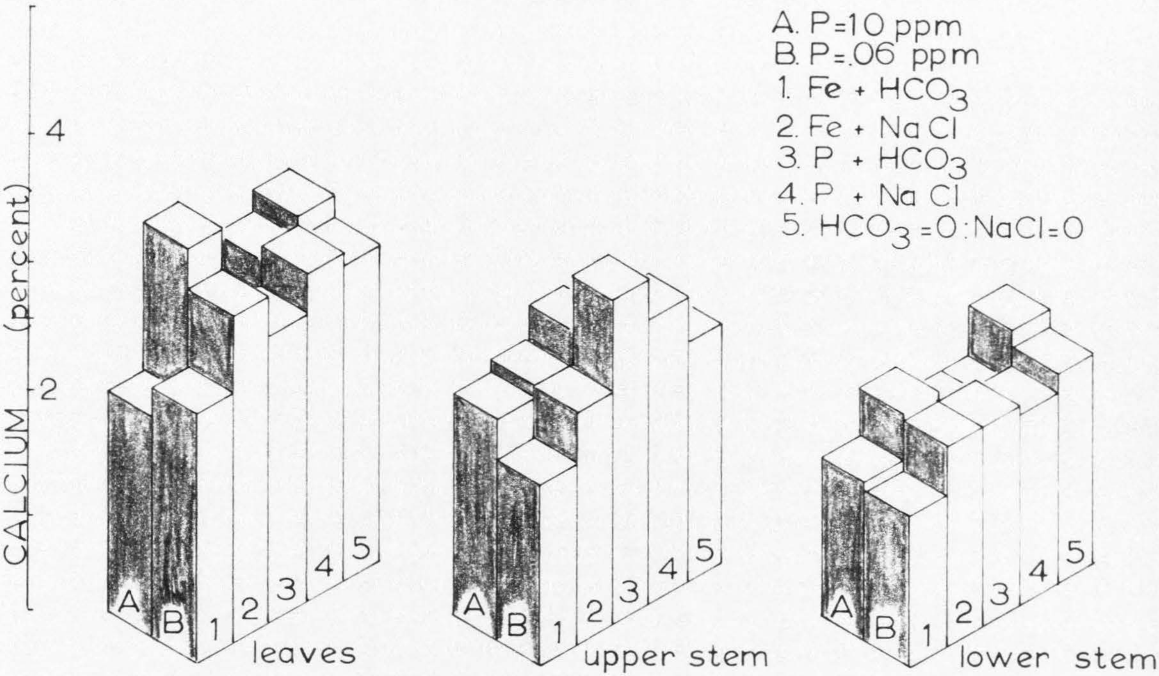

Figure 4. A comparison of the amount of calcium in the lower stem, upper stem, and leaves of Red Kidney beans when the plant was subjected to different treatments

the calcium content in the upper stems. DeKock (1955) and Wadleigh and Brown (1952) found no differences in the calcium content of chlorotic and green leaves.

Ratios of various elements

Phosphorus to iron ratio.--For the present experiment the ratio of phosphorus to iron was higher in the high phosphorus treatment than it was in the low phosphorus treatment (Fig. 5). This is because of the greater absorption of phosphorus in the high phosphorus treatment.

If the results of the upper stems and lower stems were combined, the phosphorus to iron ratio would be greater for those treatments containing the bicarbonate. This is in accordance with statements made by DeKock (1955) and Warnock (1952).

Potassium to calcium and calcium to iron ratios.--There are no obvious correlations between the ratios of potassium to calcium and calcium to iron in this experiment (Fig. 6 and 7). One possible explanation is that calcium and potassium were available in all the pots. However, Brown *et al.* (1955) showed that chlorosis was not correlated with any particular cation or with the ratio of potassium to calcium but with monovalent cations.

