THESIS

IRON CHLOROSIS IN SILVER MAPLE

(*Acer saccharinum*)

Submitted by

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for the Degree of Master of Science

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY ROBERT L. MORRIS

ENTITLED IRON CHLOROSIS IN SILVER MAPLES (*Acer saccharinum*)

BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF

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ABSTRACT OF THESIS

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Correctives for iron chlorosis have been applied to soil, sprayed on leaves and injected into woody plants. Some success in woody plants has been achieved from soil applied chelated Fe and injected Fe chelates. Silver maple has been unusual in that little response to chelated Fe was found. Experiments were devised to inject silver maples with different Fe chelate formulations, comparing results with a soil applied EDDHA treatment as well as chlorotic and green controls.

Silver maple, for the most part, failed to respond to any of the chelate treatments applied. Foliar analyses revealed high levels of foliar Ca in chlorotic leaves compared to green leaves. When the soil producing green and chlorotic plants was analyzed, it was found that total soil Fe levels were higher for green control plants than chlorotic control plants. This increased level of total soil Fe was not reflected in the foliar analysis. There was no major distinction in foliar Fe levels between green and chlorotic tissue.

Xylary sap pH and E_h were also recorded for silver maples growing in various solution cultures. Solution cultures were prepared to simulate various types of Fe stress capable of causing Fe chlorosis. Although the solution culture pH varied widely, xylary pH stayed

relatively constant. Xylary E_h measurements maintained an even narrower "buffered" range. When pH and E_h were combined on a similar scale (pH + pe), the xylary sap pe + pH was observed to stay in a narrow range even when solution pe + pH varied widely.

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CHAPTER I

INTRODUCTION

In 1975, chlorotic silver maples in the city of Fort Collins were selected for treatment with encapsulated ferric ammonium citrate. Previous work in other areas of the country indicated that this chemical was successful in regreening chlorotic oak (151) and maple (191). Results of this treatment on Fort Collins silver maple were, for the most part, poor. However, questions were generated from these results. Are silver maples unsuited to grow in calcareous soils? Are obstacles to green growth located at the roots? Are there obstacles in the xylem or the leaf? Is there something about the transpirational stream that prevents iron from being transported efficiently?

With these questions in mind, two experiments were devised to answer some of these questions. The first experiment involved treating different silver maples with numerous chelates. Some of the chelates had never been injected directly into the xylary stream before. Others have been successful on other woody plants. Chelates that had never been injected before were chosen on the basis of their pH-stability. The pH-stability diagrams of Norvell (154) were used to determine which chelates might effectively transport Fe in the pH range of silver maple xylary sap. Soil, growth, and foliar parameters were observed closely to aid in the final analyses.

The second experiment was devised to measure the pH and reduction potential (E_h) of silver maple. Solution cultures were used so that varying amounts of Fe and H^+ might be present. Xylary pH and E_h were determined so that any changes in the xylary fluid might be noted.

The objective of these experiments was to provide some information about the cause and cure of iron chlorosis in silver maple.

CHAPTER II

REVIEW OF THE LITERATURE

A. Introduction

Chlorosis is a broad term which implies the lack of chlorophyll in, what would normally be, photosynethetic tissue. Iron chlorosis refers to a deficiency of chlorophyll which can be corrected by the presence of physiologically active iron. Iron chlorotic plants are found mainly on alkaline and calcareous but occasionally on acid soils. Iron chlorosis symptoms are relatively easy to spot because of the characteristic whitish-yellow tissue that results from a lack of chlorophyll. Early symptoms occur in the newest leaves because of iron's relative immobility once incorporated into organic compounds. The new leaves become increasingly symptomatic as the chlorosis advances until only the veins remain green and the lamina becomes light yellow. Advanced stages of this disorder are evidenced by interveinal necrotic areas followed, eventually, by death. A continuous supply of Fe is needed throughout the growing season to eliminate these symptoms.

Addition of Fe to chlorotic crops improves crop yields. Compared with macronutrients, Fe is needed in smaller quantities. A 150 bushel corn crop optimally requires about 300 pounds of nitrogen (N) per acre. In contrast, that same corn crop requires only 3 pounds of Fe (150). In perennial crops, chronic Fe chlorosis contributes to twig dieback

and general decline in plant vigor. This decline opens the plant to insect or pathogen attack and eventual early death.

Iron chlorosis has been attributed to a variety of conditions. Mortvedt (149) claimed that iron chlorosis results primarily from poor soil drainage; manifestations of which are poor soil aeration, restricted root growth, decreased Fe uptake and soil salinity. Other contributing factors include (a) low soil Fe, (b) high soil levels of calcium carbonate, (c) bicarbonate (HCO_3^-) in the soil or irrigation water, (d) over-irrigation or a high water condition, (e) high soil phosphates, (f) high heavy metal levels such as manganese (Mn), copper (Cu) or zinc (Zn), (g) low or high soil temperatures, (h) high light intensities, (i) high levels of soil nitrate (NO_3^-), (j) unbalanced cation-anion ratios, (k) poor aeration, (l) the addition of certain organic matter to the soil, (m) viruses, and (n) root damage by nematodes or other organisms (150).

Other factors have been suggested. The HCO_3^- ion has received much attention in the literature as a contributing factor towards Fe chlorosis (12,19,34,57). Besides competition from anions like HCO_3^-, metal cations can compete with Fe when Fe becomes a limiting element. Mn (16), nickel (Ni) (20), and Cu (35) can be factors in Fe chlorosis. Some have suggested that a difference in the ratio of uptake of metal cations can differ between plant species and influence the degree of chlorosis (13). Thorne et al. (200) have suggested that factors contributing to iron chlorosis work to inactivate Fe inside the plant rather than prevent its uptake.

B. Uptake and Translocation

1. Plant uptake of iron

Soil minerals are made available to the plant through root interception, mass flow of the soil solution and diffusion through the soil matrix. Banin and Navrot (5) have calculated that if a plant absorbs 10 ppm Fe, the plant's total Fe needs will be satisfied. A survey of Colorado soils (73) showed an average concentration of about 2% Fe or 20,000 ppm. However, the availability of Fe for plant uptake is highly pH dependent and governed by the solubility products of Fe oxide complexes (154). Total available Fe (Fe^{++} plus Fe^{+++}) decreases below the 1% level at a pH = 5 (158). O'Conner has demonstrated that the diffusion pressure generated by 1% available Fe cannot supply adequate Fe to a plant's roots (158). DeKock (57) found that the amount of Fe reaching a plant shoot is related to the Fe available in a soluble form in the medium.

Maximum absorption of Fe occurs in a region within a few millimeters of the root apex (107). The root hairs contribute very little to absorption. Pea and corn cross sections, however, showed an accumulation of Fe in the root hairs and epidermis (116). Young lateral roots have been reported to absorb more Fe than mature roots (2,28) and Fe stress stimulates lateral root production (28).

Kliman (116) and Ambler et al. (2) have shown that plants absorb Fe^{+2} more readily than Fe^{+3}. The plant was shown to actively affect the level of Fe^{+2} surrounding the root (2,5,27,28,38,49,53,75,109,140, 166,211,216). The condition of the plant influences Fe availability. During Fe stress response, plants have been reported to secrete a

reducing substance (2,27,38,53,75,211,216) which has been purported to change the Fe^{+3} to Fe^{+2} prior to absorption. Brown and Jones (38) reported that more Fe and Mn were absorbed by chlorotic Fe efficient soybean roots than nonchlorotic roots of the same cultivar. The same was true of corn (53). It has been suggested that this reducing substance may be a flavin excreted by roots (53,75,216). In support of this, increased riboflavin production has been noted in Fe deficient tobacco (216). Riboflavin is synthesized in leaves and root tips (17) and can be released to the nutrient medium under conditions of Fe deficiency (213,236). The amount of riboflavin released is positively correlated to the H^+ which leads to an increased membrane permeability due to the substitution of calcium from the membrane. A high pH nutrient solution stimulates riboflavin production but limits its excretion (211).

A "mucigel," jell-like coating on the outer surface of the roots, has been identified by Jenny and Grossenbacher (109) and suggested to be important in nutrient availability. This has been discounted by Hodgson et al. (93).

Others have suggested that the root secretes protons (H^+) which lowers the rhizosphere pH and increases Fe availability (53,140,211). Changes in nutrient availability have been noted when the pH of the nutrient solution was altered (25,39). Clark and Brown (53) reported that Fe efficient corn produced greater amounts of protons and "reductants" and reduced more Fe at the surface than Fe inefficient corn.

Kanan and Witwer (113) suggested that the uptake of Fe in isolated leaf cells of iron chlorotic soybean is an energy-dependent

process. Evidence has pointed towards a plant's respiratory activities contributing towards an increase in Fe uptake by an alteration in the electropotential of the solution surrounding the rhizosphere (5).

2. Internal carriers of iron

Upon entry inside the plant, Fe^{+2} seems to be bound by some carrier (22,31,43,49,54,145,183,199,202). Organic acids have been indicated as transporters of Fe inside the stem (31,54,145,183,201, 207). Citrate (22,26,30,43,54,202,207), malate (62,100,205) and trans-aconitate (54) affect Fe transport in several species. In corn, the relative concentration of organic acids found in the roots theoretically capable of Fe transport were: malate > aconitate > citrate (238). However, stem exudates showed that ^{59}Fe moved toward an anode as ^{59}Fe citrate (54). Tiffin (201) demonstrated that citrate has a higher affinity for Fe than malate. Besides this, Fe efficient plants produce more citrate under Fe stress than Fe inefficient plants (31,235). High levels of Fe depressed the citrate content of soybean stems under some conditions (201). Tiffin reported (201) that the lowest citrate levels corresponded to the lowest Fe treatments while intermediate Fe levels gave the highest citrate levels. Decreasing the Fe available to soybeans also decreased the citrate level in stem exudate (30).

Part of this might be explained by Brown (22) who has reported that Fe stress in soybeans promoted the accumulation of citrate in root sap but without a concomitant increase of citrate in the stem exudate. He noted that a relationship existed between the Fe and citrate (Fe:citrate ratio) translocated in stem exudate but that no

relationship existed in the root exudate. Clark et al. (54) has shown that the citrate level in either the cortical or xylary cells was too low to account for efficient transport of Fe to the xylem from the root. They concluded that there must be other factors involved in the transfer of Fe from cortical cells to the xylary passageway.

Brown (22) referred to an accumulation of citrate in the root as a "citrate pool." The root citrate pool level controlled the translocation of Fe in the stem (37) but the total amount of Fe in the roots had little relation to chlorosis (57). The Fe:citrate ratio is not a constant, but varies because of Fe stress and nutrition (37). Wallace and Mueller (225) related susceptibility of Fe-inefficient soybeans to Fe deficiency as a result of the plant's failure to translocate Fe from the root rather than a failure of the root to accumulate Fe.

Different types of Fe chlorosis can affect the levels of total organic acids and the levels of individual organic acids. Su and Miller (102) divided Fe chlorosis into four types based on levels of organic acids found in chlorotic leaf tissue: (a) Fe deficiency chlorosis, (b) HCO_3^--induced chlorosis, (c) high P-induced chlorosis, and (d) high Mn-induced chlorosis. They reported that HCO_3^--induced chlorotic leaves of soybean had much higher levels of malonic acid than the control leaves. However, total organic acids remained unchanged. They also found HCO_3^--induced chlorosis promoted higher levels of total organic acids in stem exudates, than the level of organic acids caused by iron deficiency. Clark (52) found that total organic acid content rose whenever any mineral became deficient but evidently not to the degree found in HCO_3^--induced chlorosis. DeKock and Morrison (62) found that all types of chlorotic leaves (with the exception of

chlorotic tissue produced by virus yellows in sugarbeet) contained more citric acid relative to malic or oxalic acid. Mn-induced chlorosis had a lowering effect on malonic acid content while total organic acids remained constant (199).

3. Plant translocation of iron and phosphorus interference

Rediske and Biddulph (171) stated that the rate of translocation of Fe within a plant is dependent on three factors. First, the Fe concentration in tissues helps to regulate Fe translocation. As Fe levels in the tissues decrease, Fe translocation rates increase. Second, generally as the level of P in the tissues and exudate decrease, Fe mobility increases. Third, as the pH of the exudate is lowered, Fe mobility increases.

Mineral interferences causing or inducing Fe chlorosis have been investigated thoroughly. Phosphorus has been reported to have an effect on Fe uptake and/or utilization (13,25,35,39,59,60,74,75,80). DeKock (57) used the P:Fe ratio as a guide to the plant's Fe efficiency. Brown and Jones (39) reported that the P concentration was about 20% higher in Fe-inefficient sorghum than in Fe-efficient varieties. They hypothesized that the Fe-inefficient varieties had more efficient mechanisms for taking up P than the Fe-efficient varieties.

Hale and Wallace (80) claimed that in an acid pH, low levels of P precipitated Fe in the roots and caused a slight decrease of Fe in the leaves of soybean. Biddulph and Woodbridge (13) found that increasing levels of P in the nutrient medium resulted in increased levels of P in the roots of bean while N and Fe levels decreased. These authors

claimed that Fe and P form a precipitate and cause a barrier to the uptake of Fe.

Phosphorus and Ca were shown to inactivate Fe in the above ground parts of soybean while P alone decreased the absorption and translocation of Fe (45). Bennett (10), on the other hand, reported that adding excess P to nonchlorotic plum trees did not produce chlorosis. He also found high levels of P in nonchlorotic trees and concluded that high levels of P may not necessarily precipitate Fe and cause chlorosis.

Brown (25) suggested that P can effectively compete with the plant for Fe by acting as a catalyst in iron's oxidation process. Competition for Fe was demonstrated when HCO_3^- and $H_2PO_4^-$ were shown to competitively inhibit the accumulation of Fe from an Fe chelate (80).

Iron levels were related to the exudation of reducing substances. The Fe level was negatively related to flavin exudation while levels of P and flavin secretion in the nutrient solution were shown to be positively related (75).

4. Plant translocation of iron and manganese interference

High levels of manganese depress Fe absorption (186,193,194,209) apparently because of an "antagonism" between them (186,194). Because of this antagonism, plants with adequate Mn levels are usually more chlorotic than those with low Mn levels (209). In another demonstration of this antagonism is that Mn deficiency was brought about by increased Fe levels in nutrient solution.

Ouellette (139) claimed that a proper Fe:Mn ratio somewhere between 5:1 and 100:1 was needed for soybeans. This ratio was appropriate only if the Mn level remained somewhere between 0.1 and

2.5 ppm. Ouellette (165) discovered that the rate of Mn uptake increased with increasing levels of nutrient Mn while Fe peaked at 300 ppm. In one case, Mn uptake was independent of the Fe concentration in the medium (180).

Interference between Fe and Mn in nutrient solutions occurred at equimolar levels (138) and at Fe:Mn ratios in nutrient solutions below 1.5:1 and above 2.5:1 (193,194). Somers et al. (193) found that the highest levels of CO_2 evolution occurred when the ratios of soluble Fe to soluble Mn in plant tissues were the same ratio as in the nutrient medium, i.e., 1.5:1 to 2.5:1.

Twyman (209) hypothesized reasons for Mn/Fe interference. He claimed that: (a) Fe and Mn could compete for the same transporter, (b) they are "antagonistic towards each other so that Mn slows up the entry of Fe and encourages the formation of inactive Fe chelators," or (c) there is a toxic action of Mn on Fe metabolic processes.

Sideris and Young (188) proposed the possibility that Fe deficiency may arise from competition between Mn and Fe in a porphyrin chlorophyll precursor. Gerretsen (76), after finding that Mn stimulated oxygen uptake in a chloroplast study, concluded that Mn induced chlorosis could be due to the photo-oxidation of accessory protective pigments normally associated with chloroplasts.

Hewitt (84) made the point that simultaneous deficiency symptoms of Fe and Mn can exist in the same plant and that symptoms vary enough to indicate independent functions. On soybeans and sunflowers, Fe deficiency symptoms appeared as a result of high Mn levels, but the reverse was not true (233).

Commercial chelates have demonstrated Fe and Mn relationships. Iron EDDHA, when used on calcareous soils, can induce low Mn levels in plants (219). Because of the relative instability of Mn EDTA in the presence of Fe at a specific pH, Mn EDTA intensified the chlorosis in Mn-deficient beans (117). The use of Fe EDDHA can correct an internal Fe/Mn imbalance where the Mn level is too high by increasing internal levels of Fe (151,152).

Other cations can influence the status of Fe. The presence of Cu (41), Ca (162), Mg, K and Rb (138) were reported as being inhibitory to Fe uptake. Opposed to this, all of the cations so far mentioned, with the exception of zinc (Zn), enhanced the Fe content of the stem exudate when supplied at low levels (138). Zinc was reported to have an inhibitory effect on Fe uptake (1,138). Copper was suggested to have a synergistic effect with P on Fe utilization in wheat, colora rice and soybeans (35).

The Fe status can have an effect on the uptake of other minerals. DeKock and Inkson (60) grew mustard at various Fe levels and recorded the mineral content of the leaves. As Fe levels increased, the Mn:Fe, P:Fe and K:Ca ratios decreased. DeKock and Hall (59) found that the same kind of P:Fe ratio and Ca:K ratio existed in chlorotic tissue regardless of the type of chlorosis. Absorption of Zn, Ca, Rb, and P remained constant in chlorotic tissue of Fe efficient soybeans though absorption of Fe and Mn rose (38).

5. Plant translocation of iron and HCO_3^- interference

Lime-induced chlorosis (caused by excess HCO_3^-) has been distinguished from other types of chlorosis (137). Leaves of pear and

apple affected by lime chlorosis were high in K and low in Ca and Mg (137). Soybeans supplied with Fe EDDHA in a nutrient solution high in HCO_3^- resulted in decreased Fe accumulation (80), but did not impede Fe translocation from the roots. Miller (147) observed that sodium bicarbonate ($NaHCO_3$) inhibited respiration in excised roots of plants which were susceptible to HCO_3^- chlorosis but had little effect on the respiration of nonsusceptible plants. Bicarbonate ions competitively inhibited Fe accumulation by competing with anionic iron chelate for absorption on an absorption site (80). Wallihan (232) reported that no detectable Fe moved to the tops of orange seedlings under HCO_3^- stress.

The substrate pH is affected by HCO_3^- absorption. Absorption of HCO_3^- anions leads to the exudation of hydroxyl ions (OH^-) by the plant roots and increases the substrate pH (57). The absorption of HCO_3^- leads to internal organic acid formation by the plant, buffering the plant sap at about a pH = 6 (57).

6. Medium pH

Cation uptake can have a stimulatory effect on Fe uptake by reducing the pH of the nutrient solution (25,39,74). Excess cation uptake was reported to decrease the pH of the nutrient medium because of an efflux of H^+ from the plant's roots (161) which becomes important in chelate-metal dissociation. Dodge and Hiatt (65) implied that plant-induced pH changes can be caused by a differential uptake of cations over anions and by the form of nitrogen absorbed. This reduction in substrate pH enhances the dissociation of an Fe chelate (161). Iron efficient corn under Fe stress produced higher amounts of

H^+ and reductant and therefore reduced more Fe at the root surface than Fe-inefficient corn (53). Corn was incapable of regreening under the same conditions that allowed sunflower to regreen. It was hypothesized that relatively high levels of P in corn, as well as its reduced capacity to lower substrate pH, contributed toward corn's chlorosis (114). Brown and Jones (39) reported that within 4 and 5 days, Fe-efficient soybeans increased the pH of the solution more than Fe-inefficient soybeans. This was possible due to the differential uptake of K (39).

