EFFECT OF CHELATE COMPOUNDS ON THE IRON NUTRITION OF THE BEAN, *PHASEOLUS VULGARIS* L.

by

JOHN LESLIE MASON

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APPROVED:

[redacted]

Associate Professor of Botany

In Charge of Major

[redacted]

Chairman of Botany and Plant Pathology Department

[redacted]

Chairman of School Graduate Committee

[redacted]

Dean of Graduate School

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INTRODUCTION

Iron deficiency has been the cause of considerable economic losses in crops grown on both extremely acid and extremely alkaline soils. Although it was the first micro-element deficiency to be recognized, and has been the subject of countless experiments, it has remained until very recently one of the most baffling deficiencies to study and to correct.

However, in 1951, Jacobson (31, p. 413) showed that the ferric potassium salt of ethylenediaminetetraacetic acid (EDTA) was very satisfactory as a source of iron for plants growing in nutrient solutions. Shortly after, in 1952, Steward and Leonard (47, p. 566) reported the successful cure of iron deficiency chlorosis in citrus orchards with this material.

The first successes with citrus were obtained on acid soils. Unfortunately, when EDTA was tried on alkaline soils, it frequently failed to correct iron deficiency because the iron complex was not sufficiently stable under alkaline conditions. Recently, several new compounds have been developed by making substitutions in the chains attached to the ethylenediamine nucleus. Some of these compounds show promise as complexing agents for iron for the nutrition of plants under alkaline conditions.

The objectives of this study were to develop rigidly controlled methods of growing plants in nutrient solutions at alkaline

pH's, to determine the critical, optimum and toxic levels of two of the new compounds, the iron complexes of N-hydroxyethylethylene-diaminetetraacetic acid (HEEDTA) and diethylethylenetriamine-pentaacetic acid (DTPA), as sources of iron for plants grown in nutrient solutions at high pH, to determine the effect of pH, phosphorus and calcium concentration of the nutrient solution on yield and iron uptake of plants, and to measure the interactions between iron and phosphorus, and iron and calcium when iron was supplied as chelate complexes.

REVIEW OF LITERATURE

Causes of Iron-Deficiency Chlorosis

Antognini (2, p. 47) considered that iron chlorosis of plants could be caused by an actual deficiency of iron, to soil reaction, or to a lack of available iron in the soil due to high manganese, copper or lime. Hewitt (52, p. 143) stated that iron deficiency can be caused by high lime content of calcareous soils, deficiency of other elements, or excess of phosphorus, manganese, zinc or copper. He thought that simple deficiency of iron probably occurred only under pot conditions. Porter and Thorne (44, p. 373) considered that high moisture, low temperature, high lime content, and high bicarbonate ion concentration in the soil were associated with chlorosis. Haertl (18, p. 86) stated that extremely acid or basic soil frequently leads to iron-deficiency chlorosis. Holley and Cain (25, p. 173) noted that the same type of chlorosis found on calcareous soils can also result from the presence of nickel or cobalt which apparently interferes with the function of iron. De Kock (34, p. 172) grew mustard plants in nutrient solutions under oxygen and bicarbonate treatments using several chelate compounds. With radioactive iron and the autoradiograph technique, he showed that in the chlorotic plants there was little iron in the interveinal tissue but considerable concentrations in the conducting elements. It may be noted throughout these reports that actual

deficiency of iron is seldom credited with causing iron-deficiency chlorosis.

Effect of Iron Deficiency on Plants

In extreme studies of iron-deficiency chlorosis by systemic analyses of the leaves of normal and chlorotic plants, Iljin studied the nitrogen distribution, the carbohydrate and organic acid metabolism, and the ash content of seventeen species of European plants over a period of four years.

His studies of nitrogen metabolism (27, p. 295) included total nitrogen, soluble nitrogen, amino nitrogen, ammonium nitrogen, amide nitrogen, and residual nitrogen. He found that the total nitrogen content of young leaves of healthy plants was highest in the spring and gradually decreased after the leaves were fully formed. The total nitrogen content of young leaves of chlorotic plants at the same time was considerably greater. At this stage, the soluble nitrogen content of chlorotic leaves might be five to fifteen times as high as that of healthy ones. Soluble nitrogen is rapidly assimilated in healthy leaves. This was retarded in chlorotic leaves where amino nitrogen might be several times higher than in healthy ones by early summer. Ammonium nitrogen in normal and chlorotic tissues in the spring was low and about the same in both. Amide nitrogen might be ten or more times higher in chlorotic leaves than in healthy ones. Insoluble nitrogen formed ninety-four to ninety-

six per cent of the total nitrogen in healthy, fully-developed leaves, whereas only thirty to forty per cent was insoluble in chlorotic leaves. Iron deficiency evidently interferes with the conversion of soluble nitrogen compounds to the insoluble compounds. "Residual nitrogenous compounds" (unidentified compounds) were absent in healthy foliage or present in small amounts only, whereas in chlorotic plants they accounted for forty to sixty-five per cent of the soluble nitrogen.

Holley and Cain (25, p. 172), investigating the residual nitrogenous compounds that Iljin described, studied chlorotic and healthy blueberry, apple and magnolia leaves and identified the free amino acids by paper chromatography of the eighty per cent alcohol extract. They determined arginine by a colorimetric method as a check on the chromatographic method and found that an accumulation of free arginine is characteristic of plants affected with iron-deficiency chlorosis, and that the arginine disappears with recovery from chlorosis. Chlorotic blueberry leaves contained about 25 micrograms of free arginine per square centimetre whereas green leaves contained only 1 microgram per square centimetre.

In studies of carbohydrate and organic acid metabolism, Iljin (28, p. 350) analyzed the sap of the same seventeen species of European plants during four seasons for total organic acids as well as for certain specific acids such as citric, malic and tartaric. He considered that the increased carbohydrate content of diseased

foliage over healthy foliage might result from impaired translocation of carbohydrates out of chlorotic leaves. In those species which he considered able to metabolize citric acid, he found several times more citric acid in the leaves of chlorotic plants than in those of healthy ones, the amount being directly correlated with the severity of the disease.

In studies of the ash content of leaves, Iljin (29, p. 26) found that the mineral content of the sap of chlorotic leaves exceeded that of normal ones. He also found that the mineral content of normal leaves increased gradually from spring to autumn, whereas irregular fluctuations occurred in chlorotic leaves. There was more potassium in the sap of chlorotic leaves than in the sap of healthy leaves. Potassium content decreased progressively throughout the growing season in normal leaves, but there was a marked increase in midsummer in chlorotic leaves. Chlorotic leaves usually contained more calcium than healthy ones and the ratio of potassium to calcium in chlorotic leaves was higher than that in healthy leaves. The magnesium content increased with increasing chlorosis.

Wallace and Hewitt (52, p. 159) considered that the outstanding characteristic of the inorganic status of plants suffering from lime-induced chlorosis was the high content of potassium and the low calcium to potassium ratio. They suggested that this condition was an effect of chlorosis rather than a cause.

Baxter and Belcher (5, p. 34) titrated suspensions of ground leaves and roots of citrus from chlorotic and healthy trees grown

on both alkaline and acid soils. In the chlorotic plants, they found a higher pH, a higher buffer capacity of the root sap, and a higher concentration of calcium and carbonate ions of the roots than in the healthy plants. They considered that the accumulation of bicarbonate ion in roots unfavorably affected carbon dioxide excretion and thus internal pH, and was the main factor in the metabolic disturbance leading to iron deficiency.

Effect of pH of the Substrate on Plant Growth

Arnon and Johnson (4, p. 525) studied the effect of the pH of the nutrient solution on the growth of tomato, lettuce and Bermuda grass. They found that complete failure of growth occurred at pH 3. In no case did root growth occur at this pH. At the other extreme, injury and a marked reduction of growth were observed at pH 9. Best growth of plants, as measured by fresh weight, occurred at pH 5, 6, and 7. There was considerably reduced growth at pH 4 and 8.

In a general review of the influence of pH of the nutrient solutions on plant growth, Hewitt (21, p. 98) stated that the majority of efficient nutrient solutions had a pH between 4.5 and 6. The work of a large number of investigators showed that, in general, injury occurred for a wide range of plants at about pH 3.5 to 4 on the acid side and pH 8.5 on the alkaline side. In tests of the pH of the plant sap, Arnon and Johnson (4, pp. 534-535) found that neither the pH of the nutrient solution in the range of pH 4 to 9,

nor the concentration of calcium in the nutrient solution had any effect on the pH of the plant sap.

Arnon and Johnson (4, p. 533) also studied the effect of pH and calcium concentration of the nutrient solution on the weight of plants grown in acid nutrient solutions. They found that in lettuce and tomato higher calcium concentrations increased growth at pH 4, increased growth somewhat less at pH 5, and did not increase growth at all at pH 6. Hewitt (21, p. 99) pointed out that at high pH values, precipitation and non-utilization of iron, calcium and phosphorus and in some instances manganese can take place.

Biddulph and Woodbridge (7, pp. 433-435) investigated the effect of pH of the nutrient solution on the absorption of phosphorus by the bean plant. They employed pH values of 4, 5, 6 and 7 combined with iron supplied at 1 p.p.m. as ferric nitrate. Their data show that there was a reduction of phosphorus content of trifoliate leaves between pH 6 and 7, a gradual reduction of phosphorus in cordate leaves between pH 4 and 7, and a gradual reduction of phosphorus content of stems between pH 4 and 6.

Translocation of Minerals in Plants

According to Biddulph (6, p. 263) minerals obtained by the plant root from the environment are absorbed by the epidermal cells of the root, transferred cell-to-cell across the cortex to the cells immediately surrounding the xylem, and secreted into the

xylem where they ascend to the aerial portions of the plant. From the xylem, minerals can be captured by adjacent cells such as cambium and young phloem, move laterally through the rays to actively metabolizing cells, deposit in leaves, or move to apical primordia. The direction of movement is influenced by netabolic use and transpiration.

Biddulph (6, p. 267) utilized radioactive tracer elements to study the precipitation of iron and phosphorus at the junction of the root and nutrient solution and at the junction of vein and mesophyll of bean plants. He found that the presence of a large amount of precipitated iron on the root surfaces inhibited the uptake of additional iron. The composition of the precipitate on the root was predominantly ferric phosphate below pH 6.0. At pH 6.0, some calcium was present and at pH 7.0 calcium was an important constituent. He found also that as precipitates of iron and phosphorus increased on the roots, the movement of phosphorus as well as iron into the top was impaired. He concluded that precipitation reactions on the roots might influence the ready entrance of iron and phosphorus into the root and hence into the plant as a whole, and that this precipitation depended in large measure on the pH of the growing medium.

A condition similar to the precipitation on the surface of the roots might exist at the xylem extremities where actively metabolizing cells obtain minerals from the xylem. Although prior precipitation at the root surface might provide some protection, it

seemed valid to him to assume that precipitation might also occur at the xylem extremities.

Phosphorus-Iron Interrelations

Biddulph and Woodbridge (7, pp. 431-443) have made comprehensive studies of the interrelations of phosphorus and iron in the bush bean grown in nutrient solutions at three levels of phosphorus (0.2, 1, and 20 molar times 10^{-5}) and three levels of iron (0, 1, and 2 molar times 10^{-5}) at pH 6. At the high phosphorus level, phosphorus uptake was not affected by the level of iron supplied. At the medium phosphorus level, phosphorus uptake increased in the roots and decreased in the tri-foliate leaves as the iron level of the substrate increased. At the low phosphorus level, the effect on the roots was about the same, but was intensified on the tri-foliate leaves. The iron content of the tri-foliate leaves at the medium phosphorus level was not affected by the iron level in the substrate, but that of the roots increased sharply as the iron supply increased.

De Kock (34, pp. 167-172) grew mustard plants in nutrient solutions at pH 7.8 under varying oxygen and bicarbonate levels and with three different sources of chelated iron. He found that the degree of chlorosis was related to the iron content of the leaves but that the relation was not "clear-cut". However, he recalculated the phosphorus and iron values as the phosphorus to

iron ratio and obtained values of 60 to 70 for chlorotic plants and 40 to 50 for healthy plants. The degree of chlorosis was then correlated with this ratio as a "well-marked" series.

Bolle-Jones (8, p. 170) found that chlorosis in potato plants grown under limiting iron supply was induced or accentuated by the addition of phosphate.

Manganese-Iron Interrelations

Leach and Taper (35, p. 561) grew beans, tomatoes and onions in nutrient solutions varying in iron and manganese concentration. In one experiment with onions, they supplied iron and manganese at 5 and 25, 5 and 10, 5 and 5, 10 and 5, and 25 and 5 p.p.m. levels. The amounts of iron and manganese absorbed in twenty-four hours varied directly with the concentration supplied. However, the combined amount of iron and manganese absorbed for any one concentration was quite constant though the ratio of the two might be very different.

Potassium-Iron Interrelations

The interrelations of potassium and iron have been studied by Bolle-Jones (8, p. 170) working with the potato plant. He found that when iron supplies were limiting, iron-deficiency chlorosis was induced or accentuated by a reduction of potassium. He also found that iron deficiency chlorosis was cured by addition of high

levels of potassium. This action was attributed to increased mobility of iron in the plant associated with reduction of iron accumulation in the roots. The status of iron in the plant was found to govern the distribution of potassium within the plant, with high iron levels favoring its translocation to the tubers at the expense of the young shoot regions.

Relations between Iron and the Carbon Dioxide-Carbonate-Bicarbonate System

Yaalon (59, pp. 356-363) discussed the physical-chemical relations of calcium carbonate, carbon dioxide and pH. His work was based on the use of experimentally determined constants in equations derived from the law of mass action. He demonstrated that calcium carbonate solubility and the pH of the resulting solution are determined by the carbon dioxide pressure in the gaseous phase of the system. The equilibrium was affected relatively little by temperature or neutral salts. He considered that 0.0003 atmosphere (the carbon dioxide concentration of the earth's atmosphere) and 0.05 atmosphere to be the minimum and maximum values of carbon dioxide likely to be found in the soil atmosphere. The possible extreme values of pH at these carbon dioxide concentrations in a solution saturated with calcium carbonate are from pH 7.6 to 8.7 at 0.0003 atmosphere carbon dioxide and from pH 6.7 to 7.6 at 0.05 atmosphere carbon dioxide.

Lindsay and Thorne (39, p. 278) investigated the relations of bicarbonate ion concentration and oxygen level to chlorosis and iron uptake. They studied the soil solutions expressed from fresh soil samples by pressure membrane apparatus. The expressed solution was collected under liquid paraffin and analyzed for bicarbonate by the carbon dioxide volumetric method. They found that the bicarbonate and calcium concentrations of these soil solutions were significantly higher in areas producing chlorotic plants than in adjacent areas where the plants were green. Gypsum or sulfur application to the soil decreased the bicarbonate ion concentration, but did not cure the chlorosis in the raspberries growing on it.

In greenhouse studies, the same investigators (39, pp. 273-276) grew beans in Hoagland's nutrient solution. The bicarbonate treatment was made by adding 10 milliequivalents of sodium bicarbonate per litre to the nutrient solution. The treatments with no bicarbonate received 10 milliequivalents of sodium chloride per litre. The solutions were aerated with controlled mixtures of air and nitrogen to control the oxygen level but no attempt was made to control the carbon dioxide content of the air used. The pH of the solutions was kept at 7.8. The bicarbonate treatment at the low oxygen level reduced both the chlorophyll content and the total weight of the plants. Increasing the oxygen content of the aerating mixture caused further decreases in growth. The bicarbonate treatment greatly retarded the movement of radio-iron to stems and

leaves, but oxygen level had no effect. The same treatments were used in second experiment with the addition that iron was supplied from two sources, soluble iron phosphate and EDTA. When EDTA was the source of iron, there was less reduction of growth and chlorophyll content than when the source was soluble iron phosphate.

Porter and Thorne (44, p. 381) later investigated the interrelations of carbon dioxide and bicarbonate ion in plant chlorosis. They grew beans and tomatoes in Hoagland and Arnon's nutrient solution using soluble ferric phosphate as the source of iron. The solutions were aerated with 25 litres per day of mixtures of air containing 1, 3, and 5 per cent carbon dioxide. Two series of treatments were made, the first with pH held constant and bicarbonate varied from 0.3 to 50 milliequivalents per litre and the second with bicarbonate held constant and the pH varied from 7.3 to 8.3. When the pH was held constant, leaf chlorophyll decreased as bicarbonate increased and when the bicarbonate was held constant, leaf chlorophyll decreased as pH increased. The former effect was much more marked than the latter. Increasing concentrations of bicarbonate resulted in increasing leaf iron content even though chlorosis increased also.

De Kock (34, p. 172) grew mustard in nutrient solutions and supplied iron in the form of four chelate compounds. Some treatments received 10 milliequivalents of sodium bicarbonate per litre and others 10 milliequivalents of sodium chloride. Some were aerated with 20 per cent oxygen and some with 1 per cent oxygen.

The 1 per cent oxygen resulted in less chlorosis than the 20 per cent oxygen. Bicarbonate intensified the chlorosis. Plants from the 1 per cent oxygen treatment contained more iron in the stems and roots than those treated with 20 per cent oxygen but the leaf content was about the same. Bicarbonate slightly depressed the iron content of the leaves.

Nichel-Iron Interrelations

Crooke, Hunter and Vergnano (15, p. 311) investigated the influence of varying levels of iron and pH on nickel toxicity in oats grown in sand and water cultures. They found that toxicity symptoms were less severe when the iron concentration in the nutrient solution was high. Iron uptake was reduced by both nickel and increasing pH and resulted in chlorosis at pH's above 5.5.

Crooke (14, p. 173) found that nickel as nickel EDTA applied at the rate of 2.5 p.p.m. nickel was unavailable to plants, whereas ionic nickel produced toxicity symptoms.

Iron Content of Plants Having Iron-Deficiency Chlorosis

Iljin (30, p. 195) found that the quantity of total iron in plants was not correlated with chlorosis. He stated that in yellow leaves the quantity of total iron could be less than, greater than, or equal to that in green leaves.

However, other investigators have indicated that there was a

relationship between iron content and degree of iron-deficiency chlorosis. Hill (22, p. 166) found that blueberry plants with iron-deficiency chlorosis contained less iron than normal plants. Wallihan (56, p. 104) found that iron chlorosis of citrus in southern California was caused by a simple deficiency of iron. Leonard and Stewart (36, p. 50) found that correction of chlorosis in citrus was accompanied by substantial increases in total iron. After reviewing the literature, Wallace and Hewitt (52, p. 153) stated that values of the total iron content of both green and chlorotic plants from the data of different investigators varied greatly, and that it was generally recognized that a division of the iron into "active" and "inactive" iron was necessary. They noted the difficulty of strict comparisons because different investigators used different methods of fractionation of iron.

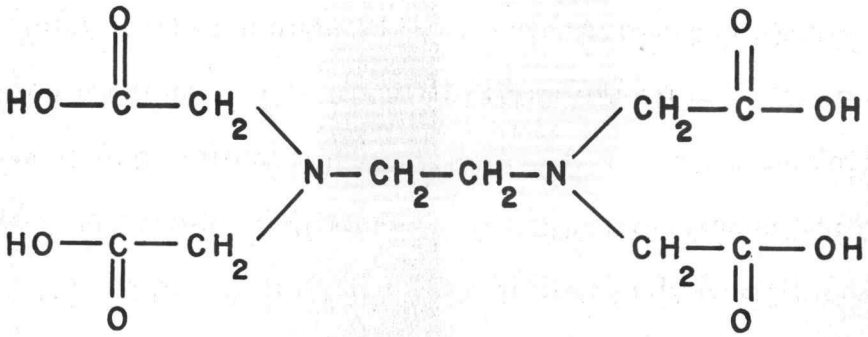
Mason (41, pp. 104-107) thought that dust might affect values found from iron content and measured the effect of dust contamination on the iron content of apple leaves. He found that dust collected near the sampled trees contained 3.5 per cent iron. He made tests of the effectiveness of five leaf treatments to remove the dust contamination: 1. wiping with dry muslin; 2. wiping with wet muslin; 3. scrubbing with a soft nylon toothbrush for ten seconds while immersed in water; 4. scrubbing as above while immersed in 0.3 normal hydeochloric acid; 5. scrubbing as above while immersed in Teepol (a detergent). The iron values he found after treatment were: 1. 160 p.p.m. 2. 150 p.p.m. 3. 113 p.p.m. 4. 107 p.p.m.

5. 108 p.p.m. It is evident that considerable surface contamination of the leaves occurred with respect to iron. Washing had no effect on the other nutrient elements. He also showed that these washing techniques had no effect on the iron values of leaves protected by growing under cellophane bags or in a greenhouse. Since most or all of the older literature failed to recognize this factor, the iron analyses reported are correspondingly open to question.

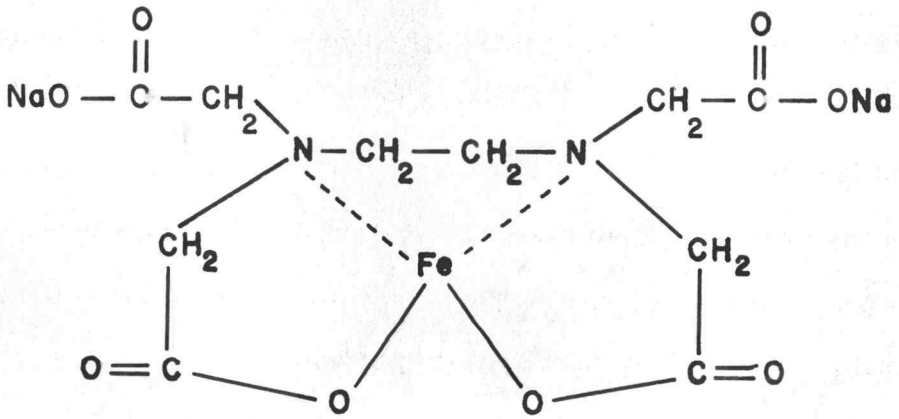
Chelates and the Iron Nutrition of Plants

Characteristics of a chelate compound. A chelate compound is described by Haertl (19, p. 6) as: "...any compound which will inactivate a metallic ion with the formation of an inner ring structure in the molecule, the metallic ion becoming a member of the ring."

The first synthetic chelating agent generally used in plant nutrition and the parent of the large family of chelates now used, was ethylenediaminetetraacetic acid. For convenience, it is generally referred to as EDTA. Haertl pictures its structure as follows:



According to Haertl, EDTA is not water-soluble and is therefore prepared as the sodium salt. This sodium salt reacts with metallic ions such as iron to form a chelate having the following structure.



Stewart and Leonard (48, p. 795) stated that the chelation reaction proceeds according to the law of mass action in which the constant K of the reaction equals the ratio of the concentration of the metal chelate compound to the concentration of the metal in the ionized state times the concentration of the chelating agent.

When K is very large, the ion concentration is very small. The K value is commonly referred to as the stability constant and is a useful guide to predict certain properties of chelates since the higher the K value, the more stable is the complex. Since at high pH values, precipitation reactions may compete with the chelate for the ion, a high K value with its correspondingly lower concentration of unattached ion will make the chelate more stable.

Method of action of chelate compounds. Holmes and Brown (26, p. 178) grew soybeans in seventeen naturally calcareous soils and used five chelate compounds not containing iron but "tagged" with carbon 14 to mobilize soil iron. Autoradiographs showed that the chelates made iron in the soil (iron 55 added two weeks earlier) available to the soybeans. The radioactive iron in the plant was concentrated in the leaf and flower primordia, whereas the carbon 14 from the chelate was uniformly distributed through the plant. These results suggest that the chelate compound enters the plant, but that separation occurs with utilization of iron by the metabolic processes of the plant.

Weinstein et al. (57, p. 421) investigated the absorption, translocation and metabolism of EDTA by split-root cultures. They used four treatments: (a) whole roots in one container with iron supplied as ferrous sulfate; (b) split-root culture with iron supplied as ferrous sulfate in one container and no iron or EDTA in the other; (c) split-root culture with iron supplied as ferrous

sulfate in one container and EDTA in the other; (d) whole root culture with iron and EDTA supplied together. All treatments were run at pH 5.0 and pH 7.0 and the nutrient solutions were changed every two days. All treatments produced normal growth at pH 5.0. However, at pH 7.0 both treatments without EDTA produced chlorotic plants, while the treatment with iron in one container and EDTA in the other and the treatment with iron and EDTA in the same container produced normal plants. These results indicate that EDTA made iron available to the plants or assisted in the absorption and distribution of iron.

