EVALUATION OF IRON SOURCES FOR TREATMENT OF CHLOROSIS IN CITRUS TREES

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Iron chlorosis in Citrus

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ABSTRACT

A young grapefruit orchard in Bouar, showing symptoms of iron deficiency, was treated with four different chemicals with the purpose of estimating their effectiveness as sources of iron for correcting iron chlorosis. The chemicals used were NaFe-EDTA, ferrous sulfate, Greenz 26 and chel 330, all were used at two levels. With the exception of chel 330, all were used as soil application, and apart from NaFe-EDTA, the rest were used as foliar sprays. In addition, acid water (pH=5) and tap water were also sprayed. Each treatment was replicated four times. Leaf samples were collected before applying the treatments and six months later and were analyzed for iron, nitrogen, phosphorus, potassium, calcium, magnesium, and manganese, with the intention of finding some possible relation of iron chlorosis to those elements.

Results of said analysis indicated that ferrous sulfate at the rate of 14 grams per liter and ten grams per liter, and Greenz 26 at the rate of eleven grams per liter gave a significant increase in iron content of the leaves. In general foliar sprays showed significantly better results as compared to soil applications in treating iron chlorosis, indicating that the amounts applied in the soil were probably low. No relation was found between iron content of the leaves and the degree of iron chlorosis. Most of the chemicals applied showed a greening effect on the leaves; this may be

ascribed to the activation of the iron already present. There was no relation between iron and nitrogen content of the leaves; phosphorus, calcium and manganese, however, were present at high levels and this excess was suspected as being one of the major causes of iron chlorosis. Potassium content was in the normal range, while magnesium was slightly low. Although iron content of the leaves was high, it might have been that part of it was in the physiologically inactive state.

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INTRODUCTION

Iron is one of the essential elements required in small quantities by plants. Its deficiency has been noticed in different parts of the world for some time. A special pattern of chlorosis in the leaves makes its deficiency symptoms different from symptoms of other micro-element deficiencies. Chlorotic trees have a lighter bloom than healthy trees, and consequently there is a reduced production of fruit. Fruits from iron deficient trees mature early and are of poor quality (12). Chlorotic plants are weak and more subject to winter injury and to the attack of plant diseases and insects (12). Under severe cases of chlorosis, the tree may even die (12,37,38,69,72). This makes iron nutrition an important factor in fruit production.

A great amount of research has been carried on during the past 50 years attempting to solve the problem of iron chlorosis; out of this work excellent results were achieved with some treatments for particular locations. It was not possible to duplicate the successful treatments in some areas due to differences in soil conditions under which the plants were growing.

Many iron compounds have already been tested in an attempt to correct for iron chlorosis; the most commonly used iron salts were ferrous sulfate and tartarate, ferric malate, phosphate and citrate as well as others. The said compounds

were tried as soil applications, foliar sprays and by trunk injections. Recently chelates have been used as carriers of iron for plants, but even said materials were not effective under all conditions and no consistent results have been obtained by any one of them. All these findings indicate the importance of iron chlorosis and the need for more research in said field.

In Lebanon any trained observer can notice in most orchards, that there is a chlorosis problem. The farmers are aware of the fact and ascribe it to several factors like excess water or lack of nitrogen, while others consider it a disease; however very few of them have attempted to correct the deficiency. This lack of attempt is mainly due to improper guidance as to how the problem of chlorosis could be solved.

The present study was initiated in June 1958 with the purpose of trying out different chemicals claimed to be effective in controlling iron chlorosis, and finding out the best suitable material under the conditions of the experiment. Chemical tissue analysis of some elements other than iron was carried on with the intention of trying to find out the relation of iron to those elements.

REVIEW OF THE LITERATURE

I. Iron supply to plants.

Iron is the third most abundant mineral element in the earth's crust (61) and is not deficient in most soils. Wallace (68) believes that iron occurs in soils in the form of its oxides and it is these compounds that are mainly responsible for the red and brown colors in soils. Brown (10) considers that variations in the capacity of plants to absorb and accumulate iron must be due to factors other than the level of this element in the soil; its deficiency is usually a consequence of its insolubility rather than its abscence (43). The findings of Brown (10) indicate that the availability of any supply of iron in the growth media is dependent upon both the plant species and the growth media. He further states that most iron compounds occurring naturally in soils are, to a great extent, insoluble while the ferrous ions which are soluble, are unstable in aerated soils with a pH of six or above.

II. Functions of iron in plant nutrition.

Iron is indispensable as a catalyst for the synthesis of chlorophyll in green plants (43,68). It is considered that the state in which iron exists in plant tissues is often a determining factor in chlorophyll synthesis (43,68). Physiologically active iron is in the ferrous state, and al-

though it may be absorbed as ferric, the latter is rapidly reduced within the cell. Manganese seems to be an oxidizing agent for iron and an excess of it may induce iron deficiency symptoms by converting the available iron into ferric iron (43). Iron is also thought to be a catalyst for the process of oxygen carrying in respiration (68).

Inorganic iron salts possess the properties of an oxidase and of an electron transporter. Some of the cytochromes are oxidases and function as activators of atmospheric oxygen; others are electron transporters (10). The oxidation reduction mechanism of the cytochromes is not fully known, however the only noticeable difference between the oxidized and reduced forms lies in the valency of the iron atom (2).

$$Fe^{+++} + e^{-} \longrightarrow Fe^{++}$$

Cytochromes occur widely in the plant kingdom and especially in embryonic or juvenile organs (27). Hewitt (27) reports that all the known enzymes that depend on iron involve porphyrin molecules and ferrous porphyrin compounds are less stable than the ferric; consequently iron would be replaced more readily in a ferrous complex than in a ferric complex. This supports the commonly held view that ferrous iron is the active form in chlorophyll production. Therefore some of the enzymes that operate in respiration are iron compounds, examples of which, are the catalase, peroxidase, cytochrome oxidase and the cytochromes (43).

Another function of iron is its effect on root growth. Following an application of Fe-EDTA by Ford (15) to trees showing extensive root damage induced by iron deficiency, a pronounced new growth of roots took place.

Ford et al (16) studied the relation between the degree of chlorosis and the amount of root damage after the application of Fe-EDTA. The feeder root system of severely chlorotic trees increases from one to nine grams, in a square foot column five feet deep, six months after an application of Fe-EDTA. Trees showing moderate symptoms of iron deficiency had nine grams of feeder roots per column, while nearby treated trees had 16 grams. Trees with mild symptoms had 18 grams while treated trees had 21 grams. Healthy trees had 23 grams. These findings are a good illustration of the effect of iron on root growth.

III. Mechanism of iron absorption.

Several theories were intended to explain the mechanism of absorption of the different elements. The cation exchange theory is the one accounted for in the absorption of the larger portion of cations (43,52). Exchange seems to take place either between the cations adsorbed to the root tips, mainly H^+, and those in solution, or between those adsorbed to root tips and those adsorbed to soil colloids. The latter type of exchange is termed "Contact exchange". Another less important process of absorption is the diffusion

of ions.

In his review on the mechanisms of absorption, Robertson (52) stated that combination is an important mechanism of absorption. When iron enters into the formation of a compound, resulting in a decrease in iron activity in solution, a gradient favorable to the entry of further ions of the same kind is created. Work done by Kliman (52), presents evidence that iron is absorbed in the reduced form (ferrous iron), this form being unstable in soils with a pH of six to eight. This finding indicates that the root is provided with a mechanism for reduction.

IV. Possible causes of iron deficiency.

One of the obvious causes of iron deficiency in plants is its absence from the soil. This condition however is very rare and has been seldom met with. It has not been mentioned in any of the reviewed articles and in fact the opposite was always mentioned (43). This shows that iron deficiency in plants is a result of its unavailability to plants rather than its actual absence (5,10,43).

One of the factors that contribute to iron unavailability is high manganese in the soil. Brown (10) reported that both low levels of iron and high levels of manganese in solution cultures resulted in a low activity of catalase and cytochrome oxidase in the plant. He also stated that high manganese in plants or soils, may oxidise iron to the in-

active state. Manganese even at 75 ppm in solution showed no determinal effect on growth (57), and it was reported (58) that manganese causes lesser effects than zinc or copper. Iron deficiency is not common on acid soils and when it occurs it will be the result of high ratios of manganese to iron (68).

Copper is another element whose excess causes iron deficiency (58,68). It was reported that copper is fifty times as toxic as manganese and twelve to fifteen times as toxic as zinc, to orange seedlings grown in solution cultures (10). Smith and Specht (10,57) suggested that the cause of iron deficiency in Florida citrus orchards is an accumulation of copper in the soil. Reuther and Smith (49) concluded that copper is more potent in producing iron chlorosis than either zinc or manganese. Plants receiving 2.5 ppm copper in the solution were stunted and chlorotic while those receiving 10 ppm stopped growth completely (57).

