AVAILABILITY AND UPTAKE OF IRON: EFFECT OF pH CHANGES DURING UPTAKE OF MACRONUTRIENT IONS

by

Adu Asabere Opoku

A dissertation submitted in partial satisfaction of the requirements of a graduate program in Soil Science for the degree of Doctor of Philosophy

June, 1970

University of California, Riverside

DISSERTATION ACCEPTANCE AND DEPOSIT FORM

NAME Adu Asabere Opoku

DEGREE Ph. D. FIELD OF STUDY OR MAJOR Soil Science

DATE June, 1970 FORM Typed and bruning

TITLE OF DISSERTATION Availability and uptake of iron: Effect of pH changes during uptake of macronutrient ions

Approved:

Office of the Dean of the Graduate Division

Date

Physical format of dissertation approved and accepted for deposit in the University Library:

Office of the Reference Librarian

Date

The dissertation of Adu Asabere Opoku is approved:

Committee Chairman

University of California, Riverside

June, 1970

ABSTRACT OF THE DISSERTATION

Availability and Uptake of Iron: Effect of pH Changes During Uptake of Macronutrient Ions

by

Adu Asabere Opoku

Doctor of Philosophy, Graduate Program in Soil Science

University of California, Riverside, June 1970

Professor Jakob J. Oertli, Chairman

Long-term greenhouse experiments with corn (Zea mays) established that uptake of Fe from nutrient solutions may be significantly enhanced by increasing the supply of potassium. A mechanism was proposed to describe how uptake of macronutrient ions may in turn influence availability and uptake of Fe and other elements from their chelated and sparingly soluble compounds. It was hypothesized that since differential accumulation of cations is accompanied by a net release of H ions, and excess anion accumulation accompanied by a net release of OH ions, uptake of Fe, for example, will be enhanced by increasing supply of salts favoring differential accumulation of cations: The released H ions will acidify the root free space, root surfaces, and the immediate root environment; minerals on root surfaces will be dissolved and

chelated molecules will be dissociated; both reactions will liberate ions most of which will diffuse through a relatively high H ion environment to absorption sites in the roots.

The hypothesis was tested in short-term absorption experiments in which corn seedlings were allowed to absorb Fe from FeEDDHA or Fe-hydroxide sols in the presence of increasing concentrations of salts of K, Na, Li, Mg, or Ca. Uptake of Fe was highest in the presence of salts from which excess cation accumulation is known to occur and in which the largest pH drop was observed. Increasing the concentration of K, as KCl or K_2SO_4, from 0.0 to 5.0 or 10.0 mM K in unbuffered solutions or exchange resin suspensions at pH $\geq$ 6.9 resulted in considerable pH decline and increased Fe uptake. The pH decline correlated with the enhancement of Fe uptake. However, acidification to below pH of about 4.7 tended to reduce further Fe uptake. No detectable pH changes occurred in $CaCO_3$ plus Fe-hydroxide suspensions containing increasing supply of K, yet Fe uptake was enhanced during accumulation of K. Since essentially all Fe supplied as Fe-hydroxide occurred as colloidal particles or precipitates, enhancement of Fe uptake was attributed to a three-ion-contact effect: Plants accumulating excess cations released H ions which decomposed or dissolved the Fe sols or precipitates on root surfaces, thus freeing Fe ions for absorption. Theoretical calculations indicated that increasing H-ion concentration in the immediate root environment will significantly enhance liberation of Fe^{+3} from the stable chelate FeEDDHA.

Old hypotheses claiming that potassium ions in plants are directly

involved in reactions favoring translocation and utilization of Fe were replaced with one relating K uptake to subsequent reactions in the substrate.

ACKNOWLEDGEMENTS

My sincere thanks and appreciation to Dr. Jacob J. Oertli, my mentor in the very classical sense.

I am also indebted to Drs. Ellis F. Wallihan and Albert L. Page for being very easily accessible and helpful.

My heartfelt thanks to Abenaa Fosuhemaa ne ne mma atofo.

I extend my gratitude to Mrs. Marilyn A. Asbell for her diligent typing of the dissertation.

In these days when nearly all leaders or officers of institutions are accused of being impersonal and unsympathetic to the problems of the individual, I make some exceptions and extend my gratitude to representatives of the following institutions:

In the U.S.A.:

1. My sponsors, The African-American Institute (A-A.I.), for a generous fellowship and keen interest in my well-being;

2. The University of California, Riverside:

- The Graduate Division for a fellowship to cover tuition, and for administering the A-A.I. fellowship;

- Department of Soils and Plant Nutrition for making facilities available and for all types of help and friendliness.

In Ghana:

1. The Government of Ghana on whose behalf the A-A.I. fellowship was awarded, and who also paid part of my transportation;

2. The University of Ghana:

- The Vice-Chancellor for granting me study leave and much needed

financial help;

- The Dean of the Faculty of Agriculture, Dr. Seth La Anyane, and the Senior Research Officer, A.R.S.-Kade, Dr. Frank Pospisil, for their kind help.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

FeEDTA	Ferric chelate of ethylenediamine tetraacetic acid
H_4EDDHA*	(Also called EHPG by others) Ethylenediamine di(O-hydroxyphenylacetic acid)
FeEDDHA	Ferric chelate of EDDHA
ATP	Adenosine triphosphate

---

*I am very grateful to Geigy Chemical Company, Ardsley, New York, for a generous gift of this product.

INTRODUCTION

Many plant physiologists and soil scientists have sought to explain why some plants develop symptoms of Fe chlorosis even though they may be growing in substrates such as soils which are replete with Fe-bearing compounds. Many factors are now recognized as, or suspected of, influencing either absorption of Fe or Fe nutrition within the plant itself. These include the following: oxidative states of Fe, types of Fe compounds, pH, and interactions of certain ions with Fe-ions in either the substrate or the plant tissues.

Studies on interactions between Fe and other nutrient ions have often been limited to P, Ca, and the micronutrient metals. Potassium has generally earned much less attention and, even when it has been considered, the reported influence has invariably been attributed to a supposed effect on translocation and utilization within the plant. To my knowledge, the possibility that the effect of K might be indirect and limited to reactions in the substrate has never been investigated.

We therefore considered it worthwhile to investigate uptake of Fe from a point of view that plants accumulating excess cations over anions modify the acidity of the medium and that this in turn affects the amount of Fe that is absorbed by the plant. In common Soil Science parlance, our hypothesis may be re-stated simply that the accumulation of ions indirectly influences the "availability of Fe" to plants. Expressions such as "availability of Fe" and "available Fe" will be used but we wish to point out that we do so merely because such expressions

appear acceptable even though the quantity so described cannot be satisfactorily characterized or measured.

LITERATURE REVIEW

The causes and conditions of Fe chlorosis in plants have been reviewed extensively in the literature. The reviews of Thorne, et al. (1950), Brown (1956, 1961, 1963), and Hewitt (1963) adequately cover the subject and elucidate on deficiencies in our present understanding of the phenomenon.

Thorne, et al. (1950) in particular pointed out how failure to distinguish between causes and effects has led to various hypotheses concerning the reported influences of various ions in Fe metabolism in plants (Somers and Shive, 1942; Wallace and Hewitt, 1946; Brown, 1963; Hewitt, 1963). Chlorotic leaves generally contain more Mg and K, but less Ca, than healthy leaves (Wallace and Hewitt, 1946; Iljin, 1952; Kock and McDonald, 1960). This may be a result of impaired metabolism due to lack of Fe. Olsen (1958) is of the opinion that although Fe deficiency slows down plant growth, uptake of other salts continues. The elements become associated with a smaller quantity of dry matter and hence their higher concentrations in tissues. A higher concentration of K or other alkali or alkaline earth metals in chlorotic leaves would therefore be a result, rather than a cause, of the chlorosis.

But there are reports claiming that K is directly involved in the metabolism of Fe in plants. Hewitt and his associates have been the leading exponents of the idea (Wallace and Hewitt, 1946; Hewitt, 1963).

The following review will be limited to the reported effects of K on Fe nutrition. Attention will be paid to reports dealing with

experiments specifically set up to study these effects, but we will occasionally allude to incidental observations pertaining to the subject. This will lead to the proposition of a mechanism to describe how absorption of K may influence absorption of Fe, rather than the influence of K on the metabolism of Fe in a plant.

Effects of K on Fe Nutrition

The influence of K on Fe nutrition has generally been reported to be both beneficial and detrimental (Hewitt, 1963; Teng, 1965). But there have also been reports indicating that Fe nutrition of plants is indifferent to the K status of the growing medium or the plant except insofar as adequate supply of K contributes to the general satisfactory performance of the plant (Ward, 1959).

Beneficial effects

Between 1923 and 1926, Hoffer and his coworkers reported that corn plants growing in K-deficient soils exhibited symptoms of Fe deficiency, and that these plants had accumulations of Fe and Al in the nodal tissues. Applications of K fertilizers eliminated the Fe accumulation but had no effect on the Al (Hoffer and Carr, 1923; Hoffer and Trost, 1923; Hoffer, 1926). Hart (1934a,b) also reported similar accumulation of Fe in nodes of sugar cane plants grown in sand cultures. These were also eliminated by application of K. She noted that the K application caused a substantial decrease in the percentage of Fe in the roots and stems, and a slight increase in the blades. She suspected that the accumulation of Fe blocked xylem vessels and therefore hindered both

transpiration and translocation. The observed remedial or preventive effect of K application was attributed to increased transpiration rate, Fe being carried along with the transpiration stream.

Between 1949 and 1955, Hewitt and his co-workers in Long Ashton, Wales, reported on a number of sand culture experiments on the effects of K supply on Fe nutrition in potato plants (Jones and Hewitt, 1949; Jones, 1950; Bolle-Jones and Notton, 1953; Hewitt and Bolle-Jones, 1953; Bolle-Jones, 1952, 1955). A summary of their findings and conclusions was later presented by Hewitt (1963). The findings emphasized a general beneficial effect of K on Fe metabolism in the following ways:

(1) Plants growing in low Fe solutions (10^{-4} to 6.7×10^{-4} mM Fe, Fe as ferric-citrate) showed Fe deficiency symptoms much earlier in low K solutions (0 to 0.08 mM K) than plants growing in high K solutions (0.8 to 8.0 mM K).

(2) Iron chlorosis was less severe in the presence of adequate supply of K (over 0.8 mM K).

(3) Generally, K supply determined the incidence of Fe deficiency at high levels of P.

(4) Increasing the supply of K and Fe together increased the chlorophyll contents of leaves.

From these findings, Hewitt (1963) concluded that the effect of increasing K supply on Fe nutrition was due to a complex response to several effects which include the following:

(1) Increased efficiency of utilization of Fe in the synthesis of chlorophyll.

(2) Decreasing inorganic P concentration by promoting

phosphorylation in phosphokinase enzyme systems.

(3) Decreasing uptake of P, though organic P is not affected to the same extent.

(4) Increasing the translocation of Fe from roots where it was possibly immobilized by phosphate.

Contrary to Hewitt's (1963) suggestion, Franklin (1969) has reported that the presence of K in nutrient solution enhanced uptake of P from inorganic sources.

Hewitt's explanations are essentially based on how the presence of K may prevent P from occurring in plants in inorganic forms. This fits well into reports that Fe may be inactivated in plants as insoluble Fe-phosphates (Rogers and Shive, 1932; Biddulph, 1953).

Hewitt's group also noted that the Fe status of the plant governed the distribution of K within the plant and the inception of K deficiency symptoms (Bolle-Jones, 1952; Hewitt, 1963). This opinion and some others of the group are contradicted by the findings and conclusions of other workers (Ward, 1959; Wojciechowski, et al., 1967).

Detrimental and indifferent effects of K

There have also been reports indicating that increasing K supply may be detrimental to Fe metabolism, including chlorophyll synthesis. Horn and Pritchett (1962) found that springtime Fe chlorosis of centipede grass in Florida was caused by heavy applications of N and K; autumn Fe chlorosis was caused by increasing rates of K which ranged from 0.41 to 1.64 kg per 100 sq. meters. Subsequently, Teng (1965) reported that low night temperatures (-1.1°C) caused Fe chlorosis in

the grass. The severity of this chlorosis was increased by increasing K application which also decreased Fe uptake as well as the concentrations of organic acids, amino acids, and reducing sugars.

Khadr (1965), working with Poncirus trifoliata grown in nutrient solutions, reported that, in general, high K combined with low Mg decreased Fe contents of the citrus leaves. Ten years earlier, Wallihan (1955) reported that chlorotic citrus leaves were not only higher in K content but also there was a consistent increase in K with increasing Fe chlorosis. The chlorotic leaves were, however, lower in Ca content, but there was no consistent variation of Ca content with grades of chlorosis.

Wojciechowski, et al. (1967) noted that "contrary to the results of others," increasing K supply to two varieties of potatoes tended to lower the chlorophyll a contents of both varieties, and also lowered chlorophyll b content of one variety.

Some reports of detrimental effects of K supply on plant Fe concentrations, and vice versa, may have been due to a failure to recognize or emphasize evidence of dilution effects of increasing applications of K or Fe. Ward (1959) showed that increasing K supply (0 to 10 mM) to potatoes growing in nutrient solutions progressively increased vegetative growth, tuber production, and K concentrations in tissues but decreased the concentrations of Na, Ca, Fe and Cu. In 20-hour absorption studies involving soybean plants, Lingle, et al. (1963) found that at low concentrations of the major cations the effects of increasing K, Ca, Mg, or K+Rb on Fe uptake and transport was similar to the well-known Viets effect (Viets, 1944): Fe uptake and transport increased with increasing cation concentrations up to certain values beyond which

further increases resulted in reduced uptake and transport of Fe. Lingle, et al.'s report is the only one we know that was based on short-term absorption of K and Fe. All the other reports mentioned thus far in this review were based on long-term field or greenhouse experiments lasting several weeks or months. This may explain some of the differences in the various observations and conclusions.

All the workers reporting evidence of beneficial effect of K on Fe nutrition dwelt on the possible influence of K on forms and translocation of Fe as well as metabolism of Fe in plants. We find it difficult to accept the suggestions, particularly Hewitt's (1963) hypotheses on how the K status of plants may control the ratio of organic to total plant P. The last account of the studies by Hewitt's group appears in a review paper published in the well-known treatise, "Plant Physiology," volume 3, edited by F. C. Steward (Hewitt, 1963). Thus the explanations have been highly publicized and possibly accepted without much question. We therefore consider it worthwhile to examine how absorption of K may influence the forms and translocation of Fe in plant tissues.

Translocation and Forms of Fe in Plants

We again recall that Hoffer's group and Hart reported evidence of accumulation of Fe in nodes of corn, while Hewitt's group believed that Fe was immobilized in roots, not on roots, and that in both instances increasing the supply of K resulted in improved translocation of Fe. Such an involvement of K in Fe translocation is probably plausible because of the following reasons not considered by earlier workers.

Organic acid metabolism during ion absorption is to some extent

part of a natural mechanism to maintain electroneutrality in plant tissues. Equivalent amounts of organic acids disappear when the tissues accumulate excess anions over cations, while tissues accumulating excess cations synthesize almost stoichiometrically equivalent amounts of organic acids to balance the internal electrochemical charges (Ulrich, 1941, 1942). Such organic acids as citric and malic appear in large quantities during accumulation of K (Ulrich, 1941, 1942; Kholdebarin, 1969). In a recent review on iron compounds in plant nutrition, Price (1968) called attention to reports that Fe in xylem fluid is in association, if not in combination, with citrate or other organic ligands. On subjecting the xylem fluid to electrophoretic separation, the Fe moves together with the organic ligands to the anode (Tiffin and Brown, 1962; Lingle, et al., 1963; Brown, 1966; Tiffin, 1966a,b). Besides the citrate, malate, and other organic ligands, plants contain numerous organic compounds that are also capable of complexing or chelating Fe and other heavy metal ions. The list of such organic compounds includes proteins, amino acids, purines, flavines, nucleotides, and nucleic acids.

In view of the abundance of natural chelating agents in plants, what was described as accumulation of Fe in the nodes of corn and sugar cane may not have been deposits of inorganic Fe. This also applies to reports claiming that Fe and other heavy metals may be inactivated in plant tissues as precipitated inorganic compounds, particularly as Fe-phosphates (Rogers and Shive, 1932; Biddulph, 1953; Hewitt, 1963). Läuchli and Schwander (1966) used x-ray microanalyzer to investigate the occurrence of P, K, and other elements in vascular bundles of midribs of five-week-old corn plants. They were unable to detect any accumulation

of inorganic precipitates of P, K, or Fe. What little "deposition" of P they found was considered to be organically bound in the cell walls. Price's review (Price, 1968) on iron compounds in plant nutrition is completely silent on the possibility of the occurrence of Fe as inorganic precipitates. The physiological pH of the sugar cane plants used by Hart (1934b) was 5.2 to 5.6. Under these conditions, Fe ions in excess of about 10^{-12} M would readily precipitate were it not for the presence of natural chelating agents.

On the question of unexpectedly high concentration of Fe in chlorotic leaves (Rogers and Shive, 1932; Thorne and Wallace, 1944; Teng, 1965), Jacobson and Oertli (1956) have shown that this is because the chloroplasts deteriorated prior to the arrival of Fe in the leaves; chloroplasts so deteriorated may not be rejuvenated, at least not by a belated supply of copious amounts of Fe.

Comparisons of healthy and chlorotic leaves indicate that the latter contain more organic acids (Iljin, 1951b; Kock and Morrison, 1958b), amino acids (Iljin, 1951a; Kock and Morrison, 1958a), and K (Iljin, 1952; Kock and McDonald, 1960). Teng (1965) has shown that high rates of K and of Fe stimulate production of organic acids in centipede grass. Also, Hart (1934b) showed that K starvation of sugar cane plants did not initially affect nitrate reduction but protein synthesis was curtailed after formation of amino acids. Prolonged starvation eventually resulted in lowering the concentrations of amino acids, reducing sugars, and sucrose in stems. Nevertheless, it is unlikely that K starved plants are necessarily starved, or devoid, of most of the numerous natural chelating agents. In short, we cannot talk of

deficiencies of natural chelating agents but of deficiencies of Fe in what Price calls the "iron space" (Price, 1968). This is a situation in which the concentration of natural chelate ligands exceeds that of Fe available for chelation or complexing, hence the relatively small amount of Fe may be inactivated in the sense that its chemical activity will be considerably reduced. If such inactivation can be visualized, then a case can also be made for how excess absorption of K may lead to inactivation of Fe in plants. This will occur if the plant contained only a small amount of Fe and the rate of absorption of Fe were extremely slow. The excess organic acids synthesized during accumulation of K can be imagined to reduce the activity of the small amounts of Fe present or entering the plant.

The foregoing discussions have been limited to the possible influences of K on forms and translocation of Fe within plants, as has been suggested by investigators in the field. It is, however, possible that the influence of K on Fe nutrition may be explicable by reactions in the substrate following absorption of excess K by the plant. This possibility is discussed in the following pages.

Theories of Fe Availability

The main theories seeking to explain the availability of Fe to plants are (1) the solubility theory, (2) the CO_2 theory, (3) the chelation theory, and (4) the contact mechanism theory. All four have been examined in detail by Jenny and his co-workers (Jenny, 1961; Glausser and Jenny, 1960a,b; Grunes and Jenny, 1960; Charley and Jenny, 1961). Our discussions will be limited to how the presence and absorption of K

may influence reactions reported to occur in the applications of these theories. Since the presence of K per se will not influence the solubility of the sparingly soluble Fe-bearing minerals in soils, we shall not separately describe the solubility theory. Our hypothesis will consider how K uptake will indirectly influence the solubility of the minerals.

The CO_2 theory

The concentration of CO_2 in atmospheric air is 0.03%, but the concentration in soil air may be from 0.2 to 2% or higher, and still much higher in the rhizosphere (Griffin, 1963). Under some circumstances, such a high concentration of CO_2 leads to a lowering of pH due to formation of carbonic acid. Significant reductions of pH in the rhizosphere would result in some dissolution of Fe-hydroxides and oxides and may contribute enough Fe for plant growth even in calcareous soils (Charley and Jenny, 1961).

The sources of the extra CO_2 in soil air are aerobic respiration and decomposition of roots and soil micro-organisms. Application of K fertilizers may influence CO_2 production in two ways: "salt respiration" i.e. increased aerobic respiration during salt absorption by roots and microbes, and increased plant growth which will eventually furnish more organic matter for microbial decomposition. The contribution of K to both sources of CO_2 should, however, be relatively unimportant in the CO_2 economy of the rhizosphere.

