THE ABSORPTION AND TRANSLOCATION OF IRON

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Introduction

Iron has been recognized as an essential element for quite some time, yet much of the data concerning the uptake and translocation of this element by plants is conflicting or incomplete. Three factors have emerged, however, as being highly influential in the process of iron absorption. These factors are pH, phosphorus concentration, and the chemical form of the iron.

OLSEN (**7**) has indicated that high pH and high phosphorus concentration of the nutrient solution induce iron deficiency chlorosis. He also suggested that too high a phosphorus concentration could cause a precipitation of iron along the veins of the leaf. FRANCO and LOOMIS (**2**) somewhat more recently have shown that iron absorption from cultures is reduced by phosphorus, especially at pH 6 or higher. The effect of various forms of iron on iron uptake has been investigated in a relative sense, the data being concerned primarily with those forms of iron giving the best growth under a given set of conditions.

Iron has long been commonly referred to as a non-mobile element. Indeed, GILE and CARRERO (**4**) at a very early date conducted an experiment with rice which demonstrated this immobility. However, since that time, the work on iron translocation has been sparse and incomplete.

The present paper is concerned with those factors which have been shown to effect the absorption and translocation of iron, namely, pH, phosphorus concentration and the amount and chemical form of the iron used. An attempt has been made to define the effects of these factors in a quantitative sense and to determine interrelations between them.

Materials and methods

Red Kidney bush beans (*Phaseolus vulgaris* L.) were used as the experimental plants throughout this investigation. They were obtained from Burpee's standard commercial stock.

The plants were cultured in 6-liter enameled refrigerator pans according to the technique outlined by BIDDULPH (**1**). The culture period in the refrigerator pans normally lasted 12 days. The nutrient solution used consisted of the following salts and concentrations: $Ca(NO_3)_2$ 0.0025 M, KNO_3 0.0025 M, $MgSO_4$ 0.0010 M, KH_2PO_4 as indicated for each experiment,

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H_3BO_4 0.5 p.p.m. B, $CuSO_4$ 0.02 p.p.m. Cu, $MnCl_2$ 0.5 p.p.m. Mn, $ZnSO_4$ 0.05 p.p.m. Zn and Fe as indicated for each experiment. During the first 4 days nutrient solution of half strength was used. At the beginning of the next 4-day period, the solutions were changed to full strength and 0.5 p.p.m. of iron was included. The solutions were again changed for the final 4-day period, full strength solutions again being used. The various experimental conditions were produced in the nutrient solutions during this last 4-day period.

At the conclusion of the final 4-day experimental period the plants were harvested. Those upon which analyses were to be made were divided into trifoliate leaves (blades only), primary leaves, stems (and petioles), and roots. The separated material was then placed in an oven at 70° C to dry. That material used for autoradiographs was pressed between newspaper folds and quickly dried in an oven at 70° C.

After the plant material had dried for at least 2 days, the ovens were turned off and allowed to reach room temperature. The plant materials were then removed, weighed, and carefully placed in 200-ml. Kjeldahl flasks through smooth-surfaced paper funnels. Digestion of plant parts was carried out as a modification of a method described by PIPER (**8**) using sulfuric and perchloric acids. After cooling, the contents of the flasks were quantitatively transferred to 250-ml. volumetric flasks. Suitable aliquots were then removed for analysis.

Total iron was determined by a modification of the thiocyanate method of SANDELL (**11**). The color was developed under standard conditions and the transmittance was determined with a Cenco Photelometer using a 10-ml. cell 1 cm. thick and a green filter. The readings were compared with a curve prepared from a set of standards made up according to the same procedure as the unknown samples, including the digestion.

It has been found in working with radioiron that the isotope Fe^{55} offers numerous advantages for this type of work over the better known isotope Fe^{59}. Among these advantages are the longer half-life necessitating fewer decay corrections, the greater specific activities obtainable, and the lower energies of the radiations eliminating the necessity for extra shielding. Therefore, the tracer work to follow was done with this isotope. The stock solution of iron was prepared by adding a tracer amount of Fe^{55} to a solution of $FeCl_3$ of known concentration. This solution will hereafter be referred to as Fe^*Cl_3. The resulting activity of Fe^{55} in the nutrient solution was 0.1 μc./l.

In preparation of the samples for radioiron determination, use was made of a technique (**9**) similar to the thiocyanate method described for total iron. In this case, however, the ferric thiocyanate complex was extracted with isoamyl alcohol and an aliquot evaporated on a stainless steel counting disc. The discs were then placed in a Nucleometer (Radiation Counter Laboratories) and "counted." By utilization of a previously prepared relationship between activity and total iron in the radioactive solution, the marked iron content of the unknown samples could be determined.

The translocation studies were carried out by a phloem injection technique described by BIDDULPH (1). The radioiron solution which was injected into the second trifoliate leaf from the base assayed 0.46 mc. of Fe^{55}/ml. and contained 1000 p.p.m. of carrier iron as $FeCl_3$.

After harvest, autoradiographs of the injected plants were prepared to determine the extent of translocation. This was done by placing the pressed and dried plants directly on a 10×12 inch sheet of Eastman no-screen x-ray film. These were placed in an x-ray exposure folder and allowed to expose for 15 days. The films were then developed. The numerous steps in exposure, development and reproduction were maintained uniform throughout so that semi-quantitative comparisons between autoradiographs might be made.

Results and discussion

THE NUTRIENT RELATIONSHIP OF VARIOUS IRON COMPOUNDS

In order to complete an effective study of iron uptake from a nutrient medium, it was considered necessary to have an appreciation of the of the reaction of various types of iron compounds in the nutrient solution to be used. Therefore, a series of experiments was conducted whereby the rate of formation of iron precipitates could be followed.