Lime induced chlorosis is the most common chlorosis encountered in orchards, and it is one of the most difficult to cure (10,43,68). It was noticed that an appreciable part of the absorbed iron is combined with other soil materials and is not available to plant cells (54). Soil and plant conditions associated with excess lime favor the oxidation of iron to the less active ferric state and its fixation in compounds of low biological activity (10).

Chlorosis was often associated with soils containing

carbonates, and there seems to be an important relation between some irrigation waters and the degree of chlorosis of plants using this water. In some areas where chlorosis was most severe, the irrigation waters came from a source with a bicarbonate concentration of 200 ppm or more. Subsequent irrigation with water low in bicarbonate tended to alleviate the condition (10). The same author reported that soil compaction, poor drainage, addition of green and barnyard manure to soils, all indicated that bicarbonate may be a causative factor of chlorosis in calcareous soils.

Soil pH is another important factor that causes iron deficiency. Insoluble forms of iron are brought into solution by the action of acids and iron availability increases with acidity and is greatly reduced by high pH values (61, 68). A test using vermiculite as a growing medium showed that when the pH was maintained around seven, growth was normal even with high amounts of copper, zinc or manganese; but when the pH was decreased and kept at four, stunting and chlorosis developed when copper or zinc were present in the growing media (57). Ferrous ions were found readily converted to $Fe(OH)_2$ in an alkaline medium and then readily oxidized to the less available $Fe(OH)_3$ (26). Therefore both the reaction and oxidation-reduction state of the soil are responsible in the transformation of iron to unavailable forms, and a high content of (Fe^{++}) in the soil is an indication of a need for lime or a state of poor aeration of the soil.

Excess phosphates were also found to have an effect in causing chlorosis. The hydrous oxides of iron are effective in removing ($H_2PO_4^-$) at low pH values because the stability of the basic phosphate is greater than that of the hydrous oxide at the low pH.

$$Fe(OH)_3 + H_2PO_4^- \longrightarrow Fe\begin{matrix}OH\\OH\\H_2PO_4\end{matrix} + OH^-$$

A shift in the pH of the soil toward alkalinity will shift the equilibrium toward a greater stability of the hydrous oxide and a release of phosphate (44). Phosphates precipitated iron in the conducting tissues of bean plants (10,37). No differences were found, in the composition of iron and phosphorus in stunted spinach leaves as compared with normal plants. This shows that the greater portion of iron in the chlorotic leaves was in an unavailable form. On the other hand Bingham and Martin (6) reported that iron was not antagonized by the addition of phosphorus fertilizers.

In acid soils, iron deficiency is common where zinc prevails, and excess zinc may show the same effect on highly calcareous soils (68). At 25 ppm zinc was slightly toxic to roots; chlorosis and stunting occurred when there were 75 ppm of zinc in the nutrient solution (57). Smith and Reuther reported that excess zinc in the nutrient solution will cause iron chlorosis in the foliage without reducing the iron content of the leaves.

Excessive amounts of potassium were reported to displace iron in the leaves from an active enzyme surface and consequently disrupt metabolic processes (10). The same author reported that low iron levels in a medium deficient in potassium induced chlorosis in potato plants and that this type of chlorosis was cured by the addition of high levels of potassium. It was believed that potassium increased iron utilization in chlorophyll formation. Iron mobility is affected by several factors among which potassium deficiency is an important one (6). Russel (54) further stated that iron deficiency can be induced as a result of potassium deficiency.

Excess soil moisture was also reported to have a bearing on chlorosis. Crawford (12) stated that excess water and lack of oxygen are important causes of iron deficiency. There is a marked increase in chlorosis after frequent irrigation treatment, therefore soil aeration has something to do with chlorosis. Boynton and Compton (9) indicated that a slight depression in oxygen, in a nutrient solution, reduced the number and size of roots produced. From the above mentioned findings one may conclude that lack of oxygen hampers the cytochrome system, responsible for respiration, and thus the rate of absorption is reduced.

Brown (10) in reporting the work of Thorne et al. stated that an unbalanced cation content of plant tissues may lead to disrupted synthetic activities with an abnormal

accumulation of certain organic acids. He also reported that, in chlorotic citrus leaves, the nitrogen is higher than in green leaves. It is believed that this increase in soluble nitrogen in chlorotic plants results from protein disintegration (10). Iljin (29) reported that in spring the leaves of chlorotic plants had a considerably higher nitrogen content than those of healthy plants. This abnormality in nitrogen content may be expected to result in physiological disturbance which will affect chlorosis.

Wander and McBride (71) suspected three materials as being possible causes of chlorosis near a superphosphate manufacturing plant, namely, Phosphoric acid, Sulfur dioxide, and volatile materials containing fluorine. Sprays of hydrofluoric acid, fluosilicic acid and phosphoric acid were applied at a concentration of one tenth normal to four-year old grapefruit trees. After seven applications, during a period of two months, a chlorotic pattern similar to that produced near the superphosphate plant appeared. This occurred on the trees which had received the hydrofluoric and fluosilicic acid sprays.

The rootstock was reported to have some influence in causing chlorosis. Galet (18) noted that the American varieties of grapevine and their hybrids, though resistant to phylloxera are more subject to chlorosis in various degrees than the European varieties. Lapedagne (35) stated that lime induced chlorosis of peach on peach rootstock may be overcome

by inarching with almond stocks. It was reported that iron was high in trees on rough lemon and low in trees on grapefruit rootstock (11). Haas (25) analyzed rootlets from several species of citrus and reported that the inorganic composition of the rootlets appeared to reflect their rootstock influence on the inorganic composition of the scion.

Crawford (12) reported that below normal temperature is unfavourable for chlorophyll formation, especially in early spring. Wallace (68) stated that iron deficiency in plants may result from high concentration of Cobalt, Nickel or Chromium in the nutrient solution.

Chlorotic leaves were found to contain an accumulation of malonic, succinic, fumaric and isocitric acids. When leaves were supplied with radioactive carbon dioxide, an organic acid component, containing much radioactive carbon, was present in green plants but essentially absent in chlorotic plants (51). McGeorge (42) also found an accumulation of citric acid in chlorotic leaves. This finding shows that there is some factor that causes a block in the organic acid metabolism in lime-induced chlorotic plants.

Inheritance also plays a role in causing iron chlorosis. In 1942 at Messina, Ruggieri (53) noted a sweet orange tree one of whose three main branches showed pronounced leaf chlorosis. The same condition remained in 1943. Buds were taken from the chlorotic branch and worked on strong seedlings of sour orange. Two years later, all the chlorotic

characters reappeared in the shoots from these buds.

V. Detection of iron deficiency.

The first approach employed in the detection of iron deficiency in plants is by use of visual symptoms. Iron deficiency is characterized in young seedlings by chlorosis which becomes more pronounced with each succeeding leaf flush. The last leaves to appear in the last flush are ivory white with tip scorch and leaf curl (23,68). Many authors have described the symptoms of iron deficiency on citrus leaves. In mild cases, the leaf blade between the veins become light green, while the veins remain dark green. As the malnutrition advances, the interveinal areas become yellow, and later the entire leaf may become ivory white. In extreme cases defoliation occurs and causes the dieback of many limbs. Chlorotic trees have a lighter bloom than non-chlorotic trees, consequently resulting in a state of reduced production of fruits which is poorly colored. Severely affected trees may die (12,37,38,69,72). Crawford (12) adds that affected leaves do not attain normal size. Fruits are small in size, mature early and are of poor quality. He further adds that chlorotic plants are weak and more subject to winter injury and to the attack of plant diseases and insects.

McGeorge (42) noted that chlorosis may appear on peaches, apples and grapes in the spring as the leaves unfurl. However, in most cases, it appears first during summer on the

topmost leaves or on the terminal leaves of outside shoots which represent the summer growth. The manner in which chlorosis appears indicated when the deficiency was induced, usually active iron is present in the spring following the winter dormancy to supply the first leaves that appear. As the season advances, additional leaf and twig growth are produced by the tree causing a state of deficiency of said element.