Chelation theory

Soil microbiologists tend to emphasize the possible involvement of soil microorganisms in availability of micronutrient elements to higher

plants (Alexander, 1961). Jenny and his associates, however, tend to de-emphasize this in consideration of the availability of Fe: They have shown that alfalfa plants can obtain enough Fe from sterile sand culture containing $Fe(OH)_3$ and $CaCO_3$ (Anton, et al., 1965; Pozuelo and Grossenbacher, 1965). Nevertheless, we cannot dismiss the possible importance of microbial decomposition products and similar compounds in soils.

Soils contain organic compounds that can dissolve minerals and complex the ions, thus making them available to plants. There are three main sources of these compounds, namely, (1) soil organic matter and their decomposition products, (2) excretions from soil microorganisms, and (3) excretions from living roots.

Excretion of chelating compounds by roots and microbes may be influenced by the K content of the substrate, but there is no reported information to this effect. We have stated that higher plants accumulating excess cations over anions synthesize large amounts of organic acids (Ulrich, 1941, 1942; Kholdebarin, 1969). Soil algae and other soil microorganisms may react in the same manner. It is therefore possible that both the higher plants and the microbes will excrete some organic acids into the growth media. Citric, malic, and malonic acids will account for the bulk of the excretions. But these are very susceptible to decomposition by other microorganisms; therefore their importance must be limited to the contact zone between soil particles and root surfaces.

Some chelating agents improve availability of heavy metal nutrient ions in soils perhaps because the chelated compounds diffuse more easily and faster than free ions whose mobility tends to be retarded by

interactions with other ions and charged surfaces (Hodgson, et al., 1967).

There used to be two schools of thought on absorption of metal ions provided to plants as chelated molecules such as FeEDTA and FeEDDHA. The first school represented by Wallace, et al. (1955) and Hill-Cottingham (1957) claimed that the entire molecule enters the roots for plants appeared to contain equimolecular amounts of the metal and the ligand. The opposing school, represented by Brown and his associates, claimed that plants in fact absorb more of the metal than the ligand leaving the bulk of the ligand behind (Tiffin and Brown, 1959; Brown and Tiffin, 1960). The laboratories of the leading exponents of the first school have more recently obtained data indicating that plants exposed to FeEDDHA absorb more Fe than the ligand (Jeffreys, et al., 1961; Hill-Cottingham and Lloyd-Jones, 1965); but some add that this is true only in the case of Fe-starved plants (Hill-Cottingham and Lloyd-Jones, 1965). These observations have led to the more generally accepted mechanism involving separation of the metal ion from the ligand somewhere on the roots and that perhaps the ligand is absorbed separately and more slowly than the metal ion (Tiffin and Brown, 1959; Brown and Tiffin, 1960; Hodgson, 1968, 1969).

Later we will discuss how K supply to plants can indirectly influence the separation of the metal ion from the ligand.

Contact mechanism theory

The contact exchange theory as proposed by Jenny and Overstreet (1938, 1939) postulates the direct exchange of ions on colloidal

surfaces brought into intimate contact. Ions on one surface exchange for others when oscillation volumes of both sets of ions overlap. Water may be present in the system but it is not essential; all that is necessary is the intermingling of the oscillation volumes (Jenny, 1953). The theory covers all colloidal systems. Soil colloids and plant roots together form just another colloidal system to which the theory has been applied, specifically, to describe transfer of ions from soils to plant roots. Thus, according to the theory, nutrient ions are transferred from soil colloids to root surfaces without the help of the soil solution or soil water, and therefore uptake of adsorbed ions is essentially independent of the water content of the soil (Jenny, 1953). However, recent studies of Jenny (1966) indicate that liquid boundary layers, Nernst films, surrounding roots modify ion uptake phenomenon and that contact effect is a limiting case, being important only in infertile soils or very dilute nutrient solutions.

The importance of root-soil contact in plant nutrition is demonstrated by experiments indicating that roots absorb more cations from clay suspensions than from equilibrium filtrates (Scheuring and Overstreet, 1961; Jenny, 1966). Glauser and Jenny (1960a) have also shown that Fe nutrition of alfalfa plants in calcareous soils is related to the number of Fe hydroxide or oxide particles which are in close proximity to the root surface. The mode of ion transfer to roots in these instances may be by contact exchange as postulated by the original theory and/or liquid diffusion. In the case of Fe uptake from calcareous soils, the soil solid phases would not bear exchangeable Fe ions (Thorne and Wallace, 1944); therefore the importance of solid-root

contact deserves special consideration.

The early reports on the cation exchange theory schematically pictured plant roots as being saturated with H-ions which are replaced by K, Na, Mg, or Ca ions adsorbed on adjacent clay particles (Jenny and Overstreet, 1938, 1939; Jenny, 1953). Jenny and his associates have also examined interactions between H-saturated colloids and surfaces of sparingly soluble Fe minerals not necessarily covered with exchangeable Fe ions. With the aid of x-ray and differential thermal analyses, they demonstrated that H-amberlite resin (the H saturated form of Amberlite IR 100) easily flocculated and "decomposed" Fe-hydroxide sols (Grunes, 1951; Grunes and Jenny, 1960). The amberlites of K, Na, and Mg did not "decompose" the sols. Hydrogen-roots, dead alfalfa roots saturated with H-ions, "decomposed" the Fe-hydroxide sols even in the presence of $CaCO_3$ (Grunes and Jenny, 1960; Charley and Jenny, 1961). Furthermore, the freed Fe-ions diffused into the roots but diffusion rates were lower in the presence of $CaCO_3$.

Although the early reports on the contact exchange phenomenon recognized that H ions on growing roots were metabolically produced, there has not been any attempt to relate H-ion production to availability and absorption of Fe and other nutrient ions from solid minerals.

CATION ACCUMULATION AND AVAILABILITY OF IRON: A HYPOTHESIS

We have referred to organic acid metabolism in tissues as part of a mechanism to maintain electroneutrality during differential accumulation of ions. Excess accumulation of anions is accompanied by the release of OH and/or HCO_3 ions, while excess accumulation of cations is accompanied by release of H-ions into the growth medium (Ulrich, 1941, 1942).

The Hypothesis

For plants accumulating excess K, the pH of an unbuffered nutrient solution will decrease considerably; whereas such pH changes would not be noticeable in a well-buffered medium such as a soil, the H-ions would nevertheless influence the stabilities of compounds in micro-regions in roots free space and between roots and the medium: Minerals will be "decomposed" and metal ions will separate from the minerals or chelated molecules. Ions such as Fe^{+3} will be freed and made more susceptible to absorption by plants. We propose that this is how increasing the supply of readily absorbed cations enhances availability and absorption of Fe and other micronutrient heavy metals from solids and chelated compounds. The effect of the major cations is indirect; it is exerted only through the action of H-ions released during their differential accumulation.

Discussion on the Hypothesis

The hypothesis deals with only the action of H-ions in the release and transport of ions from somewhere on the root surface to a hypothetical absorption site in the roots. Other mechanisms may be employed to describe the actual absorption process.

Since solid soil particles do not permeate into the root free space, the contact zone is placed at the surface of the root mucigel which covers the root. Jenny and Grossenbacher (1965) have published electron micrographs showing this. They also suggested that the root surface and soil particles are bound together through carboxyl groups of the cell walls or mucigel. Metabolically produced H-ions will cause separation of metal ions from chelated compounds or minerals. Metal ion separation from minerals is here considered to be the same as mineral decomposition or dissolution but not an exchange reaction. Some freed cations will diffuse into the soil system and precipitate again, but a relatively large amount will reach the root surface because of its close proximity. The cation may first become an integral part of the roots, and subsequently move by diffusion, exchange and/or solution, through the cell wall to absorption sites. Re-precipitation will be minimal since the short diffusion path will be a relatively high H-ion environment if H-ions are being released from the roots.

There is no satisfactory method for measuring the H-ion activity or concentration within the short diffusion path or at the zone of root-solid contact. Measuring pH on root surfaces by touching roots with electrodes gives questionable results because of the influence of the

junction potential on the measurement (Williams and Coleman, 1950; Jenny, et al., 1950). Furthermore, the pH of the medium of growth is not representative of the H-ion environment on the roots: The H-ion concentration of the medium represents only the dilute portion of the electric double layer on the negatively charged root surface. According to Burd (1947), the normal escape of CO_2 from plant roots does not decrease pH of the soil solution, but it enhances the acidity of the root-soil interface.

The reports of Riley and Barber (1969) and Farr, et al. (1969) give an idea of the pH and ionic environment of soils near roots absorbing various ions. Riley and Barber (1969) compared the pH and ionic concentrations of water extracts of the following samples from potted soil containing soybean plants: non-rhizosphere soil, i.e., original soil, rhizosphere soil, and root plus rhizoplane soil, also called the root-soil interface. The root-soil interface showed a high accumulation of HCO_3 and an increase in pH as compared to the non-rhizosphere soil. These were closely related to the level of NO_3 in the soil solution and were attributed to greater uptake of anions than cations. Farr, et al. (1969) measured ion concentration gradients in small sections of soils near roots of onions manured with KCl and $CaCl_2$. The concentration of exchangeable H increased toward the root axis while exchangeable K and Ca decreased in that direction. The existence of such concentration gradients must also be due to root activity; K and Ca being absorbed while H-ions are being released into the medium. The concentration of exchangeable H in the first millimeter from the root axis was about six times the concentration prevailing in the bulk soil and must also have

been much higher in the free space and contact zone. We need not emphasize the influence of a high H-ion concentration on solubility of such Fe minerals as Fe-hydroxides and oxides. The stability of FeEDTA is also greatly influenced by pH changes (Oertli, 1956; Chaberek and Martell, 1959). We shall therefore discuss only the stability of FeEDDHA, another widely-used chelate compound.

Liberation of Fe from FeEDDHA

Chelating agents or ligands such as $EDDHA^{-}$ have a strong affinity for both H and metal ions and both cations compete for the ligand (Chaberek and Martel, 1959; Korman, 1960). But increasing the H-ion concentration in the system results in displacement of the metal ion from the chelate molecule according to the general equation

$$Fe^{+3} + H_4A \rightleftarrows FeA^{-} + 4H^{+}$$

where H_4A here represents the acid form of the ligand $EDDHA^{-4}$ and FeA^{-} represents the chelate specie in the system. The acid dissociation constants of all four forms of A^{-4} are available (Frost, et al., 1958; Chaberek and Martell, 1959). The stability constant of FeEDDHA is taken to be 10^{33} (after Lindsay, et al., 1966). The compound is said to remain "essentially" stable throughout the pH range of about 4 to 8.3 encountered in agricultural soils (Kroll, 1957; Lindsay, et al., 1966). But Bhan, et al. (1962) and Jeffreys, et al. (1961) have shown that plants obtain more Fe from FeEDDHA at pH 4.5 or 5.0 than at 7.0 or 8.5.

Tiffin and Brown (1959) have demonstrated that Fe separates from the ligand somewhere on the roots, and the cation is taken up separately

from the ligand. They have further suggested that susceptibility of plants to Fe deficiency is related to the metabolic process involved in the separation of Fe from the chelate compound (Brown and Tiffin, 1960). We suggest that H-ions released from roots will favor dissociation of FeEDDHA on root surfaces. One can calculate the concentration of Fe^{+3} released at various equilibrium pHs. This has been done for FeEDTA and other chelate compounds (Oertli, 1956; Chaberek and Martell, 1959). Details of a procedure are given in Appendix 1. We have used the procedure to calculate Fe^{+3} in equilibrium with two different systems, i.e., preparations, of $9x10^{-5}$ M FeEDDHA:

System #1 represents a preparation made by reacting equal concentrations of Fe^{+3} and $EDDH^{-4}$;

System #2 represents one made by reacting $9x10^{-5}$ M Fe^{+3} with 1% excess $EDDHA^{-4}$.

The results of the calculations are presented below:

	Equilibrium Conc. of Fe^{+3}, M/liter	
pH	System #1*	System #2*
3	$3x10^{-6.56}$	$10^{-6.12}$
4	$3x10^{-8.56}$	$10^{-10.12}$
5	$3x10^{-10.56}$	$10^{-14.12}$
6	$3x10^{-12.56}$	$10^{-18.12}$
7	$3x10^{-14.22}$	$10^{-21.44}$
8	$3x10^{-15.72}$	$10^{-24.44}$
9	$3x10^{-17.04}$	$10^{-27.08}$

*System #1: Prepared by reacting $9x10^{-5}$ M Fe^{+3} with $9x10^{-5}$ M $EDDHA^{-4}$.

System #2: Prepared by reacting $9x10^{-5}$ M Fe^{+3} with $9.09x10^{-5}$ M $EDDHA^{-4}$. Note that we begin with 1% excess ligand.

For System #1, the Fe^{+3} concentration at pH 7, for example, is about a millionth of the concentration at pH 4. The data, therefore, indicate that decreasing pH enhances the liberation of Fe from the apparently stable FeEDDHA and can therefore enhance uptake of Fe, especially if the liberation occurs on the root surface or within the root free space. Our hypothesis therefore reconciles the following observations in plant nutrition:

(1) differential accumulation of cations is accompanied by release of H-ions from roots,

(2) increasing H-ion concentration enhances uptake of Fe from both minerals and chelate compounds,

(3) Fe chelate compounds liberate Fe ions on root surfaces prior to absorption of the cation.

MATERIALS AND METHODS

Seedlings of corn, Zea mays var. Golden Gross Bantam Sweet Corn, were used for all studies. Seeds were purchased from the Burpee Seed Company, Riverside, California.

The studies are grouped into long-term and short-term experiments.

The long-term experiments were conducted in a temperature-controlled greenhouse, and the absorption periods lasted from seven days to as long as four weeks, not counting the duration of any pre-treatment absorption.

The short-term experiments were carried out in a temperature controlled laboratory, and the duration of the absorption period was never more than 48 hours.

The materials and methods used in the two conditions are separately described below, but some methods and materials common to both environments are first described.

Demineralized water was used for preparing and topping nutrient solutions in the greenhouse studies. Double distilled water, that is, laboratory distilled water re-distilled in a Pyrex glass still, was used for preparing all solutions used in the short-term studies and for all chemical analysis.

Reagent grade salts were used throughout the studies.

Polyethylene containers were used as culture solution containers. Glass containers were used for only short periods during the pre-treatment culture prior to the short-term absorption studies.

Corks, wooden lids, and all metal and wooden parts of plant holders

were coated with water-repellent marine varnish and were always scrubbed and rinsed before every use.

Filtered compressed air, through small nylon tubes, provided continuous aeration in all culture solutions.

Two types of Beckman pH meters were used to measure pH of nutrient solutions: a portable type was used in the greenhouse studies while the Expandomatic type was used in the short-term laboratory studies.

All treatments or their combinations were run in duplicate.

Long-term Greenhouse Experiments

Soaked corn seeds were placed on perlite and left in a mist (tap water) chamber for periods ranging from 7 to 14 days. Identical seedlings were transplanted into culture solutions as described below.

Roots were carefully washed free of the perlite particles and the seedlings inserted into a cork. A piece of water repellent cotton wool held the stem to the cork. The cork was inserted into a hole in a wooden lid placed on top of a polyethylene container containing the nutrient solution.

The experiments covered in this section were conducted between May 15 and September 1, 1968.

Composition of treatment nutrient solutions

Potassium and Fe levels were the variables in the first two experiments while P levels were additional variables in a third. K was supplied as KNO_3, Fe as FeEDTA, and P as $NH_4H_2PO_4$. The treatment solutions were 54 liters of half-strength Hoagland Solution #2 containing

combinations of various levels of salts of K, P and Fe. The composition of the complete Hoagland Solution #2 is as follows (Hoagland and Arnon, 1950):

Salt	Concentration mM
KNO_3	6
$NH_4H_2PO_4$	1
$Ca(NO_3)_2$	4
$MgSO_4$	2

The concentrations, in ppm, of the micronutrient elements, excepting Fe but including chloride, are as follows:

Boron	0.5
Manganese	0.5
Zinc	0.05
Copper	0.02
Molybdenum	0.01
Chloride	3.0

The pH of the 54 liters treatment nutrient solution was set at 6.5 and was adjusted daily with 1 N NH_4OH.

Details of each of the three experiments are presented below.

Experiment #1. Sets of four seven-day-old seedlings were transplanted into nutrient solutions consisting of all 12 possible combinations of four levels of K and three levels of Fe. The levels were as follows:

K_1 - 0.075 mM K^+ Fe_1 - 0.5 ppm Fe

K_2 - 0.15 mM K^+ Fe_2 - 2.5 ppm Fe

K_3 - 0.30 mM K^+ Fe_3 - 5.0 ppm Fe

K_4 - 3.00 mM K^+

The plants were harvested 28 days later.

Experiment #2. Sets of three 14-day-old seedlings were raised in seven liters of full strength Hoagland Solution #2 for 14 more days; Fe was provided at a concentration of 2.5 ppm. Sets of two seedlings were then transplanted into treatment nutrient solutions of the same levels (of K and Fe) and combinations as in Experiment #1 above. The plants were harvested 11 days later.

Experiment #3. Sets of four eight-day-old seedlings were raised in nine liters of approximately full-strength Hoagland Solution #2 for 21 more days. The solutions also contained Fe at a concentration of 5.5 ppm. Sets of two seedlings were then transplanted into treatment nutrient solutions containing each of all 12 possible combinations of three levels of P, and two levels of K and of Fe. The levels of the nutrients were as follows:

P_1 - 0.5 mM H_2PO_4 K_1 - 0.15 mM K Fe_1 - 5.0 ppm Fe

P_2 - 2.0 mM H_2PO_4 K_2 - 3.00 mM K Fe_2 - 7.5 ppm Fe

P_3 - 4.0 mM H_2PO_4

The plants were harvested eight days later.

Harvesting and handling

The youngest four leaves of sets of plants in a container were sampled together for analysis; each leaf was split along the midrib and one-half placed in a paper bag and marked for potassium determination. The other half was scrubbed with detergent solution (Joy), rinsed several times in running demineralized water, and placed in a paper bag. These washed half-leaf samples were dried for determination of Fe.

Roots and the remaining parts of the tops of the sets of plants in a container were placed in separate paper bags for drying. All samples were dried in a forced draft oven at a temperature of 60°C for at least 48 hours, then weighed to the nearest tenth of a gram.

Chemical analysis

Potassium. Leaf samples for K determination were ground in a Wiley mill. Thirty mg samples were weighed into 60 ml test tubes and extracted with 50 ml of double distilled water for 12 hours. K in the filtrate was determined on a Beckman D.U. flame photometer.

Iron. An adaptation of the O-phenanthroline method of Sandell (1950) was used to determine Fe in leaf samples.

The washed half-leaf samples were ground in a chromium-plated mill and 200.0 mg samples weighed into 20 ml acid washed test tubes calibrated at 10 ml. One ml aliquots of concentrated H_2SO_4 were added and the samples digested on a hot plate. Occasional additions of drops of H_2O_2, followed by heating, hastened the digestion. The clear digests were allowed to cool to room temperature and the walls of the test tubes washed down with 2 ml of double distilled water.

The red iron-O-phenanthroline color was developed by adding 1 ml of each of 10% NH_2OH-HCl and 0.1% aqueous O-phenanthroline solution, and then enough concentrated NH_4OH to raise the pH to 5-6. The solution was allowed to cool to room temperature and finally made up to 10 ml. The absorbance of the solution was read, 30 minutes later, at 508 mμ using a Bausch-Lomb Spectronic 20 colorimeter. The concentration of Fe was calculated from a standard curve. The Fe content of the leaves will be presented as ppm Fe.

Short-Term Experiments

The short-term absorption studies were carried out in a large laboratory with a temperature of 29°C ± 1° and a relative humidity of about 40%. Although these temperature and humidity values often prevailed throughout the short absorption periods proper, occasional breakdown of the air conditioning system during the pre-treatment raises some questions about comparisons between data from differing cropping periods.

Bunches of six ten- to eleven-day-old corn seedlings were used for the nutrient absorption. They were raised and prepared as follows:

Seeds were washed and soaked in continuously aerated double distilled water for 24 hours, then placed between two layers of blotting paper on a perforated polyethylene sheet mounted on a wooden frame. The frame was placed on top of a 12 liter polyethylene container filled with about eight liters of water so that the seeds were held about 2.5 cm above the water level and were kept moist by the blotting papers, the margins of which dipped into the water. The set-up was covered with a wooden lid. The seeds remained in this assembly for 48 hours, after

which those with radicles measuring more than 2.5 cm long were transplanted into perforated polyethylene platforms resting on eight liters of solution in a polyethylene dishpan. The polyethylene sheet just touched the solution.

The solution contained

0.2 mM $Ca(NO_3)_2$

0.1 mM $MgSO_4$

0.1 mM $NH_4H_2PO_4$.