One liter of nutrient solution of a composition previously indicated containing 0.0005 M phosphorus was placed in a flask. To this was added 1.0 p.p.m. of iron as the particular compound being studied. The pH was adjusted to the desired level with *N* KOH or H_2SO_4, and the flask was covered and placed in darkness at room temperature. Samples of the supernate were subsequently taken at 1-, 3-, and 7-day intervals by pipetting from the top of the solution. The solutions were otherwise undisturbed so that the aliquot contained soluble iron as well as any particles of precipitate small enough to remain in colloidal suspension. The aliquots were reduced in volume by evaporation and the iron content determined by the thiocyanate method.

The results are indicated in figure 1. It will be noted that ferric phosphate is very insoluble and the solubility is little affected by pH. Ferric chloride and ferric nitrate are very similar in reaction. They too become quite insoluble and are not influenced appreciably by pH. The organic salts of iron—that is, oxalate, tartrate, and citrate—are very similar in that they are somewhat more soluble at a pH of 4.0 than pH 7.0, making their use practicable at a lower pH This increase in solubility over the inorganic salts may be due to complex formation with the organic anion. Ferric humate (5) was found to be the most promising iron compound to use in experiments conducted over a wide pH range because of its constant and relatively high solubility. Ferrous sulfate was found to be unique in its reaction to pH being relatively soluble at pH 4.0 but quite insoluble at pH 7.0 with corresponding gradations between. Both potassium ferrocyanide and potassium ferricyanide were included in the above experiment, but no

precipitation of these compounds was found within the range of experimental conditions imposed.

As a part of this experiment, 10 groups of plants were grown in nutrient cultures each group replicated once. Each culture incorporated one of the various forms of iron as the source of this element. After 6 days in the

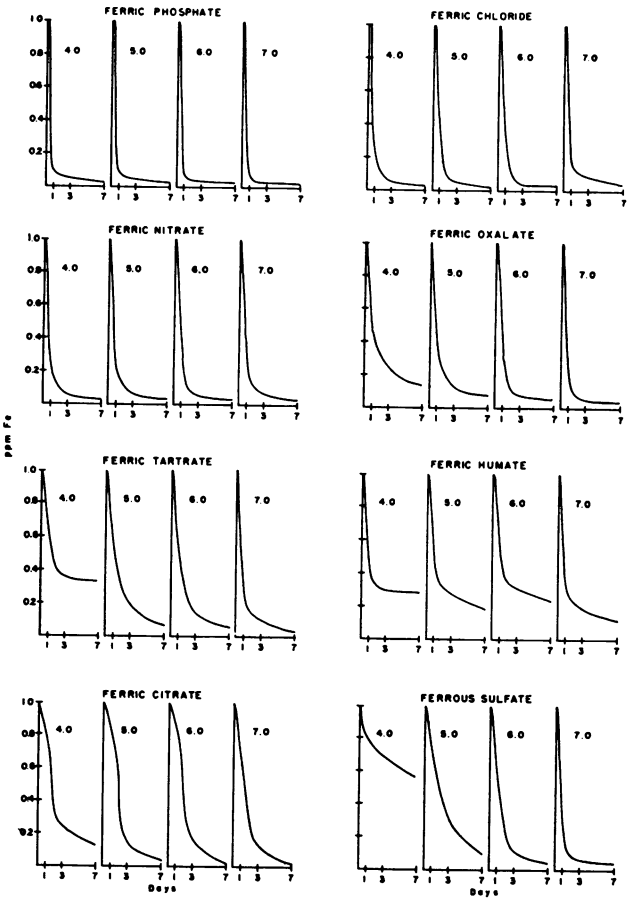

FIG. 1. The amount of iron remaining in solution at various pH values during time intervals of one, three, and seven days. Ten forms of iron were employed, two forms explained in text. Phosphorus concentration of the solution was 0.0005 M.

solutions the plants were removed and the various parts subjected to an analysis for total iron. Figure 2 presents the results obtained.

It can be seen that the amount of iron associated with the roots varies with the compound introduced into the nutrient solution. It is also apparent that the concentration of iron in the leaves is independent of the solubility of the iron compound employed. Finally, there is no apparent dependence of the concentration of iron in the leaves on the concentration of iron associated with the roots.

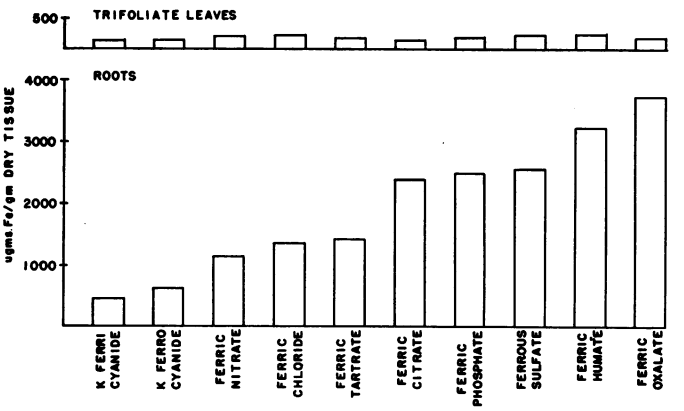

FIG. 2. The effect of ten iron compounds on the absorption of iron at pH 4.0. The phosphorus concentration was 0.0005 M

THE EFFECT OF pH ON IRON UPTAKE

The uptake of iron from a nutrient solution is considerably influenced by changes in pH. As later evidence will indicate, it is quite probable that this influence is exerted through an increased mobility of iron as well as an increase in adsorption on the root surfaces at low pH values. Table I tabulates the data of an experiment conducted to show the effect of pH on iron uptake. It is clearly seen (fig. 3) that as the hydrogen ion concentration increases the accumulation of iron associated with the roots also increases. Likewise, the uptake into the trifoliate leaves increases. This relationship is found when 1.0 p.p.m. of iron is present as ferric nitrate. A similar relationship is found when ferric humate is used (**10**).