Tissue analysis is another means for detecting iron deficiency. The mineral content of a leaf reflects the effect of the total environment in which the tree is thriving (30). The same authors state that tissue analysis should be and is a guide to fertilizer need of plants. Wallihan (69) however found that tissue analysis was not as useful in determining iron chlorosis, because through years of comparisons that have been made between chlorotic and healthy leaves, the results have failed to show consistent differences in iron content. This lead to the conclusion that part of the iron in the leaves was not available for the manufacture of chlorophyll. However Wallihan (69) reported that considerable light was shed by Jacobson on this subject. He noticed that if careful washing of the leaves was exercised before analysis for iron, a close relationship between iron content and chlorophyll content of the leaves was established. Brown (10) mentioned that soil, dust and spray residues may be difficult to remove from the surface of plant material and this pres-

ents some difficulties to the analyst. In the same article it was reported that green leaves washed consecutively in a detergent solution, dilute hydrochloric acid and distilled water, consistently contained more iron than similarly washed chlorotic leaves from the same area. However the iron content in the leaves was not consistent from orchard to orchard and it was not possible to determine a critical value that could be used as a measure in differentiating sufficient from chlorotic leaves. Wallihan (69) noticed that most of the samples classified as extremely chlorotic contained less than 30 ppm iron, while those only moderately chlorotic contained up to about 70 ppm and those without chlorosis pattern ranged up to 100 ppm Fe. Jones et. al (31) reported that iron is deficient when its concentration is less than 35 - 50 ppm and is in excess when its concentration is more than 250 ppm. Brown (10) reported the classification of leaf iron by Kuykendall who divided it into 3 categories as follows: below 60 ppm iron, between 60 and 90, and above 90 ppm iron.

From the findings of the different workers, one tends to conclude that there is no sharp line of demarcation in iron content between chlorotic and healthy leaves. Large concentrations of iron in the leaves do not necessarily indicate a healthy condition, because the iron may be in the physiologically non active ferric state. Tissue analysis therefore gives an indication concerning the condition in which iron exists in the soil or plant. If iron is low in

chlorotic leaves it may either be tied up or deficient in the soil. If it is high in chlorotic leaves, then the deficiency symptoms are caused by inactivation of iron. Furthermore the leaves collected for analysis should be thoroughly cleaned before drying to eliminate contamination.

VI. Treatment of iron chlorosis.

1. Soil application: Among the minor elements, iron is the one that has caused most troubles and on which much work has been done. Early research workers (32) resorted to the use of iron salts such as ferrous sulfate as soil application. They obtained rapid temporary correction by applying four pounds of ferrous sulfate in holes around the tree. Crawford in 1933 (12) listed ferrous tartrate, ferrous sulfate, ferric malate, ferric phosphate and ferric citrate as soil additives. The chemicals were used in dry form and in water solution at the rate of one pound of the salt for each inch of tree diameter. The acid or acid forming materials used in combination with iron and aluminum sulfate proved to be very effective as a permanent remedy for certain plants. The solution used consisted of, one quart sulfuric acid, twenty gallons water, two pounds iron sulfate, and two pounds of aluminum sulfate (12).

Wallace (68) considers that adequate supplies of iron may be insured by dressings of sulfur that may lower the pH. Wiebosch (73) noticed in North Holland that organic manures

have an important effect in correcting iron chlorosis. This was confirmed by Wallace (68) who considered that organic matter increases the carbonic acid content of the soil and thus decreases the pH in the vicinity of plant roots. In an experiment by Guest (24) plants were able to absorb iron from finely ground magnetite but not from magnetite with coarse particles. It was suggested that iron salts react with alkaline silicates to form solutions of ferri-silicic complexes that are stable within a pH range of 3.5 to 12 (14,54).

Regardless of the salts applied, iron will sooner or later be rendered unavailable in the soil. It combines with oxygen to form iron oxide which is insoluble. Two decades ago, chemists found a new series of compounds that have the ability of surrounding iron atoms and protecting them from other chemicals in the soil (70). The said new compounds are called chelates. Many naturally occurring substances have been listed by Wallace (65) as natural chelating compounds such as, Ascorbic acid, humic acid, citric acid, tartaric acid and amino acids. But such chelates have low stability constants. The first chelating agent that was produced commercially is ethylenediamine tetra-acetic acid (EDTA); other chelating agents were developed later, of which Diethylenetriaminepentaacetic acid (DTPA), Hydroxyethylenediaminetriacetic acid (HEEDTA), and Cyclohexane trans 1,2-diaminetetraacetic acid (CDTA) are the most

common (33). Wallace (65) stated that there is evidence indicating that chelating agents without metals can enter the plant and reactivate iron previously precipitated. A great number of experiments have been conducted eversince chelates were discovered; each of them gave different results depending on many factors that are likely to interfere. Leonard and Stewart (38) found that 25-100 grams iron per tree, as Fe-EDTA-OH produced excellent recovery on calcareous soils, while 300 grams of iron as Fe-EDTA were required to bring about the same effect. In another experiment, the same authors (39) found that 20 grams iron as Fe-EDTA per tree corrected chlorosis on acid soils within two months. Wallihan et al. (70) applied up to ten pounds Fe-EDTA per tree without obtaining successful results under same California soil conditions. This was ascribed to poor drainage and high calcium carbonate in the soil. Bingham and Beutel (5), got no response with Fe-DTPA, while they got encouraging results with chel 138-HFe. Ford et al. (16) found a significant increase in feeder roots of trees treated with FeEDTA and the root growth was rapid. Geigy Co. (22) recommends one sixth to one third pound NaFe-EDTA per tree under Florida conditions. Alexander and Walsh (1) got good results with 20 grams Fe-EDTA per tree. Hilgeman (28) reported that sequestrene 330 Fe produced satisfactory regreening at the rate of two pounds per tree. Van horn (64) got very good color and growth by using one half to one

pound Sequestrene-138 HFe. Although chelates look to be promising as correctives of iron chlorosis, yet no inexpensive chelating agent is found to correct chlorosis on calcareous soils. Further the process of removing the metal from the chelate, and the metabolic fate of the chelate in the plant are still not well understood, and work with tagged chelates is being carried on by a number of research workers.

2. Foliar application: The earliest successful use of ferrous sulfate sprays was in 1916 and 1924 when it was found that 2.8 percent solution of ferrous sulfate gave temporary recovery from chlorosis (8). Leaves are the most important structures in the absorption of nutrients from sprays. Witwer et al. (74) determined that a twelve-year old apple tree provides a leaf area equal to one tenth of an acre, including both surfaces of the leaves. The leaves are not covered with a continuous waxy coating. Pectinaceous substances are interspersed with cutinized areas. Such substances have a great absorbing capacity for water and nutrients (74). Young leaves are more efficient than old leaves and Witwer (74) reported that opening and closing of stomata is of little significance in nutrient absorption from sprays. The bark may absorb nutrients when cracks, ruptures or splits in leaf scars are present. Boynton (8) lists several factors that affect absorption of nutrients sprayed on the leaves; these are: surface wetting, paths of entry like pectinaceous substances, temperature and humidity, age and

nitrogen status of absorbing leaves, and chemical composition of the nutrient spray.

Iron sprays have never been very satisfactory for any one crop, reported Steward and Leonard (59), and broadleaf evergreens that are iron chlorotic form only small green spots on the leaves upon spraying with iron salts. Wallihan et al. (70) got the same results by using chelate sprays and when higher concentrations were used, not only did the iron fail to spread better, but marked injury began to occur. Following a foliar application of iron chelate to rose plants, White (72) noticed considerable injury to the young growth resulting in burning and malformation of the leaves. Reitz (46) recommends the use of two pounds ferrous sulfate dissolved in 100 gallons water. The Crown Zellerbach Co. (13) recommends the use of a solution containing ten to fifteen pounds Greens 26 to 100 gallons water which is equivalent to 170-250 grams iron and Hayfron (23) prefers spraying with one percent ferrous sulfate solution. It has been noted that wetting of the two surfaces of the leaf is very important in spraying. When comparing the spraying efficiency, it was found that more greening of leaves was achieved when the lower side of the leaf was sprayed as compared to the upper side (46).

3. Injection: This method is of no commercial value because it requires time and labor as well as the dosage being critical (70). Wallihan et al. (70) tried Fe-EDTA at a

rate of less than half an ounce of the chelate per citrus tree, severe injury resulted. It was later determined that the injury was caused by the specific combination of iron and the chelating agent. Demolon and Batisse (14) described the technique for injection. At 10 to 20 cm above the soil, a hole with a diameter one tenth that of the stem is bored to a depth three quarters that of the stem. The hole is inclined downward and is filled with ferri-silicic complex in a powder form, and is then stopped with grafting wax. Crawford (12) observed that iron citrate and iron phosphate are more satisfactory than iron sulfate for injection.

Russel (52) advocates the injection of pellets of iron citrate or tartrate. Injection by solution has been reported effective. The iron salts are used at the rate of one ounce per gallon of water; if leaves are on the tree, the concentration should be dropped one fourth that of the leafless trees (4).