Wadleigh and Robbins (212) reported that at a constant pH, corn on high K cultures were slightly chlorotic and high Ca and Mn cultures yielded moderately chlorotic plants, while high P and nitrate (NO_3^-) cultures were severely chlorotic. Franco and Loomis (74) claimed that a drop in the solution pH used in growing corn, brocolli, soybeans, tomato, sunflower, cotton and rice was due to the preferential absorption of ammonium (NH_4^+) while absorption of NO_3^- resulted in an increased solution pH. The use of ammonium nitrate (NH_4NO_3) has resulted in the prevention of Fe chlorosis in susceptible plants (74).

Brown (25) found that the plant's recovery of Fe from chelators and insoluble precipitates depended on the soybean's ability to exude H^+. He found that roots of Fe-efficient soybean released more H^+ than Fe-inefficient soybean. The amount of Fe translocated seems to be related to the amount of Fe^{+3} reduced to Fe^{+2} near the root (125). Corn was found to be ineffective in altering the pH of the medium, and so the pH of the medium affected the amount of Fe released to the plant root from EDDHA (11).

7. Internal pH

Confusion exists concerning internal plant pH and the nutrient medium. Bennett (10) reported that exuded plant sap from chlorotic tissue was found to have a higher pH than nonchlorotic tissue. But Chapman (50), in a study involving the tracheal sap of hawthorne, apple, elm, pear, and pines, found no relationship between the sap pH and chlorosis. Oserkowsky (164) found a relationship between the trachael sap pH and tracheal sap Fe levels in pear leaves from the same tree, which showed variations in chlorosis between branches. Oserkowsky (164) did find that the pH is lowest and Fe levels are highest at the beginning of a growing season. Diurnal changes in Fe solubility correspond to day/night changes of the pH in composite tissue fluids (102,172). Lowest pH values for tissues occurred in the xylem, with highest pH values occurring in the phloem (172). Steep pH gradients always occurred between xylem and phloem (172) while Fe accumulation always occurred in high pH tissues lying adjacent to relatively low pH tissues (172). An increase in the pH of lime-induced chlorotic leaves occurs as a result of cation-anion imbalance and results in a precipitation of Fe due to competitive chelation (231). DeKock (57), using mustard plants, found that the pH of expressed sap of roots, stems and leaves maintained a pH of 6 irrespective of the medium pH.

It has been demonstrated (170) that the pH in plant cells is relatively constant, despite large changes in extracellular pH, due to "pH regulating mechanisms" probably contained in the plasmalemma.

The form of nitrogen taken up can influence the plant sap pH, and the level of Fe accumulated as well as the pH of the medium. The nitrogen form absorbed can influence the degree of chlorosis (212), the

availability of Fe (189), mineral composition of the plant (4,152), the organic acid content of the plant (4,52,152), the pH of the expressed plant sap (212), and the amount of Fe translocated (189).

8. Aeration

The amount of aeration can affect chlorosis. The Fe content of mustard showed a progressive decline when the aeration of the medium increased from 1% to 20% air (57). Lindsay and Thorne (136) showed that chlorosis increased in HCO_3^- treated cultures at high oxygen levels. They pointed out that this was accompanied by reduced movement of Fe to the leaves, lower chlorophyll levels, and reduced growth. They suggested that poor aerating conditions around roots contributes to chlorosis through increased CO_2 levels and a raised HCO_3^- level at the root.

9. Plant differences in Fe uptake and utilization

The literature has reported differences in Fe absorption and utilization between varieties as well as species (1,26,29,34,35,91,108, 140,146,39,53). Intervarietal differences have been reported in soybean (1,35,108), sunflower (114,140), corn (26,29,53,72,114), sorghum (39,146), tomato (31), peanut (83), rice and wheat (35). Iron-efficient soybeans were found to transport and accumulate more Fe than Fe-inefficient varieties. This was attributed to a greater accumulation of organic acids in the efficient variety (37,39). This difference is genetically controlled within the plant (235) and probably mechanically controlled by the root (238,239). Himes et al. (91) reported that the water soluble leaf extracts of oak and maple solubilized different amounts of Fe from ferric hydroxide. Brown and

Holmes (36) pointed out that different levels of Fe were needed to prevent chlorosis in soybeans, wheat and corn. Sunflower and corn differed greatly in lowering the pH of the nutrient medium and in the absorption of P (114). Sunflower's superior performance over corn at low Fe levels was attributed to its efficiency for absorbing lower concentrations of anions as well as its superiority in lowering the pH of the medium (114).

Wheat, corn and soybeans differed in susceptibility to Cu induced Fe deficiency (36).

From these observations, it has been concluded that the Fe status of a plant is determined by the plant species or variety and influenced by the mineral nutrition of the soil (36).

When two cultivars of soybeans, one efficient and the other inefficient, were grown in the same solution, the inefficient variety inhibited Fe uptake by the efficient variety (108). The efficient variety, however, didn't seem to affect the inefficient varieties (108). This hypothetical inhibitory substance appeared to be an excreted compound from inefficient varieties (69). This was disclaimed (1). The disclaimor maintained that no inhibitory substance was involved, but rather, the inefficient variety did not utilize Fe as efficiently as the efficient variety and so allowed more Fe to become available for absorption by the efficient variety. Reasons have been proposed for discrepancies between plants. Brown and Bell (29) proposed that Fe-inefficient plants: (a) have less reducing capacity at the root, (b) require more Fe stress for maximum uptake of Fe, (c) are less efficient in taking Fe from solution, (d) are less efficient at lowering the pH of the solution, or (e) less tolerant to the effects

due to P. They and others (29,146) hypothesized that P may have a direct effect on the metabolic utilization of Fe. Similar conclusions have been made by Brown and Ambler (26). Clark and Brown (53) have reported that efficient varieties of corn produced larger amounts of H^+ and reductant in nutrient solutions and reduced more Fe at the root surface than inefficient varieties. When efficient and inefficient varieties of corn were grown in a mixed culture, they concluded the reason that inefficient varieties weren't benefited by a substance excreted by the efficient variety was because Fe efficiency was controlled inside the root. Mikesell et al. (146) found the reduction potential of Fe-efficient sorghum lines to be much lower than sorghum or corn. Others (1,114) found that the mixed soybean genotypes did not interfere with Fe uptake in a combined culture medium.

Difference in Fe usage are not restricted to the genera. Christ (51) noted that monocotyledonous plants, as a general rule, needed a higher level of nutrient Fe to sustain optimum growth than do dicotyledonous plants.

10. Chelates and iron uptake

Chelates have been reported to increase the uptake (36,94,144, 179,220,223,225) and, in some cases, improve the translocation of Fe (47). Plant species differ in their response to a chelate (36,41,45, 51) and the effectiveness of a chelate varies on different soils (36, 255). It was first reported that both the metal and the chelating agent were absorbed (8,61,90,108,224,225,227,228) but later it was discovered that a nonequivalent uptake of the metal and chelate occurred (23,190,203,204,206,228,229). Plant roots and chelates appear

to "compete" for available Fe (81,107,223). This indicates that a chelation-type reaction may be involved in the absorption of Fe at the root-soil interface (46). Simons et al. (190) reported that when a chelate was supplied in excess of the metal, the ligand was readily taken up by the root. When chelates were present without Fe, in solution, soybeans were unable to grow well if the Fe^{+3} stability constant of the chelate was above 10^{25}.

C. Metabolism Within Iron Chlorotic Plants

1. Chlorosis and water relations

Chlorosis in plants, whether it is from a lack of Fe or a result of pathological disease, alters the metabolism of the tissue affected. Physiological changes occur which are detrimental to the plant. Iron deficiency in Fe-inefficient soybeans decreased growth by 37%, decreased photosynthesis 33%, but had no effect on transpiration rates (115). Hutchinson (98), after working with HCO_3^--induced chlorosis in several seedling species, concluded that chlorosis reduces the capability of an affected plant to resaturate after a water deficit. Water deficits as low as 10% caused permanent damage in chlorotic tissue while normal tissue could tolerate deficits from 60-85% before resaturation ability was impaired. These findings correspond well to field observations about increased dessication damage to plants grown on a calcareous soil. Crafts (55) has shown that chlorotic foliage transpires more rapidly than green foliage during both stomatal and cuticular transpiration. Also, stomatal closure occurs at greater water deficits in chlorotic tissue. Deficient plants exposed to severe drought cannot regain normal metabolism even though full turgidity is

regained (101). With this in mind, small water deficits can put cholortic plants at a physiological disadvantage.

2. Chlorosis and organic acids

Earlier mention was made concerning the accumulation of organic acids during Fe chlorosis. DeKock and Morrison (62) found that the citric acid: (malic and oxalic acid) ratio varies as the P:Fe and K:Ca ratios vary. The total amount of organic acid varied inversely with the P content of the leaf. Iljin (101) supports this work but has drawn a positive correlation between the Ca level and total organic acid level in the plant sap. Carbohydrate levels in affected tissue was found by Iljin (101) to be higher than healthy tissue, probably due to an impairment in carbohydrate translocation in chlorotic tissue. The literature is still not clear whether this rise in organic acid content is a cause or is a result of iron chlorosis. Hsu and Miller (199) attribute the accumulation of citric acid to a decreased aconitase activity. Venkat Raju et al. (211) assumed that this accumulation of citrate anions occurs within the root cells and causes an inhibition in the uptake of anions, but stimulates cation uptake and, as a result, the pH of the external solution decreases. They were, however, unable to detect any release of organic acids to the external medium and concluded that organic acid release by the roots was not responsible for a decrease in the pH of the medium.

3. Cation-anion balance

Cation and anion uptake in plants is associated with H^+ and OH^- pumps, respectively (103,170). Release of H^+ to the root's exterior results in OH^- accumulation inside the root and this is thought to

stimulate internal organic acid synthesis ("pH stat") (56). Wallace et al. (327) have pointed out that there are at least three ways in which unbalanced cation-anion uptake can modify the Fe status in plants via the pH: (a) excess cation over anion uptake can result in acidification of the external medium and make Fe more available (32,65, 114,140,160,221); (b) excess cation over anion uptake can lead to an increased pH inside the plant and precipitate Fe, or (c) the presence of NO_3^- could result in an increased amount of H^+ which would make Fe more mobile.

Pierce and Appleman (167) have shown that plants take up a large excess of inorganic cations over inorganic anions and that this anion deficit is made up by internal organic acid production. Inorganic ions were taken up in varying amounts depending on the plant species. Plants supplied with NO_3^- nitrogen take up more cations than those given NH_4^+ nitrogen and have a higher internal organic acid or salt concentrations than ammonium supplied plants (218). The literature does not explain whether the NO_3^- source is the direct cause of anion accumulation which then causes Fe chlorosis or whether a disturbed Fe metabolism causes organic anion accumulation (58). It was suggested that there is a specific concentration of organic acids needed for optimum growth (63,64) and that this is species dependent (119). Nelson and Selby (152) have shown that decreases in dry matter production were significantly correlated with anion concentrations.

4. Chlorophyll and mineral balance

Chlorotic plants were reported to have an increased capacity to accumulate Fe (90,126,140,221). This results in higher levels of Fe in

chlorotic leaves than control leaves (101,106,126,137). Others (104, 192,232) have found good correlations between Fe levels and chlorophyll content. However, reports, even after leaf surface contamination was eliminated, were conflicting (106,233). Oserkowsky (164) claimed that good correlations were obtained when Fe was extracted with 1.0 N. HCl. Leeper (126) stated that if Fe is supplied at a uniform rate, then good Fe/chlorophyll correlations are obtained. But if plants undergo a preliminary period of Fe deficiency, then no correlation between Fe and chlorophyll content is found. Iljin (101) reported that generally the salt content of chlorotic leaves exceeds normal leaves, the difference increasing as the severity of the chlorosis increases. The salt content of normal foliage tends to increase gradually from spring until autumn, whereas there are irregular changes in the salt content of chlorotic leaves throughout the growing season. Iljin (101) also recorded that chlorotic plant leaves have considerably greater total nitrogen content than healthy leaves. The amount of NH_4^+ nitrogen stayed relatively constant but amine and amide nitrogen as well as soluble proteins were much higher in chlorotic tissue (101). McCalla and Woodford (143) found that a drastic alteration of individual nutrients inside the plant did not affect the balance between total cations and anions. This balance was always in favor of the anions.

Even though amino acids have been eliminated as a carrier for iron (183), they have been demonstrated to be present in xylary exudate. They have also been shown to increase in leaves of plants under stress (10,62,99). DeKock and Morrison (62) found that Fe deficient chlorotic leaves of ten different species contained an overall increase in total amino acids and that this increase was not dependent on the

type of chlorosis. Young leaves typically showed an increase in amino acid levels as compared to mature tissue (62). DeKock et al. (62) did point out a positive correlation between the P:Fe ratio and the total amino acid content.

5. Chlorosis and respiration

The exudation of H^+ and reducing substances coupled to the uptake of Fe imply an active metabolic process (113). Glenister (77) reported that the respiration rate was depressed in young chlorotic leaves of Fe deficient plants. He reported that stem exudate Fe levels increased as the plant matured. He also found that Fe levels decreased as one sampled the plant in the distal directions. Banin and Navrot (5) have suggested a coupling between Fe uptake and the respiration activity of roots. They further suggested that this coupling adjusts Fe uptake to suit the plant's roots. Young and Wallace (238) noted that Fe deficiency significantly reduced corn root mitochondrial ATPase activity. In the presence of K^+ and Mg^{++} ions, Fe^{+2} stimulated ATPase activity, especially when plants were Fe deficient.

6. Iron and catalytic activity

Iron, as an important component of respiratory enzymes, has been well established. Cytochromes are powerful oxidases which rely on the oxidation-reduction potential of inorganic ions to mediate electron transport or activate oxygen. Weinstein and Robbins (234) pointed out many natural chelating materials that possess catalytic activity: chlorophyll, cytochromes, catalase, peroxidase, cytochrome oxidase, polyphenol oxidase and ascorbic acid oxidase. They found catalase to be the most sensitive to Fe deficiency, followed closely by cytochrome c

and ferredoxin. Peroxidase is probably the least affected followed closely by cytochrome oxidase (168). Low levels of catalase and cytochrome oxidase were found in green and albino leaf tissues of plants grown with low levels of nutrient Fe or high levels of Mn (233). Manganese has substituted for Fe in horseradish peroxidase resulting in a lower activity (233). In another report, Fe levels and catalase activity were higher in nonchlorotic than chlorotic tissue (94). Brown and Steinberg (40) reported that ascorbic acid oxidase in tobacco was unaffected by iron deficiency while peroxidase and catalase activity was low (19,33). Others reported ascorbic acid oxidase remained unchanged during Fe deficiency but instead it was a good indicator of Cu deficiency (19,33). Elstrom and Howard (69) used root peroxidase activity as an indication of Fe nutrition in soybeans. They found that peroxidase activity increased at both sufficient and extremely low levels of Fe. Bicarbonate was shown to decrease respiration and protein synthesis as well as decrease oxidase activity (196).

Iron is needed in protein synthesis. Perur et al. (166) found that Fe chlorosis caused an 82% reduction in the protein content of the leaf chloroplastic fraction. Early Fe deficiency can cause a disfigurement of the chloroplast which is related to protein synthesis. This disfigurement was thought to result from a lack of Fe which was needed in some early requirement of protein synthesis like transcription or translation (15). Possibly there is some relation to the oxidation and reduction of inorganic nitrogen which is regulated by Fe metabolites, and subsequent incorporation into proteins. Nitrate reductase is linked to the activity of cytochromes (153).

Iron and chlorophyll synthesis are undoubtedly related. It is generally agreed that chloroplasts contain the majority of Fe in a photosynthesizing cell (88). Price and Carell (169) found that the rate of chlorophyll synthesis in *Euglena gracilis* was a linear function of the total Fe content of the cell. Marsh et al. (141) claimed that Fe reduced the rate of synthesis of aminolevulonic acid (ALA) which suppressed chlorophyll synthesis. Synthesis of the chlorophyll precursor after ALA proceeded just as rapidly in Fe deficient as in normal tissue when ALA was supplied to Fe deficient tissue.

From papers previously mentioned, some conclusions can be drawn about iron's makeup in the cell: (a) some Fe is bound in the lamellar matrix, (b) more than 90% of the Fe is bound as lipoprotein, (c) much of the Fe is contained in phosphoproteins, (d) the cell nucleus contains very little iron, and (e) Fe can accumulate in the nuclei of root cells.

D. The Treatment of Iron Chlorosis

1. Introduction

In early attempts to cure Fe chlorosis, Bennett (9) listed several methods that were commonly used: (a) spraying, (b) trenching, (c) liquid injection of an Fe compound into plants, and (d) dry salt injection. Spraying has been done using Fe^{+2} sulfate in conjunction with a sticker. More recently, sprays using an assortment of Fe chelates (and wetting agents like Triton X (128), and sodium diotylsulfosuccinate with Fe polyflavonoid complexes (195) have been used. Foliar penetration is enhanced if an efficient surfactant is used (66). Kanan (111) reported that Fe from iron sulfate penetrated

through a leaf cuticle faster than Fe from an Fe chelate without a surfactant.

The trench method mentioned by Bennett (9) involves the digging of several trenches 1 or 2 feet deep, concentrically around the tree and placing ferrous sulfate to the sides of the trench. A modification of trenching was developed using a soil auger to bore holes about 2 inches in diameter. More recently, soil applications have included trenching (6,175), liquid soil injection (184), and application of chemicals to irrigation furrows (176). Chemicals used in soil applications have been iron sulfate, sulfur and an assortment of chelating material usually of the polyaminocarboxylic acid types. The trenching method has been used in ornamentals but is associated with obvious aesthetic disadvantages.

Liquid injection into plants has been done since the mid 1800's. In the past, a short pipe was screwed tightly into predrilled holes of a tree. A reservoir was attached above the pipe and the solution was taken up in the transpirational stream (9). Chemicals most commonly used were solutions of iron sulfate, iron chloride, iron nitrate and Fe^{+2} or Fe^{+3} citrate (9). More modern techniques have included placing solutions of iron sulfate or iron chelates under pressure, forcing the plant to transport the solution more rapidly. Dry salts have been used for tree injection methods as well. It was discovered by Mokrezecki (148) that it was unnecessary to dissolve iron salts in water before feeding it to a tree. Fe^{+2} and Fe^{+3} citrate have been applied in holes, bored into a tree below the soil line. The bored hole was covered over with grafting wax and the soil was replaced (148). More recently, a

plastic encapsulation method was developed which incorporates ferric ammonium citrate into the tree in a similar manner (151).

2. Chemicals used for treating Fe chlorosis

Van Driel (210) reported that iron sulfate was more effective for correcting chlorotic tomato vegetative growth than Fe EDTA. In the literature, this has been the exception rather than the rule. Iron sulfate's low cost has been a main factor in its widespread use in the past. However, with the advent of chelates, micronutrient fertilization has become economical. The high cost of chelate chemicals has been offset, somewhat, with the low application rates needed for effective results.