It is not precisely understood why the accumulation of iron associated with the roots in solution culture should increase as the hydrogen ion concentration increases. This phenomenon is undoubtedly associated with the type of particle formed in the precipitation of the iron, since nearly all of the iron becomes insoluble at those pH levels studied. A good portion of the precipitate formed will be of the ferric oxide type. Ferric oxide particles are pH sensitive in formation, the rate of coagulation being particularly influenced. Variations in the rate of coagulation can result in the formation of crystalline hematite, gelatinous hydrated ferric oxide particles or the suspended hydrosol itself when coagulation is at a minimum. It is postulated that the increase in iron concentration on the roots as the hydrogen ion concentration increases is due to the difference in the ability of these various types of iron particles to adsorb on the roots. It is assumed that most of the iron associated with the roots under the above conditions is adsorbed iron. The very high concentrations found, about 10 times that of the trifoliate leaves, indicate that it is not true absorption. Such abnormal concentration ratios of root over leaves would not be expected if all the iron associated with the roots in this case was absorbed iron.

In those samples where no iron was added to the nutrient solution, the principal source of iron was from the cotyledons (**12**). There was also approximately 0.004 p.p.m. in the nutrient solution added as impurities in the salts. Under these conditions chlorosis readily occurred at pH 7.0 and 6.0, rarely at 5.0 and never at 4.0. This indicates that at pH 4.0 more iron from the cotyledons was utilized by the plants. This fact is also evidenced by the slightly higher content of iron in the tissues at the lower pH values. In referring to the data of table I it is found that a slightly greater amount of iron is found in the spent nutrient at the lower pH values evidently having

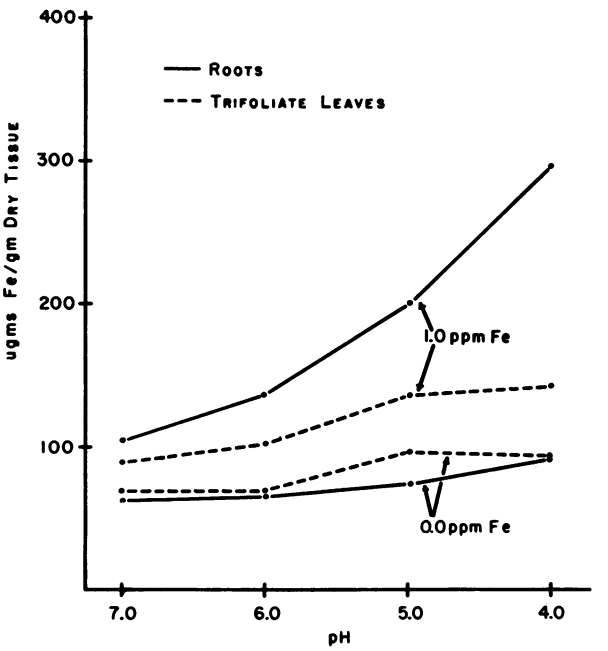

FIG. 3. The effect of pH on uptake of iron from a nutrient solution. Ferric nitrate was used as the source of iron for that series having iron. The phosphorus concentration was 0.005 M. The concentration of iron on the roots of the 1.0 p.p.m. series should be multiplied by ten.

leached from the plant. This could be caused by an increased mobility of iron or a difference in membrane permeability with varying pH.

INFLUENCE OF IRON CONCENTRATION ON IRON UPTAKE

The concentration of iron in the nutrient solution does not appear to have a proportional effect on the uptake of this element over the range studied. Figure 4 indicates that in general the uptake increased as the concentration increased, but the greatest rate of uptake only doubled when the concentration of iron was increased as much as 10 times. At the lower concentrations of iron (0.004 to 0.1 p.p.m.) the rate of increase of iron uptake is very small regardless of the pH. This can probably be attributed to the

TABLE I

THE EFFECT OF PH ON UPTAKE AND GROWTH AT TWO IRON LEVELS USING FERRIC NITRATE. THE UPTAKE IS EXPRESSED AS μGM. FE/GM. OF DRY TISSUE. THE DRY WEIGHT IS THAT OF SIX PLANTS. THE DUPLICATES SHOWN ARE THOSE OF TWO DIFFERENT PANS.

		p.p.m. Fe in spent nutrient solution	Primary leaves		Trifoliate leaves		Stems		Roots	
			Dry wt. gm.	Fe Conc.	Dry wt. gm.	Fe Conc.	Dry wt. gm.	Fe Conc.	Dry wt. gm.	Fe Conc.
	pH 4.0	.15	1.69	375	1.16	159	1.27	51	.69	2970
		.15	1.58	163	1.16	123	1.09	40	.70	2920
	pH 5.0	.29	1.74	174	1.47	114	1.23	34	.76	2000
		.37	1.80	316	1.52	153	1.30	45	.73	1200
1.0 p.p.m. Fe										
	pH 6.0	.42	1.76	141	1.44	99	1.30	30	.75	1280
		.37	1.73	122	1.52	100	1.29	30	.74	1430
	pH 7.0	.52	1.44	113	1.28	92	1.15	34	.64	1040
		.50	1.92	117	1.28	86	1.32	32	.74	1020
	pH 4.0	.015	1.61	102	1.19	100	1.10	36	.68	93
		.012	1.65	99	1.22	86	1.13	25	.69	90
	pH 5.0	.023	1.33	109	1.44	91	1.20	39	.79	66
		.015	1.73	106	1.44	99	1.17	25	.80	80
0.0 p.p.m. Fe										
	pH 6.0	.009	1.55	103	1.04	49	1.25	30	.82	64
		.011	1.84	122	1.36	85	1.30	30	.90	67
	pH 7.0	.009	1.74	90	.95	60	1.23	19	.89	69
		.010	1.89	131	1.03	77	1.34	20	.90	54

fact that most of the iron used by the plant comes from the cotyledons when such a small concentration is available through the nutrient solution. The total amount of iron available from the cotyledons would overshadow that obtained from the nutrient environment (**12**). But as the concentration is increased to 1.0 p.p.m. there is a substantial increase in the rate of uptake at pH 4.0. The observed rate of uptake was not proportional to the increase in iron concentration in the nutrient solution. However, the accumulation of iron on the roots increased greatly between 0.1 and 1.0 p.p.m. of iron added to the nutrient solution at pH 4.0 and approaches a proportional relationship.