Results obtained by the author of this thesis (45) indicated that injection of lemon trees with half a liter ferric citrate at a concentration of seven tenths percent gave a significant increase in iron concentration in the leaves. In addition, ferrous sulfate at the same concentration came second in effectiveness.

VII. Inorganic leaf composition of Citrus.

Visual evaluation of deficiency symptoms is suffi-

ciently reliable for trained observers, provided the condition is fairly severe and there are no complications with other deficiencies or excesses. In some cases symptom expression of one element may be modified by variations in the level of another element. Boron deficiency, for instance, is more severe under low magnesium levels than when magnesium is high (50). From the above discussion it is apparent that visual observations are not enough as such for determination of the nutrient status of plants. Another diagnostic tool to be used with visual symptoms is tissue analysis mainly leaf analysis. However there are certain factors to consider while evaluating tissue analysis data. The first of said factors is the age of leaves. Reuther and Smith (50) reported that the concentration of nutrient elements changes rapidly during the first month as growth is rapid, and then more gradually during the next two or three months. The second factor is the growth cycle because most citrus species have three distinct flushes or cycles of growth and it is reported that leaf samples from different flushes do not differ radically in composition at a comparable age. The season has a definite influence on growth. Leaf samples obtained just after a flush of growth contained less nitrogen than samples obtained just before a flush. Nitrogen, phosphorus and potassium are mobile elements and their concentrations fluctuate more with a season in relation to growth, fruiting and fertilizer application. Iron and calcium appear to be among

the least mobile (50). The scion variety has shown definite influence on composition of leaves. Leaves of Shary grapefruit on Cleopatra mandarin stock are higher in potassium, calcium, sodium, and boron content than leaves of comparable Valencia leaves on the same stock (50). The rootstock influences the concentration of the major elements in leaves appreciably, but affects the concentration of micro-nutrient elements even more.

Many attempts were made to establish standard concentrations for individual elements in leaf tissue but no concrete figures could be set due to the many factors that influence the composition of the tissue. However by summerizing some of the published data on leaf analysis a general indication as to optimum concentration was achieved (50). The said leaf analysis figures are shown in Tables one and two.

Table 1. Summary of inorganic composition of valencia spring-flush foliage from Florida, Texas, Arizona and California orchards by Reuther and Smith (50)

Region	Age of leaves months	Range	%N	%P	%K	%Ca	%Mg	Fe ppm	Mn ppm
Florida	4-5.5	Max.	3.00	0.159	2.20	5.50	0.510	102	85
		Min.	2.15	0.111	0.95	2.32	0.293	36	20
		Mean	2.66	0.139	1.56	3.44	0.390	61	37
Texas	3.5	Max.	2.50	0.147	1.18	5.90	0.355	105	48
		Min.	2.02	0.100	0.46	3.28	0.264	40	11.6
		Mean	2.30	0.120	0.73	5.14	0.318	69	28
Arizona	2.5-3	Max.	2.68	0.165	1.65	5.40	0.640	47	34.0
		Min.	1.82	0.115	0.72	2.38	0.264	18	7.1
		Mean	2.16	0.145	1.22	4.24	0.351	36	12.8
California	2.5-3	Max.	2.75	0.165	1.53	4.80	0.328	64	17.0
		Min.	1.92	0.115	0.31	2.69	0.180	30	8.7
		Mean	2.42	0.140	0.71	3.95	0.265	45	12.5

Table 2. Mineral content of fruit plants (55)

Fruit	Element	% element in dry matter showing deficiency symptoms	No symptom
Grapefruit	N	2.3	2.3
	K	1.16	1.16 - 2.00
	Mg	0.11 - 0.30	0.22 - 1.08
	Na	----	0.09 - 0.11
Orange	Mn	0.0020	0.0022 - 0.0085
	Fe	0.0020-0.0055	0.0060 - 0.0150
	P	0.075	0.10 - 0.125
	Ca(sand)	0.14 - 0.20	1.48
	Ca(field)	-----	9

Summarized from "Fruit nutrition" pp.844-845

MATERIALS AND METHODS

The experiment was conducted in a citrus orchard situated in Bouar, about 45 km North of Beirut on the Beirut-Tripoli road. The orchard is 250 to 300 meters from the sea with an altitude of about thirty meters; it occupies a small depression sloping East-West. The plot used for this esperiment has brown clay soil, with a pH of seven and three tenths, which is stony and deep. During summer, after irrigation, the manager claimed that white deposits are noticed on the surface of the soil. North winds are prevalent in the area but do not cause any serious injury to the trees and no windbreaks were used.

The trees were planted in the spring of 1955 and comprise grapefruit scions on sour orange rootstock. Training and pruning were restricted to the removal of drooping branches close to the ground. The orchard management practices consisted of cultivating with the use of a tractor to a depth of 20 to 25 centimeters. Starting in April the trees were irrigated every 15 days and after every irrigation the land was hand cultivated to remove weeds; this resulted in four to five cultivations a year. Trees are planted on the four meter square system and irrigated, by use of a two-meter basin, with water from the Nahr Ibrahim river.

As to fertilizers, a mixture of equal weights of superphosphate (18-20 percent P_2O_5) and ammonium sulfate

(20 percent N) was used. Half a kilogram was applied in April for every tree, and another half in June. Every other year, three tin cans (20 liter capacity) of goat manure were applied per tree in November.

The insect and disease control program consisted of spraying with Demol (75 grams per 20 liters), dusting with sulfur in February and March, spraying with wettable sulfur in June (75 grams per 20 liters) and spraying with oil in August (100 grams per 20 liters).

Although the orchard did not show a severe condition of iron deficiency, it was chosen for this study because of political troubles occurring at that time and the difficulty of finding a more suitable place.

The treatments used for this study consisted of different chemicals, each of which was given the same reference number in all replications. The treatments were as follows:

		Soil application	
1.	NaFeEDTA	444 grams/tree	40 grams Fe/tree
2.	NaFeEDTA	888 grams/tree	80 grams Fe/tree
3.	Ferrous sulfate	906 grams/tree	181 grams Fe/tree
4.	Ferrous sulfate	1812 grams/tree	362 grams Fe/tree
5.	Greenz 26	679 grams/60 liters/tree	30.5 grams Fe/tree
6.	Greenz 26	1132 grams/100 liters/tree	50.9 grams Fe/tree

Spray application

7.	Chel 330	4.25 grams/liter	0.05 percent Fe
8.	Chel 330	5.95 grams/liter	0.07 percent Fe
9.	Greenz 26	11.11 grams/liter	0.05 percent Fe
10.	Greenz 26	15.55 grams/liter	0.07 percent Fe
11.	Ferrous sulfate	10 grams/liter	0.2 percent Fe
12.	Ferrous sulfate	14 grams/liter	0.28 percent Fe
13.	Acid water	pH = 5	
14.	Tap water		
15.	Control	No treatment	

The orchard was divided into four blocks, A, B, C and D. In each block 15 trees were chosen as uniformly as possible and they were given random numbers, so that tree number five would receive treatment number five whether in block A, B, C or D.

Before applying any treatment, leaf samples were taken from each tree. Great care was taken to collect a random sample with leaves of the same age (eight months old) (46,60). The leaves were chosen as normal leaves, free from diseases, malformations or any other disorder such as adhering foreign material. Every sample consisted of 30 to 40 leaves depending on the size of the leaves.

During June 19 and 20, 1958, after collecting the leaf samples, the treatments were applied.

On July 15 the orchard was sprayed with wettable

sulfur by the owner and on August 12 with oil. Goat manure was added in November.

During the month of October, leaves of the fall flush were punctured and on December 15, 1958, leaf samples were collected from the punched leaves, following the same leaf collecting procedure previously described. Further it was found that some of the trees under experimentation started bearing fruit. The said trees appear in Table 3.

Table 3. Amount of fruits borne by the grapefruit trees of this experiment in the fall of 1958

Tree number	Number of fruits	Tree number	Number of fruits
A_3	3	B_{15}	10
A_7	3	C_1	5
A_8	1	C_2	1
A_{10}	23	C_5	36
A_{11}	1	C_{12}	9
A_{15}	2	D_1	2
B_1	Heavy bearing	D_5	5
B_2	" "	D_7	26
B_3	21	D_9	19
B_4	16	D_{10}	9
B_7	3	D_{11}	4
B_8	17	D_{13}	3
B_9	15	D_{14}	15
B_{11}	Heavy bearing	D_{15}	12
B_{12}	" "		

Visual observations on the experimental trees were recorded during September and December of 1958.

