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The Effect of Nutrient Levels in Nutrient Cultures on the Translocation of Foliar Applied Nutrients

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THE EFFECT OF NUTRIENT LEVELS IN NUTRIENT CULTURES ON THE TRANSLOCATION OF FOLIAR APPLIED NUTRIENTS

by

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INTRODUCTION

Since man first grew crops on calcareous soils he has probably been troubled with what we today call lime-induced chlorosis. This chlorosis has determined whether he grew certain desirable ornamentals or crops or whether he had to substitute others which were less desirable.

Lime-induced chlorosis is spread world wide. It has been reported in the vine and fruit growing regions of Europe, in the chernozem soils of Russia, and many other areas where the rainfall is relatively low and the soil is relatively high in calcium carbonate. In the United States it most frequently occurrs where the average annual rainfall is less than 30 inches. The conditions causing chlorosis are not stable, for it varies from year to year and even from week to week with changing conditions in climate and soil.

An estimated 500,000 acres of crops grown in the western United States on calcareous soils are subject to moderate to severe chlorosis. This physiological malady has challenged the technical ingenuity of outstanding plant and soil scientists. The exact cause or causes have never been isolated; consequently no permanent preventative measures or cures can, as yet, be recommended.

Lime-induced chlorosis, more correctly called lime-induced-iron chlorosis, for iron deficiency symptoms are the characteristics found in the plant, is characterized by an interveinal yellowing of the leaves at the meristematic region combined with reduced vigor of the plant as a whole. Once green, the leaf never turns yellow due to a lack of iron.

Thus a leaf chlorotic due to a lack of iron must have always been chlorotic. Even though chlorophyll development is not normal in the chlorotic leaf, unless the condition is critical, the leaf size and stem growth are not seriously effected. If the condition is critical, necrosis will set in and the leaf will drop from the plant. If such a condition is not corrected either through natural or artifical means, some portion or all of the affected plant will die.

The plants susceptible to lime-induced-iron chlorosis are many and varied. Thorne and Wann (1953) have listed a great many plants and their relative susceptibility to lime-induced-iron chlorosis.

Certainly, as pointed out as early as 1879 by Church (1879) and more recently by Thorne and Wann (1950), not all chlorosis in plants is due to iron deficiency. A shortage of such plant nutrients as manganese, nitrogen, zinc, and magnesium will cause chlorosis, a failure of proper chlorophyll formation in the leaves and possibly in the stems of the growing plant.

Lime-induced-iron chlorosis is unique in that, with exceptions, examinations show ample amounts of iron in the afflicted plants for normal plant growth (Wallace, 1928). The iron appears to be inactivated within the plant so as to impair active functioning. Oserkowsky (1933) found a close association between the active iron content of the plant and chlorophyll development.

No single factor has been found to adequately explain this physiological disease although many factors have been associated with it. Thorne, Wann, and Robinson (1950) have observed that calcareous soils characterized by fine texture, high moisture content, poor aeration, and cool temperature intensify the development of chlorosis in plants.

Vyunov (1937) presents the hypothesis that all plants having acid root secretions are immune to chlorosis.

Biddulph (1951) and Rediske (1950) found that the pH, iron, and phosphorus content of the growing solution influenced the movement of iron in red kidney beans. Gile and Carrero (1920) observed that excessive uptake of calcium from calcium carbonate by pineapple plants induced chlorosis. Somers and Shive (1942) and McGeorge (1946) found that the pathological symptoms produced when manganese was in excess in the nutrient substrate were identical with those of iron starvation. Hewitt (1948) found that additions of zinc to sand cultures in which he was growing sugar beets induced a type of iron deficiency in the plants. Rediske (1950) has suggested that iron chlorosis might be caused by excessive or toxic quantities of such elements as copper, zinc, and manganese as well as phosphorus.