The assembly was placed under fluorescent light giving about 480 foot candles on top of the container. The solution was replaced two days later. Four days later (i.e., seven to eight days after germination) sets of six identical seedlings in the third leaf stage were removed and bunched together at the base of the stems with masking tape. Each bunch was inserted into a polyethylene lid and fastened to it with more tape. The assembly was then placed on 900 ml wide mouth mason jar filled with 850 ml solution containing

0.02 mM/liter $Ca(NO_3)_2$

0.01 mM/liter $MgSO_4$

0.01 mM/liter $NH_4H_2PO_4$

0.01 mM/liter KCl

plus 0.15 ml of Hoagland's micronutrient stock solution.

The assembly was placed under a bank of cool light tubes giving 1800 ft. candles. The plants remained in this set-up until the third day when they were transplanted into the treatment culture solutions (see below). At this time, the roots measured about 40 cm and the tops 25 cm. Roots were rinsed in two changes of double distilled water just

before transplanting into the treatment solutions.

Preparation of nutrient solutions

Iron was provided in treatment solutions as either FeEDDHA or $FeCl_3$ labelled with ^{59}Fe at activities ranging from 0.1 to 2 μc per two liters. The other salts in the solutions will be mentioned at the appropriate places.

The volume of treatment solutions was two liters. The solutions were prepared at least six hours before commencement of absorption, but pH adjustments were delayed until just before commencement of the experiments. Initially, dilute NaOH solution was used to raise solution pH to what will be called initial pH of solutions and KOH was used where Na was a treatment ion, but toward the end of the studies saturated $Ca(OH)_2$ were used in all pH adjustments. Except for one experiment, no attempts were made to adjust pH of solutions during the absorption period.

The absorption experiments were always carried out under the fluorescent light giving 1800 foot candles.

Preparation of resin suspensions

In one experiment, we studied the influence of an ion exchanger and K supply on uptake of Fe. The exchanger was Bio-Rex 70, a carboxylic type cation exchange resin with cation exchange capacity of 10.2 meg. per gram dry weight and an actual wet mesh of minus 325. It was supplied by Calbiochem, Los Angeles, California. Samples of the Na-resin were converted to the H-resin by washing twice, by centrifugation and decantation, with a total of 30 symmetries of HCl. Excess HCl was

removed by several washings with double distilled water. The resin was assumed free of HCl when the supernatant water tested free of Cl as determined by reaction with $AgNO_3$ solution. A water suspension of the H-resin was made and aliquots used in preparing nutrient suspensions described below.

An aliquot of acidified $FeCl_3$ solution calculated to give 10^{-4} M Fe per liter was pipetted into continuously aerated 1900 ml of water. This was followed by an aliquot of $^{59}FeCl_3$ solution. The mixture was allowed to equilibrate for about one hour, then the required aliquot of resin suspension was added to give 1.0 or 5.0 meg resin per two liters. This was followed, an hour later, by $CaCl_2$ solution to give 0.025 mM Ca per liter, and then by KCl solution to give the desired K concentration which ranged from nil to 5.0 mM. The pH of the nutrient suspension, which at this point were 3.8, was raised to 7.0 with $Ca(OH)_2$ and the solution made up to two liters. Aliquots were then pipetted into tin planchets* and test tubes. The suspensions were centrifuged and aliquots of the supernatant solutions pipetted into tin planchets. Qualitative tests for Fe were also made on aliquots of the supernatant solutions: The tests were intended to find out whether any Fe, radioactive or not, remained in the aqueous phase of the suspensions.

Harvesting, handling, and digestion

In some experiments, leaves and stems of bunches were harvested and handled together, while the two parts were handled separately in other

---

*My sincere thanks to the White Cap Co., a division of Continental Can Co., for a generous gift of special tin caps which adequately served as planchets.

experiments. The lower ends of the stems were always washed in running tap water and then rinsed in distilled water. Roots were washed in approximately 20 liters of running tap water and handled separately from the other parts. The tap water contained about 1 to 2 mM Ca per liter. Plant parts were placed in manila envelopes and dried at a temperature of 80°C for at least 48 hours. They were then cut into pieces (while still in the envelopes) with a pair of scissors, weighed to the nearest tenth of a milligram, and carefully transferred into 50-60 ml test tubes calibrated at 40 ml. One ml of concentrated H_2SO_4 was added, and the samples digested on a hot plate. Additions of drops of H_2O_2 hastened the digestion.

Toward the end of the studies, it was realized that the amount of H_2O_2 used could be considerably reduced by first ashing the materials in a mixture of H_2SO_4 and HNO_3. The method was therefore modified by first adding 2 ml of concentrated HNO_3 immediately after the addition of H_2SO_4. The mixture was then heated on a hot plate until the digest turned black. Two separate additions of 1 ml of the HNO_3, each followed by heating, turned the digest clear brown. Drops of H_2O_2 were used to complete the digestion to obtain a clear solution. Control blanks were run to check on possible contamination.

Iron on root surfaces

The amount of Fe retained on root surfaces was determined by exposing dead roots to radioactive Fe solution, and then attempting to wash them free of Fe. Roots of bunched seedlings (six seedlings per bunch) were killed by 12 hours' exposure to ether vapor in a closed

container. Sets were then placed in wide-mouth mason jars containing 100 ml of 10^{-5} M FeEDDHA solution labelled with ^{59}Fe; the root samples were exposed to the solutions for two hours. They were then washed in running water until the washings tested free of ^{59}Fe. They were then transferred into jars containing 100 ml of the following: (1) 0.01 M NaEDDHA, (2) 0.01 M HCl, or (3) water. One set of samples was shaken on a mechanical shaker for 30 minutes; another was left in the treatment solutions for 30 minutes. All samples were finally washed in running water, dried, weighed, and digested for Fe extraction.

Extraction of Fe from plant digests

Since the available radiation detector, a Nuclear-Chicago's Low Background Counter Model 1043, is able to detect and count only beta isotopes, it was considered necessary to minimize, if not completely eliminate, incidence of self absorption. This was accomplished by extracting the Fe in the plant digest by the Bathophenanthroline method of Diehl and Frederick Smith (1965). The method is described below:

The walls of the test tube containing the clear plant digest were washed down with 3 ml of double distilled water followed by two ml of 10% NH_2OH-HCl solution, shaken, and allowed to cool to room temperature. Four ml of 0.001 M Bathophenanthroline solution (in 1:1 ethyl alcohol and water) was added and shaken. This was followed by enough concentrated NH_4OH to raise the pH to 5-6, as indicated by a piece of Congo Red test paper. The red solution was then covered and allowed to cool to room temperature. Nine and one-half ml of iso Amyl alcohol was then added and the content shaken vigorously. Enough water was then added to

bring the total liquid volume to 40 ml, shaken and left undisturbed for about 10 minutes.

The volume of the clear red alcoholic phase containing the Fe measured exactly 11.0 ml. Four ml aliquots were pipetted into tin planchets and evaporated to dryness in a hood. The dried contents of the planchets were essentially free of all salts except the red Fe-bathophenanthroline salt which occurred as a thin red film. Radiations on the planchets were counted twice, 10 minutes each. Aliquots of the radioactive nutrient solutions and blanks, taken prior to exposure to plants, were always counted with the samples of the Fe-bathophenanthroline extracts. The amounts of solution Fe taken up by the plants or occurring in a plant part were calculated and are presented as moles of Fe per gm dry weight.

Extraction tests on plant digests to which ^{59}Fe solution had been added indicated that the first extraction was so complete that a second extraction was unnecessary. Also, comparisons of 4, 2, and 1 ml aliquots of the 11 ml alcoholic extract showed practically no evidence of self absorption.

As stated above, the total volume of the red alcoholic Fe-bathophenanthroline extract and the aqueous phase together was 40.0 ml. Appropriate aliquots of the aqueous phase were also taken and diluted for determination of K on a Beckman D.U. flamephotometer.

RESULTS AND DISCUSSIONS

We will first present and discuss the long-term greenhouse experiments and then turn to the short-term experiments.

Plant yields and mineral compositions of plant parts are expressed on an air dry weight basis.

Long-Term Greenhouse Experiments

The results of the three greenhouse experiments, #1, #2 and #3, are presented in Tables 1, 2, and 3, respectively. The yields of the plants are given as weights of tops and roots. The tops represent the stems and leaves. The data in the three separate tables are not comparable absolutely, but it is evident that all treatment levels and combinations influenced plant growth. Differences between some of the values in Tables 1 and 2 are significant at the 5% level. These are indicated for data on top yields, Fe and K contents but not for root yields. The root yields generally followed the top yields, hence the decision not to present statistical information or discussions on roots. The coefficients of variability of the top yield data are 118.3, 28.5, and 67.6% for Experiments #1 (Table 1), #2 (Table 2), and #3 (Table 3), respectively.

Symptoms of Fe chlorosis appeared on most plants in Experiments #1 and #2 (Tables 1 and 2) and were limited to leaf Fe concentrations of less than 60 ppm.

Experiment #1

Table 1 shows that increasing solution Fe concentration from 0.5 to 2.5 ppm always resulted in considerable increases in plant growth. Some of the top yield differences were significant. Further increase in solution Fe from 2.5 to 5.0 ppm resulted in insignificant increases in plant growth at all levels of K. Symptoms of Fe deficiency were most apparent and serious at the lowest Fe level. Gradations of chlorosis were evident only at the 2.5 ppm Fe level.

Increasing Fe concentration from 0.5 to 2.5 ppm resulted in significant increases in leaf Fe, but further increase to 5.0 ppm did not. Increasing solution Fe from 0.5 to 2.5 ppm resulted in significant decrease in leaf K only at the 0.15 and 0.30 mM K levels. The decreases in leaf K were generally about 50%. These corresponded with the extent of increase of plant yields attributable to increased Fe supply.

This is similar to what Hewitt's group interpreted as evidence of Fe interference with K translocation (Jones and Hewitt, 1949; Hewitt and Bolle-Jones, 1953). We consider the evidence as an example of dilution effect of Fe application on K content analogous to the effect of increasing K supply on vegetative growth and tissue Fe content of potatoes described by Ward (1959). No dilution effects of Fe on leaf K were evident at the 3.0 mM K level, even though the first increment of Fe supply more than tripled plant growth.

Increasing solution K concentration, on the other hand, resulted in higher leaf Fe contents at the 2.5 and 5.0 ppm Fe levels when K concentration was increased to 3.0 mM. It is of interest that the increased leaf-Fe content occurred along with significant increase in plant growth.

TABLE 1. Yield and leaf contents of 35-day-old corn plants grown at various concentrations of K and Fe.[1]

Fe Conc. ppm		K Concentration, mM 0.075	0.15	0.30	3.00
0.05	Tops, gms	6.2 d*	5.4 d	12.1 cd	11.0 cd
	Roots, gms	0.8	0.5	1.3	1.1
	K, %	1.89	4.35x	4.45x	6.70w
	Fe, ppm	38	32	32	36
2.50	Tops, gms	11.8 cd	16.6 bcd	22.2 bc	36.5 a
	Roots, gms	1.4	1.3	2.2	3.5
	K, %	0.85	1.37	2.25	6.40w
	Fe, ppm	50 s	50 s	62 r	86 g
5.00	Tops, gms	16.4 bcd	21.0 bc	23.4 b	35.6 a
	Roots, gms	1.2	3.0	2.4	3.4
	K, %	0.70	1.66	2.00	6.40w
	Fe, ppm	60 rs	57 rs	67 r	88 g

[1]Mean yield of set of four plants; K and Fe contents of youngest four leaves.

*Values designated by the same letter do not differ significantly at P=0.05, as determined by the Duncan multiple range test. (These are not indicated for roots.)

This may be considered evidence of the beneficial effect of K supply on Fe uptake or nutrition, especially since some gradations of chlorosis were visible. This is also similar to the effects of K (on Fe) as reported by others (Jones and Hewitt, 1949; Jones, 1950; Bolle-Jones, 1952, 1955; Bolle-Jones and Notton, 1953).

Experiment #2

The treatments used in Experiment #1 influenced plant growth (Table 1), so we repeated the experiment with the following modification: 28-day-old plants were exposed to the treatment solutions for 11 days. The results are presented in Table 2. The treatments still influenced yields, as well as Fe and K contents of leaves. Differences between some of the yield and leaf Fe data are significant at the 5% level. Differences between leaf K contents are not significant. Increasing Fe supply did not result in very large increases in growth so the dilution effect of Fe supply on leaf K contents is not pronounced, and is not consistent (compare data in Tables 2 and 1). Increasing K supply had some significant influence on leaf Fe at 2.5 and 5.0 ppm Fe levels but not at the 0.5 ppm level. The grades of chlorosis at 2.5 ppm Fe level also followed the supply of K.

Experiment #3

The effects of various rates of P, K and Fe on plant yields and Fe contents are presented in Table 3. Differences between the values are not significant at the 5% level. Nevertheless, the following comments are still pertinent. The effect of P supply on leaf Fe was pronounced only at the low K level combined with low Fe level. Increasing P supply

TABLE 2. Yield and leaf contents of 39-day-old corn plants exposed to various concentrations of K and Fe for 11 days.[1]

Fe. Conc. ppm		K Concentration, mM			
		0.075	0.15	0.30	3.00
0.05	Tops, gms	14.2 d*	14.6 cd	16.0 bcd	17.2 bcd
	Roots, gms	2.8	2.3	2.4	2.8
	K, %	2.71	2.45	3.52	4.85
	Fe, ppm	26 z	33 z	36 z	35 z
2.50	Tops, gms	16.2 bcd	15.1 bcd	18.2 bcd	21.5 ab
	Roots, gms	2.5	2.1	2.7	3.3
	K, %	1.62	3.12	3.75	5.73
	Fe, ppm	39 yz	40 yz	52 xy	55 x
5.00	Tops, gms	16.2 bcd	20.2 abc	20.9 ab	24.0 a
	Roots, gms	2.0	2.5	2.4	3.2
	K, %	2.52	1.97	3.31	5.27
	Fe, ppm	57 x	60 wx	64 wx	72 w

[1]Mean yield of set of two plants; K and Fe contents of youngest four leaves.

*See footnote, Table 1.

from 0.5 to 4.0 mM $H_2PO_4^-$ reduced leaf Fe content, but raising K concentration corrected the detrimental effect of high P. Again, this occurred in spite of the K increment resulting in some increased growth. This agrees with the reported beneficial effect of K in correcting Fe deficiency induced by abnormal concentrations of phosphorus (Hewitt and Bolle-Jones, 1953; Hewitt, 1963). Our data, however, do not support the explanation that this beneficial effect of K might have been due to mobilization of Fe immobilized as phosphates in the corn roots or any other tissues (Hoffer and Carr, 1923; Hoffer, 1926; Hart, 1934a; Hewitt and Bolle-Jones, 1953; Hewitt, 1963).

As discussed in the Literature Review section, it is difficult to accept the hypotheses that K in the plant may accelerate translocation of Fe or help lower the concentration of inorganic P which would otherwise precipitate or immobilize Fe in plant tissues. With respect to absorption of Fe, it seems that the specific effects of K or any other cation can be better studied in short-term experiments.

The findings in the greenhouse experiments can be summarized as follows: Increasing Fe supply tended to stimulate plant growth which diluted the K concentrations in the leaves. Increasing K supply tended to result in increased leaf Fe concentration in spite of the fact that the treatments also resulted in increased plant growth. While this beneficial effect of K supply on leaf Fe content appears, the accompanying differences in plant growth make it extremely difficult, if not impossible, to assign or define a mechanism involving K in Fe nutrition. It can only be said that timely arrival of sufficient Fe in chloroplasts averts incidences of Fe chlorosis. Leaves of plants in the low Fe

TABLE 3. Yield* and Fe content** of 35-day-old corn plants exposed to various concentrations# of P, K, and Fe for seven days.

		Fe_1		Fe_2	
		K_1	K_2	K_1	K_2
P_1	Tops, gm	27.0	35.5	26.5	31.0
	Roots, gm	3.0	4.2	3.2	4.0
	Fe, ppm	75	75	71	75
P_2	Tops, gm	31.0	35.0	27.5	34
	Roots, gm	3.5	4.5	3.0	4.0
	Fe, ppm	64	70	70	74
P_3	Tops, gm	31.0	32.5	30.0	39.5
	Roots, gm	3.8	4.5	3.0	4.0
	Fe, ppm	51	73	62	74

*Mean yield of set of two plants.

**Youngest four leaves only.

#P_1 - 0.5 mM $H_2PO_4^-$ K_1 - 0.15 mM K Fe_1 - 5.0 ppm Fe
P_2 - 2.0 mM $H_2PO_4^-$ K_2 - 3.0 mM K Fe_2 - 7.5 ppm Fe
P_3 - 4.0 mM $H_2PO_4^-$

solutions did not receive much Fe, and consequently they showed acute symptoms of Fe chlorosis whether the K content of the growth medium or the leaves were high or low.

Short-Term Experiments

We recall that K was deliberately omitted from the pre-treatment solutions until the last three days before commencement of Fe absorption when it was supplied as 0.01 mM KCl. The leaves contained about 0.19 mmoles K per gram at the end of the pre-treatment period. Calcium and Mg were supplied throughout the pre-treatment period, first as 0.2 mM $Ca(NO_3)_2$ and 0.1 mM $MgSO_4$, respectively, and later as a tenth of the respective initial concentrations. Attempts to raise corn seedlings in Ca and Mg free solutions yielded very poor plants.

Seedlings were slightly chlorotic at the end of the pre-treatment periods and symptoms disappeared or accentuated during treatments. No yield differences resulted from the 48-hour experiments.

The stems always reflected the same treatment effects as the leaves. A similar observation was made on tomatoes by Riekels and Lingle (1966). This indicates that, at least for short-term studies of Fe uptake involving the use of ^{59}Fe, there may not be any exceptional accumulation of Fe in stem tissues in a manner described by Hoffer's group and by Hart (Hoffer and Carr, 1923; Hoffer, 1926; Hart, 1934a). We will therefore very often present the stem and leaf composition data as the concentration of plant tops. Toward the end of the studies we became more satisfied that leaf composition data alone were sufficient for assessment of treatment effects.

Iron on roots

It is known that Fe from both chelated and inorganic sources may precipitate on root surfaces (Biddulph, 1953; Bhan, et al., 1962). We tried to find out whether surface Fe could be removed by washing with various solutions.

Table 4 shows the concentrations of Fe remaining in association with dead roots after two hours' exposure to 10^{-5} M FeEDDHA followed by 30 minutes' washing with tap water, 0.01 M HCl, or 0.01 M NaEDDHA. Some of the root samples were shaken on a mechanical shaker during the washing process. Roots washed with only tap water retained the largest amounts of Fe, followed by those washed with 0.01 M HCl. Washing with 0.01 M NaEDDHA was the most efficient, and still more so if the suspension were shaken. This, however, did not rid roots of all the Fe acquired during the exposure to the FeEDDHA solution.

Washing with HCl was not much more effective than washing with tap water. Nevertheless, this is not contradictory to our proposed mechanism which states that H-ions from roots make Fe in solids or chelates more available for absorption. Iron entering the HCl solution or water may have been re-precipitated or re-retained by the dead roots, while Fe entering the NaEDDHA solution was chelated against re-retention by roots. Brown and his associates suggested that EDDHA and roots compete for Fe ions (Brown and Tiffin, 1960; Brown, et al., 1960, 1961). The effectiveness of EDDHA in removing much Fe from roots (Table 4) is perhaps analogous to excess ligand winning much of the competition.

Failure of the washing solutions to remove all the Fe from the dead roots may also be an indication of the affinity between Fe and roots.

TABLE 4. Concentrations of Fe remaining in association with roots washed with various solutions.

Treatments		Fe on Roots
Washing Solution	Shaken	10^{-8} moles/gm
H_2O	No	0.425
H_2O	Yes	0.351
0.01 M HCl	No	0.363
0.01 M HCl	Yes	0.349
0.01 M NaEDDHA	No	0.285
0.01 M NaEDDHA	Yes	0.185

The nature of the bonds between roots and Fe is not well understood, but Jenny and Grossenbacher (1965) suggest they may be chemical bonds between the Fe and the pectic carboxyl groups on the roots.

Franklin (1969) showed that considerable amounts of P (from radioactive $2x10^{-5}$ M KH_2PO_4 solution) were "adsorbed or precipitated" on roots (barley, corn, or soybeans) pre-treated with 1.0 mM $FeCl_3$ or $AlCl_3$ solution for one minute. Much less P were retained by roots similarly pre-treated with chlorides of Fe^{+2}, Ca, Mg, K, Na, or H. The adsorbed P was described as the "difference in retention of P between roots given a final H_2O wash and roots given a final 0.01 M HCl wash." This quantity was considerable on barley and corn roots pre-treated with Al^{+3} (Fe was not included in the comparison). The rate of subsequent P "uptake" was greatest for roots pre-treated with Al^{+3}, Fe^{+3}, and Fe^{+2} as a group. A one-minute pre-treatment was effective for more than 20 minutes. We do not agree with Franklin's distinctions between adsorbed, precipitated, and absorbed P in connection with Al and Fe pre-treated excised roots; nevertheless, his data give an idea of the extent and probable importance of trivalent cations retained on root surfaces.