At pH 7.0 the uptake of iron into the trifoliate leaves is at a uniform and slightly increasing rate in the range of 0.004 to 1.0 p.p.m. of nutrient iron.

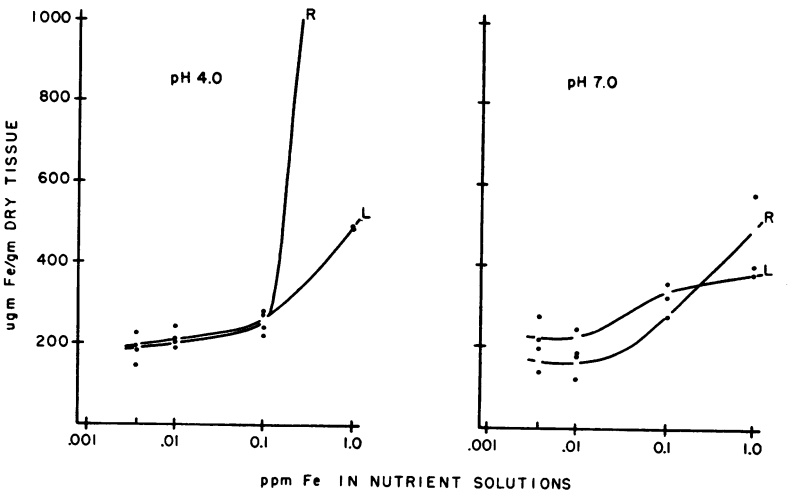

FIG. 4. The effect of iron concentration in a nutrient solution on iron uptake using ferric nitrate as the source of iron. The phosphorus concentration was 0.0005 M. R—roots, L—trifoliate leaves.

This probably is a result of the low availability of iron at high pH values. Likewise, the root accumulation is not heavy and increases only at a low rate within this range of nutrient iron concentrations.

At pH 4.0 the cultures at all concentrations of iron were healthy and green whereas at pH 7.0 only those cultures containing 1.0 p.p.m. of iron did not sustain a chlorosis. Thus, generally speaking, it has been found that 0.1 p.p.m. of iron in a nutrient medium is sufficient iron for normal green growth of the Red Kidney bean plant grown at pH 4.0 and 5.0, but 1.0 p.p.m. of iron sems to be necessary at 6.0 and definitely at 7.0 providing the phosphorus concentration is 0.0005 M or less.

THE EFFECT OF PHOSPHORUS CONCENTRATION ON IRON UPTAKE

Of all the conditions affecting iron uptake that were studied, the effect of phosphorus concentration seemed to be the most complex. Previous

authors (**3, 7**) have investigated the effect of higher concentrations of phosphorus on iron uptake; however, variations from the accepted reaction have been found for lower concentrations of phosphorus.

In figure 5 can be seen the results of three experiments conducted over a range of phosphorus concentrations from 2×10^{-6} to 5×10^{-3} M all at pH 6.0. The plants were grown in a greenhouse where such ambient conditions as sunlight, temperature and humidity were not easily controlled. Because

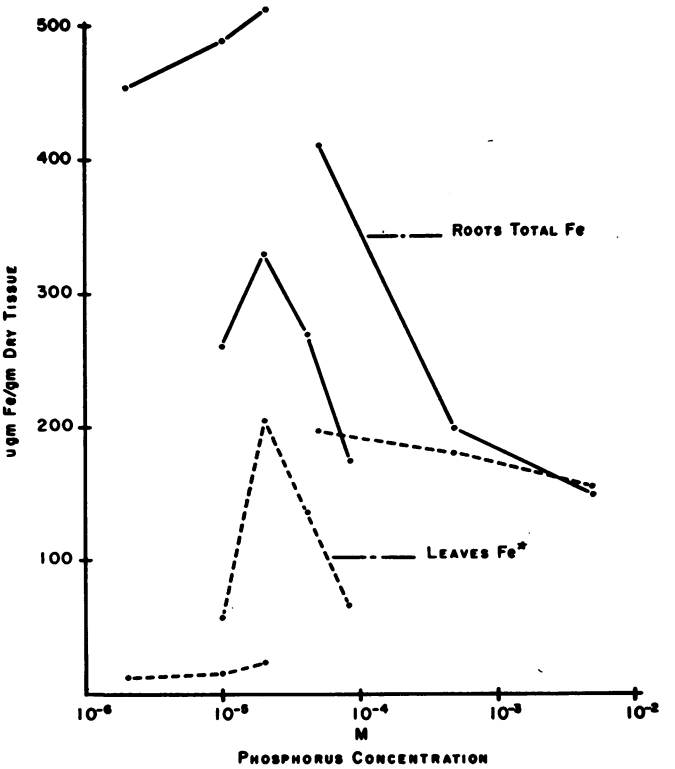

FIG. 5. The effect of phosphorus concentration in a nutrient solution on iron uptake at pH 6.0 using Fe^*Cl_3 as the source of iron during the experimental period. The leaves represent marked iron absorbed during experimental period, whereas the roots represent total iron. Multiply root concentrations by ten. The three groups of data were obtained as separate experiments run at different times.

of this, some variation in absolute values between experiments was obtained since each experiment was presumably conducted under different ambient conditions. These data, however, illustrate one important point, that iron uptake from a nutrient solution was at a maximum when the phosphorus concentration was equimolar with iron; *i.e.*, 2×10^{-5} M. It may also be pointed out that the iron associated with the roots (absorbed and adsorbed iron) was at a maximum at 2×10^{-5} M phosphorus. A second experiment covering the entire range in phosphorus concentration from 5×10^{-6} to

5×10^{-3} M was conducted at a pH of 6.0. Again it was found that uptake into the leaves was at a maximum at the phosphorus concentration nearest to that equimolar with iron; *i.e.*, 5×10^{-5} M. But in contrast to the previous experiments, the accumulation of iron by the roots was greater at the lowest nutrient phosphorus level instead of at the equivalence point between iron and phosphorus (fig. 6). Thus the amount of iron associated with the roots may either increase or decrease as phosphorus concentrations are decreased from those equimolar with iron in the nutrient solution.