Chelate chemistry has been discussed by several authors (24,42,79, 127,133,134,142,200,215) and will not be discussed in any detail by this author. Chelation is a ubiquitous biological mechanism important in soil reactions as well as biochemical reactions (67,157). Chaberek and Martell (48) have defined a chelate as a complex involving a metal ion with an organic molecule (ligand) that can donate electrons from at least two parts of the same molecule. Generally such donation involves the metal ion's positive charge which draws electrons away from the organic molecule's reaction centers (67). In biochemical reactions, the metal ion exerts most of its effects while in a bound form (178). Modification of the ligand may occur through a change in pH or intermediate metabolic reactions so that the chelated metal ion concentration can vary (178). Changes in the electromotive force of the medium surrounding the chelate can alter the bound metal ion concentration in equilibrium with the free ligand in accordance with the following reaction:

$$e^- + FeL^- \rightleftarrows Fe^{+2} + L^{4-}$$

Chelate-metal stabilities for Fe are highly dependent on the oxidation state of Fe. Ferrous iron generally forms stable compounds with organic anions containing oxygen, nitrogen and sulfur while Fe^{+3} iron show stability with oxygen containing ligands (168).

In forming a chelation hypothesis involving plant nutrition, Williams (237) first assumed there was an "excess of every type of organic ligand competing for a limited number of cations." Price (168) goes on to explain that if this is true, then the complexes being formed will depend on the equilibria between competing ligands and metals. He also mentions that the "spin" (measure of extent of electron pairing between ligand and metal) affects the chelate-metal interaction as well. Low spin chelates tend to exchange slowly between the free ions and the chelates while high spin chelates have a rapid exchange.

The extent of chelation and species of chelate formed is governed by stability constants:

$$K = \frac{(\text{metal chelate})}{(\text{free metal ion}) \times (\text{free chelate})} .$$

K depicts the molar ratio of the metal chelate to the free metal ion and free chelate. The chelate's stability constant and the pH of the medium are the most important factors in selecting a chelate for use in plant nutrition (127,234). Naturally occurring chelating materials are present in humus which influence metal solubilities (96,131,182) and chelates are exuded by plant roots (216).

In the past, there has been controversy whether the Fe is absorbed in chelated form (89,214) or if the plants are capable of selectively

absorbing the Fe from the chelate, leaving the chelate outside the root (24,190,203). The most popular concept is that both mechanisms occur but vary with plant species (206). The stability constant of the metal chelate was shown to have a direct effect on the rate of regreening. Simons et al. (205) found that the rate of regreening of soybean leaves was related to the stability constant of the metal chelate, i.e., weaker chelates gave a better response at lower concentrations than stronger chelates (46). Without chelation and in the pH range acceptable for plant growth, Oertli and Jacobsen (160) found that the plant requires more Fe than is thermodynamically possible owing to its solubility product at this pH range.

Elgawhary et al. (68) believed that the greatest benefit obtained from chelated micronutrients was the increased movement of these ions towards the plant root through mass flow and diffusion. Root exudates, natural chelates or synthetic chelates, added near the plant root, helped to supply increased amounts of nutrients for absorption. Not only did these chelates improve transport, but also increased the solubility of micronutrient cations which in turn increased diffusion gradients (158).

A popular concept developed by Wallace (215) is called the "competitive chelation hypothesis." This hypothesis states that other metals besides Fe "compete with Fe for Fe-binding sites, both in cells in leaves and at the root surface." If one further assumes also the "ligands such as OH^-, $H_2PO_4^-$, HCO_3^- and chelating agents can compete with Fe-binding sites for Fe," then Fe chlorosis can be explained. There is evidence that other metals can displace Fe from the chelate (79) and that these reactions are predictable (82,134,155,156). The root was

reported to act in competition with the chelate for the micronutrient (44,107). When the chelate-iron stability constant was particularly high, the chelate effectively competed with the plant for Fe and caused chlorosis (81).

There have been proposals about the contribution chelates make toward movement of the metal-chelate complex towards the roots. Hodgson (92) proposed and O'Conner (157) provided evidence for the build up of concentration gradients of dissociated ligands near the root surface. These gradients aid diffusion in soils with a small capacity factor. This uncomplexed ligand then diffused away from the root where it complexed with a cation and diffused back towards the root (an area of low chelate-metal concentration). O'Conner et al. (159) concluded that the presence of a chelate may raise the solubility of Fe to a point where mass flow may aid in absorption.

Limitations in long term effectiveness of chelates in soil application may be caused by leaching (71), photodensitization (87), microbial degredation (224), exchange reactions with other metals (218), clay fixation (218), and alkaline hydrolysis of the metal chelate (218).

3. EDDHA

EDDHA, in the past, has been called APCA, EHPG, and Chel-138 in the literature (206). Competitive interactions between EDDHA, DTPA and EDTA showed that EDDHA had the strongest affinity for Fe^{+3} (44). Fe EDDHA gave a weak absorbance at 480 nm. provided that there weren't any interfering compounds in the stem exudate of zinnia, soybean and sunflower (206). Halvorson and Lindsay (82) developed diagrams depicting the Fe EDDHA complex stability over a wide pH range. They

found that it remained strongly associated in the pH range from 4 to 9 even when in competition with EDTA and DTPA. It was hypothesized that this extreme stability contributed to a competition between the chelate and the plant for Fe.

The formation constant for Fe^{+3} EDDHA has been calculated to be (82):

$$Fe^{+3} + EDDHA \rightleftarrows FeEDDHA \qquad pK^{c}_{.01} = 35.07.$$

It will take at least a week for a chelate to reach equilibrium with its surroundings. Lindsay et al. (134) found that the relative stability concentrations ranged from Fe > Mn > Zn. If this chelate influenced mass flow and diffusion in a soil application, then an increased amount of Fe could be expected at the plant root. However, for species which developed chlorosis in photosynthetic tissue even at elevated levels of Fe, a chelated soil application may not be sufficient to overcome the deficiency (218).

Soil applied EDDHA has been noted to increase Fe levels in plants (230). Yield increases as much as 210% have been noted when FeEDDHA was applied to peanuts growing on calcareous soils (83). FeEDDHA's success against lime-induced chlorosis has been attributed to its stability in alkaline soils (121). FeEDDHA has been noted as the most effective chelate for correcting chlorosis in pear (78,122,174), sorghum (112), citrus (6,86), soybeans (226) and pin oak (184). Peach has responded with FeEDDHA alone (118,173,174,184) but not with low levels of soil applied FeEDDHA plus a nitrogen source (108). Excess EDDHA has caused a range in plant injury from no detrimental effects at high rates (226) to toxic effects when uncomplexed and under laboratory conditions (229).

It has been implied that FeEDDHA, when used in a foliar application, caused a scorching of the leaf surface (14). It is also reported to degrade readily in sunlight (222). Both of these factors discourage its use in foliar applications.

Rogers (173) has reported that the addition of FeEDDHA as a soil amendment increased foliar Fe and decreased foliar Mn and nitrogen. Phosphorus, Zn and K levels remained the same. DeKock et al. (60) reported no treatment effect on the nitrogen content of pear leaves while P and K levels were reduced except in very low levels of soil applied FeEDDHA.

4. EDTA

EDTA does not bind Fe as strongly as EDDHA or DTPA. Unlike EDDHA, Cu, Mn, Ca, and Zn can displace Fe from the complexes at higher pH's (82). In a pH range of 6 to 7, increased levels of Zn can decrease the effectiveness of FeEDTA. Above a pH of 6, FeEDTA becomes unstable. Above a pH of 7 in calcareous soils, Ca effectively competes for the EDTA ligand (82).

The formation constant for Fe^{+3} EDTA has been calculated, using the equation (82):

$$Fe^{+3} + EDTA^{4-} \rightleftarrows FeEDTA^{-} \qquad pK^{c}_{.01} = 26.27.$$

Upon adding FeEDTA to a soil with a pH of 8.5, all of the Fe was lost from the chelate during the first 4 hours (134). EDTA added to the soil without complexed Fe, did not result in an increase in the amount of soil solution Fe through an increase in Fe solubility (134).

Losses of EDTA in the soil have been reported (88,197,228). Because of the chelate-Fe complex instability in alkaline soils, foliar applications of FeEDTA are more popular than soil applications or foliar applications of FeEDDHA (94), even though sunlight is thought to decrease the effectiveness of FeEDTA (87). Foliar sprays and limb injections in pear trees of regent grade EDTA, without Fe, has resulted in recovery from chlorosis (85) although the response was slower than when chelated Fe was used.

Rather than the development of small green spots like those produced by ferrous sulfate sprays, FeEDTA has caused a complete regreening of the leaf (129). FeEDTA has consistently given good greening on chlorotic citrus in Florida's acid soils (129). Foliar application of FeEDTA has been used on plums and peaches with some success (18). EDTA in a soil application increased the dry weight of rye and stimulated the uptake of Fe, Cu and Mn as the level of EDTA increased (123). FeEDTA was a good source of Fe for plants growing in solution culture (105) without causing an Fe stress to develop due to chelate-plant competition as would occur with EDDHA (82).

Manganese EDTA, when applied to deficient bean plants, intensified the Mn deficiency (117). FeEDTA has been found to be mildly toxic at normal rates (229) and moderately toxic at the rate of 200-400 pounds per acre (228) EDTA, when applied internally through injection, EDTA has been found to affect respiration (147), chromosomal fragmentation (208), RNAse activity (120), and mitochondrial activity through an uncoupling of oxidative phosphoryalation.

5. DTPA

DTPA's primary use is to supply Fe to plants through soil applications (156). DTPA was reported giving better results on beans in a soil application when compared to EDTA (82).

FeDTPA begins to lose its stability at a pH about 7.8, following the reaction (82):

$$Fe^{+3} + DTPA^{4-} \rightleftarrows FeDTPA \qquad pK^{c}_{.01} = 28.86.$$

FeDTPA's formation constant places it between FeEDDHA and FeEDTA at a low pH. At pH values above 7.0, Zn can displace Fe from the chelate and reduce the effectiveness of FeDTPA (82,135). A heavy soil may increase the effectiveness of FeDTPA near the plant roots at a pH equal to about 8.0 (135).

As with other chelates, the effectiveness of DTPA is reduced in calcareous soils. Competition for the ligand can come from other cations as well. Micronutrients present in high concentrations can effectively compete for the DTPA ligand at a high pH due to its decreased stability. The chelate itself can compete with the plant for Fe. When the molar concentration of DTPA exceeded Fe in a nutrient solution, corn, wheat and okra could not effectively compete for Fe and, therefore, developed chlorosis (46). Other plants (soybeans and lupines) were more competitive and could obtain the Fe (46). Concentrations of Ca, Mg, P and boron (B) increased in chlorotic leaves as the level of nutrient DTPA increased.

DTPA can stimulate the uptake of cations other than Fe. The sodium salt of DTPA was found to induce Fe chlorosis in plants under

some conditions while at the same time increasing the uptake of heavy metals other than Fe (217).

Losses other than through absorption can sometimes be a problem. DTPA in a soil application was lost rapidly in the first few days (88) through absorption to soil particles (89). Losses of DTPA also occur through microbial decomposition (88).

6. Citrate.

Neely (151) found that regreening occurred in pin oak when encapsulated ferric ammonium citrate was injected into the tree. Other treatments (95,197) gave less satisfactory results. Hopkins and Wann (95) found that Fe citrate could keep Fe in solution in fairly alkaline conditions (197). Encapsulated ferric ammonium citrate gave increased growth and regreening in eastern white pine suffering from Fe chlorosis (191). Citrate, as a carrier for Fe, is severely limited due to the presence of aluminum at a low pH and Ca and Mg at a higher pH. As a soil application, these limitations would be too overwhelming to be effective (154) and is reflected in its low formation constant (154).

$$Fe^{+3} + Cit \rightleftarrows FeCit \qquad pK^{c}_{.01} = 12.5.$$

There have been implications that a low formation constant may be an advantage (190) so that the chelate will not compete with the plant for the nutrient.

ABSTRACT OF MINERAL AND CHLOROPHYLL CHANGES IN LEAF TISSUE OF *Acer saccharinum* AFTER TREATMENT WITH IRON CHELATES

Chlorotic silver maples (*Acer saccharinum*) were treated with soil applied EDDHA, and encapsulated ferric ammonium citrate, EDTA and DTPA. Foliar levels of Ca were higher in chlorotic tissue than green tissue. Chlorophyll levels and twig growth were not significantly different from chlorotic controls after treatment. Soil Fe levels were different for chlorotic and green control trees. However, foliar Fe analyses demonstrated that Fe levels were not different in green and chlorotic leaf tissue.

CHAPTER III

MINERAL AND CHLOROPHYLL CHANGES IN LEAF TISSUE OF *Acer saccharinum* AFTER TREATMENT WITH IRON CHELATES

Introduction

Neeley (15) and Smith (21) reported that trunk implantation of encapsulated ferric ammonium citrate (FAC) gave effective regreening of chlorotic pin oak and eastern white pine, respectively. Soil applied NaFeEDDHA (sodium ferric ethylenediamine di-(O-hydroxyphenylacetate)) gave consistent regreening of peach (17), citrus (3), pin oak (19), and pear (17). This regreening was reflected in both total chlorophyll content (21) and visual observations by means of a chlorosis index (19).

Increased Fe levels in tissues have not always corresponded to Fe treatments (10). Some have found a Fe/Mn ratio corresponding to Fe chlorosis (23). Others have suggested that various other factors may be involved, e.g., high P levels (6), high Ca levels (4), a Ca/K ratio (7) and interference from other micronutrients such as Cu (6) and Zn (1).

Preliminary work has indicated that FAC (18% Fe) did not give consistent regreening in mature silver maples (*Acer saccharinum*) implanted as a capsule. The objective of this study was to determine the effect of seven Fe treatments on silver maples growing in the landscape.

Materials and Methods

Seven Fe treatments were applied to 41 silver maples which were selected at random. Of these trees, 36 were chlorotic. Two controls were selected. One control was a green control (nonchlorotic foliage) and the other a chlorotic control (chlorotic foliage). The remaining trees all had chlorotic foliage.

Fe treatments and rates applied to chlorotic silver maples (*Acer saccharinum*) are as listed in Table 1. Soil applications were evenly applied beneath the tree canopy. Trunk implantations were made with soluble capsules places in the xylem of the tree. Capsules were arranged spirally around the tree, spacing them every three inches. Applications were made as listed in Table 2. Application was made in May, 1977, during bud break. Pretreatment chlorophyll levels were determined in July, 1976. Post treatment chlorophyll measurements were made in July, 1977, and September, 1977.

Leaf samples were taken from current (1977) and previous (1976) year's growth. Ten branches were selected from the tree's canopy and sampled. The leaves were washed thoroughly with a commercial detergent and rinsed. The leaves were then dried in a forced-air oven at 70°C for 24 hours. Dried tissue was ground in a stainless steel Wiley mill using a 40 mesh screen. Chlorophyll extractions were done according to Ross (18). Extracted chlorophyll was measured with a Bausch and Lomb Spectronic 20 spectrophotometer set at 652 nm. Chlorophyll levels were also measured in July and September, 1977, using a portable reflectance meter (25).

Leaf N was determined by the Kjeldahl method (5). Other elements (P, K, Ca, Fe, Mn) were ascertained by the Inductively Coupled Plasma

TABLE 1. Treatment, rate and method of application used on silver maple

Treatment	Rate	Application Method	Trees per Treatment
Green Control	--	--	5
Chlorotic Control	--	--	6
FeEDDHA (ethylenediamine di-O-hydroxyphenyl acetic acid)	1 lb./tree	Soil Application	6
FAC 18 - 18% Fe (ferric ammonium citrate)	0.8 gm/ capsule	Trunk Implant	6
FAC 28 - 28% Fe (ferric ammonium citrate)	0.8 gm/ capsule	Trunk Implant	6
FeEDTA (ferric ethylenediaminetetraacetic acid)	0.9 gm/ capsule	Trunk Implant	6
FeDTPA (ferric diethylenetriaminepentaacetic acid)	0.75 gm/ capsule	Trunk Implant	6

TABLE 2. Number of capsules implanted for each inch of trunk diameter

Diameter Brest Height	No. of Capsules
3-5 inches	2-3
6 inches	4
7 inches	5
Each Additional Inch	1

(ICP) method (11). Soil P and Ca extractions were done using perchloric and hydrofluoric digestions. Fe was extracted with DTPA and measured with ICP. Shoot growth was recorded in cm. and analysis of variance and correlations were calculated using GLIMAP (2).

Results and Discussion

A. Chlorophyll Levels From Fall 1976 (Pretreatment) and Fall 1977 (Post treatment)

Figure 1 demonstrates that even though all of the treatments showed some increase in chlorophyll levels over time, none of the treatments approached the chlorophyll levels of leaves from green control plants.

There are several reasons why encapsulated chelates may not have regreened chlorotic tissue. During the test year, water rationing was begun by the city council in an effort to conserve water for a dry summer. This imposed water restriction may have had a negative effect on growth and turgor in chlorotic plants (10). Lack of response from soil applied FeEDDHA was a surprise and may be indicative of a poor response in silver maple to artificially elevated levels of soil Fe.

FeEDTA has a relatively high stability at the xylary pH common to silver maple, e.g., pH ≈ 6.0 (16). This relatively high stability of FeEDTA may have led to competition between the chelate ligand and sites of Fe release from the chelate (24). If sites of Fe release from the ligand were in areas of lower pH than the xylem pH, competition could be intensified (16).

FeDTPA could have increased the problem since its pK is higher than FeEDTA's pK (16) and maintains this Fe stability at a higher pH (16). It has been suggested that Fe chelates with a pK below 25 are needed to

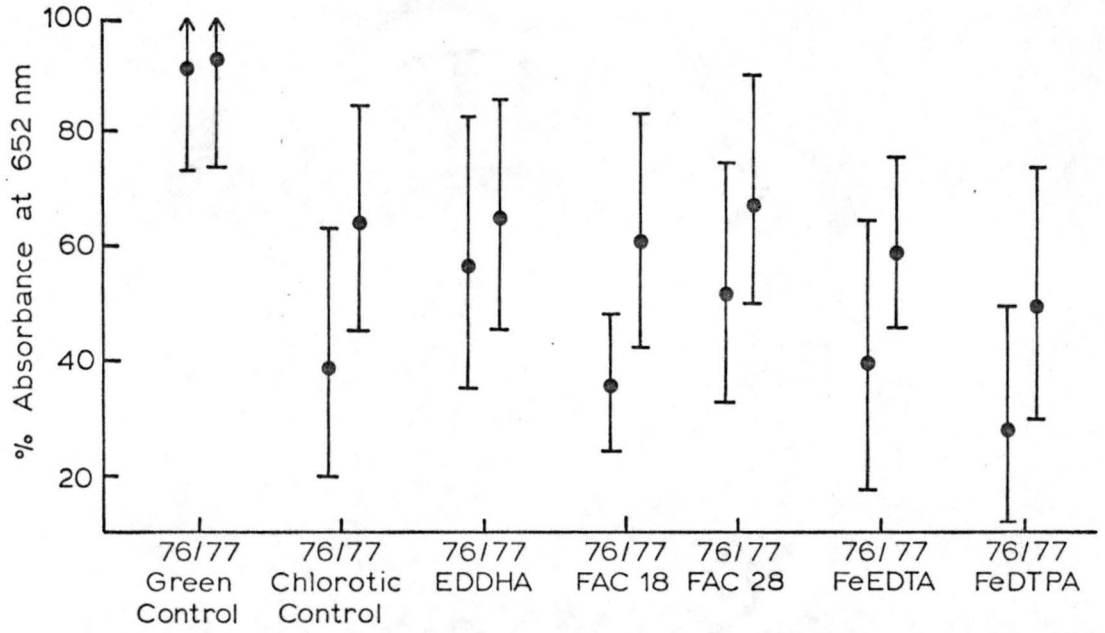

Fig. 1. Fall 1976 (pretreatment) chlorophyll levels and fall 1977 (post treatment) chlorophyll levels with 95% confidence limits.

prevent competition within the plant (20). Accordingly, applications of Fe citrate (FAC), with a pK much lower than 25 should have regreened chlorotic tissue. However, this study has not been supportive of this concept for silver maples.