De Kock (33, p. 473) found that a water extract of peat used in one container of a split-root culture was as effective as EDTA in mobilizing iron from the other container. Indeed, even humus-like substances synthesized from reagent grade sucrose were equally effective.

De Kock and Strmecki (32, p. 512) grew mustard plants with lignite of less than 0.15 millimetre fineness added to the cultures. They found that availability of iron was increased by the humic acid from the lignite.

Hill-Cottingham (23, p. 347) made an interesting discovery on the effect of light on iron chelates. He found that three chelates that were completely stable in the dark were decomposed on exposure to daylight. He suggested that reduction of iron chelates may take place in the leaves of plants to yield the less stable ferrous compound which could then be removed more readily by complex forma-

ation with proteins.

Origin and development of use. The first report of the use of EDTA as a source of iron in plant nutrition was made by Jacobson (31, p. 413) in 1951. He grew tomato, sunflower, corn and barley in Hoagland's solution with FeEDTA. He found that the compound supplied iron satisfactorily at a concentration of 5 to 10 p.p.m. of iron and that only one addition was needed in eight weeks, whereas other iron compounds become unavailable in a few days.

Steward and Leonard (47, p. 566) developed the use of EDTA for control of iron deficiency chlorosis in citrus trees. Their first approach was high pressure injection of ferrous sulfate into the tree trunks. This treatment corrected the deficiency but a more practical method was needed. Placing the roots of chlorotic trees in solutions of iron sulfate resulted in no iron uptake. When citric acid was added to the solutions, the leaves of the trees became considerably greener, but when the solution was applied to the soil around chlorotic trees, no response occurred because the citric acid was quickly broken down by soil organisms. When, as a result of Jacobson's work, EDTA was tried as a soil application, good results were obtained.

While EDTA has been satisfactory on acid soils, it has not been satisfactory on alkaline soils, and new derivatives similar to EDTA have been developed for use under alkaline conditions. Holmes and Brown (26, p. 168) reported the use of N-hydroxyethylethylene-

diaminetetraacetic acid (HEEDTA), diethylenetriaminepentaacetic acid (DTPA), cyclohexanediaminetetraacetic acid (CDTA), and an aromatic polyaminocarboxylic acid of unknown formula (APCA). These compounds are reported to form more stable complexes with iron at higher pH values than EDTA. Presumably, these compounds could function at higher soil pH values since the iron would still remain complexed.

Resistance to breakdown in the soil. Haertl (19, p. 6) stated that EDTA had not given any indication of being decomposed by microbial action. Perkins and Purvis (43, p. 329), however, found that wheat and sunflower plants receiving sodium EDTA as the only source of added nitrogen made more growth than those receiving no nitrogen, but less than those receiving sodium nitrate. Apparently, nitrogen from EDTA was utilized by the plants. They do not give evidence or speculate as to whether the EDTA broke down in the plant, or in the medium before it was absorbed by the plant.

Decomposition at alkaline reactions. According to Haertl (19, p. 6) FeEDTA begins to become unstable at pH 6.5 and the iron precipitates as the hydroxide, whereas the iron complexed with HEEDTA is available up to pH 9.

Effect of chelated iron compounds on the soil. Perkins and Purvis (43, p. 329) studied the effect of NaEDTA on the soil. They treated soil samples with 25 to 5000 pounds of the compound per 2,000,000 pounds of soil (acre, six inches deep). The 5000

pound rate increased extractable iron to 32 times the original, and extractable manganese to 15 times.

Effectiveness of chelate compounds used on calcareous soils. Leonard and Steward (37, p. 109) determined that FeEDTA applied at the rate of twenty grams of iron per tree on acid soils turned chlorotic leaves green in six weeks. However, five to fifteen times as much was needed on calcareous soils. Bould (9,p94) stated that FeEDTA was not effective in alkaline soils at the rates effective in acid soils. In another publication, he stated (10, p. 55) that ten to twenty grams of iron as EDTA corrected copper-induced chlorosis in acid soils, but that one hundred to three hundred grams were required to correct lime-induced chlorosis. Holmes and Brown (26, p. 178) found two chelates, DTPA and APCA, that were effective in correcting chlorosis on calcareous soils, APCA being the most effective. APCA decreased the uptake of manganese and copper while increasing the uptake of iron. These compounds were applied without iron and therefore mobilized soil iron previously unavailable.

MATERIALS AND METHODS

Growth Chamber

All plants were grown in a chamber specially constructed to provide uniformity of temperature, light intensity and daylength. The exterior of this chamber and its relation to the main Horticulture building are shown in Fig. 1.

The chamber was constructed separate from other buildings to ensure freedom from ethylene gas and other substances toxic to plants at extremely low concentrations. Ethylene is a normal decomposition product of fruits and is frequently present in toxic concentrations in buildings such as the nearby Horticulture building in which fruits are stored, examined or processed.

The chamber is twelve feet square and eight feet high. It is divided into three rooms, two growth rooms six by eight feet and one header room four by twelve feet. The header room provides an air lock between cold outside air and the plants in the growth room in the winter, and is also used to house the electrical service fixtures and various supplies needed for the operation of the chamber. The building is insulated with three-inch rock wool batts in the walls, floor and ceiling. Only one room is currently fitted with a bank of lights suitable for a full growth chamber. The interior of this room is shown in Fig. 2. The other room has a small light bank and is used for growing young plants until they can be put into the nutrient solutions.

Fig. 1. An exterior view of the growth chamber used in these experiments.

Fig. 2. An interior view of the room fitted as a full growth chamber showing the light bank and plants growing in five-gallon glazed crocks.

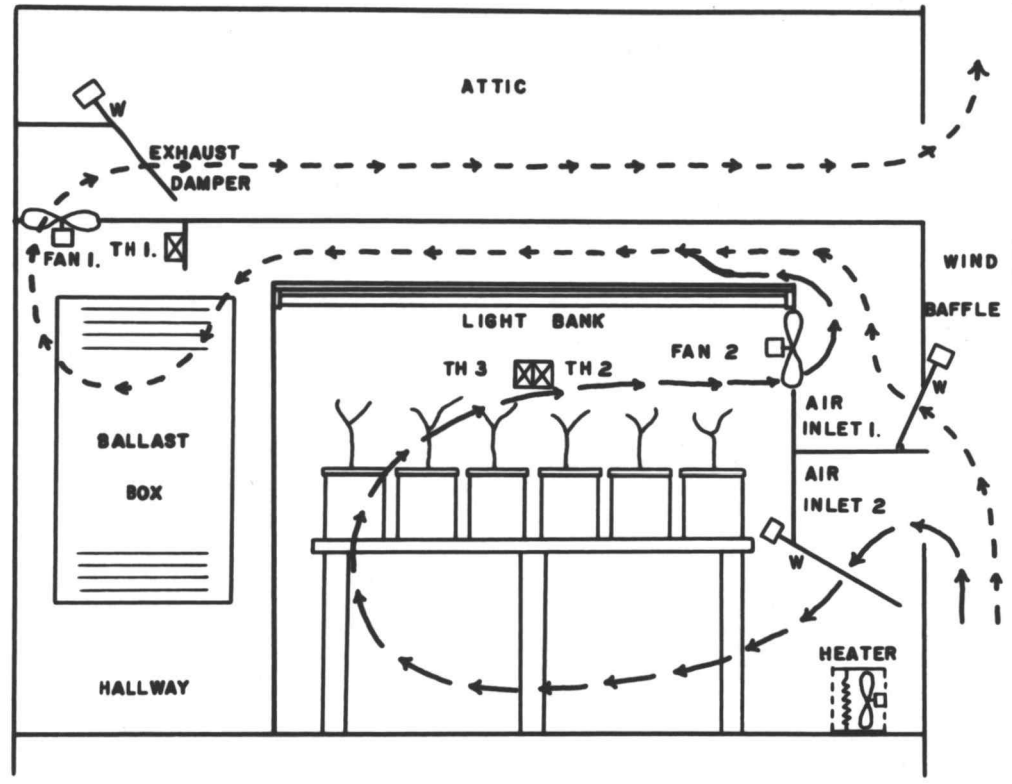


Fig. 3. A diagram of the plant growth chamber showing the ventilation and temperature control system. TH- thermostat. W- damper. Thermostat 1 controls Fan 1, etc.

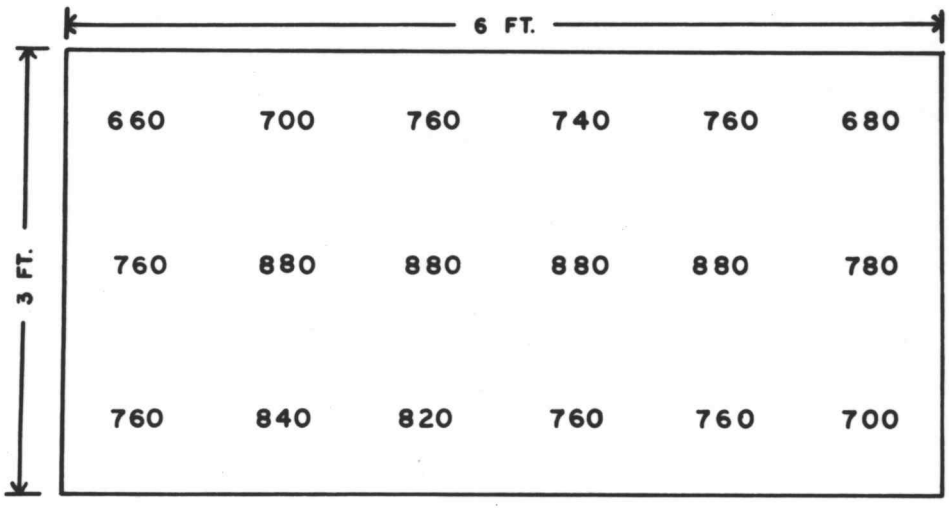


Fig. 4. Distribution of light intensity in foot-candles under the light bank measured eighteen inches below the tubes.

The room fitted as a full growth chamber has a light bank eight feet by three feet consisting of a steel box four inches deep with white vitreous enamel on the lower side supporting eighteen eight-foot General Electric F96T12CW cold-cathode cool-white fluorescent lamps. Eight twenty-five watt incandescent lamps supplement the red end of the spectrum, because the insufficient red emission of the fluorescent lamps causes abnormally shortened internodes in plants. Nine General Electric 59G464-430 milliampere ballasts to supply power to the fluorescent lamps are placed in a louvred steel box separately ventilated on a wall in the header room. The light bank supplies a light intensity of about eight hundred foot-candles eighteen inches below the lamps. The distribution of light intensity above the crocks is shown in Fig. 4. The walls of the growth chamber are painted flat white to reflect as much light as possible.

The temperature in the growth chamber is controlled by exhaust fans and electric heaters operated by thermostats. When the temperature is too high, the exhaust fans remove hot air and cool air flows in through flap dampers to replace it. When the temperature is too low, electric heaters supply more heat. The air exhaust and heater system is shown in diagrammatic form in Fig. 3. The temperature was held at 70 to 75 degrees F. during these experiments.

Daylength is controlled by time clock. A daylength of eighteen hours was used in all experiments. The time clock turned the

lights on at eight in the evening and off at twelve noon. In this way, the peak heat emission from the light bank occurred during the coolest part of the night, making it possible to use the growth chamber a little earlier in the fall and a little later in the spring than if the maximum heat emission from the lights coincided with the hottest part of the day.

Seedling Culture

The experimental plant used was the Rival variety of the bush bean, Phaseolus vulgaris L. The bean is well suited to indoor plant nutrition studies because of its small size and rapid growth.

Seeds were dusted with Arasan, a commercial fungicide, and planted in flats in four inches of fine vermiculite, Terralite brand, manufactured specially for the growing of plants. The flats were watered with a full nutrient solution minus iron.

When the seedlings were ready to be transplanted at the young cordate leaf stage, they were gently removed from the flats and the roots washed in water to remove most of the vermiculite. Only plants with good root systems were retained. Four plants were placed in each crock.

Apparatus for Nutrient Solution Cultures

Nutrient solutions in five Imperial gallon glazed vitreous crocks were used for the early experiments, and in three gallon

crocks for later work. Plants were supported between two halves of cork stoppers grooved to hold the plant stem. The corks fitted into holes drilled in twelve by twelve inch wood frames of one inch stock. The crocks were filled to within one inch of the top with nutrient solution and replenished to that height with distilled water from time to time as required.

Air was supplied to the crocks from a portable compressor at first and later from a large automotive type compressor. The air was reduced in pressure by a standard wator-or-air pressure regulator and led to each crock by glass tubing. Air flow was restricted by means of a half inch length of 0.5 m.m. capillary tubing at the end of the aeration tube. The solutions were aerated continuously, and a large volume of air was used to ensure vigorous mixing of the contents of each crock in order that local areas of different pH should not develop.

Nutrient Solution

The nutrient solution used was one specially selected for studies in the pH range from 3 to 9 by Arnon and Johnson (4, p. 526). Phosphorus precipitates in alkaline solutions and changes the phosphorus concentration. Arnon and Johnson avoided this change by using for all cultures the concentration of phosphorus remaining in the solution at the highest pH used, pH 9.

Biddulph and Woodbridge (10, pp. 431-434) have found that nutrient solutions containing 0.00005 M phosphorus provided enough

phosphorus for continued growth of the bush bean. Since Arnon and Johnson's solution contained 0.00006 M phosphorus, it was considered that there would be little possibility of phosphorus deficiency occurring.

The composition of Arnon and Johnson's nutrient solution is:

Constituent	Concentration
	M
KH_2PO_4	0.00006
K_2SO_4	0.0015
KNO_3	0.01
$Ca(NO_3)_2$	0.002
$MgSO_4$	0.001

For micro-nutrient supply, Arnon and Johnson added iron and manganese to nutrient solutions as humates made from sucrose. In the experiments reported in this study, however, Hoagland's B4 solution of micro elements was used. The stock solution described below was added to the nutrient cultures at the rate of 0.2 millilitre per litre.

Constituent	Concentration
Boric acid	1.43 g./ l.
Manganous chloride	0.90 g./ l.
Zinc acetate	0.08 g./ l.
Copper sulphate	0.039 g./ l.

All stock solutions and cultures were made up with single-distilled water.

Chelated Iron Compounds

Two derivatives of the ethylenediamine nucleus were used: HEEDTA and DTPA. Data on these and other chelated iron compounds is presented in Table 28 in the appendix. These compounds were made up in a stock solution and added to the mineral nutrient solutions by addition of the proper amount of the diluted stock solution. Only one addition of iron solution was made during the experiment since Jacobson (31, p. 413) has shown that the chelate compounds remain available for considerable periods.

Adjustment of the pH of the Nutrient Solutions

A thirty millilitre sample of the nutrient solution was taken from each culture at daily or two-day intervals. The pH of the sample was determined with a model M Beckman glass electrode pH meter. The meter was adjusted each day with a Beckman pH 7.00 buffer solution.

The pH of the nutrient solution almost invariably became more acid. When this occurred, 0.5 per cent sodium hydroxide was added to bring the pH back to the desired value.

Buffer for Nutrient Solutions

In the first experiments reported in this study, very great fluctuations in the pH of the nutrient solutions occurred even though the pH was adjusted as often as three times a day. In addition, drifting of the pH meter due to poor buffering made it difficult to get accurate readings. The buffering capacity of Arnon and Johnson's solution is not adequate for satisfactory pH control.

Tris-hydroxymethylaminomethane has been used in the region of pH 7 to 9 as a buffer for biochemical preparations. This buffer, according to the vendor's report(2, p. 13) does not absorb carbon dioxide from the air, is compatible with calcium salts whereas phosphate and carbonate buffers are not, is stable in solution for three months at room temperature, and lacks inhibitory action for all the enzymes tested. Swim and Parker (50, p. 466) used this buffer at the rate of 2 to 3.6 grams per litre and found that embryonic kidney, lung and testicle cultures grew as well in it as in bicarbonate buffer. For this reason it was tried in these studies as a method of maintaining pH within limits that would make the experiments valid. A 0.2 molar stock solution was prepared and was used in the nutrient solutions at 0.002 molar or 0.001 molar.

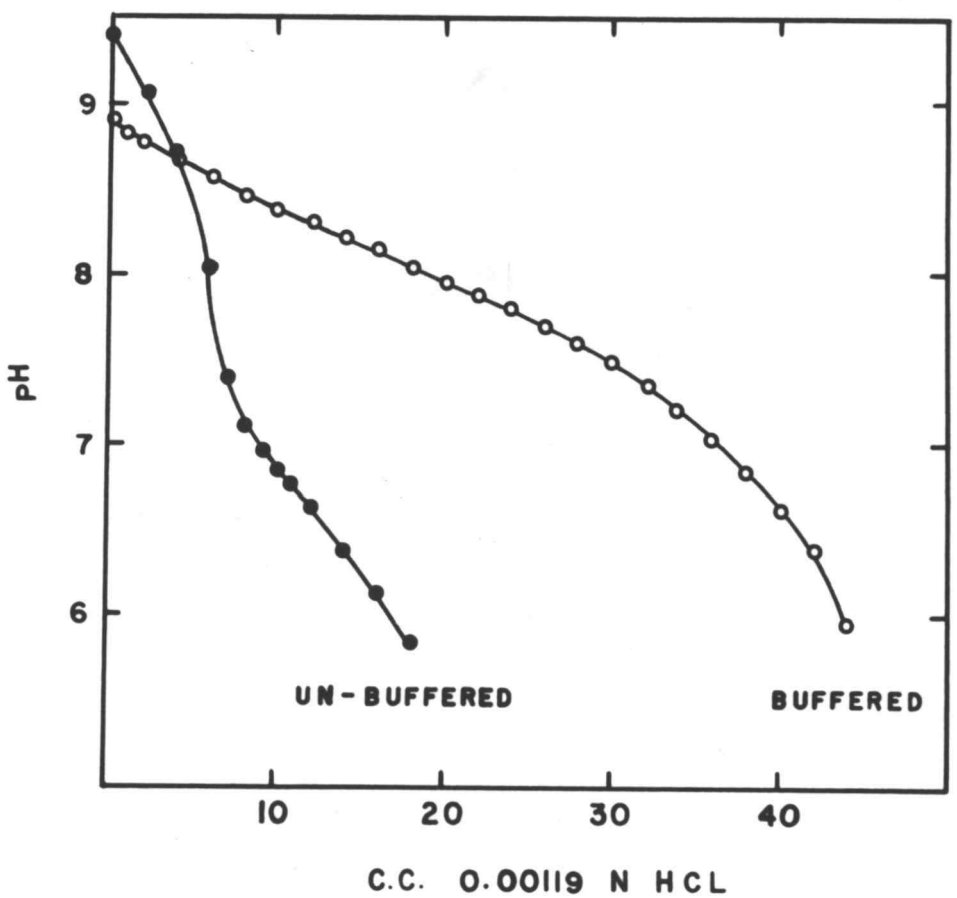


Fig. 5. Buffer curves of Arnon and Johnson's nutrient solution with and without 0.002 molar trishydroxymethyl-aminomethane buffer.

The effectiveness of the buffer was measured by making buffer curves of Arnon and Johnson's nutrient solution with and without added buffer, as shown in Fig. 5. Inspection of the curves revealed at once why pH control had been so difficult in the unbuffered nutrient solutions. Between pH 7.3 and pH 8.5 the steep slope of the curve indicated that a slight addition of alkali or acid produced a large change in pH. The solution had practically no buffering action in the vicinity of pH 7.5. Examination of the curve will show that it would be virtually impossible to maintain a pH of 7.5. The buffered solutions, however, had a much flatter curve in this region, and this was reflected in the ease of maintenance of a given pH in the buffered nutrient cultures. No toxicity was noted from the compound, and no differences in results in iron deficiency experiments were observed.

Harvest Procedure

The plants were harvested about the time that flower buds were just beginning to appear, approximately three and a half weeks after planting. In some of the early experiments, the plants were divided into leaves, stems and roots. It was observed at the time that the trifoliate leaves were affected by the treatments much more than the cordate leaves. In later experiments, therefore, the leaves were further divided into trifoliate leaves and cordate leaves. The petioles were removed from all leaves and included in the stem samples.

The plant parts were put into No. 2 brown paper bags, and air-dried at room temperature at a relative humidity ranging around thirty per cent. Later, they were oven-dried at 65 degrees C. and weighed.

Grinding Tissues

Leaf and root samples were ground with a mortar and pestle. It was found impossible to grind stems by this technique and these were cut into small pieces using plastic scissors. All of the samples were well mixed.

Dry Ashing

One-gram samples of the various tissues were dry ashed according to the method of the Association of Official Agricultural Chemists for iron (38, section 6.8, p. 95). The method was followed exactly except that only one acid extraction of the ash with a thirty minute digestion on a water bath was used.

Iron Analysis

Iron was determined by the method of the Association of Official Agricultural Chemists for plant material (38, sections 6.7 to 6.9, pp. 95-96). This an o-phenanthroline colorimetric method. The intensity of colour was measured on a Klett-Summerson colorimeter against standards and blanks prepared each day.

Statistical Analysis

All experiments (except those on vacuum impregnation and spray technique) were designed for statistical analysis. Analyses of variance were made of all sets of data and least significant differences were calculated.

RESULTS

The experiments in this study were numbered consecutively in the order that they were carried out. Since it was desirable to keep the growth chamber facilities fully utilized, frequently two or more lines of investigation were being developed concurrently, with alternate experiments being devoted to each line. This procedure allowed a little time for appraisal of the results before designing the next experiment. In some experiments, it was economical to combine two different series of treatments for the study of the interaction, or simply to determine the effect of different levels of one treatment at several levels of another treatment. One of these combined experiments was so large it had to be grown at two consecutive periods, but it is reported as one experiment.

In reporting the results, the original serial numbers of the experiments are retained, but the experiments are grouped according to the lines of study. In those cases where two or more treatments were included in one experiment, parts of the experiment are reported under several major divisions.

The records of the pH of the nutrient solutions for each experiment are presented in appendix 1. The graphs of dry weight and of iron content of tissues are drawn from the summary tables for each experiment and are presented in the body of the thesis. The summary tables are derived from the complete tables of yield and iron contents which are presented in appendices 2 and 3

respectively.

A. Determination of the Optimum Level of Iron Supplied as the HEEDTA Iron Complex in Alkaline Nutrient Solutions

Experiment 4.

This experiment was planned to determine the optimum level of iron supplied as the HEEDTA iron complex in nutrient solutions at alkaline reactions. Iron was supplied at 0. 1, and 5 p.p.m. Each treatment was replicated three times and the whole experiment was completely randomized using random numbers from the tables of Cochran and Cox (14, p. 428). The pH planned for this experiment was 8.5. However, extreme fluctuations of pH occurred, and it was found to be more practicable to maintain the pH around 7.5. Dry weights of leaves, stems and roots were determined and are presented in Table 1 and Fig. 7. Analyses of the iron content of the tissues were made and are presented in Table 2 and Fig. 7. Representative plants from the experiment are shown photographically in Fig. 6. Note the extreme chlorosis of the tri-foliate leaves contrasted with the green of the cordate leaves of the control plot grown under these conditions.

In this experiment, the optimum level of iron was found to be 1 p.p.m. However, the difference in yield between the 1 and 5 p.p.m. treatments, although fairly large, was not significant, and 5 p.p.m. cannot be ruled out of the optimum range. The iron content

Fig. 6. Experiment 4. Growth of bean plants at 0, 1 and 5 p.p.m. iron supplied as the HEEDTA complex in mineral nutrient solution at pH 7.5.

