A GREENHOUSE STUDY OF THE CHLOROSIS OF COTTON

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TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF ILLUSTRATIONS

I INTRODUCTION

Cotton is one of the most important crops in Oklahoma's agricultural economy. Cotton occupies only an average of 8.5 percent of the total cropped area in Oklahoma, but it accounts for approximately 20 percent of the total income from crops harvested. During the five year period 1950 to 1955, the average annual income from cotton lint was 57,687,667 dollars while cotton seed accounted for an additional 9,067,000 dollars (1).[1] In addition, considerable income was derived from harvesting, ginning, and processing of oils, plus supplies and machinery used in growing cotton. Primarily due to acreage allotments the area planted to cotton is on the decline, while the average cotton yields are increasing.

The yield and composition of plants have always been of prime importance to agronomists. For a number of years, only ten chemical elements were considered to be essential for the normal growth of plants, but continued research and more precise methods of investigation have enlarged the list to some fifteen plant food nutrients. Although considerable knowledge has been accumulated by previous investigations concerning the functions of the essential elements in plant growth, there are a number of nutrients which have not been assigned a definite role in the nutrition of the cotton plant.

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[1]Figures in parenthesis refer to Literature Cited.

In recent years several investigators (32, 34, 37, 46, 48) have shown that there is a definite need for more research on the "so-called" minor elements. Most soils contain an adequate total supply of the minor plant food nutrients for satisfactory crop production. However, poor physical conditions of the soil, adverse climatic conditions, and improper use of fertilizers or soil amendments may depress the availability of these minor elements until crop yields are seriously decreased. Within the past few years the need for minor plant food nutrient research has been pointed out in several areas of the United States. Recently, chlorotic conditions have developed on cotton, grain sorghums, and honey locust in some sections of Oklahoma. These chlorotic conditions have been attributed to deficiencies of iron and/or manganese.

One of the objectives of this study was to try to determine the best means of correcting this chlorosis. In a greenhouse pot experiment various chemical treatments (including iron and manganese applied in different forms and by different methods) and a physical treatment of an artificial compacted layer were used. Secondly, nutrient solution cultures were conducted to observe and record some of the symptoms of cotton caused by a lack of certain essential plant food nutrients.

II REVIEW OF LITERATURE

General Physiology of the Cotton Plant

Cotton is classified as a perennial plant; however, under the environmental conditions of the cotton belt in the United States it grows as an annual. In the tropics, some species called "tree cottons" (12) attain heights of 15 to 20 feet, while in the United States cotton grows as a soft stem shrub with many branches and varies in height from about 2 to 6 feet. The cotton plant is unique with its indeterminate growth habit, dimorphism of branches, and characteristic shedding of small floral buds and bolls (23). The branches of a cotton plant arise from the main stem and are arranged in an alternate pattern with leaves produced in a three-eights spiral. Each leaf has two buds or rudiments of buds produced in its axil. If the true axillary bud develops, a vegetative branch is formed and if the extra-axillary or lateral bud (located on either side of the true axillary bud) develops, a fruiting branch will be initiated. The proportion of vegetative and fruiting branches formed by the American upland cottons depends upon the environmental conditions. The vegetative branches are structurally similar to the main stem of the plant and may also produce fruiting branches. Vegetative branches are usually formed at the lower six to nine main stalk nodes while fruiting branches occur at successively higher nodes. The American upland cottons are considered to be day-length neutral; however, during cool, long days they will react as short-day cottons and produce only vegetative branches.

Iron

Functions of Iron in Plant Nutrition

Iron has been recognized as an essential element for proper nutrition for both plants and animals for many years. Although the exact function of iron in plants has not been clearly defined in many cases, it is generally accepted that iron is necessary for the formation of chlorophyll and in oxidation-reduction systems associated with respiration. DeTurk (21) reported that some research has indicated that iron may function in the production of a part of the chlorophyll molecule, namely the pyrrole ring. According to Sideris (68), iron is probably linked with a protein which serves as an activator of other proteins associated with the formation of chlorophyll. Iron plays an important part in photosynthesis and is a constituent of several enzymes. Iron is needed in the formation of iron-porphyrin prosthetic groups for several enzymes; namely, catalases, cytochromes and cytochrome-oxidases.

Factors Affecting the Availability of Iron in Soils

Most soils are not deficient in total iron, but variations in plant feeding power and availability of iron can cause deficiencies to occur. Iron oxides, which are largely responsible for the reddish color of many soils are the main sources of iron in soils (81). Most of these naturally occurring iron compounds are quite insoluble. The ferrous forms of iron are more soluble than the ferric forms, but the ferrous ions are unstable in soils with pH values above 6.0. In soils which have favorable drainage and aeration, relatively insoluble ferric compounds tend to predominate. In acid, water-logged and poorly aerated soils the more soluble

ferrous compounds are formed (42). However, these conditions may be modified or completely changed by the activity of soil microorganisms, organic matter content, or the presence of other ions.

Truog (78) and Olson (57) believed that soil reaction is one of the most important factors in controlling the availability of iron. In general, a pH of 6.5 to 7.0 seems to be most optimum for all nutrients. At a pH of 6.5 iron exists in the ferrous state and is available for plant use. The availability of iron for plant root absorption increases with acidity (81). Olson (57) concluded that the availability of iron was not only dependent upon the soil reaction, but also upon the quantity of iron oxides present. Gile and Carrero (29) stated that the availability of iron is related to the presence of carbonate of lime in the soil, while Bohrt and Hughes (8) found chlorotic plants on acid soils and attributed this condition to a combination of manganese, magnesium and iron deficiency. The pH of the plant sap has a definite bearing upon the soluble iron content of plants. Plants containing sap with a high pH value were low in soluble iron, while those with a low pH value of the sap were very high in soluble iron (36, 63).

Various plant food nutrients are related to the absorption and utilization of iron by plants. Wallace (81) found that soil additions of phosphates caused a decrease in the availability of iron to plants. Chapman et al. (18) found that excess phosphate in an alkaline medium for sand cultures caused deficiencies of iron which could be corrected by either lowering the pH or decreasing the phosphate level. In contrast with these findings, Speirs et al. (73) reported no significant effect from the additions of treble superphosphate on the iron content of turnip greens. Chlorosis of plants grown on highly calcareous soils or soils which have been

over-limed is referred to as "lime-induced" iron chlorosis. It was believed that the high pH of these soils caused a reduction in the availability of iron. McGeorge (47) concluded that on these calcareous type soils the excessive calcium in chlorotic barley seedlings caused an inactivation of iron in the plant. Leeper (42) reported that the change in metabolic activity of the plants grown on calcareous soils was the cause of iron chlorosis. In agreement with this view, Iljin (35) stated that a lack of iron, as such, was not the cause of lime-induced chlorosis, but that the metabolism of the plant was so disturbed that improper utilization of iron occurred. He also believed that all plant processes were affected and not just those associated with chlorophyll formation. Swanback (76) found that high concentrations of calcium would decrease the translocation of iron in the plant. In contrast with these views, Speirs et al. (73) concluded that there was no correlation between iron and calcium content of turnip greens on experiments conducted at 19 locations in the southeastern section of the United States. Hewitt (32) noted that iron deficiencies may be induced by excess copper, zinc, manganese and several other heavy metals. Chapman et al. (18) also found that an excess supply of copper and zinc may cause an induced iron chlorosis. They stated that copper decreased the availability of iron and possibly the availability of manganese on acid peat soils. This effect might be favorable depending upon the degree of oxidation as well as the iron manganese contents of these soils.

Halvorson (30) pointed out the complexities involved in the availability of iron. He reported that the solution and precipitation of iron in nature are affected by equilibrium conditions which depend on oxygen tension, carbon dioxide tension, acidity and the presence of organic

compounds. These equilibrium conditions also could be changed considerably by bacterial activity. Even under anaerobic conditions which favor the availability of iron, a decrease in solubility of iron may occur due to the formation of ferrous carbonate or, as Chapman (17) suggested, by the development of an insoluble carbonate coating on the iron particle.

Several investigators (14, 24, 29, 30, 38, 40) have noted a relationship between soil moisture levels and iron deficiencies. The availability of iron on calcareous soils appeared to be slightly greater near the optimum water content than at higher soil moisture levels (29). At high soil water percentages, a chlorosis of plants was noted. However, rice plants, which are known to be hydrophytes, did not show a chlorosis when the soil was submerged in water. Gile and Carrero (29) believed that the rice plants developed a new kind of root which could assimilate iron better than a root formed in a soil of lower water content. Lawton (40) found that compaction of the soil and maintenance of high soil moisture levels increased the extractable ferrous iron and decreased the amount of ferric iron. High moisture levels in soils tend to favor anaerobic conditions which cause the reduction of iron to the soluble ferrous state. Speirs et al. (73) concluded that there was no correlation between rainfall and iron content of turnip greens. However, they stated that irrigation during a relatively dry season caused an increase in iron content of the plants.

Jones and Tio (38) in their studies concerning "frenching" (a disease attributed to iron deficiency) of tobbaco found that there was a relationship between available iron, the activities of soil organisms and temperature. The iron content of plants was higher on soils which had lower soil temperatures. They believed that the higher temperatures

stimulated soil organisms which could compete more readily for the soluble iron than the tobacco plants. Lohnis (44) reported that exceptionally hot periods could cause the occurrence of iron deficiencies. There has been very little work done on the effect of temperature on iron deficiencies, but it is entirely possible that temperature stresses could cause variations in the uptake of iron due to reduced plant growth, altered soil organism activity or changes in the physical conditions of the soil.

Sources of Iron for Plants

According to DeTurk (21) the amount of iron needed by plants is very small and can be taken up both in the ferrous and ferric forms. Kilman (39) believed that iron is taken up as the reduced divalent form and then only when it exists in the cationic state. He found that iron existed in both cationic and anionic states in soils, but under alkaline conditions the cationic forms were precipitated and became unavailable for plant use. Therefore, the additions of iron compounds became necessary on alkaline soils.

Various methods and materials have been used to alleviate or prevent iron chlorosis of plants. Burke (15) reported that injections of ferrous sulfate salts into the trunks of chlorotic fruit trees would correct iron chlorosis, while spray and soil applications of ferrous sulfate were not effective. These iron salt injections could be either dilute solutions or solid salts. Chapman (17) stated that finely ground magnetite could be used as a successful source of iron in sand cultures while Gile and Carrero (29) concluded that ferrous sulfate, ferric citrate and ferric tartrate were satisfactory sources of iron for plants grown in nutrient

solutions. Wallace (81) found that iron chlorosis could be corrected with ferrous sulfate as foilage sprays and by injections of iron compounds into the plant stem.

Since the work of Jacobson (37) much interest has been aroused in the use of organic chelate iron complexes as a source of iron for plant growth. Chelating agents are organic compounds which combine with metals to form a ring structure and hold the metals in a usable form for plants (3, 75). In recent years, various investigators (37, 74, 79, 80) have reported the successful use of iron chelates of ethylenediaminetetraacetic acid (EDTA), N-Hydroxyethylethylenediaminetriacetic acid (HEEDTA), and diethylenetriaminepentaacetic acid (DTPA) in ameliorating or preventing the occurrence of iron chlorosis. Jacobson (37) found that 5 to 10 p.p.m. of iron as EDTA was adequate for the prevention of chlorosis in corn, tomato, barley and sunflowers grown in nutrient cultures. Wallace et al. (80) stated that HEEDTA and DTPA were more effective than EDTA in eliminating iron chlorosis on high lime soils. Holmes and Brown (34) in their study of the effect of five chelates on chlorotic soybeans grown on calcareous soils, reported that DTPA would correct iron chlorosis when applied at the rate of approximately 250 pounds per acre. However, EDTA, HEEDTA, and cylcohexanediaminetetraacetic acid (CDTA) did not cure chlorosis of soybeans. Although all the chelates used in their experiment did not alleviate chlorosis on soybeans, they tended to make soil iron more available for plants. Wallace et al. (79) noted that the iron chelates EDTA, HEEDTA, and CDTA were mildly toxic to bean plants. They believed that the toxicity was due to factors other than the iron content because the leaves of plants treated with the chelate "Fe-138" (aromatic polyaminocarboxylic acid, APCA) were higher in iron and exibited no chlorosis.