In the light of these and many other past laboratory observations, it would seem that additional work could be profitably done to determine the individual effects of various nutrient elements on the absorption and translocation of iron. The objective here will be to study the effects of varying levels of pH, phosphorus, copper, manganese, zinc, iron, calcium, and magnesium in nutrient solutions on the translocation of foliar applications of iron and phosphorus.

REVIEW OF LITERATURE

Since the objective of this research is to determine the effect of a number of elements in the plant on the translocation of iron and phosphorus from foliar applications into the plant, it would seem best to consider the elements here separately or in groups depending on their relationships. As will be observed, many of the conditions considered may be as a result of the factor causing the chlorosis and not necessarily the factor causing the chlorosis.

pH

Biddulph (1951) found iron to enter readily into bean plants from cultural solutions when the solution pH was 4.0 with a phosphorus concentration of 0.0001 molar. When the pH in the solution was 7.0 the iron entered only the veins of the bean plant but not the mesophyll. When the phosphorus concentration in the solution was 0.001 molar and the pH was 7.0 the iron would not enter the xylem from the solution but remained on or in the roots. This inhibition was interpreted to be due to the precipitation of the iron in the solution before it could enter the root. Rediske (1950) found that when the iron was applied to the bean plant as a foliar application the best absorption and translocation of the iron occurred when the pH of the nutrient solution was high with low concentrations of iron and phosphorus.

Epstein and Stout (1951) found no association between the range of hydrogen ion concentrations in a bentonite growing medium and the iron uptake from the medium by tomato plants.

Chapman, Liebig, and Vaneslow (1939), Olsen (1935), and Biddulph (1951) have observed that chlorosis can be corrected by either reducing the pH or the phosphorus levels of the growing medium. Haas (1942), Gile (1916), McGeorge (1951), and Franco and Loomis (1947), and Cain (1954) are investigators who observed chlorosis to occur when the hydrogen ion concentrated is low.

Rogers and Shive (1932), Milad (1939), and Cain (1954) observed a higher pH in the sap of chlorotic leaves than in the green leaves. Rediske (1950) cites unpublished results which were observed by Biddulph and Woodbridge indicating that the sap of bean plants is not altered by the pH of the growing medium.

Phosphorus

Chapman, Liebig, and Vaneslow (1939) found that excessive phosphate under proper conditions of pH may cause iron, zinc, or nitrogen deficiency in orange trees. Franco and Loomis (1947) noted that in culture solutions with pH values above 6, the uptake of iron from the solution by the plant is reduced by phosphorus. They found that when using the Knop type of solution chlorosis could be reduced or prevented by omitting the phosphorus from the solution and adding it separately two to four days later after the iron had been absorbed. Bennett (1945), Iljin (1952), and Warnock (1952) found that the iron chlorotic plant tissues produced on calcareous soils were higher in total phosphorus than the green tissues grown on the same soils. However, Chandler and Scarseth (1941), Lindsay (1953), and Iljin (1944) while producing peanuts, beans, and grapes, respectively, state that they found no correlation between phosphorus and chlorosis. Lindsay used solution cultures while the other investigators used soils rich in calcium. Rediske (1950) observed

that as the phosphorus concentration in the nutrient solution was increased the absorption of iron by beans from either nutrient solutions or foliar treatments was inhibited.

Calcium and magnesium

Since lime-induced chlorosis occurs on calcareous soil it would seem reasonable to believe that the calcium and magnesium of the soil might have something to do with the chlorosis that develops. Gile and Carrero (1920) found that excessive uptake of calcium from gypsum did not induce chlorosis in pineapple while excessive uptake from calcium carbonate did. They therefore concluded that the chlorosis of some plants was caused by or associated with the presence of calcium carbonate in the soil.

