SOME ASPECTS OF THE EFFECTS OF HIGH CONCENTRATIONS OF CALCIUM CARBONATE AND VARIOUS ORTHOPHOSPHATES ON THE ABSORPTION, TRANSLOCATION, AND UTILIZATION OF IRON BY *CHRYSANTHEMUM MORIFOLIUM*

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

Rufus Burr Rutland

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ABSTRACT

SOME ASPECTS OF THE EFFECTS OF HIGH CONCENTRATIONS OF CALCIUM CARBONATE AND VARIOUS ORTHOPHOSPHATES ON THE ABSORPTION, TRANSLOCATION, AND UTILIZATION OF IRON BY CHRYSANTHEMUM MORIFOLIUM

by Rufus B. Rutland

The total concentration of iron in chlorotic leaf tissue of chrysanthemums grown in solution culture under conditions of high lime was reduced to essentially that observed in chlorotic leaves resulting from an absence of iron in solutions of low lime and low pH. There were differences among cultivars in leaf content of iron associated with various stages of chlorosis, but in general levels of leaf iron above 70 ppm were found in green leaves, and lower levels were associated with chlorotic leaves. This reduction in leaf iron resulted from a reduction in the amount of iron absorbed by the roots and translocated from the roots to the leaves, apparently partially due to internal changes.

Chlorotic leaves from either minus-iron, low-lime pre-treatment or plus-iron, high-lime pre-treatment absorbed iron at rates equal to, or exceeding those of green leaves. The presence of sodium bicarbonate in foliar application solutions, or conditions of high pH (above pH 6.0) reduced absorption. Chelation of iron with ethylenediamine tetraacetate did not increase the rate of absorption.

Iron, boron, zinc, and manganese were all reduced in the leaves of azaleas as a result of high bicarbonate irrigation.

High concentration of phosphates in the root medium induced chlorosis. Chlorosis was more severe when both phosphate and

bicarbonate were high than when bicarbonate alone was high. There was a higher iron content in severely chlorotic leaves than in slightly chlorotic ones when phosphorus was in high supply, suggesting the accumulation of iron that was not functional in chlorophyll synthesis.

Chlorosis was not induced in the presence of high lime and high phosphate when iron was supplied as ethylenediamine tetraacetate. Transport of iron from roots to leaves was increased, and a concomitant decrease of phosphorus in the roots occurred. Furthermore, a more efficient use of iron in chlorophyll synthesis appeared to result from chelated iron compared to ferric ammonium citrate.

Associated with the appearance of chlorosis was a reduction in shoot growth as indexed by leaf dry weight and dry weight percentage and by shoot length. Root dry weight increase was not adversely affected, especially not by high phosphate conditions. Under high bicarbonate conditions, however, root development was abnormal as a result of death or inactivation of the root tip and subsequent excessive development of laterals.

These responses noted in solution culture were also noted under similar treatment of azaleas in peat soil culture, but not with chrysanthemums in soil culture. It is possible that the presence of high quantities of iron in the soil, even if insoluble, provided abundant and continuous contact between root and iron particle so that, as long as root development continued, an adequate supply of iron was obtained.

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REVIEW OF LITERATURE

Introduction

Green plants are dependent on chlorophyll for photosynthesis. A deficiency of certain mineral elements can result in an inadequate maintenance of chlorophyll in leaf tissues. As early as 1845 Gris (48) demonstrated that an absence of iron would result in a deficiency of chlorophyll. Furthermore, iron must be in continuous supply (20, 21, 56).

Under conditions of high pH chlorosis resulted in the presence of iron (43, 56, 86). Prevention or correction of such chlorosis is possible by acidifying the soil or nutrient solution (52, 56). Though high pH is detrimental, so also is too low a pH, primarily because of limited calcium supply (1, 3, 42, 99). Plants have an optimum pH which varies among species, as is indicated by the attention of standard texts to this point.

High soil lime appears to be the most important cause of induced iron chlorosis as indicated by the great volume of research reports and comprehensive reviews (9, 18, 122, 136). Those papers which directly relate to the question of altered plant ability to absorb, translocate, and utilize iron will be examined here.

The Nature of Lime Induced Chlorosis

When lime or calcium carbonate is high in the soil, several soil components may be affected. Obviously the concentration of calcium is high. Much of the calcium carbonate will be present in the solid state, but it dissolves in equilibrium with the dissolved CO_2 in the

soil solution (14). Gile (43) did not believe that a high concentration of calcium in the soil was the cause of chlorosis, since he grew plants in a soil of 15% $CaSO_4$ without inducing chlorosis. More recently, however, Thorne et al (122) concluded that it was possible that calcium affected the mobility of iron in plants. Lingle, et al (82) and Cain (31) showed that iron uptake was reduced when the calcium in solution reached 8 millimolar concentration, and McGeorge (87) found soil calcium to be high in chlorotic citrus groves.

Bradfield (14) pointed out that the bicarbonate concentration in the soil solution increased as $CaCO_3$ dissolved in the carbonic acid resulting from dissolved CO_2. This could be a causal factor in lime-induced chlorosis, and such a possibility has been supported by several researchers (29, 43, 54). It has been (42, 105, 130) shown that the bicarbonate effect is additive to the pH effect by comparing plant response under high HCO_3 with that under the same high pH of NaOH treatments.

Soil pH is high under conditions of high lime. Bradfield (14) demonstrated a relationship between CO_2 and $CaCO_3$ as it affected soil pH. As the CO_2 concentration in the soil atmosphere increased in the presence of insoluble $CaCO_3$, solution pH decreased; but at CO_2 levels less than 4%, the solution would be neutral or alkaline. Such low levels of CO_2 might exist in the zone except after recent incorporation of organic matter. High pH has been confirmed as a cause of chlorosis under controlled conditions using a variety of means of controlling pH (11, 36, 84, 86, 97, 105). There is, however, at least one reported case of a plant (sorgham) accumulating more iron and developing a greater chlorophyll content under alkaline conditions than

under neutral or acid conditions (77). The opposite is predominately true, though, and often the effect of excess lime has been corrected by acidifying the soil with such agents as mineral acids (52), sulfur (107), or ammonium salts (41, 54). The effect of pH as demonstrated in solution cultures is not always similar to that obtained in soil cultures (99).

The solubility of iron is reduced at high solution pH. Welcher (146) gives the solubility product for ferric hydroxide as 10^{-36} and Latimer (78) as 6×10^{-38}. At either value solubility is extremely low. Using Latimer's value, Wallace (136) calculated the concentration of ferric ions to be 3 parts per billion at solution pH 4, and it would be reduced by a factor of 1000 at pH 7. Furthermore, it is quickly oxidized in the presence of oxygen, especially in alkaline or neutral solutions (136).

Brown (18) also mentioned the instability of ferrous iron in solutions close to neutrality in reaction. The concentration of iron in solution cultures was reduced by bicarbonate (26) and by hydroxide and orthophosphate (89). At appropriate concentrations of cation and anion, floculation was instantaneous (86).

Somers and Shive (114), and Oertli and Jacobson (97) found that iron accumulation in the leaf correlated with the concentration of soluble iron in the root medium. It has been established that the use of organic acid or humic acid forms of iron prevents chlorosis by maintaining iron in solution (12, 56, 106). Jenny and Overstreet (80) found that radioactive potassium was exchanged from roots to iron-bentonite suspensions but not to ferric hydroxide colloids in their studies of that mechanism of root absorption.

Important as soluble iron might be in the prevention of chlorosis, it is not always essential. Gile and Carrero (44) grew plants successfully using dialyzed colloidal iron in suspension. The colloidal form was not as effective as ferric chloride at the same concentration of iron; but with the concentration of metallic iron in the collodial form five times that of the concentration of the chloride form, equal growth was observed. Others (34, 40, 49) have used magnetite as the source of iron in sand-solution studies and obtained healthy green plants. The use of frits (148, 149, 150, 151, 152) and other forms (72) is based on this same principle: roots can absorb iron form solid forms on contact either by direct exchange or by dissolution of iron in the microenvironment surrounding the root. Wynd and Wildon (149, 152) collected the nutrient solution leachate from frit-sand mixtures in which healthy, green plants were growing and found that the leachate did not contain enough iron to prevent chlorosis when applied to plants in sand only. Furthermore, Jenny (71) and Guest (49) demonstrated that there had to be a large number of contact sites between root and iron particle for the plant to be able to absorb sufficient iron from insoluble sources. This principle might account for the findings of Twyman (129) that one large application of ferrous sulfate was better than several small ones. Such application probably assured good distribution of iron within the treated soil mass, thus permitting more contact with a restricted root system.

In addition to these direct effects of lime in the soil there are indirect effects -- for example, the altered solubility of phosphorus. Sendory (110) noted that calcium phosphate was more soluble in serum than in saline solution and that serum contains traces of sodium bicarbonate.

This effect was confirmed in the soil solution (46). Brown et al. (23, 26, 91) also observed this and noted that high HCO_3 with high phosphorus reduced leaf-iron and produced chlorosis. This indirect effect of HCO_3 has been studied extensively as a major factor in lime induced chlorosis. Others (59, 108, 140) have shown chlorosis to be more of a problem on calcareous soil if phosphorus is also high. And still others (12, 27, 41, 98, 106, 113) have shown that high phosphorus alone can cause chlorosis. Hageman and Hartman (52) observed phosphoric acid to give poor results as an acidifying agent for HCO_3 solutions compared to sulfuric and nitric acid. On the other hand, Franco (41) observed good growth without chlorosis with high KH_2PO_4 maintaining a solution pH 5.5 in Hoagland solution. Bingham and Martin (13) had similar results with high phosphorus in three soils under conditions that did not result in an increase of soil pH.

Changes within the Plant

In addition to the direct and indirect effects on the soil solution as previously discussed, high lime is known to induce changes in the plant that may influence iron nutrition. Changes in mineral composition would of course be expected to follow changes in the soil solution. Bennett (9) in reviewing the early literature reported a consensus that the ratio of potassium to calcium in chlorotic leaves was higher than that in green leaves. In fact, many of the papers that he reviewed reported less calcium in the leaves of plants growing on a calcareous soil than those on a non-calcareous soil. Others (64, 87, 138) have reported similar observations.

Baxter and Belcher (6) observed, though, that root tissue of chlorotic citrus had more calcium than did that of green plants in the same orchard. In controlled experiments, Wadleigh, et al. (130) found that bicarbonate resulted in a decrease in leaf calcium and an increase in leaf potassium. Heller, et al. (55) and Wall, et al. (132) similarly found that bicarbonate depressed the accumulation of calcium and magnesium in plant tissues. Cain (31) varied the calcium concentration in sand-cultures and found in the leaves a high K:Ca ratio at low calcium treatments and a low K:Ca ratio at high calcium, as would be expected. Leaf phosphorus has often been observed to be higher in chlorotic plants than in green ones (9). This has been shown to be brought about by high levels of bicarbonate (37, 132). Furthermore, deKock (37, 38) pointed out that the ratio of P:Fe in leaf tissue was higher in chlorotic plants.

Internal pH of the plant can also be affected by lime. There are reports that no differences exist between the pH of expressed sap of chlorotic and green plants (101) and that the growing of plants in solutions of different pH did not alter their sap pH (3). On the other hand, there are reported differences. Baxter and Belcher (6) found root pH to be higher in chlorotic citrus plants on calcareous soil than in green plants although there were no differences between leaf pH of chlorotic and green plants. Haas (51) observed a higher pH of expressed sap from plants of clover, barley, timothy, and wheat on limed plots than of those on non-limed plots. This was true also of the root tissue of mustard, but no difference was found for corn or oats. The sap of chlorosis-susceptible cultivars of corn were found to have a higher pH than that of resistant cultivars (51). Higher pH of expressed sap of corn

and oats growing on limed plots than of those plants on non-limed plots has been reported (80). Wadleigh and Shive (131) and Colgrove and Roberts (36), studying the effects of ammonium and nitrate forms of nitrogen at different solution reaction, found tissue pH to correlate with solution pH, and also tissue pH of plants on nitrate nitrogen was higher than that of those on ammonium at the same solution pH.

Rogers, et al. (50, 109) also found differences among species as well as daily variation within a plant — lower pH during the night. Furthermore, the latter observed that there was more iron in solution in plant sap of low pH than in that of high pH. There was a higher concentration of iron in plant sap than in nutrient solution of the same pH. Furthermore, there were differences among tissues, with the pH of xylem being lower than the pH of phloem, root cortex, or leaf parenchyma. Iron apparently accumulated along pH gradients between tissues (50, 65, 109).

Differences also exist in biochemical composition of plants as a result of exposure to high lime. The organic acid content was found to be higher in chlorotic than green plants of 17 species (63). Citric acid increased in apple foliage before any visual manifestation of chlorosis developed. Increased malic acid in some species was related to increased calcium absorption resulting from increased supply. Su and Miller (119) observed higher citrate in chlorotic soybean than in green plants when the chlorosis was due either to withholding iron from the nutrient solution or to induction by high bicarbonate, phosphate, or manganese.

Clark, et al. (35) and Bedri, et al. (8) noted that roots of some plants could absorb and fix CO_2 into organic acids. Others (24, 103, 130) have noted the absorption of bicarbonate with a subsequent increase

in organic acids and more recently this has been confirmed with radioisotope labelling techniques (8, 10, 116). Jacobson (71) proposed that the synthesis of organic acids is one way in which plants maintain cation - anion equivalence when they are absorbing more cations than anions, as demonstrated when barley absorbs $KHCO_3$.

The respiration of potato disks was affected by the pH of the ambient solution, it being optimum in neutral solutions and slightly reduced at both lower and higher pH (118). Miller (94) and his students observed fairly uniform respiration rates over the pH range 4.5 to 9 for enzyme systems in vitro. However, bicarbonate reduced the activity of cytochrome oxidase and prior exposure of roots to high bicarbonate caused such reduction even when bicarbonate was not present in the substrate during respiration measurement (93). Similarly, Brown, et al. (17, 19) found catalase activity to be reduced in some species when grown on calcareous soils. Both of these research teams observed the reduction of respiration to be more pronounced in those plants susceptible to lime induced chlorosis than in resistant ones (19, 91). Furthermore, Miller (91) noted that high phosphorus reduced the activity of cytochrome oxidase in preparations from soybean root, although some phosphorus was essential for activity; and Huffaker and Wallace (62) found that high phosphate reduced phosphoenolpyruvate carboxylase and carboxykinase preparations from orange leaves and fruits.

Species of plants differ in their susceptibility to chlorosis (23, 42) as do cultivars of a species (9, 18, 23, 136), and even individuals of a cultivar (6, 80, 87, 90, 102, 122, 145). Such differences in susceptibility could be due to differences in iron requirement, but they may also be due to differences in the ability of the plant to absorb,

translocate, or utilize iron. Some evidence has been presented to relate plant differences to differential ability of roots to absorb (22, 24, 135), or translocate iron (133). Soybean cultivars differed in their absorption of bicarbonate and subsequent organic acid synthesis (8), and respiration rates of susceptible species were reduced by bicarbonate; whereas, this was not true for resistant plants (94).

There are also indirect plant effects brought about by high lime that complicate our interpretations of causes and effects. Foremost among these is the reduction in growth. This has been observed in most cases of chlorosis (9, 18, 122, 136), even when the cause is not typical of lime induced chlorosis (77). The cause of reduced growth has been attributed to high calcium concentration in the root environment (43), an adverse pH of the soil (1, 2, 3, 4, 60), high bicarbonate and CO_2 (29, 42, 55, 130), or high phosphorus (60). Root extension is reduced under high bicarbonate conditions (116). In addition to overall reduction in plant size there is reduction in dry weight of the tissues (105, 123, 136).

Effect on Absorption

Aspects of the problem so far discussed dealt with indirect evidence that internal changes in the plant as brought about by exposure to lime might possibly affect the ability of the plant to absorb and translocate iron. Such changes, if they occur, would complicate any efforts to correct or prevent chlorosis of plants growing on calcareous soils. Direct evidence for such changes is limited, but it is being developed at the present, and of particular importance in such studies is the use of radioisotopes to facilitate accurate measurements of the

small quantities of iron moved during short periods of time.

Evidence has been presented that indicates that some of the genetic difference in susceptibility is due to a differential ability to absorb iron. Brown, et al. (20, 22, 25) and Bhan and Wallace (11) found that Hawkeye soybean absorbed more iron than did PI 54619-5-1, and Brown (25) pointed out specifically that a difference in the rate of reduction of ferric iron in the ambient solution accounted at least in part for the difference in absorption of the iron.

Reference has already been made to the reduction in respiration rates of plants afflicted with lime-induced chlorosis. Branton and Jacobson (15, 16) found that when root respiration was reduced by dinitrophenol, iron absorption and subsequent accumulation in the tops of pea plants was reduced. This is consistent with earlier observations by Hoagland (57) and with Kramer's discussion (76) to the effect that an expenditure of energy obtained from metabolic processes requiring oxygen is necessary for active absorption against a concentration gradient. This is further supported by observations that aeration of nutrient solutions facilitates the absorption of such minerals as calcium, magnesium, phosphorus, nitrogen, and potassium, and that CO_2 represses such absorption (33). Linsay and Thorne (81) showed oxygen to increase iron absorption from solution unless that solution was high in bicarbonate, in which case the precipitation of iron was apparently accelerated. Dependence on high rates of respiration for iron absorption also probably accounts for the observations that low soil temperature and high soil moisture (with associated low soil oxygen) increase the susceptibility of plants to chlorosis (30, 108, 109).

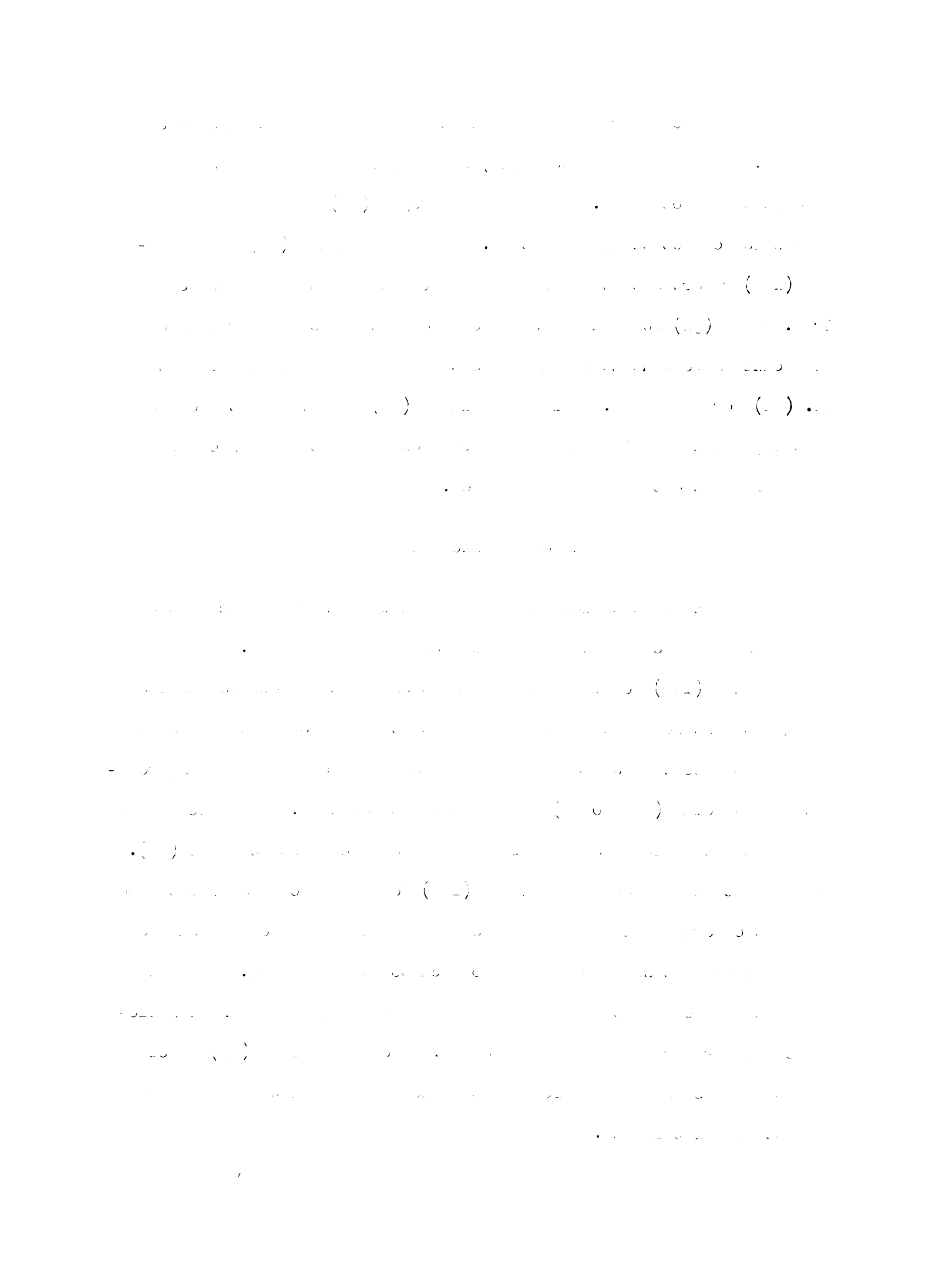
Insoluble frits (151) and magnetite (34, 71) can be sources of iron in the presence of lime, but Chapman (34) observed that chlorosis could result when the lime level was high unless the amount of finely ground magnetite was increased in the soil. He did not resolve the question of how this reduction was brought about, but it is likely that the extension of the root system was reduced under these conditions, and higher magnetite levels increased the number of iron particles to compensate for root reduction and thus maintained the necessarily high root-particle contact. Guest (49) also noted a reduction in iron absorption from a magnetite source under conditions of high calcium and magnesium carbonate.

Very little information has been developed pertaining to the effects of lime-induced chlorosis on foliar absorption of iron (133). The form of iron applied is a factor in the foliar treatments: inorganic forms are absorbed more readily but chelated forms are translocated more readily (85). In laboratory experiments inorganic forms were more effective than organic, but in field tests better recovery from chlorosis was obtained from some of the chelated forms (85). These observations were not related to the level of lime or condition of chlorosis; however, Teubner, et al. (121) have provided some information about foliar absorption in general. They found that light and warm temperatures stimulated foliar absorption. The pH of the treatment solution was a factor with different optima for different elements. Surfactants increased the wetting of the leaf but decreased total absorption because the quantity of treatment solution that would remain on the leaf was reduced. Wallace and Bedri (134) found more foliar absorption of iron from treatment solutions of low pH than from those of high pH.

There is some direct evidence that lime reduces root absorption of iron. Steward and Preston (118) observed an effect of pH on root absorption of potassium. Hoagland and Broyer (58) noted bicarbonate repression of potassium absorption. Hale and Wallace (53) and Wallihan (142) reported that bicarbonate reduced the root absorption of iron. Cain (31) found a reduction of iron absorption as a result of high calcium concentration in the rooting medium, as did Lingle, et al. (82) for magnesium. Hale and Wallace (53) observed that phosphorus, in the presence of bicarbonate, reduced root absorption of iron, whereas phosphorus without bicarbonate did not.

Effect on Translocation

The effect on translocation is not easily separated from that on absorption, but some tentative conclusions have been drawn. Rediske and Biddulph (106) concluded that absorption and translocation of iron was greater at low pH that at high, and that more iron appeared to be translocated to the tops of beans when iron and phosphorus were approximately equimolar (2×10^{-5}M) in the nutrient solution. High bicarbonate impeded the translocation of calcium and iron from root to shoot (45). Using radioactive tracers, Wallihan (142) found no iron translocated to the tops of orange plants in five hours from high bicarbonate solutions, whereas there was some from the zero bicarbonate treatment. The data were such as to permit the conclusion that both absorption and translocation were reduced by high bicarbonate. Brown and Jones (25) concluded that the resistant Hawkeye soybean translocated more iron than did the susceptible PI cultivar.



Using a split root technique, Brown, et al. (26) found that high bicarbonate in the lower root medium did not reduce translocation of iron absorbed by the higher portion of the root system, but when both bicarbonate and phosphorus were present in the lower medium there was a reduction. Similarly, there was no reduction in iron absorption from high calcium in the lower medium, but sufficient reduction from calcium and phosphorus together in the lower medium that soybean plants became chlorotic with concomitant reduction in leaf-iron (28). High phosphorus with either bicarbonate or chloride salts of sodium reduced the translocation of iron in other experiments (91).

Olsen (98) early postulated that iron was precipitated with phosphorus within the plant when grown at solution pH 6 - 7 unless the source of iron was iron humate. Thorne (122) made reference to several such reports in his review. Some evidence given for this phenomenon is that root iron is very high compared to foliar iron (83, 87, 104, 122). More direct are the observations of Hoffer (59) and Loehwing (83) that iron accumulated in the nodes of corn. Hoffer (59) indicated that liming and applying phosphorus to the soil reduced this accumulation by reducing the amount of iron absorbed; whereas, Loehwing (83) related such precipitation to a higher sap pH under the influence of high lime. Biddulph (12) demonstrated the probable internal precipitation of iron from high phosphorus in the rooting medium. Branton and Jacobson (16) also showed accumulation of iron in root cortex and in the leaf parenchyma near veinlet endings, although this accumulation was not studied in relation to lime or phosphorus induced chlorosis. High lime was found by North (93) to reduce the translocation of iron after injection into the root; but he, like Brown and Holmes (20), found no reduction in translocation when iron was introduced into the stem.