B. Treatment Effect on Twig Growth, Chlorophyll Level, and Leaf Reflectance

Twig growth. In Figure 2A it can be seen that soil applied FeEDDHA was the only treatment with twig growth comparable to the green controls. Of interest is the downward trend of twig growth from pretreatment (1976) to post treatment (1977) in treatments other than soil applied FeEDDHA. Rogers (17) compared shoot growth of treated and control peach trees and found no significant differences between treatments used to regreen trees. He did find an increase in shoot growth over chlorotic controls when soil applied FeEDDHA was used. This trend of no difference in shoot growth between treatments may have been a reflection of the small amount of rainfall received in the summer of 1977 and watering restrictions imposed on municipal watering during this droughty period.

Chlorophyll levels. Figure 2B also demonstrates that chlorophyll levels were significantly higher in the leaves of green control trees than in any other treatment. It was interesting to note that leaves of nonchlorotic control trees showed a significant increase in chlorophyll levels through the growing season.

FAC 28 showed a decrease in chlorophyll content through the season. However, FAC 18 did show a high chlorophyll content in the summer. This same downward trend in chlorophyll content from summer to fall of the leaves from FAC 18 treated trees seems to indicate that the original

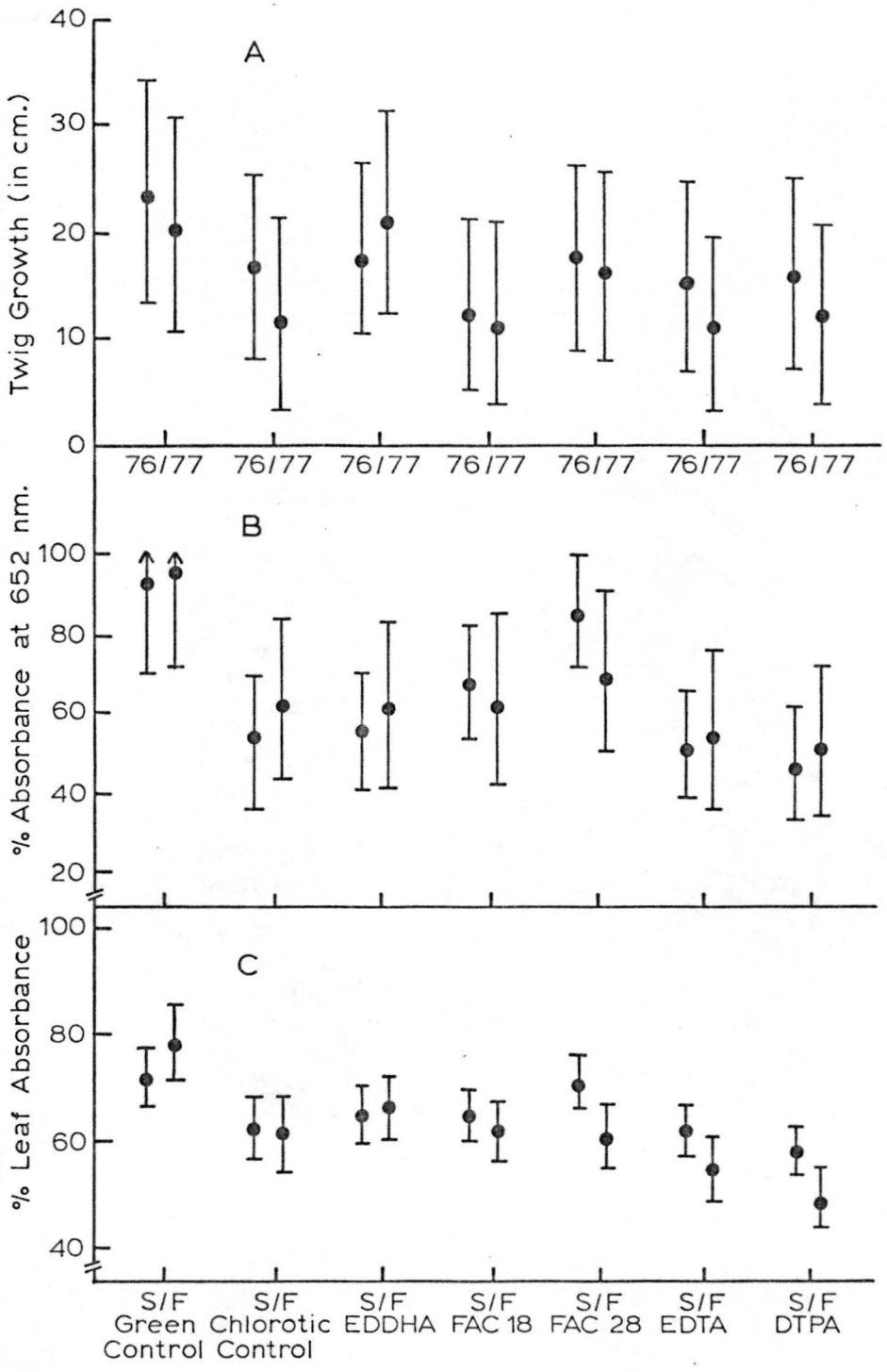

Fig. 2. 1976 and 1977 twig growth and 1977 summer and fall chlorophyll and absorbance readings of seven treatments with 95% confidence limits.

regreening effect was not continuous or that a decline in the color and photosynthesizing ability of the tree occurred.

Reflectance readings. Reflectance readings in Figure 2C demonstrate that only the green control tree leaves had a significant increase in reflectance due to treatment from summer to fall. This corresponds to increases in leaf chlorophyll content for the same period. Other treatments, with the exception of soil applied FeEDDHA, showed a trend towards decreased reflectance as the growing season progressed.

Bennett (4) found that chlorophyll levels increased through the season in healthy tissue. This is demonstrated in reflectance readings which increased through the season in green control tree leaves. With FAC 28, FeEDTA, and FeDTPA treatments, reflectance readings decreased through the season probably because of the tree's poor response to these treatments and increased drought stress. FAC 28 showed the same kind of decrease in chlorophyll content through the season in both chlorophyll levels and leaf reflectance. FAC 18 showed a relatively higher level of chlorophyll content in the spring in both leaf reflectance measurements and extraction measurements.

C. Treatment Effects on Foliar N, P, K, Ca, Fe and Mn

Nitrogen. Figure 3A shows that nitrogen followed a downward trend during the growing season. A significant downward change in N levels occurred in the FeDTPA treatment as well as the FAC 18 treatment. The nitrogen content of chlorotic tissue has been shown to decrease (4,17) or remain unaffected. The per cent total N in summer analyses ranged from 2.1-3.3 while fall analyses ranged from 1.7-2.8. This decrease in

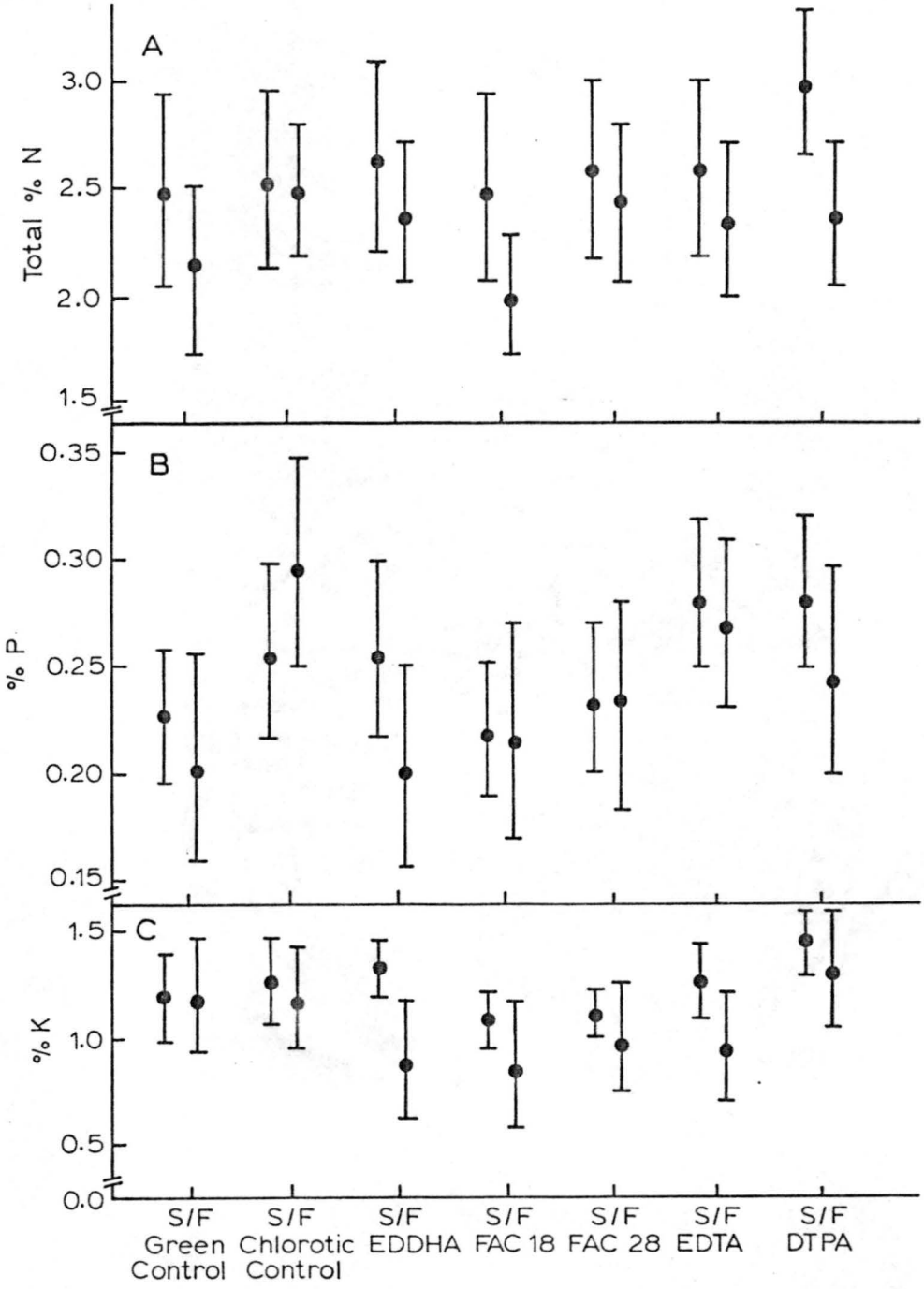

Fig. 3. Comparison of treatment effects on 1977 summer and fall foliar levels of N, P, K, with 95% confidence limits.

total N could be due to a dilution factor because of the increase in leaf volume during the season and exceptional mobility of N.

Phosphorus. Foliar P covered a wide range of levels in the fall analyses as recorded in Figure 3B. A wide and significant variation occurred between controls. However this difference was negated by the wide variation in P levels in treated but chlorotic leaves. The per cent P ranged in the summer analyses from .18-.32. Fall analyses ranged from .16-.34.

Potassium. Significant decreases in foliar K occurred from summer to fall in the FeEDDHA and FeEDTA treatments. Figure 3C shows that highest K concentrations occurred in the DTPA treatment. Controls were not significantly different from each other. The FAC treatments were significantly different from the Fe DTPA treatment but were not different from one another. Summer K levels ranged from .9-1.6 per cent. Fall levels from .60-1.6 per cent.

Calcium. Figure 4A shows that large increases in foliar calcium occurred from summer to fall analyses in all treatments except for the green control. Increases of this magnitude were significant. Calcium levels were extremely low in the green control leaves. However, no differences in summer levels appeared among other treatments. The same was true of the Ca levels found in the fall analyses. Summer Ca levels ranged from .45-1.2 per cent. Fall levels ranged in per cent from .50-2.12. Even though foliar Ca levels were within acceptable levels, green tissue had significantly lower Ca levels than chlorotic tissue.

Ca has been thought to inactivate Fe because of the carbonate or bicarbonate ion (6). Increased Ca levels can compete for ligand-carrier sites (16). This raised level of Ca is not known whether it is a result

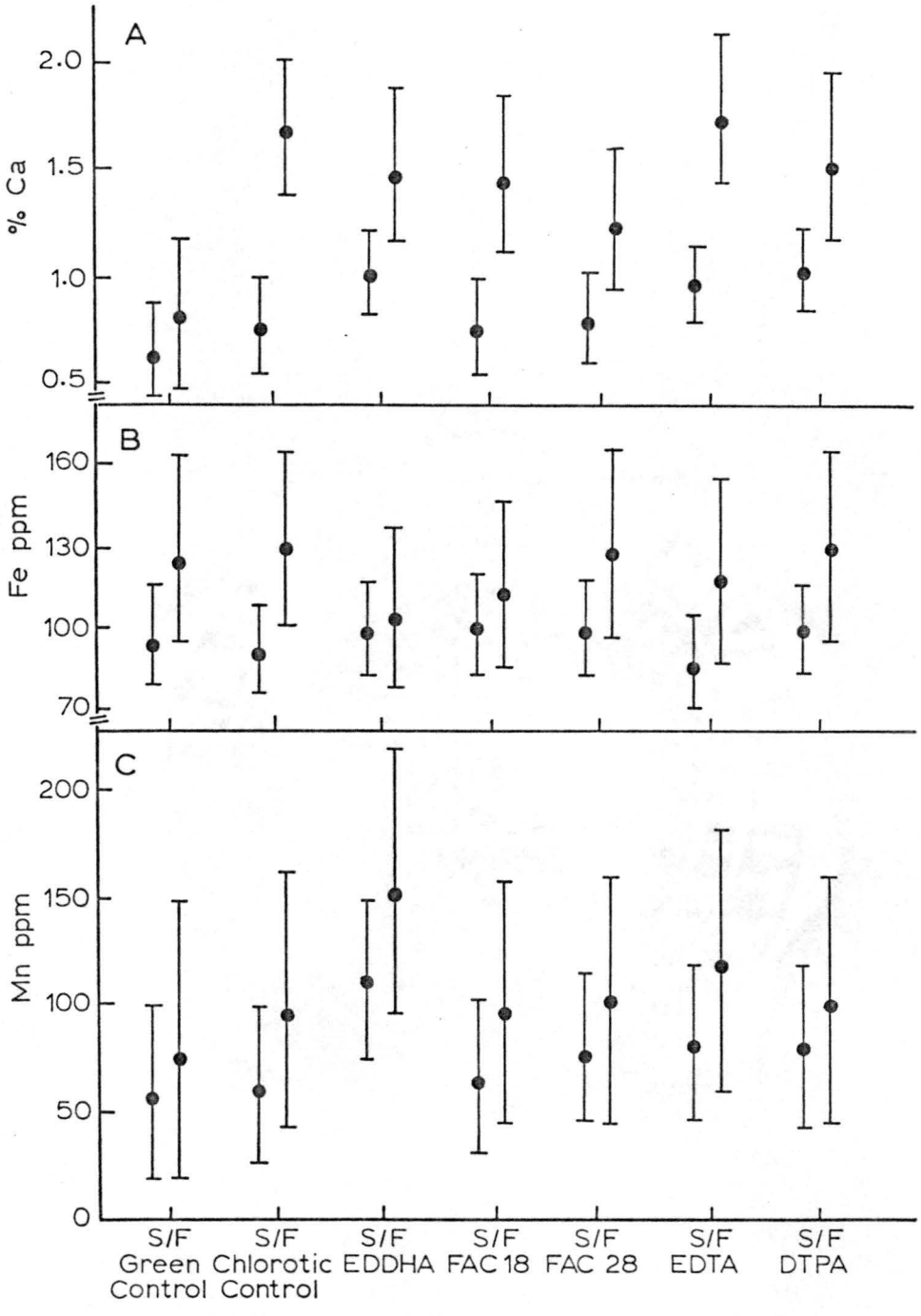

Fig. 4. Comparison of treatment effects on 1977 summer and fall foliar levels of Ca, Fe, Mn with 95% confidence limits.

of chlorosis or a cause of it. Ca is needed for healthy tissue but only in micronutrient amounts (8). Luxury consumption of Ca can occur and often does (8). Chloroplasts can contain as much as 60 per cent of the total leaf Ca (22). It has been concluded that chloroplasts can act as sites of Ca accumulation (13).

Iron. Fe had a wide range of levels within treatments. This contributed to a great deal of overlap of confidence intervals between treatments in Figure 4B. No apparent difference existed in Fe levels between treatments. No difference in Fe levels between chlorotic and green tissue seems to be necessary for adequate maintenance of chlorophyll levels. Fe levels increased through the season. This has been reported for other plants as well (14). Summer Fe levels ranged from 69-116 ppm while fall levels rose to the range of 77-165. It seems that differences in the uptake of Fe was not involved in chlorosis when the two controls were compared. It also seems that the translocation of Fe to the leaves was not hindered since foliar Fe levels were the same. A major question remains about the form of Fe found in these leaves as well as its location. It seems that green tissue usually has interveinal areas high in Fe with the veinal areas accumulating low amounts. The opposite is true of chlorotic tissue (7). Perhaps radiolabeling might provide part of this answer. Probably genetic variation in the biochemical accessibility of Fe is a major factor of this chlorosis (26). Certain individuals seem to have a biochemical advantage over other individuals.

Manganese. Like Fe, Figure 4C shows that the trend in the concentration of Mn from summer to fall was towards an increase. Soil applied FeEDDHA had a somewhat higher level of tissue Mn in summer and fall than

other treatments. Mn levels varied from summer to fall, 20-146 and 20-215 ppm, respectively. Mn has been reported to be deficient below 17 ppm in peach (17). The lower end of the Mn levels in green control tissue of silver maple dipped into this area but none of the treatments exceeded toxicity levels, e.g., 500 ppm (12).

All of the plant nutrients analyzed were within acceptable ranges of normal plant growth.