Some work has been done to determine whether there is a direct relationship between the calcium carbonate content of the soil and the degree of chlorosis that might exist. Thorne (1941) found no correlation in his studies of Utah orchards but did observe that chlorosis was related to the degree of prevailing compaction of the soil. However, McGeorge (1949) states that when he determined calcium carbonate by the A.O.A.C. method he found a definite relation to chlorotic and non-chlorotic citrus groves. McGeorge concluded that 2.5 to 3.0 percent calcium carbonate in the soil is the approximate dividing line above which chlorosis of citrus might be expected. Though Thorne and McGeorge's findings were in disagreement, Thorne found a greater solubility of calcium when extracted with carbon dioxide for chlorotic than for non-chlorotic soils. This would suggest that the soluble or near soluble calcium content of the soil might be more important from the viewpoint of chlorosis than the total calcium carbonate or some equivalent.

The chemical situation in the soil does not always reflect what might be expected in the plant tissue. Though calcium may frequently be in greater concentration in the soil solution of the chlorotic soil than in the non-chlorotic soil, it is not necessarily higher in the plant tissue. However, there are varying results. Wallace and Mann (1926), while working with apple leaves, Wallace (1928) while working with the bark of the current season's growth and the leaves of susceptible pear, plum, apple, and raspberry, and Lidner and Harley (1944) while working with pear and apple leaves found less calcium in the ash of the chlorotic tissue than in the green tissues. McGeorge (1948), using hegari seedlings, Iljin (1952) studying the sap of grape leaves, and Cain (1954) observing blueberries found more calcium in the chlorotic tissues than in the green tissues. Lindsay (1953), studying beans, found no relationship for calcium but did find the magnesium concentration increased with the increasing chlorosis. Iljin (1952) found similar magnesium relations in Sambucus nigra. Gauch and Wadleigh (1945), while growing red kidney beans in aerated solution culture, noted that as the calcium chloride concentration of the solution was increased the concentration of calcium in the total plant increased but the increases were not proportional to the amounts added. They also found that increasing the amounts of sodium sulfate resulted in a marked progressive decrease in the calcium concentration in the leaves, yet additions of sodium chloride had little effect.

Iron and manganese

The iron-manganese ratio of the nutrient substrate in which the plants grow seems to be of considerable importance. Somers and Shive (1942) and McGeorge (1946) found an optimum ratio of 2 for soybeans.

The pathological symptoms produced when the ratio was above the optimum were identical to those of a manganese deficiency. The symptoms, when the ratio was below the optimum, were identical to those of iron deficiency. The symptoms became progressively more severe as the ratio varied more from the optimum. This lead them to theorize that, since the oxidation potential of manganese was higher than that of iron, the excess manganese was higher than that of iron, the excess manganese oxidized the iron over from ferrous to ferric iron, making it essentially useless to the plant and leaving it precipitated in the plant. Gerretsen (1950) similarly concluded this following his work with oats. Camp et al. (1945) observed a similar trend in orange trees growing in soil.

Olson and Carlson (1949) did not always find a low iron-manganese ratio in soils producing lime-induced-iron chlorosis in sorghum plants. Morris and Pierre (1947) used varying quantities of iron and manganese in nutrient solutions in which lespedeza was grown. Iron concentrations up to one p.p.m. resulted in reduced manganese toxity but iron concentrations of 2.5 p.p.m. reduced growth rate regardless of the iron concentration. Morris and Pierre theorized that the beneficial effects of the iron was due to a retarded uptake of manganese. Bennett (1945) observed that increased iron in nutrient cultures reduced the uptake of manganese by tomatoes.

Epstein and Stout (1951) found that increasing increments of manganese to the bentonite medium in which tomatoes were grown displaced ferric ions from the exchange complex and raised the amount of iron taken up by the roots. However, this was followed by a reduced translocation of iron from the roots to the shoots. Sideris (1950) observed a similar effect in pineapple grown in nutrient solution.

Haas (1932) used varying levels of iron and manganese in solution cultures in which he grew lemon and orange trees. A deficiency of manganese in the solution produced plants deficient in iron. It was also found that neither iron nor manganese could substitute for the other in the plant. Hewitt (1948) grew sugar beets in sand cultures containing toxic levels of manganese and produced iron deficiency symptoms.