The exact mechanism involved in the utilization of iron from chelates is still unknown; however, recent research by Wallace et al. (80) has shown that the whole chelate molecule is absorbed by plants. It is evident, from this review, that further work must be done concerning the use of chelates for the prevention and correction of chlorosis.

Manganese

Functions of Manganese in Plant Nutrition

According to Russell (64) manganese is an invarible constituent of plants. Manganese has been shown to play numerous roles in the normal production of plants (28, 48, 49, 50, 65). McHargue (50) found direct evidence that manganese has a function to perform in the formation of chlorophyll, photosynthesis and possibly in the synthesis of proteins in the plant. He also suggested that manganese functions in the production and secretion of enzymes, hormones, and vitamins. Gerretsen (27) reported that manganese plays an important role in the oxidation-reduction processes associated with photosynthesis in plants. He also noted that manganese was associated with carbon dioxide assimilation and that lower amounts of manganese in the plants caused a reduction in the area of the root system, yield, and resistance to root invading organisms. Mulder and Gerretsen (55) stated that manganese was an activator of some enzymatic reactions associated with carbohydrate and nitrogen metabolism. Leeper (41) concluded that manganese deficiencies caused an accumulation of nitrates and McHargue (50) reported that manganese functions in the reproductive processes associated with seed production in some plants.

Factors Affecting the Availability of Manganese in Soils

Manganese may be found in various forms within the soil. According to Wallace (81) the occurrence of manganese in soils resembles that of iron since the oxides of manganese are the most important forms. Fujimoto and Sherman (25) listed several factors that affect the supply of available manganese in the soil. They stated that high temperatures, high moisture levels, reduction of pH and addition of reducing agents tends to increase the availability of manganese. Snider (71) believed that the seasons of the year and the state of composition of manganese compounds were more important in manganese absorption. He maintained that in the spring, manganese compounds were in the reduced state and were available for plant growth. Later in the year, manganese compounds became more fully oxidized and were unavailable causing a manganese deficiency in plants grown on the soil.

According to McHargue (47) manganese may be made unavailable in some soils by the addition of an excess of basic materials such as calcium carbonate. Schmehl et al. (66) concluded that additions of liming materials to acid soils caused a reduced availability of manganese and Hewitt (32) suggested that there was an antagonistic effect between levels of calcium and the uptake of manganese. With additions of calcium sulfate in sand cultures there was a decrease in manganese content of the plants. Swanback (76) stated that manganese depressed the absorption and utilization of calcium at low levels of calcium supply, while at high levels of calcium the absorption and utilization of manganese were reduced. In contrast with these findings, Morris and Pierre (53) were unable to correct manganese toxicity of lespedeza grown in solution cultures by the addition of calcium. Bortner (9) reported that additions of phosphate decreased

the available manganese in soils and Morris (52) found lower concentrations of manganese in sweet clover and lespedeza as a result of additions of phosphate fertilizers. Beeson et al. (5) concluded that high levels of phosphate increased the yields of soybeans and decreased the manganese content significantly while Morris and Pierre (53) noted that an excess of manganese resulted in reduced growth under conditions of high phosphate supply.

Wallace (81) believed that level of pH and organic matter content of soils are the most important factors affecting the availability of manganese. Maclachlan (45) observed that manganese deficiencies often occur on soils high in organic matter while Samuel and Piper (65) found that the presence of organic matter played no part in manganese deficiency symptoms of oats. Gerretsen (27) stated that manganese deficiencies were not only caused by a lack of manganese in the soil, but also by the presence of certain bacteria which attack the roots of the plants and caused a decreased absorption of the element by the plant. This view was substantiated by Quastel et al. (62) who suggested that manganese was converted to unavailable forms by the activity of soil microorganisms.

Sources of Manganese for Plants

Manganese deficiencies have been corrected in a number of ways. Gerretsen (27) reported that formalin has been used to treat the soil and has prevented the appearance of manganese deficiencies. He believed that the formalin caused an increase in root surface which improved the ability of the plant to absorb manganese. Russell (64) related that additions of manganese sulfate to the soil has prevented manganese deficiencies on many crops. However, in some conditions manganese sulfate

would not correct manganese deficiencies because the manganese was converted to an unavailable form in the soil. Maclachlan (45) and Mulder and Gerretsen (55) stated that manganese deficiencies could be alleviated by applications of manganese sulfate to the soil, spraying the foliage with dilute manganese solutions, treatment of the soil with acidifying materials and in some cases flooding the soil which causes conversion of manganic oxides to available manganese. Spraying the foliage with solutions of 0.2 to 0.5 percent manganese sulfate seemed to be the most economical and effective way to correct manganese deficiencies.

Iron-Manganese Relationships in Plant Nutrition

Since the discovery by McGeorge (46) of iron deficiencies of pineapples grown on the manganiferous soils of Hawaii, investigators have been very much interested in the iron and manganese relations in plant nutrition. Somers and Shive (72) found that manganese inactivates iron in the leaves of plants by oxidizing ferrous iron to ferric iron with a result in precipitation of iron as ferric organic complexes which cannot be utilized by the plant. They believed that there was no difference between manganese toxicity and iron deficiency or manganese deficiency and iron toxicity. However, the work of Morris and Pierre (54), Berger and Gerloff (7), Lohnis (44), Ouellette (58) and Hewitt (32) did not substantiate this idea. Somers and Shive (72) reported that the ratio of iron to manganese should be about 1.5 to 2.5 to assure optimal plant growth. In contrast, Ouellette (58) working with soybeans grown in nutrient cultures, stated that the ratio between iron and manganese was unimportant. In agreement, Carlson and Olson (16), Bennett (6) and Nicholas (56) concluded that the ratio between iron and manganese was

not the critical factor in the production of chlorosis. They believed that the absolute levels of the available iron and manganese was the most important factor in producing deficiencies of either element. Sideris and Young (69) in their study of pineapples grown in nutrient cultures, found that the absorption of iron and manganese was directly related to the levels of supply. Lohnis (44) in her extensive studies on manganese toxicity did not observe a beneficial effect of applied ferrous sulfate treatments on manganese injured plants, except, when the manganese injured plants were grown at high temperatures. This temperature factor may explain much of the controversy among various workers as to the relations between iron and manganese.

Gerretsen (28) advanced a possible explanation of the interdependence of the iron and manganese contents of the plant for optimum growth and chlorophyll production. He stated that an excess of manganese in the presence of low iron supply would increase the photo-oxidation of protein protectors of chlorophyll and cause the bleaching of chlorophyll. This view on the complementary oxidation-reduction effect has not been confirmed, but it does offer an explanation for the mutual anatagonism of iron and manganese under some conditions.

Nutrient Solution Cultures

Much of the knowledge regarding the role of mineral elements in plants has been obtained by means of nutrient solution culture studies. According to Miller (51) the earliest recorded experiment with water cultures was conducted by Woodward in 1699 who grew spearmint in various water sources to determine whether the water or solid particles furnished nourishment for plants. He concluded from his experiment that the solid

particles were the source of nourishment of plants. Various solutions have been proposed for nutrient culture studies (33). From the beginning of nutrient culture work, investigators have clearly recognized that the composition of a given nutrient solution was not superior to every other composition (33).

The type of container to use in solution culture studies depends upon the kind of plant to be grown, the length of the growing period and the purpose for which the plants are grown. Shive and Robbins (67) reported that two-quart glass fruit jars, one gallon glass candy jars or two gallon crocks were suitable containers for nutrient culture studies while Hoagland and Arnon (33) stated that one or two-quart fruit jars or five to ten-gallon earthenware jars could be used successfully. For satisfactory growth in nutrient cultures some means of renewing the solutions to maintain a better nutrient balance seems necessary. Allison and Shive (2) found that soybeans grown in cultures with a continous renewal of solutions were always superior to plants grown in cultures with intermittent renewal of solutions. Brenchley (11), also noted that barley and wheat seedlings gave a marked increase in growth in solutions that were changed frequently. Gericke and Tavernetti (26) stated that heating the nutrient solution would produce large increases in the growth and yield of tomatoes, but the work of Arnon and Hoagland (4) did not substantiate this experiment. Arnon and Hoagland (4) concluded from an experiment of growing tomatoes in soil and solution cultures under the same conditions that the water requirement was approximately the same for plants grown in nutrient solutions or soils.

Bryant (13) reported that aeration of the water cultures had a marked effect upon the growth of barley roots. He stated that plants growing in

non-aerated cultures produced about three times as many roots as plants growing in aerated solutions, but the roots growing in aerated cultures were three times as long and were about 15 percent greater in diameter. Clark and Shive (19) found that roots of tomato plants grew throughout the container in aerated solutions while they grew only near the surface in unaerated solutions. They also concluded that lack of aeration tended to make the plants mature earlier than plants in aerated solutions. Arnon and Hoagland (4) stated that tomato plants grown in aerated solutions gave significant improvement in growth and yields as compared to plants grown in non-aerated cultures.

III MATERIALS AND METHODS

This investigation was divided into three phases; greenhouse pot studies, laboratory tests and nutrient solution cultures. The objective of the greenhouse pot studies was to ascertain the effect of chemical and physical treatments on the yields of cotton. The purpose of the laboratory tests were to characterize the soils and to determine the effect of various treatments on the iron and manganese contents of the soils as well as the plants. The nutrient culture phase was conducted to observe and record the symptoms of the cotton plants caused by a lack of the various essential elements.

Greenhouse Pot Studies

Soils Used in the Pot Culture Experiment

The soils collected for this study were a McLain loam from the Cotton Experiment Station at Chickasha, Oklahoma and the Brownfield soil from the Sam Holmberg farm at Erick, Oklahoma. Two bulk samples of each soil, one from an area exibiting no chlorosis and one from an area showing chlorosis, were brought to the greenhouse. Samples of the McLain loam exibiting no chlorosis and showing chlorosis will hereafter be referred to as soil A and soil B, respectively. In the same order, the Brownfield soil will be designated soil C and soil D.

The Brownfield series[1] is comprised of loose sandy soils with reddish friable subsoils and no zone of carbonate accumulation. This series

[1]Established Series Division of Soil Survey, BPISAE, ARA, USDA, Unpublished data.

occupies undulating to billowy upland on the high plains of Texas and adjoining states. Drainage is free both externally and internally. The native vegetation is composed chiefly of shin oak and coarse grasses (largely little bluestem and sand dropseed) with some scattered sand sage and yucca. In some places, black grama, hairy grama and triple awn grasses occur extensively. These soils are used largely for grazing, but some areas are farmed chiefly to corn, cotton, grain sorghums and other feed crops. These soils are very drought resistant, but are susceptible to wind erosion and fertility is depleted very rapidly.

The McLain series[1] is a youthful Reddish Prairie soil developed on reddish calcareous alluvium. This series occupies level stream terraces lying 5 to 20 feet above the present flood plain along the Washita, Canadian and Red Rivers. These soils have distinct color profiles and have the free carbonates removed to a depth of several feet, but they lack a distinct textural profile. Surface drainage is slow. Internal drainage is moderate but is very favorable for crop growth. These soils were originally forested with oak, elm, pecan, hackberry and ash but have been cleared for cultivation. The chief crops grown on these soils are corn, cotton, alfalfa, small grains, sorghums and broomcorn. These soils are very fertile and are highly productive.

Greenhouse Procedure and Soil Treatments

Two-gallon, glazed, non-porous pots were used. Each pot was thoroughly washed and rinsed with distilled water. The drain holes were closed with rubber stoppers and 8,500 grams of soil were placed in each pot.

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[1]Extablished Series, Division of Soil Survey, BPISAE, ARA, USDA, Unpublished data.

After studying the analyses of the soils, it was decided that applications of nitrogen, phosphorus and potassium were necessary to prevent them from becoming a limiting factor. Analytical grade salts of ammonium nitrate, mono-calcium phosphate and potassium chloride were used in making the solutions for N, P and K applications. Enough of each solution was applied in cross-hatch bands three inches below the surface in each pot to bring the N, P and K to an optimum level. These basal applications were applied approximately one week before the date of seeding.