D. Treatment Effects on Fe/Mn, P/Fe, Ca/K, and Ca/P Ratios Measured in Summer and Fall

None of the ratios analyzed were significantly different (Figures 5 and 6) from treatment to treatment with the exception of Fe/Mn. The fall Fe/Mn ratio for the green control leaf tissue increased significantly over most other treatments (Figure 5A). However, two other factors were noticed. First, the range of the confidence limits from summer to fall increased dramatically for all treatments and in all of the ratios examined. Secondly, fall confidence limits were much higher than summer confidence limits. In some cases as much as ten times higher. The average Fe/Mn ratio for fall leaf tissue from green control plants (4.7) was much higher than average Fe/Mn ratios of other treatments measured in the fall. The fall Ca/K and Ca/P ratios of the leaf tissue from green control plants was somewhat lower than other treatments but still questionable as to its significance.

E. Differences in Chlorotic and Green Controls for Soil Fe, Soil P, and Soil Ca

Although there were no significant differences in soil Ca and soil P levels for chlorotic and green controls, soil Fe levels differed as shown in Figure 7. However, the trend in both cases of soil P and

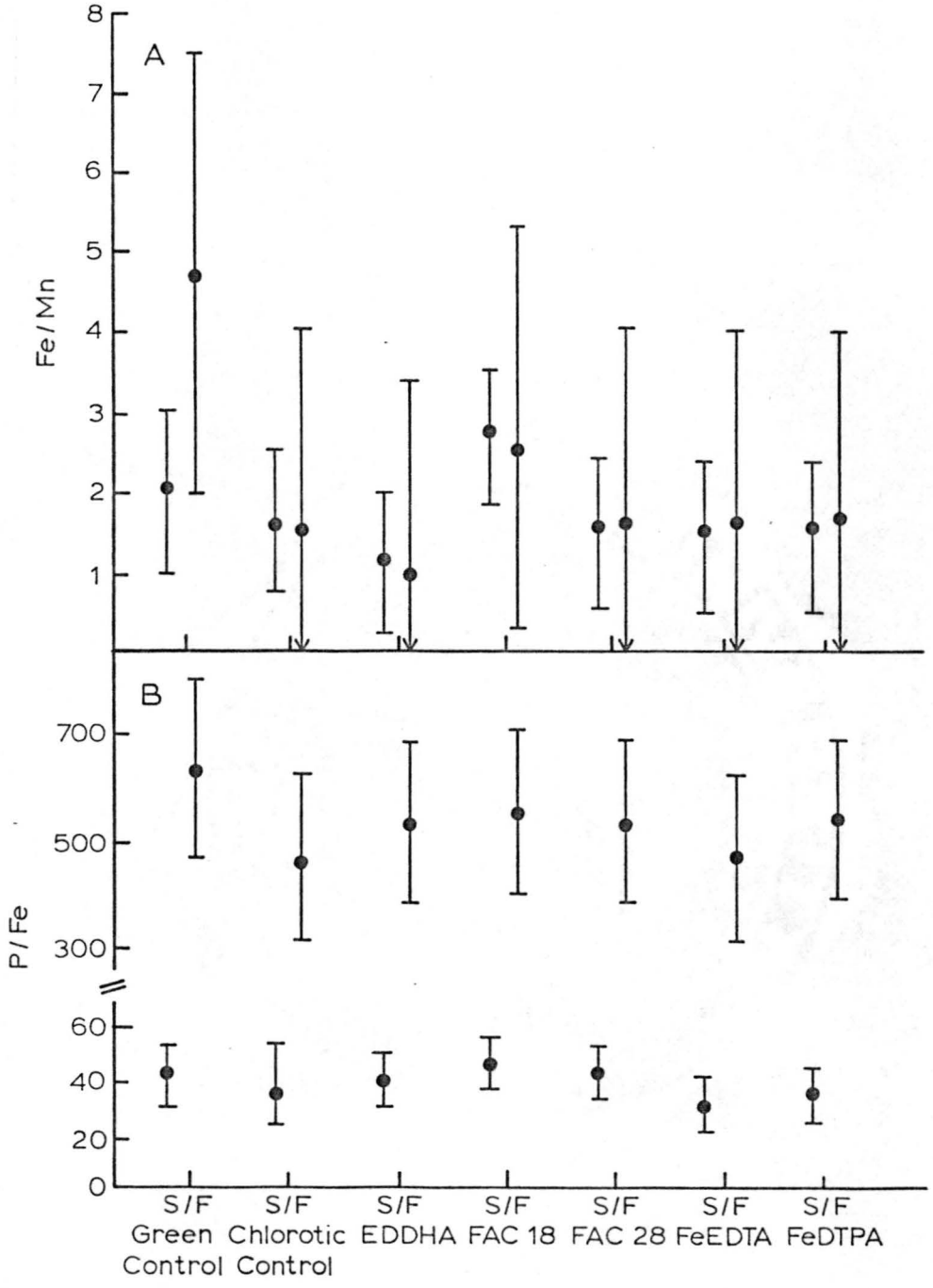

Fig. 5. Treatment effects on the foliar ratios of Fe/Mn and P/Fe in summer and fall samples.

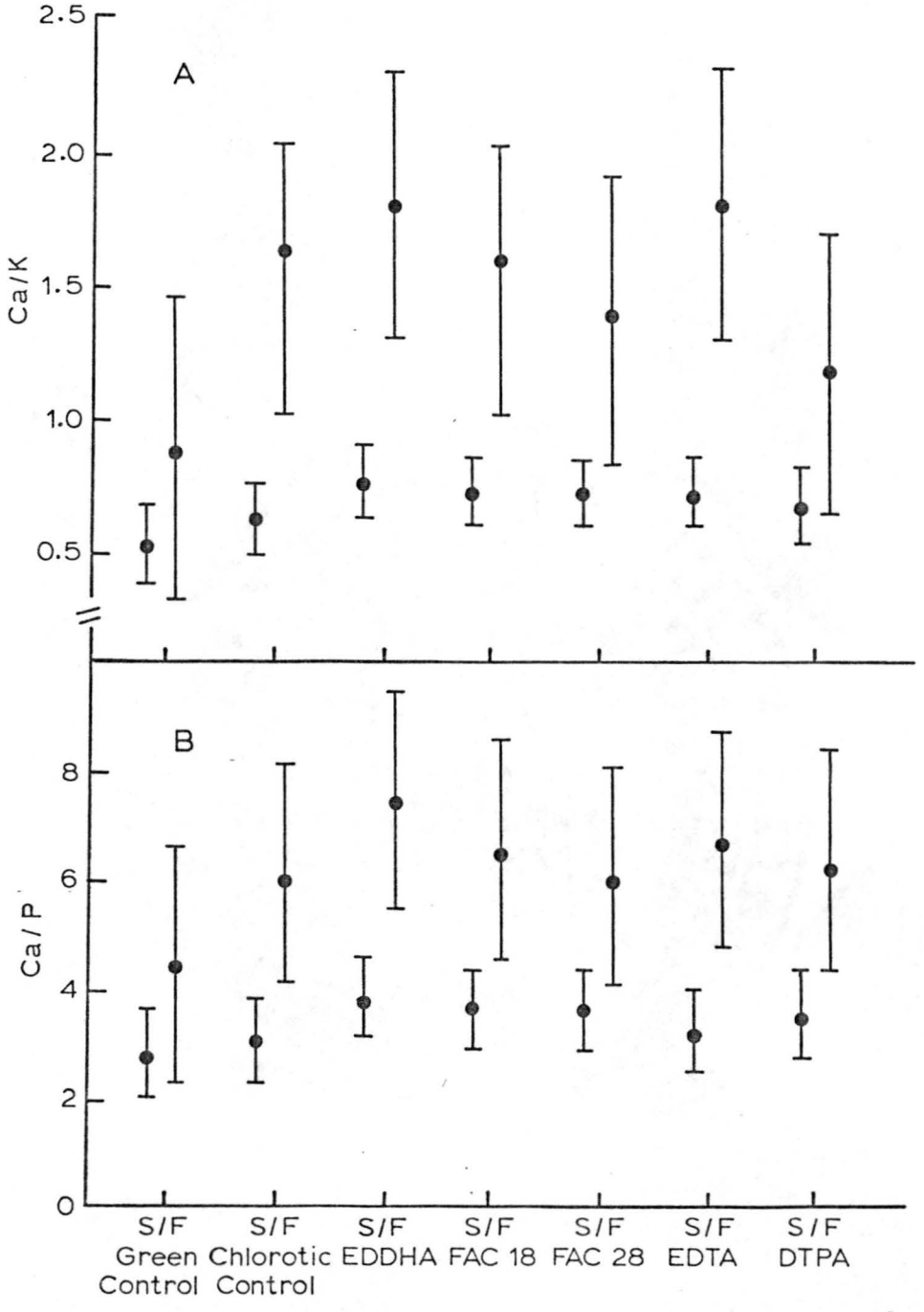

Fig. 6. Treatment effects on the foliar ratios of Ca/K and Ca/P in summer and fall samples.

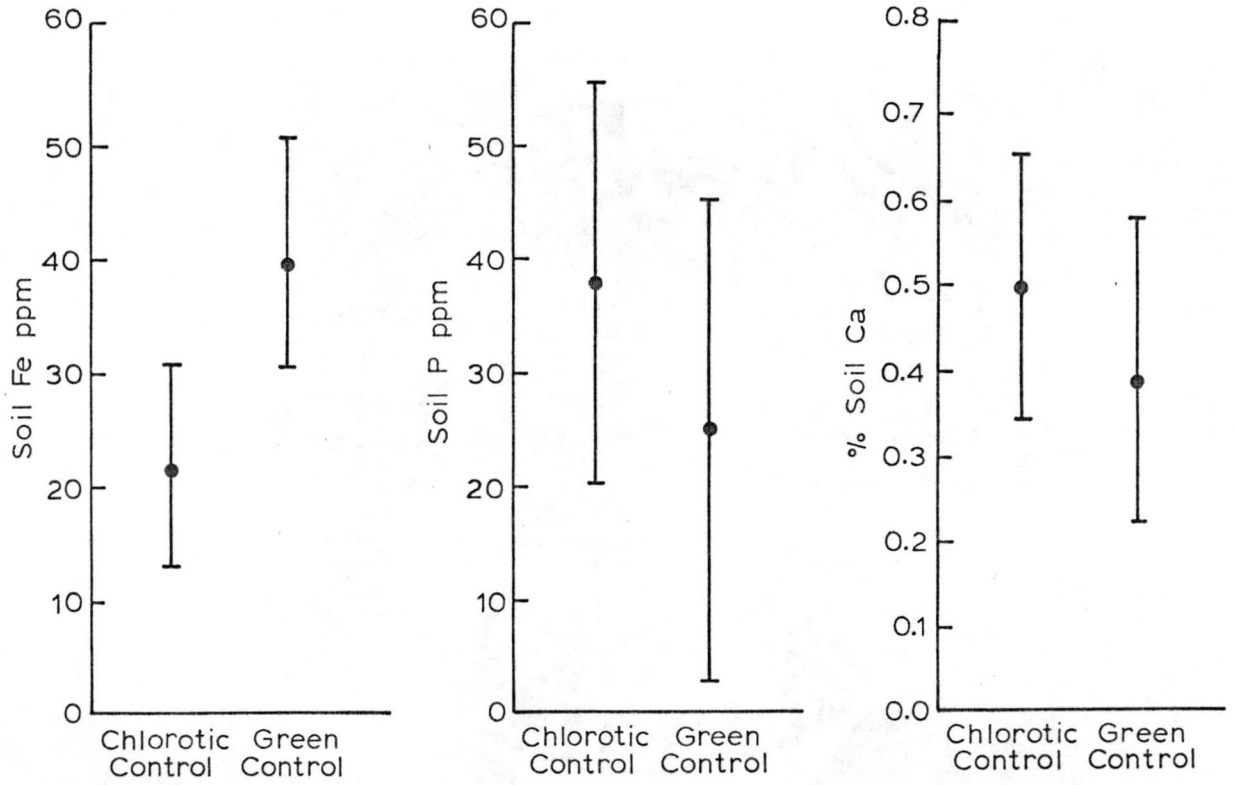

Fig. 7. Differences in soil Fe, P, and Ca for chlorotic and green control trees with 95% confidence limits.

soil Ca indicate a lower concentration in the soils in which green controls were growing. Differences in soil Fe levels growing green and chlorotic trees are obvious but neither are in the deficient range (< 2 ppm). It appears that differences in the soil Fe levels between controls does not contribute to differences in chlorophyll levels since this difference in soil Fe levels is not reflected in foliar Fe levels.

F. Correlations for Different Mineral and Growth Parameters

Correlations were made of those mineral and growth parameters listed in Table 4. As a general rule, correlations which were significant at one time of the year were no longer significant at another time of year. The only exception was the Ca/P ratio and reflectance correlation. In the summer this correlation was linear and significant at the 5% level. In the fall, the linear correlation became significant at the 1% level, gaining some correlation to a curvilinear relationship at the 5% level. Of particular importance is the foliar K and foliar P linear correlation which was significant in summer samples at the 0.1% level. Foliar Ca and foliar P were correlated to a linear model in summer samples at the 1% level. A curvilinear relationship was demonstrated of the reflectance and Ca/K ratio in fall samples. Fe and reflectance responded to a curvilinear correlation at the 5% level and only in fall treatments. This may have been because of the artificially induced higher Fe/ligand levels present in summer tissue. As the season progressed, chlorophyll levels and leaf Fe levels had a longer period of equilibration.

TABLE 3. P-Values for determining linear and curvilinear correlations of mineral and growth parameters

Correlations	P-Values*			
	Summer		Fall	
	Linear[a]	Curvilinear[b]	Linear	Curvilinear
Soil Fe, Foliar Fe (controls)	.9295	.2277	.0989	.1530
Soil Ca, Foliar Fe (controls)	.7102	.6457	.6887	.0966
Soil P, Foliar Fe (controls)	.9117	.4623	.2370	.7131
Foliar Fe, Foliar Ca	.1949	.3252	.1819	.1616
Foliar Fe, Foliar N	.6405	.8547	.9305	.4523
Foliar Fe, Foliar Mn	.8078	.3986	.1600	.8969
Foliar Ca, Foliar P	.0057	.1175	.1844	.2838
Foliar K, Foliar Ca	.0005	.0963	.1619	.2761
Foliar Fe, Reflectance	.2654	.9739	.5628	.0209
Foliar Fe, Chlorophyll	.1729	.5822	.3541	.5074
Fe/P, Reflectance	.7949	.4645	.4408	.9793
Fe/Mn, Reflectance	.1659	.7184	.0225	.5785
Ca/P, Reflectance	.0125	.7660	.0028	.0418
Ca/K, Reflectance	.0761	.6458	.2920	.0037

*P-Values less than .05000 indicate acceptance of given correlation.

a - $\eta = b + mx$

b - $\eta = b + mx + ny^2$

G. Conclusions

The use of encapsulated FAC 18, FAC 28, FeEDTA and FeDTPA gave no significant improvement to twig growth, reflectance readings and chlorophyll content in the leaves of silver maple. Leaf Ca levels were significantly higher in chlorotic leaf tissue than in green tissue. Ca may interfere with the utilization of Fe at the chloroplastic level since foliar Fe levels were not different between treatments. Most of the significant correlations concern Ca or P, either of which can interfere with Fe utilization.

REFERENCES

1. Ambler, J. E., J. C. Brown, and H. G. Gauch. 1971. Sites of iron reduction in soybean plants. Agron. Jour. 63:95-7.

2. Angleton, G. M., and J. C. Simpson. 1977. Linear model analysis techniques using the CRHL general linear model analysis package (GLIMAP). Collaborative Radiological Health Lab., College of Vet. Med. and Biomedical Sciences, Colo. St. Univ., Fort Collins, Colo.

3. Basiouny, F. M., C. D. Leonard, and R. H. Biggs. 1970. Comparison of different iron formulations for effectiveness in correcting iron chlorosis in citrus. Fla. St. Hort. Soc. Proc. 83:1-6.

4. Bennett, J. P. 1945. Iron in leaves. Soil Sci. 60:91-105.

5. Bremner, J. M. 1965. Inorganic forms of nitrogen in methods of soil analysis, Part 2. In, C. A. Black (ed.) p. 1179-1237. Chemical and Microbiological Properties. Amer. Soc. Agron. Madison.

6. Brown, J. C. 1959. Iron chlorosis. Adv. Agron. 13:329-69.

7. DeKock, P. C. 1958. The nutrient balance in plant leaves. Agr. Progress 33:88.

8. Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. Wiley and Sons. New York.

9. Hale, V. Z., and A. Wallace. 1960. Bicarbonate and phosphorus effects on uptake and distribution in soybeans of iron chelated with EDDHA. Soil Sci. 89:285-7.

10. Hutchinson, T. C. 1970. Lime chlorosis in seedling establishment on calcareous soils. New Phytol. 69:261-8.

11. Jones, J. B. 1977. Elemental analysis of soil extracts and plant tissue ash by plasma emission spectroscopy. Comm. in Soil Sci. and Pl. Anal. 8:349-65.

12. Jones, J. B. 1972. Plant tissue analysis for micronutrients. In, J. J. Mortvedt, P. M. Giordano, and W. L. Lindsay (eds.) p. 319-41. Micronutrients in Agriculture. Soil Sci. Soc. of America. Madison.

13. Larkhum, A. W. D. 1968. Ionic relations of chloroplasts in vivo. Nature 218:447-9.

14. Leal-Diaz, J. 1964. The uptake of ferrous and ferric iron by *Phaseolus vulgaris*, L., var. Red Kidney. Ph.D. Thesis. Univ. Calif., Davis.

15. Neely, D. 1973. Pin oak chlorosis: Trunk implantations correct iron deficiency. Jour. of Forestry. 71:340-2.

16. Norvell, W. A. 1974. Equilibria of metal chelates in soil solution. In J. J. Mortvedt, P. M. Biordano, and W. L. Lindsay (eds.) p. 115-38. Micronutrients in Agriculture. Soil Sci. Soc. of America. Madison.

17. Rogers, E. 1971. The effects of different sources and rates of iron chelate on Red Haven peach trees. Colo. St. Univ. Progr. Rep. 71-25.

18. Ross, C. W. 1974. Plant Physiology Laboratory Manual. Wadsworth Publ. Co., Belmont, Calif.

19. Schoenweiss, D. F. 1973. Correction of lime-induced chlorosis of pin oak by liquid soil injection. Hortsci. 8:333-4.

20. Simons, J. N., R. Swidler, and H. M. Benedict. 1963. Kinetic studies of regreening of Fe deficient soybeans. Pl. Phys. 38:667-74.

21. Smith, E. m. and C. D. Mitchell. 1977. Chlorosis of red and silver maple. Weeds, Trees, and Turf. July, p. 32,36.

22. Stocking, C. R. and A. Ongun. 1962. The intracellular distribution of some metallic elements in leaves. Amer. Jour. Bot. 49:284-9.

23. Twyman, E. S. 1951. The Fe and Mn requirements of plants. New Phytol. 50:210-26.

24. Wallace, A. 1971. Regulation of the Micronutrient Status of Plants by Chelating Agents and Other Factors. Arthur Wallace, publisher. Los Angeles.

25. Wallihan, E. F. 1973. Portable reflectance meter for estimating chlorophyll concentrations in leaves. Agron. Jour. 65:659-62.