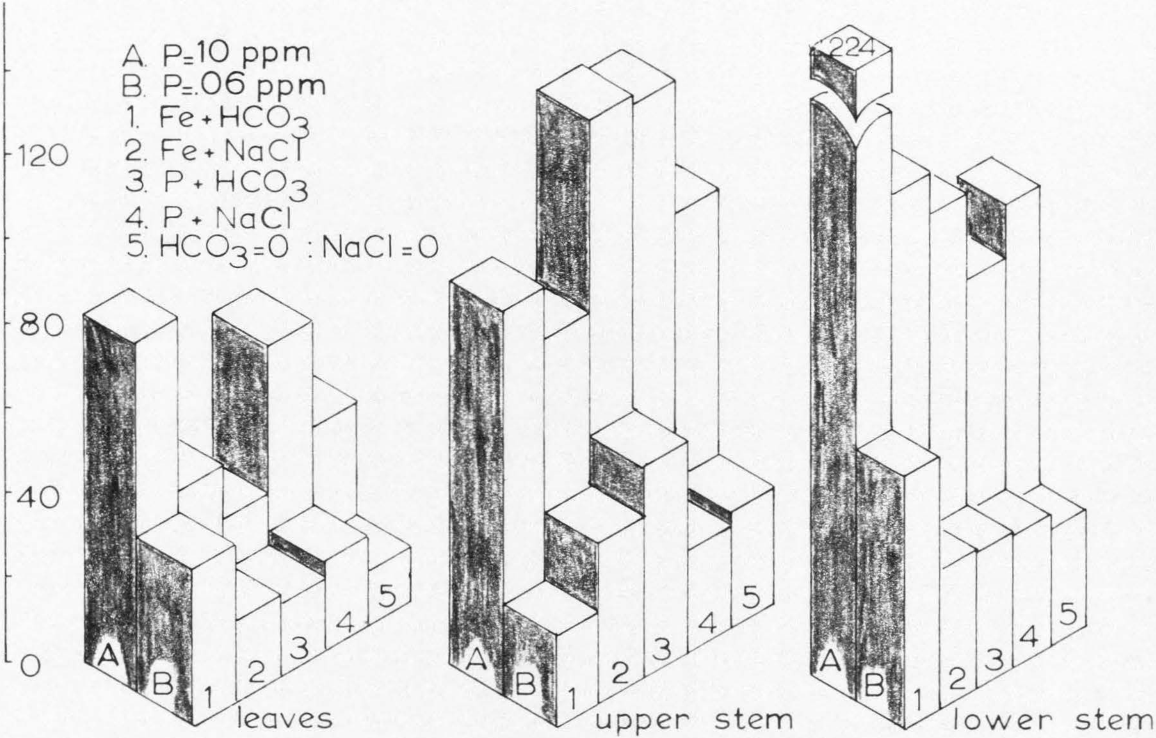

Figure 5. A comparison of the phosphorus to iron ratio in the lower stem, upper stem, and leaves of Red Kidney beans when the plant was subjected to different treatments