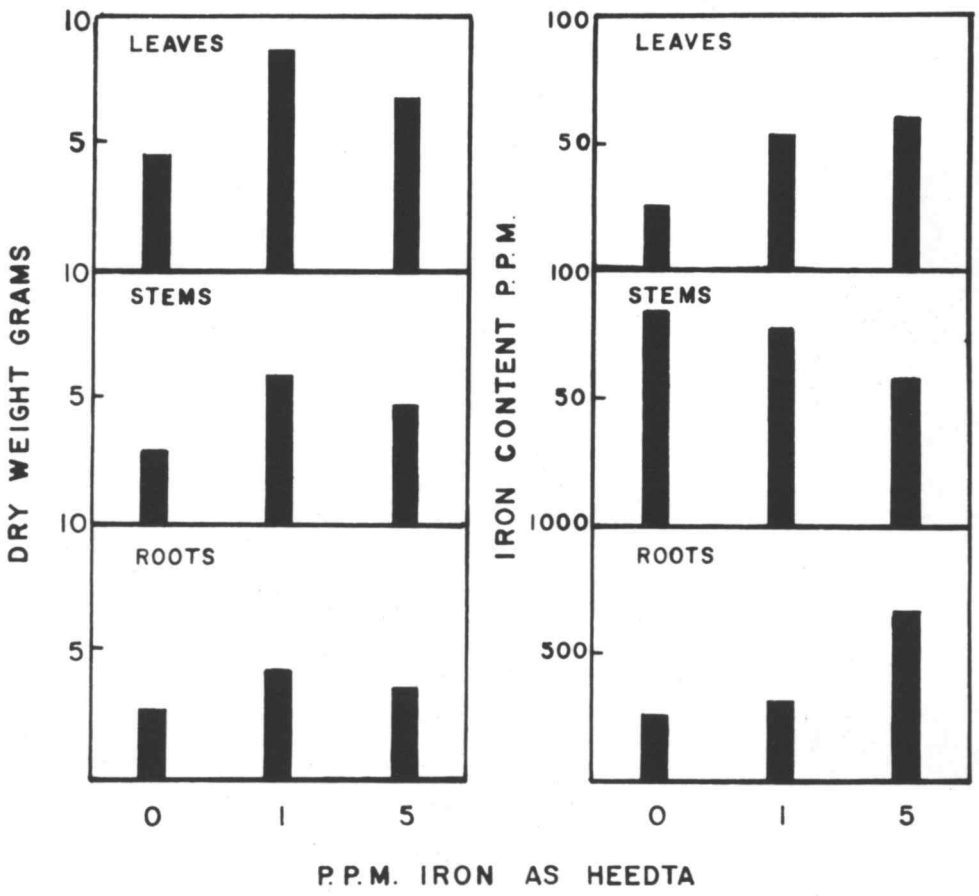


Fig. 7. Experiment 4. Dry weight and iron content of root, stem and leaf fractions of bean plants grown at varying levels of iron supplied as the HEEDTA complex in nutrient solutions at pH 7.5.

Table 1. Experiment 4. Dry weights of leaves, stems, and roots of bean plants grown at varying levels of iron supplied as the HEEDTA complex in nutrient solutions at pH 7.5.

Plant part	P.p.p. iron as HEEDTA complex			L.S.D.
	0	1	5	.05
		grams		
Leaves	4.48	8.62	6.73	2.75
Stems	2.88	5.72	4.63	1.71
Roots	2.52	4.22	3.63	0.95

Table 2. Experiment 4. Iron content of leaves, stems, and roots of bean plants grown at varying levels of iron supplied as the HEEDTA complex in nutrient solutions at pH 7.5.

Plant part	P.p.m. iron as the HEEDTA complex			L.S.D.
	0	1	5	.05
		p.p.m.		
Leaves	24	53	59	N.S.
Stems	83	77	57	N.S.
Roots	226	313	651	N.S.

of leaves from this treatment was about 50 p.p.m. The iron content of the other tissues, particularly of roots, were quite variable resulting in no significance between means.

Experiment 8

Experiment 4 showed that 5 p.p.m. iron as HEEDTA complex depressed growth and that 1 p.p.m. iron resulted in maximum growth. However, it did not show what level between 0 and 1 p.p.m. is optimum. Experiment 8 was planned to determine what levels between 0 and 1 p.p.m. gave optimum growth.

Iron was supplied at 0, 0.1, and 0.3 p.p.m. as HEEDTA iron complex. The pH of the nutrient solutions was held near 8.5. There were three replicate crocks in each treatment, each crock containing four plants. The whole experiment was completely randomized using random numbers from the tables of Cochran and Cox (14, p. 428).

The young bean plants were transferred to crocks on May 5. The plants were arrayed in order of size and divided into four groups. One plant from each group was placed in each crock. At the first three-leaf stage of growth on May 18, all crocks showed iron deficiency and iron as HEEDTA was added. When harvested, the plants were divided into leaves, stems and petioles, and roots. The samples were oven-dried and weighed. Mean dry weights are presented in Table 3 and Fig. 9. Mean iron contents are presented in Table 4 and Fig. 9. Representative plants are shown photograph-

Fig. 8. Experiment 8. Growth of bean plants at 0, 0.1 and 0.3 p.p.m. iron supplied as the HEEDTA complex in mineral nutrient solutions at pH 8.5.

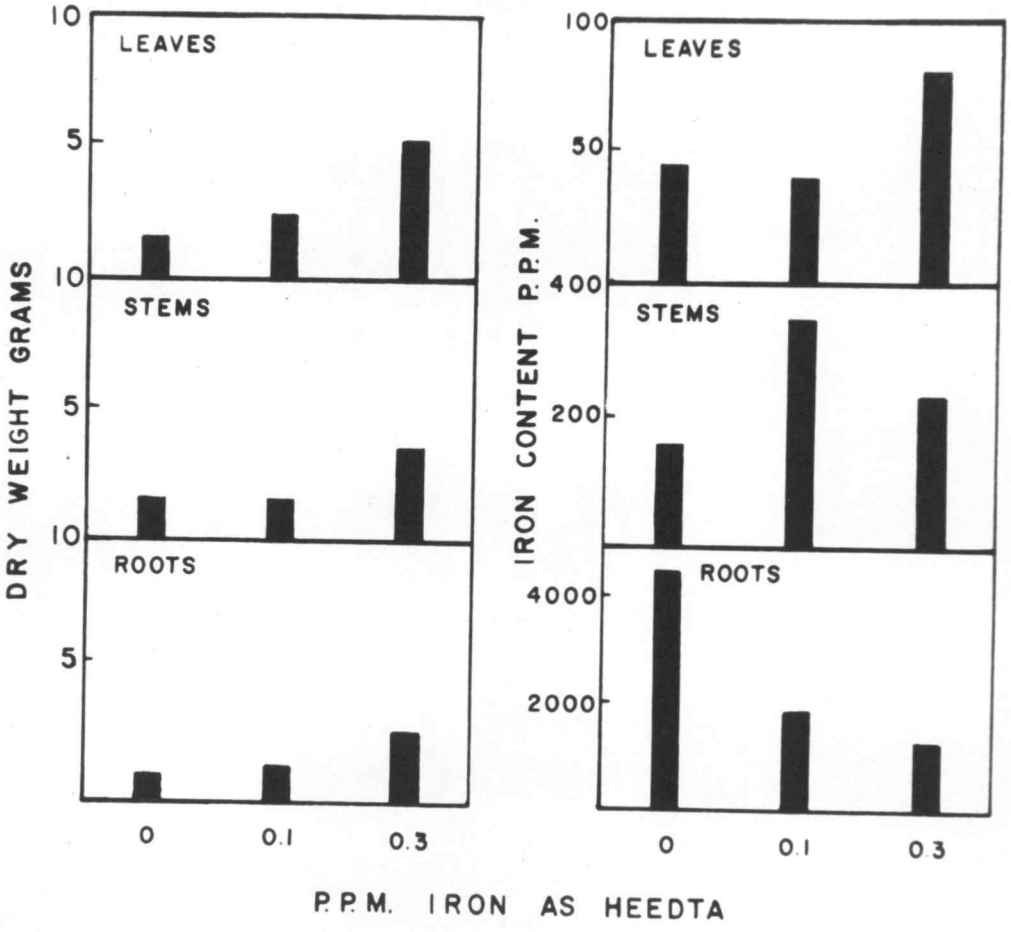


Fig. 9. Experiment 8. Dry weight and iron content of roots, stems and leaves of plants grown at varying levels of iron supplied as the HEEDTA complex in nutrient solutions at pH 8.5.

ically in Fig. 8. Note especially the difference in size of the control plant contrasted to the 0.1 p.p.m. plant in view of the similar iron content of the leaves in both treatments (44 and 39 p.p.m. respectively).

The 0.3 p.p.m. level appeared to result in normal growth, but the 0.1 p.p.m. level was insufficient. The iron content of the leaves from the 0.3 p.p.m. treatment was almost double that of the other two treatments. A large variance in the iron content of the stems and roots caused the differences to be non-significant.

Table 3. Experiment 8. Dry weights of leaves, stems, and roots of bean plants grown at varying levels of iron supplied as the HEEDTA iron complex in nutrient solutions at pH 8.5.

Plant part	p.p.m. iron as HEEDTA iron complex			L.S.D.
	0	0.1	0.3	.05
		grams		
Leaves	1.53	2.39	5.18	1.50
Stems	1.51	1.46	3.48	0.76
Roots	0.86	1.26	2.60	0.48
Whole plant	3.90	5.11	11.26	2.66

Table 4. Experiment 8. Iron contents of leaves, stems, and roots of bean plants grown at varying levels of iron supplied as the HEEDTA iron complex in nutrient solutions at pH 8.5.

Plant part	p.p.m. iron as HEEDTA complex			L.S.D.
	0	0.1	0.3	.05
		p.p.m.		
Leaves	44	39	79	17
Stems	150	341	225	N.S.
Roots	4523	1822	1253	N.S.

Experiment 15

Experiment 4 showed that 5 p.p.m. iron as HEEDTA complex depressed growth and that 1 p.p.m. produced optimum growth. Experiment 8 showed that 0.3 p.p.m. was adequate and 0.1 p.p.m. was insufficient. This experiment was planned to span the range covered by the two previous experiments. Levels of 0.2, 0.5, 1, 2, 5, and 10 p.p.m. iron were supplied. The experiment was designed to give information also on the interaction of phosphorus with iron, but that part of the data is reported in a more appropriate section. The six levels of iron were combined with three levels of phosphorus, 0.00006 molar, 0.0006 molar, and 0.006 molar. The eighteen treatment combinations were replicated twice. Since the growth chamber holds only eighteen crocks, the two complete replicates were run separately. Each replicate was completely randomized by taking

numbers from consecutive cards of a thoroughly shuffled group of eighteen library cards numbered from 1 to 18.

The first replicate was seeded on January 25, placed in crocks on February 7, and harvested on February 21, 1956. The second replicate was seeded on February 12, put in crocks on February 21 and harvested on March 6. The plants were divided into trifoliate leaves, cordate leaves, stems and petioles, and roots, and the dry weight and iron content of each sample were determined. Dry weights are presented in Table 5 and graphically in Fig. 10. Iron contents are presented in Table 6 and Fig. 10. The appearance of the plants is shown in Figs. 23, 24, and 25 under section E, experiment 15.

The range from 0.2 to 2 p.p.m. of iron as HEEDTA iron complex was found to be optimum for yield. The iron content of the trifoliate leaves at these levels of supply was about 40 p.p.m. The 5 and 10 p.p.m. treatments reduced growth. The trifoliate leaves from these treatments contained 64 and 94 p.p.m. of iron. However, optimum yield was attained at 2 p.p.m. with an iron content of 68 p.p.m. This level cannot be designated toxic, although the 94 p.p.m. level might be.

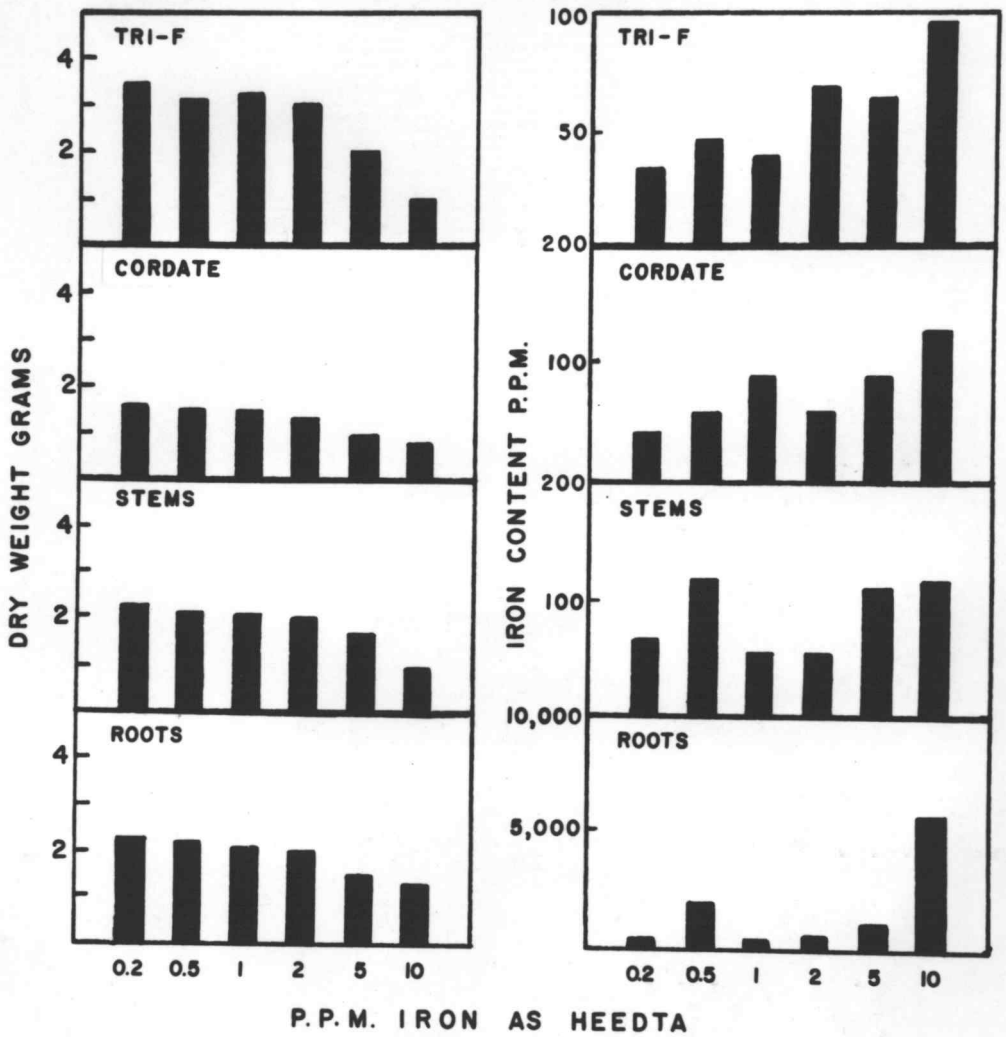


Fig. 10. Experiment 15. Dry weight and iron content of roots, stems, cordate leaves and tri-foliate leaves of bean plants grown at varying levels of iron supplied as the HEEDTA complex in nutrient solutions at pH 7.5.

Table 5. Experiment 15. Dry weights of trifoliate and cordate leaves, stems, and roots of bean plants grown at varying levels of iron supplied as the HEEDTA complex in nutrient solutions at pH 7.5.

Plant part	p.p.m. iron as HEEDTA iron complex						L.S.D.
	0.2	0.5	1	2	5	10	.05
	grams						
Tri-foliate leaves	3.41	3.07	3.23	3.01	2.02	1.00	0.77
Cordate leaves	1.59	1.45	1.40	1.30	0.92	0.78	0.25
Stems	2.29	2.10	2.11	2.10	1.66	0.97	0.61
Roots	2.25	2.19	2.06	2.02	1.47	1.26	0.34
Whole plant	9.55	8.82	8.79	8.42	6.07	4.00	1.56

Table 6. Experiment 15. Iron content of trifoliate and cordate leaves, stems, and roots of bean plants grown at varying levels of iron supplied as the HEEDTA complex in nutrient solutions at pH 7.5.

Plant part	p.p.m. iron as HEEDTA iron complex						L.S.D.
	0.2	0.5	1	2	5	10	.05
	p.p.m.						
Trifoliate leaves	33	45	37	68	64	94	28
Cordate leaves	41	59	89	61	90	128	50
Stems	67	115	52	52	108	114	N.S.
Roots	376	1925	375	533	1128	5735	86

B. Optimum Level of Iron Supplied as DTPA Complex in Alkaline Nutrient Solutions

Experiment 9 was planned to determine the optimum level of iron as DTPA in nutrient solutions at pH 7.5. Five levels of iron were supplied: 0, 0.01, 0.03, 0.1, and 0.5 p.p.m. There were two replicate crocks in each treatment and the whole experiment was completely randomized using random numbers from the tables of Cochran and Cox (14, p. 428). The plants were seeded on September 16, put in crocks on September 29 and harvested on October 17, 1955. At harvesting, the plants were divided into trifoliate leaves, cordate leaves, stems and roots. It was observed in this experiment that the cordate leaves did not suffer from iron deficiency even when the trifoliate leaves showed extreme deficiency. For this reason, it was felt that the trifoliate leaves would make a better index of iron nutrition than the combined leaf sample used in earlier experiments. Hence, the leaves were harvested as separate trifoliate and cordate leaf samples. The dry weights of the tissues are presented in Table 7 and the iron contents in Table 8.

The results of this experiment are evident from the photograph, Fig. 11, where it may be observed that poor growth was found in all of the treatments. Only the highest concentration showed very much green colour and none of the samples showed much growth. The highest iron content of the trifoliate leaves was 36 p.p.m., which is inadequate for optimum growth according to the evidence from

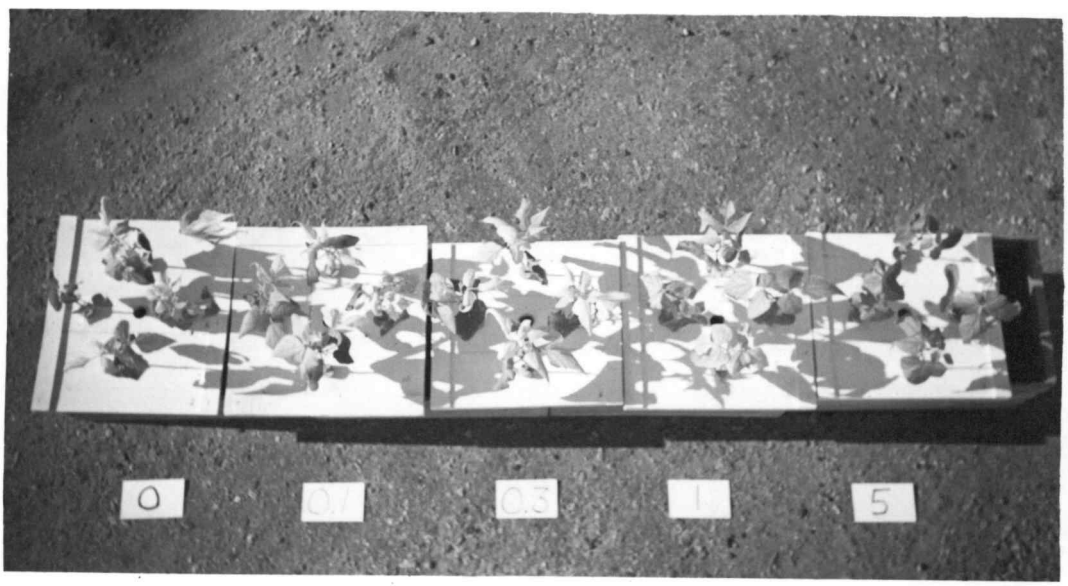


Fig. 11. Experiment 9. Growth of plants in 0, 0.01, 0.03, 0.1 and 0.5 p.p.m. iron supplied as the DTPA complex in mineral nutrient solutions at pH 7.5. (numerals on photograph indicate p.p.m. actual DTPA)

other experiments.

Table 7. Experiment 9. Dry weights of tri-foliate leaves, cordate leaves, stems and roots of bean plants grown at varying levels of iron supplied as the DTPA iron complex.

Plant part	p.p.m. iron as DTPA iron complex					L.S.D.
	0	0.01	0.03	0.1	0.5	.05
	grams					
Tri-foliate leaves	0.98	1.10	1.30	1.64	1.12	0.26
Cordate leaves	1.46	1.25	1.68	1.03	0.90	0.30
Stems	1.93	1.82	1.75	1.62	1.36	0.22
Roots	1.28	1.14	1.31	1.82	1.90	0.35
Whole plant	5.66	5.32	5.54	6.12	5.29	N.S.

Table 8. Experiment 9. Iron contents of tri-foliate and cordate leaves, stems and roots of bean plants grown at varying levels of iron supplied as the DTPA complex in nutrient solutions at pH 7.5.

Plant part	p.p.m. iron as DTPA iron complex					L.S.D.
	0	0.01	0.03	0.1	0.5	.05
	p.p.m.					
Tri-foliate leaves	23	31	24	25	36	N.S.
Cordate leaves	27	37	29	34	51	N.S.
Stems	106	112	98	72	87	N.S.
Roots	7285	4467	3100	2385	7080	N.S.

Experiment 11

This experiment was planned to determine the optimum level of iron as DTPA in nutrient solutions at pH 7.5, since experiment 9 did not provide sufficient information. In experiment 9, increasing yield of tri-foliate leaves was obtained up to 0.5 p.p.m. iron. Levels in this experiment included 0.5, 1, 2, 5, 10, and 20 p.p.m. iron. Each treatment was replicated three times, and the experiment was laid out in three blocks, each block consisting of one row of six crocks running the full length of the light bank. Treatments within the blocks were completely randomized by the use of random numbers from the tables of Cochran and Cox (14, p. 428). The plants were seeded on November 22, placed in crocks on December 2 and harvested on December 16. The dry weights of tissues are shown in Table 9 and Fig. 13. The iron contents of tissues are presented in Table 10 and Fig. 13. A photograph of representative plants is shown as Fig. 12.

The graph of the weight of tri-foliate leaves shows that 0.5 to 2 p.p.m. iron as DTPA was optimum, and that a large reduction in growth occurred at 10 and 20 p.p.m. The optimum level of iron in the leaves was found to be 50 to 85 p.p.m. Levels of 120 and 130 p.p.m. iron in the leaves resulted in markedly less growth.

Fig. 12. Experiment 11. Growth of bean plants at 0.5, 1, 2, 5, 10 and 20 p.p.m. iron supplied as the DTPA complex in mineral nutrient solutions at pH 7.5.

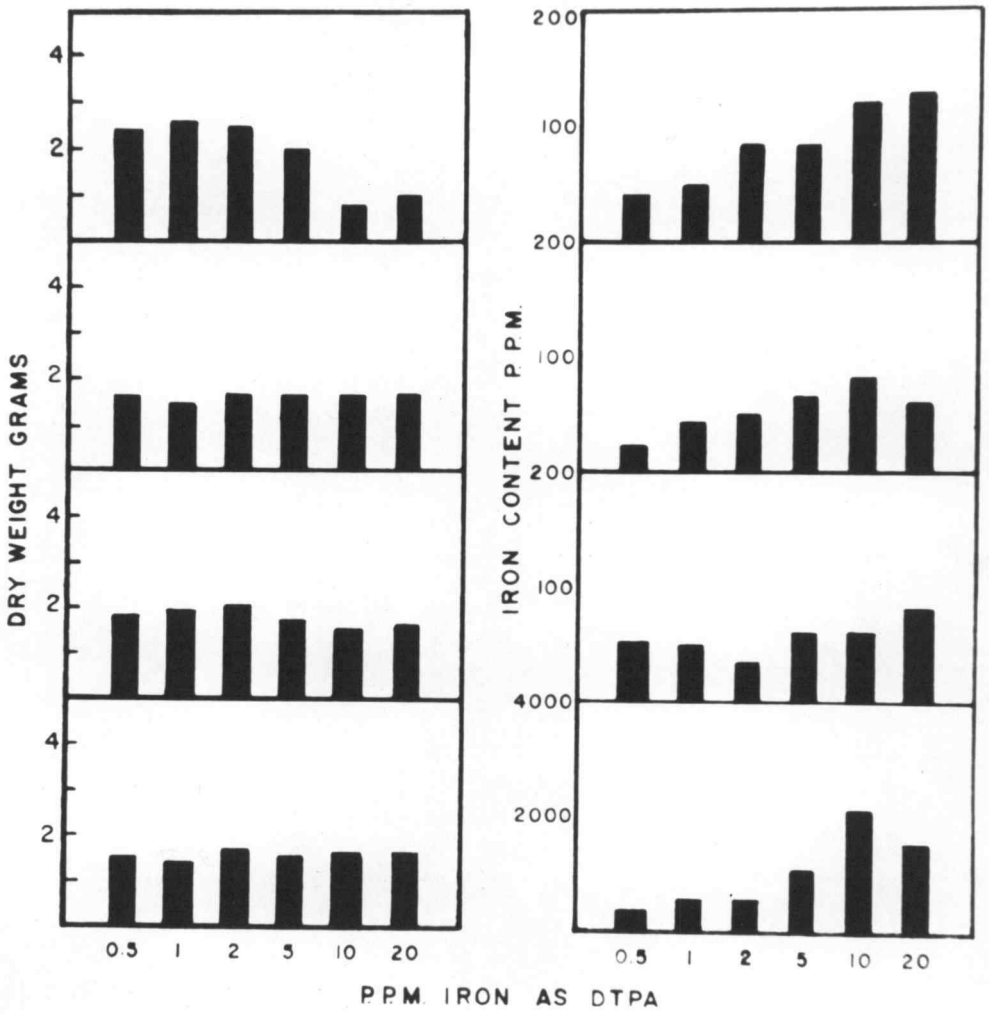


Fig. 13. Experiment 11. Effect of concentration of iron supplied as DTPA complex in nutrient solutions at pH 7.5 on dry weight and iron content of roots, stems, cordate and trifoliate leaves of bean plants.