26. Weiss, M. G. 1943. Inheritance and physiology of efficiency in iron utilization in soybeans. Genetics. 28:253-8.

ABSTRACT OF pH AND E_h MEASUREMENTS OF THE XYLARY SAP OF SILVER MAPLE (*Acer saccharinum*) GROWN UNDER DIFFERENT STRESSES ASSOCIATED WITH IRON CHLOROSIS

Silver maple, grown in solution cultures adjusted to create different iron stresses, maintained relatively constant xylary E_h and pH values. Even though the solution media varied greatly in pH, Fe quantities and HCO_3^- levels, the sum of the pH plus E_h (converted to pe) was maintained in a narrow range.

CHAPTER IV

pH AND E_h MEASUREMENTS OF THE XYLARY SAP OF SILVER MAPLE (*Acer saccharinum*) GROWN UNDER DIFFERENT STRESSES ASSOCIATED WITH IRON CHLOROSIS

Introduction

High soil pH, high levels of soil Ca, and high levels of soil HCO_3^- as well as low levels of soil Fe have been cited as causes of Fe chlorosis (2). Fe is presumed transported in a chelated form, probably as Fe citrate (12). The stability of this complex is determined by two factors: pH and the reduction potential of the tracheal sap. Oserkowsky (8) found a relationship between tracheal sap pH and tracheal sap Fe levels in pear leaves. Some investigators (1) have shown that exuded sap from chlorotic tissue was found to have a higher pH than nonchlorotic tissue while others reported no difference (3). The pH of the medium surrounding Fe will affect its solubility and its complex formation (7). This is important in Fe transport.

Steep pH gradients between xylem and phloem tissue have been noted to cause a deposition of Fe in the more alkaline tissue (9). Wallace et al. (14) claimed that an increase in the pH of lime-induced chlorotic tissue occurs as a result of cation-anion imbalance and results in the precipitation of Fe due to "competitive chelation."

Chlorotic leaves will sometimes contain more Fe than comparable green leaves. This could be due to an increased capacity to accumulate

Fe in chlorotic plants (5). Jacobson and Oertli (4) substantiated this view when they observed that chlorotic plants supplied with increased levels of Fe usually failed to regreen. An increased accumulation of polyvalent ions, like Fe, could lead to a change in the reduction potential of the medium surrounding internal ions (11). This alteration in electropotential could have a beneficial or detrimental affect on Fe complex formation and chelate stability (7). Alterations in the reduction potential of the xylary fluid can alter Fe complex stability and contribute to its unavailability (7,11).

The purpose of this study was to measure changes in the pH and E_h of the xylary sap of silver maple grown under various stresses causative of Fe chlorosis.

Materials and Methods

Eighteen inch silver maple were grown in a greenhouse in solution cultures. Growing solutions were adjusted as in Table 1. Each medium was prepared according to Table 2 (10). Four plants were grown in each 5 gallon container with 3 containers used per treatment. Air was bubbled through the media. After 32 days, pH and E_h measurements were made on the nutrient media and xylary fluids.

Plant samples for extraction of xylary fluids were cut from the treated plants and placed in a pressure bomb. Compressed nitrogen was used to force the xylary fluid from the xylem into a reservoir formed by a piece of inert plastic tubing fitted at the distal end of the stem segment. pH measurements of the exuded sap were made with a micro pH electrode and an Orion 801 pH meter. E_h measurements were made with a Beckman platinum electrode referenced with a Ag/AgCl reference electrode.

TABLE 1. pH and E_h of xylem fluid and culture solution fluid under different Fe stresses

Treatment	Xylary			Solution		
	pH	E_h	pe	pH	E_h	pe
High pH, + Fe	5.68±.19	251±14	4.18±.23	8.43	169	2.82
High pH, - Fe	5.83±.04	236±11	3.93±.18	8.73	118	1.97
Low pH, + Fe	5.76±.23	228±26	3.80±.43	6.67	197	3.23
Low pH, - Fe	5.93±.14	216±15	3.60±.25	6.57	250	4.17

TABLE 2. Nutrient solutions mixed for high and low pH, with and without iron

Stock Solution	Compound	Concentration
A	$Ca(NO_3)_2 \cdot 4H_2O$	1.0 M.
B	KNO_3	1.0 M.
C	$MgSO_4 \cdot 7H_2O$	1.0 M.
D	KH_2PO_4	1.0 M.
E	$MnCl_2 \cdot 4H_2O$	1.81 g/l.
	H_3BO_3	2.86 g/l.
	$ZnSO_4 \cdot 7H_2O$	0.22 g/l.
	$CuSO_4 \cdot 5H_2O$	0.08 g/l.
	$H_2MoO_4 \cdot H_2O$	0.09 g/l.
F	Na Fe EDTA	0.005 g Fe/l.

Treatment	Stock Solutions Added
High pH, + Fe	A, B, C, D, E, F
High pH, - Fe	A, B, C, D, E
Low pH, + Fe	A, B, C, D, E, F
Low pH, - Fe	A, B, C, D, E

Results and Discussion

A pH difference in the growing solution media pH varied by 2.16 pH units (Table 3) while the average xylary pH varied only 0.10 of a pH unit in the opposite direction. Thus, xylary pH readings were buffered by the plant near a pH = 5.68 - 5.93. Standard deviations of xylary pH readings reflect a small variation from the mean.

In each of the treatments outlined in Table 2, xylary E_h readings were maintained by the plant between 212-251 mv. When E_h is converted to the same negative, logarithmic scale as pH ($pe = \frac{E_h}{59.2}$) then a corresponding small variation from the mean pe was observed (Table 1). Figures 1 and 2 point out the stability at which the xylary solutions are kept even though fluctuations of the growing solution's E_h and pH were high. It should be noted that when the growing solution pH was raised artificially (Figure 1), the E_h of the same solution lowered (Figure 2). This has been demonstrated to occur in soils.[1] The end result of this is a buffering system consisting of the sum, pe + pH. When the growing solution pH is compared with the growing solution E_h, but translated to the same relative scale (pH vs pe), the variation in pH and pe imposed on the growing solution was approximately the same (mean difference in pH = 2.16, mean difference in pe = 2.2). It is important to realize this relationship because when the pe and pH were added, as a tool for measuring the stability of mineral elements in equilibria solutions, it was found (Table 3) that this sum was held

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[1] W. L. Lindsay, June, 1978, Personal Communication.

TABLE 3. Average xylary and solution fluid pe + pH affected by HCO_3^-, pH, and Fe treatment after 32 days

Treatment	Xylary pe + pH	Solution pe + pH
High pH, + Fe	9.86	11.25
High pH, - Fe	9.76	10.70
Low pH, + Fe	9.56	9.90
Low pH, - Fe	9.53	10.74

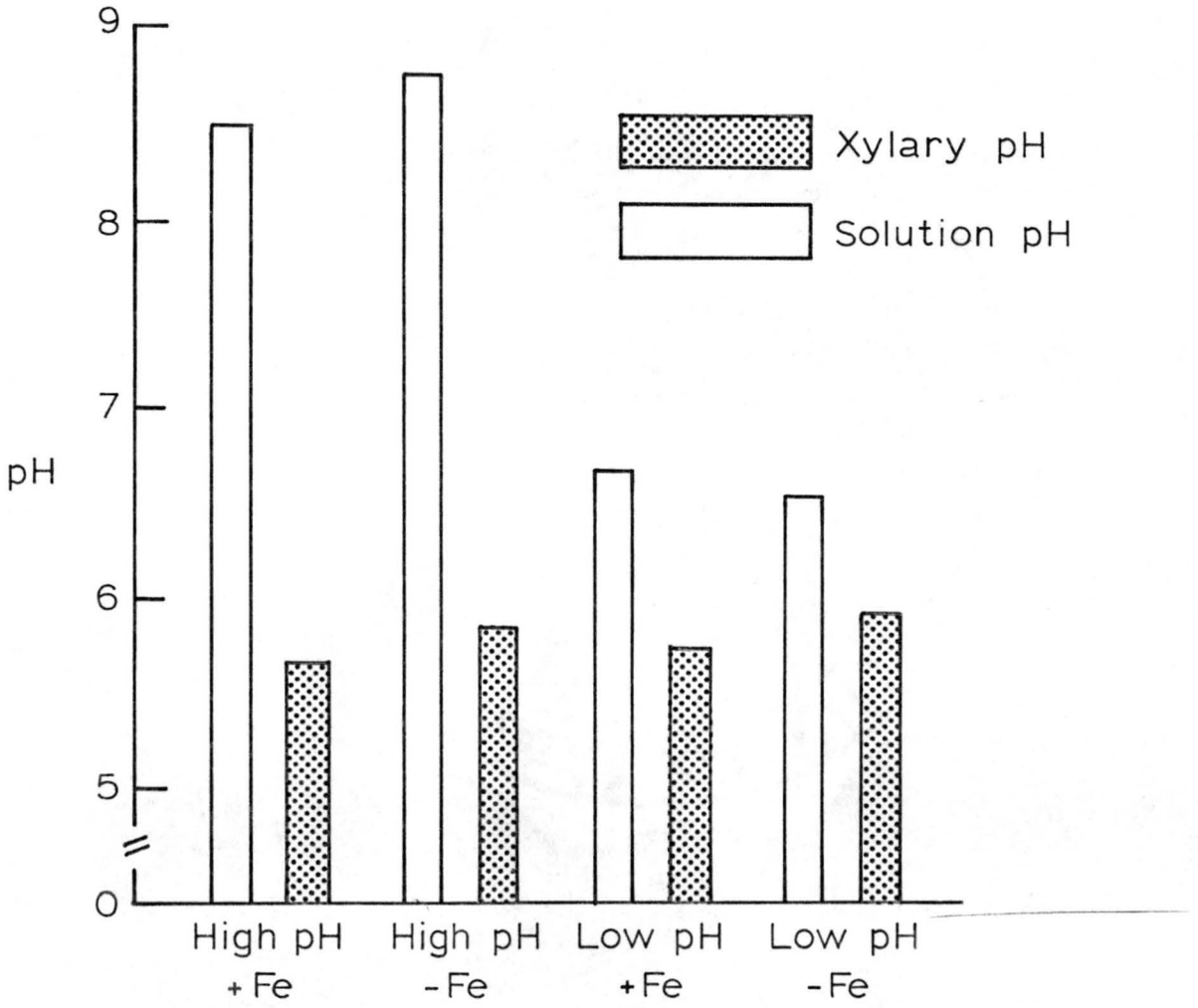

Fig. 1. pH of silver maples grown under various iron stresses.

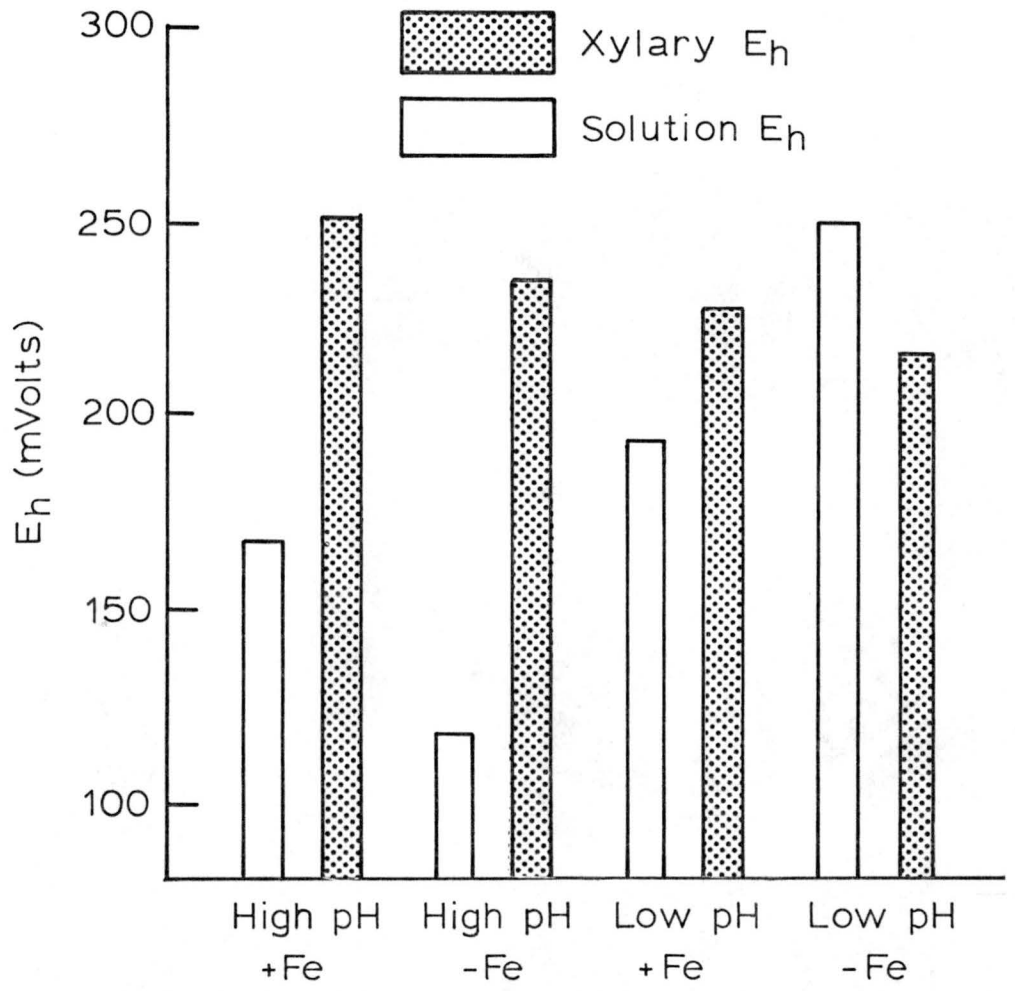

Fig. 2. Reduction potential of silver maples grown under various iron stresses.

within a narrow range (9.35-9.86; 0.51 units) while the growing solution pe + pH sum was not as constant (9.90-11.25; 1.35 units). Levitt (6) states that reducing power is generated photosynthetically as an increased ratio of NADPH/NADP or an accumulation of reduced ascorbic acid. In nonphotosynthesizing tissue such as the xylem, increased reduction potential is accomplished because of a physical barrier to oxygen erected by chlorophyll-containing cortical cells. Reduction energy must be transferred somehow from cells capable of generating this power.

Riboflavin and other reducing substances have been suggested as influencing the reduction potential of the rhizosphere with an accompanying increased Fe transport (13). It was not made clear whether this increase in flavin production resulted from, or was the cause of, increased Fe availability.

Shone (11) outlines a number of processes which might contribute to a potential difference between the xylary sap and the growing solution as follows: 1) a diffusion potential arising from a concentration gradient of ions between the xylem and the medium; 2) a "Donnan" potential which results from charged groups biologically inherent in the vascular system; 3) an electro-osmotic potential generated by moving ions and water through charged vessels; 4) a carrier potential generated by the movement of charged macromolecules; and 5) a transport potential caused by energy transporting processes moving ions across the root at different rates.

Reduction potential has been reported originating from the plant and possibly extending its sphere of influence into the area surrounding

the root. This leakage of reducing compounds may explain why the aerated culture solution had an E_h comparable to a reduced medium.

The plant seems to have an inherent capability for keeping the xylary reduction potential and pH at constant values. This phenomenon may aid the plant in maintaining a mineral transporting passageway in a constant range of pe + pH. This constant pe + pH would allow the maintenance of a constant equilibrium between Fe species and relative stability of Fe while it was being transported. This would be true provided other cation concentrations were kept relatively constant.

A change in Fe levels between treatments as well as pH changes and increased HCO_3^- levels was thought to change the reduction potential of the xylary system. Perhaps an increase in uptake of other polyvalent cations made up for this lack of Fe or perhaps the production of increased levels of organic acids which was not reflected in a change in xylary pH. It seems that the absence of Fe from the solution surrounding the roots, a rise in solution pH and increases in HCO_3^- levels does not lead to changes in xylary pH or E_h.

Conclusions

Alterations of Fe and pH in the culture solution of silver maple does not lead to changes in the xylary pH or E_h. Rather, the plant seems to have the capability of maintaining a constant value for xylary pH, E_h and, of course, their sum. This phenomenon may aid or hinder cation transport by affecting the ion's equilibrium with its surroundings.

REFERENCES

1. Bennett, J. P. 1945. Iron in leaves. Soil Sci. 60:91-105.

2. Brown, J. C. Iron chlorosis. Adv. Agron. 13:329-69.

3. Chapman, G. W. 1931. Relation of iron and manganese to chlorosis in plants. New Phytol. 30:266-83.

4. Jacobson, L. and J. J. Oertli. 1956. Relation between iron and chlorophyll content in sunflower leaves. Pl. Phys. 31:199-204.

5. Leeper, G. W. 1952. Factors affecting availability of inorganic nutrients in soils with special reference to micronutrient metals. Ann. Rev. Pl. Phys. 3:1-16.

6. Levitt, J. 1972. Responses of Plants to Environmental Stresses. p. 217-221. Academic Press. New York.

7. Norvell, W. A. 1974. Equilibria of metal chelates in soil solution. In J. J. Mortvedt, P. M. Giordano, and W. L. Lindsay (eds.) p. 115-138. Micronutrients in Agriculture. Soil Sci. Soc. of America. Madison.

8. Oserkowsky, J. 1932. Hydrogen-ion concentration and iron content of tracheal sap from green and chlorotic pear trees. Pl. Phys. 7:253-9.

9. Rogers, C. H. and J. W. Shive. 1942. Factors affecting the distribution of Fe in plants. Pl. Phys. 7:227-252.

10. Ross, C. W. 1974. Plant Physiology Laboratory Manual. Wadsworth Publ. Co., Belmont, Calif.

11. Shone, M. G. T. 1969. Origins of the electrical potential difference between the xylem sap of maize roots and the external solution. Jour. of Exper. Bot. 20:698-716.

12. Tiffin, L. O. 1966. Iron translocation. II. Citrate/iron ratios in stem exudates. Pl. Phys. 41:515-18.

13. Wallace, A. 1969. Root excretions in tobacco plants and possible implications on the iron nutrition of higher plants. Indian Jour. Hort. 26(1 and 2):47-50.

14. Wallace, A., R. A. Wood, and S. M. Soufi. 1976. Cation-anion balance in lime-induced chlorosis. Comm. in Soil Sci. and Pl. Anal. 7:15-26.

REFERENCES

1. Ambler, J.E. and J.C. Brown. 1972. Iron-stress response in mixed and monocultures of soybean cultivars. Pl. Phys. 50:675-678.

2. Ambler, J.E., J.C. Brown, and H.G. Gauch. 1971. Sites of iron reduction in soybean plants. Agron. J. 63:95-97.

3. Angleton, G.M. and J.C. Simpson. 1977. Linear model analysis techniques using the CRHL general linear model analysis package (GLIMAP). Collaborative Radiological Health Laboratory, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.

4. Arnon, D.I. 1939. Effect of ammonium and nitrate nitrogen on the mineral composition and sap characteristics of barley. Soil Sci. 48:295-307.

5. Banin, A. and J. Navrot. 1972. Pattern of iron distribution in the soil-plant system and its possible relation to iron chlorosis. Comm. in Soil Sci. and Pl. Anal. 3(3):177-182.

6. Basiouny, F.M., C.D. Leonard, and R.H. Biggs. 1970. Comparison of different iron formulations for effectiveness in correcting iron chlorosis in citrus. Fla. St. Hort. Soc. Proc. 83:1-6.