In this experiment there were eight treatments with three replications of each treatment. Both chemical and physical treatments were used. The treatments and rates of application are given in Table I.

The compacted layer used in treatments 7 and 8 was synthesized by removing 6 inches of soil from the pots and placing a sheet of waxed paper over the remaining soil. Then a 2 inch layer of soil was put on the waxed paper, wetted with distilled water and packed with a tamping rod about three inches in diameter. Another sheet of waxed paper was placed over this layer to retain as much moisture as possible in the wetted zone. The remainder of the soil was returned to the pots and after 48 hours, the top 4 inches of soil plus the piece of waxed paper were removed. The compacted zone was again wetted, packed and dried. This step was repeated three times to insure the formation of a dense pan. After drying and hardening, the top four inches of soil were returned to the pots and the other steps in the procedure were carried on.

The chemical treatments were added as solutions made from analytical grade reagents. They were applied in cross-hatch bands two inches deep so that the seeds could be planted without direct contact of the fertilizer salts.

TABLE I

TREATMENTS USED IN GREENHOUSE POT STUDIES

NUMBER	TREATMENT	RATE OF APPLICATION (lbs./A.)
1	Check	
2	Minor element mixture	
	Fe as $FeSO_4 \cdot 7H_2O$	21.6
	Mn as $MnSO_4 \cdot H_2O$	21.6
	B as $Na_2B_4O_7 \cdot 4H_2O$	4.0
	Zn as $ZnSO_4 \cdot 7H_2O$	1.5
	Cu as $CuSO_4 \cdot 5H_2O$	1.5
	Mo as H_2MoO_4	0.5
3	Chelated iron (HEEDTA) N-hydroxyethylethylenediamine-triacetic acid	21.6
4	Manganese as $MnSO_4 \cdot H_2O$	21.6
	Chelated iron (HEEDTA)	21.6
5	Iron as $FeSO_4 \cdot 7H_2O$	21.6
	Manganese as $MnSO_4 \cdot H_2O$	21.6
6	Iron as $FeSO_4 \cdot 7H_2O$	21.6
	Manganese as $MnSO_4 \cdot H_2O$ (Spray application)	21.6
7	Compacted layer plus chelated iron (HEEDTA)	21.6
8	Compacted layer	

On December 8, 1955, six seeds of Stoneville 62 cotton (59) were planted in a circle two inches from the outside of the pot and one inch deep. Stoneville 62 was used as the test plant because it is a well established and proven variety. The pots were arranged in a randomized block design along the east bench in the greenhouse. To eliminate light and temperature differences, the blocks were rotated every two weeks. When a complete stand was assured, the cotton plants were thinned to three per pot. Distilled water was used to water the cultures throughout the experiment. On May 28, 1956, the cotton bolls were harvested and yield weights were recorded. The yields were analyzed statistically according to the methods of Snedecor (70) and Duncan (22).

Laboratory Tests

Initially, the four soils were characterized by chemical and physical analyses. A sufficient quantity of each soil was air-dried and then processed by crushing the soil aggregates with a brass roller and sieving through a 20 mesh screen. Determination of the soil texture was made by the Bouyoucos hydrometer method using a 100 gram sample (10). The reaction of the soil was measured with the Beckman glass electrode pH meter using the procedure outlined by Peech and English (60). The organic matter content and total nitrogen were determined by the methods of Piper (61). Available phosphorus and available potassium were run according to the methods outlined by Harper (31). The cation exchange capacity and total exchangeable bases were measured by the A.O.A.C. methods (43). Exchangeable calcium and magnesium were determined with the Beckman quartz spectrophotometer. Available iron and manganese were determined essentially by the methods of Peech and English (60) which were modified for

use with the Cenco photelometer. These procedures used sodium acetate as the extracting agent. The amount of iron was measured by developing the color with ortho-phenanthroline and the amount of manganese was determined by developing the color with sodium bismuthate. Peech and English believed that this method measured the exchangeable and water soluble forms which were available for plant use. The results of these analyses are shown in Table II.

At the conclusion of the greenhouse experiment, soil samples were taken with a hand probe from each pot which received an iron or manganese treatment. These samples were taken to the laboratory, air-dried and processed for analysis. Available iron and manganese were determined by the methods of Peech and English (60) which were modified for use with the Cenco photelometer.

Plant samples from both the pot and nutrient culture experiments were prepared for analysis by thoroughly washing with distilled water and drying in a forced-draft oven at 65°C. These samples were then ground in a silica ball mill to prevent iron contamination. Each sample was ashed with a 3:1 nitric-perchloric acid mixture and diluted to 100 milliliters. Iron and manganese were determined according to the methods of Toth et al. (77). Iron was measured by developing the color with ortho-phenanthroline and reading the percent light transmission with a Cenco photelometer. Manganese was determined by developing the color with potassium periodate and measuring the light transmission with the Fisher colorimeter.

TABLE II

ANALYSES OF THE FOUR SOILS USED IN THE GREENHOUSE POT STUDIES

ANALYSIS	SOIL A	SOIL B	SOIL C	SOIL D
Mechanical Analysis	44.5% Sand 38.25% Silt 17.25% Clay	48.5% Sand 36.5% Silt 15.0% Clay	84.75% Sand 6.45% Silt 8.80% Clay	90.5% Sand 3.7% Silt 5.8% Clay
Textural Class	Loam	Loam	Loamy Sand	Sand
Soil Reaction (pH)	6.3	6.9	6.3	6.5
Percent Organic Matter	1.51	1.76	0.46	0.32
Percent Total Nitrogen	0.07	0.07	0.02	0.02
Cation Exchange Capacity (m.e./100 grams)	9.8	10.4	4.8	3.3
Total Exchangeable Bases (m.e./100 grams)	8.35	9.10	4.01	2.34
Exchangeable K (m.e./100 grams)	.88	.99	.35	.20
Exchangeable Mg (m.e./100 grams)	2.43	2.79	1.10	.78
Exchangeable Ca (m.e./100 grams)	4.09	4.43	1.89	1.30
Available P (lbs. per acre)	72.3	78.7	5.8	7.4
Available Fe (lbs. per acre)	5.1	1.3	3.3	1.4
Available Mn (lbs. per acre)	70	78	40.5	45

Nutrient Solution Cultures

In this phase, Stoneville 62 cotton plants were grown in various nutrient solutions containing known essential elements. The solutions were made with distilled water and analytical grade reagents. The following stock solutions were used for preparing the various nutrient solutions.

(1) 1.0 Molar calcium nitrate ($Ca(NO_3)_2.4H_2O$).

(2) 0.05 Molar mono-calcium phosphate ($Ca(H_2PO_4)_2$).

(3) 0.01 Molar calcium sulfate ($CaSO_4$ soluble anhydrite).

(4) 1.0 Molar potassium nitrate (KNO_3).

(5) 1.0 Molar potassium di-hydrogen phosphate (KH_2PO_4).

(6) 0.5 Molar potassium sulfate (K_2SO_4).

(7) 1.0 Molar magnesium sulfate ($MgSO_4.7H_2O$).

(8) 1.0 Molar ammonium di-hydrogen phosphate ($NH_4H_2PO_4$).

(9) Minor element mixture of:

boric acid (H_3BO_3)	2.86 gms./liter.
manganese chloride ($MnCl_2.4H_2O$)	1.81 gms./liter.
zinc sulfate ($ZnSO_4.7H_2O$)	0.22 gms./liter.
copper sulfate ($CuSO_4.5H_2O$)	0.08 gms./liter.
molybdic acid (H_2MoO_4)	0.02 gms./liter.

(10) Minor element mixture (same as above except without manganese).

(11) 0.5% iron solution made from an iron chelate (N-hydroxyethylethlylenediaminetriacetic acid, HEEDTA).

The nutrient solutions were made according to the directions given by Hoagland and Arnon (33). There were eight different solutions used with four replications of each treatment. The treatments are given in Table III.

The solution containers were three-liter, glass battery jars which had been reinforced with sheet aluminum and painted on the outside with

aluminum paint to eliminate as much light as possible. The containers were covered with one-half inch plywood covers to prevent as much contamination as possible.

TABLE III

TREATMENTS USED IN NUTRIENT SOLUTION CULTURES

Treatment No.	Treatment
1	Complete
2	Minus Nitrogen
3	Minus Potassium
4	Minus Phosphorus
5	Minus Calcium
6	Minus Magnesium
7	Minus Manganese
8	Minus Iron

Healthy plants for the cultures were obtained by starting the seedlings in silica sand. First attempts to start seedlings in vermiculite were futile due to heavy seedling disease infections even though the seeds were treated. Although the process for making vermiculite is essentially a sterilization process, the handling and bagging methods employed probably permits excessive contamination. After one week, the plants started in the silica sand were about three inches high and appeared to be growing normally. Plants with two good cotyledon leaves were selected for transfer to the nutrient cultures. On March 29, 1956, the stems of the desired plants were wrapped with glass wool and suspended through the plywood covers into the nutrient solution containers.

The nutrient solutions were aerated by means of a small air compressor which pumped air through a series of rubber and glass tubing, which is illustrated in Figure 1. Aeration was first attempted through extraction thimbles, but this method proved unsatisfactory. A three inch piece of capillary tubing on the tip of each aerator was found to be very suitable. Screw type pinch clamps were used to adjust the flow of air bubbles into each container. The air compressor was powered by a one-half horsepower electric motor which was connected to an electric time switch. The switch was set for 75 minute time intervals, thus, the solutions were aerated for 75 minutes followed by a 75 minute rest period.

All cultures were changed at weekly intervals for the first four weeks and twice weekly for the remainder of the study in order to maintain a better ionic balance among the nutrients. The pH was checked and adjusted daily to a value of 6.0 to 6.8 using external indicators. The pH of most of the solutions shifted continously. Some solutions became acid in 24 hours while others turned basic. Only a few solutions remained in the neutral range.

Daily observations were made and pictures were taken periodically to record any deficiencies that occurred. The plants were grown in the nutrient solutions for a period of two months. During the later part of May, the plants began to wilt even though the battery jars contained a sufficient amount of solution. The experiment was terminated on May 28, 1956, because of the extreme temperatures in the greenhouse.

Fig. 1. Aeration system of the nutrient solution cultures.

IV RESULTS AND DISCUSSION

Greenhouse Pot Studies

Effect of all Soils on Cotton Yields

The yields of cotton grown on four soils in the greenhouse are found in Table V. These data indicated that there was a difference among soils. The analysis of variance test showed that the total yields of cotton displayed a difference due to effect of soils at the 5 percent level of confidence (Table VI). The multiple range test for the differences in soils at the 5 percent probability level, given in Table VII, indicated a significant difference between each soil. Cotton yields on soil D were significantly higher than the yields on the other three soils. The yields of cotton on the chlorotic soils from a given location were significantly higher than the yields on the non-chlorotic soils from the same area. Confidence intervals for the true difference between soils at the 5 percent probability level are shown in Table IV.

TABLE IV

CONFIDENCE INTERVALS (5% P-level) FOR
THE TRUE DIFFERENCE BETWEEN SOILS

Soils	Lower Limit	Upper Limit
Soil D minus Soil C	.8554	2.3206
Soil D minus Soil B	2.4054	3.8706
Soil D minus Soil A	3.4924	4.9576
Soil C minus Soil B	.8174	2.2826
Soil C minus Soil A	1.9044	3.3696
Soil B minus Soil A	.3544	1.8196

TABLE V

THE EFFECT OF VARIOUS TREATMENTS ON COTTON YIELDS (Expressed in grams per pot) OBTAINED FROM FOUR SOILS IN THE GREENHOUSE

Treatments	Soils				
	A	B	C	D	TOTALS
1	25.4*	29.9	23.5	36.0	114.8
2	25.0	25.4	17.9	23.4	91.7
3	24.6	29.8	27.6	28.9	110.9
4	22.1	30.3	26.7	30.9	110.0
5	24.6	18.2	22.4	26.3	91.5
6	17.8	29.2	27.6	20.2	94.8
7	17.6	11.7	19.4	26.1	74.8
8	19.4	23.1	20.1	18.5	81.1
Totals	176.5	197.6	185.2	210.3	

*Each figure represents an average of three plants per pot and an average of three replications.