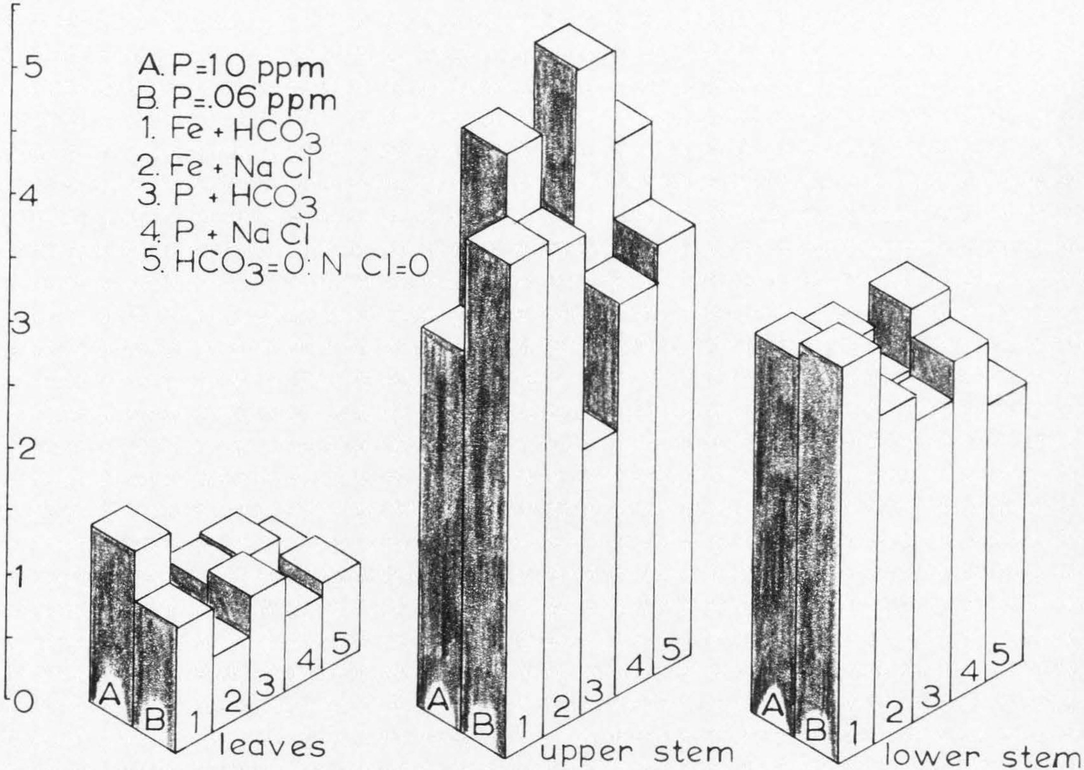

Figure 6. A comparison of the potassium to calcium ratio in the lower stem, upper stem, and leaves of Red Kidney beans when the plant was subjected to different treatments

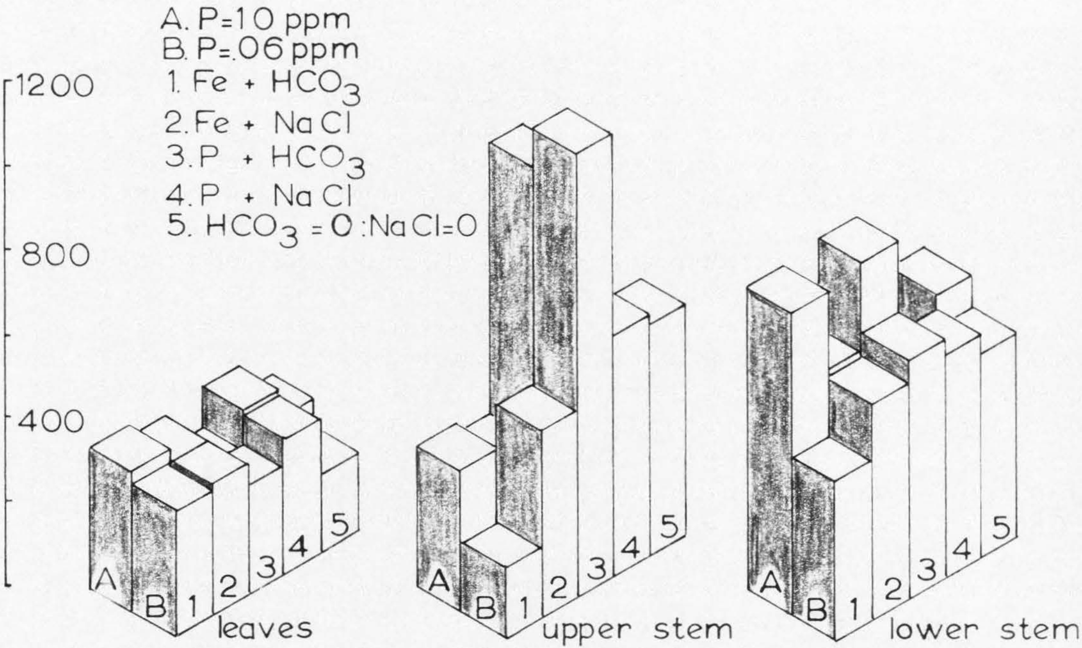

Figure 7. A comparison of the calcium to iron ratio in the lower stem, upper stem, and leaves of Red Kidney beans when the plant was subjected to different treatments

GENERAL DISCUSSION

In the split-root experiment the bean plants developed chlorosis only when the bicarbonate was present with the iron. The chlorosis was more severe when the high phosphorus treatment was used, but chlorosis did develop when the low phosphorus treatment was used. These results would indicate that the effect of bicarbonate in causing chlorosis is a reaction with the iron and not a phosphorus-bicarbonate interaction.

It was also observed that a precipitate formed in the iron-bicarbonate solution. The bicarbonate could be inducing chlorosis by precipitating the iron and preventing its absorption (Wadleigh and Brown, 1952; Marcour, 1952). However, the total percentage uptake of iron by the plant in the present experiment indicates that the iron content of the plant is not lower than that of the normal green plant. The percentage of iron was greater in the upper stems and less in the leaves in plants which developed chlorosis than in plants which did not develop chlorosis. This indicates that the plant was able to absorb iron but that the iron was inactivated in the upper stem. Since the phosphorus content was also higher in this section it is postulated that iron inactivation is with the phosphorus. This effect could be due to an increase of phosphorus taken up by the plant with enrichment in the upper stem. The bicarbonate ion being with the iron would reduce the uptake of iron but would not effect the uptake of phosphorus. The ratio of phosphorus to iron in the upper stems could be so large that subsequent iron taken up would be precipitated in this area (Rediske and Biddulph, 1956).