Johnson (1917) suggested that excess manganese in noncalcareous Hawaiian Island soils was responsible for iron-chlorosis in pineapple. Holmes and Brown (1957) attributed some of the chlorosis in oranges grown on acid, sand soils in Florida also to manganese or copper excesses created by numerous sprayings for insect control.

Zinc and copper

Studies have led some to believe that zinc and copper might cause chlorosis with an iron deficiency pattern in some plants. Chapman, Liebig, and Vaneslow (1939) observed that under a variety of nutrient conditions an excess of zinc brought on an iron chlorosis in lemon and orange plants. A limited number of observations also indicated to them that slight excesses of copper induced iron chlorosis. Christensen (1948) observed a yellowing of the terminal leaves of dwarf Alderman garden peas grown on saline soil in every case where he made an application of copper sulfate. Christensen observed that the chlorosis occurred least rapidly where the soil sodium chloride concentration was the highest. The copper apparently had no antagonistic effect on the sodium chloride but the sodium did reduce the copper toxicity. Chapman, Liebig, and Vaneslow (1939) found also that excessive phosphorus under proper conditions of pH may cause both iron and zinc deficiency. Thus an excess of zinc can be offset by an application of phosophorus.

Hewitt (1948) found that young leaves of sugar beets grown in purified sand culture with excesses of cobalt, copper, zinc, and chromate (CrO_4^{--}) developed chlorosis. The zinc and chromate ions gave results similar to those when iron was omitted from the solution. The cobalt and copper gave the most severe chlorosis. Painting the leaf surfaces with 0.25 percent iron sulfate mixed with a wetting agent cured the chlorosis completely within five days, showing that the chlorosis was due to a failure of iron metabolism. Holmes and Brown (1957) theorize that iron-chlorosis which has appeared in orange trees in acid sandy soils of Florida may be due to excess copper in the soil. The excess copper is probably due to the years of use of copper as sprays.

Summary of literature review

A study of the literature shows many relationships between iron-chlorosis and plant nutrition. The pH of the growing medium has been shown to effect the hydrogen ion concentration of the plant sap by some investigators while others observed no change. Some investigators have shown the mobility of iron to be effected by the pH of the growing medium. Excesses of phosphorus was often cited as related to iron immobility. High levels of calcium and magnesium have often been found in chlorotic plant tissue. No definite levels of calcium carbonate in the soil seemed to correlate with the incidence of lime-induced-iron chlorosis.

The iron and manganese levels in growing medium have been variously correlated with iron chlorosis. The high oxidation potential of manganese has been often used to explain the inhibiting effects of manganese on iron absorption by plants. High levels of zinc and copper in the growing medium have been cited as possible causes for inhibited

absorption and translocation of iron by the roots of iron-chlorosis susceptible plants.

Only one collection of research, that of Rediske (1950) under the direction of O. Biddulph, was found which dealt with the effects of nutrient levels in the growing medium upon the absorption and translocation of foliar applied iron. The use of iron as a foliar spray to temporarily correct iron-chlorosis is not new. Yet nearly all of the research reviewed dealt with effects of various nutrient levels and combinations in the root environment on root absorption and translocation of iron.

EXPERIMENTS PERFORMED

A number of experiments were performed in different situations such that a general discussion of the experimental procedures used cannot be readily made together. The methods used will be discussed under each of the respective experiments.

Experiment I. Greenhouse work determining iron mobility in beans

Method. A supply of standard commercial grade Red Kidney bush beans (Phaseolus vulgaris) was obtained from Burpee's Seed Company. The seeds were sorted for uniformity. They were dusted lightly with Semasan and germinated in a bed of sterile, coarse sand.

When the seedlings were about four inches tall, they were selected for uniformity and placed in the nutrient culture. The nutrient culture was placed in one gallon wide-mouthed glass milk jars which had been previously painted on the outside with black paint followed by aluminum paint. Three plants were used per jar.