7. Bell, W.D., L. Bogoard, and W.J. McIlrath. 1958. Response of the yellow-strip maize mutant to ferrous and ferric ion. Bot. Gaz. 120:36-39.

8. Benedict, H.M., J.N. Simons, and R. Swidler. 1962. The absorption of iron and chelating agents by soybean in split root experiments. Pl. Phys. 37(Suppl.):x/vii-x/viii, No. 831.

9. Bennett, J.P. 1931. Treatment of lime-induced chlorosis with iron salts. Univ. Cal. Agr. Exp. Sta. Circ. 321.

10. Bennett, J.P. 1945. Iron in leaves. Soil Sci. 60:91-105.

11. 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 EDDHA. Agron. J. 54:119-121.

12. Biddulph, O. 1951. The translocation of minerals in plants. p. 261-278. In E. Truog (ed.) Univ. Wisconsin Press, Madison.

13. Biddulph, O. and C.G. Woodbridge. 1952. Uptake of phosphorus by bean plants with particular reference to the effects of iron. Pl. Phys. 27(3):431-444.

14. Birch, P.D.W. 1972. Iron heptonate - a new chelate for controlling iron deficiency in plants. J. Hort. Sci. 47:1-3.

15. Bogorad, L., G. Pires, H. Swift, W.J. McIlrath. 1959. The structure of chloroplasts in leaf tissue of iron deficiency Xanthium. Brookhaven Symp. 11:132-136.

16. Bolle-Jones, E.W. 1955. The effect of varied nutrient levels on the concentration and distribution of Mn within the potato plant. Plant and Soil 6:45-60.

17. Bonner, J. and R. Dorland. 1943. Some observations concerning riboflavin and panthothenic acid in tomato plants. Ann. Bot. 30:414-418.

18. Bould, C. 1955. Chelated iron compounds for the correction of lime-induced chlorosis in fruit. Nature 175:96-91.

19. Brown, J.C. 1953. The effect of the dominance of a metabolic system requiring iron or copper on the development of lime-induced chlorosis. Pl. Phys. 28:495-502.

20. Brown, J.C. 1954. Some observations on the reduction of 2,3,5-triphenyltetrazolium chloride in plant tissue as influenced by mineral nutrition. Pl. Phys. 29:104-107.

21. Brown, J.C. 1963. Interactions involving nutrient elements. Ann. Rev. Pl. Phys. 14:93-106.

22. Brown, J.C. 1966. Iron and calcium uptake as related to root-sap and stem-exudate citrate in soybeans. Phys. Plant. 19:968-976.

23. Brown, J.C. 1967. Differential uptake of iron and Ca by two corn genotypes. Soil Sci. 103:331-338.

24. Brown, J.C. 1969. Metal chelation in soils. Soil Sci. Soc. Amer. Proc. 33(1):59-61.

25. Brown, J.C. 1972. Competition between phosphate and the plant for iron from Fe^{2+} ferrozine. Agron. J. 64:240-243.

26. Brown, J.C. and J.E. Ambler. 1970. Further characterization of iron uptake in two genotypes of corn. Soil Sci. Soc. Amer. Proc. 34:249-252.

27. Brown, J.C. and J.E. Ambler. 1973. "Reductants" released by roots of Fe-deficiency soybeans. Agron. J. 65:311-314.

28. Brown, J.C. and J.E. Ambler. 1974. Sites of iron reduction in tomato. Phys. Plant. 31:221-224.

29. Brown, J.C. and W.D. Bell. 1969. Iron uptake dependent upon genotype of corn. Soil Sci. Soc. Amer. Proc. 33:99-101.

30. Brown, J.C. and R.L. Chaney. 1970. Effect of iron on the transport of citrate into the xylem of soybeans and tomatoes. Pl. Phys. 47:836-840.

31. Brown, J.C., R.L. Chaney, and J.E. Ambler. 1971. A new tomato mutant inefficient in the transport of iron. Phys. Plant. 25:48-53.

32. Brown, J.C. and R.B. Clark. 1972. Factors controlling differential iron uptake by maize inbreds. Agron. Abs. p. 104.

33. Brown, J.C. and S.B. Hendricks. 1952. Enzymatic activities as indications of copper and iron deficiencies in plants. Pl. Phys. 27:651-660.

34. Brown, J.C. and R.S. Holmes. 1955. Iron the limiting element in a chlorosis: Part I. Pl. Phys. 30:451-456.

35. Brown, J.C., R.S. Holmes, and A.W. Specht. 1955. Iron the limiting element in a chlorosis: Part II. Pl. Phys. 30:457-462.

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

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

38. Brown, J.C. and W.E. Jones. 1962. Absorption of iron, manganese, zinc, calcium, rubidium, and phosphorus ions by soybean roots that differ in their reductive capacity. Soil Sci. 94:173-179.

39. Brown, J.C. and W.E. Jones. 1975. Phosphorus efficiency as related to iron inefficiency in sorghum. Agron. J. 67:468-472.

40. Brown, J.C. and R.A. Steinberg. 1953. Iron and copper enzymes in leaf lamina of tobacco when deficient in micronutrients or grown on calcareous and organic soils. Pl. Phys. 28:488-494.

41. Brown, J.C. and L.O. Tiffin. 1959. 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. 39:8-15.

42. Brown, J.C. and L.O. Tiffin. 1962. Properties of chelates and their use in crop production. J. Agr. Food Chem. 10:192-195.

43. Brown, J.C. and L.O. Tiffin. 1965. Iron stress as related to the iron and citrate occurring in stem exudate. Pl. Phys. 40:395-400.

44. Brown, J.C., L.O. Tiffin, and R.S. Holmes. 1960. Competition between chelating agents and roots as factors affecting absorption of iron and other ions by plant species. Pl. Phys. 35:878-886.

45. Brown, J.C., L.O. Tiffin, R.S. Holmes, A.W. Specht, and J.W. Resnicky. 1959. Internal inactivation of iron in soybeans as affected by root growth medium. Soil Sci. 87:89-94.

46. Brown, J.C., L.O. Tiffin, A.W. Specht, and J.W. Resnicky. 1961. Iron absorption by roots as affected by plant species and concentration of chelating agent. Agron. J. 53:81-85.

47. Carmock, C. and A. Wallace. 1963. Ethylendiamine di(O-hydroxyphenylacetate) influence on iron uptake and translocation in bush beans. p. 147-155. In A. Wallace (ed.), Solute Uptake by Intact Plants, Los Angeles.

48. Chabarek, S. and A.E. Martell. 1959. Organic Sequestering Agents. John Wiley and Sons, New York. 616pp.

49. Chaney, R.L., J.C. Brown, and L.O. Tiffin. 1972. Obligatory reduction of ferric chelates in iron uptake by soybeans. Pl. Phys. 50:208-213.

50. Chapman, G.W. 1931. Relation of iron and manganese to chlorosis in plants. New Phytol. 30:266-283.

51. Christ, R.A. 1974. Iron requirements and iron uptake from various iron compounds by different plant species. Pl. Phys. 54:582-585.

52. Clark, R.B. 1968. Organic acids of maize (Zea mays, L.) as influenced by mineral deficiencies. Crop Sci. 8:165-167.

53. Clark, R.B. and J.C. Brown. 1974. Internal root control of iron uptake and utilization in maize genotypes. Plant and Soil 40:669-677.

54. Clark, R.B., L.O. Tiffin, and J.C. Brown. 1973. Organic acids and iron translocation in maize genotypes. Pl. Phys. 52:147-150.

55. Crafts, A.S. 1968. Water deficits and physiological processes. In T.T. Kozlowski (ed.), Water Deficits and Plant Growth. Academic Press.

56. Davies, D.D. 1973. Control of and by pH. Symp. Soc. Exp. Biol. 27:513-529.

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

58. De Kock, P.C. 1958. The nutrient balance in plant leaves. Agr. Progress 33:88.

59. De Kock, P.C. and A.W. Hall. 1955. The phosphorus-iron relationship in genetical chlorosis. Pl. Phys. 30:293-295.

60. De Kock, P.C. and R.H.E. Inkson. 1962. Manganese content of mustard leaves in relation to iron and major nutrient supply. Plant and Soil 17(2):183-190.

61. De Kock, P.C. and R.L. Mitchell. 1957. Uptake of chelated metals by plants. Soil Sci. 84:55-62.

62. De Kock, P.C. and R.I. Morrison. 1958. The metabolism of chlorotic leaves. Biochem. J. 70:272-277.

63. De Wit, C.T., W. Dijkshoorn, and J.C. Noggle. 1963. Ionic balance and growth of plants. Versl. Landbouwk. Onderz. 69:15.

64. Dijkshoorn, W. 1969. The relation of growth to the chief ionic constituents of the plant. p. 201-213. In Rorison, I.H. (ed.), Biological aspects of the mineral nutrition of plants. A Symp. Brit. Ecol. Soc., Sheffield, 1-5 April, 1968, Blackwell.

65. Dodge, C.S. and A.J. Hiatt. 1972. Relationship of pH to ion uptake imbalance of varieties of wheat. Agron. J. 64:476-481.

66. Dybing, C.D. and H.B. Currier. 1962. Foliar penetration by chemicals. Pl. Phys. 36:169-174.

67. Eichhorn, G.L. 1963. Metal ion catalysis in biological systems. Adv. Chem. Ser. 37:37-55.

68. Elgawhary, S.M. 1970. Effect of complexing agents and acids on the diffusion of zinc to a simulated root. Soil Sci. Soc. Amer. Proc. 34:211-214.

69. Elstrom, G.W. and F.D. Howard. 1969. Iron accumulation, root peroxidase activity, and varietal interactions in soybean genotypes that differ in iron nutrition. Pl. Phys. 44:1108-1114.

70. Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. Wiley and Sons, New York.

71. Essington, E.H. and H. Nishita. 1966. Effect of chelates on the movement of fission products through soil columns. Plant and Soil 24(1):20.

72. Estes, G.O. and T.F. Brietsch. 1973. Physiological aspects of iron-phosphorus nutrition in two varieties of maize: I. Uptake and accumulation characteristics under greenhouse and field conditions. Soil Sci. Soc. J. 37(2):243.

73. Follett, R.H. and W.L. Lindsay. 1970. Profile distribution of zinc, iron, manganese, and copper in Colorado soils. Colo. Exp. Sta. Tech. Bull. 110.

74. Franco, C.M. and W.E. Loomis. 1947. The absorption of phosphorus and iron from nutrient solutions. Pl. Phys. 22:627-632.

75. Frolich, E.F. and A. Wallace. 1966. Flavin excretion by roots of iron-deficient tobacco. p. 10-11. In A. Wallace (ed.), Current Topics in Plant Nutrition, Los Angeles.

76. Gerretsen, F.C. 1950. Manganese in relation to photosynthesis. II. Redox potentials of illuminated crude chloroplast suspensions. Plant and Soil 2:159-193.

77. Glenister, P.R. 1944. Effects of iron deficiency on respiration of sunflower plants. Bot. Gaz. 106:33-40.

78. Guennelson, R. 1965. Iron chelate in treatment of pear tree chlorosis. Compt. Rend. Acad. Agr. (France) 48:209-13. Cf. Chem. Abstr. 63(2):2131c.

79. Guinn, G. and H.E. Joham. 1963. Displacement of iron from ferric ethylenediaminetetracetic acid and ferric hydroxyethylenediaminetriacetic acid by copper and zinc. Soil Sci. 95(2):101-104.

80. Hale, V.Q. and A. Wallace. 1960. Bicarbonate and phosphorus effects on uptake and distribution in soybeans of iron chelated with EDDHA. Soil Sci. 89:285-287.

81. Halvorson, A.D. 1970. Predicted and measured competition between chelating agent and roots in solution culture. Agron. Abstr. p. 121.

82. Halvorson, A.D. and W.L. Lindsay. 1972. Equilibrium relations of metal chelates in hydroponic solutions. Soil Sci. Soc. Amer. Proc. 36:755-761.

83. Hartzook, A., D. Karstadt, M. Navek, and S. Feldman. 1974. Differential iron absorption efficiency of peanut (Arachis hypogaea, L.) cultivars grown on calcareous soils. Agron. J. 66:114-115.

84. Hewitt, E.J. 1948. Relation of manganese and some other emtals to the iron status of plants. Nature 161:489-490.

85. Higdon, R.J. 1957. Pear tree chlorosis with special reference to its correction with chelated metals. Amer. Soc. Hort. Sci. Proc. 69:101-109.

86. Hilgeman, R.H. 1969. Correction of iron chlorosis with iron chelates in Arizona. Citrograph 54(10):406, 426-428.

87. Hill-Cottingham, D.G. 1955. Photosensitivity of iron chelates. Nature 175:347-348.

88. Hill-Cottingham, D.G. 1957. A spectrophotometric method of analysis of chelate solutions and its application to the study of iron chelates in soils and plants. Soil Sci. 84:43-49.

89. Hill-Cottingham, D.G. and C.P. Lloyd-Jones. 1958. Behavior of iron chelates in calcareous soils. II. Lab experiments with some further chelating agents. Plant and Soil 9:189-201.

90. Hill-Cottingham, D.G. and C.P. Lloyd-Jones. 1965. Behavior of iron-chelating agents with plants. J. Exptl. Bot. 16(47): 233-242.

91. Himes, F.L., R. Tejeira, and M.H.B. Hayes. 1963. The reactions of extracts from maple and oak leaves with iron and zinc compounds. Soil Sci. Soc. Amer. Proc. 27(5):516-519.

92. Hodgson, J.F. 1968. Theoretical approach for the contribution of chelates to the movement of iron to roots. 9th Int. Congr. Soil Sci. Trans. (Adelaide, Australia, 1968). I:229-241.

93. Hodgson, J.F., 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. 31:410-413.

94. Holmes, R.S. and J.C. Brown. 1955. Chelates as correctives for chlorosis. Soil Sci. 80:167-179.

95. Hopkins, E.F. and F.B. Wann. 1925. The effect of the H-ion concentration on the availability of iron for Chlorella sp. J. Gen. Phys. 9:205-210.

96. Horner, K.C., D. Burk, and S.R. Hoover. 1934. Preparation of humate iron and other humate metals. Pl. Phys. 9:663-669.

97. Hsu, W.O. and G.W. Miller. 1968. Iron in relation to aconitase hydratase activity in Glycine max. Biochem. Biophys. Acta 151:711-713.

98. Hutchinson, T.C. 1970. Lime chlorosis in seedling establishment on calcareous soils. New Phytol. 69:261-268.

99. Iljin, W.S. 1951. Metabolism of plants affected with lime-induced chlorosis: Nitrogen metabolism. Plant and Soil 3:239-256.

100. Iljin, W.S. 1951. Metabolism of plants affected with lime-induced chlorosis. II. Organic acids and carbohydrates. Plant and Soil 3:339-351.

101. Iljin, W.S. 1952. Metabolism of plants affected with lime-induced chlorosis (calciose). III. Mineral elements. Plant and Soil 4:11-28.

102. Ingalls, R.A. and J.W. Shive. 1931. Relation of H-ion conconetration of tissue fluids to the distribution of iron in plants. Pl. Phys. 6:103-125.

103. Jackson, P.C. and H.R. Adams. 1963. Cation-anion balance during potassium and sodium absorption by barley roots. J. Gen. Phys. 46:369-386.

104. Jacobson, L. 1945. Iron in the leaves and chloroplasts of some plants in relation to their chlorophyll content. Pl. Phys. 20:233-244.

105. Jacobson, L. 1951. Maintenance of iron supply in nutrient solutions by a single addition of ferric potassium EDTA. Pl. Phys. 26:411.

106. Jacobson, L. and J.J. Oertli. 1956. Relation between iron and chlorophyll content in sunflower leaves. Pl. Phys. 31:199-204.

107. Jacobson, L. and R. Overstreet. 1947. A study of the mechanism of ion absorption by plant roots using radioactive elements. Am. J. Bot. 34(8):415-420.

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

109. Jenny, H. and K. Grossenbacher. 1962. Root-soil boundary zones, as seen by the electron microscope. Calif. Agr. 16(10):7.

110. Jones, J.B. 1972. Plant tissue analysis for micronutrients. p. 331-337. In J.J. Mortvedt, P.M. Giordano, and W.L. Lindsay (eds.), Micronutrients in Agriculture. Soil Sci. Soc. Amer., Madison.

111. Kanan, S. 1969. Penetration of iron and some organic substances through isolated cuticular membranes. Pl. Phys. 44(4):517-521.

112. Kanan, S. and B. Joseph. 1975. Absorption and transport of iron and manganese in germinating sorghum. Pl. Phys. 55:1006-1008.

113. Kanan, S. and S.H. Wittwer. 1967. Absorption of iron by enzymically isolated leaf cells. Phys. Plant. 20:911-919.

114. Kashirad, A. and H. Marschner. 1974. Effect of pH and phosphorus on iron nutrition of sunflower and corn plants. Agrochimica 18(6):497-508.

115. Kleinkopf, G.E., A. Wallace, and T. Hartsock. 1976. Lime chlorosis on photosynthesis and transpiration on iron inefficient soybeans. Comm. in Soil Sci. and Pl. Anal. 7(1):97-99.

116. Kliman, S. 1937. The importance of ferrous iron in plants and soils. Soil Sci. Soc. Proc. 2:385-392.

117. Knezek, B.D. and H. Greinert. 1971. Influence of soil iron and manganese EDTA interactions upon the iron and manganese nutrition of bean plants. Agron. J. 63:617-619.

118. Kochan, W.J. 1962. Iron chelate control of chlorosis in peach trees. Idaho Agr. Exp. Sta. Bull. 384.

119. Kostic, M., W. Dijkshoorn, and C.T. De Wit. 1957. Evaluation of the nutrient status of wheat plants. Neth. J. Agr. Sci. 15:267-280.

120. Kotaka, S. and A.P. Krueger. 1969. Effect of EDTA on nucleic acid content of green barley leaves. 11th Int. Bot. Congr. Abstr. (Seattle) p. 115.

121. Kroll, H. 1957. The ferric chelate of EDDHA for treatment of lime-induced chlorosis. Soil Sci. 84:51-53.

122. Kuykendall, J.R., R.H. Hilgeman, and C.W. Van Horn. 1957. Response of chlorotic citrus trees in Arizona to soil applications of iron chelates. Soil Sci. 84:77-86.

123. Lachica, M.A., A. Aguilar, and C. Gonzalez. 1967. Effect of EDTA on the uptake of some trace nutrients: II. Response of rye to different concentrations of Na_2EDTA as a fertilizer. Agrochimica 11(6):571-579.

124. Larkhum, A.W.D. 1968. Ionic relations of chloroplasts in vivo. Nature 218:447-449.

125. Leal-Diaz, J. 1964. The uptake of ferrous and ferric iron by Phaseolus vulgaris, L. var. Red Kidney. Ph.D. Thesis. Univ. Calif., Davis.

126. Leeper, G.W. 1952. Factors affecting availability of inorganic nutrients in soils with special reference to micronutrient metals. Ann. Rev. Pl. Phys. 3:1-16.