An increase in the amount of phosphorus in the upper stems of the kidney beans when bicarbonate is present in the solution culture is in agreement with the results of Doney (1959). He found that the total amount of phosphorus taken up by the bean plant was decreased when bicarbonate was present, but that the amount of phosphorus in the upper stem was more when the bicarbonate was present than it was when the bicarbonate was absent. In his experiments the bicarbonate, phosphorus, and iron were all together in the nutrient medium. In the present experiment the bicarbonate was with the iron and both were separated from the phosphorus. Any interactions of phosphorus and iron could only be inside the plant. In the soil experiment this was substantiated when it was observed that when the kidney beans were grown on non-calcareous soil the upper stems contained less phosphorus than either the lower stems or the leaves. However, when the beans were grown on calcareous soil the upper stems contained more phosphorus than either the lower stem or the leaves.

It appears that when the bicarbonate ion is present with the iron, the bicarbonate has some effect upon the metabolism of the plant which causes an increased accumulation of phosphorus in the upper stem of the plant. This increased phosphorus causes the iron to become inactivated or precipitated in the upper stem and in turn induces chlorosis.

In the split-root experiment, when the bicarbonate ion was present there tended to be a decrease in the amount of phosphorus, iron, calcium, and potassium taken up and also a decrease in the size of the plants. This depression in absorption and growth caused by the bicarbonate ion seems to be typical of lime-induced chlorosis and has been observed by many others (Stolwijk and Thimann, 1957; Jackson and Coleman, 1959; and Goss, 1957).

In the soil experiment the results show that the amount of phosphorus was decreased, and with a few minor exceptions, the amount of potassium was increased when the plants were grown on calcareous soil. The greatest increase in potassium was found in the kidney beans. The amount of calcium taken up was slightly increased in the tomatoes and soybeans but slightly decreased in the kidney beans. The increase in potassium with a slight change in calcium content is typical of lime-induced chlorosis. Wadleigh and Brown (1952) 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.

In both the soil and split-root experiments the leaves of the plants which developed chlorosis contained more potassium and less calcium than the leaves of the plants which did not develop chlorosis. It is suggested that the bicarbonate ion is responsible for the lowered iron "activity" and calcium content of the leaves and the enhanced potassium content (Wadleigh and Brown, 1952). It seems that an influence of the bicarbonate ion is through its effect on the protoplasmic consistency of the absorbing cells of the roots so that bean plants show an accentuated accumulation of monovalent cations and a depressed accumulation of divalent cations, and, that the bicarbonate ion, when present with the iron, allows an increase of phosphorus in the upper stem. This increase of phosphorus causes a precipitation or inactivation of iron which in turn causes chlorosis.

SUMMARY

The objective of this research was to study the mineral content of various plant sections--upper stems, lower stems, and leaves--as influenced by conditions associated with lime-induced chlorosis. The investigation was made from two approaches. One approach was to grow plants--tomatoes, Hawkeye soybeans, and Red Kidney beans--on a calcareous soil known to produce chlorosis and on a non-calcareous soil which does not produce chlorosis. The second approach was to grow Red Kidney beans in nutrient solutions using a split-root technique and to use the bicarbonate ion and phosphorus concentration as the chlorosis-causing factors. The split-root technique provided a way to separate iron and phosphorus.

In the split-root experiment two levels of phosphorus were used--10 ppm and 0.06 ppm. Sodium bicarbonate was either present or absent. Sodium chloride was used in some of the treatments so that the bicarbonate effect could be separated from the sodium effect.

After the plants were harvested they were dissected into sections and analyzed for phosphorus, iron, potassium, and calcium. The concentrations of each element in the plant sections were compared.

When the tomatoes, soybeans, and kidney beans were grown on the calcareous soil their growth and the uptake of phosphorus was decreased. In the tomatoes and soybeans there was a slight increased uptake of potassium and calcium; in the kidney beans the uptake of potassium was increased even more, and that of calcium slightly decreased when the plants were grown on the calcareous soil.