All leaf samples collected were taken to the laboratory where they were washed with detergent (10,69). Every leaf was scrubbed with a cheesecloth on both surfaces. Then the leaves were rinsed in water, scrubbed with 0.1 N HCl (10), rinsed in tap water and twice in distilled water (10). Excess water was shaken off the washed leaves that were then placed in tagged cheesecloth bags and dried in a forced-draft oven at 70°C ± 1°C for at least 24 hours (46,60). The said bags were washed the same way as the leaves.

Samples were ground in a porcelain mortar to pass a 60 mesh copper sieve in order to avoid any possible iron contamination. The ground material was stored in screw top sampling bottles and before analysis they were dried in a vacuum oven at 70°C ± 1°C overnight (14-16 hours) and cooled in a dessicator before weighing samples for analysis. Duplicate analysis were made and the results calculated on a dry weight basis. Besides iron, nitrogen, manganese, magnesium, phosphorus, potassium and calcium were determined. With the exception of nitrogen, the rest of the inorganic constituents were determined by the Toth et al. method (62). Nitrogen was determined by the Kjeldahl method (62). When duplicate results had a relative error of more than six percent, the analysis were repeated.

$$\frac{x - \bar{x}}{\bar{x}} \times 100 \leqslant 6$$

The pairing method was adopted for the statistical analysis of the results because it accounts for the initial as well as the final condition of the trees (75). In the tables the letter t is followed by two numbers; the upper is the number of degrees of freedom for the error (and this is obtained by subtracting the degrees of freedom of the treatments and the degrees of freedom of the replicates from the total degrees of freedom) while the lower number is the level at which t is considered. When the calculated value for t exceeds the respective 0.05 tabulated t, it is significantly different at the 0.05 level or 95 percent level and if it exceeds the respective 0.01 tabulated t, the difference is highly significant at the 0.01 level or 99 percent level. The following signs were used to indicate significance: (x) sig. at 0.05 level and (xx) sig. at 0.01 level. The critical difference was calculated as follows:

$$c.d. = \sqrt{S^2 \left(\frac{1}{r_1} \quad \frac{1}{r_2} \right)} \times t$$

S^2 = Variance

r_1 and r_2 = number of replicates

t = tabulated value of t in the "t" distribution table with the same degrees of freedom of the error at the respective 0.05 and 0.01 levels.

When the difference between any two values is greater than the critical difference, then these two values are significantly different at the respective levels; and if it is less then they fall in the same statistical group.

RESULTS AND DISCUSSION

Results of the inorganic leaf analysis are presented in tables four to eleven which include the concentration of each of the following elements: iron, nitrogen, phosphorus, potassium, calcium, magnesium and manganese. Since leaf samples from each of the treated trees were analysed once before applying the treatments and six months later, two sets of concentrations are presented in each table, one as the initial concentration and the second as final concentration. This procedure was used to show the change in any one element in each of the individual trees following the application of the treatments. The differences between means of the first analysis results and the final analysis results of each element within each treatment were statistically analysed to secure a basic figure of the effect of each treatment and reduce the variability between trees due to the small number of replications per treatment.

In addition to the chemical analysis, visual observations were made, in September and later in December of 1958, on the general status of individual trees. To facilitate the reading of the tables each treatment was given the same number in each of the tables. Following is a key where each numbered treatment is described.

The iron concentration in the leaves calculated on a dry weight basis was expressed as parts per million, here-

Key to treatments showing tree number and the corresponding treatment received

Treatment number	Tree numbers	Treatment received
	Soil application	
1	A_1, B_1, C_1, D_1	444 grams NaFe-EDTA/tree
2	A_2, B_2, C_2, D_2	888 grams NaFe-EDTA/tree
3	A_3, B_3, C_3, D_3	906 grams Ferrous sulfate/tree
4	A_4, B_4, C_4, D_4	1812 grams Ferrous sulfate/tree
5	A_5, B_5, C_5, D_5	679 grams Greenz 26 /tree
6	A_6, B_6, C_6, D_6	1132 grams Greenz 26 /tree
	Spray application	
7	A_7, B_7, C_7, D_7	Chel 330 4.25 grams/liter
8	A_8, B_8, C_8, D_8	Chel 330 5.95 grams/liter
9	A_9, B_9, C_9, D_9	Greenz 26 11.11 grams/liter
10	$A_{10}, B_{10}, C_{10}, D_{10}$	Greenz 26 15.55 grams/liter
11	$A_{11}, B_{11}, C_{11}, D_{11}$	Ferrous sulfate 10 grams/liter
12	$A_{12}, B_{12}, C_{12}, D_{12}$	Ferrous sulfate 14 grams/liter
13	$A_{13}, B_{13}, C_{13}, D_{13}$	Acid water pH = 5
14	$A_{14}, B_{14}, C_{14}, D_{14}$	Tap water
15	$A_{15}, B_{15}, C_{15}, D_{15}$	Control, No treatment

Table 4. Visual observations on the general condition of the trees in September and December of 1958

Tree number	Condition in September	Condition in December
A_1	green	green
A_2	green	green
A_3	green	green
A_4	green	green
A_5	green	green
A_6	green	partial yellowing of veins
A_7	green	green
A_8	green	yellow spots caused by scales
A_9	green	symptoms of magnesium deficiency
A_{10}	green	green
A_{11}	general yellowing of veins	general yellowing caused by scales
A_{12}	green	partially defoliated and stunted
A_{13}	green	green
A_{14}	green	green
A_{15}	green	green
B_1	green	green
B_2	dark green	green
B_3	green	green
B_4	light green	light green

Table 4. Cont'd

Tree number	Condition in September	Condition in December
B_5	green	green
B_6	dark green	green
B_7	green	partial yellowing
B_8	green	slight general yellowing of new growth
B_9	green	green and curled leaves
B_{10}	green	green and curled leaves
B_{11}	green	green
B_{12}	green	green
B_{13}	general yellowing	green
B_{14}	green	green
B_{15}	partial yellowing	green
C_1	green	yellowing of new growth
C_2	yellowish and weak	small curled leaves
C_3	small leaves and stunted tree	stunted tree, rosetting, small leaves
C_4	yellowish weak tree	green
C_5	green	green
C_6	green	partial yellowing
C_7	yellowish	partial yellowing
C_8	yellowish	green
C_9	green foliage, weak tree	stunting, rosetting, weak tree

Table 4. Cont'd

Tree number	Condition in September	Condition in December
C_{10}	yellowish	general yellowing, rosetting, small leaves
C_{11}	green	slight chlorosis
C_{12}	green	green
C_{13}	green	green
C_{14}	yellowish	green
C_{15}	weak tree, small leaves	rosetting and small leaves
D_1	dark green	green
D_2	green	general defoliation and rosetting
D_3	green	green
D_4	green but weak tree	defoliation, rosetting, small leaves
D_5	green	green
D_6	green	partial yellowing
D_7	green	green
D_8	green but weak tree	general yellowing of new growth
D_9	weak tree, green	slight defoliation and rosetting
D_{10}	green	slight defoliation
D_{11}	yellowish and weak	yellow, rosetting, defoliation

Table 4. Cont'd

Tree number	Condition in September	condition in December
D_{12}	dark green	green
D_{13}	green	green
D_{14}	green	green
D_{15}	green	green

Definitions of the terms used in Table 4.

Green = All the leaves on the tree were green.

Dark green = leaves were dark green in color.

Light green = Leaves were light green in color.

General yellowing of veins = The veins of the leaves were yellow.

Partial yellowing of veins = Some of the leaves had yellow veins.

Partial defoliation = A large amount of leaves had dropped.

Stunting = Not much new growth.

Rosetting = small leaves that are close to each other.

Weak tree = Tree with poor new growth and smaller number of branches than others

Trees number A_{12}, C_3, C_9 and C_{15} are all located along the irrigation ditch and it seems that the said trees were stunted because of water logging.

after referred to as ppm, and is recorded in Table four. Before applying the treatments, the concentration of said element ranged from 55 to 117.5 ppm. These values do not coincide with values given by Jones et al. (31) who considered that an iron deficient tree contains 35 to 50 ppm iron in its leaves. Some of the values however are in accordance with the classification followed by Wallihan (69) who found out that iron chlorotic leaves may contain from 30 to 70 ppm iron. Brown (10) reported the work of Kuykendall who found that leaves containing up to 90 ppm iron are regarded as medium in their iron content. McGeorge (42) found iron chlorotic leaves with as high as 250 ppm iron, he further quoted Overkowsky's work who found no apparent relation between percent total iron and chlorophyll, however the said worker found a close relation between the amount of iron extracted from the leaves by normal HCl and their chlorophyll content. The amount of iron extracted from green leaves by normal HCl was higher than that from the chlorotic leaves. On this basis, iron soluble in normal HCl was called "active iron". Meyer and Anderson (43) stated also that not all iron in the leaves is active; only the ferrous iron has physiological activity. Therefore the total iron is not a satisfactory indication of iron availability to plant functions. However in this study iron content of the leaves was used to indicate the efficiency of certain sources of said element in supplying iron to the plant.