The plants were held in place by wrapping a small wrap of cotton just below the cotyledon and placing between the two halves of a split one-hole cork. The plant assemblies were placed in the three holes which were made in standard waxed milk jar caps which were fitted on the tops of the culture jars.

The growing solution used contained 0.25, 1.25, 1.25, and 0.5 millimolar concentrations of potassium acid phosphate (mono-basic), potassium nitrate, calcium nitrate, and magnesium sulfate respectively. Double this concentration of major elements was tried, but unless the cultural

solution was mixed on the previous day, the primary leaves would wilt badly and large parts of the leaf would dry up. This complication was not observed when the lower concentrations were used. The concentrations of trace elements in parts per million were boron, 0.5; manganese, 0.5; zinc, 0.5; copper, 0.25; molybdenum, 0.01; and iron, 1.0. The pH was adjusted to near 6.

The nutrient solutions were kept continuously aerated by a means of a source of compressed air. The air was bubbled through water before being admitted to the culture solutions.

An effort was made to keep the greenhouse temperature at approximately 80° F. during the approximately 16-hour light period. The night temperature was allowed to go to 65° F. During the winter months the light hours were supplemented through use of blue and white fluorescent lights hung three feet above the top of the benches. Difficulty was experienced during the summer months in keeping the temperature constant since adequate thermostatic controls and air conditioning were not available.

After the plants had been in the culture for four days, the solutions were replaced with new solutions of the same original concentrations. On the eighth day the solutions were again replaced. At this time the experimental variables were applied according to table 1. The pH's were adjusted daily, using one normal hydrochloric acid and sodium hydroxide from dropper bottles.

On the 12th day a framework was erected on which the oldest trifoliate leaf was held flat. To the middle leaflet, towards its base but on both sides of the midrib, was applied a total of 50 lamda of 1000 p.p.m. of iron as ferric chloride or Fe-DTPA tagged with approximately

Table 1. Concentrations of salts and other experimental variables used in the nutrient cultures*

Treatment No.	pH	$Ca(NO_3)_2$	KNO_3	$MgSO_4$	KH_2PO_4	Cu	Mn	Zn	Fe	Foliar application of Fe^{+++} as
		mM	mM	mM	mM	p.p.m.	p.p.m.	p.p.m.	p.p.m.	
1	5.0	1.25	1.25	0.5	0.500	0.02	0.5	0.05	0.005	Cl^-
2	5.0	1.25	1.25	0.5	0.500	0.02	0.5	0.05	0.005	DTPA†
3	7.5	1.25	1.25	0.5	0.500	0.02	0.5	0.05	0.005	Cl^-
4	7.5	1.25	1.25	0.5	0.500	0.02	0.5	0.05	0.005	DTPA
5	5.0	1.25	1.25	0.5	0.005	0.02	0.5	0.05	0.005	Cl^-
6	5.0	1.25	1.25	0.5	0.050	0.02	0.5	0.05	0.005	Cl^-
7	5.0	1.25	1.25	0.5	5.000	0.02	0.5	0.05	0.005	Cl^-
8	5.0	1.25	1.25	0.5	0.005	0.02	0.5	0.05	0.005	DTPA
9	5.0	1.25	1.25	0.5	0.050	0.02	0.5	0.05	0.005	DTPA
10	5.0	1.25	1.25	0.5	5.000	0.02	0.5	0.05	0.005	DTPA
11	7.5	1.25	1.25	0.5	0.005	0.02	0.5	0.05	0.005	Cl^-
12	7.5	1.25	1.25	0.5	0.050	0.02	0.5	0.05	0.005	Cl^-
13	7.5	1.25	1.25	0.5	5.000	0.02	0.5	0.05	0.005	Cl^-
14	7.5	1.25	1.25	0.5	0.005	0.02	0.5	0.05	0.005	DTPA
15	7.5	1.25	1.25	0.5	0.050	0.02	0.5	0.05	0.005	DTPA
16	7.5	1.25	1.25	0.5	5.000	0.02	0.5	0.05	0.005	DTPA
18	5.0	1.25	1.25	0.5	0.005	0.20	0.5	0.05	0.005	Cl^-
21	5.0	1.25	1.25	0.5	0.005	0.20	0.5	0.05	0.005	DTPA
24	7.5	1.25	1.25	0.5	0.005	0.20	0.5	0.05	0.005	Cl^-
27	7.5	1.25	1.25	0.5	0.005	0.20	0.5	0.05	0.005	DTPA
30	5.0	1.25	1.25	0.5	0.005	0.02	5.0	0.05	0.005	Cl^-
33	5.0	1.25	1.25	0.5	0.005	0.02	5.0	0.05	0.005	DTPA
36	7.5	1.25	1.25	0.5	0.005	0.02	5.0	0.05	0.005	Cl^-
39	7.5	1.25	1.25	0.5	0.005	0.02	5.0	0.05	0.005	DTPA
42	5.0	1.25	1.25	0.5	0.005	0.02	0.5	0.50	0.005	Cl^-
43	5.0	1.25	1.25	0.5	0.005	0.02	0.5	5.00	0.005	Cl^-
45	5.0	1.25	1.25	0.5	0.005	0.02	0.5	0.50	0.005	DTPA
46	5.0	1.25	1.25	0.5	0.005	0.02	0.5	5.00	0.005	DTPA