Translocation from, and mobility within the leaf has been studied to a very limited extent. Brown (18) in his review concluded that the mobility of iron in the leaf varied with the form of iron applied. Such reports as that of Biddulph (12) support the commonly held position that iron is not translocated readily from the leaf, even when introduced by infusion through the cut leaf. Wallace and Bedri (134), however, determined that there was some translocation of foliar applied iron and that the amount translocated was proportional to the amount absorbed, being in the range of one to ten percent. Lunt and Kohl (85) found that translocation varied with the form of iron applied to the leaf. Translocation of radioactive iron from the leaf to which it was applied was reduced by high calcium, magnesium, phosphorus, copper, manganese, and iron, itself, in the rooting medium (95). Growing plants under high bicarbonate for a few days prior to foliar application of iron increased the amount translocated from the site of application (39). And although it was translocated from the site of application to the leaf, iron remained in the petiole and stem under the influence of prior treatment with high bicarbonate, high pH or high phosphorus, as much as 40% of the iron translocated out of the treated leaf remaining in the conducting tissue, contrasted to 28% under low pH, bicarbonate, and phosphorus treatments.

In spite of such evidence for the precipitation of iron within and contiguous to conducting tissues, attempts to correct chlorosis by infusion or injection of chelating agents have not been successful in redissolving and mobilizing that iron to the extent that chlorosis was corrected (111).

Tiffin and Brown (126, 127) presented evidence of malic and malonic acids acting as carriers of iron in the conducting tissues of soybeans. Jones and Eagles (73) showed that $C^{14}O_2$ was fixed by leaves as products which were translocated to other leaves. Primarily translocation of these products was to young immature leaves of the plants and to the less mature portions of mature leaves. To some extent it was translocated to older leaf tissue, but only into the veins and veinlets, accumulating so as to show by autoradiography the veinal pattern of the leaf in a manner closely resembling patterns observed in iron translocation studies (15). Papers dealing with the root fixation of CO_2 and bicarbonate have reported specifically that malic and malonic acids were increased in the roots under the influence of high bicarbonate (35, 69, 130). Others (29, 63, 130) have shown an increased organic acid content in chlorotic leaves -- namely, malic, citric, oxalic, and tartaric acids. Wallace, et al. (137) even found a higher content of citric and malic in the chlorotic portion of variegated leaves of ivy and camellia than in green portions. A possible expectation as a result of these observations would be that chlorotic plants growing under high bicarbonate conditions would have a high production of organic acids in the root system, and these organic acids would facilitate the translocation of iron absorbed through the roots. However, Bedri, et al. (8) found that the C^{14} fixed in the roots after being absorbed as CO_2 did not move upward into the shoots and leaves.

Iron has generally been considered not to be redistributed from old to young tissues, since deficiency symptoms appear in the youngest tissues first. However, plants which have been growing with an adequate iron supply for some time often can produce considerable normal-appearing

leaf tissue on a medium devoid of iron (100, 128). Young azalea plants were grown in sand-solution culture involving iron-free solutions for 210 (100) and 150 days (128) before chlorosis developed. For plants to have grown that long without showing symptoms of iron deficiency there either had to be an internal iron reserve which could have been translocated to the meristematic tissues, or sufficient iron was present in the nutrient solutions used to maintain threshold supply.

Iron Utilization

There are numerous reports (64, 80, 87, 90, 101, 113) of chlorotic leaves containing as much iron as green leaves, and this has been considered evidence that some of the iron in the leaf was inactive in chlorophyll synthesis.

Oserkowsky (102) developed this concept to a considerable extent by correlating acid-extractable leaf iron with chlorophyll. By extrapolating to zero chlorophyll, he established the amount of leaf iron that was not functional in chlorophyll synthesis, and this fraction he called "inactive." McGeorge (87) concurred in principle. Acid-soluble iron was decreased by bicarbonate, whereas total iron was not (29). Brown (18) concluded that "active" iron was that iron soluble in normal hydrochloric acid. The form in which the "inactive" iron occurs has not been established, although several workers (12, 26, 37, 98) have suggested iron phosphates.

Bennett (9), on the other hand, argued that most of the iron in the plant is not in solution but is absorbed on protein (enzymes) and as such is not extracted by acid but is metabolically active. Not all protein-iron would be involved in chlorophyll synthesis, but a considerable

portion of it would be. Therefore, he did not accept the hypothesis that acid-extractable iron was the fraction active in chlorophyll synthesis. Jacobson (66) also stated that some of the iron that he found to be inactive in chlorophyll synthesis was possibly active in other processes in the leaf cell.

Direct studies of the role of iron in chlorophyll synthesis have just begun. Cheberek and Martell (32) cited evidence for the role of iron in the condensation of pyrrole groups to form urophophyrin and in coordinating bonds in the cyclic condensation of porphobilinogen groups as they assume the spacial configuration of chlorophyll. Magnesium displaces iron from the cyclic molecule, and thereafter the iron is available for further condensation of pyrrole or porphobilinogen groups. Granick's pathway (46) for chlorophyll synthesis from protoporphyrin - 9 indicates a similar role for iron. As these roles of iron are further defined, our understanding of the effect of bicarbonate in the root medium should be improved.

Jacobson (66) demonstrated the importance of cleaning leaves before analysis. The iron content of leaves washed in 0.3 N hydrochloric acid had only 6% the iron content of non-washed leaves. Others (18, 122, 120, 130) have further established this point by comparing different methods of washing. Several later reports (66, 68, 79, 97, 111, 130, 141) have indicated that with careful sample preparation good correlations were found between the total concentrations of iron and chlorophyll in the leaf. Some (66, 97, 111, 122) have correlated iron with chlorophyll but indicated that it was not possible to establish a "critical" level, since incipient chlorosis occurred at different levels of iron in different orchards, species, or clones. More consistent differences between green

and chlorotic leaves have sometimes been found when the iron content was expressed on a leaf area basis (111). Porter and Thorne (105) on the other hand, found that total iron within the plant was reduced by bicarbonate, but not the ratio of iron to leaf dry matter, since the dry weights of plants were also reduced under high bicarbonate conditions.

In addition to careful sample preparation, it is important that the iron supply be constant. Iron in leaves increased greatly after treating deficient plants with available iron (68, 79, 80). This would indicate that fluctucations in iron supply result in fluctuations in iron concentration in the leaves, and probably leaf iron fluctuates more rapidly than chlorophyll content. Oertli and Jacobson (97) were able to establish a correlation between the iron concentration in the leaf and that in the root medium by using large volumes of nutrient solution for each root system and by changing the solutions frequently, in contrast to reports of no correlation (106).

Synthetic Chelates

Synthetic chelates have been studied as a means of correcting lime-induced chlorosis, and hence offer some information about the nature of the disorder. Early investigations established that chelation with organic acids and humates improved the stability of iron at high pH (7, 12, 38, 61, 84), but not to the extent of making it absolutely stable at any solution reaction (11, 37, 85). Bear (7) indicated that ferric ethylenediamine tetraacetate (EDTA-Fe) was stable to pH 8 or 9, and ferric diethylenetriamine pentaacetate (DTPA-Fe) to pH 11. Jacobson (67) found 100 per cent of initial EDTA-Fe still in solution after 90 days at pH 6, 82 per cent at pH 7, 70% at pH 8, and 10 per cent at pH 9. Furthermore,

the chelate-metal system tends to maintain a constant concentration of free metal in solution (103).

This increased solubility of iron at high pH undoubtedly accounts for some of the success obtained in correcting or preventing chlorosis under high lime conditions (37, 60, 98, 99, 133, 140, 144), though this would not likely be the case in the acid, sandy soils of Florida where chelates have also been successfully used (79, 114).

Stabilizing iron in this manner also makes possible observations about the effects of soil conditions on absorption and translocation of iron independent of its precipitation from the soil solution. By using EDDHA, Bhan and Wallace (11), and Jeffreys, et al. (70) showed that high pH reduced root absorption of iron, as did the nitrate form of nitrogen relative to ammonium. This was evident from the accumulation of iron in both the root and tops of the plants. High bicarbonate reduced the accumulation of EDTA-Fe in leaves and stems but not in roots, i.e., translocation of the absorbed iron was reduced (81).

Some evidence has been presented which indicates that synthetic chelates increase the translocation of iron within plants, and this might account for the improved response under high bicarbonate conditions. Weinstein, et al. (143) reported higher leaf-iron and lower root-iron in plants receiving EDTA-Fe relative to those receiving ferrous sulfate. The same authors (144) noted that supplying the sodium salt of EDTA to one portion of the root system with the other portion in ferrous sulfate prevented chlorosis under conditions that induced chlorosis when EDTA was not supplied. Chelation with ethylenediamine di-(o-hydroxy phenylacetate) - EDDHA - and with DTPA increased the translocation of root-absorbed iron, even when the chelating acid was supplied either before or after tagged

iron sulfate.(70).

Wallace, et al. (139) measured the relative absorption and translocation of the EDTA molecule and the chelated iron and found essentially equimolar accumulation. This indicated that the iron was absorbed and translocated as part of the chelated molecule. Lunt and Kohl (85) also found increased translocation of iron when it was applied to rose leaves in a chelated form. By contrast, Neher, et al. (95) observed no increase in the translocation of chelated iron over that of ferric chloride.

More recent work by Tiffin and Brown (124, 125, 126, 127) has questioned the possibility of translocation of an intact, chelated iron molecule. Tiffin, et al. (125) first reported that stem exudate of soybean contained more total iron than EDDHA-chelated iron when EDDHA-iron was supplied to the rooting medium, and concurrently free EDDHA was found to accumulate in the nutrient solution as iron was absorbed. At the same time EDDHA was found to be absorbed since the chelating ability of the exudate increased when the roots were supplied the free-acid form of EDDHA. In a later paper the same authors (127) showed that C^{14} labelled chelates were absorbed, but the electrophoretic migration was not the same as that of the intact molecule, indicating that some degradation had taken place within the plant. Wallace and North (138) reported that the nitrogen from N^{14}-labelled chelate molecules remained in the residue of plant tissue after extraction with water, alcohol, and acid, which also suggested that the chelate molecule was not intact within the plant.

MATERIALS AND METHODS

Iron Solubility in Nutrient Solution

Experiment I: Stability of Iron in Non-aerated Nutrient Solution

The stability of iron in nutrient solution was determined using radioiron (Fe^{59}). Half-strength Hoagland's number one solution (Appendix Table 1) was prepared and adjusted to pH 4, 5, 6, 7, or 8 by adding either HCl or NaOH. Aliquots of 50 ml to which were added 0.4 uc of $Fe^{59}Cl_3$ were placed in each of three test tubes and stoppered with sterile cotton. This gave an initial acitivity of 1000 cpm per ml as determined with a gas-flow Geiger-Mueller tube used with a VersaMatic scaler.

A one-milliliter aliquot was removed from each test tube after one-half, 1, 3, 6, and 12 hours, and after 1, 2, 3, 4, 5, 9, 11, 18, and 25 days. It was evaporated to dryness on a stainless steel planchet and its radioactivity determined.

The percentage of iron remaining in solution was calculated by dividing the activity of each sample aliquot by the activity of the initial aliquot and multiplying by 100.

The pH of each treatment was determined on an aliquot of nutrient solution at each sampling time by means of a Beckman Zeromatic pH meter and was not observed to change.

Three replications were provided in a completely randomized design.

Experiment II: Influence of Lime and Ammonium Citrate on Iron Stability in Aerated Nutrient Solution

The influence of ammonium citrate on iron stability in aerated nutrient solution of pH 5 and low lime concentration was contrasted with that at pH 7 with high lime.

In this experiment, as well as in those that follow, the term "low lime" refers to a solution to which no carbonates or bicarbonates were added. The pH was adjusted to pH 5.5 by adding ammonium sulfate and the solution was aerated with compressed air. A trace of bicarbonate was probably present since the air can be assumed to have contained approximately 0.03% carbon dioxide. The term, "high lime", refers to the concentration of calcium bicarbonate which resulted from suspending finely ground calcium carbonate in the solution and aerating with air enriched with carbon dioxide to a final carbon dioxide concentration of 2% by volume. Calcium carbonate dissolved in equilibrium with the dissolved carbon dioxide and resultant carbonic acid as calcium bicarbonate. A calcium bicarbonate concentration of approximately 3 millimolar was assumed on the basis of equilibrium studies reported by Bradfield (14). After the system had come to equilibrium, solution pH was 7.0 and remained constant as long as solid calcium carbonate was present. "High lime", therefore, refers to the combined effects of calcium and bicarbonate at approximately 3 millimolar concentration and solution pH 7. These conditions are indicated in all instances when the terms "low lime" or "high lime" are used in this paper.

Iron was added to each 50 ml aliquot of nutrient solution in aerated test tubes after the pH had stabilized at the desired pH.

Ferric chloride was added as one form of iron in sufficient quantity to have 3 ppm iron in solution. In the other iron treatment, a stock solution of ferric chloride was combined with ammonium citrate at a molar ratio of 1 iron to 6 ammonium citrate and allowed to stand for a few minutes before being added to the nutrient solutions to give a final concentration of 3 ppm iron. The instantaneous development of green color in the solution of ferric chloride and ammonium citrate indicated that a reaction had occurred between the two compounds and thus the ferric ammonium citrate complex was formed.

The solutions were analyzed colorimetrically after 20 hours by the O-phenanthroline method described in Methods of Analysis (5).

The experiment was repeated for the two treatments containing ferric chloride, so that observations could be made after 1, 2, 5, and 7 hours by preparing fresh solutions of those treatments and analyzing five-milliliter aliquots removed from each test tube at each time of observation.

Three replications of each treatment were provided in a completely randomized design.

Experiment III: Influence of Lime and Phosphate on the Stability of Iron in Aerated Nutrient Solution

The stability of iron as ferric ammonium citrate in aerated nutrient solution containing either high bicarbonate or high phosphate or both was determined by sampling nutrient solutions in which chrysanthemums were being grown as part of Experiment XVIII, which is described in detail on page 43. The solution treatments are

The solution treatments are described in brief in the table below.

pH	HCO_3	H_xPO_4	Aeration
5.5	0	0.5mM KH_2PO_4	Air
7.2	0.5mM K_2CO_3 excess $CaCO_3$	0.5mM KH_2PO_4	2% CO_2 in air
6.8	0	0.5mM KH_2PO_4 1.5mM K_2HPO_4	Air
7.2	0.5mM K_2CO_3 excess $CaCO_3$	0.5mM KH_2PO_4 1.5mM K_2HPO_4	2% CO_2 in air

The base solution was half-strength Hoagland's number one with Hoagland's "A" solution (Appendix Table 1) for minor elements. Ferric ammonium citrate was used to provide a concentration of 2 ppm iron.

Aliquots were removed from the crocks of aerated nutrient solution in which young chrysanthemum plants were growing at intervals of 2, 24, and 48 hours after fresh solutions were added to the crocks. Iron in solution was determined by the o-phenanthroline method.

Four replications were provided in a randomized complete block design.

Lime-Induced Chlorosis in Chrysanthemums and Azaleas

Experiment IV: Effects of High Lime on Growth and Chlorosis of Chrysanthemums

A comparison was made of plant growth, development of chlorosis, and iron content of leaves of chrysanthemums resulting from high-lime induced iron stress with that resulting from iron stress caused by withholding iron from the nutrient solution. Rooted cuttings of

the cultivar Beauregard were selected for uniform size (four expanded leaves) and well developed roots. Two plants were transferred to each two-gallon, glazed crock after washing the roots to remove the rooting medium. The crocks were filled with half-strength Hoagland's number one solution containing Hoagland's "A" solution for minor elements and modified as indicated below to maintain the treatment conditions. Aeration was provided by forcing air through aquarium aerating stones. A masonite cover supported the plants by means of polystyrene plugs fitted in the holes through which the root system and basal stem passed.

These plants, as well as those of the other experiments reported here, were grown in a greenhouse having a minimum night temperature of 60° F. A double layer of cheesecloth was suspended over the plants along the south side of the experimental area, this shade being maintained for all experiments conducted during the months of May through August. The natural photoperiod was extended when necessary to 16 hours with supplemental lighting of 20 f. c. intensity to maintain plants in a vegetative state.

The terminal bud was pinched out of each plant after one week of growth and three lateral shoots near the apex were allowed to develop.

Three treatments were provided, as follows:

(a) pH 5.0, no lime, 2 ppm iron as ferric ammonium citrate

(b) pH 5.0, no lime, no iron

(c) pH 6.8, high lime, 2 ppm iron as ferric ammonium citrate

The low pH treatments were obtained by adding ammonium sulfate at 0.5 millimoles per liter to Hoagland's number one base solution. The high pH, high lime treatment was obtained by adding 0.5 millimoles per liter of potassium carbonate and suspending finely ground C. P. grade calcium carbonate in the solution and aerating with air enriched to 2% CO_2.

Four replications were provided in a randomized complete block design.

The plants had attained a height of about 18 inches and produced about 22 nodes, after six weeks under these treatments. At that time they were rated for degree of chlorosis and necrosis. The relative scale for chlorosis was:

1. None
2. Slight yellowing
3. Moderate yellowing with green vein pattern
4. Severe yellowing with some white areas

The scale for necrosis was:

1. None
2. Slight, along margins
3. Moderate
4. Severe, with entire leaf affected in some cases.

At that time the experiment was terminated. The non-necrotic leaves from all shoots of each plant in each crock were removed and combined as one sample. They were washed in 0.1 normal hydrochloric acid in distilled water containing 0.5 ml per liter Tween-20 as a wetting agent and were rinsed twice in distilled water. The leaves were then dried in a forced-draft oven at 70° C. and their dry weights

determined as an index of growth. The roots were also removed and dry weights determined.

The dried leaf samples were ground to pass a 20-mesh screen in a Wiley Mill fitted with bronze blades. For total iron the tissue was extracted by ashing at 500° C. and dissolving the ash in hydrochloric acid and analyzed colorimetrically by the o-phenanthroline method.

Experiment V: Effects of High Lime and Iron Chelation on Chlorosis and Mineral Composition of Chrysanthemums

The effects of EDTA (ethylenediamine tetraacetic acid) chelation of iron on iron accumulation in the leaf and prevention of chlorosis under high pH and high lime conditions were determined. Four treatments were established, as follows:

(a) pH 5.5, no lime, 2 ppm iron as ferric ammonium citrate

(b) pH 5.5, no lime, no iron

(c) pH 7.0, high lime, 2 ppm iron as ferric ammonium citrate

(d) pH 7.0, high lime, 2 ppm iron as ferric EDTA

The methods of controlling solution pH were the same as in Experiment IV.

Two plants of Chrysanthemum morifolium, cv. Legal Tender, were grown in each two-gallon glazed crock under the same methods of culture as in Experiment IV.

Three replications were provided in a randomized complete block.

The degree of chlorosis that had developed in four weeks was rated, and the leaves that had developed on all shoots of both plants

in each crock were removed and prepared for analysis. The leaf samples were analyzed by spectrographic methods to determine leaf concentration of calcium, magnesium, phosphorus, iron and manganese. This analysis was performed in the tree fruit nutrition laboratory of the Department of Horticulture of Michigan State University under the supervision of Dr. A. L. Kenworthy (75).

Experiment VI: Effects of Bicarbonates in Irrigation Water on Growth Chlorosis, and Mineral Composition of Azalea

The effects of pH, bicarbonate, and associated cation in the irrigation water on the growth and chlorosis of azaleas was determined. The azalea was selected because of its known sensitivity to high soil pH and bicarbonate concentration. Young seedling plants grown from hybrid seed lot #49, a Mollis type produced by Dr. W. J. Haney, were grown in four-inch clay pots containing German peat moss which had an initial pH 5.2 and was of the type used extensively by Michigan azalea growers because of its low pH and high buffer capacity. Plants were fertilized weekly by applying 200 ml of a nutrient solution that was twice the concentration of Twigg and Link's azalea solution (125). They were irrigated daily by applications of 200 ml per pot of the appropriate solution listed below:

(a) Distilled Water

(b) Tap Water with 0.1 ml concentrated H_2SO_4 per liter

(c) Tap Water with 0.25 ml H_3PO_4 per liter

(d) Tap Water

(e) Distilled Water with 5 me $Ca(HCO_3)_2$ per liter

(f) Distilled Water with 5 me $NaHCO_3$ per liter

The tap water used was that at the Plant Sciences greenhouse and had a pH of 7.4 and an electrical conductivity of 45 mho x 10^{-5} due primarily to dissolved bicarbonates of calcium, magnesium, and sodium. The calcium bicarbonate solution was prepared by suspending 5 me $CaCO_3$ per liter in distilled water and bubbling carbon dioxide through it until the carbonate dissolved. The sodium bicarbonate solution was prepared from chemically pure sodium bicarbonate and distilled water. The quantities of acid added to the tap water were sufficient to neutralize it.

The pH of the first 50 ml of leachate from the pot was determined at least once every two weeks. The additions of acid to the tap water was adjusted as necessary to maintain the same pH as occurred in the distilled water treatment, pH 5.5. The pH of the treatments receiving tap water, calcium bicarbonate, and sodium bicarbonate remained within the range pH 7.0 to 7.4 throughout the duration of the experiment.

The plants were subjected to treatment conditions from July 26 until October 7 in a randomized complete block design with three replications. They were soft-pinched to encourage lateral shoot development. By October the shoots had terminated in dormant buds. The leaves that developed during the experiment were rated for chlorosis on the relative scale, removed, washed, and analyzed spectrographically for calcium, magnesium, potassium, sodium, phosphorus, iron, manganese, boron, and zinc. Leaf dry weight was determined and used as an index of growth.

The cold requirement of the plants was satisfied by subjecting them to 34° F. for a period of 12 weeks (October 7 to January 5).

Then they were returned to the greenhouse and grown at 60° F. minimum night temperature. By May 5, shoot extension was complete and leaves had matured on the new shoots. The plants were segmented into leaf tissue, lateral stems, and primary stems. Each tissue segment was weighed after drying and dry weight used as an index of growth. The tissues were analyzed for phosphorus and iron by spectrographic means. The data were analyzed by analysis of variance with emphasis on the effects of the irrigation solutions on the accumulation of these elements in the various tissues, and particularly to determine if iron accumulated in stems in association with phosphorus.

Root Absorption and Distribution of Radioiron

Experiment VII: Effect of High Lime on the Absorption and Distribution of Iron in Chrysanthemum

Three groups of rooted cuttings of C. morifolium, cv. Legal Tender, were grown for two weeks in serated Hoagland's number one solution plus Hoagland's "A" solution under the following conditions:

(a) pH 5.5 and 2 ppm iron as ferric ammonium citrate

(b) pH 5.5, and no iron

(c) pH 7.0, high lime, and 2 ppm iron as ferric ammonium citrate

Low-pH conditions were maintained by adding ammonium sulfate, and high-pH, high-lime conditions were maintained by adding calcium carbonate and aerating with 2% carbon dioxide as described for Experiment II.

After two weeks of treatment, the plants under treatment "a" were green, but those under "b" and "c" were slightly chlorotic.

At that time the plants were moved from the greenhouse to the laboratory for the second phase of the experiment. Radioiron was used to determine the effects of these treatments on root absorption and distribution of iron.

The same treatments were continued during the 24- and 36-hour absorption periods, utilizing fresh solutions. Radioiron ($Fe^{59}Cl_3$) stabilized with ammonium citrate was added to these solutions in the amount of 57 uc/l. Stable iron was also included at 2 ppm iron as ferric ammonium citrate. Absorption was allowed to occur under continuous illumination of 800 f. c. and at a temperature of 28° C.

A split plot design of five replications was employed with treatment as main plot and time as sub-plot.

At the end of each absorption period, the plants were removed from the solutions and segmented into: (a) upper four leaves, (b) lower four leaves, (c) stem, and (d) roots. The lower stem sections with roots attached were washed by repeated dipping in acidified Tween-20 solution and then in distilled water. This was followed by soaking 30 minutes in Na-EDTA solution, flushing with running tap water for two minutes, and rinsing in distilled water. They were then blotted dry on absorbant tissues and the roots cut from the stem. Each tissue sample was dried at 70°C. overnight and weighed.

The dry tissue samples were crushed in the bottom of paper cups to give a uniform distribution on the bottom surface and their radioactivity was determined with a Geiger-Mueller tube using a Nuclear-Chicago scaler. The relationships of radioactivity and iron concentration of the treatment solutions were determined by counting

one-milliliter aliquots. Total iron absorbed by each plant was determined by assuming a constant relationship of radioactivity to iron concentration, and the percentage of the total amount that was in each plant segment was calculated.

Experiment VIII: Effect of High Lime on the Absorption and Distribution of Iron by Chrysanthemum Plants after Transfer to a Low-Lime Solution

A determination was made of the effect of prior treatment with high-lime conditions, and subsequent development of chlorosis, on the absorption and translocation of iron by chrysanthemum plants after their transfer to a solution of low-lime conditions.

Young rooted cuttings of C. morifolium, cv. Legal Tender, were treated for two weeks under the same three conditions as described in the previous experiment. Then the plants were transferred to the laboratory and allowed to absorb radioiron under the same conditions of light and temperature as before. Only one solution treatment was used during the 22-hour absorption period: pH 5.5 obtained by adding 0.5mM ammonium sulfate to half-strength Hoagland's number one solution. Iron was supplied at 2 ppm as ferric ammonium citrate and radioiron at 57 uc per liter as ferric chloride stabilized with ammonium citrate. The absorption procedure, sampling and determination of radioactivity was identical to that described in Experiment VII.

A completely randomized design with five replications was used. The data for each tissue segment were analyzed separately by analysis of variance and Duncan's Multiple Range Test for evidence of effects of pre-treatment.

Experiment IX: Effects of Lime on Long-Term Distribution of Iron in Chrysanthemum

The redistribution, or long-term distribution of root-absorbed iron in chrysanthemums was examined in this experiment.

Uniform rooted cuttings of C. morifolium, cv. Legal Tender, were selected and allowed to absorb radioactive iron from half-strength Hoagland's number one solution modified with 0.5mM ammonium sulfate. After 12 hours in this solution under continuous illumination at 800 f.c and at 30° C., the plants were removed. Their roots and basal stems were washed by flushing under running tap water for 10 minutes, dipping in distilled water, and draining dry on tissues.