It will be noticed elsewhere that living roots exposed to the same concentration of Fe but various concentrations of other cations may respond to the latter by acquiring more Fe. A large portion of the root Fe may be inside the roots, having been actually absorbed, but there is no satisfactory means of distinguishing between the amount of Fe within the root tissues and the amount retained on root surfaces. We cannot, therefore, unequivocally identify Fe data from root analysis as "absorbed Fe" or "Fe contents of roots." Instead, we shall identify any such data

as "Fe associated with roots" or "root-Fe."

Also, it is a common practice to consider values of the ratio "Fe in tops:Fe in roots" as a measure of translocation of Fe (Riekells and Lingle, 1966). Because of our observations and comments on the retention of Fe on roots, we find it difficult to place much importance on any such measures. We will therefore refrain from distinguishing between absorption and translocation of Fe.

Uptake of Fe from FeEDDHA systems

The effects of various concentrations of salts of K, Na, Li, Ca and Mg on uptake of Fe from 10^{-5} M FeEDDHA solutions were studied in a number of experiments. Except where Ca was the treatment ion, and unless otherwise stated, the treatment solutions contained additional 0.025 mM $CaCl_2$ as a source of Ca to help maintain the integrity of root cells. Also, unless otherwise stated, the initial pH of each treatment solution was 6.9, and the age of plants was 10 to 11 days.

The results and discussions of these experiments will show that increasing K supply has a greater and definitely beneficial effect on Fe uptake which can be related to H-ion release from roots.

Effects of various salts. The effects of the sulphate, chloride or nitrate salts of K, Na, Li, Ca and Mg on uptake of Fe were compared in one experiment. Each cation was provided at a concentration of 1.0 mM. The seedlings were 12 days old, and the absorption period lasted 48 hours. Figure 1 shows pH changes in the solutions, the concentrations of Fe appearing in the tops of seedlings, and the concentrations of Fe associated with the roots. Fe appearing in tops of plants exposed to

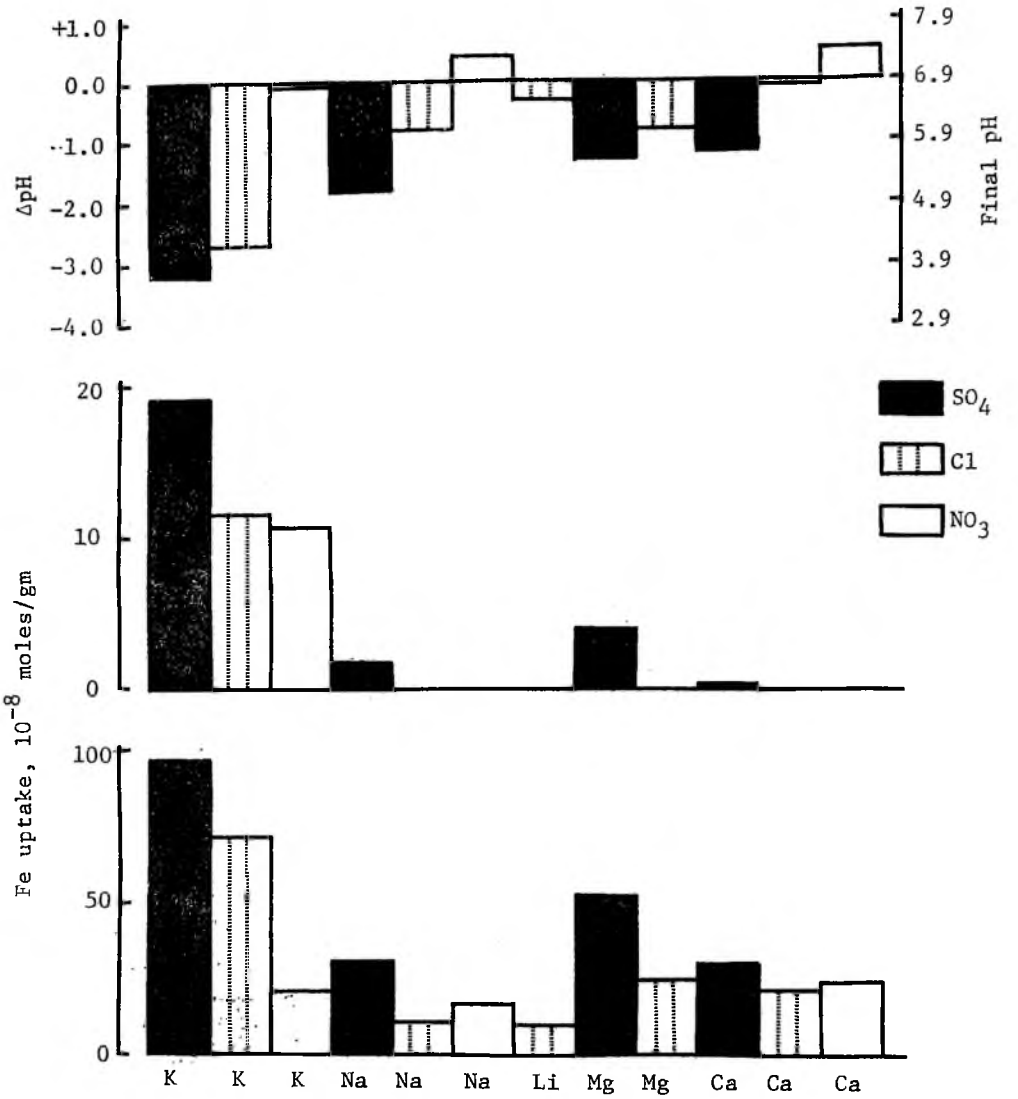

Figure 1. Effects of various salts (1.0 mM cation) on pH and uptake of Fe from 10^{-5} M FeEDDHA. (Initial pH of 6.9, absorption time of 48 hours.) Top: Final solution pH. Middle: Fe in tops. Bottom: Fe associated with roots.

the chlorides and nitrates of Na, Li, Mg, and Ca were not detectable.

The results (Figure 1) indicate that the salts have different but related effects on pH and Fe uptake. The highest uptake of Fe into tops occurred in cases where the solution pH declined considerably from the initial value of 6.9. The figure shows that the highest pH decline occurred in the sulphate solutions, followed by the chlorides, then the nitrates. The pH of the KNO_3 solutions declined only by a few tenths of a unit; those of the $NaNO_3$ and $Ca(NO_3)_2$ solutions rose slightly above the initial value.

Uptake of Fe generally reflected the pH changes, the highest uptake occurring in the salt treatments resulting in the greatest pH decline. Thus, considering only the three anions, the highest Fe uptake occurred in the SO_4 solutions, followed by the Cl, then lastly the NO_3 solutions. This is essentially the order in which the solution pH declined or changed.

Considering the cations of the sulphate salts, the highest amounts of Fe in tops were found in plants exposed to K salts followed by Mg, Na, and then Ca. The figure depicting Fe associated with roots also shows essentially the same order of the influence of the cations. Thus the order of the influences of cations on Fe uptake can be represented as

$$K > Mg > Na > Ca.$$

Although we do not have data on uptake of the ions of the treatment salts, our findings can be explained on the basis of reported relative rates of uptake of the cations and the associated anions. The order of decreasing rate of uptake of the cations can be represented by

$$K > Na > Mg > Ca$$

(Hoagland, 1923; Bange, 1959; Maas, et al., 1969). This differs from our order of their influence on Fe uptake only in the relative positions of Na and Mg.

The order of the final pH of the K solutions was

$$NO_3 > Cl > SO_4.$$

This order also reflects the rates of uptake of the anions themselves as well as their influence on uptake of associated cations (Ulrich, 1941; Hiatt, 1968; Maas, 1969). Thus, for example, the order of the rate of uptake of K or Ca from nitrate, chloride, and sulphate solutions is the same as depicted above. However, since K for example is absorbed much faster than the sulphate, this results in ionic imbalance in the cells and leads to H-ion release. Fe uptake is therefore dependent on extent of ionic imbalance rather than the rate of uptake of the cations per se. This can be argued from the fact that although the rate of uptake of K would be highest in KNO_3 solution, the highest pH decline and uptake of Fe occurred in the SO_4 solutions from which the rate of K uptake would be lowest.

Finally, we conclude that the influences of the various salts on uptake of Fe can be related to pH changes accompanying accumulation of ions. This point has been discussed in relation to our hypothesis and will be examined again in subsequent discussions on experiments in which various rates of salts were supplied to corn seedlings.

Effects of Na and Li salts. The influences of Na and Li on Fe uptake were compared in one experiment. Some plants were exposed to NaCl solutions for 24 hours while others were exposed to LiCl solutions for 48 hours. The concentrations of the salts ranged from nil to 5.00 mM.

Figure 2 shows the concentrations of Fe associated with roots of plants that had been exposed to the solutions. The amounts of test ^{59}Fe appearing in the tops of the plants were only a few counts above the background and are therefore considered undetectable. The lowest pH values were recorded in the nil and the very dilute solutions which were essentially $CaCl_2$ solutions. These were 5.4 and 5.9 for nil and 0.05 mM Na and 5.9 for nil Li. The other values were 6.0 to 6.4 for Na and 6.3 to 6.7 for Li, and were not related to the concentration of the solutions or Fe associated with the roots. It is evident in Figure 2 that Na supply has a slightly higher effect on uptake of Fe by roots, but reference to Figure 1 will show that the influences of both Na and Li are insignificant when compared with K. Corn plants prefer K to Na even if the ions are supplied separately (Bange, 1959), and Li is known to be toxic to most plants (Evans and Sorger, 1966).

Effects of Ca and Mg salts. Three sets of studies are covered in this section. Seedlings were exposed to nutrient solutions containing increasing concentrations of $CaCl_2$ or $MgCl_2$.

Magnesium supply: Table 5 shows the influence of increasing $MgCl_2$ supply, 0.05 to 5.0 mM, on the concentration of Fe appearing in leaves and stems. The final pH of the nutrient solutions are also included in the table.

Except for the 0.05 mM $MgCl_2$ level, the concentrations of Fe in leaves were almost identical with the concentrations in the stems. We will therefore refer to Fe concentration in plant tops, rather than in leaves and stems. Increasing $MgCl_2$ concentration from 0.05 mM to 0.5 mM increased Fe concentration in tops, but further increases resulted in a

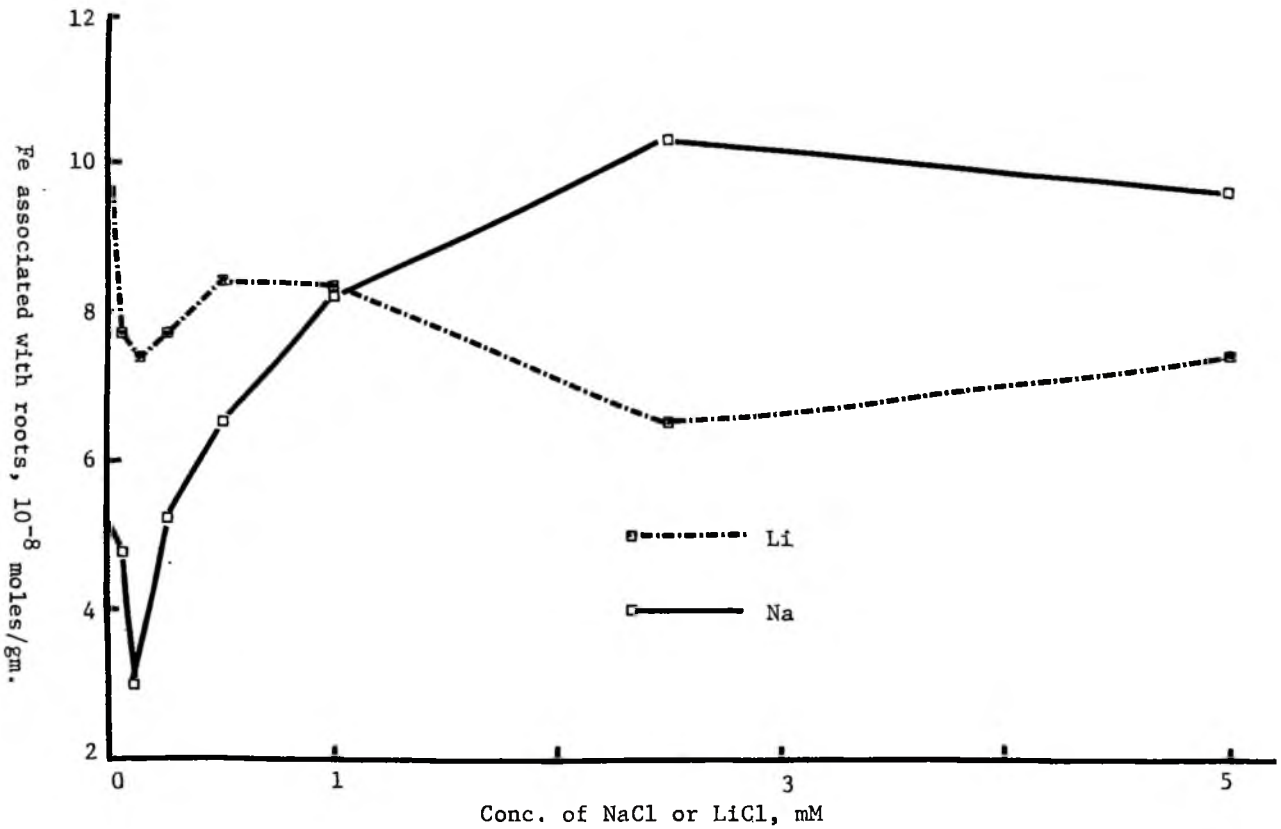

Figure 2. Effects of increasing supply of Li as LiCl, and Na as NaCl on uptake of Fe from 10^{-5} M FeEDDHA. Absorption times were 48 hours for Li and 24 hours for Na.

TABLE 5. Effects of $MgCl_2$ on Fe appearing in leaves and stems of corn. (Absorption time of 48 hours; 10^{-5} M FeEDDHA.)

Treatments $MgCl_2$,mM	Final* pH of Solution	Fe Conc., 10^{-8} moles/gm Leaves	Stems
0.05	6.1	36.8	25.9
0.50	5.4	39.2	37.1
1.00	5.3	30.6	31.1
5.00	6.2	8.2	8.6

*Initial pH of 6.9.

decline in Fe concentration. The concentration of Fe in tops of plants in the 5.00 mM treatment was about a fourth the concentration found in plants exposed to 0.05 mM. The Fe concentrations did not clearly reflect the pH of the solutions. The observations may be related to the relative rates of uptake of Mg and Cl, the latter being more readily absorbed, especially as the concentration of $MgCl_2$ is increased. This would lead to excess anion accumulation which would not favor H-ion release and consequently decreased Fe uptake.

Comparisons of effects of 1.0 mM KCl and $CaCl_2$: The effects of 1.0 mM KCl and $CaCl_2$ on the distribution of Fe in stems and leaves were compared in one experiment. Two absorption periods, 24 and 48 hours, were used. Table 6 shows the final pH of the solutions and the concentrations of Fe appearing in leaves and stems. These data also show the relationship between pH decline and uptake of Fe. Plants in the KCl solutions acidified the substrates much more than plants in the $CaCl_2$ solutions and consequently acquired much more Fe in their leaves and

TABLE 6. Effects of 1.0 mM Ca and K on Fe uptake by corn seedlings. Fe supplied as 10^{-5} M FeEDDHA.

Conc. of salt, 1.0 mM	Absorption Time, Hours	Final* Solution pH	Fe Conc. 10^{-8} moles/gm	
			Leaves	Stems
$CaCl_2$	24	6.9	6.0	8.5
	48	6.6	23.3	27.6
KCl	24	4.3	28.5	22.2
	48	4.1	69.7	32.2

*Initial pH of 6.9

stems during the two absorption periods. The differences between the concentrations of Fe in leaves and in stems are important only insofar as they reflect the rate of accumulation of Fe into leaves; uptake from the KCl solutions was more rapid and consequently considerably more Fe accumulated in leaves than in stems of plants exposed to treatment solutions for 48 hours.

Calcium supply: The data in Figure 1 and in Table 6 indicate that compared with K, the influence of Ca salts on Fe uptake is relatively insignificant. The concentration of Ca used in these studies was 1.0 mM $CaCl_2$. We therefore studied the effect of increasing $CaCl_2$ on uptake of Fe in more detail. We chose $CaCl_2$ salt because the least solution pH change occurred in this solution (Figure 1). Maas (1969) has also reported a similar observation on studies involving corn roots.

Figure 3 shows the effects of increasing concentrations of Ca (from 0.01 to 5.00 mM $CaCl_2$) on Fe appearing in plant tops, and Fe in association with roots. The final pH of the 0.01 mM $CaCl_2$ treatment was 5.9, while pH of the others ranged from 6.5 to 6.7 bearing no definite relationship to either the increasing $CaCl_2$ concentration or the amounts of Fe arriving on the tissues.

Figure 3 shows that the effect of increasing concentration of $CaCl_2$ on Fe uptake is not very definable, except that the highest Fe in tops occurred in the lowest $CaCl_2$ treatment, 0.01 mM, which also had the lowest final pH. The failure to accumulate more Fe with increasing $CaCl_2$ concentration must have been the result of a slightly higher accumulation of Cl over Ca as found by Maas (1969). The low pH of the 0.01 mM $CaCl_2$ solutions can be attributed to a higher cation accumulation

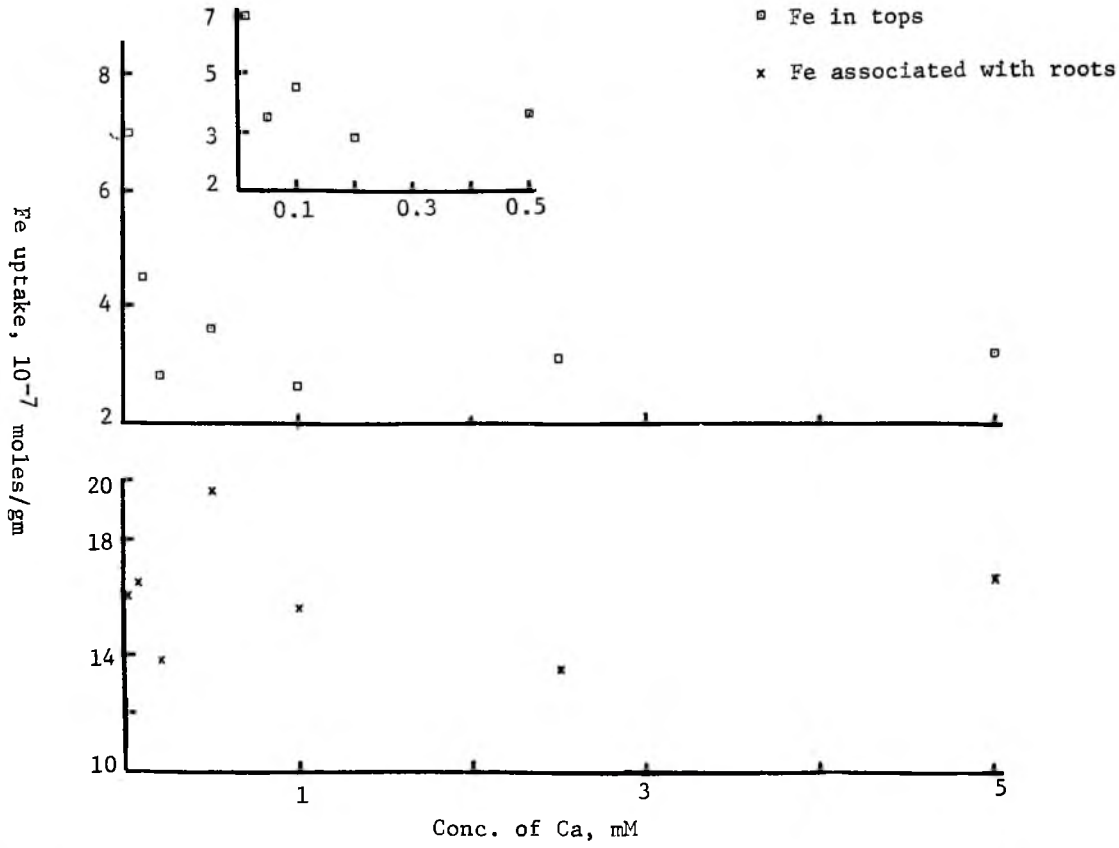

Figure 3. Effects of increasing supply of Ca, as $CaCl_2$, on uptake of Fe from 10^{-5} M FeEDDHA. (Absorption time of 48 hours. Initial pH 6.9.)

during the pre-treatment period, and the possibility that accumulation of Cl (from the 0.01 mM $CaCl_2$ solution) failed to lead to excess accumulation of total anions. It is recalled that the immediate pre-treatment solution was provided with 0.01 mM KCl.