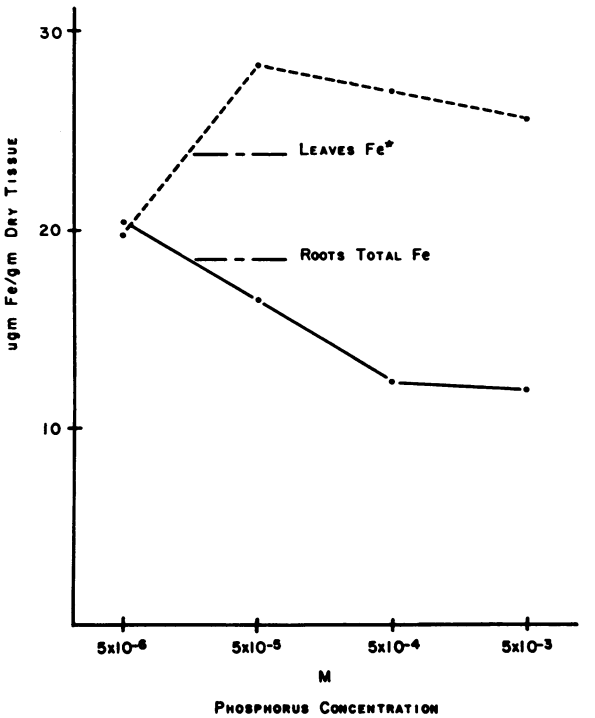

FIG. 6. The effect of phosphorus concentration in a nutrient solution on the uptake of iron at pH 6.0 using 1.0 p.p.m. of Fe^*Cl_3 as the source of iron. The leaves represent marked iron absorbed, whereas the roots represent total iron. Multiply root concentrations by ten.

It appears that when there is more phosphorus than iron in the nutrient medium, the predominant iron precipitate is a ferric phosphate. Analyses have indicated that under these conditions iron and phosphorus appear in the precipitate in an approximate stoichiometric relationship. However, at phosphorus concentrations below those equimolar with iron, the precipitate has been found to be of a ferric oxide type. This latter type of compound may exist in a number of forms, as a hydrosol, a coagulated hydrosol or as crystalline hematite. Obviously, in going from a high phosphorus concentration, 10^{-3} M, to a low phosphorus concentration, 10^{-6} M, one would proceed through various combinations of these two compounds. At the higher

phosphorus concentrations the precipitate would probably be a fine suspension of ferric phosphate, a type of precipitate which apparently is not adsorbed on the roots as well as a gelatinous type. As the phosphorus concentration was decreased, more and more hydrated ferric oxide would be formed which could accumulate on the roots and incorporate that ferric phosphate which was formed with it.

An obvious conditioning factor is a consideration of the isoelectric point described by MATTSON (**6**). If in varying the concentration of phosphorus, the isoelectric point of the particulate material is attained a coagulation and accumulation of the material on the roots will result. Sufficient data is not available to determine the mechanism of this reaction.

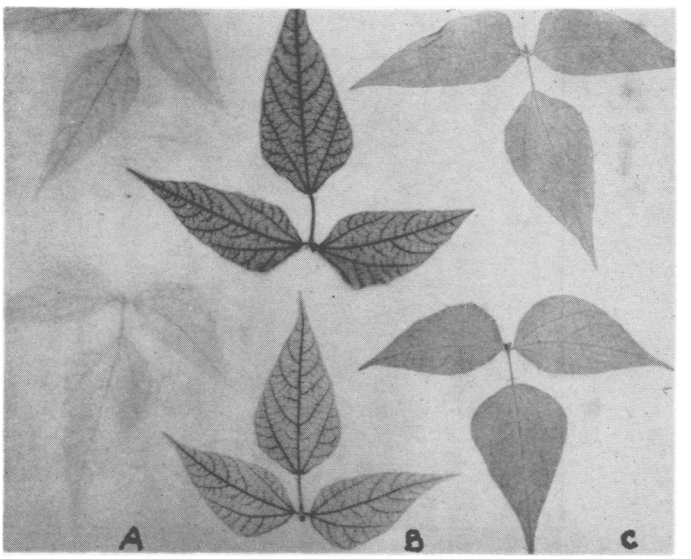

FIG. 7. Autoradiograph of trifoliate leaves of bean plants grown under three different experimental conditions using Fe^{55} as a tracer. Group A—0.001 M P, 1.0 p.p.m. Fe as Fe^*Cl_3, pH 7.0; group B—0.0001 M P, 1.0 p.p.m. Fe*, pH 7.0; group C—0.0001 M P, 1.0 p.p.m. Fe* and pH 4.0.

The uptake of iron into the trifoliate leaves tends to parallel this accumulation on the roots for high nutrient phosphorus concentrations. This may indicate that that which is adsorbed is ultimately available for absorption and consequent translocation. But as the phosphorus concentration is reduced to less than equimolar with iron, the precipitate becomes predominately a ferric oxide type. GILE and CARRERO (**3**) have shown that ferric oxide is absorbed by plants with difficulty. Thus, less uptake would be expected as the ferric oxide concentration increased. If the precipitate was a crystalline hematite it would account for little accumulation on the roots. Whereas, a gelatinous hydrated ferric oxide type, formed at low nutrient phosphorus levels, causes a heavy deposition of iron on the roots. In either case the uptake into the trifoliate leaves is small.