To determine initial distribution, nine plants were selected at random and segmented into: (a) leaves, (b) stems, and (c) roots. These were dried at 70° C., weighed, and their radioactivity determined by counting by the methods described earlier. Other plants were prepared for autoradiography by cutting the stem above the roots, cutting each leaf petiole from the stem, and pressing each severed shoot portion between blotter papers. After drying at 70° C., each plant shoot was mounted on fresh blotter paper with each leaf in the same position as it had grown on the stem. (Roots were not mounted, since previous experience had proven that radioactivity of the roots was so high, relative to that of the stems and leaves, that the film would be greatly overexposed in the area contacting the roots.) The mounted plants were placed in an exposure box with only a thin film of plastic between the plant parts and a sheet of x-ray film. A sheet of masonite

and a 3/16 inch thick sheet of steel separated each specimen from the one above it as they were stacked in a light-proof box. After two weeks, the exposed film was removed and developed in fresh x-ray developer for 60 seconds, fixed, washed, and dried. The image on the x-ray film was copied on photographic film and printed using constant exposure and development.

Long-term distribution was established by utilizing the remaining rooted cuttings and transferring them to half-strength Hoagland's number one solution containing Hoagland's "A" solution for minor elements and modified for treatment conditions, as indicated:

(a) pH 5.0, no lime, 2 ppm iron as ferric ammonium citrate

(b) pH 5.0, no lime, no iron

(c) pH 7.0, high lime, 2 ppm iron as ferric ammonium citrate

As in similar earlier experiments, the low pH treatment was provided by adding ammonium sulfate at 0.5mM, and the high pH/high lime treatment was obtained by adding 0.75mM potassium carbonate and equilibrating solid calcium carbonate with 2% CO_2.

Three plants were grown in each one-gallon crock. Each crock was considered that unit of replication, and there were six replications in a randomized complete block design.

The plants were pinched to remove the terminal bud after two weeks, and immediately thereafter the lower lateral buds were removed leaving only the upper three to develop. At that time one plant was removed from each crock and autoradiograms prepared.

At the end of the ninth week, one plant from each crock was removed and the roots and lower stems were again washed in running

tap water for ten minutes and blotted dry. The plants were segmented into: (a) leaves from lateral shoots grown after pinching, (b) stems of lateral shoots, (c) leaves from the shoot below the point of origin of laterals, (d) stem below the point of origin of laterals, and (e) roots. These tissue samples were dried at 70° C. to constant weight and dry weight recorded, then ashed at 500° C. The ashed samples were dissolved in 50% hydrochloric acid, transferred to paper cups, evaporated at room temperature, and radioactivity was determined. An indication of long term distribution or redistribution was obtained by calculating the per cent of the total radioactivity of each plant that was attributed to each segment of that plant.

Autoradiograms were prepared using the remaining plant from each crock. Because of the size of the plants, it was not possible to include the entire plant on one autoradiogram film (8" x 10"). Separate autoradiograms were prepared of the lower stem with its associated leaves and of the lateral stem with its associated leaves plus the leaf from whose axil it had developed.

Experiment X: Effect of Lime on Long-Term Distribution of Iron in Azalea.

Uniform rooted cuttings of the Rhododendron hybrid, Alice Mueller, an evergreen azalea of the type grown as a florist pot plant, were allowed to absorb Fe^{59} through their roots from nutrient solution. Eleven cuttings were provided with 80 ml of solution containing 26.9 uc Fe^{59} (concentration 340 uc/l). They remained in this solution for 72 hours in full sunlight on a greenhouse bench. The roots were

washed by dipping in a container of water and then flushing under running tap water. The rooted cuttings were then potted in clay pots filled with number four quartz sand, assigned to two treatments with five replications in a completely randomized design.

One treatment consisted of a base solution of Twigg and Link's (128) azalea solution of pH 5.5. The other treatment received nutrient solution with sufficient calcium bicarbonate (obtained by aerating a calcium carbonate suspension with carbon dioxide) to maintain pH 7. The treatments were continued for twelve weeks.

At the termination of the experiment, the plants were segmented into: (a) leaves of shoots which developed from apical buds that were present at the time of Fe^{59} absorption, (b) stems of the new shoots, (c) leaves which were present on the cuttings, (d) stem within the zone of leaf attachment of the original cutting, (e) basal stem, and (f) roots. These tissue segments were dried and their radioactivity determined. Autoradiograms were prepared of representative plants of each treatment. Photographs were made of the mounted plants and of the x-ray film image of those plants whose autoradiograms were selected for presentation.

Foliar Absorption of Iron

The method of treatment in the series of experiments on foliar absorption was similar to that described by Jyung (74). Absorption was determined on detached leaves with the blade placed in a designated treatment solution contained in a Petri dish (50 ml) and held in a submerged position with a glass rod support. The cut end of the

petiole was coated with lanolin and supported on a glass rod of large enough diameter to keep the end of the petiole out of the treatment solution. During the absorption period, the leaves were illuminated on a laboratory bench with fluorescent lamps (600 f.c.), and the air and solution temperatures were maintained at 20° C. The top of the dish was covered with a piece of rigid plastic which had been notched to accommodate the protruding petiole. This cover reduced evaporation and consequent change in salt concentration for the solutions.

The leaves were removed from the treatment solutions at the end of the absorption period and washed in a standardized manner. Each leaf was washed in a separate series of three containers of wash solution. The first solution was 50 ml of distilled water containing one drop of Tween-20. The second was a 0.06 millimolar solution of Na-EDTA. The third was distilled water. The washing was accomplished by dipping the blade in the wash solution and removing it, repeating ten times. In addition, the leaf was left to soak in the chelate solution for one hour. Before changing the leaf from one solution to the next the excess solution on the leaf was drained onto absorbant tissue paper. The leaf was blotted dry between absorbant tissues at the end of the third wash.

To provide an area basis for expressing absorption, the leaf area was determined by tracing its outline and measuring the enclosed area with a planimeter. In most cases an alternate method was used, wherein a plug of 2.27 square centimeters in area was removed with a cork borer. The sample was then dried at 70° C.

Radioactivity of the whole treated portion of the leaf blade was determined by placing the dried samples in paper cups and using a

Geiger-Mueller tube with a VersaMatic Scaler. That of the sample plugs was determined by placing the dried plugs on stainless steel planchets under a gas-flow Geiger-Mueller tube fitted with a mica membrane window and employing the VersaMatic Scaler.

Experiment XI: Effect of Age of the Leaf on Rate of Foliar Absorption of Iron

In the development of chlorosis the first leaves to be affected are those being formed in the terminal meristems, and when chlorosis is corrected by re-establishing an iron supply in the nutrient medium, the leaves expanding from those meristems develop normal chlorophyll content. Mature, chlorotic leaves on a lower position of the stem do not recover. If this is due to their not receiving a sufficient amount of the iron being translocated through the xylem, and not due to an inability to utilize that iron in chlorophyll synthesis, then by supplying iron by foliar application to those leaves, their recovery could be induced. The first experiment relating to foliar absorption was designed to examine the question of the effect of the age of the leaf on its ability to absorb foliar-applied iron.

Chrysanthemum plants, cv. Yellow Delaware, which had been grown in a complete nutrient solution for six weeks were used. Leaves of different physiological age were selected as follows: (a) young newly expanded leaves from near the growing point, (b) mature fully expanded leaves from the central portion of the stem (six to eight nodes from the apex), and (c) old leaves from the base of the shoot. All leaves appeared healthy and no chlorosis was present.

Iron absorption from a solution containing 0.3 millimolar ferric chloride labeled with 10 uc Fe^{59} as ferric chloride plus 0.01% Tween-20 at pH 3.2 was observed. No precipitation of iron from the treatment solution occurred during the duration of the absorption period. Furthermore, no change in pH occurred, so a buffered system was considered unnecessary and was not used. The radioactivity of this solution was 617 cpm/umole of iron, as determined on the same instruments used to determine radioactivity of the leaf samples. The absorption period was 24 hours, 15 minutes.

A completely randomized design with five replications was used. The absorption rate for each sample was determined in terms of mumole iron per square centimeter per hour.

Experiment XII: Effect of Chlorotic Condition on Rate of Foliar Absorption of Iron

Effects of pre-treatment to induce chlorosis on the capacity of the leaves to absorb foliar-applied iron was next determined. Chrysanthemum plants of the cultivar, Legal Tender, were grown under the following nutritional environments:

(a) pH 5.5, no lime, 2 ppm iron, as ferric ammonium citrate

(b) pH 5.5, no lime, no iron

(c) pH 7.0, high lime, 2 ppm iron as ferric ammonium citrate

These conditions were maintained in aerated nutrient solution of Hoagland's number one solution in the same manner as described in earlier experiments. The plants under "a" were green and vigorous, those under "b" and "c" were slightly chlorotic and slightly stunted at the time that leaves were utilized for the foliar absorption studies.

One leaf of comparable physiological age was selected from each of five plants grown on each of the above treatments. The selected leaves were free of mechanical injury, insect or disease infestation and were selected from among those which had expanded while the plants were subjected to differential nutritional conditions.

The five leaves of each treatment were allowed to absorb radioiron according to the detached-leaf method described previously. The treatment solution in this case was 0.3mM ferric chloride in 0.01% Tween-20, deionized water, containing $Fe^{59}Cl_3$ at 20 uc/l. Forty milliliters of this solution was provided in each Petri dish. The absorption period was 18 hours, 20 minutes with continuous light.

A completely randomized design with five replications was used.

Experiment XIII: Effects of pH, Sodium, and Bicarbonate on the Rate of Absorption of Iron

The following treatments were employed to determine the effects of solution pH, sodium concentration and bicarbonate concentration on the rate of foliar absorption of iron by chrysanthemum.

	pH	sodium (mM)	bicarbonate (mM)	pH control
(a)	5	0	0	(Tris*)
(b)	5	3	0	(sodium acetate)
(c)	7	0	0	(Tris)
(d)	7	3	0	(sodium acetate/Tris)
(e)	7	3	3	(sodium carbonate)

* Tris is tris-hydroxy, methoxy methane

Green leaves of the cultivar, Legal Tender, were allowed to absorb iron in the above solutions at a concentration of 0.3mM as ferric chloride labelled with $Fe^{59}Cl_3$ at 24 uc/liter. The period of absorption was 4.33 hours under conditions of continuous illumination of 600 f.c. and temperature of 28° C. Leaf disks were removed, dried, and their radioactivity determined by counting in a scintillation well using a VersaMatic counter.

Five replications were provided in a completely randomized design.

Experiment XIV: Effect of Chelation on Stability of Iron in Foliar Treatment Solutions containing Bicarbonate

The effects of form of iron on rate of absorption by chrysanthemum from solutions of high bicarbonate and high pH was next determined by observing the absorption of Fe^{59} from the following solutions:

	pH	sodium (mM)	Bicarbonate (mM)	iron (form)	pH Control
(a)	5	3.0	0	$FeCl_3$	(sodium acetate)
(b)	7	3.0	3.0	$FeCl_3$	(sodium carbonate)
(c)	7	3.2	3.2	FeAC*	(sodium carbonate)
(d)	7	3.6	3.6	FeEDTA**	(sodium carbonate)

* FeAC is ferric ammonium citrate

** FeEDTA is ferric ethylene diamine tetraacetate

Radioiron as $Fe^{59}Cl_3$ was added to give a concentration of 2.4 cpm/mumole of iron, and where chelation was desired the chelating agent (ammonium citrate or ethylene diamine tetraacetate)was added to give 1.5 moles for each mole of iron. Tween-20 at 0.01% was included in each treatment.

Forty-milliliter aliquots of each treatment solution were allowed to stand in a Petri dish under conditions typical of an absorption experiment except without a leaf. At the prescribed time these aliquots were transferred to paper cups, and, after the precipitate had settled to the bottom, five one-milliliter aliquots were taken for determination of radioactivity.

The treatments were replicated five times in a completely randomized design. The absorption period was 17 hours, 15 minutes.

Experiment XV: Effect of Chelation on Rate of Foliar Absorption from Solutions of Low pH.

This experiment was conducted to determine the absorption rate of chelated iron by leaves at pH 3.0 and in a solution devoid of sodium or other salts.

Radioiron was chelated with ammonium citrate and EDTA and its absorption contrasted with that of non-chelated radioiron ($FeCl_3$). The iron concentration was 0.3 millimolar in deionized water containing 0.01% Tween-20. The method of treatment was the same as for the other detached-leaf experiments. The stability of iron in the treatment solutions was determined by removing one-milliliter aliquots from a 40 ml aliquot of each treatment solution that was allowed to stand in a Petri dish without a leaf during the course of the experiment. The radioactivity of each aliquot taken upon terminating the experiment was compared to the activity of the initial aliquot removed at the beginning of the experiment, and the percentage of iron remaining in solution was calculated.

Leaves were selected from plants, cv. Legal Tender, which had been grown for a few days under low-iron conditions and were showing slight chlorosis. Five replications were provided in a completely randomized design. The absorption period was 14 hours.

Experiment XVI: Effect of pH on Foliar Absorption of Iron

To examine more fully the influence of pH in the absence of bicarbonate, the absorption of Fe^{59} by green chrysanthemum (cv. Legal Tender) leaves was followed from solutions at pH 3, 4, 5, 6, 7, and 7.8 using Tris (tris-hydroxy, methoxy methane). The iron was not chelated so that differences in stability at the given pH value could be observed.

Five replications were provided in a completely randomized design. Absorption was allowed to proceed for 16 hours under continuous illumination at 600 foot-candles and a constant temperature of 29° C.

High Lime and Phosphate Effects

Experiment XVII: Effects of High Phosphate Concentration

In the first experiment of this series, the effects of increasing concentrations of soluble phosphate in the root medium on the absorption of iron and maintenance of leaf chlorophyll were studied. Four phosphate levels were established using Hoagland's number one solution as the base: (a) 0.5 mM, (b) 1.0 mM, (c) 2.0 mM, and (d) 4.0 mM as KH_2PO_4. Potassium chloride was added in sufficient quantities to treatments

(a), (b), and (c) to provide equal concentration of potassium. Iron was supplied in all treatments as ferric ammonium citrate at a rate of 2 ppm iron. Minor elements were supplied by adding Hoagland's "A" solution.

Two cuttings of Chrysanthemum morifolium, cv. Mermaid, were grown in each two-gallon crock with pinching and pruning to three stems for eight weeks under a photoperiod of 16 hours. At the end of the experiment, leaf and root samples were collected, washed, and prepared for phosphorus and iron analysis.

The tissue samples were analyzed for phosphorus by the ammonium molybdate method and for iron by the o-phenanthroline method (5). The extract for both analyses was prepared as specified for phosphorus analysis.

Experiment XVIII: Effects of Various Forms of Orthophosphates at High Concentration on Growth, Chlorosis, and Tissue Accumulation of Iron and Phosphorus by Two Cultivars of Chrysanthemum.

The effects of the form of phosphate on growth, chlorosis, and iron and phosphorus absorption were studied.

Four treatments were provided as follows:

(a) 0.5mM phosphate as KH_2PO_4

(b) 4.0mM phosphate as $(NH_4)H_2PO_4$

(c) 4.0mM phosphate as KH_2PO_4

(d) 4.0mM phosphate as K_2HPO_4

The base solution was Hoagland's number one with Hoagland's "A" solution for minor elements. Iron was supplied at 2 ppm as ferric ammonium citrate.

The nutrient solutions were changed each week, and the pH of each was checked daily.

Two cultivars of Chrysanthemum, cv. Oregon (chlorosis-susceptible) and Yellow Delaware (resistant) were selected as test material. Two plants of each cultivar were grown in each crock. Four replications were provided in a split plot design with form of phosphate as main plot and assigned in a randomized complete block design, and with cultivar as sub-plot.

The plants were soft pinched, pruned to three stems, and grown for seven weeks under a 16-hour photoperiod. The plants were rated at the time of harvest on the basis of chlorosis, using the relative scale described earlier. Leaves produced under the treatment, and roots were removed and analyzed for phosphorus and iron. Leaf and root dry weights were determined and used as criteria of growth.

Experiment XIX: Effects of High Lime, High Phosphate, and Their Combined Effects on Growth, Chlorosis and Tissue Accumulations of Iron and Phosphorus in Chrysanthemum

The individual and combined effects of high phosphate and high bicarbonate on growth, the development of chlorosis, and iron uptake and distribution were studied in this experiment by providing the

following treatments:

(a) 0 bicarbonate, 0.5 mM phosphate

(b) 0 bicarbonate, 2.0 mM phosphate

(c) 3 mM bicarbonate, 0.5 mM phosphate

(d) 3 mM bicarbonate, 2.0 mM phosphate

The low level of phosphate was 0.5 millimolar monobasic potassium phosphate, which was the level of that salt in the base solution (Hoagland's number one). The high level was obtained by adding 1.5 millimoles dibasic potassium phosphate to the base concentration of 0.5 mM monobasic potassium phosphate. The low bicarbonate was essentially zero, since no carbonates or bicarbonates were added and the solutions were aerated with air. High bicarbonate was obtained by adding potassium carbonate to yield a concentration of 0.75 millimolar plus excess solid calcium carbonate and aerating with 2% carbon dioxide. An equal concentration of potassium was maintained in all treatments by adding potassium chloride as necessary. Ammonium sulfate was added to the low bicarbonate, low phosphate treatment for a concentration of 0.5 millimolar to maintain a solution pH of 5.5.

Four plants of the cultivar, Legal Tender, were grown in each crock with four replications in a randomized complete block design. They were soft pinched after two weeks and pruned to four branches. After eight weeks the plants were rated on the basis of chlorosis and harvested. Stem length of the lateral shoots produced under treatment, and leaf and root dry weights were determined and used as criteria of growth. The leaf and root tissues were analyzed for iron and phosphorus.

Experiment XX: Effects of Lime, Phosphate, and Iron Chelation on Chlorosis and Tissue Accumulation of Iron and Phosphorus

A comparison was made between ferric ammonium citrate and ferric EDTA in the presence of low and/or high levels of bicarbonate and phosphate. Plants were subjected to the following treatments:

(a) 0 bicarbonate, 0.5 mM phosphate, ferric ammonium citrate (2 ppm Fe)

(b) 0 bicarbonate, 0.5 phosphate, ferric EDTA (2 ppm Fe)

(c) 0 bicarbonate, 2.0 mM phosphate, ferric ammonium citrate (2 ppm Fe)

(d) 0 bicarbonate, 2.0 mM phosphate, ferric EDTA (2 ppm Fe)

(e) 3 mM HCO_3, 0.5 mM phosphate, ferric ammonium citrate (2 ppm Fe)

(f) 3 mM HCO_3, 0.5 mM phosphate, ferric EDTA (2 ppm Fe)

(g) 3 mM HCO_3, 2.0 mM phosphate, ferric ammonium citrate (2 ppm Fe)

(h) 3 mM HCO_3, 2.0 mM phosphate, ferric EDTA (2 ppm Fe)

A modified nutrient solution was used in this experiment so that the pH of the low bicarbonate, low phosphate treatment would be 5.0 or lower, while providing the same base solution for all treatments. The salts used and their concentration in the base nutrient solution were as follows:

0.5 mM monammonium phosphate

1.0 mM ammonium nitrate

2.0 mM calcium nitrate

1.0 mM magnesium sulfate

0.5 ml Hoagland's "A" per liter (minor element)

Potassium was supplied as the phosphate, carbonate, or choloride salts as appropriate in establishing the treatment conditions.

The low bicarbonate treatments received no carbonate or bicarbonate salts and were aerated with compressed air. High bicarbonate treatments received potassium carbonate at 0.75 mM and excess solid calcium carbonate and were aerated with 2% carbon dioxide in air. The low phosphate treatments had dibasic potassium phosphate added for a concentration of 1.5 millimolar, which with the 0.5 mM ammonium phosphate gave a total orthophosphate concentration of 2.0 mM. Potassium chloride was added as necessary to equate the concentration of potassium in all treatments at 4.5 mM.

Four plants of C. morifolium, cv. Legal Tender, were grown in each crock. They were soft pinched after one week and pruned to three lateral shoots, and grown under treatments for seven weeks. Four replications were provided in a randomized complete block design.

Leaves were removed from the lateral shoots and roots from the stem when the experiment was terminated. The tissue samples were washed and analyzed for phosphorus and iron.

Experiment XXI: Effects of Form and Level of Iron Applied to the Soil on Growth, Chlorosis, and Iron Concentration in Leaves of Chrysanthemum

The effects of high soluble phosphates and high bicarbonate irrigations on the availability of iron of different forms from a greenhouse soil was next determined. A soil mix of equal parts of loam, coarse sand, and Michigan peat moss was used. This soil mix was characterized by low fertility and a pH of 6.2.

Iron was supplied as either ferrous sulfate, glassy frit, or EDTA-Fe. Ferrous sulfate was used at 65, 130, 195 mg per four-inch

pot. The glassy frit (8% iron) was used at 2, 4, and 6 g per four-inch pot. Those materials were applied by mixing weighed portions with batches of soil of sufficient volume to fill a pot. EDTA-Fe was applied at rates of 5, 10, and 15 mg per four-inch pot by dissolving the appropriate quantity in 100 ml distilled water and applying the solution after the plant was established in the pot of soil.

Two cultivars of C. morifolium were used, Oregon and Yellow Delaware. The plants were grown single-stem in four-inch clay pots (one plant per pot) under a 16-hour photoperiod in a greenhouse at 60° minimum night temperature for 12 weeks. Four replications of each treatment and cultivar were provided in a randomized complete block design.

The plants were fertilized with a mixture of 2.5 g of 25-0-15-5Ca-3 Mg plus 2.5g of monobasic potassium phosphate per liter of tap water. This solution was applied at the rate 150 ml per pot at the end of the second and sixth weeks. In the 10th and 11th weeks, half-strength Hoagland's number one solution was applied. Tap water containing moderately high concentrations of bicarbonates (pH 7.4 and electrical conductivity of 45×10^{-5} mho/cm) was used for irrigating.

Frequent checks of soil pH were made by collecting leachate after applying distilled water and determining its pH. Soil leachate pH greater than 7.0 was maintained in all treatments, and by the end of the experiment it was 7.8 to 8.4. Soil pH determined by preparing a 1:2 soil-to-water extract after the plants were removed closely agreed with that of the leachate determined just before termination.

When the experiment was terminated, all leaves that had developed under the treatments (20 to 24 per plant) were removed, washed, dried, weighed, and analyzed for iron and phosphorus.

Experiment XXII: Effects of Bicarbonates and Phosphate on the Availability of Native Iron of a Greenhouse Soil

The effects of high bicarbonate and high soluble phosphate applications on the availability of naturally occurring soil iron was established. A single plant of C. morifolium, cv. Legal Tender was grown in a four-inch clay pot filled with soil identical to that used in the preceding experiment. This soil contained six ppm iron and five ppm phosphorus as indicated by the Spurway test (115).

Five treatments were established as follows:

Phosphate	Major Fertilizer	Irrigation
(a) native (5 ppm)	23-0-22	distilled water
(b) superphosphate	25-0-15-5-3	distilled water
(c) superphosphate	25-0-15-5-3	tap water
(d) ammonium phosphate monopotassium phosphate	12-31-14 calcium nitrate	distilled water
(e) ammonium phosphate monopotassium phosphate	12-31-14 calcium nitrate	tap water

Superphosphate was used at a rate of 1.5 grams per four-inch pot, which is equivalent to five pounds per 100 square feet, 6 inches deep. The soluble fertilizer, 12-31-14 and calcium nitrate were applied at a concentration of one-half ounce each in three gallons water. The material 25-0-15-5-3 (5% calcium, 3% magnesium) was applied at a concentration of 2.5g/l water. Approximately 150 ml of fertilizer solution was applied to each pot twice a month. Soil leachate was pH 6.0 to 6.5 in treatment "a" and 7.0 to 7.5 in the remaining four treatments.

Five replications were provided in a randomized complete block design. The experiment was conducted for a 10-week period. The plants

were grown single-stem under 16-hour photoperiods and at 65° F. minimum-night temperature.

At the end of the experiment, leaves were collected from the upper third of the shoot and were washed, dried, weighed, and analyzed for iron and phosphorus.

RESULTS

Experiment I: Stability of Iron in Non-aerated Solution

The rapid precipitation of iron, when supplied as ferric chloride, is indicated in Figure 1. Precipitation was very rapid at pH 7 or pH 8, with 50% of the initial concentration remaining in solution after only five hours. Precipitation at pH 6 was alos rapid, but 50% of initial iron was still in solution after five days and 12% remained after 25 days. At pH 5, ferric ions were more stable and 50% of the initial concentration was in solution after seven days, with 35% remaining at the end of 25 days. At pH 4, an equilibrium apparently was reached after eight days and 70% of the initial concentration remained in solution thereafter until the 25th day.

Experiment II: Influence of Lime and Ammonium Citrate on Iron Stability in Aerated Nutrient Solution

The rate of precipitation of iron when supplied as ferric chloride in aerated nutrient solutions was rapid, even at pH 5 (Table 1). After 24 hours, less than 1% of the original iron, of that form, remained in solution, there being no difference between the high-lime and low-lime treatments.

In the presence of ammonium citrate, iron remained in solution to a greater extent but was subject to more rapid precipitation in the presence of dissolved calicum bicarbonate than in its absence.

The rate of precipitation of ferric iron in aerated Hoagland's nutrient solution can be followed in Figure 2. Only traces of iron

Figure 1. Solubility of Fe^{59} ($Fe^{59}Cl_3$) in non-aerated, half-strength Hoagland's number one nutrient solution adjusted to various pH levels. Concentration of iron is expressed as per cent of original concentration remaining in solution at each time of observation.