Table 9. Experiment 11. Dry weights of tri-foliate and cordate leaves, stems, and roots of bean plants grown at varying levels of iron as DTPA complex in nutrient solutions at pH 7.5.

Plant part	p.p.m. iron as DTPA complex						L.S.D.
	0.5	1	2	5	10	20	.05
				grams			
Tri-foliate leaves	2.33	2.59	2.44	1.96	0.79	0.97	0.55
Cordate leaves	1.60	1.42	1.66	1.61	1.63	1.62	N.S.
Stems	1.77	1.85	1.99	1.74	1.46	1.58	0.34
Roots	1.50	1.34	1.66	1.52	1.55	1.56	N.S.
Whole plant	7.20	7.20	7.75	6.83	5.44	5.90	0.87

Table 10. Experiment 11. Iron contents of tri-foliate and cordate leaves, stems, and roots of bean plants grown at varying levels of iron supplied as DTPA complex in nutrient solutions at pH 7.5.

Plant part	p.p.m. iron as DTPA iron complex						L.S.D.
	0.5	1	2	5	10	20	.05
				p.p.m.			
Tri-foliate leaves	41	48	84	85	120	130	N.S.
Cordate leaves	22	42	49	67	84	60	N.S.
Stems	52	49	34	60	61	81	N.S.
Roots	357	552	518	1041	2152	1543	N.S.

Experiment 16

Further information on the optimum level of iron supplied as the DTPA complex was obtained in this experiment which, however, was designed primarily to give information on the effect of calcium concentration of the nutrient solution on yield and iron uptake and on the interaction between calcium and iron. Those aspects of the experiment are reported under sections G and H, experiment 16.

Iron was supplied as DTPA 0.5, 2, and 10 p.p.m. and calcium was supplied at 4, 8, and 16 milliequivalents per litre. Each treatment combination was replicated twice, making eighteen cultures. One complete replicate was run in each of two blocks, and treatments were assigned within blocks by the use of random numbers from the tables of Cochran and Cox (14, p. 428). The plants were seeded on February 29, placed in crocks on March 9, and harvested on March 23, 1956. At harvest, they were divided into tri-foliate leaves and cordate leaves, stems, and roots. Dry weights were determined on all samples and iron contents on the leaf samples only. Dry weights are presented in Table 11 and in Fig. 14. Iron contents are presented in Table 12, and in Fig. 14. Photographs of representative plants are presented in Figs. 28, 29 and 30.

The 0.5 and 2 p.p.m. treatments both gave optimum yields. The 10 p.p.m. treatment reduced yield somewhat. The tri-foliate leaves in the optimum yield range contained about 50 p.p.m. iron,

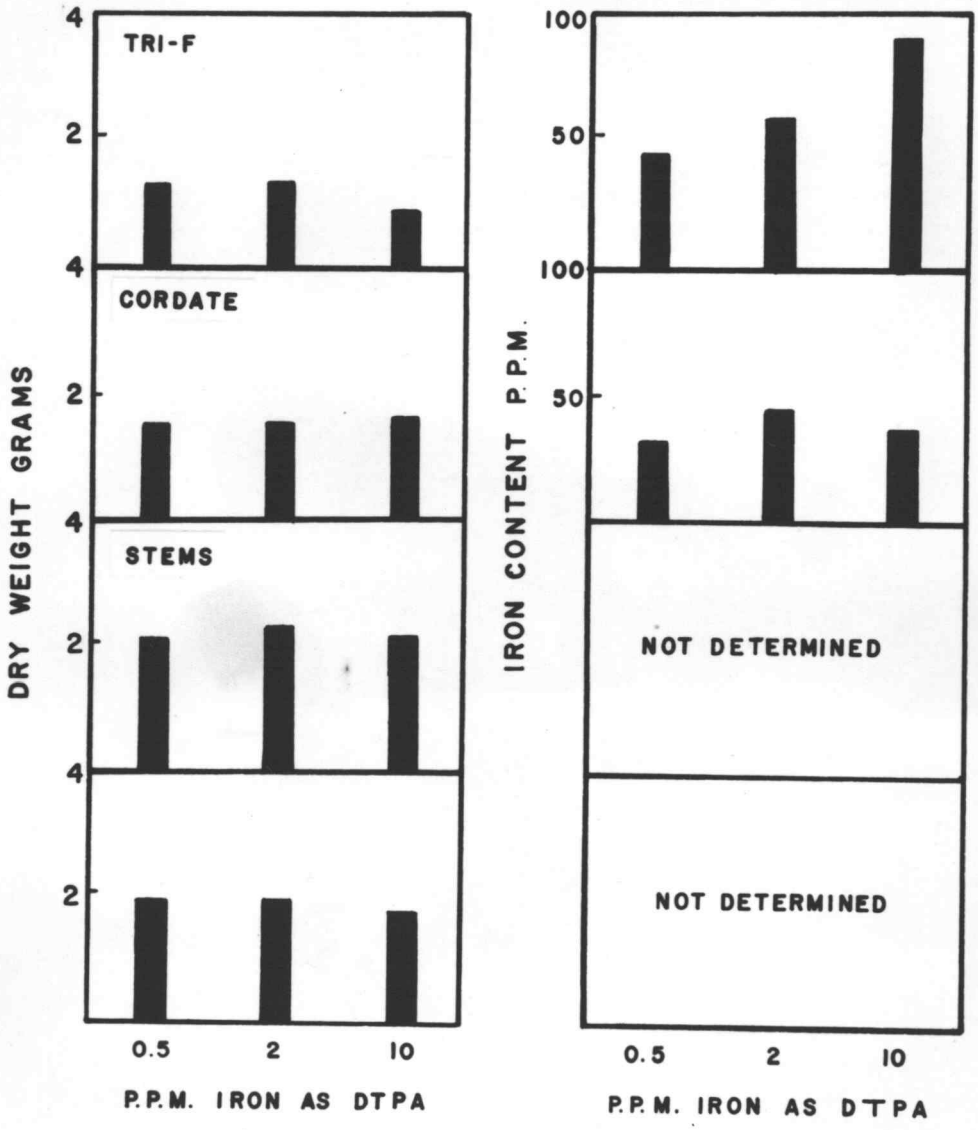


Fig. 14. Experiment 16. Effect of iron concentration of the substrate on dry weights and iron contents of tri-foliate leaves, cordate leaves, stems and roots of bean plants grown in nutrient solutions at pH 7.5.

but where yield was reduced by excessive iron supply, the tri-foliate leaves contained 90 p.p.m.

Table 11. Experiment 16. Dry weights of tri-foliate, and cordate leaves, stems, and roots of bean plants grown at varying levels of iron in nutrient solutions at pH 7.5 when the iron was supplied as DTPA complex.

Plant part	p.p.m. iron as the DTPA complex			L.S.D.
	0.5	2	10	.05
		grams		
Tri-foliate leaves	1.30	1.38	0.93	0.29
Cordate leaves	1.48	1.56	1.57	N.S.
Stems	2.08	2.26	2.07	N.S.
Roots	1.98	1.98	1.81	N.S.
Whole plant	6.85	7.17	6.37	N.S.

Table 12. Experiment 16. Iron contents of tri-foliate and cordate leaves, stems, and roots of bean plants grown at varying levels of iron as DTPA in nutrient solutions at pH 7.5.

Plant part	p.p.m. iron as DTPA complex			L.S.D.
	0.5	2	10	.05
		p.p.m.		
Tri-foliate leaves	45	58	89	N.S.
Cordate leaves	32	43	37	N.S.

C. Spraying and Vacuum Impregnation as Methods of Applying Chelated Iron to Plants

Experiment 5

This experiment was planned to test the effectiveness of introducing chelated iron into leaves by impregnation under vacuum as previously described. Single plant plots were used with three replicates. The plants were at the stage of half-grown cordate leaves when they were treated. They were lifted from the vermiculite seedbed, treated, and re-planted in vermiculite in eight ounce glass jars. There were two check treatments: one with no treatment except transplanting, and another including vacuum impregnation with distilled water. The stock HEEDTA solution contained 2.3 per cent iron. The first group treated on March 28, 1955, included three treatments: 230 p.p.m. (a 1:100 dilution of the stock solution) and the two checks. In the 230 p.p.m. treatment, the leaves wilted and fell off the plants, indicating serious damage. However, no damage occurred in either of the check treatments, showing that the chelate and not the method of treatment caused the damage. The second group was treated on March 29 with a 23 p.p.m. iron solution. A little damage resulted. The third group was treated on April 3 with a 4.6 p.p.m. solution. There was still slight damage. No more plants were available at the time and work was necessarily suspended.

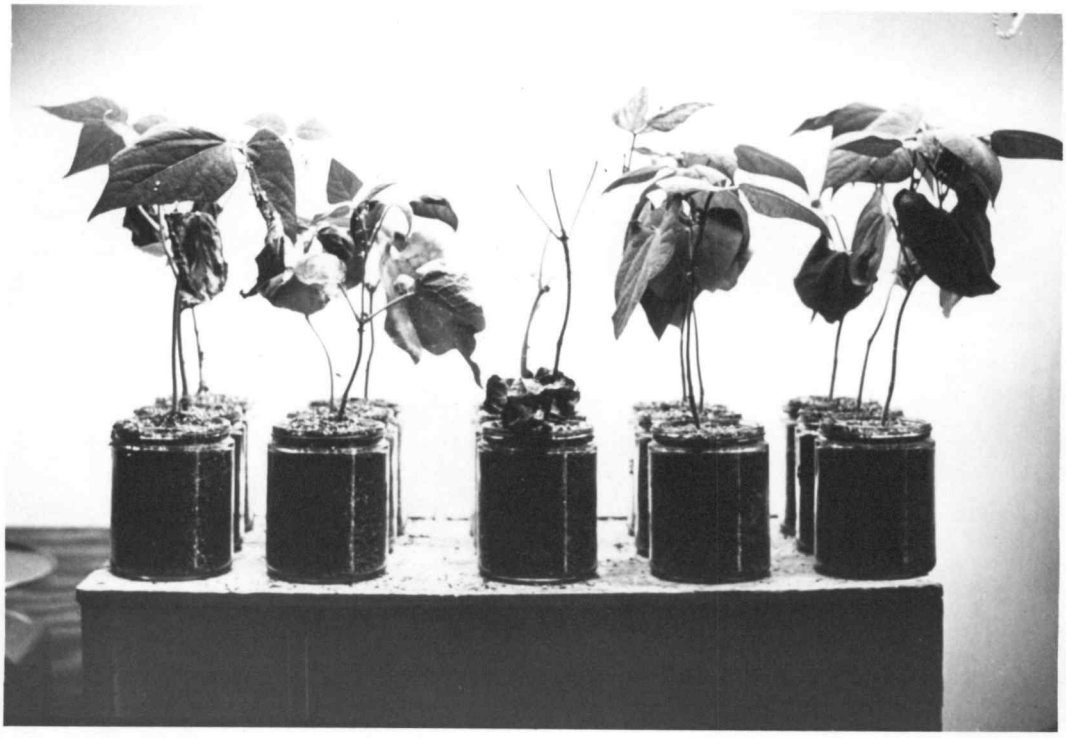


Fig. 15. Experiment 5. Effect of impregnation of cordate leaves under vacuum with solutions of iron supplied as the HEEDTA complex. From left to right: 1. 4.6 p.p.m. 2. 23 p.p.m. 3. 230 p.p.m. 4. impregnation with water. 5. no treatment.

The results of the first series are shown photographically in Fig. 15. It seems rather peculiar that damage resulted from this procedure since presumably the amount of iron complex introduced by such a procedure would be considerably less than that taken up through the roots from culture solutions.

Experiment 14

Experiment 5 showed severe damage at 230 p.p.m. iron as HEEDTA, and slight damage at 4.6 p.p.m. iron. This experiment was planned to clarify the toxic level of solutions for impregnation by vacuum still further. Treatments used were 1000, 100, 10, 1, and 0.1 p.p.m. iron as HEEDTA applied by vacuum and by dipping. The dip treatment was included to compare the toxicity as HEEDTA on the outside of the leaf and the inside of the leaf. The dip treatment included a wetting agent (Dreft) to ensure complete wetting of the leaf surface. Each treatment was replicated twice. Dry weights and iron content would have had relatively little meaning in this experiment and were not determined.

The results are shown photographically in Fig. 16 for vacuum impregnation and in Fig. 17 for dipping. There was no damage from the 0.1 and 1 p.p.m. treatments under vacuum impregnation. However, the 10, 100, and 1000 p.p.m. treatments damaged the tissues. Indeed, the 1000 p.p.m. treatment caused complete defoliation. There was no damage in the dipped plants from the 0.1, 1, or 10 p.p.m. treatments. Only the 100 and 1000 p.p.m. levels caused

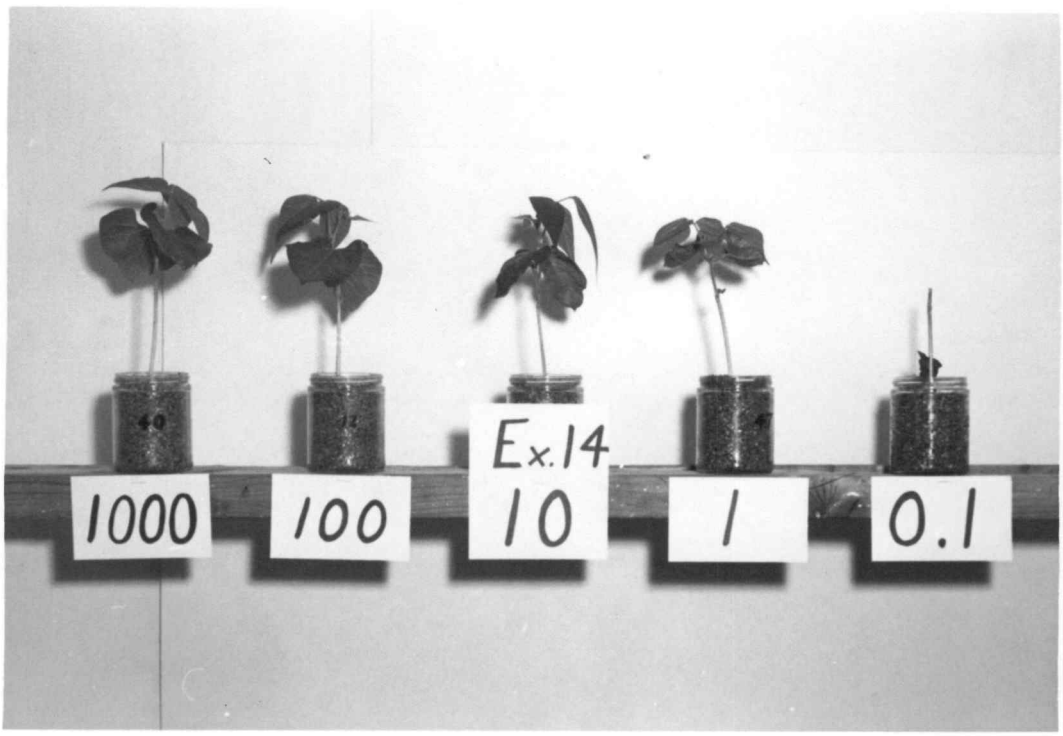


Fig. 16. Experiment 14. Effect of impregnation of cordate leaves under vacuum with solutions of iron supplied as the HEEDTA complex at 0.1, 1, 10, 100 and 1000 p.p.m. iron. (Labels on photograph are in reverse order)

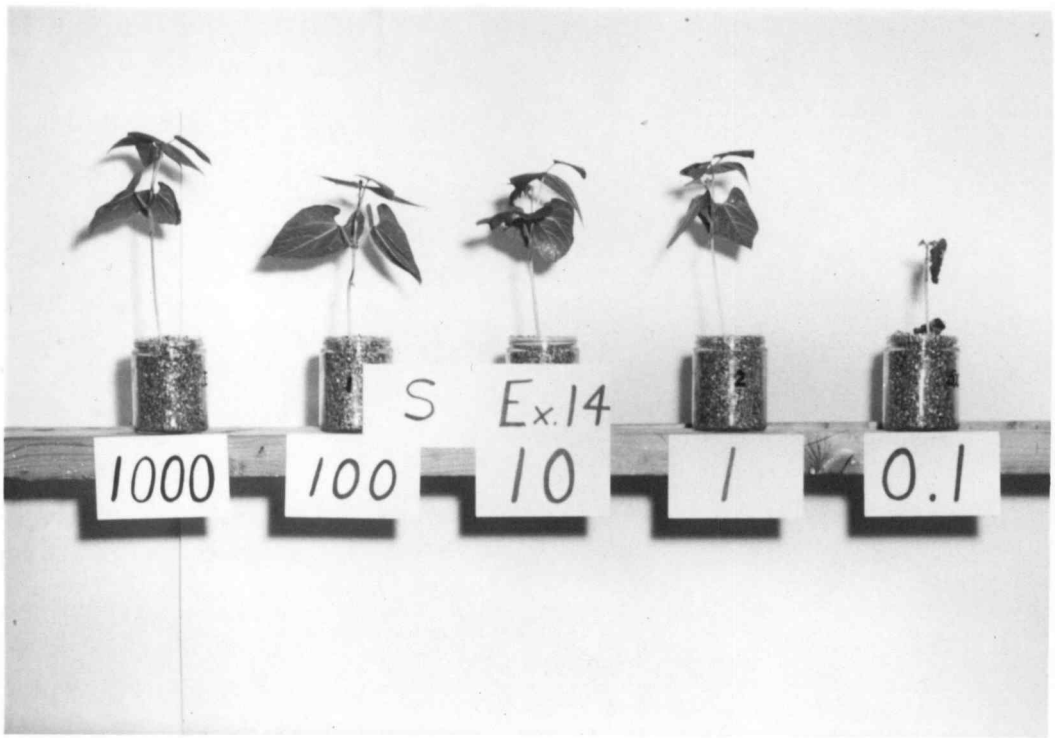


Fig. 17. Experiment 14. Effect of spraying of cordate leaves with solutions of iron as HEEDTA complex at 0.1, 1, 10, 100 and 1000 p.p.m. iron. (labels on photograph are in reverse order)

damage to the tissues, the 1000 p.p.m. treatment causing almost complete defoliation. Both methods were satisfactory for introducing chelated iron into plants as judged by green color of the plant.

D. Effect of pH of the Nutrient Solution in the Alkaline Range on Growth and Iron Uptake of Bean Plants

Experiment 13

Experiment 13 was planned to provide information as to the effect of pH in the alkaline range on the iron nutrition of the bean plant. There were six pH levels: 7.0, 7.5, 8.0, 8.5, 9.0, and 9.5. Iron was supplied to all treatments uniformly at 1 p.p.m. iron as the HEEDTA complex. Phosphorus was supplied at the low level as required in Arnon and Johnson's nutrient solution. Each treatment was replicated three times and the whole experiment was divided into three blocks each containing six crocks. The plants were seeded on January 4, put in crocks on January 13 and harvested on January 31.

The effect of the treatments is shown photographically in Fig. 18 where the reduced growth at higher pH's is readily seen. A graph of the dry weights and iron contents of tissues is shown in Fig. 19. The dry weights of the tissues are presented in Table 13 and the iron contents in Table 14.

The optimum yield was obtained at pH 7.5. Growth was progres-

Fig. 18. Experiment 13. Effect of pH of the nutrient solution on growth of bean plants when iron was supplied at 1 p.p.m. as the HEEDTA complex.

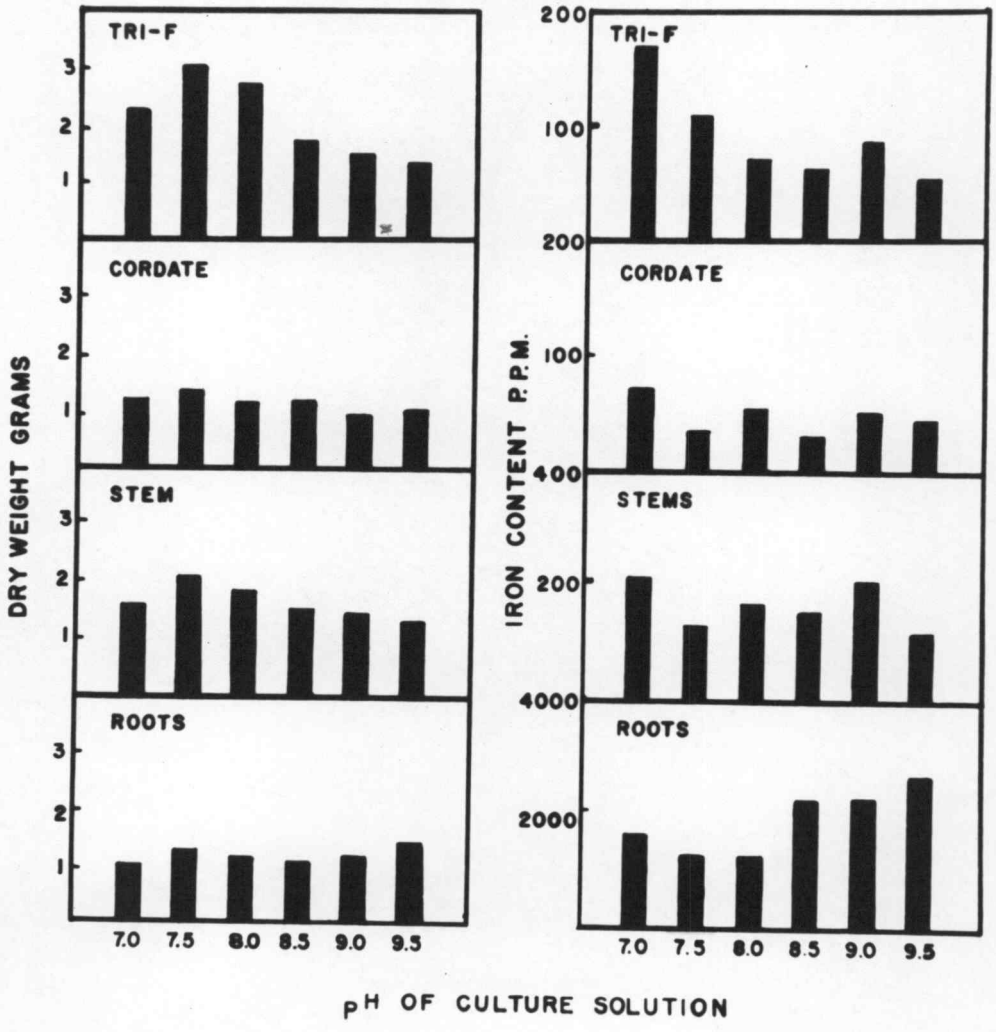


Fig. 19. Experiment 13. Effect of pH of the culture solution on dry weight and iron content of the roots, stems, cordate and tri-foliate leaves of bean plants when iron was supplied at 1 p.p.m. as the HEEDTA complex.

sively less at higher pH's, as well as at the lower pH of 7.0. The iron contents were progressively less from pH 7.0 to 8.0. The reduced yield at pH 7.0 might readily be attributed to the high iron content of the plants, 170 p.p.m. It is worthy of note that maximum yield was obtained with 108 p.p.m. iron content, a level found to be toxic in other experiments.