PHOSPHORUS-INDUCED CHLOROSIS

Considering the preceding work on phosphorus-iron relationships, it is evident that phosphorus exerts a marked influence on the uptake of iron. But not in all cases of iron-deficiency chlorosis does the primary influence seem to be on iron uptake. Rather, the ultimate effect appears to be a blocking of the use of iron after it has been absorbed. Incidents of this nature have been reported by many workers. Upon examination of chlorotic leaves, they were often found to have a higher iron content than normal green leaves. OLSEN (**7**) has described this condition and thought that perhaps it could be merely a precipitation of iron in the veins of the leaf as ferric phosphate. His contentions were upheld experimentally by the use of the Prussian blue test which indicated a heavy deposition of iron in the veins of the chlorotic leaf.

Confirmation of Olsen's work was obtained with Red Kidney bean plants grown in a nutrient solution using radioiron. Figure 7 is an autoradiograph of the trifoliate leaves of bean plants grown under three different experimental conditions. Leaves of group A were taken from plants grown in a nutrient solution containing 0.001 M phosphorus, 1.0 p.p.m. of iron as Fe^*Cl_3, and at pH 7.0. These leaves were fully chlorotic. The phosphorus concentration and pH were high enough to prevent iron in significant quantities from reaching the leaves. This is evidenced by the light exposure obtained on the autoradiograph.

Leaves of group B were taken from plants grown in a nutrient solution containing 0.0001 M phosphorus, 1.0 p.p.m. iron as Fe^*Cl_3, and at pH 7.0. These leaves had a chlorotic mesophyll but were green along the veins, similar to the plants described by Olsen. Upon examination of the autoradiograph of these leaves, it can be seen that there is a heavy deposition of iron in the veins while the mesophyll is relatively free of iron. In this case, the phosphorus concentration of the nutrient solution was low enough to allow absorption of iron at pH 7.0, but the uptake of phosphorus was still great enough to cause precipitation of iron in the veins.

Leaves of group C were taken from plants grown in a nutrient solution containing 0.0001 M phosphorus, 1.0 p.p.m. iron as Fe^*Cl_3, and at pH 4.0. In this case the uptake of iron was rapid and the distribution was uniform enough to produce a normal green leaf. It is obvious that a pH of 4.0 in the nutrient solution in some way inhibits the formation of a ferric phosphate precipitate in the veins of the leaves providing the phosphorus level is maintained below 0.0001 M. At a pH of 4.0 sufficient iron can also be translocated from the cotyledons of the germinating seed to maintain a normal green plant.

This experiment tends to substantiate the supposition that certain types of chlorosis are caused by the immobilization of iron in the veins of the leaf. This type of chlorosis is characterized by a chlorotic mesophyll with green veins. Both the pH and the phosphorus content of the nutrient may play a part in its immobilization.

The distribution of iron

In the preceding experiments, the general distribution of iron has been studied on a gross anatomical basis. It was the purpose of this investigation to determine the distribution of iron within each of the plant parts. To accomplish this, use is made of autoradiographs of whole plants that have been grown with 4 different regimes of iron administration. Plants were

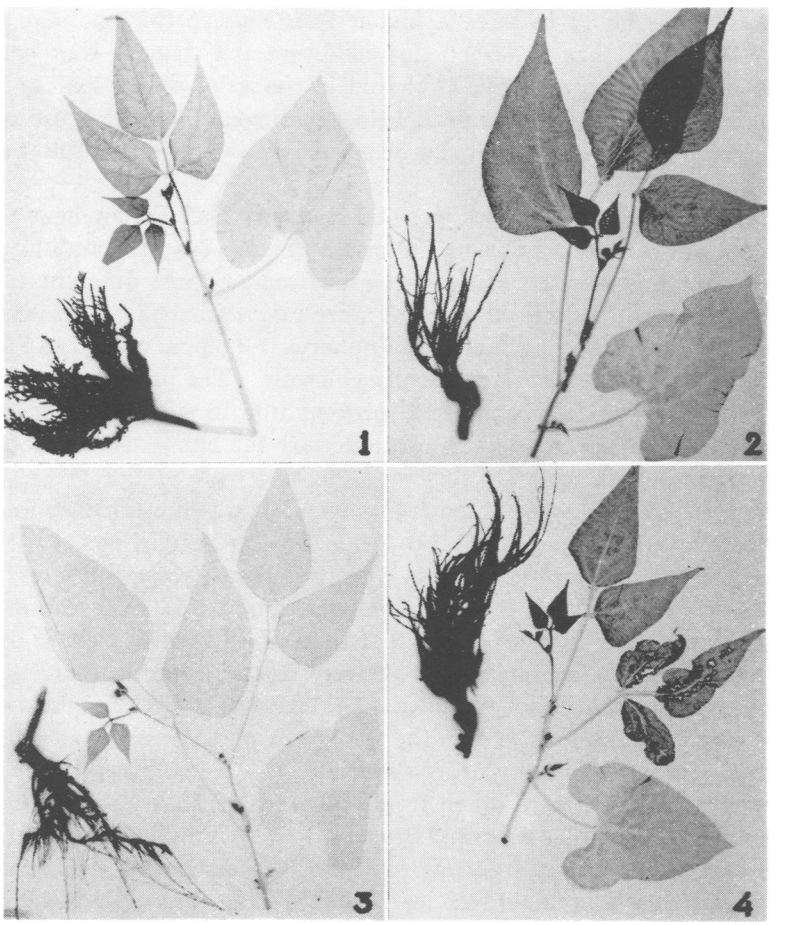

Fig. 8. Autoradiographs of whole bean plants when grown in a nutrient solution containing 1.0 p.p.m. of Fe^*Cl_3. Group 1—10 days with Fe*; group 2—15 days with Fe*; group 3—10 days with Fe* then 5 days with no Fe; group 4—10 days with no Fe then 5 days with Fe*.

grown in 4 groups with 1.0 p.p.m. of iron as Fe^*Cl_3 available as the source of iron. The phosphorus concentration in each case was 0.0002 M. Group 1 was allowed to grow in a nutrient solution with iron for 10 days at which time it was harvested, 1 plant being prepared for an autoradiograph (fig. 8). The remaining 7 plants were dried for analysis. Group 2 was also allowed to grow with iron available for the 10-day period plus an additional 5 days

as well. The plants were then harvested in the same manner as group 1. The plants in group 3 received the same treatment for the initial 10 days. At this time as much of the adsorbed iron as possible was removed from the roots with an HCl solution of a pH of 3.0. The plants were then placed in a nutrient solution containing no iron for the remaining 5 days. They were then harvested at the end of the 15-day period according to the same plan as group 1. Group 4 was grown without iron for the initial 10-day period causing a rather severe chlorosis of the first trifoliate leaf. It was then placed in a nutrient solution containing iron for the remaining 5 days. The harvest and preparation for analysis were the same as for group 1. Autoradiographs of these plants are shown in figure 8. Complete analyses were performed and concur with the conclusions based on the autoradiographs.