Table 1. (Continued)

Treatment No.	pH	$Ca(NO_3)_2$	KNO_3	$MgSO_4$	KH_2PO_4	Cu	Mn	Zn	Fe	Foliar application of Fe^{+++} as
		mM	mM	mM	mM	p.p.m.	p.p.m.	p.p.m.	p.p.m.	
48	7.5	1.25	1.25	0.5	0.005	0.02	0.5	0.50	0.005	Cl^-
49	7.5	1.25	1.25	0.5	0.005	0.02	0.5	5.00	0.005	Cl^-
51	7.5	1.25	1.25	0.5	0.005	0.02	0.5	0.50	0.005	DTPA
52	7.5	1.25	1.25	0.5	0.005	0.02	0.5	5.00	0.005	DTPA
54	5.0	1.25	1.25	0.5	0.005	0.02	0.5	0.05	0.050	Cl^-
55	5.0	1.25	1.25	0.5	0.005	0.02	0.5	0.05	0.500	Cl^-
56	5.0	1.25	1.25	0.5	0.005	0.02	0.5	0.05	5.000	Cl^-
58	5.0	1.25	1.25	0.5	0.005	0.02	0.5	0.05	0.050	DTPA
59	5.0	1.25	1.25	0.5	0.005	0.02	0.5	0.05	0.500	DTPA
60	5.0	1.25	1.25	0.5	0.005	0.02	0.5	0.05	5.000	DTPA
62	7.5	1.25	1.25	0.5	0.005	0.02	0.5	0.05	0.050	Cl^-
63	7.5	1.25	1.25	0.5	0.005	0.02	0.5	0.05	0.500	Cl^-
64	7.5	1.25	1.25	0.5	0.005	0.02	0.5	0.05	5.000	Cl^-
66	7.5	1.25	1.25	0.5	0.005	0.02	0.5	0.05	0.050	DTPA
67	7.5	1.25	1.25	0.5	0.005	0.02	0.5	0.05	0.500	DTPA
68	7.5	1.25	1.25	0.5	0.005	0.02	0.5	0.05	5.000	DTPA
69	5.0	25.00	1.25	0.5	0.005	0.02	0.5	0.05	0.005	Cl^-
71	5.0	25.00	1.25	0.5	0.005	0.02	0.5	0.05	0.005	DTPA
73	7.5	25.00	1.25	0.5	0.005	0.02	0.5	0.05	0.005	Cl^-
75	7.5	25.00	1.25	0.5	0.005	0.02	0.5	0.05	0.005	DTPA
77	5.0	1.25	1.25	10.0	0.005	0.02	0.5	0.05	0.005	Cl^-
79	5.0	1.25	1.25	10.0	0.005	0.02	0.5	0.05	0.005	DTPA
81	7.5	1.25	1.25	10.0	0.005	0.02	0.5	0.05	0.005	Cl^-
83	7.5	1.25	1.25	10.0	0.005	0.02	0.5	0.05	0.005	DTPA