Table 13. Experiment 13. Dry weights of bean plants grown at varying pH values in nutrient solutions supplied with 1 p.p.m. iron as the HEEDTA iron complex.

Plant part	pH of the nutrient solution						L.S.D.
	7.0	7.5	8.0	8.5	9.0	9.5	.05
	grams						
Tri-foliate leaves	2.23	2.98	2.67	1.72	1.47	1.33	0.50
Cordate leaves	1.16	1.24	1.10	1.16	0.92	0.92	N.S.
Stems	1.59	2.08	1.80	1.49	1.44	1.34	0.33
Roots	1.01	1.29	1.14	1.10	1.19	1.44	N.S.
Whole plant	6.00	7.59	6.71	5.47	5.03	5.04	1.35

Table 14. Experiment 13. Iron contents of bean plants grown at varying pH values in nutrient solutions supplied with 1 p.p.m. iron as the HEEDTA iron complex.

Plant part	pH of the nutrient solution						L.S.D.
	7.0	7.5	8.0	8.5	9.0	9.5	.05
	p.p.m.						
Tri-foliate leaves	169	108	72	64	87	56	56
Cordate leaves	71	36	54	30	50	44	N.S.
Stems	208	130	170	149	208	117	N.S.
Roots	1652	1282	1231	2327	2351	2742	N.S.

E. Effect of Phosphorus Concentration in the Nutrient Solution on Growth and Iron Content of Bean Plants Grown at pH 7.5.

Experiment 12

Experiment 12 was planned to show the effect of the phosphorus level of the nutrient solution on the uptake of iron from HEEDTA and DTPA iron complexes in the nutrient solution. Three levels of phosphorus were used: 0.00006 molar, 0.0006 molar and 0.006 molar. Both chelate compounds were supplied at the rate of 1 p.p.m. iron and the pH was maintained at pH 7.5. Each treatment combination was replicated three times. The whole experiment was laid out in three blocks, each containing one complete replicate. Treatments were assigned to crocks by use of a table of random numbers from Cochran and Cox (14, p. 428). The plants were seeded on December 16, transplanted to crocks on December 23 and harvested on January 10.

Fig. 20. Experiment 12. Growth of bean plants in nutrient solutions at pH 7.5 at low, medium and high phosphorus levels when iron was supplied at 1 p.p.m. as the HEEDTA complex.

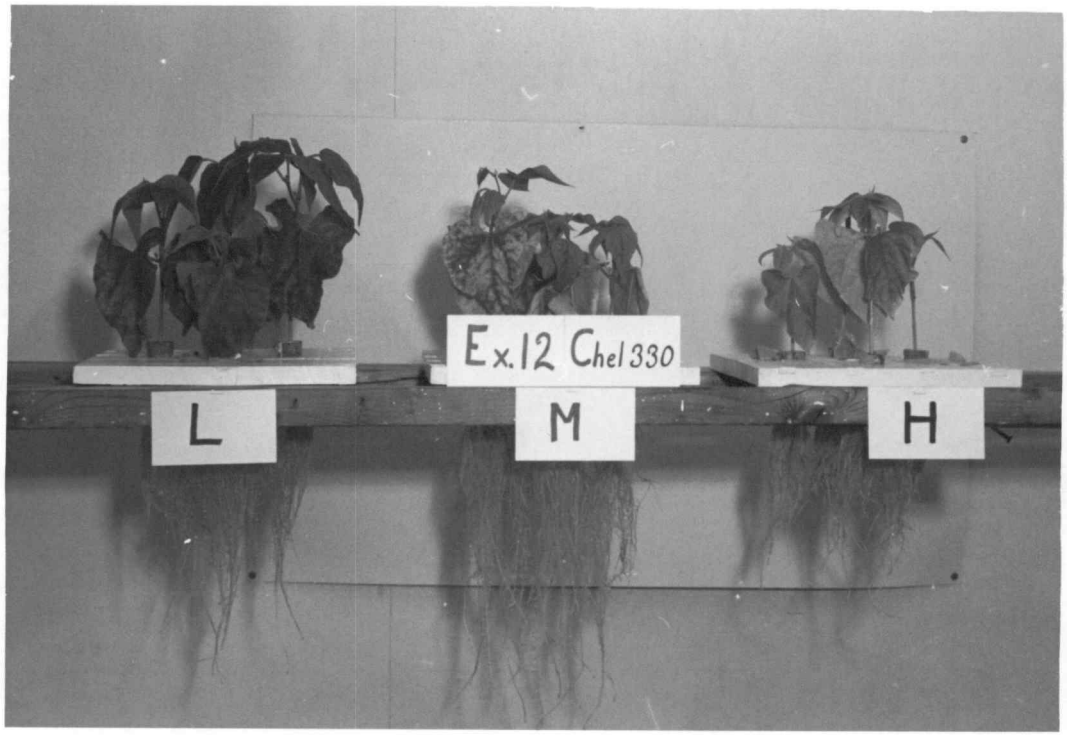


Fig. 21. Experiment 12. Growth of bean plants in nutrient solutions at pH 7.5 at low, medium and high phosphorus concentrations when iron was supplied at 1 p.p.m. as the DTPA complex.

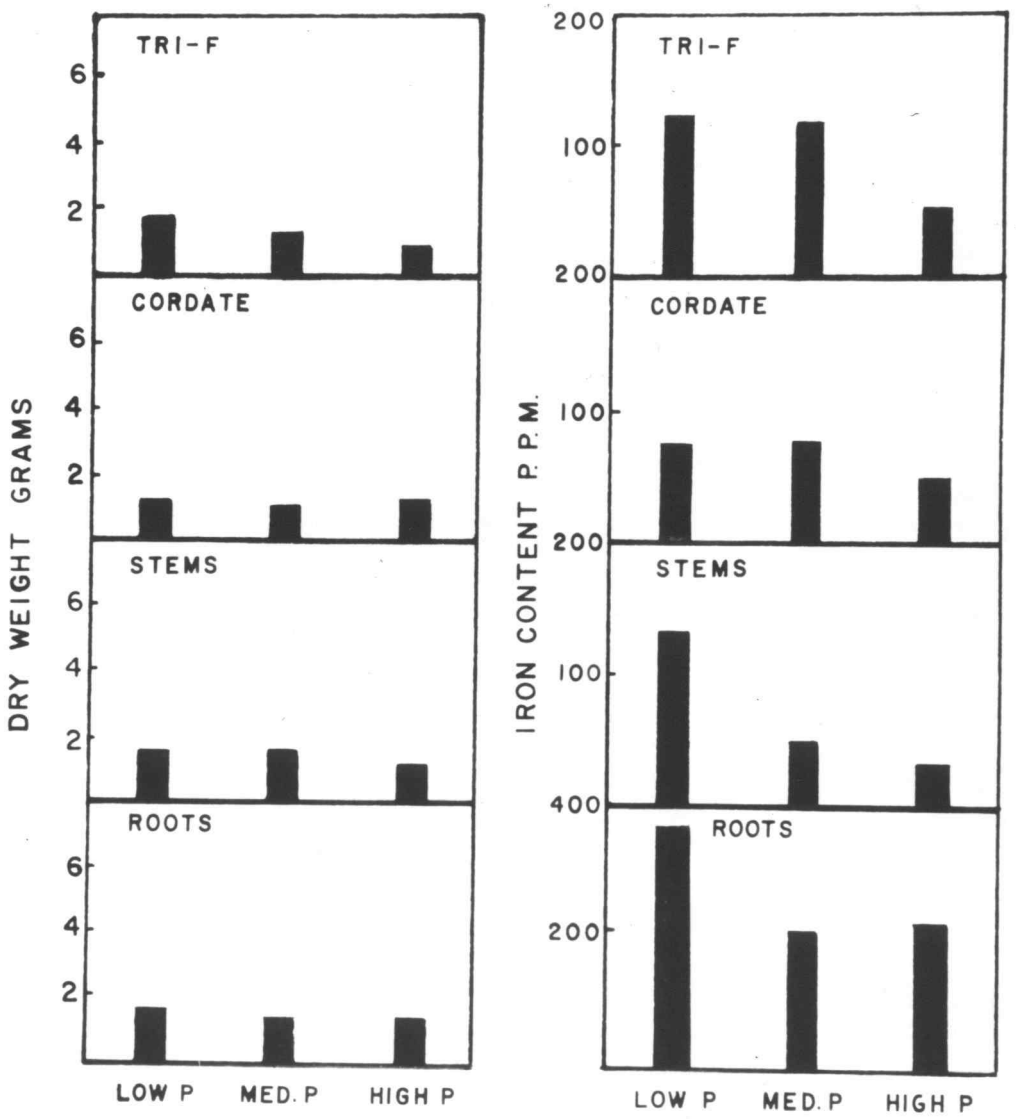


Fig. 22. Experiment 12. Effect of phosphorus level of the nutrient solution on dry weights and iron contents of bean plants grown in nutrient solutions at pH 7.5 when iron was supplied as the DTPA complex.

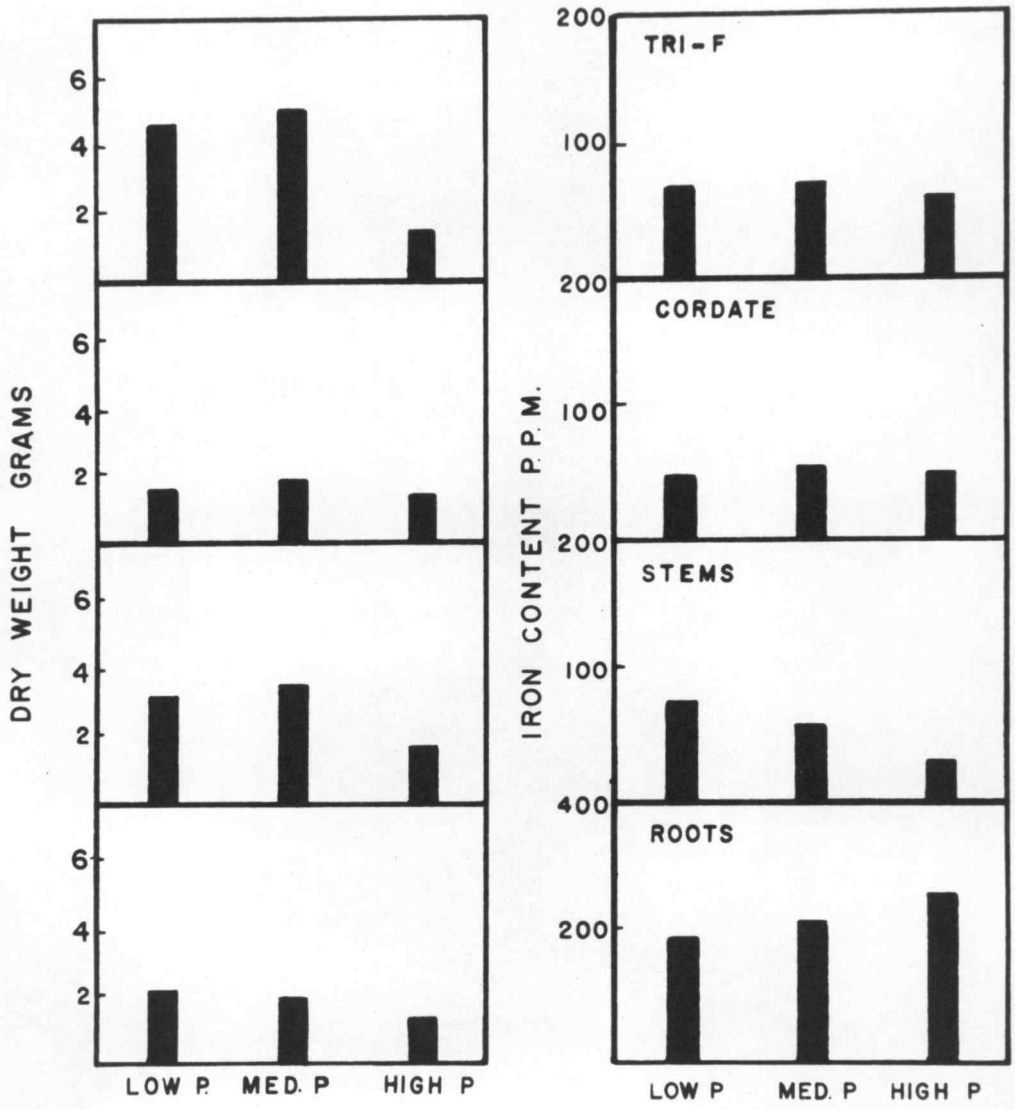


Fig. 23. Experiment 12. Effect of phosphorus level of the nutrient solution on dry weights and iron contents of bean plants grown in nutrient solutions at pH 7.5 when iron was supplied at 1 p.p.m. as the HEEDTA complex.

Dry weights and iron contents of the tri-foliate and cordate leaves, stems and roots were determined.

In this experiment, it was observed that the plants that developed moderate chlorosis in the high phosphorus treatments were affected in the cordate leaves as much as in the tri-foliate leaves. This is in contrast to the effect of iron deficiency observed in earlier experiments in which the cordate leaves were not affected, but the tri-foliate leaves were seriously affected.

The highest phosphorus treatment reduced both dry weight and iron content markedly, and caused a mild overall chlorosis of leaves. The DTPA treatments resulted in much less growth and much higher iron contents at all levels of phosphorus.

Table 15. Experiment 12. Dry weights of bean plants grown in nutrient solutions at varying levels of phosphorus with iron supplied at 1 p.p.m. as the HEEDTA and DTPA iron complexes.

Plant part	Chelated compound					
	HEEDTA			DTPA		
	low P	med P	high P	low P	med P	high P
	grams					
Tri-foliate leaves	4.70	5.30	1.52	1.84	1.43	0.66
Cordate leaves	1.63	1.75	1.45	1.54	1.43	1.46
Stems	3.31	3.52	1.70	1.77	1.54	1.23
Roots	2.25	1.81	1.39	1.68	1.42	1.45
Whole plant	11.89	12.39	6.05	6.83	5.83	4.80

Table 16. Experiment 12. Iron contents of bean plants grown in nutrient solutions at varying levels of phosphorus when supplied with iron as the HEEDTA and DTPA complexes at 1 p.p.m. iron.

Plant part	Chelated compound					
	HEEDTA			DTPA		
	low P	med P	high P	low P	med P	high P
	p.p.m.					
Tri-foliate leaves	70	72	62	123	121	52
Cordate leaves	47	55	49	74	77	48
Stems	75	58	32	127	49	36
Roots	193	219	261	368	215	227

Table 17. Experiment 12. Effect of phosphorus level in the nutrient solution on dry weight of tissues for both chelate compounds combined.

Plant part	Phosphorus level			L.S.D.	Chelate		L.S.D.
	Low	Med	High	.05	HEEDTA	DTPA	.05
Tri-foliate leaves	3.27	3.37	1.09	1.20	3.84	1.31	0.98
Cordate leaves	1.59	1.59	1.45	N.S.	1.61	1.48	N.S.
Stems	2.54	2.53	1.46	0.58	2.84	1.51	0.47
Roots	1.97	1.62	1.42	0.32	1.82	1.52	0.27
Whole plant	9.36	9.11	5.43	1.97	10.11	5.82	1.61

Table 18. Experiment 12. Effect of phosphorus level of the nutrient solution on iron content of tissues for both chelates combined.

Plant part	Phosphorus level			L.S.D.	Chelate		L.S.D.
	Low	Med	High	.05	HEEDTA	DTPA	.05
				p.p.m.			
Tri-foliate leaves	98	98	58	N.S.	68	99	N.S.
Cordate leaves	62	67	50	N.S.	50	67	N.S.
Stems	102	54	35	45	55	71	N.S.
Roots	281	218	245	N.S.	224	270	N.S.

Experiment 15

The design of this experiment has been described under section A, experiment 15. One of the features of the design was the effect of phosphorus level in the nutrient solution on growth of plants and uptake of iron. Three levels of phosphorus were supplied: 0.00006, 0.0006, and 0.006 molar.

Dry weights and iron contents of tri-foliate leaves, cordate leaves, stems and roots are shown in Tables 19 and 20 and in graph form in Fig. 27. The results are shown photographically in Figs. 24, 25 and 26.

The results show that 0.006 molar phosphorus, which is a normal phosphorus level for nutrient solutions, seriously retarded growth and reduced the iron content of tri-foliate leaves, whereas

Fig. 24. Experiment 15. Growth of bean plants in nutrient solutions at pH 7.5 when iron was supplied at 0.2, 0.5, 1, 2, 5 and 10 p.p.m. as the HEEDTA complex, and phosphorus was supplied at 0.00006 molar.

Fig. 25. Experiment 15. Growth of bean plants in nutrient solutions at pH 7.5 when iron was supplied at 0.2, 0.5, 1, 2, 5 and 10 p.p.m. as the HEEDTA complex and phosphorus was supplied at 0.0006 molar.

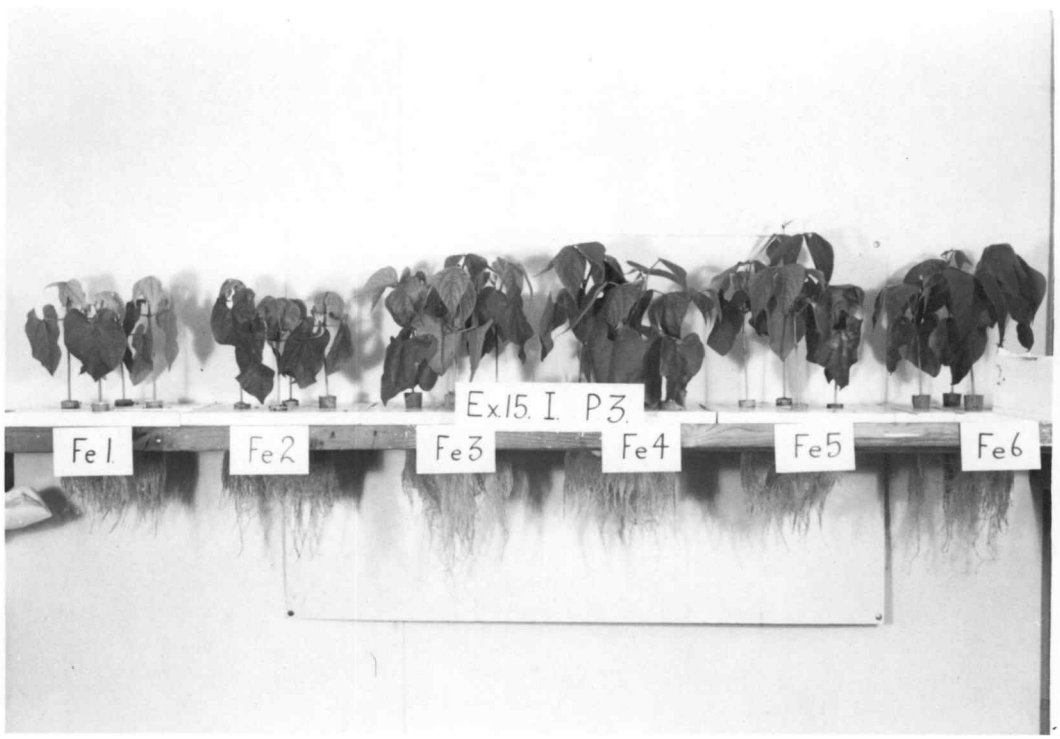


Fig. 26. Experiment 15. Growth of bean plants in nutrient solutions at pH 7.5 when iron was supplied at 0.2, 0.5, 1, 2, 5 and 10 p.p.m. as the HEEDTA complex and phosphorus was supplied at 0.006 molar.

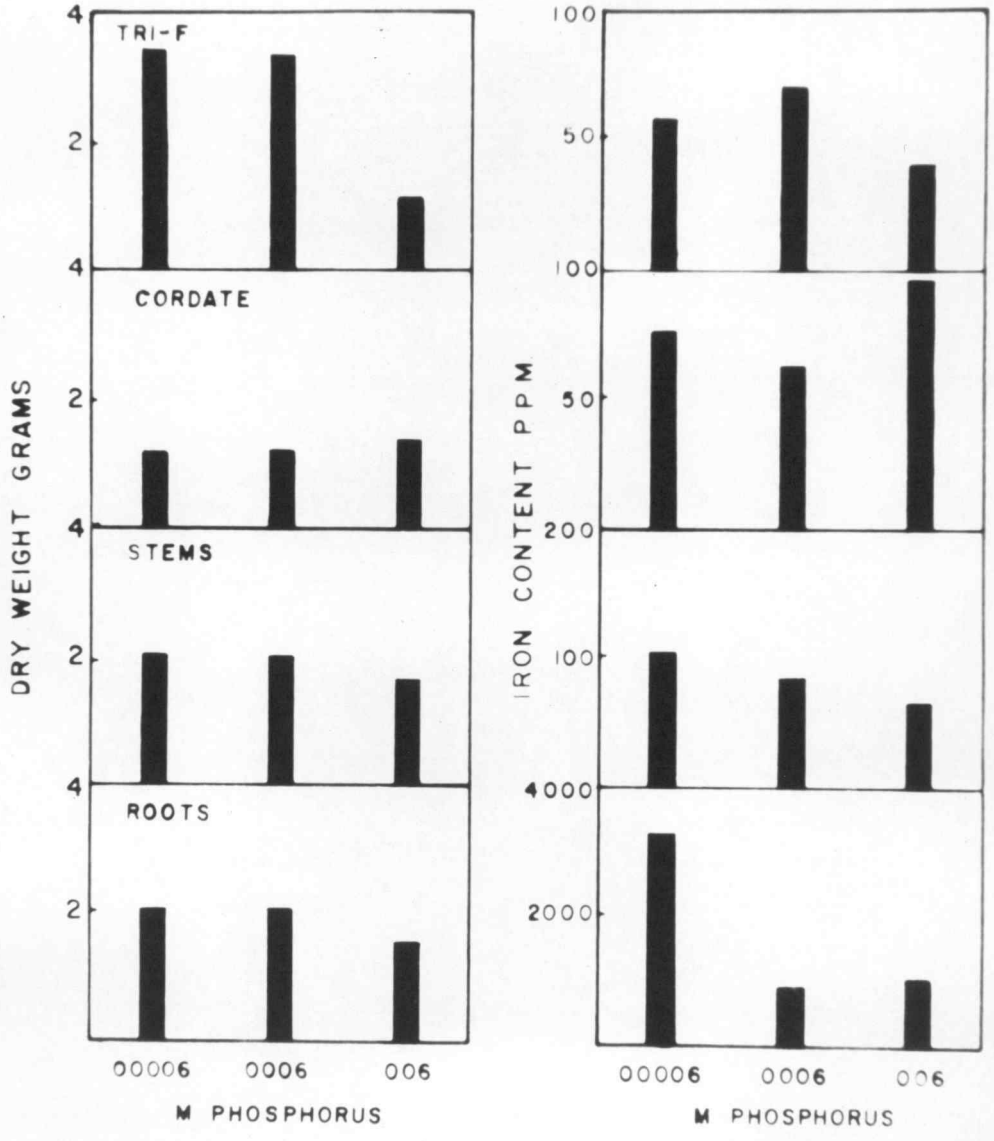


Fig. 27. Experiment 15. Effect of phosphorus concentration of the nutrient solution on dry weight and iron content of tri-foliate and cordate leaves, stems and roots of bean plants grown in nutrient solutions at pH 7.5.

0.0006 molar phosphorus did not retard growth or affect leaf iron content. The plants in the high phosphorus treatment, as in experiment 12, developed a mild chlorosis which was evident in the cordate leaves as well as in the tri-foliate leaves.

Table 19. Experiment 15. Effect of phosphorus level of the nutrient solution on the dry weight of the tissues when iron is supplied at the rate of 1 p.p.m. as the HEEDTA iron complex.

Plant part	Phosphorus concentration--molar 0.00006	0.0006	0.006	L.S.D. .05
		grams		
Tri-foliate leaves	3.43	3.38	1.06	0.54
Cordate leaves	1.17	1.16	1.39	0.18
Stems	2.03	1.97	1.62	0.43
Roots	2.05	2.06	1.51	0.24
Whole plant	8.68	8.57	5.58	1.11

Table 20. Experiment 15. Effect of phosphorus level of the nutrient solution on the iron content of tissues when iron is supplied as the HEEDTA iron complex at the rate of 1 p.p.m. iron.