About Fe appearing in tops, our findings agree with those of Wallace and Mueller (1962) on bushbeans but differ from those of Lingle, et al. (1963) on soybean exudates. Wallace and Mueller used 100 ml of 0, 0.1, and 1.0 mM $CaCl_2$, while Lingle, et al. used one liter of equimolar concentrations of $CaCl_2$ and $CaSO_4$ plus a fairly high concentration of all other nutrient ions. Lingle, et al. found that increasing the Ca concentration from 0.625 to 5.0 mM enhanced exudate production and "Fe uptake and translocation" but further increase resulted in drastic decline in both. They also made similar observations on the effects of Mg and of K+Rb on uptake of Fe. The use of a fairly complete nutrient solution can be justified, but the presence of ions which can separately and independently influence uptake of Fe makes interpretation of their results rather difficult.

Effects of K salts. Since the rate of uptake of K is known to be much higher than uptake of Cl or SO_4, one can expect excess accumulation of cations in plants exposed to KCl or K_2SO_4 solutions. The case of uptake from KNO_3 solutions will be examined elsewhere.

The following results and discussions deal with the effects of increasing concentrations of K on uptake or acquisition of Fe by excised corn roots and intact corn seedlings.

Excised roots: Figure 4 shows the effects of increasing concentration of K, as K_2SO_4, on the amounts of Fe and K acquired by excised corn

roots in a 24-hour period. The final pH of the solutions are also provided in the figure. The initial pH was 7.0. Calcium was provided as 0.5 mM $CaSO_4$. The concentrations of K ranged from 0 to 5.0 mM.

There is a close relationship between the parameters presented in Figure 4, namely, root K concentration, Fe associated with roots, and the final pH of the nutrient solutions. The concentrations of K in roots increased sharply as the concentration of K in the solution was increased from zero to about 0.5 mM K, and remained essentially unchanged even as the K concentration was raised to 5.00 mM. The concentration of Fe associated with roots followed the same pattern. The pH of the nutrient solutions dropped rapidly as the K concentration was increased to about 0.5 mM and seemed to drop more slowly as the K concentration was increased to 5.00 mM. It is our opinion that absorption of K indirectly affected Fe absorption or surface retention through reactions involving H ions released from the roots during accumulation of K ions.

Intact plants. pH adjustments: In nutrient solution studies of metal ion (e.g., Fe) absorption, it is a customary practice to continually adjust the pH. This is merely an attempt to minimize, or eliminate, influences of pH variation on the absorption process. We employed the practice in our greenhouse studies, but only once in the short-term studies for we were primarily interested in the effects of the pH changes per se. Furthermore, since some of our treatments always resulted in pH decline, adjusting the pH would have involved the introduction of cations in concentrations that might have interfered with the studies. We therefore decided on buffering some solutions with $CaCO_3$; these experiments will be described elsewhere, but in the

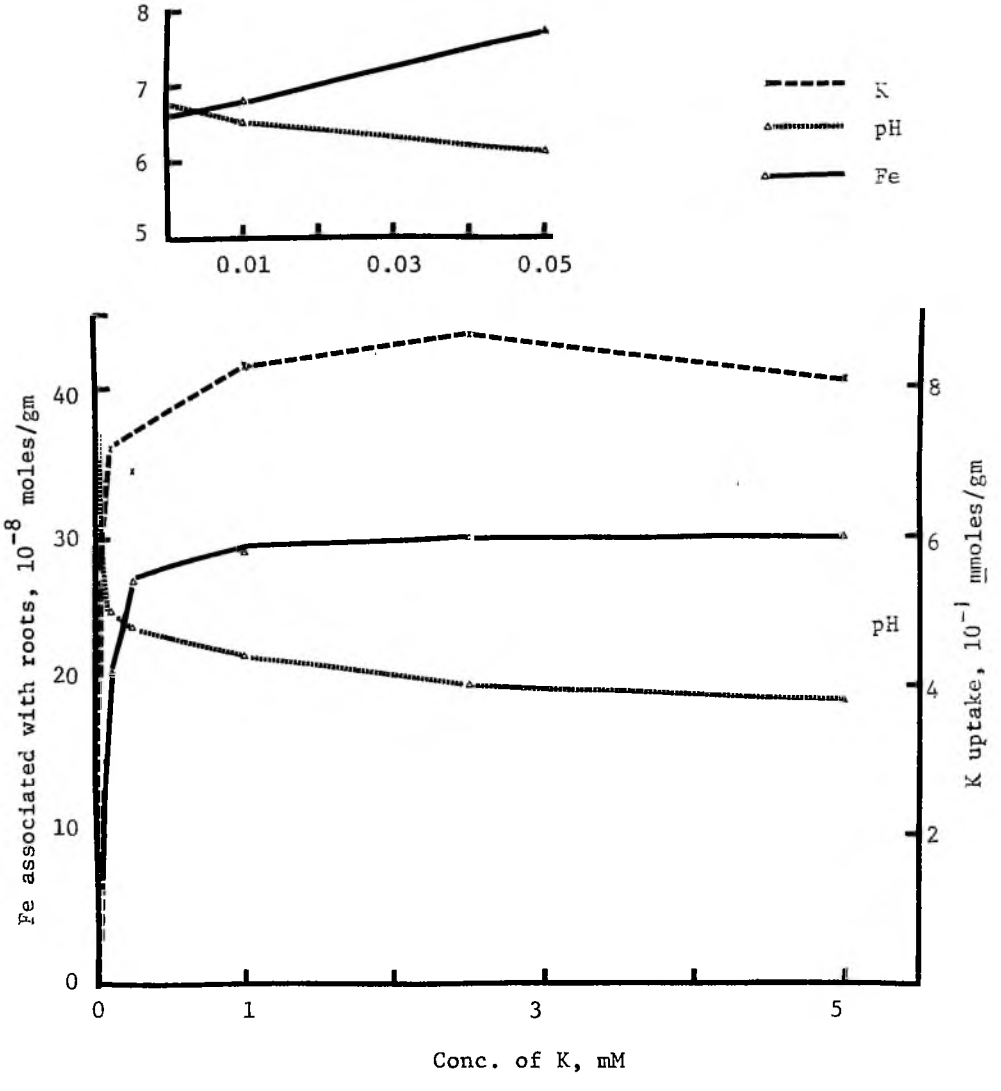

Figure 4. Effects of increasing supply of K, as KCl, on pH changes and uptake of K and Fe by excised corn roots. Absorption time of 24 hours. Fe supplied as 10^{-5} M FeEDDHA.

meantime we shall describe the one experiment in which pH was occasionally adjusted with NaOH.

Figure 5 represents data from a 12-hour absorption experiment in which the pH was adjusted every three hours, with NaOH, to 6.9. The figure represents Fe concentrations appearing in plant tops at the end of 12 hours. The concentration of K, as KCl, ranged from 0.05 to 10.0 mM.

The three hourly pH adjustments prevented the pH from falling below 6.0 during the first nine hours. Raising the pH to 6.9 at the end of the ninth hour, however, did not prevent the pH of some of the solutions (specifically those containing more than 0.5 mM K) from reading about 5.8 at the end of the 12th hour.

The figure does not indicate any remarkable influence of K supply on uptake of Fe. This may have been due to either the influence of the Na added or the shortness of the absorption period. The latter is examined below.

Absorption time. Table 7 shows the concentrations of Fe appearing in corn plant parts during 6, 12, and 24-hour absorption of Fe from solutions containing 0.5 mM $CaSO_4$ plus 0.05, 0.50, or 5.00 mM KCl. The concentrations of Fe in the tops were calculated from values obtained for leaves and stems. The initial pH of the solutions was 6.9 and was not adjusted during the absorption periods. The final pH readings are provided in the table. The data indicate that except for the 5.0 mM K treatment leaves did not contain more Fe than stems (on weight basis) until during the last 12 hours. At the end of 24 hours, Fe concentrations of the 0.5 mM K plants were nevertheless higher than in the 5.00

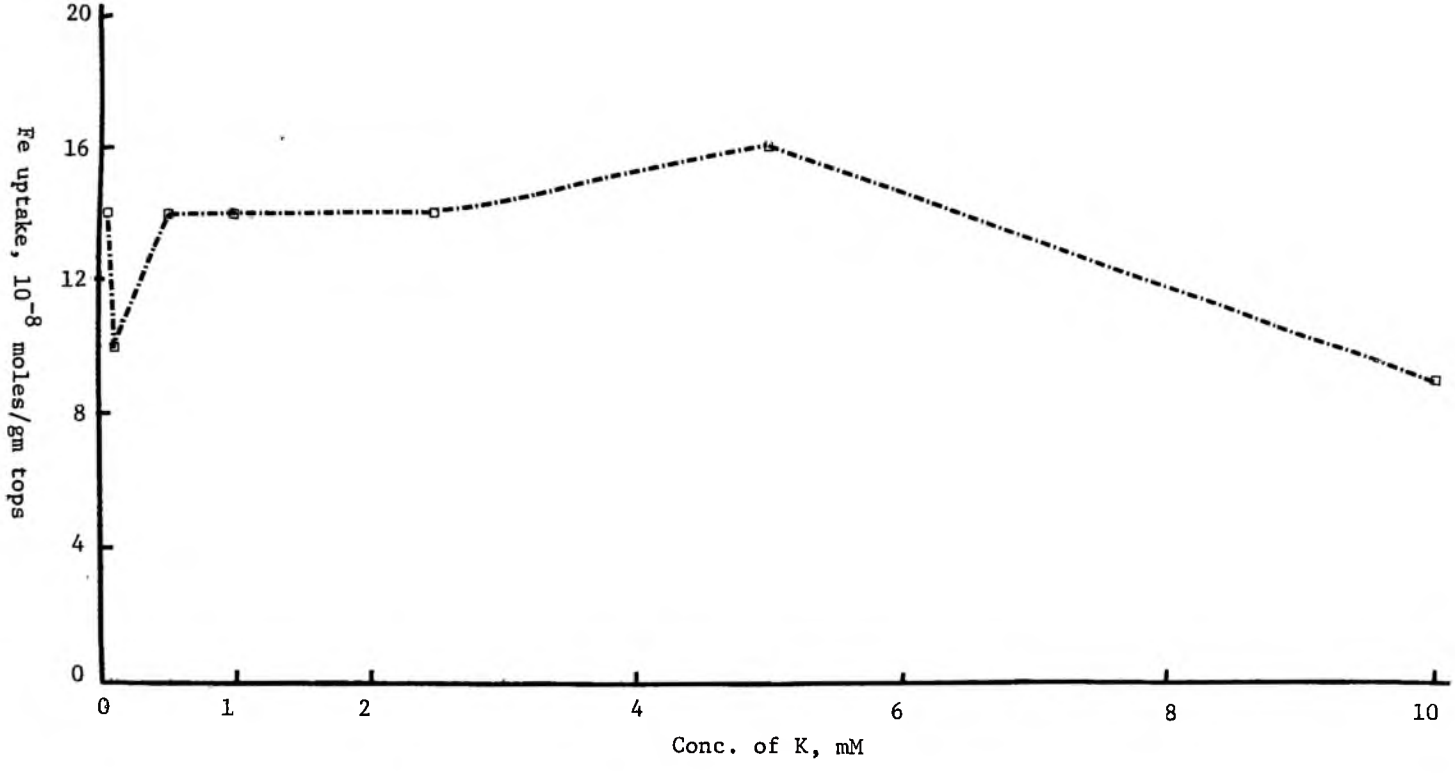

Figure 5. Effects of increasing concentration of K, as KCl, on uptake of Fe in 12 hours during which pH of solution was adjusted to 6.9 every three hours.

TABLE 7. Effects of three concentrations of potassium on the concentrations of iron appearing in corn plant parts in 6, 12 and 24 hours. (Potassium supplied as KCl and iron as 10^{-5} M FeEDDHA.)

Treatments mM K	Final* pH	Concentration of Iron 10^{-8} moles/gm tissue			
		Leaves	Stems	Tops	Roots
		6 hours			
0.05	6.8	8.0	9.4	8.6	50
0.50	6.3	9.3	9.7	9.5	50
5.00	6.2	10.6	10.4	10.5	57
		12 hours			
0.05	6.7	22.5	24.0	23.3	69
0.50	5.4	26.0	23.5	24.8	80
5.00	5.1	30.2	20.0	25.1	81
		24 hours			
0.05	6.7	52.4	46.5	49.5	101
0.50	4.7	65.4	54.0	59.7	158
5.00	4.3	55.4	41.0	48.2	91

*Initial pH of 6.9

mM K plants. Both the lower root pressure and reduced transpiration rates in the 5.00 mM treatment cannot be important since the roots acquired less Fe in 24 hours. That the low uptake of Fe in the 5.00 mM treatment occurred after the first 12 hours may have been due to the influence of the high acidity prevailing in this solution. Although higher acidity of substrate is well known to favor Fe availability and uptake (Truog, 1946; Oertli, 1956; Jeffreys, et al., 1961), the data in Table 7 indicate that Fe absorption became impaired below pH 4.7. Marschner, et al. (1966) have reported evidence of cellular and metabolic impairment of corn root tips exposed to pH less than 4.75 for brief periods. The impairment was accompanied with loss of K. Jacobson, et al. (1950) had earlier reported that impairment of injury of barley roots by high H concentration was accompanied with losses of some organic and inorganic constituents of the roots. These injuries and disturbances were reported to be reduced by the presence of Ca ions. We supplied Ca as 0.5 mM $CaSO_4$, but it seems that absorption of Fe in the 5.0 mM K solution still became impaired.

Potassium supply. The effects of increasing K concentration on the concentration of Fe appearing in tops of intact plants and in association with roots are presented in Figure 6. The figure also shows the pH of the nutrient solutions at the end of the absorption period of 24 hours. K was supplied as KCl and the concentrations ranged from 0.01 to 10.00 mM. The data indicate that the concentrations of Fe in tissues increased as K supply was increased to 0.1-0.2 mM and pH declined to about 4.7. Further increase in K concentration reduced solution pH to about 4.0. Fe in plant tissues declined, although not in direct relation

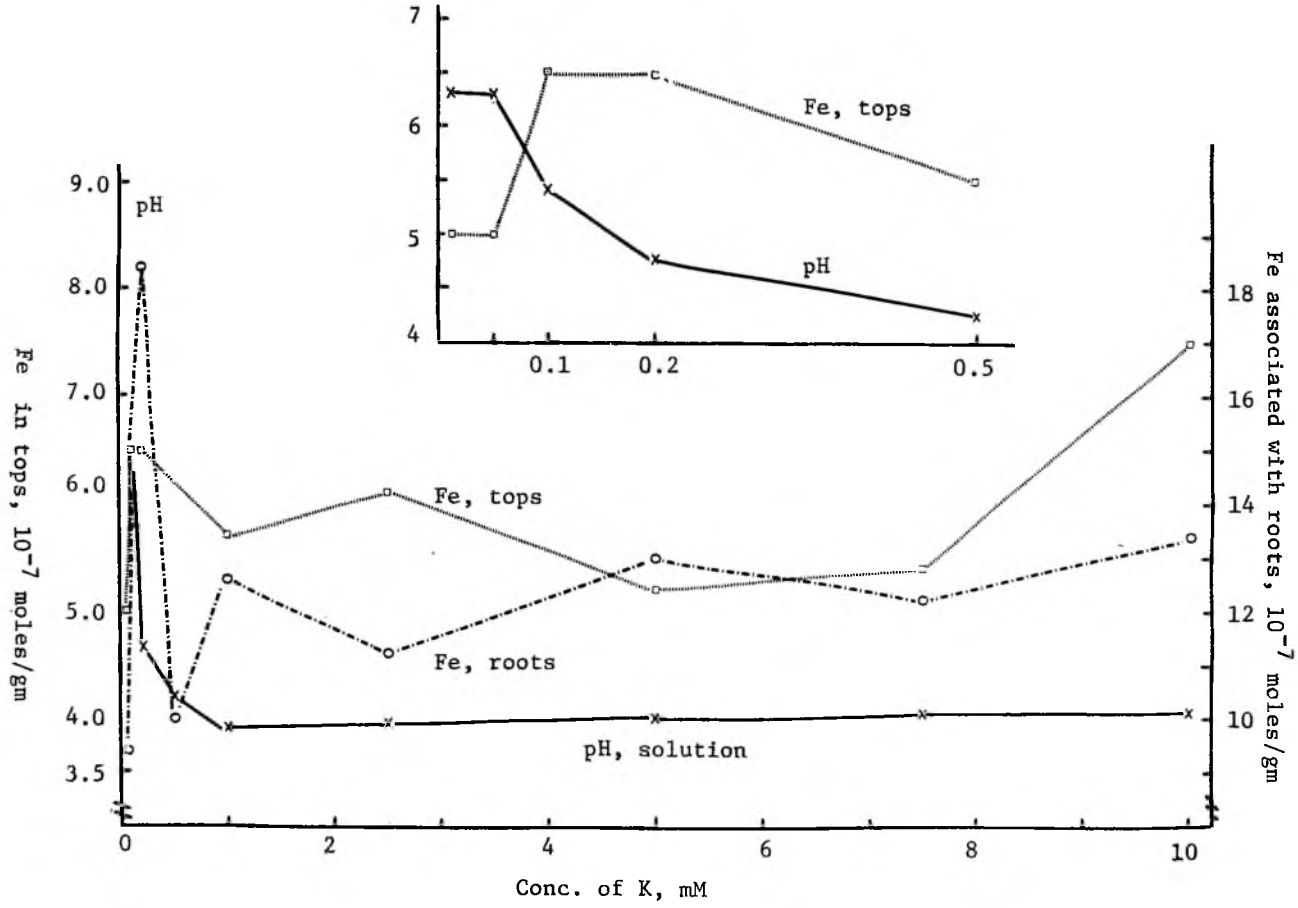

Figure 6. Effects of increasing supply of K as KCl on uptake of iron from 10^{-5} M FeEDDHA in 24 hours. Initial pH of 6.9.

to pH decline, until 5 mM K, but increased again as K concentration was raised to 10 mM while pH remained at about 4.0. The increased uptake of Fe between 7.5 and 10.0 mM K may be related to the recovery from H ion disturbance on ion uptake (Arnon, et al., 1942).

The studies on the influence of various salts indicate that the supply of K has a highly beneficial effect on uptake of Fe from FeEDDHA (Figure 1). The effect of K is, however, not always well defined. We attributed this to a possible detrimental effect of a high H concentration. Thus, although our hypothesis involves increased H ion concentration in the dissociation of FeEDDHA, the high H concentration (pH less than 4.75) itself apparently interferes with ion uptake.

We therefore studied the influence of K on uptake of Fe from inorganic Fe sources in systems of initially higher pH. The absorption period was 48 hours, so that in most cases the period would be long enough for plants to recover from any temporary H injuries.

Uptake of Fe from Fe-hydroxide systems

The following presentations deal with the influence of K supply on uptake of Fe from inorganic Fe in substrate with initial pH of 7.0 or higher. We supplied the Fe as 10^{-4} M $FeCl_3$, but centrifuging aliquots of nutrient solutions and testing for both radioactive and non-radioactive Fe in the supernatant solutions showed that essentially no Fe ions remained in the aqueous phase. This indicates that the Fe occurred in the medium as precipitates or sols of Fe-hydroxide. Most of the sols settled on the roots within an hour after transplanting and remained even when plants had reduced substrate pH to as low as 4.0. This is understandable since 10^{-4} M Fe^{+3} would begin to precipitate at about pH 2.7. We will therefore presume that

the plants were obtaining Fe from solid Fe-hydroxide precipitates and that the intimacy of contact between the solids and roots favored decomposition of the sols by H released from the roots. This assumption is based on reported evidence of decomposition of Fe-hydroxide sols by H-resins and by H saturated roots (Grunes, 1951; Grunes and Jenny, 1960).