The points of interest gained from this experiment include the following: The distribution of iron in the leaf tissues of healthy plants is surprisingly uniform throughout, with the exception of a slightly higher concentration in the terminal and lateral buds. The rate of uptake of iron by iron-deficient plants is greater than for normal plants at least for an initial period. This is evidenced by plants of group 4 which accumulated as great a concentration of iron during a 5-day period as the normal plants of group 2 accumulated during a 15-day period. The concentration of nutrient iron in the primary leaves of the plants of group 1 appears to be less than in the trifoliate leaves even though the data of table I indicates the primary leaves nearly always have the greater concentration. Thus the initial supply of iron must come from the cotyledons with a later slow uptake from the nutrient solution. The roots are covered by a heavy uniform deposition of iron. If such plants are removed to a solution containing no iron, the iron accumulation is not redeposited on new root growth, but the new leaves formed during this period acquire ample supplies of nutrient iron.

The translocation of iron

The translocation of iron, and the factors affecting it, has long intrigued workers because of its possible close relationship with chlorosis. A number of studies of these factors have been made, particularly the effect of phosphorus. Its effect on the translocation of iron from the roots to the leaves has been most frequently considered, and little attention has been given to its possible effect on the redistribution from the leaf to other parts of the plant. It was the purpose of the following experiments to determine if translocation of iron from the leaf is possible, and if so, to outline some of the factors influencing it.

In this study use is made of the leaf flap injection method of Biddulph (1) whereby iron can be injected into a leaf in such a manner that downward redistribution will occur through the phloem. A number of experimental conditions were applied to the nutrient solutions during the growth of the plants to determine which of those factors known to influence uptake might also influence redistribution of iron.

The iron solution to be injected contained 0.46 mc./ml. of Fe^{55} and 1000

p.p.m. of carrier iron and was injected into a veinlet of the second trifoliate leaf of the bean plant. A 10-minute injection period was allowed. A 24-hour "migration" period was allowed between injection and harvest. Autoradiographs were made of the pressed and dried plants to determine the extent and relative amounts of redistribution that were obtained.

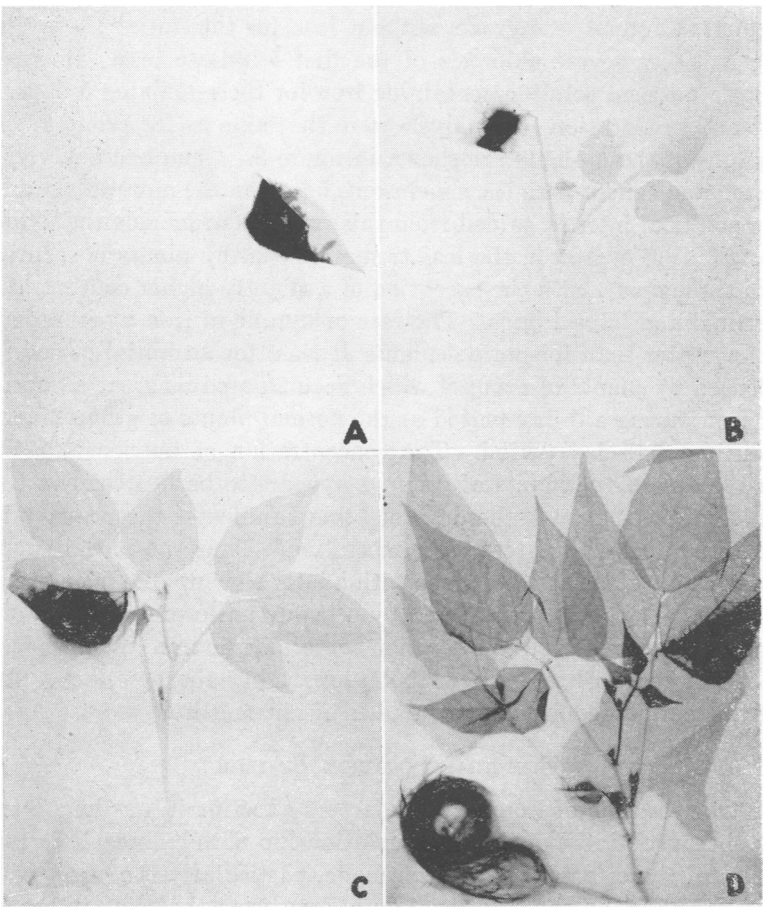

FIG. 9. Autoradiographs of whole bean plants after injection with Fe^*Cl_3 in the second trifoliate leaf when grown in the following nutrient conditions: (A) pH 7.0, P—0.001 M, Fe—1.0 p.p.m.; (B) pH 4.0, P—0.0001 M, Fe—1.0 p.p.m.; (C) pH 7.0, P—0.00001 M, Fe—0.0 p.p.m.; (D) pH 4.0, P—0.000005 M, Fe—0.0 p.p.m. The site of injection is indicated by the intensely exposed area.