Plant part	Phosphorus concentration--molar 0.00006	0.0006	0.006	L.S.D. .05
		p.p.m.		
Tri-foliate leaves	58	71	42	20
Cordate leaves	77	62	96	N.S.
Stems	106	83	64	N.S.
Roots	3266	900	953	61

F. Interaction of Iron Complexed as HEEDTA and Phosphorus When Supplied in Mineral Nutrient Solution at pH 7.5.

Experiment 15

The design of this experiment has been described under section A, experiment 15. The main effects of iron level and phosphorus level have already been described. The remaining factor to be reported is the interaction between iron and phosphorus. The term interaction is used in this study exclusively in its statistical meaning of the effect of two factors together above the sum of their individual effects. Data for treatment combinations are presented as Table 22 for dry weights and Table 23 for iron contents.

The interaction effect was separated from all other effects by the analysis of variance method. The analysis of variance for the yield data for tri-foliate leaves indicates a very small interaction when compared with the very large main effects of phosphorus and iron, as shown in Table 21. Interactions for yield of cordate leaves, stems, roots and whole plants were not significant. All interactions for iron content of tissues were non-significant.

Table 21. Experiment 15. Analysis of variance of the yield of tri-foliate leaves of bean plants supplied with 1 p.p.m. iron as the HEEDTA complex, showing interaction between iron and phosphorus.

Variation due to	Sum of squares	Degrees of freedom	Mean square	F	
Iron	26.104933	5	5.220987	13.14	H.S.
Phosphorus	44.052650	2	22.026325	55.45	H.S.
Interaction	12.776617	10	1.277662	3.22	H.S.
Blocks	0.834178	1	0.834178		
Error	6.752422	17	0.397201		
Total	90.520800	35			

Table 22. Experiment 15. Mean dry weights of whole plants, roots, stems, cordate and tri-foliate leaves of bean plants grown at pH 7.5 in nutrient solutions at three levels of phosphorus and six levels of iron.

Plant part	Phosphorus Concentration	p.p.m. iron as HEEDTA complex 0.2	0.5	1	2	5	10
		grams					
Tri-foliate leaves	0.00006	4.77	4.15	4.16	3.81	2.82	0.86
	0.0006	4.60	4.32	4.07	3.88	2.25	1.20
	0.006	0.86	0.74	1.45	1.38	1.00	0.91
Cordate leaves	0.00006	1.61	1.37	1.18	1.23	0.86	0.74
	0.0006	1.44	1.30	1.34	1.23	0.89	0.75
	0.006	1.72	1.67	1.66	1.41	1.01	0.84
Stems	0.00006	2.70	2.30	2.22	2.43	1.78	0.34
	0.0006	2.60	2.28	2.38	2.26	1.80	1.04
	0.006	1.57	1.73	1.73	1.60	1.38	1.01

Table 22. con't. Experiment 15. Mean dry weights of whole plants, roots, stems, cordate and tri-foliate leaves of bean plants grown at pH 7.5 in nutrient solutions at three levels of phosphorus and six levels of iron.

Plant part	Phosphorus Concentration	p.p.m. iron as HEEDTA complex 0.2	0.5	1	2	5	10
		grams					
Roots	0.00006	2.61	2.53	2.12	2.23	1.60	1.08
	0.0006	2.50	2.19	2.11	2.14	1.53	1.32
	0.006	1.65	1.83	1.93	1.68	1.26	1.38
Whole plant	0.00006	11.69	10.36	9.69	9.71	7.06	3.53
	0.0006	11.15	10.10	9.90	9.47	6.48	4.31
	0.006	5.81	5.98	6.77	6.08	4.65	4.15

Table 23. Experiment 15. Mean iron contents of tissues of bean plants grown at pH 7.5 in nutrient solutions at three levels of phosphorus and six levels of iron.

Plant part	Phosphorus Concentration	p.p.m. iron as HEEDTA complex 0.2	0.5	1	2	5	10
		p.p.m.					
Tri-foliate leaves	0.00006	26	48	45	62	43	110
	0.0006	33	46	49	92	109	96
	0.006	28	40	17	50	40	77
Cordate leaves	0.00006	51	54	107	50	89	107
	0.0006	27	43	41	43	96	119
	0.006	43	78	78	89	85	157
Stems	0.00006	61	199	54	64	109	150
	0.0006	84	47	51	48	132	137
	0.006	56	97	49	53	81	54
Roots	0.00006	597	5563	494	941	1722	10279
	0.0006	309	376	332	305	898	3176
	0.006	220	335	297	351	762	3750

G. Effect of Calcium Concentration of the Nutrient Solution on Growth and Iron Content of Bean Plants Grown at pH 7.5.

Experiment 16

The design of this experiment has been described under section B, experiment 16. One of the features of the design was the effect of calcium level of the nutrient solution on growth of plants and uptake of iron. Three levels of calcium were supplied: 4, 8, and 16 milli-equivalents per litre.

Dry weights of tri-foliate leaves, cordate leaves, stems and roots are presented in Table 24, and in graph form in Fig. 31. Iron contents are presented similarly in Table 25 and Fig. 30. Results are shown photographically in Figs. 28, 29 and 30.

There was no difference in the effect of the various levels of calcium on the yield or on the iron uptake. The high mean iron content of tri-foliate leaves at high calcium supply was caused by a single extremely high result and may be discounted.

Fig. 28. Experiment 16. Growth of bean plants at low calcium level in nutrient solutions at pH 7.5 when iron was supplied at 0.2, 1 and 10 p.p.m. as the DTPA complex.

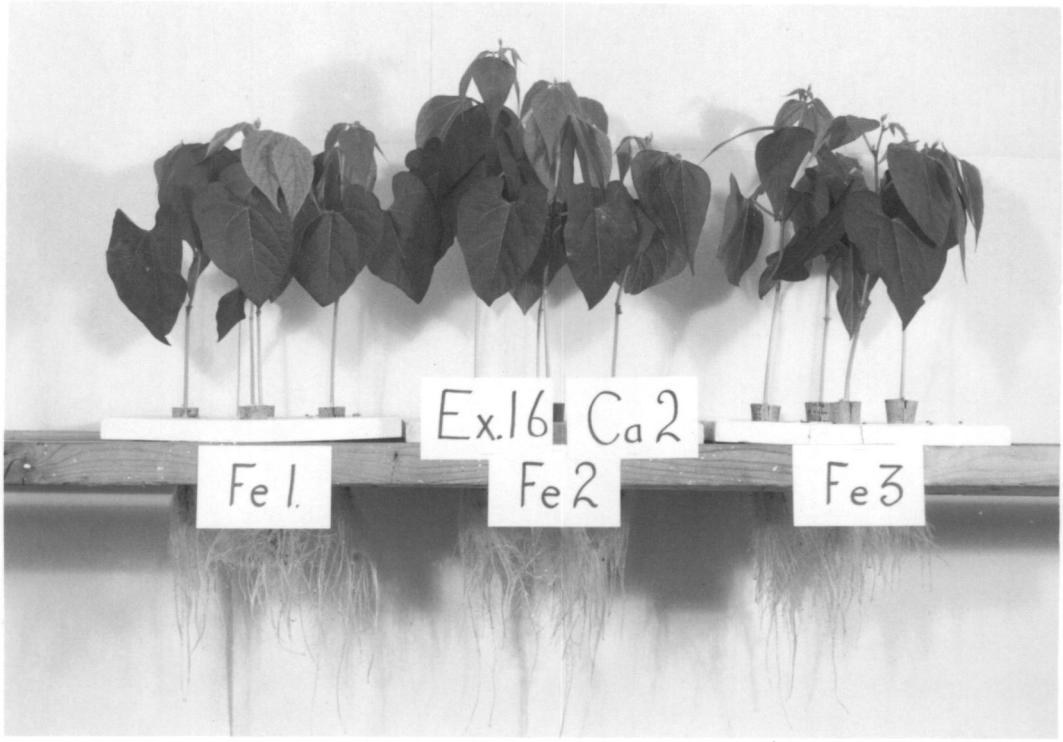


Fig. 29. Experiment 16. Growth of bean plants at medium calcium level in nutrient solutions at pH 7.5 when iron was supplied at 0.2, 1 and 10 p.p.m. as the DTPA complex.

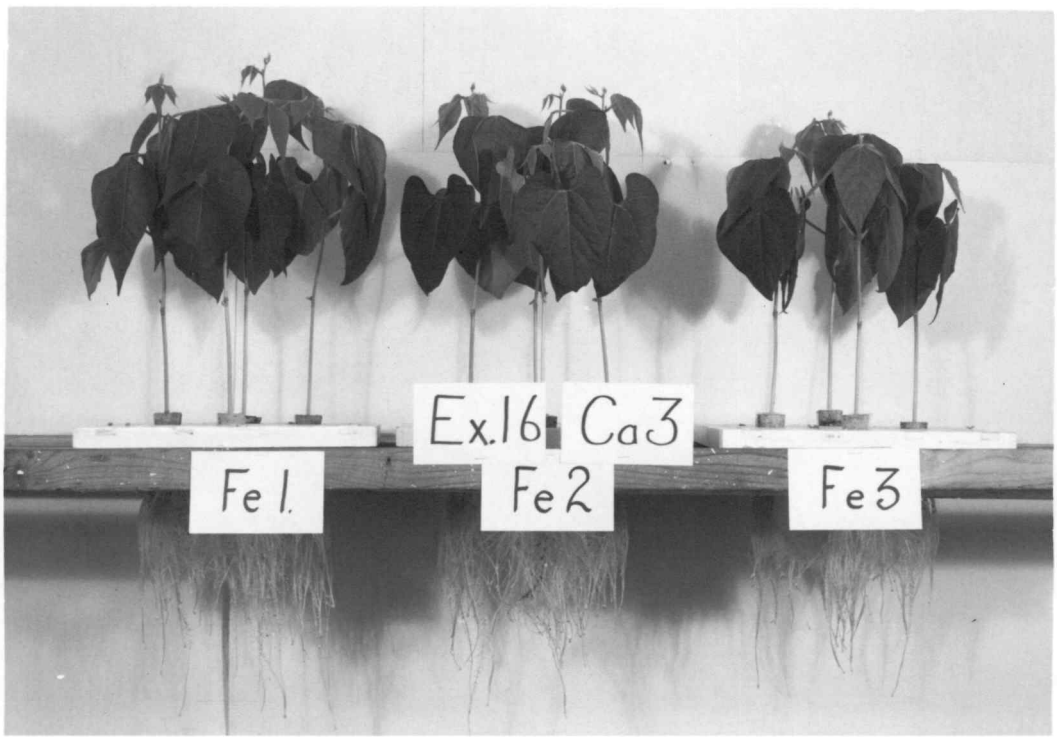


Fig. 30. Experiment 16. Growth of bean plants at high calcium levels in nutrient solutions at pH 7.5 when iron was supplied at 0.2, 1 and 10 p.p.m. as the DTPA complex.

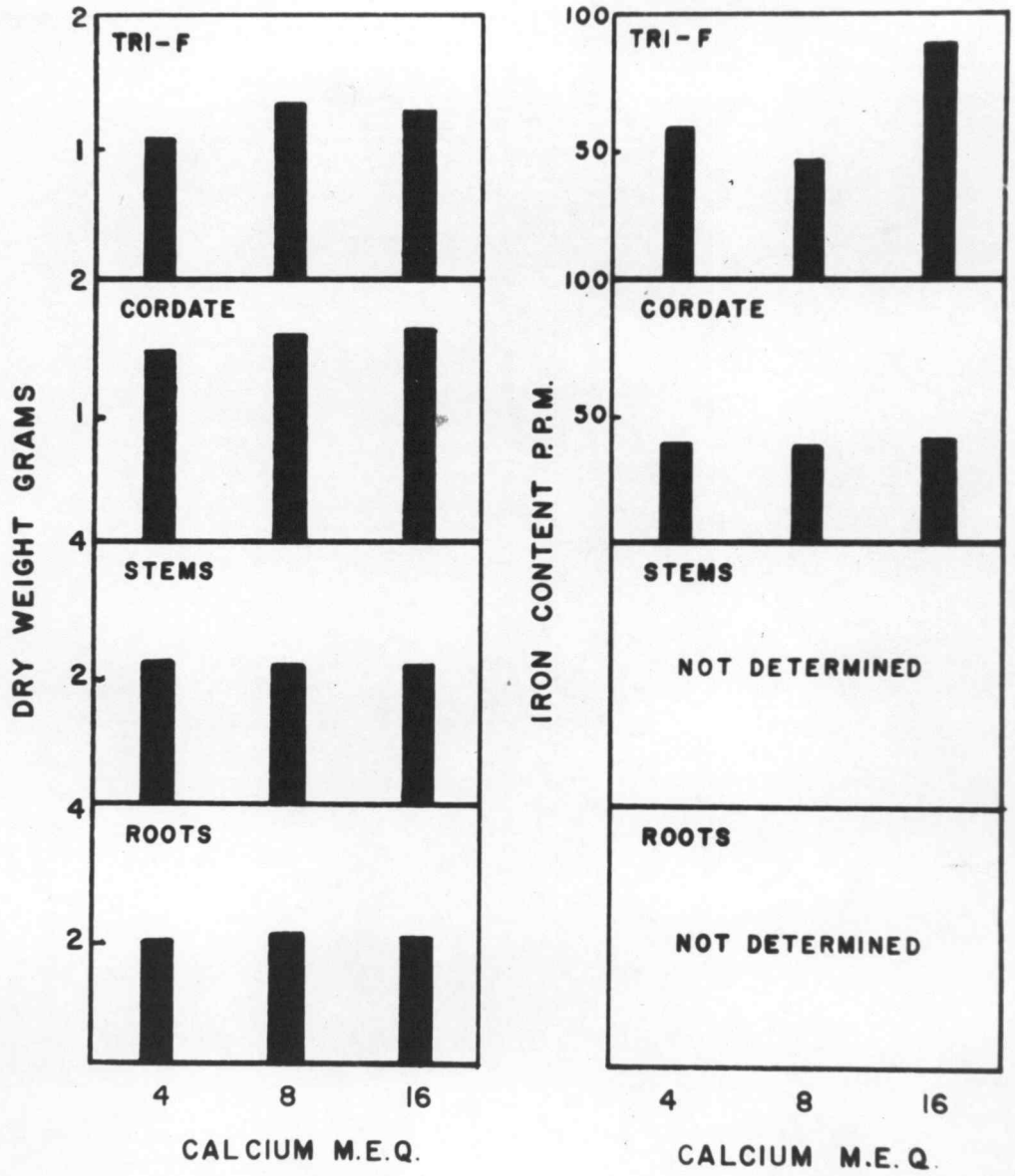


Fig. 31. Experiment 16. Effect of calcium level of the nutrient solution on the dry weight and iron content of bean plants grown at pH 7.5 at varying levels of iron supplied as the DTPA complex.

Table 24. Experiment 16. Effect of varying calcium concentration of the nutrient solution on dry weights of bean plants grown with 1 p.p.m. of iron supplied as the DTPA complex.

Plant part	Calcium concentration of cultures milli-equivalents 4	8	16	L.S.D. .05
	grams			
Tri-foliate leaves	1.05	1.32	1.24	N.S.
Cordate leaves	1.44	1.55	1.62	0.11
Stems	2.13	2.12	2.16	N.S.
Roots	1.89	1.95	1.94	N.S.
Whole plant	6.50	6.94	6.96	N.S.

Table 25. Experiment 16. Effect of varying levels of calcium in the nutrient solution on iron contents of bean plants grown with 1 p.p.m. of iron supplied as the DTPA complex.

Plant part	Calcium concentration of cultures milli-equivalents 4	8	16	L.S.D. .05
	p.p.m.			
Tri-foliate leaves	57	45	89	N.S.
Cordate leaves	37	36	39	N.S.

H. Interaction of Iron Supplied as DTPA and Calcium in Nutrient Solutions at pH 7.5.

Experiment 16

The design of this experiment has been described under section B, experiment 16. One feature of the design of the experiment was

the interaction between iron and calcium. The data for the treatment combinations was presented in Table 26 for dry weights and Table 27 for iron content of tissues.

The interaction effect was separated from all others by the analysis of variance method. The analyses of variance for both yield and iron content showed all interactions between calcium and iron to be non-significant.

Table 26. Experiment 16. Mean dry weights of tri-foliate leaves, cordate leaves, stems and roots of bean plants grown in nutrient solutions at three levels of calcium and three levels of iron supplied as the DTPA complex.

Plant part	Calcium Concentration	p.p.m. iron as DTPA iron complex 0.5	2	10
	milliequivalents		grams	
Tri-foliate leaves	4	1.26	1.16	0.72
	8	1.30	1.45	1.19
	16	1.34	1.50	0.86
Cordate leaves	4	1.30	1.55	1.46
	8	1.52	1.46	1.66
	16	1.62	1.66	1.57
Stems	4	2.09	2.25	2.04
	8	1.99	2.19	2.17
	16	2.16	2.32	1.99
Roots	4	1.87	2.03	1.74
	8	2.08	1.81	1.95
	16	1.99	2.09	1.74
Whole plant	4	13.06	14.01	11.93
	8	13.80	13.86	13.97
	16	14.24	15.16	12.34

Table 27. Experiment 16. Mean iron contents of tissues of bean plants grown in nutrient solutions at three levels of calcium and three levels of iron supplied as the DTPA complex.

Plant part	Calcium Concentration	p.p.m. iron as DTPA complex 0.5	2	10
	milliequivalents		p.p.m.	
Tri-foliate leaves	4	85	131	128
	8	83	80	109
	16	99	134	299
Cordate leaves	4	74	73	74
	8	67	81	67
	16	53	102	81

DISCUSSION

The procedures developed during the course of this study have proved to be very satisfactory for the investigation of plant mineral nutrition at high pH. The procedure used at the beginning was far from satisfactory because the nutrient solution used had such a low buffer capacity that large fluctuations in the pH occurred. The use of trishydroxymethylaminomethane as a buffer in the nutrient solutions reduced the fluctuations in pH very markedly. There was no evidence of any toxic effect, or any harmful effect on plant growth or any effect on the iron nutrition. This is believed to be the first use of this compound or an organic compound in mineral nutrient solutions solely for buffering. Close control of the pH of mineral nutrient solutions, as is required when pH sensitive compounds such as the chelating agents are utilized, is made quite practical.

Contamination of leaves by dust containing iron was shown by Mason (41, pp. 104-107) to be a serious disturbing factor in iron analysis of plants grown outdoors. In the experiments reported here, plants were grown indoors and although cool outside air was drawn into the chamber for control of temperature, it was believed that there would be little dust contamination. This contention is borne out by the levels of iron found in the leaves in experiment 9 in which all cultures proved to be iron deficient. The iron contents were 23, 31, 24, 25 and 36 p.p.m. The relative uni-

formity and the low levels of these analyses support the contention that dust contamination was low.

There was a large amount of residual variation in almost all the iron analyses, more than would be expected in such experimental material for other elements. It might be suspected that contamination of some samples did occur, but it is difficult to see how the contamination could be caused by dust, since samples from the same treatment in the same experiment frequently varied very widely in iron content. Furthermore, while leaves might be expected to be contaminated by dust, it is difficult to explain the large variation in iron content of stems on this basis, since stems present such a small surface area in relation to their weight. In the root analyses the iron present on the outside of the root in the form of precipitated iron or dried residual nutrient solution is necessarily included in the analysis. Large variation was to be expected here, since the amount of precipitate remaining on the roots on removal from the nutrient solution varied widely.

As far as can be determined from the literature, these experiments represent the first really successful attempt at growing plants at closely controlled pH levels above pH 7. Nutrient solution cultures in particular have been unsuccessful in the past because iron nutrition has presented a problem for which no adequate solution existed. Since so little was known, it was necessary to determine some rather simple points before proceeding to the more complex ones. One such point was the optimum level of iron when it

was supplied as the two chelate complexes HEEDTA and DTPA.

The optimum level of iron as HEEDTA in nutrient solutions at pH 7.5 has been found by these experiments to be in the range of 0.2 to 2 p.p.m. It was shown in experiment 8 that 0.1 p.p.m. was not adequate and in experiments 4 and 15 that 5 p.p.m. retarded growth considerably. The middle of the range 0.2 to 2 is 1.1. This is very close to 1 and therefore the optimum level is placed at 1 p.p.m.

The optimum level of iron as DTPA in nutrient solutions at pH 7.5 has been shown to be in the range of 0.5 to 2 p.p.m. In experiment 9, 0.5 p.p.m. was insufficient to prevent chlorosis. However, in experiment 11 the yield from the 0.5 p.p.m. treatment was only slightly less than the highest yield. At high levels of iron in the nutrient solution, 5 p.p.m. reduced yield slightly, but 10 and 20 p.p.m. reduced yield markedly. The middle of the range 0.5 to 2 p.p.m. is 1.25 p.p.m. Again, this is very close to 1 and therefore the optimum level is set at 1 p.p.m.

While 1 p.p.m. iron supplied as a chelate complex prevented chlorosis and resulted in normal growth at pH 7.5 in these experiments, it is worthy of note that 1 p.p.m. iron without chelate would probably not have been sufficient for normal growth and prevention of chlorosis beyond pH 5.0 or 5.5.

The range of optimum concentrations of iron supplied as the HEEDTA and DTPA complexes in nutrient solution was not wide.

Deficiency commenced at about 0.1 p.p.m. and toxicity at about 5 p.p.m. This seems to be a somewhat more limited range than has been found in the use of chelates on soil where, although the optimum application of iron as EDTA is about 20 grams per tree for citrus, toxicity is not observed until levels of application of 200 grams or more are reached. Of course, in a tree, the toxic level of iron in the tissues would be reached more slowly because of the size of the organism. During this time the chelating compound would be disappearing from the soil by leaching, actions of microorganisms, and the like. These experiments are not directly comparable to field conditions. However, these experiments do indicate a need for really critical work under field conditions before the possibility of toxicity can be discounted.

In tri-foliate leaves, when the HEEDTA iron complex was the source of iron, toxicity occurred when the iron content reached 60 to 95 p.p.m. and the optimum range was 35 to 80 p.p.m. When the DTPA iron complex was the source of iron, deficiency occurred at 30 p.p.m. and toxicity at 85 to 130 p.p.m. The optimum range was 40 to 85 p.p.m. There is general agreement between the deficient, optimum and toxic levels for the two sources of iron. Overlapping of ranges is evident, but this is a common occurrence in all nutrients in plant tissues. In general, deficiency occurred below 40 p.p.m. and toxicity above 80 p.p.m. the optimum range being from 40 to 80 p.p.m.

In cordate leaves, when HEEDTA was the source of iron, toxicity occurred at 90 p.p.m. and the optimum range was 40 to 90 p.p.m. When DTPA was the source of iron, deficiency occurred at 27 to 51 p.p.m., toxicity at 35 to 85 p.p.m. and the optimum range was 20 to 50 p.p.m. In general, deficiency occurred below 30 p.p.m., toxicity above 65 p.p.m. and the optimum range was 30 to 65 p.p.m. This range appears to be slightly lower than that for tri-foliate leaves.

In stems, when HEEDTA was the source of iron, levels of iron in deficient plants were from 85 to 350 p.p.m., in plants suffering from toxicity 55 to 105 p.p.m. and in plants having optimum iron contents 50 to 220 p.p.m. When DTPA was the source of iron, levels of iron in deficient plants were 100 p.p.m,, in plants suffering from toxicity 35 to 85 p.p.m., and in normal plants 20 to 50 p.p.m. There appears to be an inverse correlation between the adequacy of iron nutrition as judged by yield, and the iron content of stems.

In roots, when HEEDTA was the source of iron, levels of iron in deficient plants were 250 to 4500 p.p.m., in plants suffering from toxicity 650 to 5500 p.p.m., and in normal plants 300 to 2000 p.p.m. When DTPA was the source, there were 2500 to 7000 p.p.m. in the deficient plants, 1000 to 2000 p.p.m. in the toxic plants and 300 to 500 p.p.m. in the normal ones. There is no correlation between iron content of the roots and iron nutrition of the plants. This is not surprising since the roots were in many cases coated with heavy precipitates which were probably largely ferric phos-

phate, and the amount of precipitate present on the root and also the amount retained on the root on removal from the nutrient solution varied widely. The iron analyses of the roots were extremely variable, and as a result the statistical analyses seldom showed significant differences.