Key to Treatments

1. Check

2. Minor element mixture.
   21.6 #/A. of Fe as $FeSO_4.7H_2O$
   21.6 #/A. of Mn as $MnSO_4.H_2O$
   4.0 #/A. of B as $Na_2B_4O_7.4H_2O$
   1.5 #/A. of Cu as $CuSO_4.5H_2O$
   1.5 #/A. of Zn as $ZnSO_4.7H_2O$
   0.5 #/A. of Mo as H_2MoO_4

3. 21.6 #/A. of Chelated Iron (HEEDTA)

4. 21.6 #/A. of Mn as $MnSO_4.H_2O$ + 21.6 #/A. Chelated Iron (HEEDTA)

5. 21.6 #/A. of Mn as $MnSO_4.H_2O$ + 21.6 #/A. Fe as $FeSO_4.7H_2O$

6. 21.6 #/A. of Mn as $MnSO_4.H_2O$ + 21.6 #/A. Fe as $FeSO_4.7H_2O$ (Spray Application)

7. Compacted Layer + 21.6 #/A. of Chelated Iron (HEEDTA)

8. Compacted Layer

TABLE VI

ANALYSIS OF VARIANCE OF COTTON YIELDS OBTAINED FROM FOUR SOILS IN THE GREENHOUSE

Source	d.f.	S.S.	M.S.	F.
Total	93	524.01		
Block	2	60.58		
Total Treatments	31	270.20	8.7161	2.7064**
Soils	3	27.17	9.0566	2.8121*
Treatments	7	123.56	17.6514	5.4809**
Soils x Treatments	21	119.47	5.6890	1.7664*
Error	60	193.23	3.2205	

*Indicates significance at the 5% level of confidence.
**Indicates significance at the 1% level of confidence.

TABLE VII

A MULTIPLE RANGE TEST SHOWING THE SIGNIFICANT DIFFERENCES IN COTTON YIELDS DUE TO THE EFFECT OF SOILS IN THE GREENHOUSE

A. Standard Error of Mean: $\sqrt{\frac{\text{Error Mean Square}}{\text{No. of items in Soils}}}$ = .3663 (d.f. = 60)

B. Shortest Significant Ranges:

Range:		(2)	(3)	(4)
	(5% level)	P = 2.83	2.98	3.08
		RP = 1.036	1.092	1.128

C. Results:

Soils:	A	B	C	D
Means Ranked in Order:	22.063	23.150	24.700	26.288

Note: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.
A solid line underscore indicates a similarity of soils at the 5% probability level.

These confidence intervals depicted more difference between soil C and soil D than between soil A and soil B. The true difference between soil A and Soil B was less than any other two soils in this experiment.

Effect of Treatments on Cotton Yields Obtained from Individual Soils

The yield data in Table V indicated a difference among the effects of treatments on the different soils. From these data, an individual analysis of each soil seemed justifiable. The analysis of variance test of the treatments on soil A gave no significant difference in cotton yields among the treatments (Table VIII). The analysis of variance test on soil B found in Table IX disclosed significant differences in treatments at the 1 percent level of confidence. The multiple range test showed that the check, chelated iron, chelated iron plus manganese, and the inorganic iron plus manganese (spray application) treatments were similar at the 5 percent level of confidence (Table X). The chelated iron plus manganese treatment was slightly better than the check, chelated iron and inorganic iron plus manganese (spray application) treatments. At the 1 percent level of confidence the check, minor element mixture, chelated iron, chelated iron plus manganese, and inorganic iron plus manganese (spray application) treated pots were similar. The minor element mixture and compacted layer treatments were similar at the 5 percent level of confidence and were significantly lower then the chelated iron plus manganese, check, chelated iron and inorganic iron plus manganese (spray application) treatments. The inorganic iron plus manganese and compacted layer plus chelated iron treated pots were significantly different at the 1 percent level of confidence and were significantly lower than all other treatments.

TABLE VIII

ANALYSIS OF VARIANCE OF COTTON YIELDS SHOWING THE EFFECT OF TREATMENTS ON SOIL A

Source	d.f.	S.S.	M.S.	F.
Total	23	108.24		
Blocks	2	45.51		
Treatments	7	25.94	3.7057	1.8172
Error	14	28.55	2.0392	

TABLE IX

ANALYSIS OF VARIANCE OF COTTON YIELDS SHOWING THE EFFECT OF TREATMENTS ON SOIL B

Source	d.f.	S.S.	M.S.	F.
Total	23	155.25		
Blocks	2	1.10		
Treatments	7	106.32	15.1885	4.4457**
Error	14	47.83	3.4164	

**Indicates significance at the 1% level of confidence.

TABLE X

A MULTIPLE RANGE TEST OF COTTON YIELDS SHOWING
THE EFFECT OF TREATMENTS ON SOIL B

A. Standard Error of Mean: $\sqrt{\frac{\text{Error Mean Square}}{\text{No. of items in Soils}}} = 1.06715$ (d.f. = 14)

B. Shortest Significant Ranges:

Range		(2)	(3)	(4)	(5)	(6)	(7)	(8)
(5% level)	P =	3.03	3.18	3.27	3.33	3.37	3.39	3.41
	RP =	3.2335	3.3935	3.4896	3.5536	3.5963	3.6176	3.6390
(1% level)	P =	4.21	4.42	4.55	4.63	4.70	4.78	4.83
	RP =	4.4927	4.7168	4.8555	4.9409	5.0156	5.1010	5.1543

C. Results:

Treatments:	7	5	8	2	6	3	1	4
Means Ranked in Order:	11.7	18.2	23.1	25.4	29.2	29.8	29.9	30.3

Note: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.
A solid line underscore indicates similarity at the 5% probability level.
A broken line underscore indicates similarity at the 1% probability level.

The analysis of variance on soil C located in Table XI showed significant differences among the treatments at the 5 percent level of confidence. The multiple range test, indicated a similarity among the inorganic iron plus manganese (spray application), chelated iron, and chelated iron plus manganese treated pots and disclosed that they were significantly better than all other treatments (Table XIII). The check and inorganic iron plus manganese treatments were similar and were significantly better than the minor element mixture, compacted layer plus chelated iron and compacted layer treatments. The compacted layer plus chelated iron, compacted layer, and minor element mixture treated pots were similar and were significantly lower than all other treatments. The analysis of variance, on soil D gave significant differences among treatments at the 5 percent level of confidence (Table XII). The multiple range test in Table XIV showed that the check pots were significantly better than all other treatments. The chelated iron and chelated iron plus manganese treatments were similar and were significantly lower than the check. The chelated iron and inorganic iron plus manganese treatments were also similar. The inorganic iron plus manganese and compacted layer plus chelated iron treated pots were similar, but the chelated iron and compacted layer plus chelated iron were significantly different. The minor element mixture and compacted layer plus chelated iron treatments were similar and were better than the inorganic iron plus manganese (spray application) and compacted layer treatments.

TABLE XI

ANALYSIS OF VARIANCE OF COTTON YIELDS SHOWING
THE EFFECT OF TREATMENTS ON SOIL C

Source	d.f.	S.S.	M.S.	F.
Total	23	117.57		
Blocks	2	59.36		
Treatments	7	34.60	4.9428	2.9309*
Error	14	23.61	1.6864	

*Indicates significance at the 5% level of confidence.

TABLE XII

ANALYSIS OF VARIANCE OF COTTON YIELDS SHOWING
THE EFFECT OF TREATMENTS ON SOIL D

Source	d.f.	S.S.	M.S.	F.
Total	23	115.78		
Blocks	2	.49		
Treatments	7	76.17	10.8814	3.8942*
Error	14	39.12	2.7942	

*Indicates significance at the 5% level of confidence.

TABLE XIII

A MULTIPLE RANGE TEST OF COTTON YIELDS SHOWING
THE EFFECT OF TREATMENTS ON SOIL C

A. Standard Error of Mean: $\sqrt{\frac{\text{Error Mean Square}}{\text{No. of items in Soils}}}$ = .74973 (d.f. = 14)

B. Shortest Significant Ranges:

Range		(2)	(3)	(4)	(5)	(6)	(7)	(8)
(5% level)	P =	3.03	3.18	3.27	3.33	3.37	3.39	3.41
	RP =	2.2717	2.3841	2.4516	2.4966	2.5266	2.5416	2.5566

C. Results:

Treatments:	2	7	8	5	1	4	3	6
Means Ranked in Order:	17.9	19.4	20.1	22.4	23.5	26.7	27.6	27.6

Note: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.
A solid line underscore indicates similarity at the 5% probability level.

TABLE XIV

A MULTIPLE RANGE TEST OF COTTON YIELDS SHOWING
THE EFFECTS OF TREATMENTS ON SOIL D

A. Standard Error of Mean: $\sqrt{\frac{\text{Error Mean Square}}{\text{No. of items in Sums}}}$ = .9651 (d.f. = 14)

B. Shortest Significant Ranges:

Range	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(5% level)	P = 3.03	3.18	3.27	3.33	3.37	3.39	3.41
	RP = 2.9243	3.0690	3.1559	3.2138	3.2524	3.2717	3.2910

C. Results:

Treatments:	8	6	2	7	5	3	4	1
Means Ranked in Order:	18.5	20.2	23.4	26.1	26.3	28.9	30.9	36.0

Note: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.
A solid line underscore indicates similarity at the 5% probability level.

Effect of Treatment on Cotton Yields

The analysis of variance test, which is located in Table VI, indicated a significant difference in effect of treatments on cotton yields at the 1 percent level of confidence. The multiple range test, showed the significant differences in cotton yields due to the various treatments (Table XVI). The yields obtained from the treated soils were all lower than the yields produced on the check pots. The soil applications of chelated iron and chelated iron plus manganese (Treatments 3 and 4) were not significantly different from the check, although the yields were slightly reduced. The inorganic iron plus manganese, inorganic iron plus manganese (spray application) and minor element mixture treatments produced cotton yields which were not significantly different at the 1 percent level of confidence, but all of them were significantly lower than the check. The compacted layer plus chelated iron treated pots were significantly lower in yields than the compacted layer treatment at the 5 percent level of confidence, but were not significantly different at the 1 percent level. Both of these treatments were significantly lower than the check at both levels of confidence. Confidence intervals for the true difference between significantly different treatments at the 1 percent probability level are given in Table XV.

TABLE XV

CONFIDENCE INTERVALS (1% P-level) FOR THE TRUE DIFFERENCE BETWEEN SIGNIFICANTLY DIFFERENT TREATMENTS

Treatments	Lower Limit	Upper Limit
Treatment 3 minus Treatment 7	7.6471	10.4029
Treatment 4 minus Treatment 6	2.4221	5.1779
Treatment 4 minus Treatment 5	3.2471	6.0029
Treatment 1 minus Treatment 5	4.4471	7.2029
Treatment 5 minus Treatment 8	1.2221	3.9779

TABLE XVI

A MULTIPLE RANGE TEST SHOWING THE SIGNIFICANT DIFFERENCES IN COTTON YIELDS DUE TO THE EFFECT OF TREATMENTS

A. Standard Error of Mean: $\sqrt{\frac{\text{Error Mean Square}}{\text{No. of items in treatments}}} = .5180$ (d.f. = 60)

B. Shortest Significant Ranges:

Range		(2)	(3)	(4)	(5)	(6)	(7)	(8)
(5% level)	P =	2.83	2.98	3.08	3.14	3.20	3.24	3.28
	RP =	1.466	1.544	1.595	1.627	1.658	1.678	1.699
(1% level)	P =	3.76	3.92	4.03	4.12	4.17	4.23	4.27
	RP =	1.948	2.031	2.087	2.134	2.160	2.191	2.212

C. Results:

Treatments:	7	8	5	2	6	4	3	1
Means Ranked in Order:	18.700	20.275	22.875	22.925	23.700	27.500	27.725	28.700

Note: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.
A solid line underscore indicates similarity at the 5% probability level.
A broken line underscore indicates similarity at the 1% probability level.