Table 5. Total iron in grapefruit leaves (ppm) sampled in June and December 1958.

Blocks		Treatments 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	June	81.2	85.0	82.5	92.5	62.5	117.5	80.0	78.7	70.0	90.0	77.5	60.0	82.5	77.7	95.0
	Dec.	115.0	112.5	90.0	90.0	97.5	115.0	75.0	97.5	115.0	115.0	93.2	140.0	97.5	140.0	105.0
B		80.0	88.7	80.0	90.0	80.0	91.2	90.0	77.5	77.5	83.7	86.2	67.5	75.0	95.0	92.5
		90.0	82.5	75.0	75.0	97.5	90.0	71.2	77.5	82.5	86.2	77.5	101.2	110.0	101.2	90.0
C		85.0	58.7	65.5	87.5	77.5	77.5	90.0	87.5	57.5	52.5	72.5	81.2	61.2	95.0	66.7
		101.2	79.2	75.0	105.0	67.5	90.0	82.5	105.0	132.5	93.2	75.0	86.2	67.5	86.2	97.5
D		75.0	71.2	90.0	55.0	62.5	97.5	68.7	62.5	91.2	87.5	62.5	70.0	71.2	83.7	77.5
		75.0	90.0	75.0	82.5	66.2	105.0	82.5	90.0	75.0	90.0	157.5	105.0	67.5	82.5	75.0
Mean		80.3	75.9	78.7	81.2	70.6	95.9	82.2	76.5	74.0	85.9	74.7	69.7	72.5	87.8	80.9
		95.3	93.5	78.7	81.1	87.2	100.0	77.8	92.5	101.2	96.1	100.8	108.1	85.6	102.5	91.9
Diff.		15.0	17.6	0.0	6.9	16.6	4.1	-4.4	16.0	27.2	10.2	26.1	38.4	13.1	14.7	11.0

Calculated t = 5.739^{xx}

Critical difference at (a - 0.05 level = 11.18
(b - 0.01 level = 17.09

Groups at 0.05 level:
(12),(9,11),(2,5,8,1,14,13,15,10,4,6,3,7)

Groups at 0.01 level:
(12),(9,11,2,5,8,1,14,13,15,10,4,6,3,7)

Table 6. Average increase in iron content (ppm) in soil treated grapefruit trees and in spray treated trees during six months

	Soil	Spray	Check
Average increase	10.0	19.0	11.0

Calculated t = 4.67 ^{x}

Critical difference at 0.05 level = 6.78

Groups at 0.05 level (Spray), (soil, Check)

Table 7. Percent Nitrogen in grapefruit leaves sampled in June and December 1958

Blocks		Treatments														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	June	2.71	2.59	2.51	2.57	2.61	2.56	2.51	1.78	1.80	2.64	2.05	1.77	2.43	2.91	2.64
	Dec.	2.71	2.62	3.08	2.39	3.08	2.90	2.24	2.23	2.77	2.86	2.30	2.61	2.85	2.96	2.90
B		2.59	2.69	2.27	2.37	2.64	2.76	2.34	2.13	2.10	2.68	2.27	2.65	2.67	2.57	2.40
		2.70	2.61	2.29	2.29	2.95	2.67	2.76	2.38	2.45	2.45	2.23	2.43	2.59	2.85	2.61
C		2.58	2.68	2.12	1.74	2.21	2.07	1.85	2.21	2.69	1.62	2.17	2.22	1.98	2.22	2.23
		2.41	2.35	2.41	2.06	2.03	2.49	2.20	2.58	2.25	2.45	2.14	2.23	2.04	2.36	2.39
D		2.64	2.17	1.99	1.32	2.18	2.16	2.37	1.66	2.53	2.60	2.25	2.19	2.55	2.39	2.76
		2.68	2.27	2.62	2.42	2.76	2.17	2.34	2.32	2.27	2.86	3.06	2.47	2.95	2.27	2.62
Mean		2.63	2.53	2.22	2.00	2.41	2.39	2.27	1.94	2.28	2.38	2.18	2.21	2.31	2.52	2.53
		2.62	2.46	2.60	2.29	2.70	2.54	2.38	2.38	2.45	2.65	2.43	2.43	2.61	2.61	2.63
Diff.		-0.01	-0.07	0.38	0.29	0.29	0.15	0.11	0.44	0.21	0.27	0.25	0.22	0.30	0.09	0.10

Table 8. Percent phosphorus in grapefruit leaves sampled in June and December 1958

Blocks		Treatments														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	June	0.231	0.141	0.111	0.190	0.219	0.217	0.156	0.185	0.178	0.114	0.198	0.174	0.172	0.304	0.130
	Dec.	0.165	0.160	0.167	0.225	0.175	0.190	0.137	0.219	0.299	0.092	0.332	0.404	0.199	0.245	0.099
B		0.128	0.149	0.131	0.144	0.146	0.148	0.125	0.115	0.136	0.173	0.113	0.153	0.122	0.146	0.137
		0.070	0.077	0.110	0.122	0.112	0.140	0.303	0.205	0.181	0.151	0.170	0.142	0.276	0.172	0.208
C		0.160	0.172	0.325	0.153	0.117	0.153	0.137	0.153	0.347	0.233	0.135	0.199	0.140	0.152	0.295
		0.153	0.222	0.254	0.239	0.122	0.312	0.248	0.235	0.308	0.335	0.134	0.232	0.149	0.208	0.264
D		0.157	0.280	0.154	0.120	0.132	0.199	0.139	0.156	0.179	0.160	0.152	0.154	0.166	0.154	0.157
		0.170	0.445	0.371	0.304	0.187	0.371	0.164	0.291	0.413	0.245	0.239	0.160	0.405	0.213	0.308
Mean		0.169	0.188	0.188	0.152	0.153	0.179	0.139	0.152	0.210	0.170	0.149	0.170	0.150	0.189	0.180
		0.139	0.226	0.233	0.222	0.149	0.253	0.213	0.236	0.300	0.206	0.269	0.234	0.256	0.210	0.219
Diff.		-0.030	0.038	0.045	0.070	0.004	0.074	0.074	0.084	0.090	0.036	0.120	0.064	0.107	0.021	0.039

Table 9. Percent potassium in grapefruit leaves sampled in June and December 1958

Blocks		Treatments														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	June	1.81	1.11	1.15	1.34	1.14	1.47	1.36	1.09	1.79	1.36	1.21	1.80	1.45	1.68	1.32
	Dec.	1.72	1.62	1.39	1.81	1.32	1.78	1.43	1.91	1.81	0.88	2.10	1.70	1.58	1.81	1.19
B		0.79	0.81	0.90	0.71	0.73	0.96	0.71	0.71	0.73	0.89	0.62	0.94	0.63	0.98	0.79
		1.36	0.52	1.21	0.83	0.90	1.25	1.62	1.17	1.32	1.15	0.77	0.94	1.19	1.19	1.19
C		0.90	0.77	1.51	0.96	0.73	1.21	0.79	0.89	1.64	1.38	0.96	0.90	0.58	0.85	1.53
		0.78	0.62	1.85	1.79	0.81	1.60	1.88	1.46	1.89	1.96	0.83	1.32	0.66	1.43	1.58
D		0.96	1.79	1.72	1.34	0.83	1.38	0.60	1.25	1.23	1.20	1.20	1.57	1.04	1.08	0.75
		1.09	2.16	1.94	1.83	1.15	1.72	1.00	1.66	1.81	1.58	1.23	0.98	1.72	1.32	1.36
Mean		1.11	1.12	1.32	1.09	0.98	1.25	0.86	0.98	1.35	1.16	0.95	1.30	0.92	1.15	1.10
		1.24	1.23	1.59	1.56	1.04	1.58	1.48	1.55	1.73	1.38	1.23	1.23	1.29	1.14	1.33
Diff.		0.13	0.11	0.27	0.47	0.06	0.33	0.62	0.57	0.38	0.22	0.28	-0.07	0.37	0.29	0.23