FIGURE 1

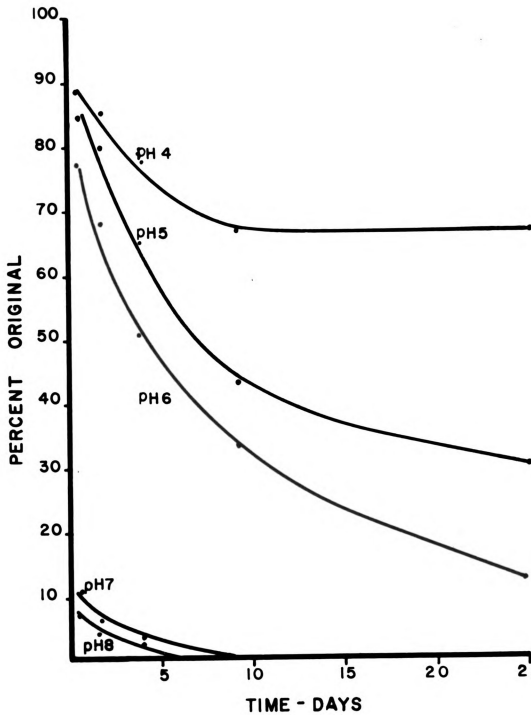

100
90
80
70
60
50
40
30
20
10
PERCENT ORIGINAL
pH 4
pH5
pH6
pH7
pH8
5
10
15
20
25
TIME - DAYS

Table 1.-Effects of Ammonium Citrate on the Stability of Iron Solutions Containing Bicarbonate.

Treatment			Fe Concentration After 20 Hours	
$NaHCO_3$	pH	Iron	Concentration	Per cent original
(ml.)		(form)	(ppm.)	(%)
0	5.0	$FeCl_3$	0.003[a]	0.7[a]
0	5.0	FeAC*	2.193[c]	73.11[c]
3	7.0	$FeCl_3$	0.027[a]	0.9[a]
3	7.0	FeAC	1.583[b]	52.78[b]

* FeAC is ferric ammonium citrate.

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence as determined by Duncan's Multiple Range Test.

remained in solution at the end of five hours in either the low-lime, pH 5.0 treatment or in the high-lime, pH 7.0 treatment. Iron appeared to be more stable in the low-lime, pH 5 treatment, but this could hardly be considered to be of significance in plant nutrition in view of the short time that it did remain in solution.

Experiment III: Influence of Lime and Phosphate on the Stability of Iron as Ferric Ammonium Citrate in Aerated Nutrient Solution

Iron as ferric ammonium citrate remained in solution in aerated nutrient solution in which chrysanthemum plants were growing for less than 48 hours (Table 2). In the presence of about 3 mM of calcium bicarbonate, either with 0.5 mM or 2.0 mM of orthophosphate, only about 50% of the supplied iron remained in solution for two hours. At pH 6.8 with 2.0 mM potassium phosphate, as much iron remained in solution for two hours as in the low pH treatment. At the end of 24 hours, less than 25% of the original iron remained in solution, and after 48 hours no iron was detected in solution in any treatment.

Examination of the precipitate accumulating on the bottom of the crock established that considerable quantities of iron precipitated from all the high pH treatments but not from the low pH treatment. This suggested the possibility that absorption of iron by the plants in solutions of low pH accounted for the removal of iron from those solutions; whereas precipitation was largely responsible for its removal from solutions of high pH. Growth response and the development of chlorosis further substantiated these observations.

Figure 2. Solubility of Fe^{59} ($Fe^{59}Cl_3$) in aerated half-strength Hoagland's number one solution in presence of high (3 mM $CaCO_3$, pH 7.0) or low (no $CaCO_3$, pH 5.5) levels of bicarbonate.

FIGURE 2

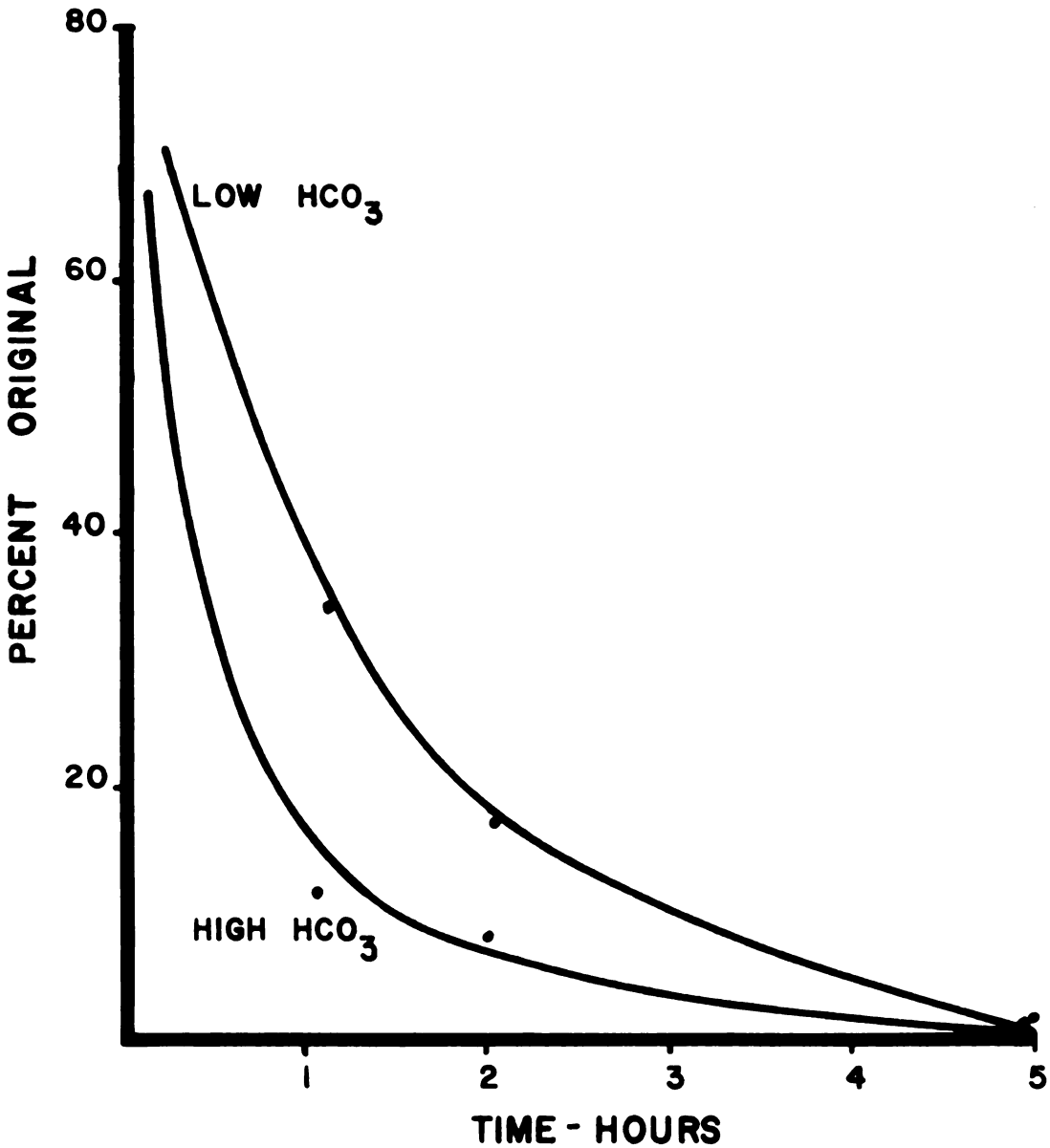

80
60
40
20
PERCENT ORIGINAL
LOW HCO_3
HIGH HCO_3
1
2
3
4
5
TIME - HOURS

Table 2. -Effects of Lime and Phosphate on the Stability of Iron in Aerated Hoagland's Number One Nutrient Solution.

Treatment			Iron Concentration After Times Indicated		
HCO_3	P	pH	2 hrs	24 hrs	48 hrs
(me.)	(me.)		(ppm)	(ppm)	(ppm)
0	0.5	5.5	2.04[a]	0.16[a]	0
3	0.5	7.2	0.82[b]	0.25[b]	0
0	2.0	6.8	1.30[a]	0.62[c]	0
3	2.0	7.2	1.13[c]	0.19[ab]	0

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence as determined by Duncan's Multiple Range Test.

Experiment IV: Effects of High Lime on Growth and Chlorosis of Chrysanthemum Plants

When chrysanthemum plants were grown in aerated nutrient solution, chlorosis resulted from both the high-lime treatment receiving iron and the low-lime treatment receiving no iron (Table 3). Associated with the development of chlorosis was the development of necrosis along the margins of the leaves which in some cases resulted in death of the entire leaf. Root growth as measured by dry weight was not affected by any of the treatment, but leaf growth was reduced in proportion to the severity of chlorosis. The accumulation of iron in leaf tissue was reduced equally in both treatments resulting in chlorosis.

Experiment V: Effects of High Lime and Iron Chelation on Chlorosis and the Mineral Composition of Chrysanthemum

Supplying iron as ferric ethylenediamine tetraacetate prevented chlorosis in the presence of high concentration of dissolved calcium carbonate. It resulted in an iron concentration in the leaf tissues which was equally as high as the low-lime, pH 5.5 treatment and which was significantly higher than the high-lime treatment receiving ferric ammonium citrate (Table 4).

The treatment which resulted in the most severe degree of chlorosis (high-lime with ferric ammonium citrate) resulted in the highest concentration of manganese in the leaf tissue.

In the low-lime, pH 5.5 treatments, the absence of iron resulted in a higher calcium concentration in leaf tissue, being as high as that in the two high-lime, pH 7.0 treatments.

Table 3.-Effects of Lime on Growth, Chlorosis and Iron Accumulation in Chrysanthemum Plants.

Treatment			Chlorosis	Necrosis	Tissue Dry Wt.		Leaf-Fe
HCO_3	pH	Fe			root	leaf	dry wt.
(ml.)		(ppm.)	(rel*)	(rel*)	(g)	(g)	(ppm)
0	5.5	2	1.3	1.0	2.15[a]	4.16[a]	59.8[a]
0	5.5	0	2.0	1.3	1.88[a]	2.96[b]	43.5[b]
3	7.0	2	3.8	3.8	1.99[a]	1.64[c]	34.5[b]

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence as determined by Duncan's Multiple Range Test.

* Degree of chlorosis and degree of necrosis are indicated by relative rating scales.

Table 4.-Effects of High Lime and Iron Chelation on Chlorosis and Mineral Composition of Chrysanthemum Leaves.

Treatment				Chlorosis	Leaf Mineral Composition				
HCO_3	pH	Iron			Fe	Mn	Ca	Mg	P
(mM)		(ppm)	(form*)	(rel**)	(ppm)	(ppm)	(%)	(%)	(%)
0	5.5	2	FeAC	1.3	66[a]	215[a]	1.20[b]	0.337[a]	0.831[ab]
0	5.5	0	-	2.0	31[b]	223[a]	2.21[a]	0.393[a]	0.825[ab]
3	7.0	2	FeAC	3.3	29[b]	521[b]	1.74[ab]	0.500[b]	0.935[a]
3	7.0	2	FeEDTA	1.3	52[a]	204[a]	1.55[ab]	0.347[a]	0.793[b]

* FeAC is ferric ammonium citrate; FeEDTA is ferric ethylenediamine tetraacetate.

** Degree of chlorosis is indicated by relative rating scale.

a Values with the same superscript letter are not significantly different at the 5% level of confidence as determined by Duncan's Multiple Range Test.

Magnesium concentration in leaf tissue was significantly higher in the high-lime, pH 7.0, ferric-ammonium-citrate treatment than in any of the others.

In the high-lime, pH 7.0 treatments, the use of ferric ethylene-diamine tetraacetate resulted in a significantly lower phosphorus concentration in leaf tissue relative to that from the use of ferric ammonium citrate. Furthermore, the highest phosphorus levels were found in leaves with the most severe chlorosis, although not significantly higher than in those of less severe chlorosis resulting from the low-lime, pH 5.5 treatments.

Experiment VI: The effects of Bicarbonates in Irrigation Water on Growth, Chlorosis, and Mineral Composition of Azaleas

From leaf dry weight data of Table 5, it can be inferred that growth was not significantly reduced by any of the treatments during the first growing season under treatment.

The plants that had been grown under low-pH irrigation were not chlorotic, as indicated by the relative scale. When phosphoric acid was added to neutralize tap water, chlorosis developed earlier and more extensively than in any other treatment. However, sulfuric acid was effective in maintaining a low soil pH and preventing chlorosis.

Iron concentration in the leaf tissue was highest in plants receiving distilled water or tap water neutralized with sulfuric acid. Green leaves had a higher concentration of iron than did chlorotic ones.

The minor elements, manganese, boron, and zinc were of lower concentration in leaves of plants under treatment with calcium or

Table 5.-Effects of Bicarbonates in Irrigation Water on Growth, Chlorosis, and Leaf Mineral Composition of Hybrid Hollis Azaleas.

Treatment		Leaf Tissue		Leaf Mineral Composition				
Water	pH	Chlo.	Dry wt.	Fe	Mn	B	Zn	P
		(rel*)	(g)	(ppm)	(ppm)	(ppm)	(ppm)	(%)
Distilled	6.0	1.0	1.12[a]	23.0[d]	45[b]	83.3[e]	44[a]	0.264[b]
Tap + H_2SO_4	5.0	1.0	1.14[a]	21.3[c]	86[a]	82.4[a]	39[a]	.507[d]
Tap + H_3PO_4	6.5	3.3	0.90[a]	12.3[a]	16[c]	66.2[bc]	23[bc]	.317[c]
Tap	7.0	1.7	1.32[a]	11.7[a]	43[b]	73.0[ab]	24[bc]	.166[a]
$Ca(HCO_3)_2$	6.8	1.7	1.25[a]	18.3[b]	23[c]	57.7[c]	27[b]	.150[a]
$NaHCO_3$	7.2	2.3	1.01[a]	15.7[b]	13[c]	57.1[c]	17[c]	.141[a]

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence as determined by Duncan's Multiple Range Test.

* Degree of chlorosis is indicated by relative rating scale.

Cont'd Table 5.

Treatment		Leaf Mineral Composition				
Water	pH	Na	Mg	K	Ca	K:Ca
		(ppm)	(%)	(%)	(%)	(ratio)
Distilled	6.0	60[a]	0.373[a]	0.660[c]	0.723[a]	0.903[a]
Tap + H_2SO_4	5.0	117[b]	.293[cd]	.693[b]	.733[a]	.947[a]
Tap + H_3PO_4	6.5	157[d]	.310[bc]	.553[d]	.620[a]	.947[a]
Tap	7.0	84[ac]	.370[ab]	.613[c]	1.086[b]	.607[b]
$Ca(HCO_3)_2$	6.8	93[bc]	.237[d]	.453[e]	1.010[b]	.457[b]
$NaHCO_3$	7.2	185[e]	.170[e]	.780[a]	0.293[c]	2.697[c]

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence as determined by Duncan's Multiple Range Test.

sodium bicarbonate or tap water with phosphoric acid than in those under distilled water or tap water with sulfuric acid. Tap water with no acid resulted in higher concentrations of manganese and boron, due possibly to the presence of these elements in the tap water.

Phosphorus accumulation in leaf tissue was lower in the high bicarbonate treatments than in the low bicarbonate ones, regardless of cation associated with the bicarbonate. Surprisingly, the highest concentration in leaf tissue of that element occurred in plants under sulfuric acid rather than phosphoric acid treatment. The phosphoric acid irrigation treatment did result in higher leaf-phosphorus than did the distilled water treatment.

The concentration of basic cations in leaf tissue was affected by the cation level in irrigation water more than by the bicarbonate ion level (Table 5). Sodium accumulated to the greatest degree in leaves of plants under the sodium bicarbonate treatment and in the two tap water treatments which received acid to lower soil pH. The lowest concentration resulted from distilled water irrigation, although it was not significantly lower than that from the tap water without acid.

Accumulation of magnesium was not strongly affected by any of the treatments except to be slightly reduced under calcium bicarbonate and greatly reduced under sodium bicarbonate.

Potassium accumulation was highest in the plants under sodium bicarbonate treatment and lowest under calcium bicarbonate. The opposite was observed for calcium accumulation, and this resulted in a much higher K:Ca ratio in plants under sodium bicarbonate

irrigations than in those under calcium bicarbonate or tap water, with intermediate ratios resulting from distilled water or tap water neutralized with either sulfuric or phosphoric acid.

The leaf composition of alkali cations was not consistently affected by bicarbonate treatment.

After the second season of growth under the same irrigation treatments, dry weight increase of both primary stem and leaf was suppressed in those treatments that resulted in chlorosis, viz., tap water with phosphoric acid, tap water without acid, and calcium and sodium bicarbonate (Table 6). The dry weights of secondary stems was not affected by these treatments. The sodium ion in combination with bicarbonate reduced growth significantly compared to the calcium ion or the mixture of sodium, calcium, and magnesium ions of tap water.

Phosphorus concentration in the plant tissues examined was not affected by these treatments. Of particular interest was the lack of evidence that phosphorus accumulated in the stem tissues under those treatments that resulted in chlorosis (Table 7).

Iron concentration in these tissues also was not consistently affected by the bicarbonate level in irrigation water (Table 7). Accumulation in the primary stem tissue was highest in the plants under distilled water, tap water with phosphoric acid, and sodium bicarbonate treatments. The concentration of iron in leaf tissue was actually highest in the tap water treatment, which resulted in chlorotic leaves. This agrees with many reported observations that chlorotic leaves can have as high an iron concentration as green ones.

Table 6.-Effects of Bicarbonates in the Irrigation Water on Growth and Chlorosis of Mollis Azalea After the Second Season of Treatment.

Treatment		Chlorosis	Plant Tissue Dry Weights		
Water	pH		Primary Stem	Secondary Stem	Leaf
		(rel*)	(g)	(g)	(g)
Distilled	5.5	1.0	4.13^{a}	2.36^{a}	5.39^{a}
Tap + H_2SO_4	5.5	1.0	3.55^{ab}	2.09^{a}	5.00^{a}
Tap + H_3PO_4	6.0	3.3	2.55^{abc}	2.44^{a}	2.23^{b}
Tap	7.5	4.0	1.45^{c}	1.50^{a}	1.00^{b}
$Ca(HCO_3)_2$	7.2	4.0	1.93^{bc}	1.97^{a}	2.49^{b}
$NaHCO_3$	7.3	2.3	1.51^{c}	1.64^{a}	2.30^{b}

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence according to Duncan's Multiple Range Test.

* Degree of chlorosis is indicated by relative rating scale.

Table 7.-Effects of Bicarbonates in Irrigation Water on Chlorosis and Concentration of Iron and Phosphorus in Leaf Tissue of Mollis Azalea After the Second Season of Treatment.

Treatment No.	Water	pH	Chlorosis	Phosphorus Concentration*		
				Primary Stem	Secondary Stem	Leaf
			(rel)	(%)	(%)	(%)
1.	Distilled	5.5	1.0	0.126[a]	0.128[a]	0.240[b]
2.	Tap + H_2SO_4	5.5	1.0	.094[a]	.112[a]	.278[b]
3.	Tap + H_3PO_4	6.0	3.3	.130[a]	.098[a]	.419[b]
4.	Tap	7.5	4.0	.096[a]	.116[a]	.411[b]
5.	$Ca(HCO_3)_2$	7.2	4.0	.146[a]	.126[a]	.190[b]
6.	$NaHCO_3$	7.3	2.3	.131[a]	.110[a]	.207[b]

[a] Values with the same superscript letter are not significantly different as determined by Duncan's Multiple Range Test at the 5% level of confidence.

* No significant difference among treatments within a plant tissue segment; concentration of P within leaf was significantly higher than in either primary stem or secondary stem; no difference between primary and secondary stem.

Table 7. (Cont'd)

Treatment	Iron Concentration			Total Iron		Distribution
No.	Primary Stem	Secondary Stem	Leaf	Shoot	Leaf	Per cent in leaf*
	(ppm)	(ppm)	(ppm)	(ug)	(ug)	(%)
1.	44^{a}	25^{a}	42^{a}	400^{a}	240^{a}	60.00
2.	27^{b}	24^{a}	43^{a}	364^{a}	213^{a}	58.52
3.	43^{ab}	27^{a}	46^{a}	242^{b}	102^{b}	42.15
4.	26^{c}	21^{a}	80^{b}	149^{c}	79^{b}	53.02
5.	23^{c}	23^{a}	50^{a}	215^{bc}	126^{b}	58.60
6.	30^{abc}	23^{a}	49^{a}	189^{bc}	110^{b}	58.20

* Per cent of total shoot-iron that was found in the leaf tissue.

Since growth had been affected to such an extent, it was considered desirable to examine thedata on t he basis of the total amount of iron accumulated in the shoot portion of the plants and in the leaf tissue. There was, on the basis of these data, more iron accumulated under low-bicarbonate and low-pH conditions than under high-bicarbonate and high-pH conditions. The lowest percentage of total shoot-iron found in the leaf tissue was observed for plants under treatment with phosphoric acid in tap water, and this might be interpreted as reduced translocation to the leaves under those conditions.

Root Absorption and Distribution of Radioiron

Experiment VII: Effect of High Lime on Absorption and Distribution of Iron in Chrysanthemums

The data of Table 8 indicate that chlorotic plants which had been grown two weeks in low-lime Hoagland's solution lacking iron absorbed several fold more iron in the first 24 hour period after adding iron tagged with Fe^{59} to the root medium than did the green plants in eitherlow-lime, low-pH solutions containing iron or the chlorotic ones in high-lime, high-pH solutions also containing iron. During this first 24 hours there was no significant difference in the total amount of iron absorbed by the green plants which had received iron and the chlorotic plants which had been under the high-lime, high-pH treatment, although absorption tended to be higher by the green plants.

During the first 36 hours of root exposure to radioiron, plants

Table 8.-The Effect of Lime on Iron Absorption by Roots of *C. morifolium*, cv. Legal Tender.

Treatment				Total Fe Absorbed
HCO_3	pH	Fe	Duration	
(mM)		(ppm)	(hr)	(ug/plant)
0	5.5	2	24	5.56[a1]
0	5.5	0	24	24.47[b3]
3	7.0	2	24	0.90[a4]
0	5.5	2	36	24.56[x2]
0	5.5	0	36	29.67[x3]
3	7.0	2	36	1.09[y4]

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence according to Duncan's Multiple Range Test. Comparisons were made among treatments within each time of observation.

[1] Values with the same superscript number are not significantly different at the 5% level of confidence according to Duncan's Multiple Range Test. Comparisons were made between times within each treatment.

under the two low-lime treatments absorbed equally high amounts of iron. The chlorotic plants under high-lime conditions did not absorb any more iron during 36 hours than they did during 24 hours, as was true also of the chlorotic plants under low-lime, minus iron conditions, although the difference between amounts absorbed was great. The green plants under low-lime conditions with iron did absorb more iron during 36 hours than during 24 hours.

These observations viewed as a whole indicate that the condition of chlorosis did not predispose the plant to low capacity for iron absorption, but prior and continuing exposure to high lime conditions did.

An indication of the effects of these treatments on distribution of iron is given in Table 9, in which the percentage of total absorbed iron that was found in each tissue segment after 24 and 36 hour absorption periods are given.

The highest percentage of iron was translocated to the youngest leaves, during either period, in the chlorotic plants exposed to conditions of low lime and iron starvation. The lowest percentage was in the chlorotic plants under high-lime conditions, this percentage being significantly lower during the 36 hour observation period.

The same differences in translocation to the lower (physiologically older) leaves were also observed. There were likewise similar differences among the percentages found in the stem tissue.

The percentage of iron remaining in the root was lowest in the chlorotic plants under low-lime treatment and highest in those under the high-lime conditions (although the latter was not significantly higher than in the green plants under low-lime treatment).

Table 9.-The Effects of Lime on the Distribution of Root-Absorbed Iron in C. morifolium, cv. Legal Tender.

Treatment				Per cent Distribution			
HCO_3	pH	Fe	Duration	Upper Leaf	Lower Leaf	Stem	Root
(ml.)		(ppm)	(hrs)	(% total absorbed iron in each segment)			
0	5.5	2	24	1.74^{a1}	2.69^{a1}	4.46^{a1}	90.56^{a1}
0	5.5	0	24	19.21^{b2}	20.50^{b2}	10.54^{b2}	49.74^{b2}
3	7.0	2	24	0.37^{a3}	0.65^{a4}	2.40^{a4}	97.01^{a4}
0	5.5	2	36	5.93^{x1}	7.34^{x1}	3.31^{x1}	83.62^{x1}
0	5.5	0	36	15.07^{y2}	13.12^{y3}	6.98^{y3}	64.83^{y3}
3	7.0	2	36	0.34^{z3}	0.34^{z4}	1.42^{z4}	97.16^{z4}

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence according to Duncan's Multiple Range Test. Comparisons were made among treatments within each time of observation.

[1] Values with the same superscript number are not significantly different at the 5% level of confidence according to Duncan's Multiple Range Test. Comparisons were made between times within each treatment.

Apparently the reduced amounts of iron found in the shoot portions of plants under high-lime treatment was due to the accumulation in the root tissue, as well as to reduced absorption.

It can be concluded from these observations with respect to translocation that the condition of chlorosis did not result in reduced translocation of iron to shoot portions and young leaf tissue, but other changes within the plant as a result of exposure to high-lime treatment must have.

An examination of the data of Table 10 reveals that there was a much higher accumulation of iron on a tissue dry weight basis in roots than in other portions of the plant, regardless of treatment. The highest accumulation in any tissue was in those plants which were under low-lime, minus-iron treatment.

In the chlorotic plants under either low-lime, minus-iron or high-lime, plus iron treatment, as much iron accumulated in upper leaf tissue in 24 hours as did in 36 hours. In the green plants under low-lime treatment more iron accumulated in upper leaves in 36 hours than in 24 hours. In those green plants iron continued to accumulate in all tissue segments over the 36 hour period.

Iron accumulated in the upper leaves of the chlorotic plants under low-lime treatment to a concentration of approximately 50 ppm within the first 24 hours, and this concentration would be almost sufficient to maintain green leaf tissue, according to earlier findings.

Table 10.-The Effects of Lime on the Accumulation of Iron in Tissue Segments of *C. pentaphyllum*, cv. Legal Tender.