These confidence intervals indicated that there was less difference between the inorganic iron plus manganese and compacted layer treatments than any others which were compared. The chelated iron and compacted layer plus chelated iron gave the greatest difference between treatments. Other yield factors being equal, this revealed the effects of the compacted layer. These intervals showed that all the compared treatments had rather wide limits for significant differences, even at the 1 percent level of confidence.

Laboratory Tests

Effect of Treatments on the Iron Content of Soils and Plants

The available iron content of the soils (before and after cropping) used in the greenhouse pot studies is shown in Table XVII. The value for the available iron content of the soils before cropping was composed of the native available iron plus 21.6 pounds per acre added in the different treatments. The native available iron content of soil A, B, C, and D was 5.1, 1.3, 3.3 and 1.4 pounds per acre, respectively. The iron amendments caused only a slight variation in the available iron content of each soil after cropping. The soils which were higher in available iron at the beginning of the study were also the highest at the termination of the experiment. The theoretical fixation of iron was slightly lower on the chelated iron treatment. The theoretical amount of iron released on the check pots was approximately equal to the amount absorbed by the plants and was slightly greater on the chlorotic soils than on the non-chlorotic soils. The chelated iron and inorganic iron plus manganese (spray application) treatments resulted in slightly

TABLE XVII

EFFECT OF TREATMENTS ON THE IRON CONTENT
(Expressed in pounds per acre)
OF SOILS AND PLANTS

Treatment	Before Cropping	Crop Removal	Theoretical Difference	After Cropping	Theoretical Fixation
2A[1]	26.7	7.8	18.9	3.0	15.9
3A	26.7	13.3	13.4	4.5	8.9
4A	26.7	7.4	19.3	3.8	15.5
5A	26.7	9.4	17.3	3.0	14.3
7A	26.7	9.4	17.3	4.1	13.2
2B	22.9	7.3	15.6	2.2	13.4
3B	22.9	12.3	10.6	3.0	7.6
4B	22.9	7.8	15.1	2.2	12.9
5B	22.9	9.2	13.7	2.1	11.6
7B	22.9	10.0	12.9	2.9	10.0
2C	24.9	7.6	17.3	3.2	14.1
3C	24.9	12.5	12.4	4.4	8.0
4C	24.9	7.2	17.7	4.1	13.6
5C	24.9	8.9	16.0	2.6	13.4
7C	24.9	9.2	15.7	4.7	11.0
2D	23.0	7.3	15.7	2.9	12.8
3D	23.0	12.1	10.9	2.9	8.0
4D	23.0	7.5	15.5	3.1	12.4
5D	23.0	8.7	14.3	3.0	11.3
7D	23.0	9.1	13.9	3.6	10.3

[1]Numbers designate treatments and letters designate soils.

higher uptake of iron by the plants. When manganese was supplied in addition to iron, the iron content of the plants was reduced except in the inorganic iron plus manganese (spray application) treatment. There were only small variations in the iron content of the plants as a result of other amendments.

Effect of Treatments on the Manganese Content Of Soils and Plants

The available manganese content of the soils, before and after cropping in the greenhouse, is given in Table XVIII. The available manganese content of the soils before cropping varied with the treatments and soils. The minor element mixture, chelated iron plus manganese, inorganic iron plus manganese and inorganic iron plus manganese (spray application) treatments had an addition of 21.6 pounds of manganese per acre. The native available manganese for soil A, B, C and D was 70.0, 78.0, 40.5 and 45.0 pounds per acre, respectively. The available manganese content of the soils after cropping showed only small variations due to the effect of treatments. There was no trend established for any particular treatment on the soils. The theoretical fixation of manganese was greater when manganese was added at the beginning of the experiment. The greatest amount of fixation occurred on soil B. The compacted layer plus chelated iron treatment resulted in slightly higher uptake of manganese by the plant. There appeared to be no particular relationship between the manganese content of the plants and any other treatments. Additions of manganese seemed to have no effect on the manganese content of the plants.

TABLE XVIII

EFFECT OF TREATMENTS ON THE MANGANESE CONTENT
(Expressed in pounds per acre)
OF SOILS AND PLANTS

Treatment	Before Cropping	Crop Removal	Theoretical Difference	After Cropping	Theoretical Fixation or Release*
2A[1]	91.6	4.2	87.4	73.0	-14.4
3A	70.0	5.2	64.8	69.0	4.2
4A	91.6	5.8	85.8	59.0	-26.8
5A	91.6	6.1	85.5	58.0	-27.5
7A	70.0	6.9	63.1	59.7	- 3.4
2B	99.6	4.2	95.4	41.3	-54.1
3B	78.0	6.0	72.0	34.0	-38.0
4B	99.6	3.8	95.8	40.7	-55.1
5B	99.6	7.3	92.3	31.7	-60.6
7B	78.0	8.8	69.2	46.7	-22.5
2C	62.1	5.2	56.9	39.3	-17.6
3C	40.5	6.5	34.0	38.7	4.7
4C	62.1	3.8	58.3	45.3	-13.0
5C	62.1	5.7	56.4	49.3	- 7.1
7C	40.5	7.9	32.6	40.3	7.7
2D	66.6	4.7	61.9	36.3	-25.6
3D	45.0	6.8	38.2	39.0	.8
4D	66.6	4.2	62.4	47.7	-14.7
5D	66.6	5.9	60.7	48.0	-12.7
7D	45.0	7.5	37.5	38.3	.8

* A negative sign indicates the amount of fixation.

[1]Numbers designate treatments and letters designate soils.

Nutrient Solution Cultures

Deficiency Symptoms

Although plants are unable to talk, they have a means of expressing their needs through abnormalities that are often called "deficiency symptoms" or "hunger signs". There are many conditions, other than nutritional, that can cause symptoms to occur, therefore, all signs of abnormalities should not be interpreted as actual nutritional deficiencies without further investigation. Hunger signs are often very complex and difficult to interpret, but their value as a guide to more detailed investigations should not be overlooked. The deficiency symptoms produced in this experiment were replicated four times and were quite uniform in all cases. Although, the deficiencies produced were probably of the most extreme nature they should be of considerable value to those who are interested in the role of various elements in cotton nutrition.

Nitrogen. The first visible signs in the deficient plants were very evident within ten days. After twenty-two days, the lower leaves began to show signs of chlorosis as the margins of the leaves appeared to become lighter in color. The leaf margins became yellow, while the remainder of the leaf turned a pale green color a few days later. By the end of the fifth week, the oldest leaves had turned yellow and the cotyledon leaves were beginning to absciss as depicted in Figure 2. Nitrogen deficiency symptoms started in the lower leaves of the plant and progressed upward as the plant became older, which indicated that nitrogen was translocated from the older leaves to the terminal leaves. The root growth of nitrogen deficient plants was reduced to about one-half that of plants grown in complete nutrient solutions and some of the roots were extremely elongated

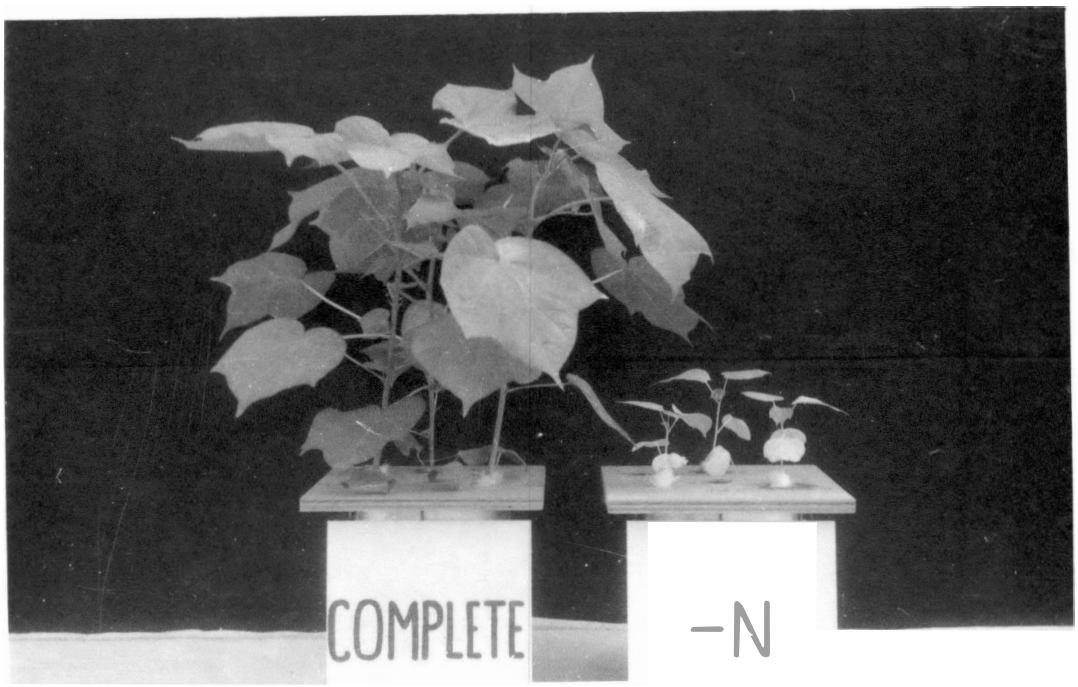

Fig. 2. Top growth of cotton plants after 38 days in a complete and a minus nitrogen nutrient solution.

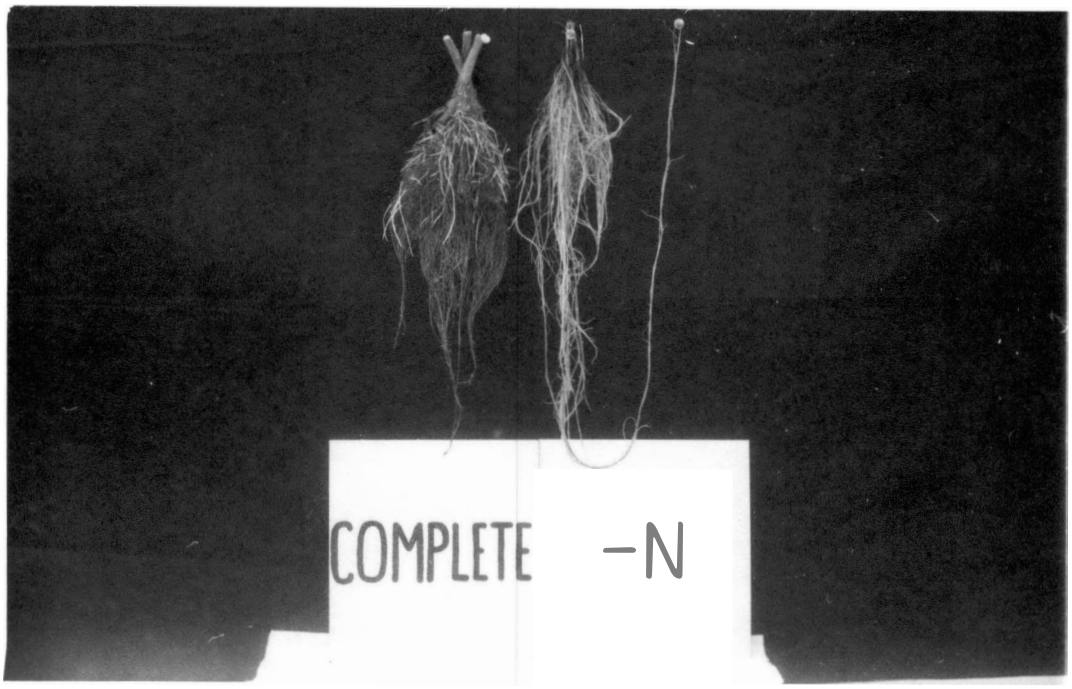

Fig. 3. Root development of cotton after 60 days in a complete and a minus nitrogen nutrient solution.

(Figure 3). It was also noticed that the lower part of the main stalk became very red and appeared to be much weaker than the stalks of plants grown in the complete solutions.