Table 10. Percent calcium in grapefruit leaves sampled in June and December 1958

Blocks		Treatments 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	June	4.00	5.20	5.20	4.45	4.60	4.45	4.30	3.85	5.20	5.02	4.60	4.20	5.20	3.25	4.75
	Dec.	5.90	5.10	5.20	4.20	5.80	4.30	4.60	4.20	4.30	5.50	3.70	3.10	4.80	3.70	4.20
B		5.60	6.10	4.90	6.20	5.90	5.20	5.60	4.15	6.10	5.35	6.50	4.75	5.90	6.10	5.20
		6.40	6.20	4.90	5.90	6.80	5.10	5.20	5.10	4.90	4.80	5.20	5.80	5.50	5.80	5.80
C		5.90	4.75	3.25	5.50	6.40	4.45	5.05	6.10	3.70	3.35	4.90	6.10	4.90	5.50	4.15
		6.40	5.10	3.30	4.80	5.50	3.90	4.60	4.90	4.20	4.20	5.60	5.50	6.10	5.20	4.45
D		5.50	4.76	4.60	3.55	5.35	3.35	5.35	3.55	4.45	5.20	4.60	5.50	4.90	4.00	5.35
		5.10	3.70	3.70	5.10	5.20	3.70	5.50	4.90	4.00	4.60	3.90	5.80	4.00	5.80	4.60
Mean		5.25	5.20	4.49	4.92	5.56	4.36	5.07	4.41	4.86	4.73	5.15	5.14	5.22	4.71	4.86
		5.95	5.02	4.27	5.00	5.82	4.25	4.97	4.77	4.35	4.77	4.60	5.05	5.10	5.12	4.76
Diff.		0.70	-0.18	-0.22	0.08	0.26	-0.11	-0.10	0.36	-0.55	0.04	-0.55	-0.09	-0.12	0.41	-0.10

Table 11. Percent magnesium in grapefruit leaves sampled in June and December 1958

Blocks		Treatments 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	June	0.287	0.269	0.234	0.247	0.241	0.239	0.254	0.227	0.227	0.210	0.214	0.247	0.216	0.225	0.237
	Dec.	0.240	0.229	0.119	0.246	0.336	0.222	0.252	0.268	0.240	0.211	0.262	0.281	0.234	0.239	0.264
B		0.254	0.217	0.214	0.217	0.251	0.212	0.206	0.140	0.174	0.137	0.151	0.151	0.205	0.147	0.170
		0.292	0.274	0.305	0.299	0.322	0.172	0.220	0.265	0.254	0.221	0.236	0.254	0.305	0.215	0.226
C		0.168	0.222	0.166	0.167	0.248	0.191	0.221	0.181	0.245	0.175	0.115	0.281	0.235	0.230	0.224
		0.276	0.271	0.281	0.259	0.254	0.276	0.259	0.265	0.276	0.242	0.224	0.277	0.232	0.213	0.235
D		0.251	0.191	0.234	0.196	0.212	0.190	0.221	0.166	0.179	0.195	0.082	0.151	0.145	0.132	0.175
		0.245	0.235	0.240	0.165	0.262	0.240	0.262	0.231	0.267	0.221	0.235	0.231	0.190	0.235	0.226
Mean		0.240	0.225	0.212	0.207	0.238	0.208	0.225	0.183	0.206	0.179	0.143	0.207	0.199	0.183	0.201
		0.263	0.252	0.236	0.242	0.293	0.227	0.248	0.257	0.259	0.224	0.239	0.261	0.253	0.225	0.238
Diff.		0.023	0.027	0.024	0.035	0.055	0.019	0.023	0.074	0.053	0.045	0.091	0.054	0.054	0.042	0.037

Table 12. ppm manganese in grapefruit leaves sampled in June and December 1958

Blocks		Treatments														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	June	37	56	47	47	85	117	37	75	37	37	66	28	75	66	66
	Dec.	207	100	80	80	80	48	92	48	48	80	68	48	92	112	100
B		75	137	75	95	95	148	95	75	117	95	117	127	127	95	117
		92	58	48	100	112	48	124	124	100	100	124	92	136	112	112
C		85	75	95	85	85	75	107	95	66	75	75	95	56	95	75
		124	136	68	124	92	80	100	124	92	136	100	124	58	100	148
D		85	75	95	56	75	95	75	66	107	107	75	117	75	95	75
		58	100	100	92	100	92	80	100	58	100	92	136	80	124	92
Mean		70	86	78	71	85	109	78	78	82	78	83	92	83	88	83
		120	98	74	99	96	92	99	99	74	104	96	100	91	112	113
Diff.		50	12	-4	28	11	-17	21	21	-8	26	13	8	8	24	30

Six months after the treatment, the iron content in the leaves ranged from 67.5 to 157.5 ppm as compared to 55 to 117.5 before applying the treatments. This concentration coincides to a certain extent with the healthy group of tissue described by Wallihan (69) and by Brown (10). This increase may not be fully due to the application of iron to the trees. There might be an increase in the iron content of the leaves as influenced by the season of growth. This increase due to the difference in seasonal growth is apparent from table four treatment 15 which comprises the trees used as checks.

Visual observations were made in late March 1955 nine months after application of the treatments and almost all the new flush appeared green. The manager of the orchard felt that his trees were noticeably healthy that year. However the trees of the experiment in general were greener than the rest of the orchard trees. In fact, it may be easily noticed from table three that the majority of the trees became green six months after the application of the treatments. This observation however does not coincide exactly with the iron content of the leaves; for example in the treatment comprising a foliar spray of Chel 330 at the rate of 4.25 grams per liter, there was a drop in iron content. However three out of four trees receiving said treatment namely trees number A_7, B_7, and D_7 were green in color and looked healthier than trees with higher iron content in leaves such

as trees number A_9, C_9, and D_9. These latter trees showed a great increase in iron content although they were stunted, partially defoliated and showed rosetting. This indicates that the increase in iron content is no indication of the condition of the tree, as was previously mentioned, it is the availability of the element that is more important. As one may notice from the results reported in Table three, there was a general greening of leaves and this may be due not only to the supply of iron but also to the activation of the element present in the leaves, by the different materials applied especially the chelates.

Among the 12 treatments where iron was applied from different sources and in different modes it is apparent from table four that some sources and some methods of treatment showed an increase in iron content in the leaves. Results of the statistical analysis for Fe indicate that some of the treatments were significantly different at 0.05 and 0.01 levels. At the 0.05 level Greens 26 at the rate of 11.11 grams per liter and ferrous sulfate at the rate of ten and fourteen grams per liter applied as foliar sprays, significantly increased the concentration of iron as compared to the check.

In comparing the two levels of every treatment from table four, it was found that the two levels of NaFe-EDTA namely 444 and 888 grams per tree were not significantly better than each other and that they both fall in the same

statistical group as the check. However by closely examining the iron concentration in individual trees in the two mentioned treatments, it is apparent that there is a consistent increase of iron content in the leaves except in trees number D_1 where there was no change and B_2 where there was a decrease. On the whole the means of the two treatments increased more than the check. This indicates that the chelate NaFe-EDTA has some effect on increasing iron in the plant when used as soil additive. Although this increase was not statistically significant, it could be ascribed to the fact that the amount applied was not sufficient under the conditions of the orchard in use. The concentration used was that recommended by the manufacturer (20,21). A similar result was obtained by Hilgeman (28) who reported no response by using two pounds of NaFe-EDTA per tree. From these results it is apparent that no one recommendation is applicable to all conditions and more research is needed for Lebanon before any solid conclusion could be drawn.

Ferrous sulfate was not effective as a soil treatment in both concentrations used namely 906 and 1812 grams per tree. In fact the iron content of the leaves was not increased over that of the check although Bennett (4) was able to supply enough iron to trees by using the same treatments.

Greenz 26 as a soil application did not show a significant increase in iron content of the leaves over the check at the rate of 679 and 1132 grams per tree. However

the mean of the iron content from trees receiving the low concentration showed an increase but not to the point of statistical significance. In general it could be stated that some of the soil treatments indicated a slight increase in iron content of the leaves over that of the check, however this increase was not significant and could be used only as a measure for future work. Under the conditions of this experiment more iron should have been applied or a larger number of applications of the same concentration during the same period.

As to spray applications, Chel 330 at a concentration of 4.25 grams per liter and 5.95 grams per liter, proved to be in the same statistical group as the check. The higher concentration however, resulted in a greater increase of iron in the leaves but not to be statistically different from the check. The two sprays consisting of Greenz 26 fall in two different groups, the lower concentration, 11.11 grams per liter, being superior to the higher concentration, 15.55 grams per liter, at the 0.05 level. Consequently Greenz 26 seems to be a promising compound for correcting iron chlorosis by spray but considerable work is required in determining the concentration to use and the number of applications. Besides being effective, it is an inexpensive by-product of the paper industry.