Treatment				Iron Accumulation			
HCO_3	pH	Fe	Duration	Upper Leaf	Lower Leaf	Stem	Root
(ml.)		(ppm.)	(hrs)	(ug Fe/g dry wt.)			
0	5.5	2	24	1.35^{a1}	0.98*	0.93^{a1}	71.44^{a1}
0	5.5	0	24	53.77^{b3}	30.58	13.90^{b3}	278.91^{b3}
3	7.0	2	24	0.05^{a4}	0.06	0.11^{a5}	14.57^{a4}
0	5.5	2	36	14.16^{a2}	8.94	2.03^{a2}	230.53^{a2}
0	5.5	0	36	48.90^{b3}	25.10	9.34^{b4}	364.49^{b3}
3	7.0	2	36	0.05^{a4}	0.04	0.10^{a5}	20.95^{a4}

a Treatment means within the same duration of treatment having the same superscript letter are not significantly different as determined by Duncan's Multiple Range Test at the 5% level of confidence.

1 Means of the same treatment contrasted between the two durations of treatment having the same superscript number are not significantly different as determined by Duncan's Multiple Range Test at the 5% level of confidence.

* Interaction between treatment and time was not significant as determined by F test with 5% level of confidence. Treatment differences averaged over both times were. Grand mean for HCO_3 - Fe was greater than those for HCO_3 + Fe and 3 HCO_3 + Fe; other contrasts were not significantly different as determined by Duncan's Multiple Range Test with 5% level of confidence.

Table 11.-The Effect of Prior Exposure to High-Lime Conditions on the Amount of Iron Absorbed Under Low-Lime Conditions by *C. morifolium*, cv. Legal Tender.

Pre-Treatment			Total Absorbed Iron
HCO_3	pH	Fe	
(ml)		(ppm)	(ug Fe/plant)
0	5.5	2	6.92[b]
0	5.5	0	8.23[a]
3	7.0	2	5.81[b]

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence according to Duncan's Multiple Range Test.

Experiment VIII: Effect of High Lime on the Absorption and Distribution of Iron by Chrysanthemum Plants after Transfer to a Low-Lime Solution

When chlorotic plants that had been grown for two weeks under either high-lime or low-lime, minus-iron conditions to induce chlorosis and green plants that had been grown in low-lime solution containing iron were allowed to absorb radioiron from solutions of low-lime and low-pH conditions, it was found that the greatest amount of iron was absorbed by the plants which were chlorotic as a result of low-lime and minus-iron pre-treatment (Table 11).

Here, as in the previous experiment, the important contrast is between the two treatments resulting in chlorosis (low-lime, minus-iron and high-lime, plus-iron). If iron-starvation chlorosis predisposes a plant to higher capacity for iron absorption than that of green plants, then it would be expected that chlorosis resulting from high lime treatment would also, unless other effects of high-lime exposure interfere. Since the amount of iron absorbed in 22 hours by the high-lime treated plants was significantly less than that of the minus-iron treated plants, then it must be concluded that the high-lime treatment did have other effects on those plants. These effects reduced the ability of the plants for some time after they were provided with a root environment of low-lime and optimum pH.

Those changes brought about by the high-lime treatment also affected the translocation of iron from roots to all segments of the shoot, as illustrated in data of Table 12.

Table 12.-The Effect of Prior Exposure to High Lime Conditions on the Distribution of Root-Absorbed Iron to Tissues of C. morifolium, cv. Legal Tender.

Pre-Treatment			Per cent Distribution			
HCO_3	pH	Fe	Upper Leaf	Lower Leaf	Stem	Root
(mM)		(ppm)	(% total absorbed iron in each segment)			
0	5.5	2	1.94[a]	0.89[a]	2.97[a]	94.20[a]
0	5.5	0	2.63[a]	1.43[b]	4.82[b]	91.11[b]
3	7.0	2	0.68[b]	0.58[a]	5.93[c]	92.72[c]

Table 13.-The Effect of Prior Exposure to High Lime Conditions on the Accumulation of Root-Absorbed Iron in Tissues of C. morifolium, cv. Legal Tender.

Pre-Treatment			Iron Accumulation			
HCO_3	pH	Fe	Upper Leaf	Lower Leaf	Stem	Root
(mM)		(ppm)	(ug Fe/g dry wt.)			
0	5.5	2	1.744[a]	0.580[a]	1.003[a]	102.02[a]
0	5.5	0	2.994[b]	1.046[b]	1.982[b]	185.10[b]
3	7.0	2	0.382[c]	0.204[a]	1.690[b]	79.78[c]

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence according to Duncan's Multiple Range Test.

There was a lower percentage of total absorbed iron translocated to both upper and lower leaf segments in the plants previously exposed to high-lime conditions than in those plants previously exposed to iron-deficient conditions (Table 12). There was less iron translocated to the upper leaves of these plants than in the green plants, which tended to be intermediate in percentage of iron translocated to the leaves.

There was a higher percentage of absorbed iron in the stems of the high-lime treated plants. There were slight differences among the percentages of iron remaining in the roots under these conditions and it is possible that the reduction in translocation to the upper leaves was the result of accumulation of iron in the conducting tissues of the stem rather than in the root tissue. The percentage of iron in the stem was highest in the high-lime treatment.

Accumulation data presented in Table 13 indicated that there was less iron absorbed and translocated by the high-lime treated plants, as expressed on the basis of tissue weight, than was by either the chlorotic plants of the iron deficient treatment or the green plants. This was particularly the case with the upper-leaf and root tissue.

Experiment IX: Effects of High Lime on Long-term Distribution of Iron in Chrysanthemums

When plants were allowed to absorb radioiron and afterward were grown under the same treatments (as in Experiment VIII) for nine weeks, it was found that considerable quantities of iron,

Table 14.-The Effects of High Lime Conditions on the Long-Term Distribution of Root-Absorbed Iron in C. morifolium, cv. Legal Tender.

Treatment			Per cent Distribution				
HCO_3	pH	Fe	New Leaf	New Stem	Old Leaf	Old Stem	Root
(mM)		(ppm)	(per cent of total in each segment)				
0	5.5	2	2.78[a]	0.48[a]	3.25[a]	4.44[a]	89.00[a]
0	5.5	0	13.15[b]	1.71[b]	6.61[b]	6.04[a]	72.49[b]
3	7.0	2	0.79[a]	0.23[a]	1.67[c]	4.91[a]	92.79[a]

which had been absorbed and remained in the root tissue initially, was translocated to new shoot tissue produced during this period. Percentages of total radioiron found in tissue segments at the termination of the experiment are recorded in Table 14. Initial distribution is not shown, since there was only a trace of radioactivity in stems and leaves after 12 hours. The radioactivity in the roots averaged 750 cpm per root.

Greater amounts of radioiron were found in the secondary leaves and stems of chlorotic plants receiving no iron during the nine weeks and maintained under conditions of low lime than in comparable tissues of chlorotic plants grown under conditions of high lime with 2 ppm iron provided or of green plants under treatment with low lime receiving stable iron. The percentage of radioiron in the primary leaves was highest in plants under the low-lime, minus-iron treatment also, which leaves were present as the young leaves near the apex of the plants during the absorption period. Differences in the percentage of iron found in the primary leaves at the end of the nine week period was considered to be due to differences in initial distribution rather than differences in translocation out of those leaves.

The difference in percentage of total radioiron in the root tissue indicates that iron was translocated from that tissue to the growing points and incorporated in new leaf and stem tissue. There tended to be an inverse relationship between the percentage of radioiron found in root tissue with that found in new leaf tissue at the termination of the experiment. There was a highly

significant difference between the percentage remaining in the roots of plants under low-lime, low-iron treatment than in roots of the high-lime treated plants which received stable iron.

Iron distribution patterns are illustrated in the autoradiograms (Figure 3-9) after two and nine weeks on designated treatments.

Exposure of the x-ray film by the radioactivity in the plants and subsequent development of the film was constant for all treatments and all times of observation. Therefore, the differences in intensity among the various tissues of a mounted specimen as well as among the plants may serve as an approximation of quantity of radioiron.

The accumulation of iron in the growing point and young leaves during the first 48 hours after the absorption period is illustrated in Figure 3. Similar patterns were observed in plants under treatment for two weeks (Figure 4, 5, and 6). There was much higher activity in the youngest leaves with very little translocation to the physiologically older leaves near the base of the plants. Furthermore, there seemed to be progressively decreasing amounts of radioiron from the youngest to the oldest leaves, an observation not well substantiated by count data of the previous experiment when an arbitrary division was made between the young and old leaves. Of further interest is the observation that essentially no differences existed among the patterns of distribution in the plants representing each treatment under study. No doubt, considerable quantities of iron had been translocated to the terminal growing points during the first 48 hours and this iron was available to

Figure 3. Initial distribution of radioiron in young chrysanthemum plant.

The autoradiogram was prepared 48 hours after absorbing $Fe^{59}Cl_3$ from half-strength Hoagland's number one solution adjusted to pH 5.5 with ammonium sulfate.

Figure 4. Distribution of radioiron after two weeks in high lime with a source of stable iron.

Autoradiogram of a chrysanthemum plant, which had absorbed Fe^{59} under identical conditions as the plant in Figure 3, and subsequently had grown for two weeks in half-strength Hoagland's number one solution at pH 7 and containing 3 millimolar calcium bicarbonate and aerated with 2% carbon dioxide in air. A source of stable iron was available to the roots.

Figure 5. Distribution of radioiron after two weeks in low lime with no source of stable iron.

Autoradiogram of a chrysanthemum plant, which had absorbed Fe^{59} under identical conditions as the plant in Figure 3, and subsequently had grown for two weeks in half-strength Hoagland's number one solution at pH 5 containing no dissolved bicarbonates and aerated with air. A source of stable iron was available to the roots.

Figure 6. Distribution of radioiron after two weeks in low lime with a source of stable iron.

Autoradiogram of a chrysanthemum plant, which had absorbed Fe^{59} under identical conditions as the plant in Figure 3, and subsequently had grown for two weeks in half-strength Hoagland's number one solution at pH 5 containing no dissolved bicarbonates and aerated with air. A source of stable iron was not available to the roots.

Figure 3

Figure 4

Figure 5

Figure 6

those leaves which developed during the first two weeks after absorption.

Some of the radioiron was translocated to new shoot tissue even after nine weeks as illustrated in Figures 7, 8,and 9. There was considerable difference in the amount reaching those tissues under the various treatments. That amount of radioiron that was translocated to new tissues was apparently evenly distributed within the new shoots with no tendency to accumulate along veins of the leaves as has been reported for other plants.

Experiment X: Effect of Lime on Long-term Distribution of Iron in Azalea

Growth of azalea plants in sand culture was poor and variable. Root systems which were not well developed at the time of potting were subject to injury from drying as the moisture level in the sand near the surface flucuated. Consequently, some plants in the low-lime treatment made very poor growth and became chlorotic. There was considerable variation in growth in the high-lime treatment, also, but the development under the high-lime treatment was inferior to that under either low-lime with iron or low-lime without iron.

Count data was erratic, and because of large variance within treatment there were no significant differences between treatments. These data are not presented because of their erratic nature.

Two autoradiograms are presented in Figures 10 and 11, which demonstrate, in comparison with the photograph of the mounted plants, that there was a strong correlation between the presence of radioiron

Figure 7. Distribution of radioiron after nine weeks in high lime with a source of stable iron.

Autoradiogram of a chrysanthemum lateral shoot and the leaf from whose axillary bud it grew. The plant had been grown for nine weeks in Hoagland's number one solution at pH 7 and containing 3 millimolar calcium bicarbonate and aerated with 2% carbon dioxide in air after having been allowed to absorb radioiron. A source of stable iron was available to the roots.

Figure 8. Distribution of radioiron after nine weeks in low lime with a source of stable iron.

Autoradiogram of a chrysanthemum lateral shoot and the leaf from whose axillary bud it grew. The plant had been grown for nine weeks in Hoagland's number one solution at pH 5, containing no bicarbonates, and aerated with air after having been allowed to absorb radioiron. A source of stable iron was available to the roots.

Figure 9. Distribution of radioiron after nine weeks in low lime with no source of stable iron.

Autoradiogram of a chrysanthemum lateral shoot and the leaf from whose axillary bud it grew. The plant had been grown for nine weeks in Hoagland's number one solution at pH 5, except containing no bicarbonates, and aerated with air after having been allowed to absorb radioiron. A source of stable iron was not available to the roots.

Figure 7

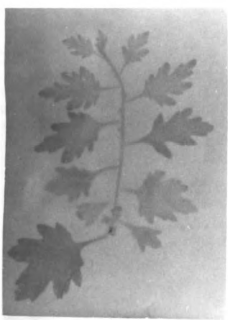


Figure 8

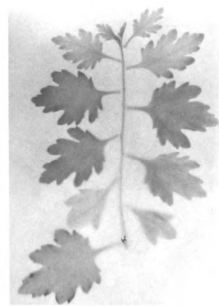


Figure 9

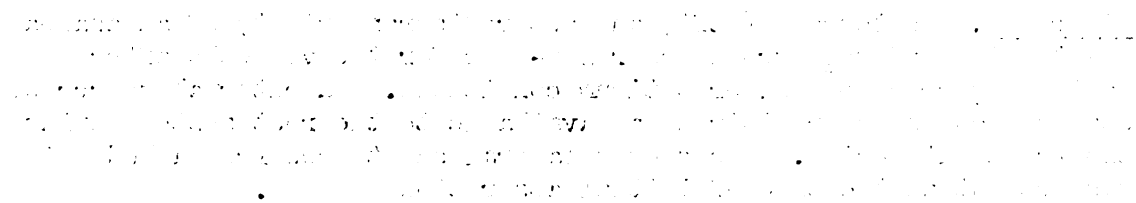


Figure 10. Photograph (left) and autoradiogram (right) of an azalea plant that had been grown under high-lime for twelve weeks after having absorbed Fe^{59} under optimum conditions. An external source of stable iron, but no radioiron was available to the root system during the treatment period. The two lateral shoots grew from buds which were present at the time of initial absorption of Fe^{59}.

Figure 11. Photograph (left) and autoradiogram (right) of an azalea plant which had been grown under low-lime for twelve weeks after having absorbed Fe^{59} under optimum conditions. An external source of stable iron but no radioiron was available to the root system during the treatment period. The single lateral shoot grew from a bud which was present at the time of initial absorption of Fe^{59}.

Figure 10

Figure 11

and chlorophyll development. Tissues were green in those areas of the leaves where radioiron accumulated. Radioiron was uniformly distributed in the leaf tissue of the green leaves corresponding with uniform green color development. Radioiron was present in the highest concentration in tissues immediately adjacent to the veins, and the interveinal pattern of chlorosis developed which is typical of this species.

Foliar Absorption of Iron

Experiment XI: Effect of Age of the Leaf on Rate of Foliar Absorption of Iron

The absorption rate of physiologically old leaves, when measured by the detached-leaf technique was found to be equal to that of young leaves and significantly higher than that of mature leaves of intermediate age (Table 15). No attempt to measure foliar absorption by leaves on the intact plant was made due to the difficulty of separating translocation from absorption processes.

Experiment XII: Effect of Chlorotic Condition on Rate of Foliar Absorption of Iron

Absorption rates of detached leaves from plants which had been subjected to either high-lime or minus-iron conditions to induce chlorosis were found to be equal to those of leaves from plants which had been grown under low-lime conditions with iron and were green (Table 16). This can be interpreted as evidence that no

Table 15.-The Effect of Age of a Leaf of C. morifolium, cv. Legal Tender on its Rate of Absorption of Iron.

Treatment	Absorption Rate
age	mu mole/cm^2/hr
young	0.900^{ab}
mature	0.744^{a}
old	1.124^{b}

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence as determined by Duncan's Multiple Range Test.

Table 16.-The Effect of Prior Exposure of Plants of C. morifolium cv. Legal Tender to High Lime Conditions on the Rate of Foliar Absorption of Iron.

Pre-Treatment			Absorption Rate
HCO_3	pH	Fe	mu mole/cm^2/hr
0	5.5	2	1.56^{a}
0	5.5	0	1.43^{a}
3	7.0	2	1.75^{a}

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence as determined by Duncan's Multiple Range Test.

changes within the leaf itself as a result of either iron starvation or exposure to high lime altered the absorption capacity.

Experiment XIII: Effects of pH, Sodium, and Bicarbonate on the Rate of Foliar Absorption of Iron

Although prior exposure of the roots of chrysanthemums to high lime did not affect the absorption rate of iron by detached leaves, the presence of bicarbonate in the foliar treatment solutions did result in reduced absorption rates (Table 17).

High concentration of sodium as sodium acetate at pH 5 did not reduce the rate of absorption relative to that at the same pH when tris was used for pH control. Foliar absorption rate was reduced at pH 7 under all three means of control used here. There were no statistical differences among the three treatments of pH 7, although there tended to be a greater reduction under sodium bicarbonate treatment. The rate under that treatment was only half what it was under the sodium acetate treatment.

Experiment XIV: Effects of Chelation of Iron in Foliar Treatment Solutions Containing Bicarbonate

When ammonium citrate and ethylenediamine tetraacetate were used to stabilize iron in solutions containing sodium bicarbonate, it was found that both agents prevented precipitation from solution. However, there was no increase in absorption rate over that resulting from high bicarbonate and iron as ferric chloride (Table 18). The absorption rates were lower in the presence of sodium bicarbonate than in its absence and at solution pH 5.

Table 17.-The Effects of Solution pH and the Presence of Sodium and/or Bicarbonate in Foliar Treatment Solution on the Rate of Absorption of Iron by Detached Leaves of C. morifolium, cv. Legal Tender.

Treatment				Absorption Rate
HCO_3	Na	pH	Control	mu mole/cm^2/hr
(mM)	(mM)			
0	0	5	Tris	4.03[a]
0	3	5	NaAc	3.94[a]
0	0	7	Tris	0.47[b]
0	3	7	Tris + NaAc	0.61[b]
3	3	7	Na_2CO_3	0.29[b]

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence as determined by Duncan's Multiple Range Test.

Table 18.-The Effects of the Presence of Bicarbonate and Form of Iron Used in the Foliar Treatment Solution on the Stability of Iron in Solution and Rate of Absorption of Iron by Detached Leaves of C. morifolium, cv. Legal Tender.

Treatment			Absorption Rate	Iron Stability
HCO_3	pH	Iron	mu mole/cm^2/hr	Per cent remaining in solution after 17 hours
(mM)		(Form)*		
0	5.0	$FeCl_3$	0.175[a]	50
3	7.0	$FeCl_3$	0.043[b]	50
3	7.0	FeAC	0.062[b]	100
3	7.0	FeEDTA	0.041[b]	100

* FeAC is ferric ammonium citrate; FeEDTA is ferric ethylenediamine tetraacetate.

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence as determined by Duncan's Multiple Range Test.

Experiment XV: Effect of Chelation on Rate of Foliar Absorption from Solutions of Low pH

Ammonium citrate and ethylenediamine tetraacetate, had no effect on the stability of iron at pH 3.0 as evidenced by the absence of precipitation when ferric chloride was used. Maximum absorption rate occurred from ferric ammonium citrate, the minimum from the ferric EDTA solution, and an intermediate rate from ferric chloride solution (Table 19).

Experiment XVI: Effect of Solution pH on Rate of Foliar Absorption of Iron

Solution pH had a marked effect on the foliar absorption rate of iron (Table 20, Figure 12). Maximum absorption occurred at pH 5 and pH 6; absorption was slightly reduced at pH 4 and considerably so at pH 3, 7, and 8.

In Table 20 absorption rates have been expressed on a leaf dry weight basis as well as leaf surface basis so that the quantities of iron absorbed can be related to the concentration reported for leaf tissue of chrysanthemum in earlier experiments.

High Lime and Phosphate Effects

Experiment XVII: Effects of High Phosphate

Phosphate concentrations as high as 4 millimolar as monobasic potassium phosphate (eight times the concentration in half-strength Hoagland's number one solution) did not reduce shoot growth (Table 21).

Table 19.-The Effect of Form of Iron in Foliar Treatment Solutions at pH 3.0 on the Rate of Absorption of Iron by Detached Leaves of C. morifolium, cv. Legal Tender.

Treatment	Absorption Rate
form of iron*	mu mole/cm^2/hr
$FeCl_3$	0.477[a]
FeAC	0.533[b]
FeEDTA	0.001[c]

* FeAC is ferric ammonium citrate; FeEDTA is ferric ethylenediamine tetraacetate.

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence as determined by Duncan's Multiple Range Test.

Figure 12. Effect of solution pH on rate of absorption of iron by chrysanthemum leaves. Solution pH is plotted on the abscissa with rate of absorption expressed as millimicromoles of iron per square centimeter leaf area per hour.

FIGURE 12

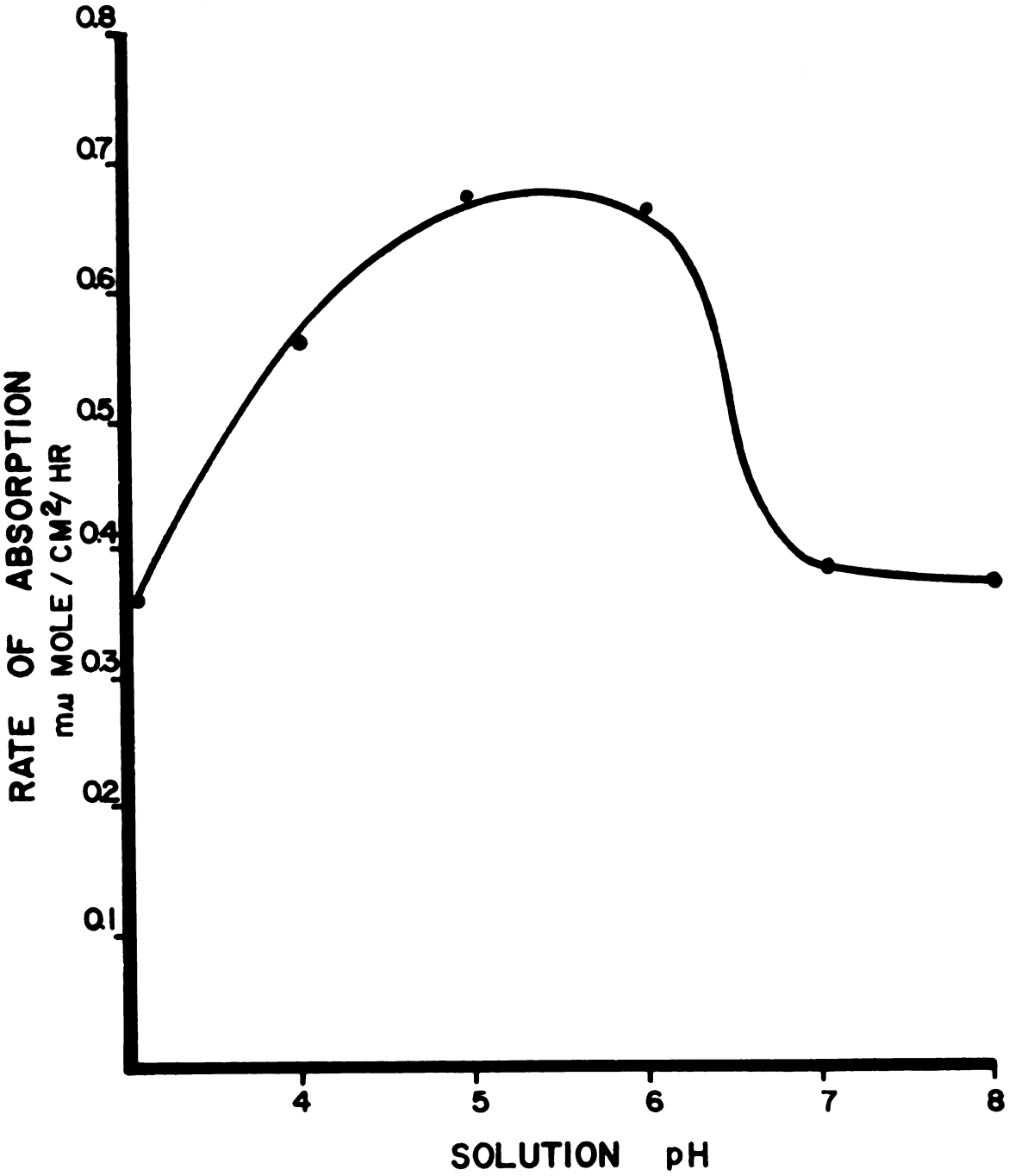

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
RATE OF ABSORPTION
mμ MOLE / CM² / HR
4
5
6
7
8
SOLUTION pH

Table 20.-The Effect of pH of Foliar Treatment Solution on the Rate of Absorption of Iron by Leaves of C. morifolium, cv. Legal Tender.

Treatment	Absorption Rate	
pH	mu mole/cm^2/hr	ug/g dry wt./hr
3	0.364[a]	7.20[a]
4	0.576[c]	9.30[b]
5	0.667[b]	11.76[d]
6	0.662[b]	14.45[e]
7	0.350[a]	8.79[bc]
8	0.373[a]	8.01[ac]

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence as determined by Duncan's Multiple Range Test.

Table 21.-The Effect of Phosphate Concentration in the Nutrient Solution on Shoot Growth and the Absorption and Accumulation of Iron and Phosphorus by *C. laurifolium*, cv. Mermaid.

Treatment	Leaf	Shoot Wt.	Root-Fe	Leaf-Fe	Root-P	Leaf-P
P conc. mM	chlo (rel)*	g. dry	(%)	(ppm)	(%)	(%)
0.5	1.0	9.93[a]	0.817[a]	84[ab]	0.479[a]	0.327[a]
1.0	1.0	10.75[a]	0.363[b]	85[ab]	0.765[b]	0.356[b]
2.0	1.3	10.10[a]	0.505[b]	91[a]	0.669[ab]	0.424[c]
4.0	1.7	8.95[a]	0.558[ab]	75[b]	0.847[b]	0.444[c]

* Degree of chlorosis is indicated by relative rating scale.