The percentage iron in the tomatoes and soybeans increased when the plants were grown on calcareous soil, but in the kidney beans the

percentage iron was increased in the lower stems and leaves but decreased in the upper stems.

The mineral distribution in the plants which did not develop chlorosis followed a general pattern. The concentration of phosphorus and iron tended to increase from lower stems, to upper stems, to leaves. The concentration of potassium was highest in the upper stems and lowest in the leaves. The concentration of calcium tended to be opposite to that of the potassium--lowest in the upper stem and highest in the leaves.

In the split-root experiment chlorosis developed in the kidney beans only when the beans were in the bicarbonate with iron treatment. This suggests an interaction between bicarbonate ion and iron and not bicarbonate and phosphorus in causing chlorosis.

When the bicarbonate ion was present either with iron or phosphorus, the plants showed decreased growth and, with a few minor exceptions, a decrease in the uptake of phosphorus, iron, calcium and potassium.

LITERATURE CITED

Aiyar, S. P.
1946 Effects of phosphate deficiency on rice. Proc. Indiana Acad. Sci. 23B: 165-193. Chem. Abstracts 40: 7473.

Arnon, D. E., W. E. Fratzke, and C. M. Johnson
1942 Hydrogen ion concentration in relation to absorption of inorganic nutrients by higher plants. Plant Physiol. 17: 515-524.

Baxter, P., and R. Belcher
1955 The role of bicarbonate ion in lime-induced chlorosis. Aust. Institute of Agricultural Sci. Jour. 21: 32-34.

Bendall, D. S., S. L. Ranson, and D. A. Walker
1958 Some effects of carbon dioxide-bicarbonate mixtures on the oxidation and reduction of cytochrome c by Ricinus mitochondria. Nature 181: 133-134.

Biddulph, O.
1951 The translocation of minerals in plants. Mineral Nutrition of Plants, E. Truog, Ed. p. 261-275. University of Wisconsin Press, Madison.

Biddulph, O., and C. G. Woodbridge
1952 The uptake of phosphorus with particular reference to the effect of iron. Plant Physiology 27: 431-444.

Bonner, W. D., Jr.
1950 The succinis oxidase system and its relation to phosphorus and bicarbonate. Nature 165: 757-758.

Brown, J. C., and R. S. Holmes
1955 Iron, the limiting element in a chlorosis: Part I. Availability and utilization of iron dependent upon nutrition and plant species. Plant Physiology 30: 451-457.

Brown, J. C., and R. S. Holmes
1956 Iron supply and interacting factors related to lime-induced chlorosis. Soil Sci. 82: 507-519.

Brown, J. C., R. S. Holmes, and L. O. Tiffin
1959 Hypotheses concerning iron chlorosis. Soil Sci. Soc. Amer. Proc. 23: 231-234.

Brown, J. C., and L. O. Tiffin
1960 Iron chlorosis in soybeans as related to the genotype of rootstock, relationship between susceptibility to chlorosis and capacity to absorb iron from iron chelate. Soil Sci. 89: 8-15.

Brown, J. W., and C. H. Wadleigh
1955 Influence of sodium bicarbonate on the growth and chlorosis of garden beets. Bot. Gaz. 116: 201-209.

Burtch, L. M., D. W. Thorne, and F. B. Wann
1948 The effect of light, soil, temperature, and soil moisture on high-lime chlorosis. Soil Sci. Soc. Amer. Proc. 13: 394-398.

Chandler, W. V., and G. D. Scarseth
1941 Iron starvation as affected by over-phosphating and sulfur treatment on Houston and Sumter clay soils. Amer. Soc. Agron. Jour. 33: 93-104.

DeKock, P. C.
1955 Iron nutrition of plants at high pH. Soil Sci. 79: 167-175.

DeKock, P. C., and Alexander Hall
1955 The phosphorus-iron relationship in genetical chlorosis. Plant Physiol. Vol. 30: 293-295.

Doney, R. C.
1959 The effect of various levels of bicarbonate, phosphorus, and pH on the translocation of iron in plants. Master's Thesis, Utah State University, Logan, Utah.

Doney, R. C., R. L. Smith, and H. H. Wiebe
1960 Effects of various levels of bicarbonate, phosphorus, and pH on the translocation of foliar-applied iron in plants. Soil Sci. 89: 269-275.