* All treatments contained 0.5 p.p.m. of boron as boric acid. Copper and zinc were applied as sulfates, manganese as a chloride, and iron as a tartrate. The pH's were adjusted with 1.0 N HCl and NaOH.

† Chelated iron as diethylene triamine penta-acetate.

200 microcuries of Fe^{59} per ml. The ferric chloride was adjusted to a pH of approximately 2. As soon as the labeled iron mixture was applied to the leaflet it was covered with a pyrex beaker to protect against insects and rapid drying. This treatment was allowed to stand for 24 hours.

At the close of the treatment time the treated leaflet, being too radioactive for analysis, was removed at the base of the leaf blade and put into shielded storage. The plants, unless used for radio-autographs, were composited in their respective parts for each pot. The parts were identified by letters as follows:

R = Roots and stems below the node of the trifoliate leaf treated, including the petioles of the primary leaves

P = Primary leaf blades

L = The two remaining untreated leaf blades of the trifoliate leaf treated and node where the treated trifoliate was attached to the stem and the petiole of the treated trifoliate

T = The treated leaflets

A = The stems and remaining apex trifoliate leaves above the node of the treated trifoliate and the buds.

Following the harvest of the bean parts they were put into paper bags and placed in a forced air oven at 75° C. for 24 hours to dry.

Those plants used for radio-autographs were taken from the nutrient solution, blotted dry with paper towels, placed in appropriate herbarium presses, and dried in the above mentioned oven. Following drying the plants were placed next to 7- x 14-inch sheets of Eastman no-screen X-ray film for a period of a half life, namely 46 days. Following this

the films were uniformly developed for comparison purposes.

Following drying of the composited plant parts, they were compressed unground under 15,000 pounds of total pressure into pellets. This was accomplished in a one-half inch diameter compaction cylinder in a Carver press. Following pelleting the samples were weighed and assayed for radioactivity in a scintillation counter.

Results. The results of Experiment I are recorded in table 2 and in figures 1 through 3. In table 2 the four treatments which are shown on a horizontal line, such as treatments 1, 2, 3, and 4, are identical except for the form of the foliar applied iron and pH.

Three plants are represented in each value for table 2 which gives the actual amounts in millimicrograms of the applied iron that was translocated to the plant parts.

From zero up to about 2,800 millimicrograms of the 50,000 millimicrograms applied to the leaflet were translocated, leaving the leaflet receiving the applied iron too radioactive to assay.

Figures 1 through 3 represent 18 different nutrient cultures and applied radioactive iron materials with treatment 5 serving as the standard.

Statistical treatment. An examination of the overall data for this experiment shows that the primary leaves, division P of the plants, received very little of the translocated iron. Due to the wide variation of the data from replication to replication nothing else is obvious. Several of the treatments involving intermediate levels of the variables did not result in obvious differences over the standard and are not included in the statistical analyses. The treatments compared were the standard (5), high phosphorus (7), high copper (18), high

Table 2. Millimicrograms of iron present in various divisions of bean plants translocated there from iron applied to the center leaflet of the first trifoliate leaf