Phosphorus. These deficiency symptoms were much slower to appear than those of nitrogen. The first signs of phosphorus deficiency were noted 21 days after transplanting. They resulted in reduced growth followed by a darkening of the leaves. In a few days, greasy appearing spots occurred along the leaf margins. The leaves felt very leathery, tough and had a glossy appearance. They then exibited an upward cupping and the greasy spots became necrotic (Figure 4). Shortly thereafter, the cotyledon leaves were shed followed by the abscission of successively younger leaves. As the deficiency symptoms became more acute, the plant assumed a spindly appearance with leaves remaining only at the top of the plant. Figure 5 illustrates the reduction in root growth and the slight elongations of the roots. Reddish areas also were found on the lower mainstalk in these plants.

Potassium. These plants developed deficiency symptoms within ten days. They were dwarfed and were much darker in color than those grown in the complete solutions. The potassium deficient leaves were much sharper at the apex than non-deficient leaves. After two weeks, necrotic areas began to occur around the margins and between the veins. As the deficiency progressed, these areas became larger and especially pronounced on the cotyledon leaves. The terminal tissue remained green which indicated that potassium was translocated from the older to the younger leaves. The symptoms moved upward with each successive leaf. The plant leaves assumed a clustered appearance due to drooping as shown in Figure 6. Root

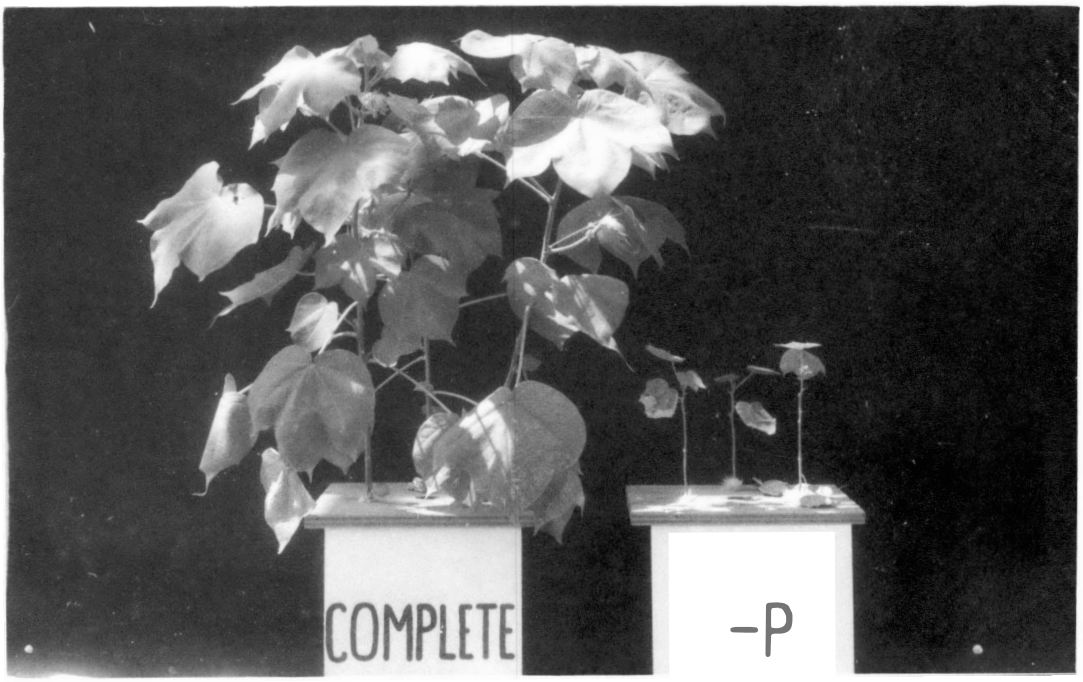

Fig. 4. Top growth of cotton plants after 60 days in a complete nutrient solution culture and one which lacked phosphorus.

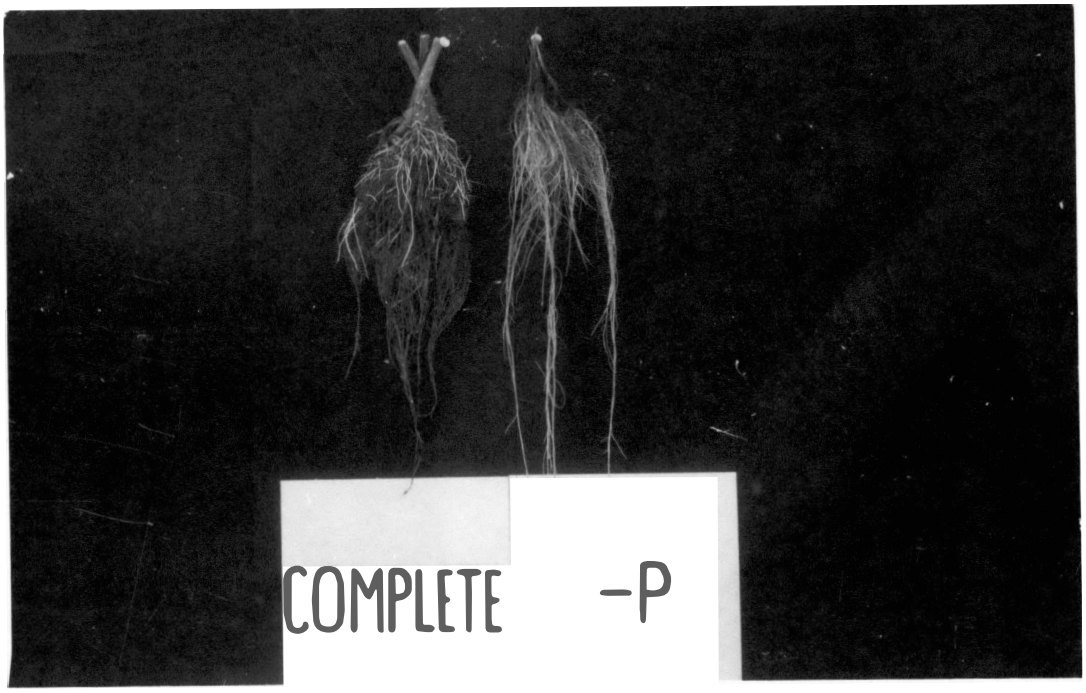

Fig. 5. Root growth of cotton after 60 days in a complete and a minus phosphorus nutrient colution.

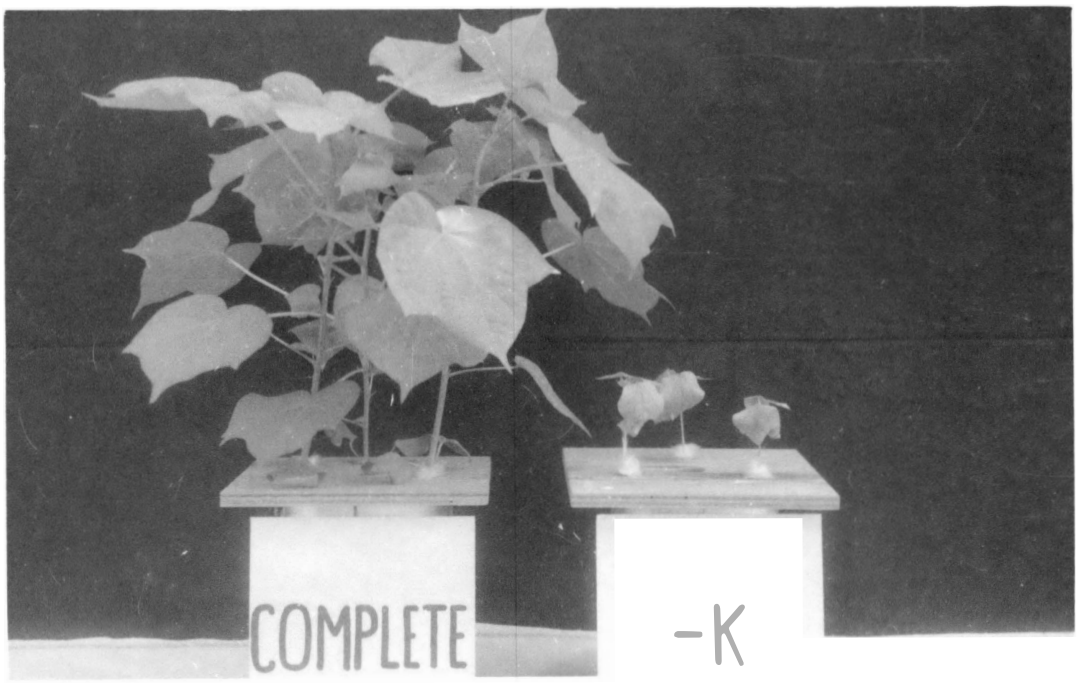

Fig. 6. Top growth of plants after 38 days in a complete and a minus potassium nutrient solution.

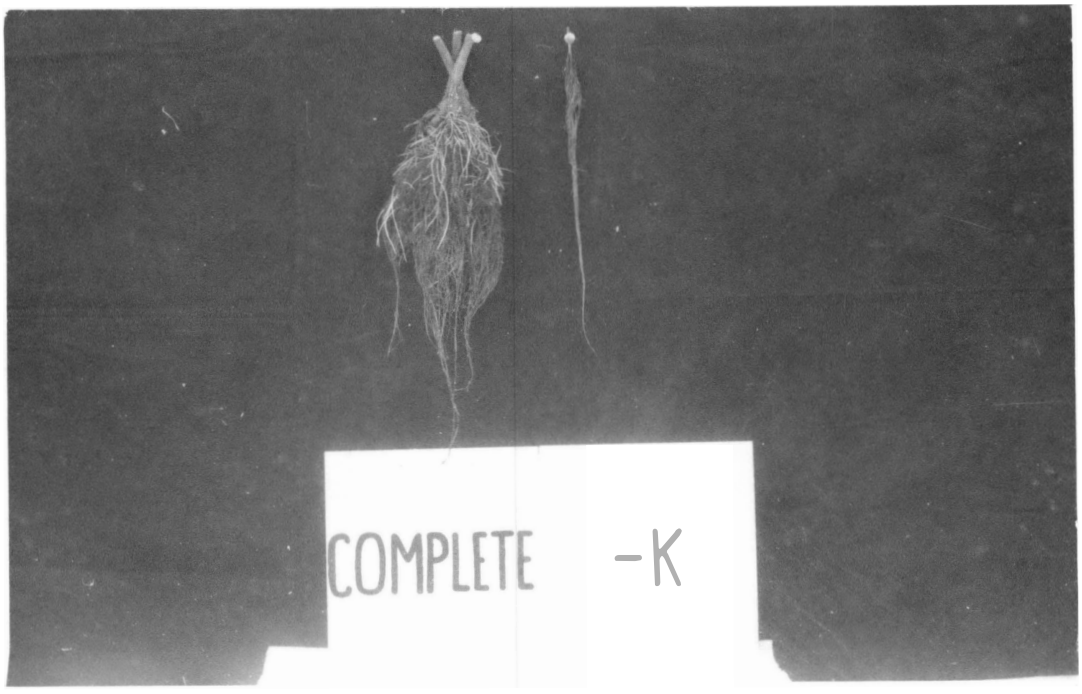

Fig. 7. Root development of cotton after 60 days in a complete and a minus potassium nutrient solution.

development in the potassium deficient plants was very restricted (Figure 7). Figure 8 is a comparison of the characteristics of cotton plants grown in complete, minus nitrogen, minus phosphorus, and minus potassium nutrient solutions.

Calcium. Deficiencies of calcium, which occurred in four days, were the first symptoms to be observed. The plants made only very limited growth and, at most, produced only two true leaves. The leaves had a wilted appearance and the petioles seemed to be very weak allowing the leaves to hang very limp. The terminal tissue turned pale green in color, soon became necrotic and died. Although the terminal tissue was almost dead, the cotyledon leaves retained their green color indicating that calcium was not translocated (Figure 9). The signs were very severe at the end of three weeks and by the fifth week the plants were dead and were beginning to decompose. The roots were very dark and made very little growth, if any, before the deficiencies occurred.