[a] Values with the same superscript letter are not significantly different at the 5% level of confidence according to Duncan's Multiple Range Test.

Furthermore, there was no consistent effect on the concentration of iron in the leaf tissue. The iron concentration resulting from 2 mM phosphate was higher than that resulting from the 4 mM treatment, but equal to that under the lower two treatments. There was a tendency for the concentration of iron in the root tissue to be somewhat less at the higher levels of phosphate than at the lowest.

The concentration of phosphorus in root tissue was somewhat higher under the three higher levels of phosphate than under the lowest level, although the concentration at 2 mM phosphate was not significantly higher than that at 0.5 or 1.0 mM.

Perhaps the most important observation is that slight chlorosis did develop under the 2 mM and 4 mM levels (one plant under 4 mM phosphate was severely chlorotic). Since this was not apparently due to a reduction in the concentration of iron in the leaf tissue, it may be concluded that the higher concentrations of phosphorus, somehow altered the utilization of iron.

It could also be concluded from these data that the absorption of iron by the roots was slightly reduced by the highest phosphate treatments, but the amount of iron translocated from the roots to the leaves was not reduced appreciably under any of these conditions.

Experiment XVIII: Effects of Various Forms of Orthophosphates at High Concentration on Growth, Chlorosis, and Tissue Accumulation of Iron and Phosphorus by Two Cultivars of Chrysanthemum

Chlorosis developed in plants of the cultivar, Oregon irrespective of the form or concentration of phosphate. Chlorosis was

slight under the low concentration of monobasic potassium and under the high monammonium phosphate treatments and severe under the high concentrations of mono- and dibasic phosphate treatments (Table 22). The chlorosis developing in this cultivar under the influence of both the low level of monobasic and the high level of dibasic potassium phosphate could be due to the high solution pH. Ammonium sulfate was not used in the low phosphate treatment as it was when this solution was used as the low-lime, low-pH treatment in other experiments, in order that the effects of concentration and form of phosphate on solution pH could be observed. The chlorosis that developed in Oregon under treatment with high monoammonium and high monobasic potassium phosphate, however, was not due to solution pH, since in both treatments the pH was well within the optimum range for chrysanthemums as established in earlier experiments. This was especially noteworthy in the ammonium phosphate treatment, in which the solution pH was 5.4 in the beginning of the experiment and decreased to 4.3 in the latter stages of the experiment.

With the cultivar, Yellow Delaware, chlorosis was not present with either low potassium phosphate or high ammonium phosphate treatment. There was slight chlorosis as a result of the high monobasic potassium phosphate and moderate chlorosis under the high dibasic potassium phosphate treatment (Table 22).

Only in the high dibasic potassium phosphate treatment (high solution pH) could reduced iron concentration in the leaf be a cause of chlorosis in either cultivar. The low concentration of iron was apparently due to reduced absorption, since there was a corresponding

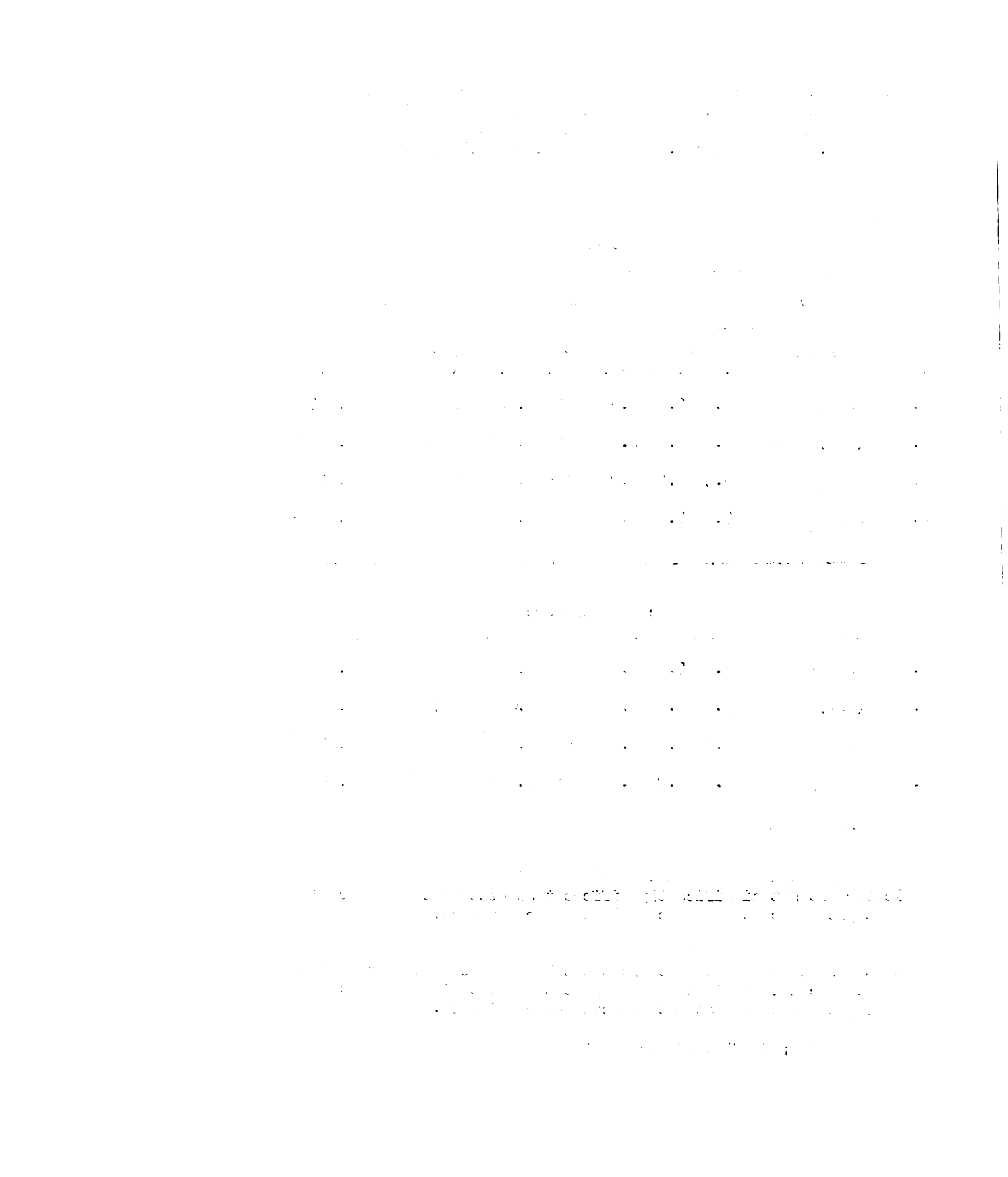
Table 22.-The Effects of High Concentration of Various Forms of Orthophosphates on the Development of Chlorosis and on Iron and Phosphorus Accumulation in Leaves and Roots of C. morifolium cvs. Oregon and Yellow Delaware.

Treatment				Leaf			Root	
Conc. (mM)	Form	pH* (T_1)	pH* (T_H)	Chlo (rel)	Fe (ppm)	P (%)	Fe (ppm)	P (%)
Oregon								
0.5	KH_2PO_4	6.0	7.0	1.8	51^{a1}	1.36^{a1}	1550^{a1}	0.267^{a1}
4.0	$(NH_4)H_2PO_4$	5.4	4.3	2.0	60^{a3}	1.59^{a2}	2260^{a3}	$.385^{a2}$
4.0	KH_2PO_4	5.4	5.7	2.2	44^{a4}	1.55^{a4}	1850^{a4}	$.373^{a4}$
4.0	K_2HPO_4	7.4	7.2	3.8	33^{b5}	2.32^{b5}	330^{b5}	1.458^{b5}
Yellow Delaware								
0.5	KH_2PO_4	6.0	7.0	1.0	94^{x2}	0.92^{x1}	8420^{x2}	0.162^{x1}
4.0	$(NH_4)H_2PO_4$	5.4	4.3	1.0	66^{y3}	1.01^{x3}	1570^{y3}	$.260^{x3}$
4.0	KH_2PO_4	5.4	4.3	1.2	60^{xy4}	1.16^{x4}	1490^{y4}	$.278^{x4}$
4.0	K_2HPO_4	7.4	7.2	2.8	40^{z5}	1.44^{x6}	330^{z5}	$.800^{y6}$

a Treatment means of the same cultivar having the same superscript letter are not significantly different as determined by Duncan's Multiple Range Test at the 5% level of confidence.

1 Cultivar means of the same treatment having the same superscript number are not significantly different as determined by Duncan's Multiple Range Test at the 5% level of confidence.

T_1 Initial Time; T_H Time of Termination



reduction in the roots (Table 22).

Associated with the reduced iron concentration in leaves was an increase in phosphorus concentration resulting from high dibasic potassium phosphate in Oregon. There was a slightly higher phosphorus concentration in leaves resulting from high monobasic potassium and high ammonium phosphate than from the low monobasic potassium phosphate, but not significantly higher.

Translocation could not be judged to have been affected by any of the treatments in either cultivar. In all cases, reduced leaf iron was associated with reduced root iron. Furthermore, the concentration of phosphorus in the roots under high phosphate was higher than that under the low phosphate treatment only in the treatment with high dibasic potassium phosphate. There was no evidence that iron and phosphorus accumulated together in the root tissue with a resultant decrease in transport of iron to the leaves.

Leaf growth in both cultivars varied inversely with the development of chlorosis. This was not true of root growth, however. Root growth was favored by the high phosphate and high pH conditions, the greatest growth being made under the influence of the high dibasic potassium phosphate treatment.

Under these conditions Yellow Delaware made greater root and leaf growth than did Oregon (Table 23). There were no statistical differences among treatment effects on leaf dry weight of Oregon, although it tended to be reduced under high phosphate, especially high dibasic potassium phosphate. This latter treatment did result in significantly less leaf dry weight of Yellow Delaware. There was

Table 23.-The Effects of High Concentrations of Various Forms of Orthophosphates on the Production of Tissue Dry Weight by *G. perfidium*, cv. Oregon and Yellow Delaware.

Treatment			Oregon		Yellow Delaware	
Conc.	Form	pH	Leaf	Root	Leaf	Root
(mM)			(g)	(g)	(g)	(g)
0.5	KH_2PO_4	7.0	3.40[a]	1.55[c]	4.59[a]	2.53[c]
4.0	$(NH_4)H_2PO_4$	4.3	2.38[a]	1.05[a]	5.19[a]	2.16[a]
4.0	KH_2PO_4	5.7	2.87[a]	1.44[b]	3.71[a]	2.22[b]
4.0	K_2HPO_4	7.2	1.94[a]	1.03[d]	2.55[b]	2.53[d]

[a] Values with the same superscript are not statistically different as determined by Duncan's Multiple Range Test at the 5% protection level on leaf dry weight data which had a significant interaction between treatment and cultivar.

not a significant interaction between treatment and cultivar in root dry weight. The average root dry weight for both cultivars was lower under high ammonium and high monobasic potassium than under high dibasic potassium phosphate. Apparently solution pH had a stronger effect on root growth than did phosphate concentration.

The important differences in response to the two cultivars, which could account for the reduced tendency of Yellow Delaware to develop chlorosis, were that Yellow Delaware absorbed more iron and less phosphorus than did Oregon under high solution pH and low-phosphate. The leaf tissue acquired more iron, but probably not related to differences in translocation. In the high ammonium phosphate treatment, Yellow Delaware absorbed essentially the same amount of iron as did Oregon, with equal concentrations accumulating in the leaf tissue, but absorbed and accumulated less phosphorus in the leaves. Under the high monobasic potassium phosphate treatment, there was no difference between cultivars in respect to either root or leaf concentrations of iron or phosphorus. Under the high dibasic potassium phosphate treatment, the differences with respect to phosphorus absorption and accumulation in leaves were essentially the same as under the low phosphate treatment, except Yellow Delaware did not absorb or accumulate more iron than did Oregon.

Experiment XIX: Effects of High Phosphate and High Lime and Their Combined Effects on Growth, Chlorosis, and Tissue Accumulation of Iron and Phosphorus in Chrysanthemum

Chlorosis was induced with high lime or high phosphate, but it

was most severe in the treatments having a high phosphate concentration (Table 24).

Growth was repressed to the greatest extent in the high-lime, high-phosphate treatment, although this repression was not significantly greater than either high-lime or high-phosphate alone except in respect to stem length.

Root growth was similarly reduced as a result of either high lime or high phosphate. Root tip activity was affected in a way that cannot be easily described by numerical means. Roots in treatments with high lime tended to be shorter and branched. Their color was dark, and the tips were black. The root tips died or ceased to give rise to new tissue and subsequently laterals developed. The laterals also ceased to elongate after attaining only a few millimeters in length. This process was repeated until a root system analagous to "witches' broom" of the shoot had developed.

The concentration of iron in leaf tissue was reduced by either high lime or high phosphate (Table 25). However, the significant effect of high phosphate under high-lime conditions was an increase in iron concentration. This was not due to a greater reduction in leaf dry weight. There was, in fact, a greater absolute amount of iron in the leaves. As indicated by the development of chlorosis, high phosphate at high lime resulted in iron accumulation in the leaf but apparently this iron was not effective in chlorophyll synthesis.

The reduction of iron in leaf tissue was not primarily due to reduced translocation. The concentration of iron in the root was reduced under high lime or high phosphate or both.

Table 24.-The Effects of Lime and Phosphate on Growth and the Development of Chlorosis in C. trifoliata, cv. Legal Tender.

Treatment			Chlo.	Leaf Weight			Stem	Root
HCO_3	pH	P		fresh	dry	dry wt. percentage	length	dry wt.
(mM.)		(mM.)	(rel*)	(g)	(g)	(%)	(cm)	(g)
0	5.5	0.5	1.0	137.5	17.8	12.9	11.6	4.34
0	6.8	2.0	4.0	70.3	6.8	9.8	5.8	3.65
3	7.2	0.5	2.5	74.2	7.4	9.9	6.5	3.92
3	7.2	2.0	4.0	65.2	6.2	9.5	5.4	3.44

* Degree of chlorosis is indicated by relative rating scale.

Significant Simple Effects*

Comparison			Leaf Weight			Stem	Root Weight
			fresh	dry	dry %	length	dry
(HCO_3)	at 0.5mM	P	yes	yes	yes	yes	yes
(HCO_3)	at 2.0mM	P	no	no	no	no	no
(P)	at 0	HCO_3	yes	yes	yes	yes	yes
(P)	at 3 mM	HCO_3	no	no	no	yes	no

*Simple effects were tested at the 5% level of confidence by the F test.

Table 25.-The Effects of Lime and Phosphate on Iron and Phosphorus Accumulation in Root and Leaf Tissue of C. morifolium, cv. Legal Tender.

Treatment			Iron Composition		Phosphorus Composition	
HCO_3	pH	P	leaf	root	leaf	root
(mM)		(mM)	(ppm)	(ppm)	(%)	(%)
0	5.5	0.5	120	3048	0.64	0.74
0	6.8	2.0	49	931	1.41	0.98
3	7.2	0.5	43	560	1.06	0.92
3	7.2	2.0	60	304	1.25	1.05

Significant Simple Effects*

Comparison				Iron Composition		Phosphorus Composition	
				leaf	root	leaf	root
(HCO_3)	in	0.5mM	P	yes	yes	yes	no
(HCO_3)	in	2.0mM	P	no	no	yes	no
(P)	in	0 mM	HCO_3	yes	yes	yes	no
(P)	in	3 mM	HCO_3	yes	no	yes	no

* Simple effects were tested at the 5% confidence level by the F test.

** Interaction between bicarbonate and phosphate was not significant at the 5% confidence level. Main effects of bicarbonate and phosphate treatments were significant with root-phosphate concentration being greater as a result of either high bicarbonate or high phosphate treatment.

Under conditions of low phosphate, there was significantly more accumulation of phosphorus in leaves at high lime than at low. However, under conditions of high phosphate, the effect of lime was opposite. The concentration of phosphorus in the roots was increased under either high lime or high phosphate, but there was not a significant interaction.

Experiment XX: Effects of Lime, Phosphate and Iron Chelation on Chlorosis and Tissue Accumulation of Iron and Phosphorus

In this experiment treatment solution pH was lower than in comparable treatments of earlier experiments. This was apparently due to using ammonium phosphate in the base solution in all treatments. Chlorosis developed in those treatments of either high lime, high phosphate, or both, when the form of iron was ferric ammonium citrate, but not when the source of iron was ferric ethylenediamine tetraacetate (Table 26). The use of the latter form resulted in a significant increase in iron concentration of leaf tissue relative to that obtained from ferric ammonium citrate under the treatments having either high lime, high phosphate, or both. There was no significant effect of chelation on the concentration of iron in leaves under the low-lime, low-phosphate treatment. Furthermore, neither high lime nor high phosphate affected leaf-iron when EDTA was used; whereas, either reduced leaf-iron accumulation when ferric ammonium citrate was used. (Figure 13)

Greater concentrations of iron were found in roots when iron was supplied as ferric ammonium citrate rather than ferric EDTA under

Table 26.-The Effects of Lime, Phosphate, and Iron Chelation on the Development of Chlorosis and on Iron and Phosphorus Accumulation in Root and Leaf Tissue of C. norifolium, cv. Legal Tender.

Treatment					Iron Composition		Phosphorus Composition	
HCO_3	pH	P	Fe	Chlo	leaf	root	leaf	root
(m.e.)		(ml.)	(form*)	(rel**)	(ppm)	(ppm)	(%)	(%)
0	4.5	0.5	AC	1.2	105	2261	1.11	0.73
0	4.2	0.5	EDTA	1.0	83	642	1.07	0.68
0	5.4	2.0	AC	2.8	58	979	1.23	1.12
0	5.4	2.0	EDTA	1.0	91	394	1.15	1.06
3	6.9	0.5	AC	3.5	61	583	1.36	0.79
3	6.3	0.5	EDTA	1.0	91	324	0.86	0.52
3	6.5	2.0	AC	3.5	74	312	1.36	0.83
3	6.0	2.0	EDTA	1.0	88	314	0.98	0.72

* AC is ferric ammonium citrate; EDTA is ferric ethylenediamine tetra-acetate.

** Chlorosis is rated on a relative scale 1 - 4, increasing severity.

Table 26. (cont'd)

F Tests for Significant Effects

Effect	Leaf-Fe	Root-Fe	Leaf-P	Root-P
HCO_3	no	yes	no	yes
P	no	yes	no	yes
Chelate	yes	yes	yes	yes
HCO_3 - P	yes	yes	no	yes
HCO_3 - Chel	no	yes	yes	no
P - Chel	no	yes	no	no
HCO_3 - P - Chel	yes	no	no	no

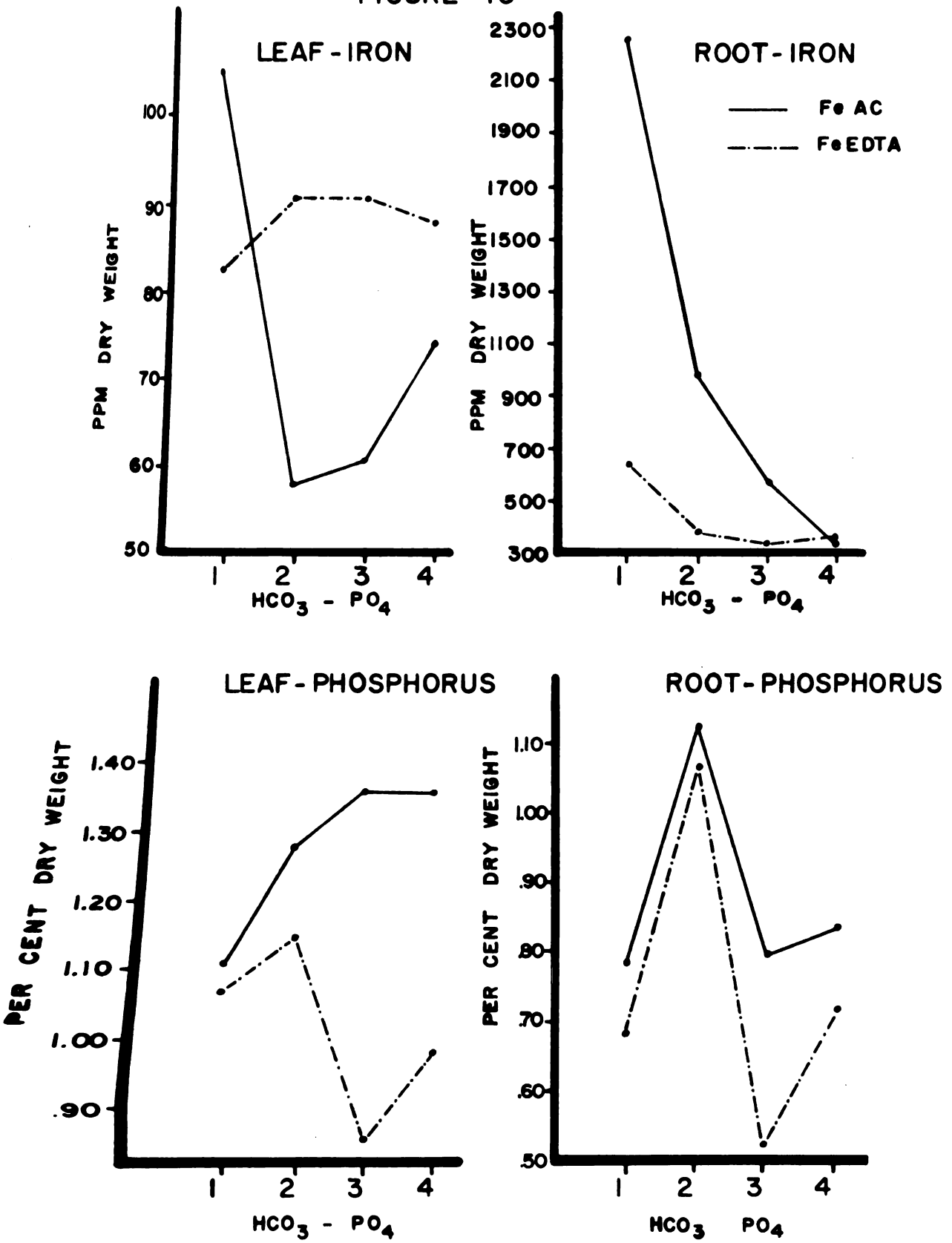
Significant Simple Effects

Leaf-Fe	Root-Fe	Leaf-P	Root-P
HCO_3 in 0.5mM P	HCO_3 in 0.5mM P	Chel in 3mM HCO_3	HCO_3 in 2mM P
P in 0mM HCO_3	HCO_3 in 2.0mM P	HCO_3 in AC	P in 0mM HCO_3
P in AC	P in 0mM HCO_3	HCO_3 in EDTA	
Chel in 2.0mM P	HCO_3 in AC		
Chel in 0.5mM P at 0mM HCO_3	AC in 0mM HCO_3		
Chel in 0.5mM P at 3mM HCO_3			
P in AC at 0mM HCO_3			

Figure 13. Effect of bicarbonate, phosphate, and iron chelation on tissue accumulations of iron and phosphorus. The concentration of iron or phosphorus in leaf or root tissue is plotted on the ordinate of the graph against treatment bicarbonate and phosphate on the abscissa. The effect of iron chelation is indicated by the use of separate lines identified as FeAC (ferric ammonium citrate) or FeEDTA (ferric ethylenediamine tetraacetate). In each graph the positions on the abscissa represent the following conditions:

1. 0 mM HCO_3 and 0.5 mM PO_4
2. 0 mM HCO_3 and 2.0 mM PO_4
3. 3 mM HCO_3 and 0.5 mM PO_4
4. 3 mM HCO_3 and 2.0 mM PO_4

FIGURE 13


LEAF - IRON
PPM DRY WEIGHT
100
90
80
70
60
50
1
2
3
4
HCO3 - PO4
ROOT - IRON
Fe AC
Fe EDTA
2300
2100
1900
1700
1500
1300
1100
900
700
500
300
PPM DRY WEIGHT
1
2
3
4
HCO3 - PO4
LEAF - PHOSPHORUS
PER CENT DRY WEIGHT
1.40
1.30
1.20
1.10
1.00
.90
1
2
3
4
HCO3 - PO4
ROOT - PHOSPHORUS
PER CENT DRY WEIGHT
1.10
1.00
.90
.80
.70
.60
.50
1
2
3
4
HCO3 PO4

each condition of lime and phosphate, except where both were high. Since high leaf-iron was associated with low root-iron under EDTA treatments, it may be concluded that the EDTA chelated form of iron was more readily translocated from the roots to the developing leaves.

Either high lime or high phosphate reduced the iron concentration in roots when iron was supplied as ammonium citrate, but not supplied as EDTA. This reduction was most pronounced when both lime and phosphate were high.

High levels of phosphorus accumulated in leaf tissue when ferric ammonium citrate was used under either high phosphate, high lime, or both. Of particular interest was the observation that no such increase resulted when EDTA-iron was used, and in fact, there was a tendency for the reverse to be true. Furthermore, the form of iron used had a stronger effect on resultant phosphorus concentration in leaves than did the concentration of phosphate in the treatment solutions.

The use of EDTA-iron also resulted in slightly reduced concentrations of phosphorus in the root tissue, under each condition of lime and phosphate concentration relative to that from the use of ferric ammonium citrate. There was a significant main effect of chelation on such accumulation, but no significant interaction with either lime or phosphate.

High lime combined with high phosphate resulted in less phosorus in the root tissue relative to low lime with high phosphate. e concentration of lime in the treatment solution had no effect on ot-phosphorus concentration when phosphate was low in the treatment lution. Furthermore, the phosphate concentration in the treatment

solution had a direct effect on root-phosphorus under low-lime conditions, but not under high-lime conditions.