The effect of pH of the nutrient solution on the yield of plants was quite large. Optimum yield was obtained at pH 7.5. Reduced yield occurred at lower and higher pH's. These results are somewhat at variance with the results of Arnon and Johnson (4, p. 525) who found that the best growth of plants occurred between pH 5.0 and 7.0 for tomato, lettuce and Bermuda grass. However, the results agree closely on the alkaline side since they found considerably reduced growth at pH 8. The depressed growth at pH 7.0 might have been caused by toxicity of iron as is evidenced by the fact that the tri-foliate leaves contained 170 p.p.m. A lower level of iron complex at this pH might have produced higher yields. The depressed growth at pH's above 8.0 might possibly have been prevented if higher levels of iron had been supplied. It is dangerous to predict this, since the leaves contained adequate iron for normal growth.

The most remarkable effect of pH, however, was on the iron content of the plant. The iron content of tri-foliate leaves dropped from 169 p.p.m. at pH 7.0 to 108 p.p.m. at pH 7.5 and 72 p.p.m. at pH 8.0. There was no further drop at pH 8.5, 9.0 and

9.5.

This is a very large drop indeed, less than one-half the content of iron being taken up by the tri-foliate leaves when pH is increased by only 1.0 pH unit. There was a smaller, though similar, effect on the cordate leaves. This would be expected since these leaves were half-formed before the differential treatments were applied. The iron content of the stems was affected very little by the treatments, but the usual large variation was present and may have obscured the effect. In the roots, there was a minimum iron content at pH 7.5 to 8.0, with higher contents at pH 7.0 and at pH 8.5 to 9.5. The trend in levels in the roots was in inverse relation to that in the tri-foliate leaves.

The level of phosphorus in the nutrient solution affected yield and iron content of the tissues very strongly. When iron was supplied as the HEEDTA iron complex, yield was markedly reduced at the high phosphorus level, even though the iron content of the tri-foliate leaves at all three levels of phosphorus was the same at about 70 p.p.m. and therefore presumably adequate for growth. The iron content of the stems was reduced and that of the roots increased as the phosphorus level increased. These results appear to be in accord with Biddulph's theory that at high phosphorus levels, iron is precipitated at the xylem extremities in the leaf as an inorganic salt and does not reach the leaf parenchyma cells. However, when iron was supplied as the DTPA iron complex, the results

were different. At low and medium phosphorus levels, iron content of the tri-foliate leaves was very high, about 140 p.p.m., but at the high phosphorus supply it dropped to 50 p.p.m. However, in the stems and roots, the iron content was high at the low phosphorus level, but low at the medium and high phosphorus levels. Yield was low at all phosphorus levels, but lowest at the high phosphorus level. These results are hard to interpret. The uptake of iron is much higher than would have been expected at a 1 p.p.m. level of supply and the yield much lower. However, careful checking has failed to reveal any errors in the level of iron supply.

It is of interest to compare this work with similar work done by Biddulph (6, p. 268) using non-chelated iron as the iron source. He found that at medium phosphorus level (0.0001 molar) at pH 4, radioactive iron entered the plant rapidly and was uniformly distributed. At the same phosphorus level, at pH 7, there was rapid entry of iron only as far as the vein system, but little or no distribution of iron in the mesophyll. At high phosphorus level (0.001 molar) at pH 7, the iron actually failed to enter the xylem due to precipitation at the root surfaces. In contrast, in these studies, when iron was supplied as chelated iron, the medium phosphorus and pH 7.5 treatment produced optimum growth. Indeed, the medium phosphorus was 0.0006 molar in comparison to Biddulph's medium phosphorus of 0.0001 molar and the pH was half a unit higher.

It seems clear that chelated iron is much more effective than

non-chelated iron in preventing iron deficiency in the leaf parenchyma due to prior precipitation in the veins in the root.

The iron level required for optimum yield was not the same at all levels of phosphorus. At the low and medium phosphorus levels, there was little difference between the yields at 0.2, 0.5, 1, and 2 p.p.m. However, at the high phosphorus level, the best yield was produced at 1 and 2 p.p.m. iron but this yield was only one-third of that produced at the medium and low phosphorus levels. Nevertheless, it indicates that the higher phosphorus levels required a higher iron supply.

Plants which were chlorotic because of excessive levels of phosphorus in the nutrient solution were affected in a different way from plants which were chlorotic because of a deficiency of iron. The iron-deficient plants developed severe chlorosis and necrosis of the tri-foliate leaves but the cordate leaves remained normal in colour and size. In contrast, the plants in the high phosphorus treatment developed chlorosis in both the tri-foliate and cordate leaves. This differential effect may be attributed to precipitation of the iron by the high phosphorus content of the tissues preventing the iron reaching the leaf parenchyma from exhibiting maximum availability.

The chlorosis in the two cases had a different appearance. Chlorosis due to iron-deficiency was characterized by a pale, clear yellow to white colouring of the leaves, whereas chlorosis

due to high phosphorus was characterized by a muddy yellow-green colour. The pale yellow chlorosis might, perhaps, be attributed to almost complete lack of formation of chlorophyll, and the muddy yellow chlorosis to bleaching of the chlorophyll present and insufficient replacement.

The effect of calcium concentration of the nutrient solution on yield and iron content of tri-foliate and cordate leaves, stems and roots was very small. The sizeable increase in iron content of tri-foliate leaves at the high calcium level can be attributed to the large variation since no significance was attained in the statistical analysis. This lack of an effect by calcium was not anticipated in view of the effects of calcium on iron nutrition that have been reported in the literature. No explanation of this finding seems to have sufficient merit to be incorporated here.

SUMMARY

1. A method for growing bean plants in mineral nutrient culture under uniform environment at alkaline pH values has been developed in order to study the effects of various synthetic chelating substances on the availability of iron under these conditions.

2. An organic buffering compound was found to give adequate control of pH without giving any symptoms of toxicity or participation in the iron nutrition of the plant.

3. At pH 7.5 it was found that about 1 p.p.m. of iron complexed with HEEDTA and DPTA gave optimum growth of bean plants. The levels of the two were found to be similar but not identical.

4. The effect of iron complex was different in the cordate as contrasted to the tri-foliate leaves, and the effects in the tri-foliate leaves were found to be more significant. Tissue levels of iron varied somewhat but were optimum about 40 to 80 p.p.m.

5. Iron applied as a complex by vacuum infiltration or by foliar surface application was toxic at fairly low levels even though the total amount of iron applied was far less than that absorbed through the roots.

6. At low and medium levels of phosphate in the nutrient solution at pH 7.5 there was little interference in iron nutrition but at high levels the interference was marked. Cordate leaves were affected the same as tri-foliate leaves.

7. When 1 p.p.m. iron was supplied as the chelate, optimum growth was found at pH 9.5 with a small decline at pH 7.0 and a marked decline at pH 8.0. The decline at pH 7.0 could have been due to iron toxicity, but at the higher pH values adequate iron seemed to be present in the leaves.

8. The interaction of iron and phosphorus was small and probably not significant. No interaction was found for iron and calcium.

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APPENDIX

APPENDIX 1. INDEX OF CHELATE COMPOUNDS

Table 28. Chemical formulae, letter designations, trade names, and manufacturers of chelate compounds.

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1. Chemical description: ethylenediaminetetraacetic acid

   Letter designation: EDTA

   Trade name: Versene — Manufacturer: Versenes Inc., Framingham Massachusetts.

   Sequestrene — Manufacturer: Alrose Chemical Co. Division of Geigy Chemical Co.

   Nullapon — Manufacturer: Antara Chemicals, New York

   Tetrine — Manufacturer: Glyco Products Co.Brooklyn New York.

   Permakleer — Manufacturer: Refined Products, Lyndhurst, New Jersey.

2. Chemical description: N-hydroxyethylethylenediaminetriacetic acid

   Letter designation: HEEDTA, EDTA-OH, NaFeEEDTA

   Trade Name: Versen-ol — Manufacturer: Versenes Inc., Framingham, Massachusetts.

   Data: A solution containing 2.3 per cent iron.

3. Chemical description: diethylethylenetriaminepentaacetic acid

   Letter designation: DTPA

   Trade name: Chel 330 — Manufacturer: Geigy Chemical Corporation, 89 Barclay St., New York

   Data: Dry material 10.5 per cent iron.

4. Chemical description: N, N'-dihydroxyethlyethlyenediaminediacetic acid

   Letter designation: HEEDTA

   Trade name: Versene-diol — Manufacturer: Bersworth Chemical Co., Framingham, Massachusetts.

Table 28 Con't. Chemical formulae, letter designations, trade names, and manufacturers of chelate compounds.

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5. Chemical description: An aromatic polyaminocarboxylic acid

   Letter designation: APCA

   Trade name: Chel 138 Manufacturer: Geigy Chemical Co.

APPENDIX 2. RECORDS OF pH OF NUTRIENT SOLUTIONS OF ALL EXPERIMENTS

Table 29. Experiment 4. Record of pH of nutrient solution.

Date	Time		Crock no. 1	2	3	4	5	6	7	8	9
March											
29	3.00 p.m.	pH	7.0	7.1	7.0	7.2	7.1	7.2	7.2	6.9	7.0
	"	Added*	15	15	15	15	15	0	15	15	15
	6.00 p.m.	pH	0	0	0	0	0	9.2	8.7	8.1	8.2
30	9.30 a.m.	pH	7.1	7.1	7.1	7.2	7.1	7.2	7.2	7.0	7.1
	"	Added	15	15	15	15	15	15	15	15	15
	4.30 p.m.	pH	7.6	7.5	7.4	7.7	7.7	7.1	7.4	7.4	7.7
	"	Added	10	10	10	10	10	10	10	10	10
31	9.30 a.m.	pH	7.0	7.0	7.1	7.2	7.3	7.3	7.3	7.3	7.2
	"	Added	15	15	15	15	15	15	15	15	15
	11.30 a.m.	pH	7.1	8.0	7.9	8.2	8.5	8.2	8.5	8.1	8.5
	"	Added	15	5	5	5	0	5	0	5	0
	4.15 p.m.	pH	7.1	7.6	7.7	7.9	7.8	7.7	7.7	7.8	7.8
	"	Added	25	10	10	10	10	10	10	10	10
April											
1	9.00 a.m.	pH	7.1	7.3	7.4	7.6	7.5	7.4	7.5	7.3	7.4
	"	Added	15	15	15	15	15	15	15	15	15
	4.00 p.m.	pH	7.1	7.6	7.7	7.9	7.8	7.6	7.7	7.6	8.0
	"	Added	15	15	15	15	15	15	15	15	15
2	9.00 a.m.	Added	15	15	15	15	15	15	15	15	15
	9.00 p.m.	pH	7.6	7.5	7.5	7.7	7.6	7.5	7.4	7.3	7.7
	"	Added	15	15	15	15	15	15	15	15	15

Table 29 Con't. Experiment 4. Record of pH of nutrient solutions.

Date	Time		Crock no. 1	2	3	4	5	6	7	8	9
April											
3	10.00 a.m.	pH	7.6	7.6	7.6	7.9	7.7	7.3	7.6	7.6	7.8
	"	Added	15	15	15	15	15	15	15	15	15
4	10.00 a.m.	pH	7.6	7.6	7.5	7.8	7.5	7.6	7.6	7.3	7.7
	"	Added	15	15	15	15	15	15	15	15	15
	4.45 p.m.	Added	15	15	15	15	15	15	15	15	15
5	9.00 a.m.	pH	7.4	7.7	7.5	7.8	7.7	7.7	7.7	7.6	7.7
	"	Added	15	15	15	15	15	15	15	15	15
	4.45 p.m.	Added	15	15	15	15	15	15	15	15	15
6	9.00 a.m.	pH	7.9	7.6	7.6	7.9	7.8	8.7	7.9	7.5	8.5
	"	Added	10	10	10	10	10	0	10	15	0
	4.00 p.m.	pH	8.1	7.7	7.6	7.9	7.7	7.7	7.7	7.6	7.8
		Added	10	15	15	15	15	15	15	15	15
7	1.00 p.m.	Added	15	15	15	15	15	15	15	15	15
	4.00 p.m.	Added	15	15	15	15	15	15	15	15	15
8	9.00 a.m.	pH	8.4	7.6	7.5	8.2	7.8	8.2	7.9	7.5	8.0
	"	Added	5	15	15	5	10	5	10	15	5
9	9.00 a.m.	pH	7.8	7.6	7.4	7.8	7.9	7.9	8.2	7.5	7.8
	"	Added	15	15	15	15	15	15	10	15	15

* Indicates ml. 0.5% sodium hydroxide added.

Table 30. Experiment 8. Record of pH of nutrient solutions. Objective, pH 8.5.

Date	Time		Crock no. 1	2	3	4	5	6	7	8	9	10
May 6	9.00 a.m.	pH	8.2	8.5	8.4	8.3	8.2	8.3	8.3	8.2	8.1	8.4
		Added*	5	2	3	4	5	4	4	5	6	3
	11.30 a.m.	pH	8.1	8.3	8.2	8.0	7.9	8.0	8.1	8.0	7.9	8.1
		Added	20	20	20	20	25	20	20	20	25	20
7	9.00 a.m.	pH	8.4	8.5	8.5	8.5	8.3	8.4	8.4	8.4	8.5	8.5
		Added	10	10	10	10	10	10	10	10	10	10
8	Cracked hydrogen electrode not relaced until May 11.											
11	2.00 p.m.	pH	8.2	8.2	8.3	8.2	8.1	8.1	8.2	8.2	8.1	8.2
		Added	20	20	20	20	20	20	20	20	20	20
12	2.00 p.m.	pH	8.3	8.4	8.4	8.2	8.2	8.2	8.3	8.2	8.1	8.3
		Added	25	25	25	30	30	30	25	30	30	25
13	11.00 a.m.	pH	8.5	8.5	8.5	8.3	8.3	8.4	8.3	8.4	8.3	8.5
		Added	15	15	15	35	35	25	35	25	35	15
14	4.00 p.m.	pH	8.3	8.4	8.3	8.2	8.2	8.4	8.3	8.3	8.3	8.3
		Added	35	35	35	50	50	35	35	35	35	35
15	10.00 a.m.	pH	8.7	8.7	8.6	8.7	8.7	8.6	8.6	8.5	8.7	8.6
		Added	0	0	0	0	0	0	0	0	0	0

Table 30 Con't. Experiment 8. Record of pH of nutrient solutions. Objective, pH 8.5

Date	Time		Crock no. 1	2	3	4	5	6	7	8	9	10
May 17	2.00 p.m.	pH	8.0	8.0	7.8	7.8	7.9	8.1	8.1	8.4	8.2	8.4
		Added	50	50	60	60	60	50	50	35	50	35
18	Air compressor motor burned out, but was replaced.											
		pH	8.8	8.5	8.5	8.4	8.8	8.7	8.6	8.5	8.7	8.6
		Added	10	25	25	25	10	10	20	25	15	20
19	2.00 p.m.	pH	8.6	8.6	8.5	8.4	8.7	8.6	8.5	8.5	8.6	8.6
		Added	20	20	25	35	20	20	25	25	20	20
20		Added	20	20	20	20	20	20	20	20	20	20
	Second air compressor motor burned out. Aeration ceased.											

* ml. 0.5% sodium hydroxide.

Table 31. Experiment 9. Record of pH of nutrient solutions. Objective pH 7.5.

Date	Time		Crock no. 1	2	3	4	5	6	7	8	9	10
September												
29	8.45 a.m.	pH	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
30	2.30 p.m.	pH	7.5	7.5	7.4	7.5	7.5	7.5	7.4	7.5	7.5	7.5
		Added*	0	0	5	0	0	0	5	0	0	0
October												
1	3.00 p.m.	pH	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
3	4.30 p.m.	pH	7.5	7.4	7.5	7.5	7.5	7.5	7.4	7.5	7.4	7.5
		Added	0	5	0	0	0	0	5	0	5	0
4	10.00 a.m.	pH	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
5	1.30 p.m.	pH	7.4	7.4	7.5	7.5	7.4	7.5	7.4	7.4	7.4	7.5
		Added	5	5	0	0	5	0	5	5	5	0
6	2.00 p.m.	7.5	7.5	7.4	7.4	7.4	7.4	7.5	7.4	7.5	7.4	7.5
		Added	0	10	10	10	10	0	10	0	10	0
7	4.30 p.m.	pH	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
9	6.30 p.m.	pH	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
11	3.00 p.m.	pH	7.4	7.5	7.5	7.4	7.5	7.4	7.5	7.5	7.5	7.5
		Added	10	0	0	10	0	10	0	0	0	0

Table 31 Con't. Experiment 9. Record of pH of nutrient solutions. Objective pH 7.5.

Date	Time		Crock no. 1	2	3	4	5	6	7	8	9	10
October												
12	2.00 p.m.	pH	7.5	7.5	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4
		Added	0	0	10	10	10	10	10	10	10	10
13	2.30 p.m.	pH	7.4	7.5	7.5	7.4	7.5	7.5	7.4	7.5	7.4.	7.4
		Added	10	0	0	10	0	0	10	0	10	10
14	3.30 p.m.	pH	7.5	7.5	7.5	7.4	7.5	7.5	7.5	7.5	7.5	7.5
		Added	0	0	0	15	0	0	0	0	0	0
15	5.00 p.m.	pH	7.5	7.5	7.5	7.4	7.5	7.5	7.5	7.5	7.6	7.5
		Added	0	0	0	15	0	0	0	0	0	0

* mls. 0.5% sodium hydroxide.

Table 32. Experiment 11. Record of pH of nutrient solutions. Objective pH 7.5.

Date	Time		Crock no. 1	2	3	4	5	6	7	8	9
December											
2	3.30 p.m.	pH	7.5	7.4	7.4	7.4	7.5	7.4	7.5	7.5	7.4
		Added*	0	10	15	15	0	15	0	0	15
3	1.30 p.m.	pH	7.5	7.6	7.6	7.6	7.5	7.6	7.5	7.4	7.6
		Added	2	0	0	0	0	0	0	2	0
6	3.30 p.m.	pH	7.4	7.5	7.6	7.6	7.5	7.5	7.5	7.4	7.5
		Added	5	0	0	0	0	0	0	5	0
7	2.00 p.m.	pH	7.5	7.5	7.6	7.6	7.5	7.5	7.5	7.5	7.5
		Added	2	2	0	0	0	0	0	2	2
9	3.00 p.m.	pH	7.5	7.5	7.6	7.6	7.5	7.5	7.5	7.5	7.4
		Added	0	0	0	0	0	0	0	0	5
12	10.00 a.m.	pH	7.5	7.4	7.6	7.6	7.6	7.5	7.6	7.5	7.4
		Added	0	10	0	0	0	0	0	0	10
14	2.00 p.m.	pH	7.5	7.5	7.5	7.6	7.5	7.5	7.5	7.5	7.4
		Added	0	0	0	0	0	0	0	0	15

Table 32 Con't. Experiment 11. Record of pH of nutrient solutions. Objective pH 7.5.

Date	Time		Crock no. 10	11	12	13	14	15	16	17	18
December											
2	3.30 p.m.	pH	7.5	7.5	7.4	7.4	7.4	7.4	7.4	7.5	7.4
		Added	0	0	10	10	15	10	10	0	15
5	1.30 p.m.	pH	7.5	7.5	7.6	7.6	7.6	7.5	7.5	7.5	7.6
		Added	0	0	0	0	0	0	0	0	0
6	3.45 p.m.	pH	7.4	7.4	7.5	7.5	7.5	7.5	7.5	7.5	7.5
		Added	5	5	0	0	0	0	0	0	0
7	2.00 p.m.	pH	7.4	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
		Added	5	0	0	0	0	0	0	0	0
9	3.00 p.m.	pH	7.4	7.5	7.5	7.6	7.5	7.5	7.5	7.6	7.5
		Added	5	0	0	0	0	0	0	0	0
12	10.00 a.m.	pH	7.5	7.5	7.5	7.6	7.6	7.6	7.5	7.6	7.5
		Added	0	0	0	0	0	0	0	0	0
14	2.00 p.m.	pH	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.6	7.5
		Added	0	0	0	0	0	0	0	0	0

* ml. 0.5% sodium hydroxide.

Table 33. Experiment 12. Record of pH of nutrient solutions

Date	Time		Crock no. 1	2	3	4	5	6	7	8	9
December											
26	3.30 p.m.	pH	7.2	7.2	7.4	7.3	7.4	7.4	7.4	7.4	7.2
		Added*	3	3	1	1	1	1	1	1	3
28	11.00 a.m.	pH	7.5	7.5	7.6	7.5	7.6	7.6	7.7	7.5	7.4
		Added	0	0	0	0	0	0	0	0	15
30	1.45 p.m.	pH	7.4	7.4	7.6	7.4	7.6	7.4	7.6	7.4	7.3
		Added	10	10	0	25	0	10	0	10	25
January											
1	3.00 p.m.	pH	7.4	7.4	7.6	7.2	7.6	7.4	7.7	7.2	7.4
		Added	15	15	0	35	0	15	0	35	25
3	3.00 p.m.	pH	7.5	7.5	7.6	7.5	7.6	7.6	7.7	7.7	7.4
		Added	0	0	0	0	0	0	0	0	25
5	11.30 a.m.	pH	7.5	7.5	7.6	7.3	7.6	7.5	7.7	7.6	7.4
		Added	0	0	0	25	0	0	0	0	35
7	3.30	pH	7.5	7.5	7.5	7.3	7.5	7.5	7.6	7.2	7.4
		Added	0	0	0	35	0	0	0	25	35
10			Harvested								

Table 33 Con't. Experiment 12. Record of pH of nutrient solutions.

Date	Time		Crock no. 10	11	12	13	14	15	16	17	18
December											
26	3.00 p.m.	pH	7.1	7.3	7.4	7.1	7.1	7.3	7.4	7.4	7.5
		Added	4	1	1	4	4	1	1	1	0
27	11.00 a.m.	pH	7.3	7.5	7.7	7.4	7.5	7.6	7.6	7.6	7.5
		Added	25	0	0	10	0	0	0	0	0
30	1.45 p.m.	pH	7.3	7.4	7.6	7.4	7.3	7.5	7.5	7.6	7.5
		Added	25	10	0	10	25	0	0	0	0
January											
1	3.00 p.m.	pH	7.3	7.5	7.6	7.4	7.4	7.3	7.2	7.6	7.5
		Added	35	0	0	15	15	25	35	0	0
3	3.00 p.m.	pH	7.5	7.4	7.7	7.5	7.5	7.7	7.7	7.6	7.5
		Added	0	10	0	0	0	0	0	0	0
5	11.30 a.m.	pH	7.5	7.2	7.6	7.5	7.5	7.6	7.7	7.7	7.5
		Added	0	35	0	0	0	0	0	0	0
7	3.30 p.m.	pH	7.5	7.4	7.6	7.4	7.5	7.5	7.8	7.6	7.5
		Added	0	25	0	15	0	0	1	0	0

* Millilitres of 0.5% sodium hydroxide.

Table 34. Experiment 13. Record of pH of the nutrient solutions

Date	Time		Crock no. 1	2	3	4	5	6	7	8	9
Required pH			7.5	9.5	7.0	8.0	9.0	8.5	8.0	7.5	8.5
January											
16	—	pH	7.6	8.3	7.1	7.7	8.0	7.8	7.7	7.6	7.8
		Added*	0	50	0	50	50	50	50	0	50
		pH	-	9.2	-	8.9	9.0	9.0	8.9	-	8.9
		Added	0	15	0	0	0	0	0	0	0
17	9.30 a.m.	pH	7.7	8.9	7.2	8.0	8.4	8.3	8.2	7.7	8.2
		Added	0	35	0	0	35	15	0	0	15
18	9.20 a.m.	pH	7.5	8.9	7.1	8.0	8.4	8.1	8.0	7.7	8.1
		Added	0	35	0	0	50	35	0	0	35
	1.30 p.m.	pH	0	9.0	-	-	9.1	8.8	-	-	8.8
		Added		50							
19	3.30 p.m.	pH	7.6	9.0	7.1	7.9	8.4	8.2	8.0	7.6	8.2
		Added	0	35	0	15	35	25	0	0	25
20	11.50 a.m.	pH	7.5	8.8	6.9	8.0	8.5	8.2	7.9	7.6	8.2
		Added	0	60	15	0	60	50	15	0	50
	3.30 p.m.	pH	-	9.2	7.3	-	9.2	8.9	8.2	-	8.9
		Added	0	35	0.5	0	6	0.5	0	0	0.5
		pH	-	-	7.0	-	-	8.7	-	-	8.7

Table 34 Con't. Experiment 13. Record of pH of the nutrient solutions.