Magnesium. The signs of chlorosis began on the ninth day for the deficient plants. As in the other hunger signs, reduced growth was one of the first noticeable characteristics. Plants grown in a nutrient solution lacking magnesium exhibited one of the most striking of all deficiency symptoms observed. The true leaves first faded to a pale green and then to yellow with the veins remaining green. As the signs became more severe, the leaves tended to elongate slightly and become necrotic around the margins. After the necrotic margins appeared, the leaves cupped upward and began to droop slightly (Figure 10). Finally, the affected leaves became entirely necrotic and started to absciss. As in the case of calcium deficiencies, magnesium is probably not mobile in plants

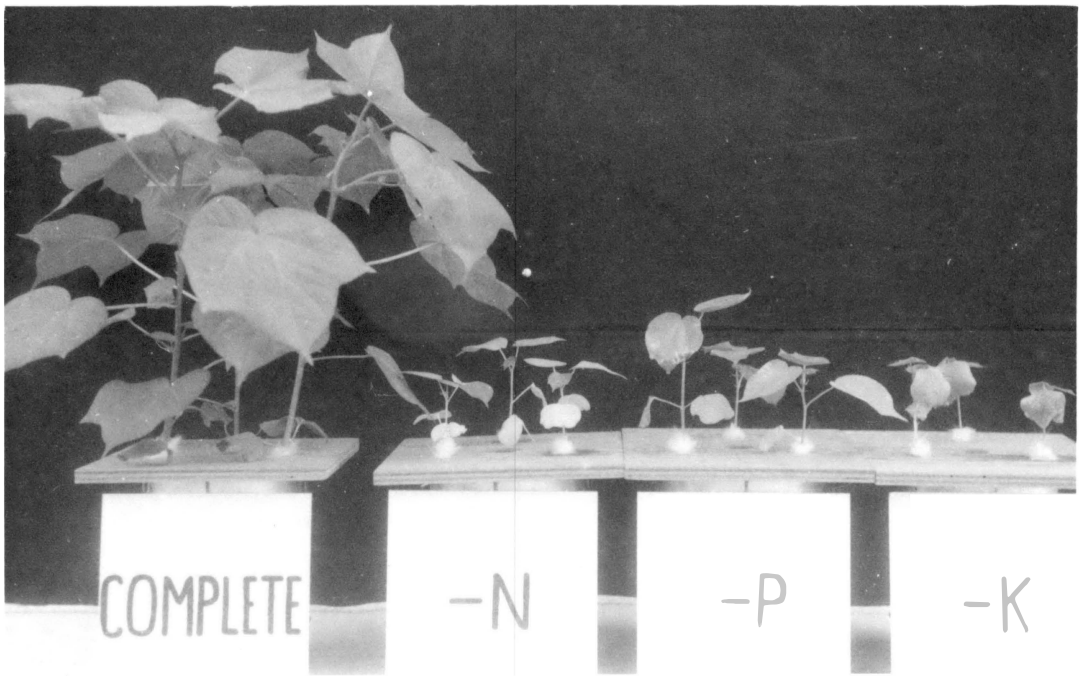

Fig. 8. Top growth of cotton plants after 38 days in a complete solution and ones lacking nitrogen, phosphorus and potassium.

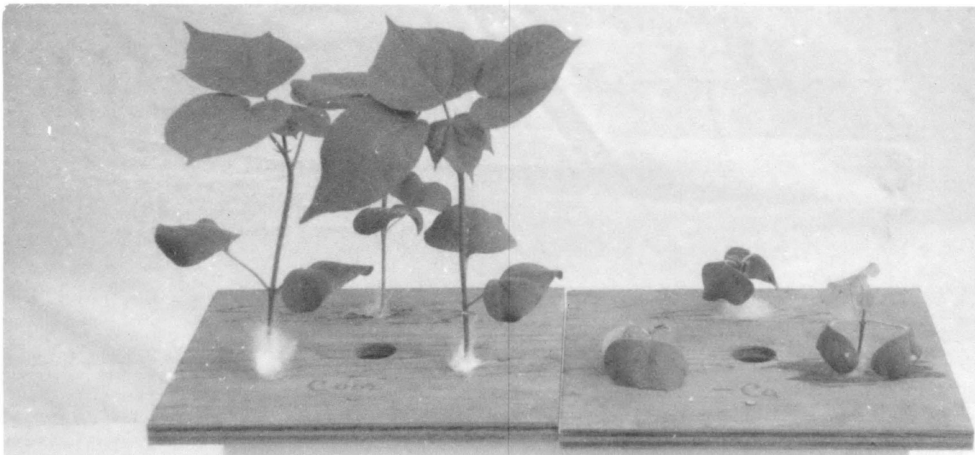

Fig. 9. Top growth of cotton plants after 16 days in a complete nutrient solution and one which lacked calcium.

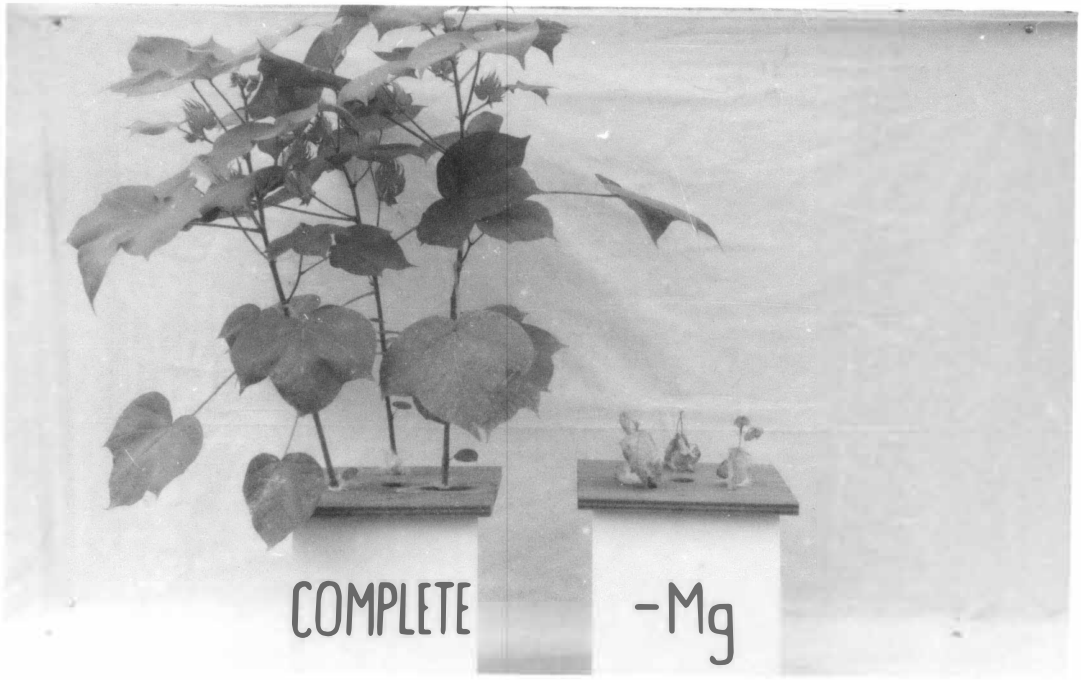

Fig. 10. Top growth of cotton plants after 60 days in a complete and a minus magnesium nutrient solution.

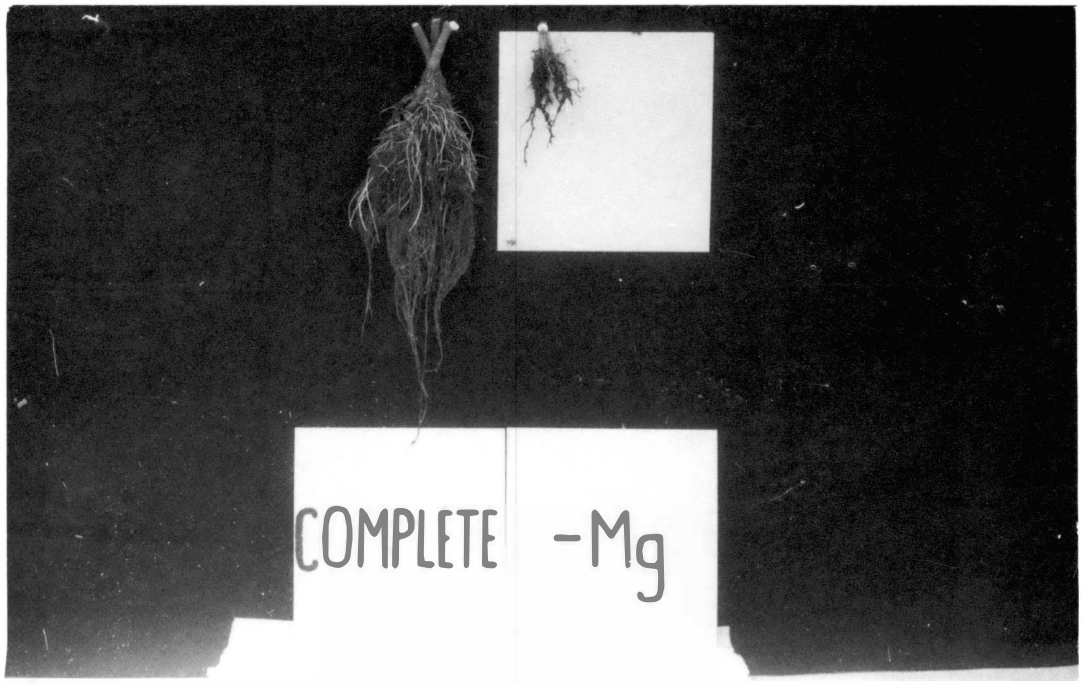

Fig. 11. Root development of cotton after 60 days in a complete and a minus magnesium nutrient solution.

since the cotyledon leaves remained green while the rest of the leaves turned chlorotic. Figure 11 portrays the retarded and very black roots of magnesium deficient plants. These symptoms differed slightly from those described by Cooper (20).

Iron. Deficiencies of this nutrient were the second ones to occur in this study. Retarded growth and a general chlorosis of the terminal tissue were noted in seven days. The new leaves were light colored and soon changed to a pale yellow which was almost white. As more terminal growth occurred, each succeeding leaf was much whiter in appearance. Within four or five days after emergence, the terminal tissue became very necrotic and ragged due to the disappearance of leaf tissue. The chlorotic leaves were very small. They cupped downward in the early stages and then tended to roll upward as the symptoms became more severe. All the growth above the cotyledon leaves was dead by the seventh week, while the cotyledon leaves retained their green color. This indicated that iron was not mobile in the cotton plant. Figure 12 illustrates the new growth which occurred from the axils of the cotyledon leaves during the eighth week. This tissue was also chlorotic, but was still living at the termination of the experiment. However, signs of necrosis were beginning to appear. Root growth in the iron deficient plants was limited, but some elongations did occur (Figure 13).