127. Lehman, D.S. 1963. Chelates in soils - a symposium. Soil Sci. Soc. Amer. Proc. 27(2):167-170.

128. Leonard, C.E. 1967. Dimethyl sulfoxide as a carried for iron in nutritional foliar sprays applied to citrus. Ann. N.Y. Acad. Sci. 141(1):148-158.

129. Leonard, C.D. and I. Stewart. 1952. Correction of iron chlorosis in citrus with chelated iron. Fla. St. Hort. Soc. Proc. 65:20-24.

130. Levitt, J. 1972. Responses of Plants to Environmental Stresses. p. 217-221. Academic Press, New York.

131. Lindsay, W.L. 1970. Cause, diagnosis and correction of iron deficiencies in calcareous soils. Agron. Abs. p. 123.

132. Lindsay, W.L. 1972. Inorganic phase equilibria of micronutrients in soils. p. 41-57. In J.J. Mortvedt (ed.), Micronutrients in Agriculture. Soil Sci. Soc Amer. Madison.

133. Lindsay, W.L. 1974. Role of chelation in micronutrient availability. p. 507-524. In E.W. Carson (ed.), The Plant Root and Its Environment. Univ. Va. Press.

134. Lindsay, W.L., J.F. Hodgson, and W.A. Norvell. 1967. The physio-chemical equilibrium of metal chelates in soils and their influence on the availability of micronutrient cations. Int. Soc. Soil Sci. Trans. Comm. II, IV. (Aberdeen, Scotland) 1966 p. 305-316.

135. Lindsay, W.L. and W.A. Norvell. 1969. Equilibrium relationships of Zn^{2+}, Fe^{3+}, Ca^{2+}, and H^{+} with EDTA and DTPA in soils. Soil Sci. Soc. Amer. Proc. 33:62-68.

136. Lindsay, W.L. and D.W. Thorne. 1954. Bicarbonate ion and oxygen level as related to chlorosis. Soil Sci. 77:271-278.

137. Lindner, R.C. and C.P. Harley. 1944. Nutrient interrelations in lime-induced chlorosis. Pl. Phys. 19:420-439.

138. Lingle, J.C., L.O. Tiffin, and J.C. Brown. 1963. Iron uptake-transport of soybeans as influenced by other cations. Pl. Phys. 38:71-76.

139. Malo, S.E. 1965. Promising methods for correcting iron chlorosis in avacados - a preliminary report. Fla. St. Hort. Soc. (Miami) Proc. 78:358-364.

140. Marschner, H., A. Kalisch, and V. Romheld. 1974. Mechanisms of iron uptake in different plant species. p. 273-281. In J. Wehrmann (ed.), Plant Analysis and Fertilizer Problems, Vol. 2, German Soc. of Plant Nutrition, Hanover.

141. Marsh, H.V., H.J. Evans, and G. Matrone. 1963. Investigations of the role of iron in chlorophyll metabolism. I. Effect of iron deficiency on chlorophyll and heme content and on the activities of certain enzymes in leaves. Pl. Phys. 38:632-638.

142. Martell, A.E. 1957. The chemistry of metal chelates in plant nutrition. Soil Sci. 84:13-26.

143. McCalla, A.G. and E.K. Woodford. 1939. Effects of a limiting element on the absorption of individual elements and on the anion:cation balance in wheat. Pl. Phys. 13:695-713.

144. Meyers, H.G..and J.N. Joiner. 1966. Effects of ferrous sulfate, chelated iron, and Versene-T levels on growth and chemical composition of centipedigrass. Fla. St. Hort. Soc. Proc. 79:464-468.

145. McGeorge, W.T. 1949. Lime-induced chlorosis: relation between active iron and citric and oxalic acids. Soil Sci. 68:381-390.

146. Mikesell, M.E., G.M. Paulsen, R. Ellis, and A.J. Casady. 1973. Iron utilization by efficient and inefficient sorghum lines. Agron. J. 65:77-80.

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

148. Mokrzecki, S. 1904. A cure for chlorosis. Gard. Chron. 35:36.

149. Mortvedt, J.J. 1975. Iron chlorosis. Crops and Soils 27:10-12.

150. Mortvedt, J.J., A. Wallace, and R.D. Curley. 1977. Iron - the elusive micronutrient. Fert. Solns. 21(1):26, 28, 30, 32, 34, 36.

151. Neely, D. 1973. Pin oak chlorosis: Trunk impantations correct iron deficiency. J. Forestry. 71:340-342.

152. Nelson, L.E. and R. Selby. 1974. The effect of N sources and Fe levels on the growth and composition of Sitka spruce adn Scots pine. Plant and Soil 41:573-588.

153. Nicholas, D.J.D. 1961. Minor mineral nutrients. Ann. Rev. Pl. Phys. 12:63.

154. Norvell, W.A. 1974. Equilibria of metal chelates in soil solution. p. 115-138. In J.J. Mortvedt, P.M. Giordano, and W.L. Lindsay (eds.), Micronutrients in Agriculture. Soil Sci. Soc. Amer., Madison.

155. Norvell, W.A. and W.L. Lindsay. 1969. Reactions of EDTA complexes of Fe, Zn, Mn, and Cu with soils. Soil Sci. Soc. Amer. Proc. 33:75-81.

156. Norvell, W.A. and W.L. Lindsay. 1972. Reactions of DTPA chelates of iron, zinc, copper, and manganese with soils. Soil Sci. Soc. Amer. Proc. 36:778-783.

157. O'Conner, G.A., W.L. Lindsay, and S.R. Olsen. 1970. The diffusion of Fe and FeEDDHA in soil. Soil Sci. Soc. Amer. Proc. 34:407.

158. O'Conner, G.A., W.L. Lindsay, and S.R. Olsen. 1971. The diffusion of iron and iron chelates in soil. Soil Sci. Soc. Amer. Proc. 35:407-410.

159. O'Conner, G.A., W.L. Lindsay, and S.R. Olsen. 1975. Iron diffusion to plant roots. Soil Sci. 119:4:285-289.

160. Oertli, J.J. and L. Jacobson. 1960. Some quantitative considerations in iron nutrition of higher plants. Pl. Phys. 35:683-688.

161. Oertli, J.J. and A.A. Opuku. 1974. Interaction of K in the availability and uptake of Fe from ferric hydroxide. Soil Sci. Soc. Amer. Proc. 38:451-454.

162. Olsen, C. 1958. Iron absorption in different plant species as a function of the pH value of the solution. Comptes Rendus Trav. Lab. Carlsberg 31:41-59.

163. Oserkowsky, J. 1932. Hydrogen-ion concentration and iron content of tracheal sap from green and chlorotic pear trees. Pl. Phys. 7:253-259.

164. Oserkowsky, J. 1933. Quantitative relation between chlorophyll and Fe in green and chlorotic pear leaves. Pl. Phys. 8:449-468.

165. Ouellette, G.J. 1951. Fe-Mn interrelationships in plant nutrition. Sci. Agr. 31:277-285.

166. Perur, N.G., R.L. Smith, and H.H. Wiebe. 1961. Effect of Fe chlorosis on protein fractions of corn leaf tissue. Pl. Phys. 36:736-739.

167. Pierce, E.C. and C.O. Appleman. 1933. Role of ether soluble organic acids in the cation-anion balance in plants. Pl. Phys. 8:224-238.

168. Price, C.A. 1968. Iron compounds and plant nutrition. Ann. Rev. Pl. Phys. 19:239-248.

169. Price, C.A. and E.F. Carell. 1964. Control by iron of chlorophyll formation and growth Euglena gracilis. Pl. Phys. 39:862-868.

170. Raven, J.A. and F.A. Smith. 1974. Significance of H^+ transport in plant cells. Cand. J. Bot. 52:1035-1048.

171. Rediske, J.H. and O. Biddulph. 1953. The absorption and translocation of Fe. Pl. Phys. 28:576-593.

172. Rogers, C.H. and J.W. Shive. 1942. Factors affecting the distribution of Fe in plants. Pl. Phys. 7:227-252.

173. Rogers, E. 1971. The effects of difference sources and rates of iron chelate on Red Haven peach trees. Colo. St. Univ. Progr. Rept. 71-25.

174. Rogers, E. 1974. The effects of different sources and rates of iron chelate on leaf color and growth of "Bartlett" pear. Colo. St. Univ. Progr. Rept. 74-9.

175. Rogers, E. 1975. An attempt to overcome iron-induced manganese deficiency in "July Elberta" peach trees with manganese chelate. J. Amer. Soc. Hort. Sci. 100:531-535.

176. Rogers, E. 1975. Mineral content and iron chlorosis of "Redhaven" peach trees as affected by iron source and rate. Hortsci. 10:519-521.

177. Ross, C.W. 1974. Plant Physiology Laboratory Manual. Wadsworth Publ. Co., Belmont, Calif.

178. Rubin, M. and J.V. Princiotto. 1963. Chelation as a basic biological mechanism. J. Agr. Food Chem. 11:98-103.

179. Salomon, E. 1970. Iron uptake and its physiological availability in citrus leaves. XVIIIth Hort. Congr. 1:185, No. 367.

180. Sanchez-Raya, A.J., A. Leal, M. Gomez-Ortega, and L. Recalde. 1974. Effect of iron on the absorption and translocation of manganese. Plant and Soil 41:429-434.

181. Sarosi, D. and L. Diofasi. 1961. Curing chlorotic vines with iron chelate sprays. Agrokem. Talajtan 10:529-538.

182. Schatz, A. 1963. Importance of metal-binding phenomena in the chemistry and microbiology of the soil: I. Chelating properties of lichens and lichen acids. Advancing Front. Plant. Sci. 6:113-149.

183. Schmid, W.E. and G.C. Gerloff. 1961. A naturally occurring chelate of Fe in xylem exudate. Pl. Phys. 36:226-231.

184. Schoenweiss, D.F. 1973. Correction of line-induced chlorosis of pin oak by liquid soil injection. Hortsci. 8:33-334.

185. Seeley, E.J. and D.R. Walker. 1969. Correction of iron chlorosis in peach trees. Amer. Soc. Hort. Sci. 66th Ann. Meetings (Pullman, Wash.).

186. Shive, J.W. 1941. Significant roles of trace elements in the nutrition of plants. Pl. Phys. 16:435-445.

187. Shone, M.G.T. 1969. Origins of the electrical potential difference between the xylem sap of maize roots and the external solution. J. Exptl. Bot. 20:698-716.

188. Sideris, C.P. and H.Y. Young. 1949. Growth and chemical composition of Ananas comosus (L.) Merr. in solution cultures with different Fe-Mn ratios. Pl. Phys. 24:416-436.

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

190. Simons, J.N., R. Swidler, andH.M. Benedict. 1963. Kinetic studies of regreening of Fe-deficient soybeans. Pl. Phys. 38:667-674.

191. Smith, E.M. and C.D. Mitchell. 1977. Chlorosis of Red and Silver Maple. Weeds, Trees and Turf. July, p. 32,36.

192. Smith, P.F., W. Reuther, and A.W. specht. 1950. Mineral composition of chlorotic orange leaves and some observations on the relation of sample preparation technique to the interpretation of results. Pl. Phys. 25:496-504.

193. Somers, I.I., S.G. Gilbert, and J.W. Shive. 1942. The Fe-Mn ratio in relation to the respiratory CO_2 and deficiency-toxicity symptoms in soybeans. Pl. Phys. 17:317-320.

194. Somers, I.I., J.W. Shive. 1942. The Fe-Mn relation in plant metabolism. Pl. Phys. 17:582-602.

195. Spiegel-Roy, P. 1968. Control of lime-induced chlorosis in fruit trees by foliar application of organic polyflavonoids. Agrochimica. 12:443-450. Cf. Chem. Abs. 69(8):58602p.

196. Steward, F.C. and C. Preston. 1941. Effects of pH and the components of bicarbonate and phosphate buffered solutions on the metabolism of potato discs and their ability to absorb ions. Pl. Phys. 16:481-520.

197. Stewart, I. and C.D. Leonard. 1952. Chelates as sources of iron for plants growing in the field. Science. 116:564-566.

198. Stocking, C.R. and A. Ongun. 1962. The intracellular distribution of some metallic elements in leaves. Am. J. Bot. 49:284-289.

199. Su, L.Y. and G.W. Miller. 1961. Chlorosis in higher plants as related to organic acid content. Pl. Phys. 36:415-420.

200. Thorne, D.W., F.B. Wann, and W. Robinson. 1950. Hypotheses concerning lime-induced chlorosis. Soil Sci. Soc. Amer. Proc. 15:254-258.

201. Tiffin, L.O. 1966. Iron translocation. I. Plant culture, exudate sampling, iron citrate analysis. Pl. Phys. 41:510-514.

202. Tiffin, L.O. 1966. Iron translocation. II. Citrate/iron ratios in stem exudates. Pl. Phys. 41:515-518.

203. Tiffin, L.O. and J.C. Brown. 1959. Absorption of iron from iron chelate by sunflower roots. Science 130:274-275.

204. Tiffin, L.O. and J.C. Brown. 1961. Selective absorption of iron from iron chelates by soybean plants. Pl. Phys. 36:710-714.

205. Tiffin, L.O. and J.C. Brown. 1962. Iron chelates in soybean exudates. Science. 135:311-313.

206. Tiffin, L.O., J.C. Brown, and R.W. Krauss. 1960. Differential absorption of metal chelate components by plant roots. Pl. Phys. 35:362-367.

207. Tiffin, L.O. and R.L. Chaney. 1973. Translocation of iron from soybean cotyledons. Pl. Phys. 52:393-396.

208. Tsarapkin, L.S. 1966. Effect of EDTA on chromosome radiation damages. Zashch. Vosstanov. Luchevykh Poviezhdeniyakh, Akad. Nauk SSSR (Russ.) p. 142-150. Cf. Chem. Abstr. 67(3):18400y.

209. Twyman, E.S. 1951. The Fe and Mn requirements of plants. New Phytol. 50:210-226.

210. Van Driel, W. 1964. The effect of EDTA on the growth and metabolism of tomato plants in water culture. Plant and Soil 20:85-104.

211. Venkat, Raju, Von K., H. Marschner, V. Romheld. 1972. Effect of iron nutritional status on iron uptake, substrate pH, and production and release of organic acid and riboflavin by sunflower plants. Zeitschrift dur Pflanzenernahrung und Bodenkunde. 132(3):177-189.

212. Wadleigh, C.H., W.R. Robbins, and J.R. Beckenbach. 1937. The relation between the chemical nature of the substrate and the degree of chlorosis in corn. Soil Sci. 43:153-173.

213. Wallace, A. 1962. A Decade of Synthetic Chelating Agents in Inorganic Plant Nutrition. A. Wallace (ed. and publishers), Los Angeles, Calif.

214. Wallace, A. 1963. Review of chelation in plant nutrition. Agr. Food Chem. 11:103-107.

215. Wallace, A. 1964. Chelation and coordination chemistry as an explanation of factors that induce iron chlorosis in plants. 16th Int. Hort. Congr. Proc. (Brussels, 1962). 4:347-351.

216. Wallace, A. 1969. Root excretions in tobacoo plants and possible implications on the iron nutrition of higher plants. Indian J. Hort. 26(1 & 2):47-50.

217. Wallace, A. 1970. Iron chlorosis patterns in some horticultural plants. Amer. Soc. Hort. Sci. Program (Miami) No. 245.

218. Wallace, A. 1971. Regulation of the Micronutrient Status of Plants by Chelating Agents and Other Factors. Arthur Wallace, publisher, Los Angeles. p. 230-239.

219. Wallace, A. and G.V. Alexander. 1973. Manganese in plants as influenced by manganese and iron chelates. Comm. in Soil Sci. and Pl. Anal. 4:51-56.

220. Wallace, A. and P.C. De Koch. 1966. Translocation of iron of tobacco, sunflower, soybean and bush bean plants. p. 3-5 In A. Wallace (ed.), Current Topics in Plant Nutrition, Los Angeles.

221. Wallace, A., E.T. Frolich, and A. El-Gazzar. 1968. Root excretions in iron deficient tobacco plants and possible effects on iron nutrition. Isotopes and Radiation in Soil Organic Matter Studies, Proc. Symp. IAEA/FAO, Wein, p. 385-395.

222. Wallace, A., E.F. Frolich, and R.T. Mueller. 1967. Effect of solution pH on sunlight stability of FeEDDHA. Agron. J. 59:88.

223. Wallace, A., V.Q. Hale, and N. Hemaidan. 1962. Chelation as a factor in nutrient uptake by plants. Pl. Phys. 37(Suppl.):x/vii, No. 830.

224. Wallace, A., R.C. Huffaker, and H.M. Vines. 1957. Absorption of metal chelates by plant roots. Pl. Phys. 32(Suppl.):38.

225. Wallace, A., R.T. Mueller, O.R. Lunt, R.T. Ashcroft, and L.M. Shannon. 1955. Comparisons of five chelating agents in soils, in nutrient solutions, and in plant response. Soil Sci. 80:101-108.

226. Wallace, A., R.T. Mueller, and R.M. Romney. 1976. Excess EDDHA in the nutrient solution on iron uptake by bush bean plants. Comm. in Soil Sci. and Pl. Anal. 7:93-96, 1976.

227. Wallace, A. and C.P. North. 1953. Lime-induced chlorosis. Calif. Agr. Exp. Sta. Circ. 7:8-10.

228. Wallace, A., C.P. North, R.T. Mueller, L.M. Shannon, and N. Hemaidan. 1955. Behavior of chelating agents in plants. Proc. of the Amer. Soc. for Hort. Sci. 65:9-15.

229. Wallace, A., L.M. Shannon, O.R. Lunt, and R.L. Impey. 1957. Some aspects of the use of metal chelates as micronutrient fertilizer sources. Soil Sci. 84:27-40.

230. Wallace, S.M. Sonfi, and G.V. Alexander. 1976. Comparison of the effects of high levels of DTPA and EDDHA on micro-element uptake in bush beans. Comm. in Soil Sci. and Pl. Anal. 7:111-116.

231. Wallace, A., R.A. Wood, and S.M. Soufi. 1976. Cation-anion balance in lime-induced chlorosis. Comm. in Soil Sci. and Pl. Anal. 7:15-26.

232. Wallihan, E.F. 1955. Relation of chlorosis to concentrations of iron in citrus leaves. Am. J. Bot. 42:101-104.

233. Weinstein, L.H. and W.R. Robbins. 1955. Effect of different iron and manganese nutrient levels on the catalase and cytochrome oxidase activities of green and albino sunflower leaf tissues. Pl. Phys. 30:27-32.

234. Weinstein, L.H., W.R. Robbins, and H.F. Perkins. 1954. Chelating agents and plant nutrition. Science 120:41-43.

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

236. Welkie, G.W. and G.W. Miller. 1960. Iron nutrition of Nicotiana tobacum L. in relation to riboflavin, riboflavin-5-P and flavin ademine dinucleotide content. Pl. Phys. 35:516-520.

237. Williams, R.J.P. 1959. p. 391-441. In The Enzymes. 2nd Ed., Vol. 1, P. Broyer, H. Lardy, K. Myrback (eds.), Academic Press, New York.

238. Young, H. and A. Wallace. 1976. Iron deficiency on corn root mitochondrial adenosine triphosphatase activity. Comm. in Soil Sci. and Pl. Anal. 7:121-124.