The previous studies on the effects of the various salts on uptake of Fe did not include data on absorption of the influencing cations. We will discuss below experiments in which uptake of both K and Fe into leaves were studied, and relate the uptake of both ions to pH changes in the substrate.

pH changes with time. In view of our conclusion on the ability of roots to recover from injury or disturbances due to high H concentration, we recorded solution pH changes during 48-hour absorption periods. Figure 7 shows pH changes of two culture solutions during two 48-hour periods. The lines represent two sets of plants from two sets of cropping periods. Potassium as K_2SO_4 was provided at a concentration of 2.5 mM, Ca as 0.5 mM $CaSO_4$ plus additional 0.42 mM added as $Ca(OH)_2$ to raise the solution pH to the initial value of 9.2. The figure is provided to give an idea of the length of time in which a plant may have been exposed to what we describe as the final solution pH during a 48-hour absorption period.

Effects of K salts. Unbuffered solutions: The relationships between uptake of K and Fe into leaves are shown in Figures 8 and 9.

Figure 8 shows the influence of increasing rates of K, 0 to 5.00 mM K^+, as K_2SO_4 on the concentrations of K and Fe appearing in leaves during 48 hours. Ca was present in solution at a concentration of 0.42

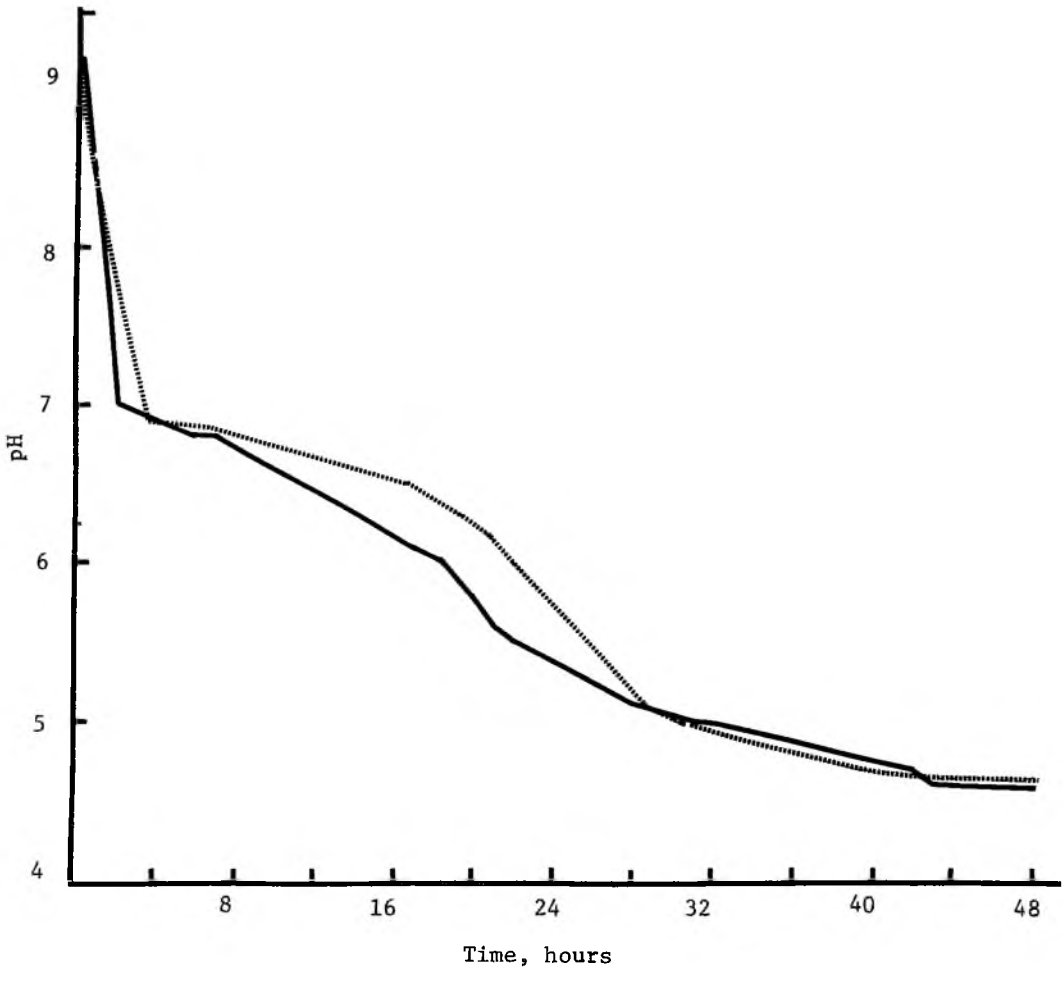

Figure 7. Solution pH changes during two 48-hour absorption periods.

mM, having been provided as $Ca(OH)_2$ used in raising solution pH to 9.2. The final pH of the solutions are provided in the figure. The data clearly show a relationship between uptake of K and Fe, and pH changes in the medium. Both uptake of K and Fe increased as K supply was increased from nil to 2.5 mM. These increases are also related to pH decline in the medium. We suggest that the pH decline accompanying increasing K uptake resulted in higher uptake of Fe through decomposition of solid Fe by H-ions released from the roots.

Figure 9 shows the effect of increasing supply of K, as 0 to 5.00 mM KNO_3, on uptake of K and Fe from half-strength Hoagland solution in which $(NH_4)_2SO_4$ was substituted for $NH_4H_2PO_4$. The initial pH of the solutions was 8.4, but the final pH readings ranged from 6.5 to 6.8 in a manner unrelated to the concentrations of K in the substrate or in the leaves. The data also show a clear relationship between uptake of K and Fe as K supply increased from nil to 0.5 mM. Thereafter, increasing supply of K resulted in increased K uptake but a decline in Fe uptake. Both observations can be explained in relation to the high concentration of NO_3 in the solutions: A high NO_3 concentration will enhance uptake of K (Maas, 1969), but the NO_3 uptake will still exceed uptake of K. The higher uptake of NO_3 will not result in a net excess accumulation of anions if the absorbed NO_3 is rapidly reduced to $-NH_2$ groups. Minotti, et al. (1968) showed that low-N wheat seedlings, exposed to KNO_3 solutions for 24 hours, were able to reduce 66% of the absorbed NO_3. Such a high rate of NO_3 reduction will favor excess accumulation of K and consequent release of H-ions which will in turn favor increased Fe uptake. This is what happened at 0 to 0.5 mM K (Figure 9).

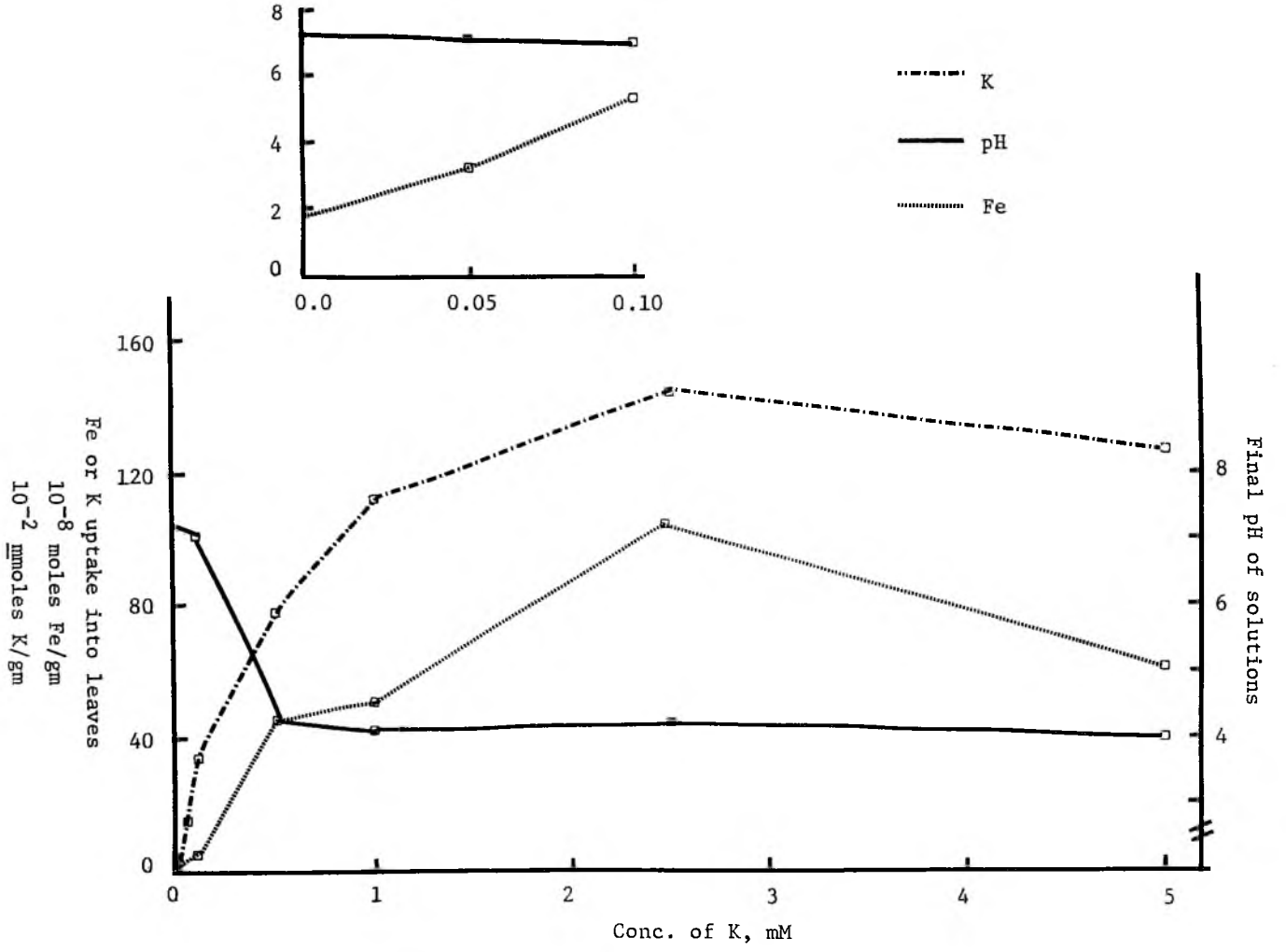

Figure 8. Effects of increasing supply of K, as K_2SO_4, on pH changes and uptake of K, and of Fe from inorganic Fe source. Initial solution pH of 9.2. Absorption time of 48 hours.

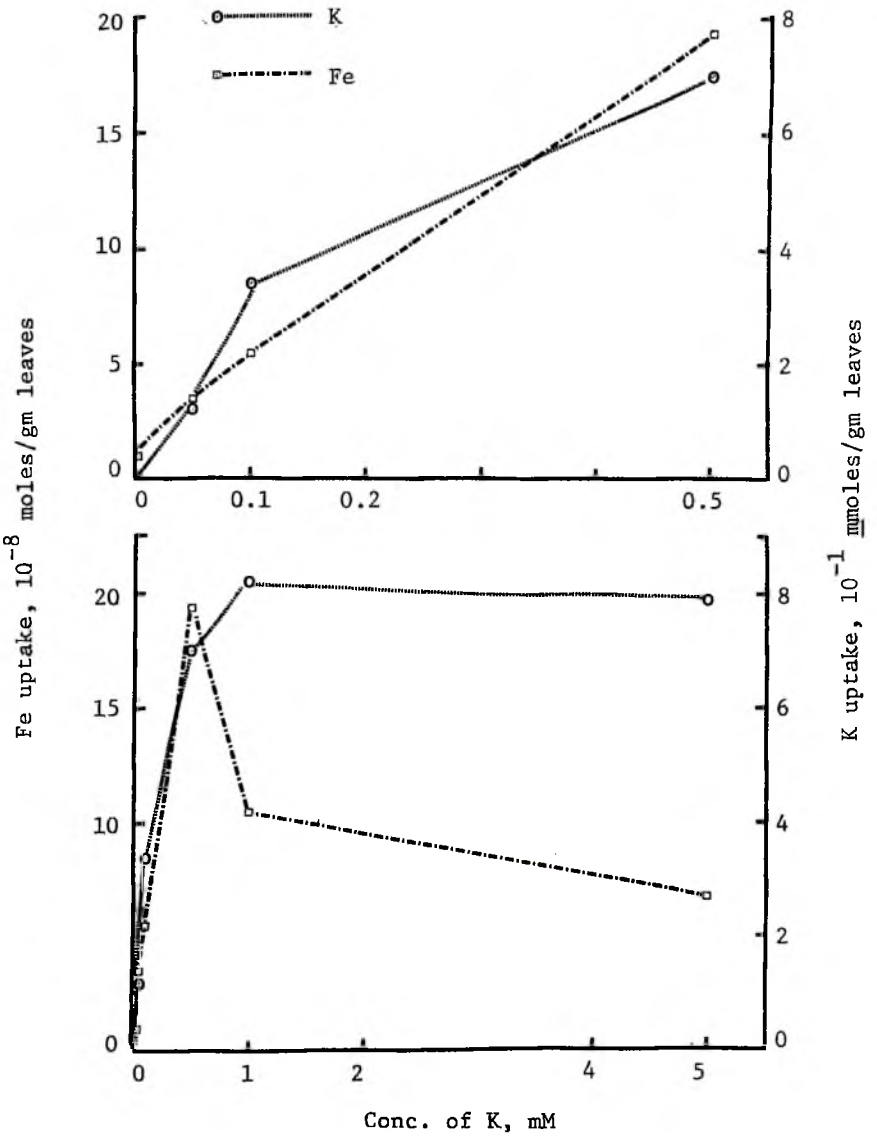

Figure 9. Effects of increasing supply of K as KNO_3 on uptake of K and Fe from half-strength Hoagland solution containing Fe-hydroxide. Absorption time of 48 hours.

The decreasing uptake of Fe at greater than 0.5 mM K concentration is explained as follows: The rate of accumulation of NO_3 may have exceeded both the rate of NO_3 reduction and the rate of K accumulation so that we had a case of excess accumulation of anions. The tissues will therefore be releasing OH and HCO_3 ions (Riley and Barber, 1969), which will decrease the availability and uptake of Fe.

$CaCO_3$ buffer systems. The effects of K supply, as K_2SO_4, on uptake of Fe from $CaCO_3$ buffered solutions are presented in Figures 10a and 10b. Figure 10a represents the results of increasing K concentration from 0.0 to 0.50 mM while Figure 10b represents results from 0.0 to 5.0 mM K. The K uptake curve in Figure 10b represents the mean of all three Fe and $CaCO_3$ treatment combinations mentioned in the figure. Iron was provided as either 10^{-4} M FeEDDHA plus 1% excess EDDHA or 10^{-4} M Fe-hydroxide. Calcium carbonate was provided at the rate of 125 mg per liter culture solution. Enough $Ca(OH)_2$ was added to the "FeEDDHA minus $CaCO_3$" treatment to raise solution pH to 9.2; the final pH of these solutions are given in the figures. They did not differ markedly from those of the "Fe-hydroxide plus $CaCO_3$" and "FeEDDHA $CaCO_3$" treatments which were 7.9 and 7.6, respectively.

The data in both Figure 10a and 10b indicate that the presence of $CaCO_3$ did not influence uptake of Fe from the chelate systems. The supply of K influenced uptake of Fe from the organic and inorganic sources. It is recalled that the FeEDDHA treatments contained 1% excess EDDHA ligand which would compete with roots for Fe (Brown and Tiffin, 1960; Brown, et al., 1960, 1961). Yet, increasing K supply increased Fe uptake. The relationship between uptake of K and Fe is very clear in

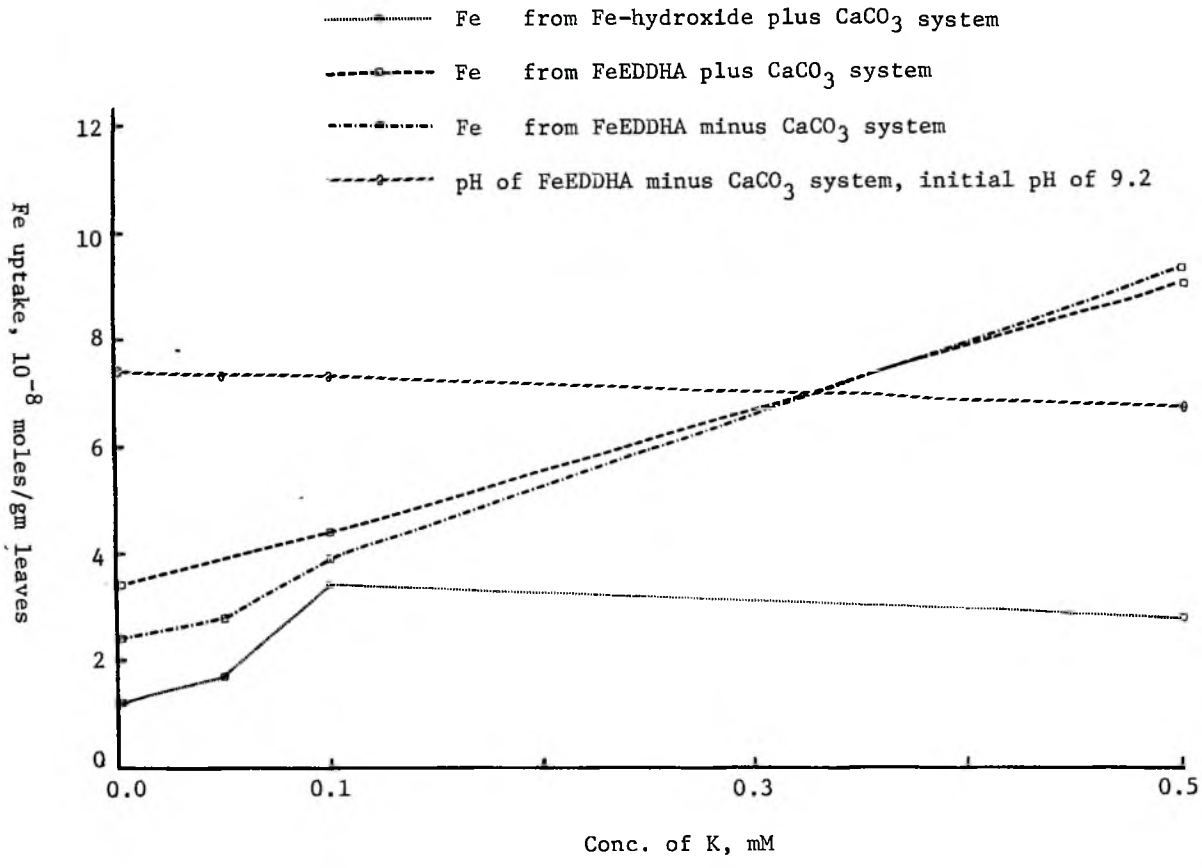

Figure 10a. Effects of $CaCO_3$ and increasing supply of K on uptake of Fe from solutions containing chelated and inorganic Fe. Absorption time of 48 hours.

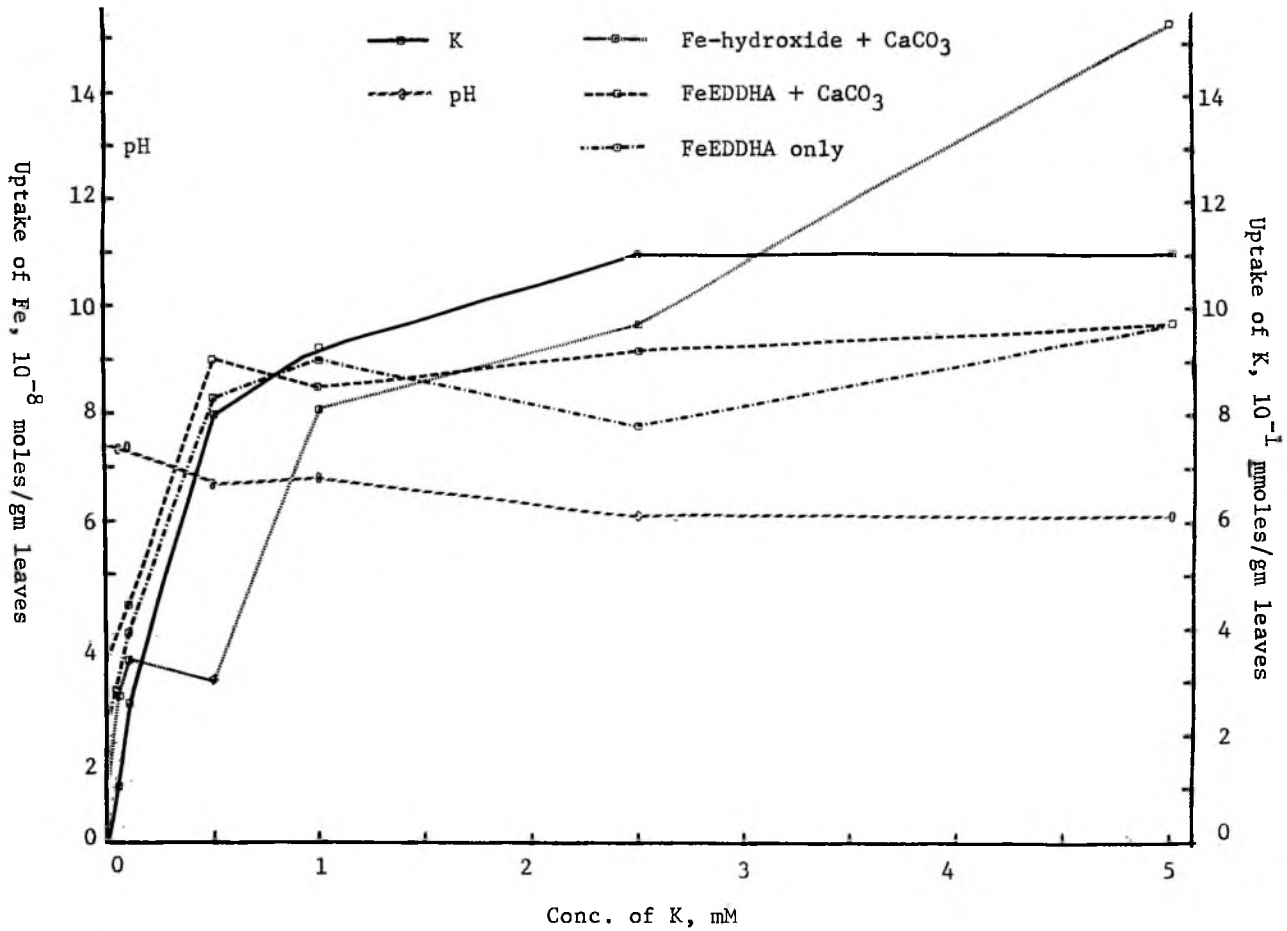

Figure 10b. Effects of $CaCO_3$ and increasing supply of K on uptake of K and of Fe in 48 hours. (See Figure 10a for more information on convensions.)