Ferrous sulfate spray at the rate of 10 grams per liter and 14 grams per liter resulted in significantly higher

concentration as compared to the check as seen in table four. Each of the two levels are in a different group. The lower concentration is in the group that is significantly better than the check at the 0.05 level, while the higher concentration is in the group that is significantly better than the check at the 0.01 level. This clearly indicates that ferrous sulfate at the two concentrations used as foliar sprays was effective, the higher concentration being always better. Ferrous sulfate is an inexpensive available compound that is obtainable and easy to apply, and with some more work it could be recommended for use by growers.

The acid water and the tap water sprays both fall in the same statistical group as the check and show no improvement in iron content.

Soil and spray applications in general as influencing Fe content of the leaves were also compared in table five. It was found that there was a significant difference at the 0.05 level between said treatments. In fact ferrous sulfate as a spray was found significantly better than soil application. Greenz 26 at the lower concentration applied as a foliar spray namely 11.11 grams per liter showed significant results at 0.05 level. When compared to soil application. This may be due to the fact that the rate of iron translocation in the plant is slow. The higher concentration of 15.55 grams per liter was not significantly different which indicates that it may have had a physiolo-

gical effect on iron absorption through the leaves. Greenz 26 is the ammonium lignin sulfonate and the basic effect of this compound on iron uptake is worth studying to find out if high concentration has any influence on the uptake of iron.

From the results of this study it was apparent that a good, effective, inexpensive source of iron for correcting iron chlorosis under some of the orchard conditions of Lebanon is ferrous sulfate. The cost of said compound will not exceed ten to fifteen piasters per tree. Also Greenz 26 was a promising compound but still it is more expensive than ferrous sulfate.

Besides iron, other elements were determined in the leaf samples collected. Nitrogen concentration in the leaves as reported in table six ranged from 1.32 to 3.08 percent, the majority falling between 2 and 2.7 percent. The tree that was lowest in iron (D_4) was also lowest in nitrogen. The said tree appeared to be weak and defoliated. From this study there was no evidence that trees high in nitrogen were also high in iron.

In general the nitrogen level in the trees studied was normal as compared to values reported by Shannon (55). It could also be noticed that there was a slight increase of nitrogen in December over June. The nitrogen data indicate that citrus orchards in Lebanon do not suffer from nitrogen deficiency especially when the trees are young. This conclusion, although based on results from one orchard, could be

applicable to the majority of similar orchards, because the nutrition program is the same in the area.

Phosphorus data reported in table seven ranged from 0.07 percent to 0.445 percent, the majority falling between 0.150 and 0.300 percent. These values are higher than normal reported values in the literature (73) and it was also noticed that there was a general increase from June to December. It was mentioned in the literature (44) that excess phosphorus is one of the causes related to iron deficiency. Results obtained show that the experimental trees were high in phosphorus and this high level may be one of the causative factors involved in iron chlorosis. However no definite conclusion could be made at this stage before further studies are conducted, where phosphorus levels applied to the tree are varied and phosphorus content in the leaves compared to that of iron.

There was a net increase in potassium in almost all the treatments as shown in table eight with the exception of the treatment receiving a spray of ferrous sulfate at the rate of 14 grams per liter. In this case there was a slight indication of a decrease. The potassium concentration ranged from 0.52 percent to 2.16 percent with the majority of samples falling between 0.7 and 1.5 percent. This conforms with results reported by Shannon (55), Reuther and Smith (50) and McGeorge (42). From these findings it seems that potassium concentration was in the normal range and has no effect on

iron. However potassium will cause iron deficiency symptoms to show up only when it is at low concentration (10). These results indicate clearly that normal to high levels of potassium do not have any effect on iron content of leaves. It was also found that there was a seasonal change in the potassium content, the potassium in the fall flush being higher than in the spring flush.

In general there was an indication of a slight drop in calcium concentration with respect to the total a mount found in the leaves as shown in table nine. Most of the samples contained four to six percent, which fall within the upper limit of the normal concentration. This high calcium may be considered a major factor in causing iron chlorosis and other chlorosis patterns found in the orchard mainly zinc. Iron chlorosis has been associated in many cases with high calcium content and the high concentration of this element in the leaves may be an indication of its availability in large amounts in the soil to the plant and one of the causative factors of iron chlorosis.

Magnesium was not high in the leaves as is indicated in table ten, on the contrary it was slightly below average when compared to work done elsewhere (42,50,55). This might be due to the excess calcium and the competition between calcium and magnesium absorption. This slightly low concentration of magnesium may be an indication of antagonism between calcium and magnesium, which may reflect that there might be

further antagonism between calcium and the other cations namely iron and zinc. The trees under experimentation showed deficiency symptoms of both latter elements. Further it was noticed that between June and December, there was a general increase in magnesium with no noticeable relation to iron content.

As to manganese concentration in the leaves reported in table eleven, there was an increase in its content with the exception of the trees in the treatments receiving a soil application of two pounds ferrous sulfate per tree, 1132 grams Greenz 26 per tree and a spray application of Greenz 26 at the rate of 11.11 grams per liter. The ferrous sulfate soil application, causing reduction in manganese was the low treatment of said material. The soil application of Greenz 26 that caused the decrease in manganese concentration was the high level of said compound applied to the soil, while the foliar spray causing the decrease was the lower of the two concentrations of the same material used as a foliar spray. These inconsistencies in effects and concentrations of the above mentioned materials make it difficult to draw a conclusion concerning the interrelationship of manganese to Greenz 26 and ferrous sulfate. On the whole manganese was high in the leaves of all trees as compared to the published data (42,50,55). This high level of manganese in the plant may be considered as one of the factors causing iron chlorosis, because manganese may act as an oxidizing agent in trans-

forming iron into ferric state which is physiologically inactive.

Although the iron in general was not low in concentration in the leaves, it may have been that only part of it was physiologically active while the rest was inactivated due to the high concentration of manganese. Leaf symptoms have indicated a general iron chlorosis at the start of the experiment and excess manganese could have been one of the causative factors.

SUMMARY AND CONCLUSION

The study reported in this thesis was conducted in an effort to find an inexpensive and efficient source of iron to treat iron deficient fruit trees. Leaves from the experimental trees were sampled and analysed for iron, nitrogen, phosphorus, potassium, calcium, magnesium and manganese to evaluate the iron uptake by the grapefruit trees from the applied sources, and find out if any relation exists between iron and other elements analyzed for. The following results were obtained.

1. Out of the chemical materials used to supply iron to grapefruit trees, ferrous sulfate as a foliar spray at the rate of 14 grams per liter gave a significant increase in iron content of the leaves at the 0.01 level.

2. Ferrous sulfate at the rate of ten grams per liter and Greenz 26 at the rate of 11.11 grams per liter as foliar sprays gave a significant increase in iron content of the leaves at the 0.05 level.

3. In general foliar sprays showed significantly better results as compared to soil applications in treating iron chlorotic trees. In some cases the latter mode of treatment indicated a slight non significant increase in iron content of the leaves.

4. The amounts applied as soil treatments, under the conditions of this experiment, seemed to be low and higher

concentrations or/and more applications should have been applied.

5. It seemed that there is no relation between iron content of the leaves and the degree of chlorosis.

6. Most of the chemicals applied showed a greening effect on the leaves. This may indicate that the materials applied activated the iron already present in the leaves and made it available for use in the plant processes. This seems to be more so in the case where chelates were applied.

7. Nitrogen content in the leaves increased in December as compared to June, with no relation to iron.

8. Phosphorus was present at a high level in the leaves and it could be that its excess is one of the causes related to iron deficiency. It was further noticed that phosphorus content showed an increase from June to December.

9. Potassium concentration in the leaves was in the normal range and had no effect on iron. There was a net increase in potassium from June to December.

10. Calcium showed a drop in its concentration in the leaves between June and December. However calcium content of the leaves was in the upper limit of the normal range and this may be another major factor in causing iron chlorosis and other chlorosis patterns found in the orchard.

11. The low amount of magnesium present in the leaves indicates clearly the competition existing between calcium and magnesium absorption. This is further illustrated by

the drop in calcium and the increase in magnesium content of the leaves from June to December.

12. There was an excess of manganese in the leaves. This element is known to oxidize iron in plant tissue and make it unavailable for the plant. This excess of manganese may be partially responsible for the iron chlorosis in the orchard. Although the total iron content of the leaves was high, it must have been that part of the iron was oxidized by the manganese.

From this study it is apparent that a rapid remedy for iron chlorosis is the use of iron sprays. The materials that showed promising results are ferrous sulfate and Greenz 26. Many treatments did not show a significant increase in iron content of the leaves, however, in a number of cases there was an indication of some uptake. Further trials may give promising results with some of those materials.

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