An investigation was made of the effect of (1) the pH of the nutrient medium, (2) the iron content of the nutrient medium, and (3) the phosphorus content of the nutrient medium, upon the mobility of injected iron. It was found that each exerted a controlling influence on the amount of iron which was translocated. The least translocation occurred when all three factors were unfavorable to translocation. They were: a high phosphorus concentration, a high pH, and 1 p.p.m. of iron in the nutrient medium. Fig-

ure 9 A represents an autoradiograph of such a plant and it can be seen that practically no iron left the leaf under these conditions. All possible combinations of high and low pH, high and low phosphorus, and high and low iron were tried. The best translocation of iron occurred when the plants were grown in a solution containing very low phosphorus (0.000005 M), no iron, and maintained at pH 4.0. Figure 9 D represents an autoradiograph obtained under those conditions of growth. It is evident that the translocation of iron has been exceptionally rapid and complete. Translocation in the plant represented by figure 9 B has been suppressed by the presence of ample iron in the tissues as a result of having grown the plant with 1 p.p.m. of iron, as well as by an adequate supply of phosphorus (0.0001 M). In the case of 9 C, only the pH of the nutrient, which was at 7.0, has retarded translocation as the plants were grown without iron and at 0.00001 M phosphorus.

It thus is apparent from these experiments that the greatest mobility of injected iron occurred in plants grown with nutrient conditions of low pH and low phosphorus and iron concentrations. The least mobility of injected iron occurred with nutrient conditions of high pH and high phosphorus and iron concentrations. This is verified by figures 9 D and 9 A, respectively. Intermediate conditions of the above factors will yield intermediate degrees of iron mobility.

The primary leaves of the bean plant have long been a curiosity to those who have used this plant for experimental purposes. The primary leaves seem able to retain that iron which they received from the cotyledons in spite of the demand from the trifoliate leaf system which may become fully chlorotic. It is also very difficult to cause a chlorosis of the primary leaves. Situations such as these have caused iron to be labelled an immobile element. However, perhaps it is not that iron is inherently immobile, but rather that conditions such as high phosphorus and high pH, as demonstrated for the trifoliate leaf system, may be operative in preventing the movement of iron from the primary leaves.

To test this hypothesis, the primary leaves of a series of plants grown under a variety of conditions comparable to those listed above, were injected with iron and autoradiographs prepared. It was found that the same conditions which govern the redistribution of iron from the trifoliate leaf system also govern redistribution from the primary leaves as well. This explains why at high pH levels the primary leaves may be green and the trifoliate leaves fully chlorotic under iron deficiency conditions. This also explains why it is almost impossible to produce an iron deficiency chlorosis of the Red Kidney bean plant in a nutrient solution of low pH and low phosphorus concentration. The mobility of iron is sufficiently great under these conditions to allow redistribution from the primary leaves in sufficient quantity to satisfy the needs of the remainder of the plant.

Radioautographs, as used in the above experiments, are perhaps only semiquantitative in scope. However, there exists such a wide difference in

results, ranging from complete immobility of iron from the leaves of plants grown with high iron, high pH and high phosphorus content, to complete mobility of iron in plants grown with low iron, low pH and low phosphorus, that valid conclusions can be drawn.

Summary

1. Red Kidney bean plants were grown in nutrient media with varying quantities of iron and phosphorus and at pH values ranging from 4.0 to 7.0 in an attempt to determine the factors associated with the absorption and translocation of iron in the plant.

2. It has been shown that the iron salt used in the nutrient media exerts a very great effect on the association (absorbed and adsorbed) of iron with the root, but no correlation was found between the actual availability of iron to the aerial parts of the plant and (1) the precipitation rate of iron from nutrient solutions containing various iron salts, and (2) the amount of iron associated with the roots.

3. As the hydrogen ion concentration of the nutrient solution increased, the iron uptake increased. The adsorbed iron on the root also increased.

4. As the iron concentration in the nutrient solutions increased, the iron absorption also increased. This was very marked at pH 4.0 but somewhat less pronounced at pH 7.0.

5. It was found that a high concentration of phosphorus in the nutrient solution retards the uptake of iron. As the phosphorus concentration was increased from that equimolar with iron (at 0.00002 M), both iron uptake into all plant parts and iron adsorption on the root decreased. As the phosphorus concentration was decreased from that equimolar with iron, iron uptake into the aerial parts also decreased, but iron adsorption on the roots was found to either decrease or increase. An explanation for the increased or decreased adsorption on the roots is offered which involves the state of the ferric oxide in the nutrient solution. It is based on the postulation that if the ferric oxide is present in the nutrient solution as a hydrosol or a crystalline precipitate, the adsorbed iron on the root decreases. But, if the ferric oxide is present as a gelatinous coagulated hydrosol, the adsorbed iron increases. The factors responsible for determining the nature of the precipitate were not fully investigated.

6. The rate of redistribution of injected iron within the plant depends on several factors as follows:

The iron concentration of the tissues is the primary factor in determining the mobility of iron. Mobility is greatest when tissue concentrations are lowest and vice versa.

The amount of phosphorus available to the plant is of secondary importance in determining iron mobility. The mobility is increased when phosphorus is low and vice versa.

The pH of the nutrient media is also of secondary importance in determining iron mobility. It is greatest when the pH is low, and least when it is high.

Each factor influencing the mobility of iron exerts its own effect regardless of the presence or absence of other factors, hence mobility is greatest at low iron concentrations, low phosphorus, and low pH. Mobility is least at high iron concentrations, high phosphorus and high pH. Intermediate conditions of the sums of all factors yield intermediate conditions in iron mobility.

7. If the phosphorus concentration of the tissues becomes too high, a chlorosis may occur which is caused by the immobilization of iron in the veins of the leaf. When this type of chlorosis occurs it is usually characterized by a persistent greening along the veins with chlorotic interveinal mesophyll. The term phosphorus induced chlorosis is a realistic designation of this phenomenon.

8. The distribution of iron in healthy bean plants tends to be uniform with slightly greater concentrations in the growing points. The accumulation of iron on the roots from a nutrient solution is heavy and may account for a large percentage of that originally added to the solution.

The Fe^{55} herein employed was obtained from the Isotopes Division of the U.S.A.E.C., Oak Ridge, Tennessee.

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