Figure 10b. This relationship also holds for uptake of Fe from Fe-hydroxide (the deviation of Fe uptake at 0.5 mM K must have been due to experimental error). Uptake of Fe from Fe-hydroxide was lower than uptake from FeEDDHA at less than 2 mM K but increasing solution K concentration to 2.5 or 5.0 mM resulted in greater uptake from the inorganic Fe source. This may have been due to the relatively higher concentration of Fe, as Fe-hydroxide sols, on the roots. H-ions from roots apparently decomposed more Fe sols than effected dissociation of the FeEDDHA molecules. This point may also be cited to explain instances where Fe uptake from inorganic sources exceeds or equals uptake from organic sources such as FeEDTA (Oertli and Jacobson, 1960) or FeEDDHA (Brown, et al., 1960, 1961).

Clark and Wallace (1962) have shown that increasing bicarbonate ion concentration to roots results in increasing uptake of the HCO_3 and corresponding increase in the root pH which may result in precipitation of Fe in the roots. No such root tissue pH increase would occur if the roots were accumulating K in quantities larger than the HCO_3 and therefore no inorganic precipitation of Fe would occur. Accumulation of K-ions in itself would be expected to hasten the conversion of carbon in the HCO_3-ions into such organic acids as malic (Jacobson, 1955).

We conclude discussion on this section by again calling attention to the beneficial effect of increasing K supply on absorption of Fe from excess $CaCO_3$ systems (Figures 10a and 10b). This is in agreement with Hoffer and Kranzt's (1949) observations in calcareous soils. We, however, believe that the beneficial effect of K in these instances is related to H release accompanying K accumulation and not prevention of

formation of Fe deposits in conducting vessels of stems.

Presence of exchange resins. Jenny and Cowan (1933) have reported that when soybean plants absorbed 1.020 meg Ca from Ca-clay suspensions, at initial pH of 6.3, the clay particles gained 0.948 meg H ions and the pH dropped to 4.30. This suggests that our hypothesis can be applied to uptake of Fe from clay systems for the pH decline must have been due to H-ion release accompanying Ca accumulation. We have demonstrated this for a system containing a synthetic cation exchange resin.

We studied the effect of increasing K supply on uptake of Fe from systems containing Fe-hydroxide and an exchanger, namely Bio-Rex 70, a carboxylic type cation exchange resin. The method of preparation of the suspension has been described on page 30. The initial pH was 7.0; absorption was 48 hours. The results appear in Table 8.

Increasing K concentration to the corn plant resulted in considerable pH decline. Comparisons of the pHs of the 1.0 mM K as well as the 5.0 mM K treatments indicate that the presence of the various amounts of resin did not influence the final pH of the substrates. The data on Fe concentrations in plant tops, however, indicate interference of Fe absorption by the resins. Interference was most serious in the presence of 5.0 meg resin.

Increasing the supply of K from nil to 0.5 mM in the 1.0 meg resin system increased Fe uptake into tops but further increase in K supply resulted in a decline in Fe uptake. This is similar to Epstein and Stout's (1951) observation on the effect of increasing Ca saturation on uptake of "adsorbed" Fe from bentonite suspensions. Both observations can be related to pH changes accompanying differential accumulation of

TABLE 8. The influence of carboxylic type exchange resin and potassium supply on absorption of inorganic iron. Fe concentration of 10^{-4} M.

Treatments			Fe Appearing	
Resin** meq/2 liters	K mM	Final* pH	in Tops 10^{-8} moles	with Roots Fe/gm/48 hours
Nil	0.00	6.6	61	420
	1.00	4.1	95	380
1.0	0.00	6.5	48	350
	0.05	6.0	97	330
	0.50	4.2	151	420
	1.00	4.0	100	390
	5.00	4.0	99	370
5.0	1.00	4.2	39	340
	5.00	4.2	41	360

*Initial pH of 7.0

**Bio-Rex 70, CEC - 10.2 meg/gm

cations; the declining Fe uptake at the highest cation supply can be attributed to the detrimental effect of resulting high H-ion concentration.

Epstein and Stout did not consider pH changes during their 24-hour absorption periods: they considered only H-ion released during preparation of the Ca-clays. Displacement of H-ions from H-bentonite by Ca was expected to decrease the exchangeability of Fe and therefore reduce Fe uptake by tomato plants. Their results contradicted their premise as also do our results (Table 8).

Qualitative tests showed that essentially no Fe ions remained in the aqueous phase of our resin suspensions. Our calculations also indicate that our supply of Fe would precipitate at about pH 2.7, while Epstein and Stout's Fe supply of 120 µgm Fe per 75 ml suspension of pH 4.5 to 7.0 would similarly precipitate at about pH 3.0. About the form of Fe in bentonite clay systems, Coleman, et al. (1964) have shown that saturating bentonite clays with hydroxides of Al or Fe readily degraded the crystalline structure of the clays. The hydroxides precipitated in the interlayer spaces and on external surfaces blocking exchange sites so that at pH 5.0 the effective cation exchange capacity of the systems was essentially zero. Therefore, because of precipitation of Fe on clay exchangers (Page and Whittig, 1961; Coleman, et al., 1964), the beneficial effects of Ca (Epstein and Stout, 1951) and K (Table 8) cannot be related to replacement of the trivalent Fe from exchangers. H-ions released from roots during accumulation of cations can decompose Fe hydroxide precipitates and thereby make Fe available for absorption. Thus, our hypothesis can be invoked to explain the beneficial effect of

K and Ca on uptake of Fe from systems containing inorganic Fe and exchangers which probably serve as mere nuclei or seeders of Fe-hydroxide precipitates.

GENERAL DISCUSSION AND CONCLUSIONS

The greenhouse experiments demonstrated that the effect of K supply on Fe nutrition may be real. In some cases (Table 1) adding more K resulted in significant plant growth, yet Fe concentrations in leaf tissues increased rather than were diluted as described by Ward (1959).

The results of the short-term experiments generally indicated that the effect of K supply can be related to enhanced uptake of Fe rather than improved translocation or utilization of Fe in plants. They also indicated that Fe uptake is highest in the presence of salts from which excess accumulation of cations is known to occur (Figure 1 and Table 6). The differential uptake of cations is accompanied by pH decline in unbuffered solutions. Uptake of Fe from such solutions is thus correlated with the pH decline (Figures 1, 4, 6, 8, and Tables 6 and 8). This suggests that the increased H-ion concentration increases the solubility of Fe-hydroxides and also increases the release of Fe^{+3} from chelated molecules.

There was no evidence of pH changes in solutions buffered with $CaCO_3$, yet increasing K supply resulted in increased uptake of Fe (Figures 10a and 10b). The fact that the ionic compositions of the solutions were conducive to accumulation of excess cations is sufficient for us to conclude that H-ions were released from the roots. The pH at the root-Fe-hydroxide interface and in the free space will be lower than recorded in the bulk solution, and will favor dissolution of the hydroxide.

It is of interest that a number of mechanisms proposed to explain ion absorption relate to the transfer of electrons and protons from the cell to the external medium. As, for example, Lundegårdh (1950) proposed that anion absorption is connected with production of electrons during respiration, "anion respiration": Anions are believed to be transported in opposite direction to the electrons with which they are repeatedly exchanged along tracks of cytochromes across the cytoplasm; cations, on the other hand, are transported inwards by repeated exchanges with H-ions which move out of the cell as a result of dehydrogenase activity. Overstreet and Jacobson (1952) have also proposed that ion absorption by roots takes place by way of exchange reactions between the culture medium and the tissues; cations are usually absorbed in exchange for H-ions from the roots while anions are absorbed by exchange with OH^- or HCO_3 ions from roots. The mechanism is represented by the following equations:

$$M^+ + HR \rightleftarrows MR + H^+ \qquad \text{(i)}$$

$$A^- + R'OH \rightleftarrows R'A + OH^- \qquad \text{(ii)}$$

where HR and R'OH represent hypothetical acidic and basic membrane carrier molecules for cations and anions, respectively.

The H^+ shown in equation (i) or OH^- in equation (ii) will end up in the substrate depending on whether cations or anions are being accumulated in the tissues.

A mechanism of transport of H and OH ions is proposed by the chemiosmotic hypothesis of Mitchell (1961) which relates the transport to the production of the energy used for metabolic activities including ion uptake. The hypothesis postulates that oxidation-reduction through the

respiratory chain of the mitochondrial system and hydrolysis of ATP by ATPase are accompanied by stoichiometric translocation of H^+ across the coupling membrane to the outside while OH^- is translocated to the other side of the membrane. The translocations of H^+ and OH^- creates a pH gradient across the membrane. But a pH gradient of 3.5 units can also cause further translocation of H^+ and OH^- from the center of activity and induce further oxidative phosphorylation to provide more energy for further absorption of ions (Mitchell, 1961; Mitchell and Moyle, 1965).

Gastric secretion of HCl is cited as an example of how protons, H-ions, leave tissues during accumulation of cations (Robertson, 1968). The presence of the acid in the stomach will increase the solubility of some sparingly soluble minerals and compounds (e.g., bones in dogs) and enhance uptake of released ions. Similarly, H-ions released from roots will enhance uptake of Fe and other ions from compounds in the immediate vicinity of roots.

Most workers tend to consider H-ion release, during long-term absorption by roots, as a nuisance to be combatted by neutralization with bases. A few, however, recognized that the H-ions may play a role in reactions leading to absorption of other ions. Hoagland (1948) and Wit, et al. (1963), for example, suggested that the released H-ions may replace metallic cations from soil exchange systems and enhance further uptake of more cations. This cannot be how the H-ions influence uptake of elements from sparingly soluble minerals such as oxides of Fe in soils. It is known that the soil solution does not contain enough Fe to support plant growth (Oertli, 1956; Oertli and Jacobson, 1960; Glausser

and Jenny, 1960; Jenny, 1966). Thomas and Coleman (1964) have further noted that little or no salt-exchangeable ferric iron is found in acid soils. Twenty years earlier, Thorne and Wallace (1944) had shown that well-aerated calcareous soils contained practically no soluble or exchangeable Fe^{+3} or Fe^{+2} ions, and that if Fe is absorbed in the ferrous form, then reduction would have to occur at the point of absorption. Indeed, Brown and Tiffin (1960) and Brown, et al. (1958, 1960) have suggested that uptake of Fe may be related to the root's ability to reduce Fe^{+3}. Grunes (1951), Grunes and Jenny (1960) have shown that both the synthetic resin Amberlite 1R 100 and dead alfalfa roots were able to reduce Fe^{+3}. The Na, K, and Mg forms of the resin, however, failed to decompose Fe-hydroxide sols but H-amberlite and H-roots did. Thus it can be said that field plants obtain Fe from solid minerals whose solubilities cannot be increased or oxidative states changed by merely increasing the concentrations of the chloride, sulphate, or nitrate salts of the major nutrient cations; neither can we attribute the beneficial effect of Ca (Epstein and Stout, 1951) and K (Table 8) to replacement of Fe from exchangers. Hydrogen ions released during excess accumulation of these cations can be expected to effect decomposition or dissolution of Fe-bearing minerals on root surfaces, and thereby increase the availability and uptake of Fe. We can therefore conclude that the effect of K on the availability and uptake of Fe is not a two-ion contact effect but rather a three-ion effect; accumulation of K brings H-ions into solution, or at least to the root surface; the H^{+} dissolves Fe in the immediate root environment, and the freed Fe-ions diffuse to absorption sites.

Although our studies and discussions have been limited to uptake of Fe, our hypothesis may also apply to uptake of other ions from their chelated and mineral compounds. As, for example, it can explain why Franklin (1969) observed that K supply enhanced uptake of inorganic P by excised roots: Burd (1947) had earlier suggested that uptake of P from sparingly soluble P compounds in soils is related to dissolution of the minerals by the relatively high concentration of H-ions at the root-soil interface; Burd, however, attributed the source of the H-ions to ionization of excreted organic acids and reaction of CO_2 with water at the interface. The findings of Ulrich (1941, 1942) and others, as well as the chemi-osmotic hypothesis suggest that the H-ions, as protons, are in fact transported directly from active sites in the cell, specifically the mitochondria. We can further speculate that H-ions reaching the root surfaces will react with all types of minerals. As, for example, the well known etching of marble by plant roots may be a result of dissolution or decomposition by H-ions from roots. Minerals of the heavy micronutrient cations may also be dissolved and made more available for absorption.

Wit, et al. (1963) and Noggle (1966) correlated organic acid contents of plant materials to yield as coupled to inorganic cation and anion balance. They concluded that nutrient treatments which increase organic anion contents of plant tissues increase plant yields, and conversely salt treatments decreasing the organic anion contents decrease plant yields. In view of our findings, the improvement of plant yields may also be related to improved availability and absorption of micronutrient metals from solid phases. The data of Riley and Barber (1969)

and Farr, et al. (1969) suggest that the pH of the immediate vicinity of roots accumulating excess anions or cations may be such as to influence the availability and uptake of some sparingly soluble compounds. It can further be argued that field plants ultimately accumulate excess cations over anions, because the rapidly absorbed anions, notably NO_3, are assimilated to a large extent in the cytoplasm and an excess of cations appears in the vacuoles (Burström, 1953). Potassium may be expected to account for a large portion of the cations in vacuoles since the other major nutrient cations, Ca and Mg, are very slowly absorbed and Ca may enter the structural components of cells while Mg may be incorporated into chlorophyll. We therefore conclude that a beneficial effect of increasing K supply on uptake of Fe can be related to H-ions released during accumulation of the K ions.

SUMMARY

Uptake of Fe by corn seedlings was studied on the premise that H-ions released during accumulation of cations improve availability and absorption of Fe from both chelated and inorganic sources. The effects of various salts of K were studied in long-term greenhouse and in short-term experiments. Effects of salts of Ca, Mg, Na, and Li were studied in short-term experiments only. The short-term experiments lasted not more than 48 hours.

The findings indicated that increasing the supply of K as KCl or K_2SO_4 in unbuffered solutions tended to result in increased Fe uptake which could be related to accompanying acidification of the substrate. However, acidification to low pH (less than about 4.7) tended to interfere with further uptake of Fe. Increasing the supply of K as KNO_3 initially enhanced Fe uptake, but further increase of KNO_3 decreased uptake of Fe. The decreased uptake was attributed to excess anion accumulation which would result in the release of OH ions, which would not favor increased Fe availability and uptake. Chlorides of Mg, Ca, Li and Na did not result in as definite increase in Fe uptake as did K salts. Increasing K supply also enhanced uptake of Fe from suspensions of both $CaCO_3$ and cation exchanger systems containing Fe-hydroxide sols. This was attributed to decomposition of the sol by H-ions released during excess accumulation of K. These H-ions were also considered to be capable of causing dissociation of chelated compounds such as FeEDDHA and thereby enhancing Fe uptake.

LITERATURE CITED

Alexander, M. 1961. Introduction to soil microbiology. John Wiley and Sons, New York.

Anton, M. R. F., K. A. Grossenbacher, and H. Jenny. 1965. Role of bacteria in the insoluble iron absorption by alfalfa plants. An. Edafol. Agrobiol. 24: 445-53.

Arnon, D. I., W. E. Fratzke, and C. M. Johnson. 1942. Hydrogen ion concentration in relation to absorption of inorganic nutrients by higher plants. Plant Physiol. 17: 515-24.

Bange, G. G. J. 1959. Interactions in the potassium and sodium absorption by intact maize seedlings. Plant and Soil 11: 17-29.

Bhan, K. C., A. Wallace, and E. J. Krohn. 1962. Effect of pH and nitrogen source on the ability of corn and soybean to obtain iron chelated with ethylenediamine di(O-hydroxyphenyl acetate). Agron. J. 54: 119-21.

Biddulph, O. 1953. The translocation of minerals in plants. In Mineral nutrition of plants, E. Truog, Editor. University of Wisconsin Press, Madison, Wis.

Bolle-Jones, E. W. 1952. A study of the interrelationships of iron and potassium in the potato plant. Trans. Intern. Soc. Soil Sci. Comm. 2: 186-92.

_____. 1955. The interrelationships of iron and potassium in the potato plant. Plant and Soil 6: 129-73.

_____ and B. A. Notton. 1953. The relative proportions of the chloroplast pigments as influenced by different levels of iron and potassium. Plant and Soil 5: 87-100.

Brown, J. C. 1956. Iron chlorosis. Ann. Rev. Plant Physiol. 7: 171-90.

_____. 1961. Iron chlorosis in plants. Adv. Agron. 13: 329-69.

_____. 1963. Interactions involving nutrient elements. Ann. Rev. Plant Physiol. 14: 93-106.

_____. 1966. Iron and calcium uptake as related to root sap and stem exudate citrate of soybeans. Physiol. Pl. 19: 968-76.

Brown, J. C. and L. O. Tiffin. 1960. Iron chlorosis in soybeans as related to the genotype of rootstock: 2. A relationship between susceptibility to chlorosis and capacity to absorb iron from iron chelate. Soil Sci. 89: 8-15.

Brown, J. C., R. S. Holmes, and L. O. Tiffin. 1958. Iron chlorosis in soybeans as related to the genotype of rootstalk. Soil Sci. 86: 75-82.

Brown, J. C., L. O. Tiffin, and R. S. Holmes. 1960. Competition between chelating agents and roots as a factor affecting absorption of iron and other ions by plant species. Plant Physiol. 35: 878-86.

_____, _____, _____, A. W. Specht, and J. W. Resnicky. 1961. Stability and concentration of metal chelates, factors in iron chlorosis of plants. Agron. J. 53: 85-90.

Burd, J. S. 1947. Mechanisms of release of ions from soil particles to plant. Soil Sci. 64: 223-25.

Burström, H. 1953. The mechanism of iron absorption. In Mineral nutrition of plants. E. Truog, Editor. University of Wisconsin Press, Madison, Wis.

Chaberek, S., and A. E. Martell. 1959. Organic sequestering agents. John Wiley, New York.

Charley, J. L. and H. Jenny. 1961. Two phase studies on availability of iron in calcareous soils. IV. Decomposition of iron oxide by roots, and Fe-diffusion in roots. Agrochimica 5: 99-106.

Clark, R. B. and A. Wallace. 1962. Effect of bicarbonate on pH in plant roots. In A decade of synthetic chelating agents in inorganic plant nutrition. A. Wallace, Editor. Arthur Wallace, Los Angeles.

Coleman, N. T., G. W. Thomas, F. H. leRoux, and G. Bredell. 1964. Salt-exchangeable and titratable acidity in bentonite-sesquioxide mixtures. Soil Sci. Soc. Amer. Proc. 28: 35-37.

Diehl, H. and G. Frederick Smith. 1965. The iron reagents. 2nd Edition. G. Frederick Smith Chem. Co., Columbus, Ohio.