Experiment XXI: Effect of Form and Level of Iron in Soil Applications on Growth, Chlorosis, and Iron Concentration in Leaves of Chrysanthemum

Growth was not judged to be affected by any of the treatments of this experiment. Leaf dry weight data indicated no differences, although the samples weighed were useful only as an approximation, since all the leaves of each plant were not weighed. Plant height and overall appearance were also judged visually to be essentially equal. Furthermore, no chlorosis developed under any of the treatments.

Essentially no differences existed between cultivars in terms of iron concentration in the leaf tissue, although Yellow Delaware tended to have a higher concentration when the highest applications of each form was used (Table 27). There was no consistent increase in leaf-iron as the level of each form of iron was increased in the soil. There was somewhat higher leaf-iron at highest ferrous sulfate and EDTA treatments as compared to that at the lowest levels; however, the opposite was observed in the case of the fritted form.

Statistical analysis of these data indicated that the soluble forms of iron (ferrous sulfate and Ferric-EDTA) were more effective in producing high leaf-iron than the fritted form. EDTA was the more effective of the soluble forms.

Table 27.-The Effect of Form and Concentration of Iron Applied to the Soil on the Concentration of Iron in Leaf Tissue of C. nerifolium, cv., Oregon and Yellow Delaware.

Treatment		Leaf-Iron Concentration	
Form	Level	Oregon	Yellow Delaware
	(rel)	(ppm)	(ppm)
$FeSO_4$	low	59	60
$FeSO_4$	med	75	62
$FeSO_4$	high	71	83
Frit	low	76	78
Frit	med	76	78
Frit	high	52	64
EDTA	low	66	72
EDTA	med	76	79
EDTA	high	81	81

Simple Effects of Form•Level Interaction

Comparison		Significant
Soluble vs frit	linear	yes
Soluble vs frit	quadratic	no
$FeSO_4$ vs FeEDTA	linear	yes
$FeSO_4$ vs FeEDTA	quadratic	no

Experiment XXII: Effects of Bicarbonate and Phosphate on the Availability of Native Iron from a Greenhouse Soil

High applications of superphosphate -- $Ca(H_2PO_4)_2$ -- did not result in a lower concentration of iron in leaf tissue, compared to the treatment receiving no additions of phosphate, unless the soil was irrigated with water high in bicarbonates (Table 28). When the more soluble form of phosphorus, monobasic potassium phosphate, was used, the resultant concentration of iron in leaves was lower than in the treatment receiving native phosphate regardless of whether bicarbonates were high or low in the irrigation water.

The concentration of phosphorus in the leaf tissue was higher under any of the high phosphate treatments than under conditions of low phosphate. However, neither the form of phosphate nor the presence of bicarbonate in the irrigation water had any significant effect on the concentration of phosphorus in the leaves.

Chlorosis did not develop under any of the treatments, and growth was not judged by visual observation to have been affected.

Table 28.-The Effects of High Bicarbonate Irrigation and High Soil Phosphorus on the Accumulation of Iron in Leaf Tissue of C. morifolium, cv. Legal Tender, Grown in Soil of Moderate Iron Content.

Treatment		Leaf Fe and P Composition	
Phosphate	Irrigation Water	Fe	P
		(ppm)	(%)
None	Distilled	91[ab]	0.278[a]
KH_2PO_4	Distilled	79[cd]	0.377[b]
$Ca(H_2PO_4)_2$	Distilled	101[a]	0.379[b]
KH_2PO_4	Tap	85[bc]	0.415[b]
$Ca(H_2PO_4)_2$	Tap	69[d]	0.398[b]

[a] Values with the same superscript letter are not significantly different as determined by Duncan's Multiple Range Test.

DISCUSSION

Solubility of Iron in Nutrient Solutions and Soil

The precipitation of iron when supplied as ferric chloride in nutrient solutions of high pH was so rapid as to indicate that the presence of other ions in those solutions had no tendency to prevent or delay its precipitation. The rapidity and extent of precipitation was essentially in agreement with reports dealing with iron precipitation from water devoid of other ions (78, 136, 146). The extreme instability of iron as ferric chloride in highly oxygenated nutrient solutions or in nutrient solutions containing dissolved lime indicated that such a form of iron would be completely unsuited to such situations (57).

Although ferric ammonium citrate was a more stable form of iron than ferric chloride, it did precipitate from solutions of high pH. Its rate of precipitation appeared to be affected by the presence and activity of microorganisms which contributed to its decomposition. And, if such decomposition was a factor under laboratory conditions, it would be expected to be of much greater importance in a soil with a well developed microbial population. Iron did not precipitate when ferric ethylenediamine tetraacetate was supplied at pH 7.2 in the presence of bicarbonate, as has been reported under other conditions (8, 67).

When the stability of ferric ammonium citrate was studied in the presence of rapidly growing plants, it was found that iron did not remain in solution for more than one or two days under conditions

of either high or low solution pH. At high pH, iron was precipitated, and the rate of precipitation was greater if the cause of high pH were high lime (calcium and potassium bicarbonate) than if it were high phosphate. However, the disappearance of soluble iron from solutions of low lime and low pH could only be attributed to adsorption and absorption by plants, since no iron precipitate was recovered from the bottom of the crocks.

Growth of chrysanthemums was rapid and leaves green under all conditions which favored the presence of soluble iron in the nutrient solution, including that of EDTA-Fe in solutions of high soluble lime or phosphate. Plants under conditions favoring rapid growth were subject to a fluctuating supply of iron, though, and when solutions were not changed each week it was necessary to add iron at weekly intervals, even when EDTA-Fe was used. Weekly additions of iron as ferric ammonium citrate to solutions of high lime or high phosphate (both having high pH) did not prevent the development of chlorosis.

It was obvious that a constant supply of soluble iron in aerated nutrient solution cultures was essential to healthy growth and green foliage. This was not so for chrysanthemums in soil cultures. Chrysanthemums in soil under the conditions of high lime and/or high phosphate were green and made apparently normal growth. Azaleas, in a peat soil, did develop chlorosis under high bicarbonate, high phosphate, or high pH conditions and were green under low pH conditions. Possibly the differences in responses of the two plants in soil culture could have been due to differences in iron supply, or differences in root growth of the two plants, or both. The chrysanthemums received

fairly high additions of iron applied by mixing with the soil before planting in one experiment. In a second experiment a soil with an high iron supply (6 ppm by Spurway test) was used. The mode of absorption might have involved direct root-iron contact phenomena similar to that described for magnetite (34, 40, 49) or glassy frit (148, 149, 152). Root growth of azaleas appeared to have been repressed more than that of chrysanthemums.

Adsorption of colloidal iron might have been the first step in absorption of iron from aerated solutions (44). Visual observations established that suspended particles did become attached to the roots in solution culture as a result of the agitation by stirring and aerating. Furthermore, this material continued to adhere to the roots until the plants were harvested for analysis, and it was removed by a washing technique used to avoid interpreting adsorbed iron as absorbed iron. Analysis of that material established that iron, phosphorus and calcium were present in it.

Lime Induced Chlorosis

Chlorotic leaves of chrysanthemums grown under conditions of high bicarbonate or high lime contained a lower concentration of total iron within the leaf tissue than did green leaves. The concentration under high-lime was comparable to that under low-lime, minus-iron. Iron concentration in chlorotic leaves varied among cultivars of the species to such an extent that the concentration in severely chlorotic leaves of plants of one cultivar can be as high as that of green leaves of another. Such variation complicated any attempt to

establish a critical level of iron for chlorophyll maintenance in leaf tissue (66, 97, 111, 122). However, the observations that chlorotic leaves contained less total iron than green leaves indicated that chlorosis developed as a result of a decreased supply of iron to the leaf rather than an inactivation of iron within the leaf when lime was the cause of chlorosis.

In all cases reported here, growth, and particularly leaf dry weight, was reduced in those plants which became chlorotic, regardless of cause of chlorosis (105, 123, 136). When leaf iron is reported as the total amount occurring in the leaves of the plants, a greater difference is usually noted than when the iron is expressed as a concentration in the dry matter. This difference between total leaf iron and leaf iron concentration on a dry weight basis was particularly evident in the case of the Mollis azalea, grown during two seasons. At the end of the first season of growth under treatment, only a slight reduction in leaf dry weight was noted and the depression in iron concentration was related to the severity of chlorosis. However, during the second season, growth reduction was severe and no difference in iron concentration in leaf dry matter was observed. The total amount of iron in the chlorotic leaves of the second season was less than that in the green leaves. The reduction in growth was greater than the severity of chlorosis.

A reduction in iron available to the developing leaves was not the only effect of high lime in the rooting medium. Spectrographic analysis of the leaf tissues of the Mollis azalea established that other important minor elements were also reduced in availability to

the leaves as a result of high bicarbonate and high solution pH. Manganese was reduced, and this element is known to have a function in chlorophyll maintenance. Boron and zinc were reduced, and these two elements have important functions in cell division and enlargement. The sodium bicarbonate treatment resulted in leaves having higher iron content than did the less severely chlorotic leaves produced under the calcium or mixed bicarbonate treatments, but leaves under the sodium bicarbonate treatment had the least concentration of zinc and the least leaf dry weight of the high bicarbonate treatments. Further, the visual symptoms developed in an evergreen azalea (data not reported) under that treatment resembled those typically ascribed to zinc more closely than those of iron deficiency. The concentration of the major cation elements were also affected by these treatments, but these changes apparently were due more to the effect of relative cation concentration in the rooting medium than to the high concentration of the bicarbonate anion. The high ratio of potassium to calcium which has been observed in many studies of the effects of high lime (9), was noted in leaves of plants under sodium bicarbonate treatment, but the ratio was low under calcium bicarbonate. Development of chlorosis was not affected by these differences, however, and this phenomenon is not considered to be a causal factor.

Phosphorus and Lime Interactions

The first indications that high phosphorus could be involved in causing chlorosis were obtained when phosphoric acid was used to neutralize the high bicarbonate of tap water. Chlorosis developed in azalea plants irrigated with tap water plus phosphoric acid while plants were green with distilled water irrigation at approximately the same soil pH. Chlorosis was induced with phosphoric acid more readily than with any of the high-bicarbonate treatments. Chlorosis was most severe under that treatment in both the first and second season of growth. During the latter season, the amount of phosphoric acid used was greater and a lower soil pH was maintained. This is consistent with the observations of Hageman and Hartman (52) that phosphoric acid was not effective in reducing the harmful effects of bicarbonate in water.

High concentrations of monopotassium phosphate in nutrient solution (four and eight times that in Hoagland's solution) induced chlorosis at solution pH 6. When various forms of phosphates were used at eight times the concentration of Hoagland's solution, chlorosis developed to the greatest extent in the treatment having the highest solution pH. However, with the cultivar, Oregon, chlorosis developed at solution pH less than 6.0, which pH otherwise promoted green foliage.

Accumulation of iron that was not functional in chlorophyll synthesis was indicated by the relatively high concentration of iron in slightly chlorotic leaves of chrysanthemum under certain conditions of high phosphate.

Dibasic potassium phosphate, on the other hand, induced chlorosis by reducing the accumulation of iron in the leaves.

By comparing the effects of high phosphate with those of high lime (or bicarbonate), it was found that chlorosis developed earlier and became more severe as a result of high phosphate. Growth reduction was most severe when high phosphate and high lime effects were combined.

Additional evidence of accumulation of inactive iron was obtained in another experiment in which leaf dry matter under high phosphate and high lime had 60 ppm iron contrasted to 49 ppm in the leaves of equally severe chlorosis and of equal dry weight under high phosphate alone, and contrasted to 43 ppm in the less severely chlorotic leaves under high lime alone.

EDTA-chelated iron prevented chlorosis under conditions of either high lime, high phosphate, or both. Concomitantly higher concentrations of iron and lower concentrations of phosphorus occurred in the leaf tissue from chelated iron than from ferric ammonium citrate.

The direct effect of high concentrations of dissolved lime (i. e. bicarbonate) on absorption of iron by roots was to reduce its stability in solution so as to reduce its availability to the roots.

Indirect effects were also noted, particularly in two experiments in which radioiron was used to measure absorption during short periods of time. In one such experiment, chlorotic plants which had been grown previously under conditions of low lime and no iron absorbed more radioiron in 24 hours than did green plants previously grown under conditions of low lime with adequate soluble iron. Chlorotic

plants grown under conditions of high lime with iron absorbed much less radioiron than did the others. Absorption, in this case, was measured under the same conditions to which the plants had been exposed for two weeks, except that the absorption period was short and the iron stabilized with ammonium citrate so precipitation was not an important factor. Iron deficiency apparently increased the capacity for iron absorption if the cause of iron deficiency was an absence of iron in the root medium and not the presence of bicarbonate.

Similar differences in capacity for iron absorption were noted when plants were grown under identical conditions but allowed to absorb radioiron under low-lime conditions. The increased capacity for iron absorption by chlorotic plants of low-lime, minus-iron pre-treatment and decreased capacity of chlorotic plants of high-lime pre-treatment was interpreted as evidence of internal changes in the plant as a result of exposure to high bicarbonate concentrations. Such internal changes reduced the plant's capacity for iron absorption, and such reduced capacity continued to affect the plant for some time after transfer to favorable conditions of low bicarbonate.

These observations regarding the rapid rate of iron absorption under conditions of low lime have further significance. When the iron supply flucuates widely in aerated solution culture, successful iron nutrition would depend on the ability of the plant to absorb most of the soluble iron during its brief exposures to it. When ferric ammonium citrate is added to aerated solutions on a weekly basis, it remains in solution for only one or two days even at low pH. The plant then grows essentially in a minus-iron solution for

the next five or six days. If it is growing rapidly during this time, a considerable amount of new tissue would undoubtedly be formed under iron stress. When iron is again made available in soluble form, that plant would absorb it at a rapid rate. Such a cycle would be repeated again and again for the plant in the low-lime solution. If, on the other hand, the plant were growing in a high-lime solution it would also have tissue lacking in iron, but other changes brought about by the high-lime effect would reduce its ability to absorb iron rapidly during the brief periods every week when iron was in solution. And since ferric ammonium citrate would remain in solution in the high-lime solution for only a few hours, the problem of availability is intensified. As a consequence of these phenomena, the plant in a low-lime solution goes through periods of incipient iron deficiency followed by periods of rapid absorption of iron and recovery from that stress; whereas, the plant in the high-lime solution develops incipient deficiency during the long absence of available iron and fails to absorb enough iron during the subsequent brief exposure to soluble iron to recover, so chlorosis develops as a permanent condition. The tendency of plants under low-lime treatment to recover from incipient chlorosis created a problem in maintaining the minus-iron treatment. Often the iron in distilled water was sufficient to bring about recovery when such water was added as a means of renewing the nutrient medium.

Plants supplied with EDTA-chelated iron do not go through these cycles of availability and non-availability of iron. The chelated iron remains in solution for a longer period of time so as to provide a continuous supply for absorption and subsequent translocation to the

developing leaves. This difference could account for the lower iron concentration observed in leaves of plants grown in solutions receiving weekly additions of EDTA-iron contrasted with that in leaves of plants grown under conditions of low-lime with weekly additions of ferric ammonium citrate. Quantities of iron in excess of that needed for chlorophyll synthesis probably were absorbed by the plants receiving periodic additions of ferric ammonium citrate, because of rapid absorption when the iron was available. The plants receiving EDTA-chelated iron never developed an iron deficiency and so did not go through periods of very rapid iron absorption which could result in excess accumulation.

Not only did bicarbonate in the rooting medium reduce iron absorption by roots, but bicarbonate in solutions applied to the leaves reduced absorption by leaves.

When the foliar treatment solution contained no salts other than iron, and when its pH was favorable for foliar absorption, such absorption proceeded at an appreciable rate even if the leaves were chlorotic as a result of prior exposure of the plant to high-lime conditions. This indicated that the condition of chlorosis, itself, did not predispose the leaf to a reduced rate of foliar absorption of iron. This was further emphasized by the fact that under certain experimental conditions the detached chlorotic leaves both from minus-iron, low-lime pre-treatment and from high-lime pre-treatment absorbed iron at a faster rate than did the green leaves.

The age of the leaf did not apparently have an effect on its rate of foliar absorption when it was detached from the plant. If

older leaves on the intact plant are capable of similar rates of absorption, then foliar application provides a means for introducing iron into those older leaves of the plant that do not receive appreciable quantities of root-absorbed iron.

The quantities of iron absorbed under optimum conditions indicate that the leaf would absorb enough iron in less than one hour to raise the iron concentration in the leaf tissue to a level equal to that which was found in green leaf tissue of most cultivars studied. This could lead to recovery from chlorosis, if other conditions for chlorophyll synthesis were favorable. It must be kept in mind, however, that leaf analysis data from earlier experiments showed that other elements were in reduced concentration in the chlorotic leaf when that leaf had been formed under high-lime conditions. Important among these is manganese, because of its role in chlorophyll maintenance. It is possible that the use of foliar sprays to correct chlorosis will not prove completely successful unless all the elements lacking in the leaves are included in the foliar spray solution and application is made at a time when enzyme systems involved in chlorophyll synthesis are still functional.

At solution pH 7, resulting from either sodium acetate, sodium bicarbonate, or Tris the rate of foliar absorption of iron was sharply reduced. Sodium bicarbonate inhibited foliar absorption more than did the sodium acetate at the same concentration, because of the higher resultant solution pH. This indicates that preparing spray solutions in tap water that is high in bicarbonates would predispose the treatment to failure because of this inhibition.

Treatment solution pH had a pronounced effect. The optimum pH was not the lowest tested, at which stability of iron in solution would be the greatest, but it was at the intermediate levels of pH 5 and pH 6. At higher pH, inhibition occurred, due primarily, of course, to precipitation of non-chelated iron from solution.

Both ammonium citrate and ethylenediamine tetraacetate reacted with ferric chloride to chelate the iron and maintain its solubility under conditions of high pH during the time periods necessary for successful foliar treatment. Ammonium citrate increased the absorption above that of the non-chelated form of iron at high solution pH, but not to the point of being equal to the absorption rate of iron at the optimum pH (pH 5). The observation that chelation with EDTA did not increase the absorption of iron under high pH conditions agrees with reports by Lunt (85) and Wallace (134). Under conditions of low pH, ammonium citrate increased the absorption relative to that of ferric chloride, but EDTA had no favorable effect.

Iron Translocation

The effect of lime on translocation of iron was best observed in experiments in which the distribution of radioactive iron to various plant parts was followed. Iron was translocated rapidly to the young leaves of plants which were chlorotic from exposure to low-lime, minus-iron conditions. A relatively high percentage of the total amount of iron absorbed by each plant was translocated to the leaves within the first 24 hours. The percentage of absorbed iron that was translocated to the young leaves of green plants was less,

and it was greatly reduced in the plants under the high-lime treatment. Count data was interpreted as evidence that essentially as much iron was translocated to the lower four leaves as to the upper four leaves under all treatment conditions, in terms of percentage translocated to each. However, the resultant accumulation in the tissues on a dry weight basis was higher in the young leaves. The interpretations from autoradiograms did not agree with that of count data. The youngest leaves evidently had more radioiron than did the older leaves, and the difference was progressive with age. If the older leaves did have as much iron as the younger ones, it was not distributed throughout the leaf tissue.

The tissue in which accumulation took place was the root. A high percentage of iron was being translocated from the roots of plants under the low-lime, minus-iron treatment during the first 24 hours. During the remainder of the observation period more iron was absorbed than translocated out of roots, so the percentage in the roots increased. And the highest percentage of iron remaining in the roots was observed in those plants under high-lime treatment.

In 36 hours the concentration of iron in chlorotic leaf tissue produced under low-lime conditions reached a level essentially equal to that observed in green leaves in other experiments. This could mean that enough iron had been absorbed and translocated to leaves to bring about a correction of chlorosis, provided that the iron-requiring mechanism for chlorophyll synthesis had not been irrevocably altered. Recovery was not observed in the short-term experiments, of course, but did occur in other experiments.

Internal changes affected the movement of iron within the plant. Exposure of plants to high-lime conditions reduced the distribution of iron from roots to leaves after transferring the plants to solutions of low-lime and low pH. Iron accumulated in stems as much as in roots.

The concentrations of iron in root tissues were less when EDTA-iron was used under high lime, or high phosphate, or both, than when ferric ammonium citrate was used. This could readily be accepted as evidence that this synthetic chelating agent prevented the accumulation of iron in the roots and favored the translocation of higher percentages of absorbed iron to the growing points.

Internal changes as a response to high lime developed rapidly. When plants were allowed to absorb radioiron under low-lime conditions, and were then transferred to high-lime conditions, they became chlorotic more quickly than did similar plants transferred to low-lime, minus-iron conditions. Very little of the iron that had been absorbed prior to treatment was translocated to the shoot apices under the high-lime conditions. On the other hand, sufficient iron was translocated from roots to developing leaves under the low-lime, minus-iron conditions to meet the requirements of those leaves for some time and thus delayed the onset of chlorosis. Such a means for supplying iron to developing leaves under low-lime could be even more important in a woody plant and could be the basis for the long delay in the development of chlorosis in azaleas as observed by Orr (100) and by Twigg and Link (128).

It was apparent from examination of autoradiograms that most of the iron was translocated from the roots to the shoot apices, with

progressively less to the more mature leaves. Much of the iron initially incorporated in the apical tissues could have been utilized in the leaf tissue that developed from them. Lateral buds do not appear in the autoradiograms to have received an appreciable amount of radioiron in the early period. Therefore, the shoots that grew from the lateral buds after the terminal buds were removed must have received radioiron from the roots, and possibly lower stem, to account for the radioiron present.

The first leaves that formed on the lateral shoots contained more radioiron than did the later ones. This observation could be interpreted as evidence that much of the iron in the lateral buds was present in the emerging leaves and more advanced primordia and was supplemented by radioiron that was being redistributed from the roots to these leaves during their enlargement. Leaves that developed later probably did so from primordia that were not present in the meristems during the period of initial distribution. After redistribution from the roots declined, there was no source of radioiron for those leaves.

Chlorosis developed in the leaves of the growing plants in relation to the decrease in radioiron as revealed on examination of the autoradiograms. There was only slight interveinal pattern of chlorosis in chrysanthemums and little restriction of radioiron to the veins. In azaleas, on the other hand, there was a pronounced interveinal pattern of chlorosis with an equally pronounced pattern of accumulation of radioiron in and near the veins as seen in the autoradiograms.

SUMMARY

Iron when supplied as ferric chloride in aerated nutrient solutions precipitated so rapidly that such a form of iron could not be considered for meeting plant requirements in nutrient solutions. When supplied in the form, ferric ammonium citrate, iron was more stable, but was subject to the precipitating effect of high pH associated with high bicarbonate, and particularly so when micro-organisms were present to hasten the decomposition of the organic molecule. As ferric ethylenediamine tetraacetate, iron was stable under all conditions examined here. Frequent additions to nutrient solutions were necessary to maintain an iron supply as plants absorbed it.

High concentrations of soluble lime (3mM bicarbonate) in nutrient solutions induced chlorosis in several cultivars of chrysanthemums. In all such cases, the total concentration of iron in leaf tissue was less than it was in green leaves. This reduction was comparable to that observed in chlorotic leaves resulting from withholding iron.

Iron concentrations in chlorotic leaves of chrysanthemums were observed to be higher than in green leaves when chlorosis was induced by high lime associated with high phosphate, or by high phosphate alone, suggesting the accumulation of iron under those conditions which was not active in chlorophyll maintenance. With the azalea plant, lower iron concentrations were observed in chlorotic leaves induced by high bicarbonate in the irrigation water or by high phosphate when phosphoric acid was used to neutralize a tap water

of high bicarbonate content. However, during the second season under the same conditions, growth of foliage was much reduced and higher iron concentrations were found in chlorotic leaves than in green leaves. A greater total amount of iron was accumulated by the leaves of the green plants than by the chlorotic plants, however, and the difference in resultant concentration of iron in the leaf tissue was attributed to the reduction in growth rather than to an accumulation of inactive iron.

Conditions of high lime which induced chlorosis in chrysanthemums reduced the capacity for root absorption of iron and reduced the translocation of absorbed iron to the developing leaves.

The chrysanthemum plant was found by radioisotope techniques to have an iron reserve capable of supplying iron to developing leaves over a period of at least nine weeks. Such a reserve apparently was in the roots, having accumulated during a brief exposure to radioiron. This root iron continued to move into developing leaves whether or not there was a continuing supply of iron to the root system, though a higher proportion of initial root iron was translocated to leaves under conditions of no iron for root absorption. Under conditions of high lime, however, very little iron initially absorbed was translocated to new shoots.

Absorption of iron by leaves of chrysanthemum plants was not adversely affected by conditions of high lime in the root medium. On the contrary, a deficiency of iron in the leaf tissue, whether caused by high lime or by an absence of iron, led to increased rates of foliar absorption of iron. If bicarbonate were present in the

foliar treatment solution, or if that solution were of high pH (greater than pH 6) by any cause, absorption of iron by the leaves was greatly reduced. There was no demonstrable effect of bicarbonate on foliar absorption other than in causing precipitation of iron from the treatment solutions. However, when iron was stabilized in such solutions as either ferric ammonium citrate or ferric ethylendiamine tetraacetate, there was little or no improvement in absorption rate. Furthermore, iron as ferric ethylenediamine tetraacetate was not absorbed from solutions of low pH as readily as was ferric chloride or ferric ammonium citrate.

Autoradiograms established that most of the iron being absorbed by healthy plants is translocated to the youngest leaves and to active terminal buds. It was found, however, that older leaves from the basal portion of plants could absorb iron from foliar treatment solution when detached from the plant as readily as could detached young leaves.

A possible interaction between bicarbonate and phosphate as causal factors in lime-induced chlorosis was suggested in experiments in which both phosphate and lime rates were varied. High lime led to increased phosphorus and decreased iron accumulation in leaves of chrysanthemums. High phosphate supply under either low-lime or high-lime conditions had similar effects. Chlorosis developed under any of these three conditions. The decrease in leaf accumulation of iron was associated with a decrease in root accumulation of iron, suggesting decreased absorption as the effect rather than decreased translocation. A decrease in root accumulation of iron was observed

when the form of phosphate was ammonium phosphate and solution pH was 4.5, as well as when dibasic potassium phosphate was used and solution pH was 7.2, which indicates that some means other than precipitation of iron from solution could account for the effect on iron availability. Precipitation from solution of high pH was observed to be a factor in inducing chlorosis.