Manganese. Symptoms of manganese starvation appeared to be the least severe of all the deficiencies in the experiment. Manganese deficiencies were evident in 16 days. Reduced growth and a slight yellowing of the leaves were the first signs noticed. At the end of three weeks the symptoms were more pronounced. The younger leaves became a pale green

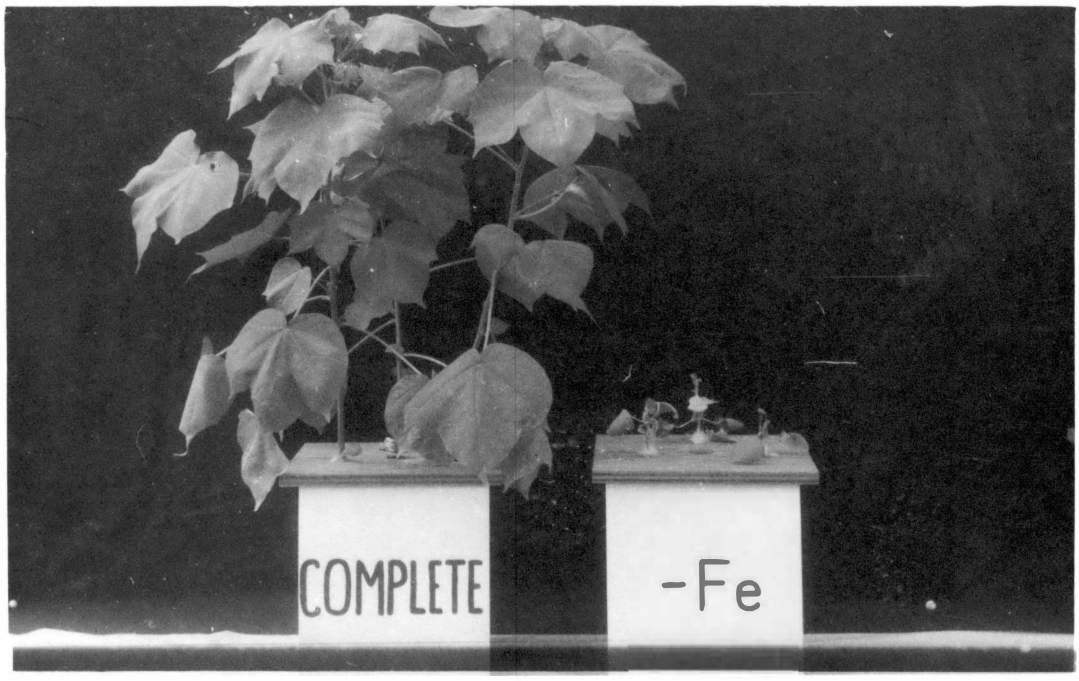

Fig. 12. Top growth of cotton plants after 60 days in a complete nutrient solution and one which lacked iron.

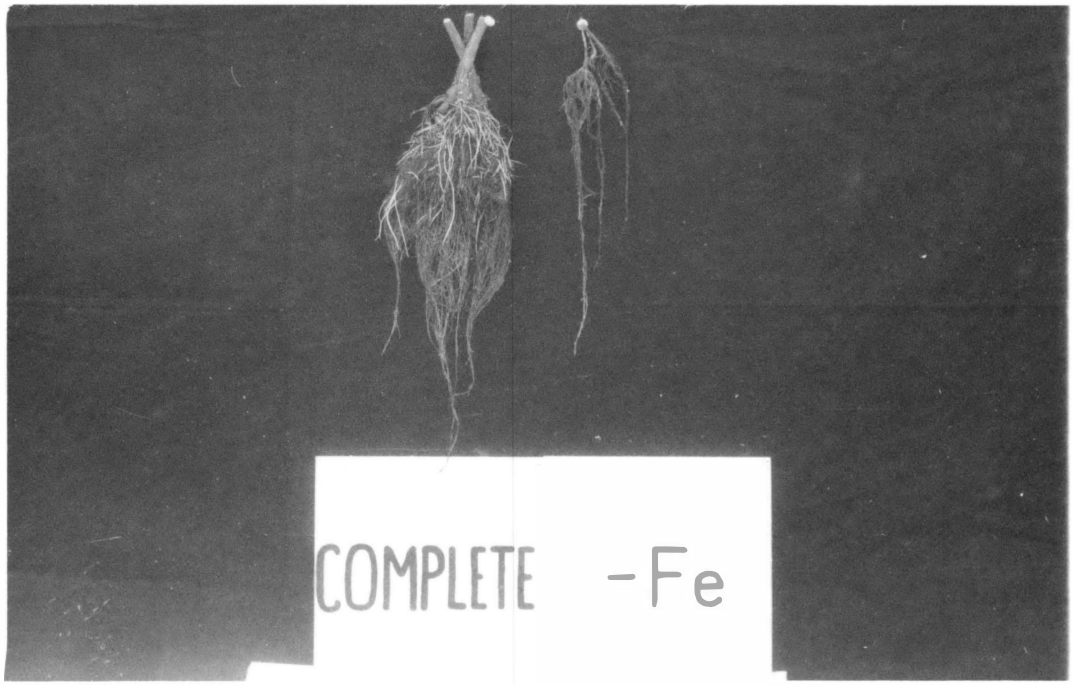

Fig. 13. Root growth of cotton after 60 days in a complete and a minus iron nutrient solution.

and were wrinkled in appearance, while the older leaves retained their dark green color (Figure 14). As the plants became older the younger leaves appeared to regain some of their green color, but they still displayed the wrinkling. At the termination of the experiment, there were no apparent differences in the color of the older and younger leaves. Figure 15 shows that the root growth in the manganese deficient cultures was approximately as good as that found in the complete nutrient solutions. With the exception of the plants grown in complete solutions the manganese deficient plants were the only ones to produce any squares.

Iron and Manganese Content of Cotton Plants Grown in Nutrient Solution Cultures

Plants grown in complete, minus manganese, and minus iron nutrient solutions were analyzed for iron and manganese contents. The results of these analyses are given in Table XIX.

These data indicated that slight variations in analyses occurred among the replications in the experiment. Plants grown in solutions which lacked manganese accumulated slightly more iron than those grown in the complete solutions. Plants grown in complete nutrient solutions contained approximately six times as much iron on a percentage basis, as those grown in iron deficient solutions. Plants grown in the minus iron solutions accumulated much more manganese percentagewise than the plants grown in complete solutions. The manganese content of the manganese deficient plants was quite variable. These manganese contents indicated that some contamination may have occurred. This could possibly explain the partial recovery of the plants grown in the minus manganese solutions.

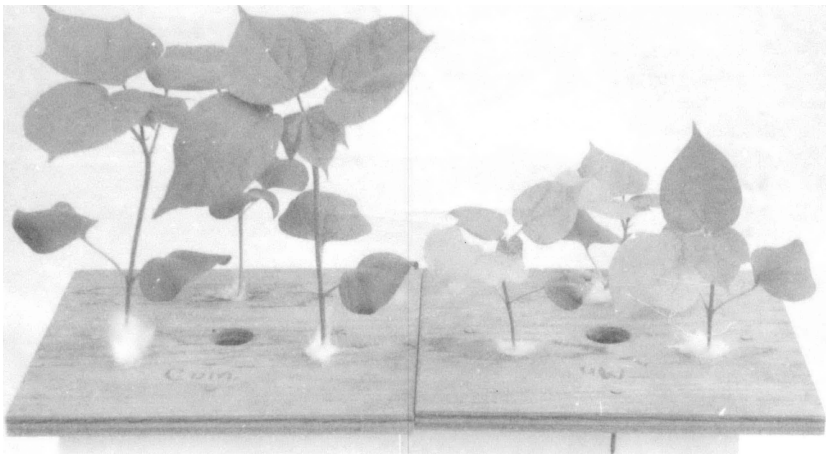

Fig. 14. Top growth of cotton plants after 16 days in a complete and a minus manganese nutrient solution.

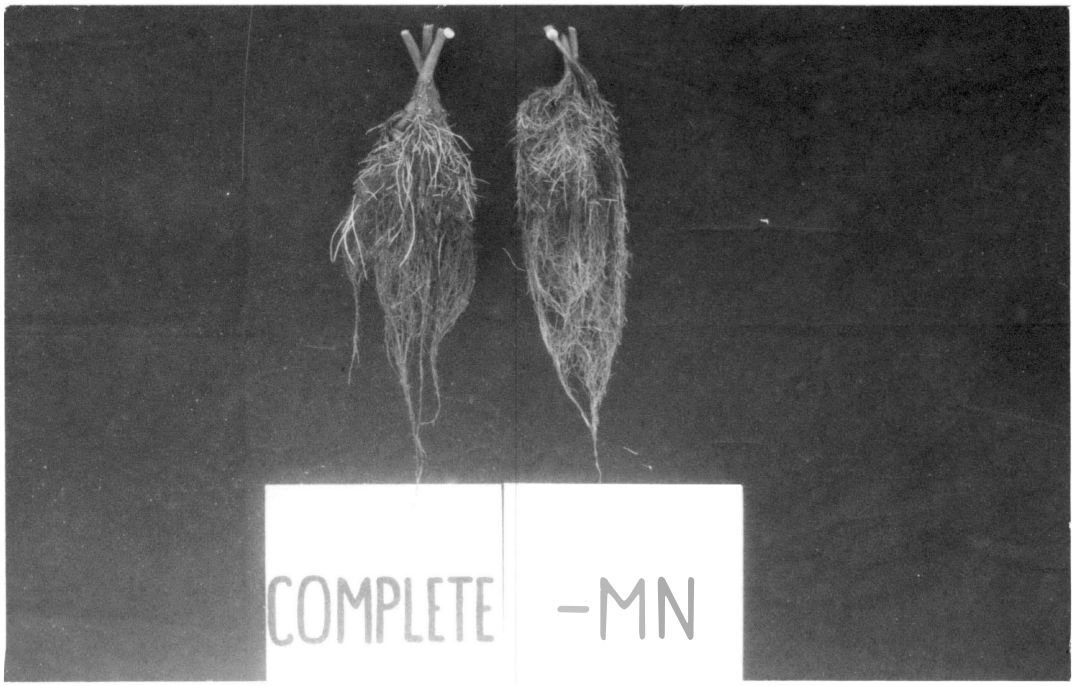

Fig. 15. Root development of cotton after 60 days in a complete nutrient solution culture and one which lacked manganese.

TABLE XIX

IRON AND MANGANESE CONTENTS OF COTTON PLANTS GROWN IN NUTRIENT SOLUTION CULTURES

Treatment	Replication	Percent Iron	Percent Manganese
Complete	1	0.186	0.395
Complete	2	0.194	0.410
Complete	3	0.173	0.430
Complete	4	0.165	0.380
Minus manganese	1	0.224	0.040
Minus manganese	2	0.206	0.050
Minus manganese	3	0.194	0.035
Minus manganese	4	0.206	0.060
Minus iron	1	0.035	3.354
Minus iron	2	0.034	2.789
Minus iron	3	0.024	1.564
Minus iron	4	0.023	1.589

V SUMMARY AND CONCLUSIONS

Greenhouse and laboratory studies were made in an attempt to determine the best means of correcting the chlorosis of cotton grown on two Oklahoma soils. The soils employed in this experiment were a McLain loam and a Brownfield soil. Various chemical amendments (including iron and manganese applied in different forms and by different methods) and a physical treatment of an artificial compacted layer were used. Nutrient solution cultures were conducted to observe and record some of the symptoms of cotton caused by a lack of certain essential plant food nutrients. From the results of these experiments, the following conclusions seem justifiable:

1. Each soil exerted a different effect on the cotton yields in the greenhouse pot studies. The yields of cotton were significantly higher on the chlorotic soils from a given area than those on the non-chlorotic soils from the same area.
2. The influence of the treatments on the yields of cotton varied with each soil. The chelated iron and chelated iron plus inorganic manganese treated pots produced yields which were similar to the checks but were significantly better than all the other treatments in the over-all analysis of the experiment. The compacted layer treatments depressed yields more than any other treatments used in this study.

3. The iron amendments caused only slight variations in the available iron content of the soils after cropping. Likewise, manganese additions created only small changes in the amount of available manganese in the soils after cropping.
4. The chelated iron and inorganic iron plus manganese (spray application) treatments resulted in slightly greater absorption of iron by the plants. Additions of manganese reduced the iron content of plants, except when inorganic iron plus manganese were applied as a spray. Slightly greater amounts of manganese were absorbed by the plants grown in the pots containing a compacted layer.
5. Iron, magnesium, and calcium were immobile in the cotton plants grown in the nutrient solutions. However, potassium and nitrogen were translocated in the deficient plants.
6. In the nutrient culture experiment, a lack of calcium or iron caused the most severe deficiency signs. The symptoms developed by the magnesium deficient plants were the most striking. Whereas, the growth of the cotton was least affected in the minus manganese solutions.
7. Under optimum conditions of temperature and moisture in the greenhouse, cotton grown on these soils did not develop chlorosis. Under field conditions, cotton chlorosis has been observed on soils with low moisture and high temperature levels. Further enlightenment on this subject could possibly be obtained through a study of varying the temperature and moisture levels as well as other soil conditions.

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