Epstein, E. and P. R. Stout. 1951. The micronutrient cations iron, manganese, zinc, and copper: Their uptake by plants from the adsorbed state. Soil Sci. 72: 47-65.

Evans, H. J. and G. J. Sorger. 1966. Role of mineral elements with emphasis on the univalent cations. Ann. Rev. Plant Physiol. 17: 47-76.

Farr, F., L. V. Vaidyanathan, and P. H. Nye. 1969. Measurements of ionic concentration gradients in soil near roots. Soil Sci. 107: 385-91.

Franklin, R. E. 1969. Effect of adsorbed cations on phosphorus uptake by excised roots. Plant Physiol. 44: 697-700.

Frost, A. E., H. H. Freedmann, S. J. Westerback, and A. E. Martell. 1958. Chelating tendencies of N,N'-ethylene bis-[2-(O-hydroxyphenyl)]-glycine. J. Amer. Chem. Soc. 80: 530-36.

Glauser, R. and H. Jenny. 1960a. Two-phase studies on availability of iron in calcareous soils. 1. Experiments with alfalfa plants. Agrochimica 4: 263-78.

_____. 1960b. Two-phase studies on availability of iron in calcareous soils. III. Contact and exchange diffusion in ionic membranes. Agrochimica 5: 1-9.

Griffin, D. M. 1963. Soil physical factors and the ecology of fungi. III. Activity of fungi in relatively dry soil. Brit. Mycol. Soc. Trans. 46: 373-77.

Grunes, D. L. 1951. Reactions between positively charged iron oxide sols and negatively charged particles of the synthetic resin, Amberlite 1R 100. Ph. D. Thesis, University of California, Berkeley.

_____ and H. Jenny. 1960. Two-phase studies on availability of iron in calcareous soils. II. Decomposition of colloidal iron hydroxide by ion exchangers. Agrochimica 4: 279-86.

Hart, Constance E. 1934a. Some effects of potassium upon the growth of sugar cane and upon the absorption and migration of ash constituents. Plant Physiol. 9: 399-452.

_____. 1934b. Some effects of potassium upon the amounts of protein and amino forms of nitrogen, sugars, and enzyme activity of sugar cane. Plant Physiol. 9: 453-90.

Hewitt, E. J. 1963. The essential nutrient elements: requirements and interactions in plants. In Plant Physiology III, 137-360. Steward, F. C., Editor, Academic Press, New York.

_____ and E. W. Bolle-Jones. 1953. Studies in iron deficiency of crops. The interrelationships of iron and potassium in the potato plant. J. Hort. Sci. 28: 185-95.

Hiatt, A. J. 1968. Electrostatic association and Donnan phenomena as mechanisms of ion accumulation. Plant Physiol. 43: 893-901.

Hill-Cottingham, D. G. A. 1957. A spectrophotometric method of analysis of chelate solutions and its application to the study of iron chelates in soils and plants. Soil Sci. 84: 43-50.

_____ and C. P. Lloyd-Jones. 1965. The behavior of iron chelating agents with plants. J. Expt. Bot. 16: 233-42.

Hoagland, D. R. 1923. The absorption of ions by plants. Soil Sci. 16: 225-46.

_____. 1948. Lectures on the inorganic nutrition of plants. Chronica Botanica Co., Waltham, Mass.

_____ and D. I. Arnon. 1950. The water-culture method of growing plants without soil. Cal. Agr. Expt. Station Cir. 347.

Hodgson, J. F. 1968. Theoretical approach for the contribution of chelates to movement of iron to roots. 9th Int. Congr. Soil Sci. Trans. 2: 229-41.

_____. 1969. Contribution of metal-organic complexing agents in the transport of metals to roots. Soil Sci. Soc. Amer. Proc. 33: 68-75.

_____, W. L. Lindsay, and W. D. Kemper. 1967. Contributions of fixed charge and mobile complexing agents to the diffusion of zinc. Soil Sci. Soc. Amer. Proc. 31: 410-13.

Hoffer, G. N. 1926. Testing corn stalks chemically to aid in determining their plant food needs. Indiana Agr. Expt. Sta. Bull. 298.

_____ and R. H. Carr. 1923. Accumulation of aluminium and iron compounds in corn plants and its probable relation to root rots. J. Agri. Res. 23: 801-23.

_____ and J. F. Trost. 1923. The accumulation of iron and aluminum compounds in corn plants and its probable relation to root rots. Agron. J. 15: 323-31.

_____ and B. A. Krantz. 1949. Deficiency symptoms of corn and small grains. In Hunger signs in crops. Amer. Soc. Agron. and Natl. Fert. Assoc., Washington, D. C.

Horn, G. C. and W. L. Pritchett. 1962. The relationship of fertilization to various turf disorders. Soil Crop. Sci. Soc. Fla. Proc. 22: 107-15.

Iljin, W. S. 1951a. Metabolism of plants affected with lime-induced chlorosis (calciose). I. Nitrogen metabolism. Plant and Soil 3: 239-56.

Iljin, W. S. 1951b. Metabolism of plants affected with lime-induced chlorosis (calciose). II. Organic acids and carbohydrates. Plant and Soil 3: 339-52.

_____. 1952. Metabolism of plants affected with lime induced chlorosis (calciose). III. Mineral elements. Plant and Soils 4: 11-28.

Jacobson, L. 1955. Carbon dioxide fixation and ion absorption in barley roots. Plant Physiol. 30: 264-69.

_____ and J. J. Oertli. 1956. The relation between iron and chlorophyll contents in chlorotic sunflower leaves. Plant Physiol. 31: 199-204.

_____, R. Overstreet, H. M. King, and R. Handley. 1950. A study of potassium absorption by barley roots. Plant Physiol. 25: 639-47.

Jeffreys, R. A., V. Q. Hale, and A. Wallace. 1961. Uptake and translocation in plants of labelled iron and chelating agents. Soil Sci. 92: 268-273.

Jenny, H. 1953. Contact phenomena between adsorbents and their significance in plant nutrition. In Mineral nutrition of plants. E. Truog, Editor. University of Wisconsin Press, Madison, Wis.

_____. 1961. Two-phase studies on availability of iron in calcareous soils. V. Kinetics of iron transfer as conditioned by ion exchange capacity and structure of roots. Agrochimica 5: 281-87.

_____. 1966. Pathways of ions from soil into root according to diffusion models. Plant and Soil 25: 265-89.

_____ and E. W. Cowan. 1933. The utilization of adsorbed ions by plants. Science 77: 394-6.

_____ and K. A. Grossenbacher. 1965. Root-soil boundary zones as seen in the electron microscope. Soil Sci. Soc. Amer. Proc. 27: 273-77.

_____, T. R. Nielsen, N. T. Coleman, and D. E. Williams. 1950. Concerning the measurement of pH, ion activities and membrane potentials in colloidal systems. Science 112: 164-67.

_____ and R. Overstreet. 1938. Contact effects between plant roots and soil colloids. Proc. National Acad. Sci. 24: 384-92.

_____ and _____. 1939. Cation interchange between plant roots and soil colloids. Soil Sci. 47: 257-72.

Jones, E. W. 1950. Experiments on iron metabolism in plants. IV. Interrelationship of iron and potassium in the potato as affected by the presence of calcium carbonate. Ann. Rept. Long Ashton Res. Sta. 71-83.

_____ and E. J. Hewitt. 1949. Experiments on iron metabolism in plants. II. The interrelationship of iron and potassium of the potato plant. Ann. Rept. Long Ashton Res. Sta. 49-57.

Khadr, A. A. 1965. Differential responses to some nutrient elements by orange and rough lemon. Ph. D. Thesis, University of California, Los Angeles.

Kholdebarin, B. 1969. Changes of organic acids during salt uptake by leaf tissues under light and dark conditions. Ph. D. Thesis, University of California, Riverside.

Kock, P. C. de, and R. I. Morrison. 1958a. The metabolism of chlorotic leaves. I. Amino acids. Biochem. J. 70: 266-72.

_____ and _____. 1958b. The metabolism of chlorotic leaves. II. Organic acids. Biochem. J. 70: 272-77.

_____ and M. A. McDonald. 1960. A relation between the ratios of iron and potassium to calcium in mustard leaves. Plant and Soil 12: 128-42.

Korman, S. 1960. The differentiation of anemia in man with an iron chelate. In Metal binding in medicine. M. J. Seven and L. A. Johnson, Editors. Lippincott, Philadelphia, Pa.

Kroll, H. 1957. The ferric chelate of ethylenediamine di(O-hydroxy-phenylacetic acid) for treatment of lime-induced chlorosis. Soil Sci. 35: 362-67.

Läuchli, A. and H. Schwander. 1966. X-ray microanalyzer study on the localization of minerals in native plant tissue sections. Experientia 22: 503-5.

Lindsay, W. L., J. F. Hodgson, and W. A. Norvell. 1967. The physico-chemical equilibrium of metal chelates in soils and their influence of the availability of micronutrient cations. Trans. Comm. II and IV. Int. Soc. Soil Sci. Soc. Aberdeen 1966, 305-16.

Lingle, J. C., L. O. Tiffin, and J. C. Brown. 1963. Iron uptake-transport of soybeans as influenced by other cations. Plant Physiol. 35: 71-76.

Lundegårdh, H. 1950. The translocation of salts and water through wheat roots. Physiol. Plant. 3: 103-51.

Maas, E. V. 1969. Calcium uptake by excised maize roots and interactions with alkali cations. Plant Physiol. 44: 985-89.

_____, D. P. Moore, and B. J. Mason. 1969. Influence of calcium and magnesium on manganese absorption. Plant Physiol. 44: 796-800.

Marschner, H., R. Handley, and R. Overstreet. 1966. Potassium loss and changes in the fine structure of corn root tips induced by H-ion. Plant Physiol. 41: 1725-35.

Minotti, P. L., Doris C. Williams, and W. A. Jackson. 1968. Nitrate uptake and reduction as affected by calcium and potassium. Soil Sci. Soc. Amer. Proc. 32: 692-698.

Mitchell, P. 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature Lond. 191: 144-148.

_____ and J. Moyle. 1965. Stoichiometry of proton translocation through the respiratory chain and adenosine triphosphate systems of rat liver mitochondria. Nature Lond. 208: 147-51.

Noggle, J. C. 1966. Ionic balance and growth of sixteen plant species. Soil Sci. Soc. Amer. Proc. 30: 763-66.

Oertli, J. J. 1956. Studies in the uptake of iron by higher plants. Ph. D. Thesis, University of California, Berkeley.

_____ and L. Jacobson. 1960. Some qualitative considerations in iron nutrition of higher plants. Plant Physiol. 35: 683-88.

Olsen, C. 1958. Iron uptake in different plant species as a function of the pH value of the nutrient solution. Physiol. Plant 11: 889-905.

Overstreet, R. and L. Jacobson. 1952. Mechanism of ion absorption by roots. Ann. Rev. Plant Physiol. 3: 189-206.

Page, A. L. and L. D. Whittig. 1961. Iron adsorption by montmorillonite systems. II. Determination of adsorbed iron. Soil Sci. Soc. Amer. Proc. 25: 282-86.

Pozuelo, M. R. E. and K. A. Grossenbacher. 1965. Uptake of insoluble iron by sterile and non-sterile plants. Agrochimica 9: 361-67.

Price, C. A. 1968. Iron compounds and plant nutrition. Ann. Rev. Plant Physiol. 19: 239-48.

Riekells, J. W. and J. C. Lingle. 1966. Iron uptake and translocation by tomato plants as influenced by root temperature and manganese nutrition. Plant Physiol. 41: 1095-1101.

Riley, D. and S. A. Barber. 1969. Bicarbonate accumulation and pH changes at the soybean (Glycine max (L.) Merr.) root-soil interface. Soil Sci. Soc. Amer. Proc. 33: 905-08.

Robertson, R. N. 1968. Protons, electrons, phosphorylation and active transport. Cambridge University Press.

Rogers, C. H. and J. W. Shive. 1932. Factors affecting the distribution of iron in plants. Plant Physiol. 7: 227-52.

Sandell, E. B. 1950. Colorimetric determination of traces of metals. 2nd Ed. Interscience Publishers, Inc., New York.

Scheuring, D. G. and R. Overstreet. 1961. Sodium uptake by excised barley roots from sodium bentonite suspensions and from their equilibrium filtrates. Soil Sci. 92: 166-172.

Somers, I. I. and J. W. Shive. 1942. The iron-manganese relationship in plant metabolism. Plant Physiol. 17: 582-602.

Teng, Jon I. 1965. The effects of potassium fertilization and night temperatures on the absorption, translocation, and assimilation of iron in centipedegrass (Eremochloa ophiuroides). Ph. D. Thesis, University of Florida.

Thomas, G. W. and N. T. Coleman. 1964. The fate of exchangeable iron in acid clay systems. Soil Science 97: 229-32.

Thorne, D. W. and A. Wallace. 1944. Some factors affecting chlorosis on high lime soils. I. Ferrous and ferric iron. Soil Sci. 57: 299-312.

_____, F. B. Wann, and W. Robinson. 1950. Hypotheses concerning lime-induced chlorosis. Soil Sci. Soc. Amer. Proc. 15: 254-58.

Tiffin, L. O. 1966a. Iron translocation. I. Plant culture, exudate sampling, iron citrate analysis. Plant Physiol. 41: 510-14.

_____. 1966b. Iron translocation. II. Citrate/ratio in plant stem exudates. Plant Physiol. 41: 515-18.

_____ and J. C. Brown. 1959. Absorption of iron from iron-chelate by sunflower roots. Science 130: 274-75.

_____ and _____. 1961. Selective absorption of iron from iron chelates by soybean plants. Plant Physiol. 36: 710-14.

_____ and _____. 1962. Iron chelates in soybean exudates. Science 135: 311-12.

Truog, E. 1946. Soil reaction influence on availability of plant nutrients. Soil Sci. Soc. Amer. Proc. 11: 305-08.

Ulrich, A. 1941. Metabolism of non-volatile organic acids in excised barley roots as related to cation-anion balance during salt accumulation. Amer. J. Bot. 28: 526-37.

_____. 1942. Metabolism of organic acids in excised barley roots as influenced by temperature, oxygen tension, and salt concentration. Amer. J. Bot. 29: 220-27.

Viets, F. G. 1944. Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots. Plant Physiol. 19: 466-80.

Wallace, A. and R. T. Mueller. 1962. Effect of calcium level on uptake of Fe^{59} from FeEDDHA by bush beans. In A decade of synthetic chelating agents in inorganic plant nutrition. A. Wallace, Editor, Arthur Wallace, Los Angeles.

_____, C. P. North, R. T. Mueller, L. M. Shannon, and N. Hemaidan. 1955. Behavior of chelating agents in plants. Proc. Amer. Soc. Hort. Sci. 51: 685-94.

Wallace, T. and E. J. Hewitt. 1946. Studies on iron deficiency of crops. I. Problems of iron deficiency and the interrelationships of mineral elements in iron nutrition. J. Pomol. Hort. Sci. 22: 133-61.

Wallihan, E. F. 1955. Relation of chlorosis to concentration of iron in citrus leaves. Amer. J. Bot. 42: 101-4.

Ward, G. M. 1959. Potassium in plant metabolism. II. Effect of potassium upon the carbohydrate and mineral composition of potato plants. Canada J. Plant Sci. 39: 246-52.

Williams, D. E. and N. T. Coleman. 1950. Cation exchange properties of plant root surfaces. Plant and Soil 2: 243-56.

Wit, C. T. de, W. Dijkshoorn, and J. C. Noggle. 1963. Ionic balance and growth of plants. Verslag. Landbouwk. Onderzoek, 68.15.

Wojciechowski, J., Z. Szczotka, and M. W. Borys. 1967. Influence of potassium, chloride, and sulphate nutrition on the potato plant. Agrochimica 11: 549-56.

APPENDIX I

Calculations of the influence of H-ion concentration on the liberation of Fe^{+3} from FeEDDHA.

Let A^{-4} represent the ligand $EDDHA^{-}$ which reacts with Fe^{+3} according to the equation

$$Fe^{+3} + A^{-4} \underset{\leftarrow}{\overset{K_{Fe}}{\rightarrow}} FeA^{-} \qquad (1)$$

The stability constant, K_{Fe}, is 10^{33} and

$$K_{Fe} = \frac{(FeA^{-})}{(Fe^{+3})(A^{-4})} \qquad (2)$$

Let Fe_t represent total iron in the system, then

$$FeA = Fe_t - Fe^{+3}$$

and

$$K_{Fe} = \frac{(Fe_t - Fe^{+3})}{(Fe^{+3})(A^{-4})}$$

$$(Fe^{+3}) = \frac{(Fe_t)}{1 + K_{Fe}(A^{-4})} \qquad (4)$$

A^{-4} in (4) represents the fully dissociated ligand in the system. The ligand may also occur as H_4A or any of three weaker acids. Let A' represent the sum of the total free ligand, i.e.,

$$A' = (H_4A) + (H_3A^{-}) + (H_2A^{-2}) + (HA^{-3}) + (A^{-4})$$

Express each of the terms on the right-hand-side in terms of (A^{-4}), and let

$$(A^{-4}) = \frac{A'}{y}$$

Substitute into (4) to obtain

$$(Fe^{+3}) = \frac{(Fe_t)}{1 + K_{Fe}\frac{A'}{y}} \tag{5}$$

where

$$y = \frac{(H_4A)'}{(A^{-4})} + \frac{(H_3A^{-})}{(A^{-4})} + \frac{(H_2A^{-2})}{(A^{-4})} + \frac{(HA^{-3})}{(A^{-4})} + 1 \tag{6}$$

Each of the terms on the right-hand-side of (6) represents the fraction of A^{-4} which occurs as the indicated acid. Express each of the fractions in terms of the indicated constants and (H^{+}) in the following reactions (Chaberek and Martell, 1959):

$$A^{-4} + H^{+} \overset{K_4}{\rightleftarrows} HA^{-3} \qquad K_4 = 10^{11.6}$$

$$HA^{-3} + H^{+} \overset{K_3}{\rightleftarrows} H_2A^{-2} \qquad K_3 = 10^{10.24}$$

$$H_2A^{-2} + H^{+} \overset{K_2}{\rightleftarrows} H_3A^{-} \qquad K_2 = 10^{8.64}$$

$$H_3A^{-} + H^{+} \overset{K_1}{\rightleftarrows} H_4A \qquad K_1 = 10^{6.32}$$

Equation (6) becomes

$$y = K_1K_2K_3K_4(H^{+})^4 + K_2K_3K_4(H^{+})^3 + K_3K_4(H^{+})^2 + K_4(H^{+}) + 1 \tag{7}$$

The Fe^{+3} concentration in the system can be calculated by considering equations (5) and (7). Equation (5) can be re-written as

$$(Fe^{+3}) = \frac{(Fe_t)}{1 + 10^{33}\,\frac{A'}{y}}$$

$$= \frac{(Fe_t)\cdot y}{10^{33}.A'} \qquad (9)$$

We show below methods of calculating (Fe^{+3}) in two selected systems:

System 1: Ferric ions in equilibrium with FeEDDHA prepared by reacting $9x10^{-5}$ M Fe^{+3} with an equal amount of $EDDHA^{-}$:

$$Fe_t = 9x10^{-5}$$

Since Fe^{+3} is released as a result of dissociation of FeA^{-} (according to equation 1), an equal amount of A^{-4} is also liberated.

$$A' = (Fe^{+3})$$

and equation (9) becomes

$$(Fe^{+3})2 = \frac{(Fe_t)\cdot y}{10^{33}} = 9x10^{-38}.y$$

and

$$(Fe^{+3}) = \sqrt{9x10^{-38}.y}$$

Example (i)

At pH 4, $y = 10^{20.88}$

$(Fe^{+3}) = 3x10^{-8.56}$ M.

Example (ii)

At pH 7, $y = 10^{9.56}$

$(Fe^{+3}) = 3x10^{-14.22}$ M.

System 2: Ferric ions in equilibrium with FeEDDHA prepared by reacting $9x10^{-5}$ M Fe^{+3} with 9.09 M $EDDHA^{-}$ so that we begin with 1% excess ligand:

$$Fe_t = 9x10^{-5} \text{ M, and } A' = 9x10^{-7} \text{ M.}$$

Substitute these values into (9) to obtain

$$(Fe^{+3}) = \frac{9x10^{-5} \cdot y}{10^{33} \cdot 9x10^{-7}} = 10^{-31} \cdot y$$

Example (i)

At pH 4, $y = 10^{20.88}$

$$(Fe^{+3}) = 10^{-10.12} \text{ M.}$$

Example (ii)

At pH 7, $y = 10^{9.56}$

$$(Fe^{+3}) = 10^{-21.44} \text{ M.}$$