The use of ferric ethylenediamine tetraacetate made possible the maintenance of a supply of soluble iron under all conditions examined, and in such cases chlorosis did not develop. The concentration of iron in leaves of plants supplied with this chelated form of iron was higher than chlorotic leaves of plants supplied with ferric ammonium citrate under high lime or high phosphate or both. Furthermore, there was less leaf accumulation of phosphorus when EDTA-iron was used than when ferric ammonium citrate was used under the same adverse conditions of lime or phosphate.

BIBLIOGRAPHY

1. Arnon, D. I. 1937. NH_4 and NO_3 nitrogen nutrition of barley at different seasons in relation to H^+ concentration, Mn, Cu and O_2 supply. Soil Sci. 44: 91-113.
2. _____. 1939. Effect of NH_4 and NO_3 nitrogen on the mineral composition and sap characteristics of barley roots. Soil Sci. 44: 295-307.
3. _____ and C. M. Johnson. 1941. Influence of H^+ concentration on the growth of higher plants under controlled conditions. Plant Physiol. 17: 525-539.
4. _____, W. E. Fratzke, and C. M. Johnson. 1942. H^+ concentration in relation to absorption of inorganic nutrients by higher plants. Plant Physiol. 17: 515-524.
5. Association of Official Agricultural Chemists. 1956. Official Methods of Analysis., 8th Ed. AOAC, Washington, D. C.
6. Baxter, P. and R. Belcher. 1955. The role of the bicarbonate ion in lime induced chlorosis. Jour. Aust. Inst. of Agric. Sci. 21: 32-34.
7. Bear, F. E., Editor. 1957. Chelates in Plant Nutrition. Soil Sci. 84: 1-97.
8. Bedri, A. A., A. Wallace, and W. A. Rhoads. 1960. Assimilation of bicarbonate by roots of different plant species. Soil Sci. 89: 257-263.
9. Bennett, J. P. 1945. Iron in leaves. Soil Sci. 60: 91-105.

10. Ehan, K. C., R. C. Huffaker, A. A. Bedri, R. T. Mueller, R. A. Jeffreys, R. M. Carmack, L. I. Fiely, and A. Wallace. 1960. Possible relationships of bicarbonate or CO_2 assimilation to cation accumulation by plant roots. Soil Sci. 89: 276-284.

11. _____, A. Wallace, and E. J. Krohn. 1962. Effect of pH and nitrogen source on ability of corn and soybean to obtain Fe chelated with ethylenediamine di-o-hydroxyphenylacetate. Agron. Jour. 54: 119-21.

12. Biddulph, C. 1947. Interrelations between Fe & P in plant nutrition. Ore. St. College Biology Colloquium, Nutrition: 24-27.

13. Bingham, F. T., and J. P. Martin. 1956. Effects of soil phosphorus on growth and minor element nutrition of citrus. Soil Sci. Soc. Amer. Proc. 20: 313-5.

14. Bradfield, R. 1941. Calcium in the soil. Physico-Chemical Relations. Soil Sci. Soc. Amer. Proc. 6: 8-15.

15. Branton, D. and L. Jacobsen. 1962. Iron transport in pea plants. Plant Physiol. 37: 539-545.

16. _____ and L. Jacobsen. 1962. Iron localization in pea plants. Plant Physiol. 37: 546-551.

17. Brown, J. C. 1953. The effect of dominance of a metabolic system requiring Fe or Cu on development of lime-induced chlorosis. Plant Physiol. 28: 495-502.

18. _____. 1955. Iron chlorosis. An. Rev. Plant Physiol. 7: 171-191.

19. _____ and S. B. Hendricks. 1952. Enzymatic activities as

indication of copper and iron deficiency in plants. Plant Physiol. 27: 651-60.

20. _____ and R. S. Holmes. 1955. Fe, the limiting element in a chlo.: Part I availability and utilization of Fe dependent upon nut. and pl. species. Plant Physiol. 30: 451-57.

21. _____. 1956. Iron supply and interesting factors related to lime-induced chlorosis. Soil. Sci. 82: 507-519.

22. _____, _____, and L. C. Tiffin. 1958. Iron chlorosis in soybeans as related to genetype of rootstock. Soil Sci. 86: 75-82.

23. _____, _____, and _____. 1959. Hypotheses concerning Fe chlorosis. Soil Sci. Soc. Amer. Proc. 23: 231-4.

24. _____, _____, and _____. 1961. Iron chlorosis in soybeans as related to genotypes rootstock. II: A relationship between susceptibility to chlorosis and reductive capacity at the root. Soil Sci. 91: 127-132.

25. _____, and W. E. Jones. 1962. Absorption of Fe, Mn, Zn, Ca, Rb, and Phosphate ions by soybean roots that differ in their reductive capacity. Soil Sci. 94: 173-179.

26. _____, C. R. Lunt, R. S. Holmes, L. C. Tiffin. 1959. The HCO ion as an indirect cause of Fe chlorosis. Soil Sci. 88: 200-6.

27. _____, and L. C. Tiffin. 1962. Zn deficiency and iron chlorosis dependent on plant species and nutrient element balance in Tylare clay. Agron. Jour. 54: 356-8.

28. _____, _____, R. S. Holmes, A. W. Specht, and J. W. Resnicky. 1959. Internal inactivity of Fe in soybean as affected

by root growth medium. Soil Sci. 87: 89-94

29. Brown, J. W. and C. H. Wadleigh. 1955. Influence of $NaHCO_3$ on growth and chlorosis of garden beets. Bot. Gaz. 116: 201-209.

30. Burtch, R. M., D. W. Thorne, F. B. Wann. 1948. The effect of light, soil temperature, and soil moisture on lime-induced chlorosis. Soil Sci. Soc. Amer. Proc. 13: 374-8.

31. Cain, J. C. 1952. A comparison of NH_4- and NO_3-N for blueberries. Proc. Amer. Soc. Hort. Sci. 59: 161-166.

32. Chaberek, S., and A. E. Martell. 1959. Organic Sequestering Agents. Proc. Amer. Soc. Hort. Sci. 75: 440.

33. Chang, H. T. and W. E. Loomis. 1945. Effect of CO_2 on absorption of water and nutrients by roots. Plant Physiol. 20: 221-32.

34. Chapman, H. D. 1939. Absorption of Fe from finely ground magnetite by citrus seedlings. Soil Sci. 48: 309-13.

35. Clark, R. B., A. Wallace, R. T. Mueller. 1961. Dark CO_2 fixation in avocado roots, leaves, and fruit. Proc. Amer. Soc. Hort. Sci. 78: 161-8.

36. Colgrove, H. S., Jr., and A. N. Roberts. 1956. Growth of azalea as influenced by NH_4 and NO_3-N. Proc. Amer. Soc. Hort. Sci. 68: 522-536.

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

38. _____, and E. L. Strmecki. 1954. An investigation into growth promoting effects of a liquite. Physiologia Plantarium 7: 503-11.

39. Dovey, R. C., R. L. Smith, H. H. Wiebe. 1960. Effects of various levels of bicarbonate, phosphorus, and pH on the translocation

of foliar applied iron in plants. Soil Sci. 89: 269-275.

40. Eaton, F. M. 1936. Automatically operated sand-culture equipment. Jour. Agr. Res. 53: 433-444.

41. Franco, C. M., and W. E. Loomis. 1948. The absorption of P and Fe from nutrient solutions. Plant Physiol. 22: 627-34.

42. Gaugh, H. G., and C. H. Wadleigh. 1951. Salt tolerance and chemical composition of Rhodes and Dallis grasses grown in sand cultures. Bot. Gaz. 112: 171-5.

43. Gile, P. L. 1920. Cause of lime-induced chlorosis and availability of Fe in the soil. Jour. Agr. Res. 20: 33-61.

44. _____, and J. C. Carrero. 1914. Assimilation of colloidal Fe by rice. Jour. Agr. Res. 3: 205-210.

45. Goss, J. A., and E. M. Romney. 1959. Effects of bicarbonate and some other ions on the shoot content of P^{32}, Ca^{45}, Fe^{59}, Rb^{86}, Sr^{90}, Ru^{106}, Cs^{137}, Ce^{144} in bean and barley plants. Plant and Soil 10: 233-241.

46. Granick, S. 1950. The structural and functional relationships between heme and chlorophyll. The Harvey Lecture Series 44: 220-45.

47. Greenwald, I. 1945. The effect of phosphate on the solubility of $CaCO_3$ and of HCO_3 on the solubility of Ca lg phosphates. Jour. Biol. Chem. 161: 697-704.

48. Gris, E. 1844. Nouvelle's experiences sur l'action des composes ferrugineux solubles appliques a la vegetation et specialment en traitment de la chlorose et de la debilite des plantes. Comt. Rend. Acad. Sci. (Paris) 19: 18-19.

49. Guest, P. 1944. Root-contact phenomena in relation to Fe nutrition and growth of citrus. Proc. Amer. Soc. Hort. Sci. 44: 43-48.

50. Gustafson, F. G. 1935. Diurnal changes in the acidity of Bryophyllum calycinum. Jour. Gen. Physiol. 7: 719-725.

51. Haas, A. R. C. 1930. Studies on the reaction of plant juices. Soil Sci. 9: 341-360.

52. Hageman, R. H., and E. L. Hartman. 1939. Injuries produced by saline and alkaline waters on greenhouse plants and the alleviation of alkaline injury by neutralization. Proc. Amer. Soc. Hort. Sci. 39: 375-380.

53. Hale, W. J., and A. Wallace. 1960. HCO_3- and P effects on uptake and distribution in soybeans of iron chelated with ethylenediamine di-o-hydroxyphenylacetate. Soil Sci. 89: 285-7.

54. Harley, C. P., and R. C. Lindner. 1945. Observed responses of apple and pear trees to some irrigation waters of north central Washington. Proc. Amer. Soc. Hort. Sci. 46: 35-44.

55. Heller, V. G., R. H. Hageman, and E. L. Hartman. 1940. Sand culture studies of the use of saline and alkaline waters in greenhouses. Plant Physiol. 15: 727-33.

56. Hoagland, D. R., and D. I. Arnon. 1938. The water culture method for growing plants without soil. Calif. Exp. Sta. Cir. 347.

57. _____, and T. C. Broyer. 1936. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. 11: 471-507.

58. _____, and _____. 1940. Hydrogen ion effects and accumulation of salt by barley roots as influenced by metabolism. Amer. Jour. Bot. 27: 173-85.

59. Hoffer, G.N. 1923. Accumulation of aluminum and iron compounds in corn plants and its probably relation to rootrots. Jour. Ag. Res. 23: 301-23.

60. Holmes. R. S. 1960. Effect of phosphorus and pH on iron chlorosis of blueberry in water culture. Soil Sci. 90: 374-9.

61. Horner, C. K., D. Burk, and S. R. Hoover. 1934. Preparation of humate iron and other humate metals. Plant Physiol. 9: 663-9.

62. Huffaker, R. C., and A. Wallace. 1959. Dark fixation of CO_2 in homogenates from citrus leaves, fruits, and roots. Proc. Amer. Soc. Hort. Sci. 74: 348-357.

63. Iljin, W. S. 1951. Metabolism of plants affected with lime-induced chlorosis (calciosis): II Organic acid and carbohydrates. Plant and Soil 3: 339-351.

64. _____. 1952. Metabolism of plants affected with lime-induced chlorosis (calciosis). Plant and Soil 4: 11-28.

65. Ingalls, R. A., and J. W. Shive. 1931. Relation of H-ion concentration of tissue fluids to distribution of iron in plants. Plant Physiol. 6: 103-125.

66. Jacobson, L. 1945. Iron in the leaves and chloroplasts of some plants in relation to their chlorophyll content. Plant Physiol. 20: 233-245.

67. _____. 1951. Maintenance of iron supply in nutrient solutions by a single addition of ferric potassium ethylene-diamine tetra-acetate. Plant Physiol. 26: 411-413.

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

69. _____, and L. Crdin. 1954. Organic acid and ion absorption in roots. Plant Physiol. 29: 70.

70. Jeffreys, R. A., V. W. Hale, and A. Wallace. 1961. Uptake and translocation in plants of labeled iron and labeled chelating agents. Soil Sci. 92: 268-73.

71. Jenny, Hans. "Plant root-soil interactions" in F. X. Zarrow, ed., Growth in Living Systems. Basic Books, Inc., New York 3. 1961. pp. 665-94.

72. Jenny, H., and R. Overstreet. 1938. Contact effects between roots and soil colloids. Proc. Natl. Acad. Sci. 53: 384-392.

73. Jones, H., and E. H. Eagles. 1962. Translocation of Carbon ^{14} within and between leaves. Annals of Botany 26: 505-511.

74. Jyung, W. H., and S. H. Wittwer. 1963. Kinetics of foliar absorption. Plant Physiol. 38 (suppl): XXVI

75. Kenworthy, A. L., and E. J. Miller. 1956. Nutrient-element analysis of fruit tree leaf samples by several laboratories. Proc. Amer. Soc. Hort. Sci. 67: 16-21.

76. Kramer, Paul J. 1957. Outer Space in Plants. Sci. 125: 633-5.

77. Kurtz, E. B., Jr., and R. H. Maier. 1960. Acid chlorosis and iron uptake by sorghum (Double Dwarf-38) grown in solution culture. Agron. Jour. 52(8): 486-487.

78. Latimer, W. L. 1952. Oxidation Potentials. 2nd Ed. Prentice-Hall, New York.

79. Leonard, C. D., and I. Stewart. 1953. An available source of iron for plants. Proc. Amer. Soc. Hort. Sci. 62: 103-110.

80. Lindner, R. C., and C. P. Harley. 1944. Nutrient interrelations in lime-induced chlorosis. Plant Physiol. 19: 420-39.

81. Lindsay, W. L., and D. W. Thorne. 1954. Bicarbonate ion and oxygen level as related to chlorosis. Soil Sci. 77: 271-9.

82. Lingle, J. C., L. C. Tiffin, and J. C. Brown. 1963. Iron Uptake-transport of soybeans as influenced by other cations. Plant Physiol. 38: 71-76.

83. Loehwing, W. F. 1928. Calcium, potassium, and iron balance in certain crop plants in relation to their metabolism. Plant Physiol. 3: 261-75.

84. Lucas, R. E., and J. F. Davis. 1961. Relationships between pH values of organic soils and availabilities of twelve plant nutrients. Soil Sci. 92: 177-182.

85. Lunt, C. R., and H. C. Kohl, Jr. 1956. Absorption and translocation of iron from various sources by rose leaves. Sym. on the use of Metal Chelates in Plant Nutrition, A. Wallace, Editor.

86. McCall, A. G., and J. R. Haag. 1921. The relation of H+ concentration of nutrient solutions to growth and chlorosis of wheat plants. Soil Sci. 12: 69-77.

87. McGeorge, W. T. 1949. A study of lime-induced chlorosis in Arizona orchards. Ariz. Ag. Exp. Sta. Tech. Bull. 117.

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

89. Mattson, S. 1930. The laws of soil colloidal behavior: III Iso-electric precipitates. Soil Sci. 30: 459-495.

90. Milad, Y. 1921. The distribution of iron in chlorotic pear trees. Proc. Amer. Soc. Hort. Sci. 21: 93-98.

91. Miller, G. W., J. C. Brown, R. S. Holmes. 1960. Chlorosis in soybean as related to Fe, P, HCO_3, and cytochrome oxidase activity.

Plant Physiol. 35: 619-625.

92. _____, and H. J. Evans. 1956. Inhibition of cytochrome oxidase by bicarbonate. Nature 178: 974-976.

93. _____, and _____. 1956. The influence of salts on activity of particulate cytochrome oxidase from roots of higher plants. Plant Physiol. 31: 357-64.

94. _____, and D. W. Throne. 1956. Effect of bicarbonate on the respiration of excised roots. Plant Physiol. 31: 151-5.

95. Meher, D. D., R. L. Smith, and H. H. Wiebe. 1956. Effect of plant micronutrient balance on the translocation of foliar applied chelated and non-chelated iron. Symp. on use of Metal Chelates in Plant Nutrition. A. Wallace, Editor.

96. North, C. P. 1956. Translocation of chelated and non-chelated iron applied to avocado seedlings. Symp. on use of Metal Chelates in Plant Nutrition. A. Wallace, Editor.

97. Oertli, J. J., and L. Jacobson. 1960. Some quantitative considerations in iron nutrition of higher plants. Plant Physiol. 35: 683-8.

98. Olsen, C. 1935. Iron absorption and chlorosis in green plants. Compt. rend. trav. lab. Carlsberg, ser. chim 21: 35-50. Abstract only.

99. _____. 1953. Iron uptake in different plant species or a function of pH value of the nutrient solution. Physiologia Plantarum 11: 889-905.

100. Orr, H. P., Tokuji Furuta, and Charles W. Bell. 1957. Azalea fertilization. Ala. Exp. Sta. Cir. 116.

101. Oserkowsky, J. 1931. Hydrogen ion concentration and iron content

of tracheal sap from green and chlorotic pear trees. Plant Physiol. 7: 353-9.

102. _____. 1933. Quantitative relation between chlorophyll and iron in green and chlorotic pear leaves. Plant Physiol. 8: 449-68.

103. Overstreet, R., S. Ruben, and T. C. Broyer. 1940. The absorption of bicarbonate by barley plants as indicated by studies with radioactive carbon. Proc. Nat. Acad. Sci. 26: 638-95.

104. Parry, R. W. Chelation and the theory of heterocyclic ring formation involving metal ions. Chem. of Coordination Compounds. Bailar, Editor.

105. Porter, L. K., and D. W. Thorne. 1955. Interrelation of carbon dioxide and bicarbonate ions in causing plant chlorosis. Soil Sci. 79: 373-82.

106. Rediske, J. H., and C. Biddulph. 1953. The absorption and translocation of iron. Plant Physiol. 28: 576-93.

107. Reuther, W., and C. L. Crawford. 1946. Effect of certain soil and irrigation treatments on citrus chlorosis in a calcareous soil: I Plant Responses. Soil Sci. 62: 477-91.

108. _____, and _____. 1947. Effects of certain soil and irrigation treatments in citrus chlorosis in a calcareous soil: II soil atmosphere studies. Soil Sci. 63: 227-52.

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

110. Sendroy, J., Jr., and A. B. Hastings. 1925. The solubility product of secondary and tertiary calcium phosphate under various conditions. Jour. Biol. Chem. 64: 509.

111. Shannon, L. M. 1956. Some chelate studies concerning the behavior of iron chlorosis. Sym. on the use of Metal Chelates in Plant Nutrition. A. Wallace, Editor.

112. 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 interpretation of results. Plant Physiol. 25: 496-506.

113. Sims, W. H., and W. H. Gabelman. 1956. Iron chlorosis in spinach induced by phosphorus. Proc. Amer. Soc. Hort. Sci. 67: 445-450.

114. Somers, I. I., and J. W. Shive. Iron-manganese relation in plant metabolism. Plant Physiol. 17: 582-602.

115. Spurway, C. H. 1933. Soil Testing, A Practical System of Soil Diagnosis. Mich. Ag. Exper. Sta. Tech. Bull. #132.

116. Stalwijk, J. A., and K. V. Thiamann. 1959. On the uptake of carbon dioxide and bicarbonate by roots and its influence on growth. Plant Physiol. 32: 513-20.

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

118. Stewart, F. C., and C. Preston. 1941. Effects of pH, and components of bicarbonate and phosphate-buffered solutions on the metabolism of potato discs and their ability to absorb ions. Plant Physiol. 16: 481-519.

119. Su, L. Y., and G. W. Miller. 1961. Chlorosis in higher plants as related to organic content. Plant Physiol. 36: 415-418.

120. Taylor, G. A. 1956. The effectiveness of five cleaning procedures in the preparation of apple leaf samples for analysis.

Proc. Amer. Soc. Hort. Sci. 67: 5-9.

121. Teubner, F. G., A. A. Wittwer, W. C. Long, and H. B. Tukey. 1957. Some factors affecting absorption and transport of foliar-applied nutrients as revealed by radioactive isotopes. Mich. Agr. Exp. Sta. Q. Bull. 39: 398-413.

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

123. _____, and A. Wallace. 1944. Some factors affecting chlorosis on high lime soil: I ferrous and ferric iron. Soil Sci. 57: 299-312.

124. Tiffin, L. C., and J. C. Brown. 1959. Absorption of iron from iron-chelate by sunflower roots. Sci. 130: 274-5.

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

126. _____, and _____. 1961. Iron chelates in soybean exudate. Sci. 135: 311-313.

127. _____, and _____, and R. W. Krauss. 1960. Differential absorption of metal chelates components by plant roots. Plant Physiol. 35: 362-7.

128. Twigg, M. C., and C. B. Link. 1951. Nutrient deficient symptoms and leaf analysis of azaleas grown in sand culture. Proc. Amer. Soc. Hort. Sci. 60: 369-375.

129. Tyman, E. S. 1959. The effect of iron supply on the yield and composition of leaves of tomato plants. Plant and Soil 10: 375-88.

130. Wadleigh, C. H., and J. W. Brown. 1952. The chemical status of bean plants afflicted with bicarbonate induced chlorosis.

Bot. Gaz. 113: 273-92.

131. _____, and J. W. Shive. 1939. Base content of corn plants as influenced by pH of substrate and form of N supply. Soil Sci. 47: 273-83.

132. Wall, R. F., and E. L. Hartman. 1948. Sand culture studies of the effect of added salts upon the composition of tomato. Proc. Amer. Soc. Hort. Sci. 40: 461-6.

133. Wallace, A. Effectiveness of iron-chelates in presence of sodium bicarbonate. Symp. on use of metal chelates in Plant Nutrition, A. Wallace, Editor. 1956.

134. _____, and A. A. Bedri. 1958. Iron and zinc foliage sprays. Calif. Agr. 12(3), p.3.

135. _____, R. A. Jeffreys, and V. J. Hale. 1962. Differential ability of two soybean varieties to take up Fe^{59} from soil added to a nutrient solution. Soil Sci. 94: 111-114.

136. _____, and C. M. Lunt. 1960. Iron chlorosis in horticultural plants, A Review. Proc. Amer. Soc. Hort. Sci. 75: 819-841.

137. _____, R. T. Mueller, D. Van Poort, and C. P. North. 1960. Dark carbon dioxide fixation of virus-diseased, iron-chlorotic, and genetic-chlorotic leaves. Proc. Amer. Soc. Hort. Sci. 76: 679-681.

138. _____, and C. P. North. 1953. Lime-induced chlorosis. Calif. Agr. 7(6): 10.

139. _____, _____, R. T. Mueller, L. L. Shannon, and M. Hamidan. 1955. Behavior of chelating agents in plants. Proc. Amer. Soc. Hort. Sci. 65: 9-16.

140. Wallace, T. 1930. Investigations on chlorosis of fruit trees.

Journ. Expl. and Hort. Sci. 7: 179-196.

141. Wallihan, E. F. 1955. Relation of chlorosis to concentration of Fe in citrus leaves. Amer. Journ. of Bot. 42: 101-104.

142. _____. 1961. Effect of sodium bicarbonate on iron absorption by orange seedlings. Plant Physiol. 36: 52-53.

143. Weinstein, R. N., E. R. Purvis, A. M. Weiss, and R. L. Uhler. 1954. Absorption and translocation of EDTA by sunflower plants. Ag. and Food Chem. 2: 421-425.

144. _____, W. R. Robbins, and H. F. Perkins. 1954. Chelating agents and plant nutrition. Sci. 120: 41-43.

145. Weiss, M. G. 1943. Inheritance and physiology of efficiency in iron utilization in soybean. Genetics 28: 253-63. Abstract only.

146. Welcher, F. J. The Analytical Uses of EDTA. Von Nostrand, Inc., New York.

147. White, H. E. 1954. Response of rose and gardenia to treatment with iron chelate and chelating agent. Proc. Amer. Soc. Hort. Sci. 64: 423-430.

148. Wildon, C. E. 1957. Growth of rose, alfalfa and tobacco plants as affected by different sources of Fe. Mich. Ag. Exp. Sta. J. Bull. 39(4): 628-634.

149. _____. 1958. The effect of prolonged leaching and of successive generations of plants on the solubility of iron and manganese in glassy frits. Proc. Amer. Soc. Hort. Sci. 72: 443-449.

150. Wynd, F. L. 1951. Availability to wheat plants of iron in very insoluble glass frits. Lloydia 14: 1-33.

151. _____, and R. A. Bowden. 1951. Glassy frits as a source

of iron for blueberry plants on calcareous soils. Hlgardia 14: 55-63.

152. _____, and C. E. Wilden. 1955. The use of glassy frits for hydroponic culture of tomato. Hlgardia 13: 109-128.

APPENDIX TABLE 1. Nutrient Solutions

I. Hoagland's Number One Solution

Compound	Stock Concentration	Dilution (ml/l)
KH_2PO_4	1 molar	1
KNO_3	1 molar	5
$Ca(NO_3)_2$	1 molar	5
$Mg\ SO_4$	1 molar	2

II. Twigg and Link's Solution

Compound	Stock Concentration	Dilution (ml/l)
KH_2PO_4	1 molar	1
$(NH_4)_2SO_4$	1.75 molar	1
$Ca(NO_3)_2$	1 molar	1
$Mg\ SO_4$	1 molar	1

III. Hoagland's "A" Solution

Compound	Stock Concentration (g/l)
H_3BO_3	2.86
$Mn\ SO_4.H_2O$	1.55
$Zn\ SO_4 \cdot 7H_2O$	0.22
$Ca\ SO_4 \cdot 5H_2O$	0.08
$H_2MoO_4 \cdot H_2O$	0.02

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