Goss, J. A.
1957 A comparison of the effects of the bicarbonate ion with other anions on the uptake of some radioisotopes by Phaseolus vulgaris and Hordenum vulgare plants. Doctor's Thesis, Botany Dept., University of California, Los Angeles.

Heller, V. G., R. H. Hageman, and E. L. Hartman
1940 Sand culture studies of the use of saline and alkaline water in greenhouse. Plant Physiol. 15: 727-733.

Jackson, W. A., and N. T. Coleman
1959 Ion absorption by bean roots and organic acids changes brought about through CO_2 fixation. Soil Sci. 87: 311-319.

Johnson, M. O.
1917 Manganese as a cause of the depression of the assimilation of iron by pineapple plants. Jour. Indus. and Eng. Chem. Analyt. Ed. 9: 47-49.

Lindner, R. C., and C. P. Harley
1944 Nutrient interrelations of lime-induced chlorosis. Plant Physiol. 19: 420-439.

Marcour, M.
1952 The uptake of iron by bean plants as influenced by age and calcium and bicarbonate ion in solution cultures. Master's Thesis, Dept. of Agron., Utah State University, Logan, Utah.

Marsh, R. P., and J. W. Shive
1925 Adjustment of iron supply to requirements of soybean in solution culture. Bot. Gaz. 79: 1-27.

McGeorge, W. T., and E. L. Breazeale
1956 Application of the Nuibauer Technique and applied potential to the study of immobilization of iron in plants. Soil Sci. 82: 329-336.

Miller, G. W.
1954 The effect of the bicarbonate ion on the respiration of excised roots. Master's Thesis, Dept. of Agron., Utah State University, Logan, Utah.

Miller, G. W., and H. J. Evans
1956 Inhibition of plant cytochrome oxidase by bicarbonate. Nature 178: 974-976.

Miller, G. W., and D. W. Thorne
1956 Effect of bicarbonate ion on the respiration of excised roots. Plant Physiol. 31: 151-155.

Olsen, C.
1935 Iron absorption and chlorosis in green plants. Compt. rend, trav. lab. Carlsberg Ser. chim. 21: 15-52.

Olsen, R. V., and C. W. Carlson
1949 Iron chlorosis of sorghums and trees as related to extractable soil iron and manganese. Soil Sci. Soc. Amer. Proc. 14: 109-112.

Oserkowsky, J.
1932 Hydrogen-ion concentration and iron content or tracheal sap from green and chlorotic pear trees. Plant Physiol. 7: 258.

Porter, L. K., and D. W. Thorne
1955 Interrelation of carbon dioxide and bicarbonate ions in causing plant chlorosis. Soil Sci. 79: 373-382.

Rediske, J. H., and O. Biddulph
1953 The absorption and translocation of iron in plants. Plant Physiol. 28: 576-593.

Sideris, C. P., H. Y. Young, and B. H. Krauss
1943 Effects of iron on the growth and ash constituents of Ananas comosus (L.) Mer. Plant Physiol. 18: 608-632.

Stolwijk, J. A., and K. V. Thiman
1957 On the uptake of carbon dioxide and bicarbonate by roots, and its influence on growth. Plant Physiol. 52: 513-520.

Thorne, D. W., F. B. Wann, and Wilford Robinson
1951 Hypotheses concerning lime-induced chlorosis. Soil Sci. Soc. Amer. Proc. 15: 256-258.

Ulrich, A.
1959 Metabolism of non-volatile organic acids in excised barley roots as related to a cation-anion balance during salt accumulation. Amer. Jour. Bot. 28: 194.

Wadleigh, C. H., and J. W. Brown
1952 Chemical status of bean plants affected with bicarbonate-induced chlorosis. Bot. Gaz. 113: 373-392.

Wallihan, E. F.
1961 Effect of sodium bicarbonate on iron absorption by orange seedlings. Plant Physiology 36: 52-53.

Warnock, Robert E.
1952 The iron content of some plants as influenced by conditions associated with lime-induced chlorosis. Master's Thesis, Dept. of Agronomy, Utah State University, Logan, Utah.

Weiss, M. G.
1943 Inheritance and physiology of efficiency in iron utilization in soybeans. Genetics 28: 253-268.

Wilde, S. A., and G. K. Voigt
1955 Analysis of soils and plants for foresters and horticulturists. Ann Arbor, Michigan: J. W. Edwards, Publishers, Inc.