Date	Time		Crock no. 1	2	3	4	5	6	7	8	9
January 22	12.45 p.m.	pH	7.3	8.7	6.8	8.0	8.5	8.2	7.9	7.6	8.2
		Added	15	90	10	0	50	15	5	0	15
23	9.20 a.m.	pH	7.6	9.2	7.4	8.0	8.8	8.3	8.0	7.6	8.2
		Added	0	50	0.5	0	35	35	0	0	35
24	10.30 a.m.	pH	7.4	9.2	7.3	8.0	8.9	8.5	8.0	7.6	8.5
		Added	15	15	0.5	0	15	0	0	0	0
26	11.00 a.m.	pH	7.7	8.8	7.0	8.0	8.5	8.3	8.0	7.6	8.2
		Added	0	35	0	0	25	15	0	0	25
27	11.00 a.m.	pH	7.7	8.9	7.0	8.0	8.6	8.4	8.0	7.6	8.3
		Added	0.5	35	0	0	25	15	0	0.25	15
28	10.30 a.m.	pH	7.5	8.9	7.0	8.0	8.6	8.4	8.0	7.5	8.3
		Added	0	50	0	0	50	25	0	0	35
29	2.30 p.m.	pH	7.6	8.8	7.1	7.9	8.6	8.4	8.0	7.5	8.3
		Added	0	120	0	5	90	30	0	0	50
30	10.00 a.m.	pH	7.6	9.2	7.2	7.9	9.1	8.5	8.0	7.5	8.6
		Added	0.2	100	0.2	10	50	30	0	0	25

* Millilitres of 0.5% sodium hydroxide.
0.5 millilitres of 1 normal sulphuric acid

Table 35. Experiment 13. Record of pH of nutrient solutions

Date	Time		Crock no. 10	11	12	13	14	15	16	17	18
Required pH			7.0	9.5	9.0	7.0	9.0	8.0	7.5	8.5	9.5
January											
16	—	pH	7.4	8.0	7.9	7.4	7.9	7.7	7.6	7.8	8.0
		Added*	0	100	100	0	100	50	0	100	100
		pH	-	9.6	9.7	-	9.7	9.0	-	9.7	9.8
17	10.00 a.m.	pH	7.4	8.8	9.1	7.5	9.0	8.2	7.7	9.2	9.3
		Added	1 *	35	0	1	0	0	0	2	15
		pH	6.9	9.4	-	7.0	-	-	-	8.7	-
18	9.38 a.m.	pH	7.3	8.6	8.6	7.3	8.3	8.0	7.6	8.2	8.8
		Added	2	50	35	2	50	0	0	35	50
	1.40 p.m.	pH	6.2	9.1	9.1	6.1	9.1	-	-	8.9	9.3
		Added	50	50	0	50	0	0	0	1	35
	3.25 p.m.	pH	8.5	9.6	-	8.4	-	-	-	8.6	9.6
		Added	2	0	0	0	2	0	0	0	0
	3.45 p.m.	pH	7.6	-	-	7.3	-	-	-	-	-
19	4.00 p.m.	pH	7.0	9.1	8.6	6.9	8.4	7.9	7.5	8.2	9.1
		Added	0	35	35	15	35	15	0	35	35
20	11.00 a.m.	pH	7.0	9.0	9.0	7.3	8.5	7.9	7.5	8.5	9.1
		Added	0	70	0	0	70	15	0	0	60
	3.37 p.m.	pH	-	9.5	-	-	9.3	8.2	-	-	9.5

Table 35 Con't. Experiment 13. Record of pH of nutrient solutions

Date	Time		Crock no. 10	11	12	13	14	15	16	17	18
January 22	12.45 p.m.	pH	6.8	8.7	8.5	7.0	8.4	8.0	7.4	7.8	8.8
		Added	10	90	35	0	50	0	5	50	90
23	---	pH	7.1	9.2	8.8	7.1	8.8	8.0	7.5	8.5	9.3
		Added	0	60	35	0	35	0	0	0	35
24	11.00 a.m.	pH	7.1	9.3	9.0	7.1	8.9	8.0	7.5	8.3	9.3
		Added	0	15	0	0	15	0	0	15	15
26	11.00 a.m.	pH	7.1	8.8	8.4	7.0	8.5	8.0	7.6	8.5	8.8
		Added	0	35	35	0	35	0	0	15	35
27	11.00 a.m.	pH	7.2	8.8	8.5	7.0	8.6	8.0	7.5	8.4	8.8
		Added	0.5	35	25	0	25	0	0	15	35
28	10.30 a.m.	pH	6.6	8.8	8.5	7.0	8.7	8.0	7.6	8.4	8.9
		Added	35	50	50	0	35	0	0	15	50
29	2.30 p.m.	pH	7.4	8.7	8.6	7.1	8.6	8.0	7.6	8.3	8.8
		Added	0.2	120	90	0	90	0	0	30	120
30	10.00 a.m.	pH	7.1	9.3	9.0	7.2	9.2	8.0	7.6	8.4	9.3
		Added	0	70	50	0.2	30	0	0	30	70

* Millilitres of 0.5% sodium hydroxide
0.5 millilitres of 1 normal sulfuric acid

Table 36. Experiment 15. Record of pH of nutrient solutions

Date	Time		Crock no. 1	2	3	4	5	6	7	8	9
February											
10	1.30 p.m.	pH	7.8	7.6	7.4	7.2	7.4	7.6	7.2	7.2	7.4
		Added*	0.5	0	15	3.0'	15	0	3.0'	3.0'	15
13	9.00 a.m.	pH	7.8	7.6	7.3	7.3	7.4	7.4	7.5	7.4	7.4
		Added	1.0	0.5	35	3.0'	30	30	0	1.0'	30
15	10.00 a.m.	pH	7.6	7.4	7.6	7.6	7.8	7.8	7.5	7.5	7.6
		Added	0	10	0	0	0	0	0	0	0
17	10.00 a.m.	pH	7.6	7.5	7.6	7.6	7.6	7.6	7.5	7.5	7.4
		Added	0	0	0	0	0	0	0	0	10
19	11.00 a.m.	pH	7.6	7.5	7.4	7.6	7.6	7.3	7.5	7.5	7.3
		Added	0	0	10	0	0	15	0	0	25

Table 36 Con't. Experiment 15. Record of pH of nutrient solutions

Date	Time		Crock no. 10	11	12	13	14	15	16	17	18
February											
10	1.30 p.m.	pH	7.2	7.2	7.2	7.6	7.4	7.4	7.5	7.5	7.5
		Added	3.0'	15	3.0'	0	15	1.0'	0	0	0
13	9.00 a.m.	pH	7.4	7.4	7.4	7.6	7.3	7.3	7.4	7.5	7.6
		Added	1.0'	15	1.0'	0.5	35	2.0'	30	0	0.5
15	11.00 p.m.	pH	7.6	7.6	7.5	7.5	7.7	7.6	7.7	7.6	7.5
		Added	0	0	0	0	0	0	0	0	0
17	10.00 a.m.	pH	7.5	7.5	7.5	7.6	7.7	7.6	7.4	7.5	7.5
		Added	0	0	0	0	0	0	10	0	0
19	11.00 a.m.	pH	7.5	7.5	7.5	7.6	7.6	7.6	7.4	7.5	7.5
		Added	0	0	0	0	0	0	15	0	0

* Millilitres of 0.5% sodium hydroxide
0.5 millilitres of 1 normal sulfuric acid
2.0' millilitres of 15% sodium hydroxide

Table 37. Experiment 16. Record of pH of nutrient solutions

Date	Time		Crock no. 1	2	3	4	5	6	7	8	9
April 12	8.00 p.m.	pH	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.2	7.3
		Added*	25	25	25	25	25	25	25	35	25
14	2.00 p.m.	pH	7.6	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
		Added	0	0	0	0	0	0	0	0	0
16	10.00 a.m.	pH	7.6	7.6	7.5	7.6	7.5	7.5	7.6	7.6	7.6
		Added	0	0	0	0	0	0	0	0	0
19	2.00 p.m.	pH	7.5	7.5	7.5	7.5	7.4	7.4	7.5	7.5	7.6
		Added	0	0	0	0	15	15	0	0	0
21	9.00 a.m.	pH	7.4	7.4	7.4	7.5	7.5	7.5	7.5	7.5	7.5
		Added	15	15	10	0	0	0	0	0	0

Table 37 Con't. Experiment 16. Record of pH of nutrient solutions

Date	Time		10	11	12	13	14	15	16	17	18
April											
12	8.00 p.m.	pH	7.6	7.1	7.0	7.4	7.2	7.4	7.2	7.3	7.2
		Added	0	50	60	15	35	15	35	25	35
14	2.00 p.m.	pH	7.6	7.5	7.6	7.6	7.5	7.5	7.6	7.5	7.6
		Added	0	0	0	0	0	0	0	0	0
16	10.00 a.m.	pH	7.7	7.6	7.7	7.6	7.6	7.6	7.6	7.5	7.6
		Added	0.5	0	0.5	0	0	0	0	0	0
19	—	pH	7.6	7.6	7.6	7.6	7.6	7.5	7.5	7.5	7.6
		Added	0	0	0	0	0	0	0	0	0
21	9.00 a.m.	pH	7.7	7.6	7.6	7.6	7.5	7.5	7.5	7.5	7.6
		Added	0.7	0	0	0	0	0	0	0	0

* Millilitres of 0.5% sodium hydroxide
0.5 millilitres of 1 normal sulfuric acid

APPENDIX 3. DRY WEIGHTS OF TISSUES, INDIVIDUAL READINGS

Table 38. Experiment 4. Oven-dry weights of tissues

Plant part	Treatment, p.p.m. Fe as HEEDTA iron complex		
	0	1	5
		grams	
Leaves	5.25	7.75	7.40
	4.60	10.45	7.85
	3.60	7.65	4.93
Stems and petioles	3.30	5.05	5.45
	2.75	6.80	5.35
	2.60	5.30	3.40
Roots	3.20	3.85	3.55
	2.50	4.45	4.00
	1.85	4.35	3.35
Whole plants	11.75	16.65	16.10
	9.85	21.70	17.20
	8.05	17.30	11.68

Table 39. Experiment 8. Oven-dry weights of tissues

Plant part	Treatment, p.p.m. Fe as HEEDTA iron complex		
	0	0.1	0.3
		grams	
Leaves	1.60	1.71	4.18
	1.48	2.31	5.04
	1.52	3.15	6.33
Stems and petioles	1.55	1.39	2.84
	1.52	1.37	3.46
	1.47	1.62	4.13
Roots	0.94	1.26	2.30
	0.88	1.20	2.45
	0.75	1.33	3.06
Whole plant	4.09	4.36	9.32
	3.88	4.88	10.95
	3.74	6.10	13.52

Table 40. Experiment 9. Oven-dry weight of tissues

Tissue	p.p.m. iron as DTPA iron complex				
	0	0.01	0.03	0.1	0.5
			grams		
Tri-foliate leaves	1.08	1.03	1.32	1.64	1.24
	0.89	1.17	1.28	1.65	1.01
Cordate leaves	1.48	1.22	1.14	0.86	0.95
	1.45	1.28	1.22	1.20	0.86
Stems	1.96	1.74	1.69	1.55	1.39
	1.91	1.91	1.81	1.70	1.33
Roots	1.46	1.14	1.37	1.79	2.00
	1.10	1.15	1.25	1.86	1.80
Whole plant	5.98	5.13	5.52	5.84	5.58
	5.35	5.51	5.56	6.41	5.00

Table 41. Experiment 11. Oven-dry weight of tissues.

Plant part	p.p.m. Fe as DTPA iron complex 0.5	1	2	5	10	20
	grams					
Tri-foliate leaves	2.59	2.41	2.05	1.32	0.73	0.74
	2.40	2.75	2.63	2.06	0.85	0.93
	2.00	2.62	2.64	2.49	0.80	1.25
Cordate leaves	1.64	1.76	1.60	1.76	1.49	1.83
	1.65	1.25	1.66	1.70	1.81	1.80
	1.51	1.25	1.71	1.38	1.60	1.24
Stems and petioles	1.75	1.77	1.80	1.57	1.46	1.54
	1.88	1.89	2.11	1.80	1.42	1.69
	1.67	1.90	2.05	1.86	1.49	1.50
Roots	1.50	1.50	1.36	1.55	1.58	1.70
	1.70	1.29	2.07	1.60	1.74	1.74
	1.30	1.22	1.56	1.40	1.34	1.25
Whole plant	7.48	7.44	6.81	6.20	5.26	5.81
	7.63	7.18	8.47	7.16	5.82	6.16
	6.48	6.99	7.96	7.13	5.23	5.73

Table 42. Experiment 12. Oven-dry weight of tissues

Plant part	Chelated compound					
	HEEDTA			DTPA		
	Low P	Med P	High P	Low P	Med P	High P
	grams					
Tri-foliate leaves	4.51	4.90	1.18	3.59	0.69	0.46
	4.36	6.58	1.76	0.90	2.41	0.90
	5.23	4.43	1.61	1.02	1.20	0.62
Cordate leaves	1.68	1.69	1.75	1.55	1.52	1.29
	1.64	1.85	1.29	1.56	1.30	1.74
	1.57	1.71	1.31	1.52	1.48	1.36
Stems	3.12	3.34	1.73	2.53	1.34	1.07
	3.09	4.28	1.69	1.28	1.89	1.39
	3.72	2.95	1.67	1.49	1.38	1.23
Roots	2.18	1.73	1.46	2.10	1.36	1.21
	1.85	1.76	1.31	1.62	1.60	1.51
	2.73	1.94	1.40	1.32	1.31	1.63
Whole plant	11.49	11.66	6.12	9.77	4.91	4.03
	10.94	14.47	6.05	5.36	7.20	5.54
	13.25	11.03	5.99	5.35	5.37	4.84

Table 43. Experiment 13. Oven-dry weight of tissues

Plant part	PH of nutrient solutions 7.0	7.5	8.0	8.5	9.0	9.5
	grams					
Tri-foliate leaves	2.47	2.46	2.61	1.45	1.51	1.20
	2.28	3.32	2.39	2.01	1.46	1.45
	1.95	3.15	3.00	1.70	1.45	1.34
Cordate leaves	1.24	1.19	0.91	0.96	0.95	1.00
	1.34	1.25	1.07	1.39	0.80	0.71
	0.90	1.29	1.31	1.14	1.01	1.06
Stems and petioles	1.80	1.81	1.70	1.39	1.26	1.31
	1.48	2.22	1.61	1.67	1.47	1.29
	1.49	2.20	2.09	1.41	1.60	1.44
Roots	1.35	1.02	1.18	1.01	1.06	1.46
	0.91	1.51	1.01	1.18	1.36	1.34
	0.78	1.35	1.24	1.11	1.15	1.52
Whole plant	6.86	6.48	6.40	4.81	4.78	4.97
	6.01	8.30	6.08	6.25	5.09	4.79
	5.12	7.99	7.64	5.36	5.21	5.36

Table 44. Experiment 15. Dry weights of tissues

Plant part	Block	Phosphorus concentration	p.p.m. iron as HEEDTA iron complex 0.2	0.5	1	2	5	10
		M			grams			
Tri-foliate leaves	I	0.00006	4.52	3.69	4.02	2.84	2.66	1.54
		0.0006	4.53	4.12	3.29	3.12	2.15	1.68
		0.006	0.41	0.57	1.24	1.71	0.95	1.44
	II	0.00006	5.02	4.61	4.30	4.78	2.98	0.19
		0.0006	4.68	4.52	4.86	4.55	2.35	0.73
		0.006	1.32	0.92	1.66	1.05	1.05	0.39
Cordate leaves	I	0.00006	1.56	1.38	1.15	1.40	0.90	0.83
		0.0006	1.50	1.13	1.18	1.34	1.26	0.85
		0.006	1.72	1.83	1.62	1.42	1.28	1.13
	II	0.00006	1.67	1.37	1.22	1.07	0.82	0.66
		0.0006	1.39	1.47	1.50	1.13	0.52	0.65
		0.006	1.72	1.52	1.70	1.41	0.74	0.55
Stems	I	0.00006	2.16	1.70	1.58	1.87	1.35	1.10
		0.0006	2.11	1.57	1.55	1.69	1.69	1.14
		0.006	1.50	1.76	1.47	1.52	1.52	1.35
	II	0.00006	3.24	2.90	2.87	2.99	2.21	0.59
		0.0006	3.10	3.00	3.21	2.84	1.89	0.94
		0.006	1.65	1.70	1.99	1.69	1.41	0.68
Roots	I	0.00006	2.70	2.63	2.42	2.18	1.91	1.28
		0.0006	2.55	2.34	2.12	2.29	1.89	1.41
		0.006	1.29	1.84	1.62	1.75	1.46	1.72
	II	0.00006	2.52	2.44	1.83	2.29	1.30	0.88
		0.0006	2.45	2.05	2.10	1.99	1.18	1.23
		0.006	2.01	1.83	2.25	1.62	1.06	1.04
Whole plant	I	0.00006	10.94	9.40	9.17	8.29	6.82	4.75
		0.0006	10.69	9.16	8.14	8.44	7.02	5.08
		0.006	4.92	6.00	5.95	6.40	5.05	5.64
	II	0.00006	12.45	11.32	10.22	11.13	7.31	2.32
		0.0006	11.62	11.04	11.67	10.51	5.94	3.55
		0.006	6.70	5.97	7.60	5.77	4.26	2.66

Table 45. Experiment 16. Dry weights of tissues

Plant part	Block	Calcium Concentration	p.p.m. iron as DTPA complex 0.5	2	10
		m.eq.		grams	
Tri-foliate leaves	I	4	1.38	1.42	0.74
		8	1.37	1.39	1.29
		16	1.73	1.50	1.16
	II	4	1.14	0.91	0.70
		8	1.24	1.52	1.10
		16	0.95	1.51	0.57
Cordate leaves	I	4	1.25	1.53	1.41
		8	1.55	1.34	1.50
		16	1.55	1.64	1.51
	II	4	1.36	1.58	1.51
		8	1.49	1.59	1.83
		16	1.70	1.69	1.63
Stems	I	4	2.14	2.39	1.94
		8	2.02	2.11	2.02
		16	2.36	2.37	2.08
	II	4	2.04	2.11	2.14
		8	1.96	2.28	2.33
		16	1.97	2.27	1.90
Roots	I	4	1.96	2.14	1.63
		8	2.27	1.74	1.76
		16	2.06	2.15	1.70
	II	4	1.79	1.93	1.86
		8	1.90	1.89	2.14
		16	1.92	2.03	1.79
Whole plant	I	4	6.73	7.48	5.72
		8	7.21	6.58	6.57
		16	7.70	7.66	6.45
	II	4	6.33	6.53	6.21
		8	6.59	7.28	7.40
		16	6.54	7.50	5.89

APPENDIX 4. IRON CONTENT OF TISSUES OF ALL EXPERIMENTS

Table 46. Experiment 4. Iron content of tissues.

Plant part	p.p.m. Fe as HEEDTA iron complex 0	1	5
		p.p.m.	
Leaves	26	89	58
	23	36	77
	23	34	42
Stems	112	46	44
	20	83	60
	118	102	66
Roots	145	178	481
	87	180	1094
	446	581	378

Table 47. Experiment 8. Iron content of tissues.

Plant part	p.p.m. iron as HEEDTA iron complex		
	0	0.1	0.3
		grams	
Leaves	40	—	80
	56	35	71
	40	44	87
Stems	106	123	146
	75	171	415
	270	730	114
Roots	6800	342	2240
	5700	3041	1024
	1068	2082	495

Table 48. Experiment 9. Iron content of tissues

Plant part	p.p.m. iron as DTPA iron complex				
	0	0.01	0.03	0.1	0.5
			p.p.m.		
Tri-foliate leaves	19	25	22	24	54
	28	37	26	26	19
Cordate leaves	20	35	34	43	40
	34	30	25	26	63
Stems	134	117	163	102	84
	78	106	132	115	177
Roots	5120	3925	3780	3080	6000
	9450	5009	2420	1690	8160

Table 49. Experiment 11. Iron content of tissues

Plant part	Treatment p.p.m. iron as DTPA iron complex 0.5	1	2	5	10	20
				p.p.m.		
Tri-foliate leaves	27	38	79	69	68	171
	53	31	81	156	200	114
	43	76	91	31	92	104
Cordate leaves	21	20	53	54	38	47
	18	76	67	113	38	78
	26	29	26	34	175	56
Stems	36	29	40	88	39	44
	36	35	34	41	34	136
	84	34	27	50	109	62
Roots	615	146	300	485	2027	1018
	177	1080	1046	584	365	1760
	278	431	209	2055	4064	1850

Table 50. Experiment 12. Iron content of tissues

Plant part	Chelated compound					
	HEEDTA			DTPA		
	Low P	Med P	High P	Low P	Med P	High P
	p.p.m.					
Tri-foliate leaves	43	86	54	56	121	37
	60	81	45	164	114	52
	108	48	86	149	129	68
Cordate leaves	53	42	59	71	69	40
	53	32	47	28	83	69
	36	90	40	123	80	36
Stems	83	46	40	145	28	26
	59	70	24	194	62	35
	82	58	32	41	56	48
Roots	233	250	342	136	176	343
	174	240	181	760	248	159
	172	166	260	207	222	179

Table 51. Experiment 13. Iron content of tissues

Plant part	pH of nutrient solutions					
	7.0	7.5	8.0	8.5	9.0	9.5
	p.p.m.					
Tri-foliate leaves	148	38	47	48	40	26
	162	137	66	87	156	89
	198	148	104	56	64	52
Cordate leaves	48	26	91	37	55	36
	59	35	37	26	39	69
	105	47	33	27	55	26
Stems	168	98	120	127	139	129
	233	57	157	224	210	122
	224	234	234	95	274	100
Roots	890	740	1074	2020	1041	2065
	2006	2058	570	3022	4952	3320
	2059	1049	2050	1940	1059	2842

Table 52. Experiment 15. Iron content of tissues

Plant part	Block	Phosphorus concentration	p.p.m. iron as HEEDTA iron complex 0.2	0.5	1	2	5	10
					p.p.m.			
Tri-foliate leaves	I	0.00006	33	64	56	69	55	87
		0.0006	34	45	70	162	169	74
		0.006	28	53	15	57	55	98
	II	0.00006	40	33	35	55	32	133
		0.0006	32	48	28	23	50	118
		0.006	28	27	20	43	25	56
Cordate leaves	I	0.00006	32	76	65	61	66	66
		0.0006	21	51	53	43	40	69
		0.006	41	102	112	107	110	92
	II	0.00006	71	33	149	40	112	149
		0.0006	33	36	29	44	153	169
		0.006	45	55	128	72	61	222
Stems	I	0.00006	27	322	74	80	72	180
		0.0006	141	60	40	62	143	174
		0.006	78	151	64	34	130	44
	II	0.00006	96	76	34	48	146	121
		0.0006	27	35	63	34	122	100
		0.006	35	44	35	52	33	65
Roots	I	0.00006	745	616	254	1058	2540	10220
		0.0006	408	342	265	296	1090	6100
		0.006	167	336	312	342	1030	660
	II	0.00006	450	10510	735	825	905	10338
		0.0006	210	411	400	314	707	252
		0.006	274	334	282	360	495	6840

Table 53. Experiment 16. Iron content of tissues

Plant part	Block	Calcium concentration	p.p.m. iron as DTPA complex 0.5	2	10
		m.eg.		p.p.m.	
Tri-foliate leaves	1	4	46	41	68
		8	35	39	59
		16	44	49	59
	2	4	39	90	60
		8	48	41	50
		16	55	85	240
Cordate leaves	1	4	40	41	39
		8	33	47	34
		16	23	26	41
	2	4	34	32	35
		8	34	34	33
		16	30	76	40