Culture solution pH 5.0						Culture solution pH 7.5					
Iron applied as $FeCl_3$			Iron applied as DTPA-Fe			Iron applied as $FeCl_3$			Iron applied as DTPA-Fe		
Treatment and division number*	Replication		Treatment and division number	Replication		Treatment and division number	Replication		Treatment and division number	Replication	
	A	B		A	B		A	B		A	B
1 - R	22.5	2.4	2 - R	71.0	12.4	3 - R	1.9	4.5	4 - R	78.7	176.3
P	0.0	0.7	P	6.3	21.2	P	0.9	1.1	P	0.3	2.5
L	2.7	3.2	L	596.0	48.7	L	99.0	5.7	L	180.8	143.4
A	183.2	0.0	A	82.0	20.8	A	1.6	2.2	A	65.1	90.5
Total	208.4	6.3	Total	755.3	103.0	Total	103.4	13.5	Total	324.9	412.7
5 - R	20.9	13.7	8 - R	17.1	187.8	11 - R	158.5	294.9	14 - R	62.4	149.0
P	0.5	2.1	P	1.8	0.2	P	0.6	5.1	P	4.8	2.3
L	28.9	14.0	L	1033.0	471.5	L	43.7	59.9	L	52.9	126.9
A	24.1	9.8	A	14.5	269.9	A	95.0	60.3	A	23.5	76.5
Total	74.4	39.6	Total	1066.4	929.4	Total	297.8	420.2	Total	143.6	354.7
6 - R	70.7	4.2	9 - R	92.5	9.2	12 - R	240.3	58.4	15 - R	11.2	129.5
P	1.8	0.5	P	1.8	4.5	P	1.7	1.3	P	0.2	0.0
L	73.8	6.3	L	461.9	1331.8	L	91.0	31.6	L	120.1	79.3
A	114.7	2.0	A	121.1	8.0	A	2423.5	8.7	A	11.3	88.1
Total	261.0	13.0	Total	677.3	1353.5	Total	2756.5	100.0	Total	142.8	269.9
7 - R	11.3	3.3	10 - R	38.9	42.2	13 - R	268.3	25.5	16 - R	124.0	59.3
P	0.6	0.8	P	2.8	1.2	P	2.6	5.0	P	3.4	1.7
L	31.3	125.3	L	980.7	109.5	L	290.7	35.3	L	1701.9	46.1
A	14.9	3.9	A	68.4	177.0	A	152.4	5.6	A	54.9	23.3
Total	58.1	133.3	Total	1090.8	329.9	Total	714.0	71.4	Total	1884.2	130.4

Table 2. (Continued)

Culture solution pH 5.0						Culture solution pH 7.5					
Iron applied as $FeCl_3$			Iron applied as DTPA-Fe			Iron applied as $FeCl_3$			Iron applied as DTPA-Fe		
Treatment and division number	Replication		Treatment and division number	Replication		Treatment and division number	Replication		Treatment and division number	Replication	
	A	B		A	B		A	B		A	B
18 - R	148.6	14.6	21 - R	89.1	9.5	24 - R	68.7	34.7	27 - R	97.3	8.4
P	1.8	9.0	P	3.8	0.0	P	1.3	2.6	P	0.8	4.3
L	258.6	936.0	L	1102.2	36.3	L	20.8	33.3	L	40.3	40.9
A	92.3	3.8	A	27.1	1.5	A	30.1	12.2	A	30.4	1.9
Total	501.3	963.4	Total	1222.2	47.3	Total	120.9	82.8	Total	168.8	55.5
30 - R	72.8	11.4	33 - R	30.5	30.0	36 - R	32.5	12.6	39 - R	132.6	12.4
P	1.4	0.6	P	0.5	0.0	P	0.3	0.0	P	0.8	55.4
L	53.1	11.0	L	154.3	168.3	L	45.4	34.7	L	349.0	1307.5
A	153.7	7.7	A	56.8	87.1	A	17.3	20.3	A	60.4	2.2
Total	281.0	30.7	Total	242.1	285.4	Total	95.5	67.6	Total	542.8	1377.5
42 - R	4.9	24.6	45 - R	37.5	62.6	48 - R	230.6	104.3	51 - R	41.8	69.4
P	0.0	0.0	P	0.0	0.0	P	1.3	0.0	P	0.6	2.3
L	13.0	62.1	L	25.2	32.0	L	96.6	40.1	L	115.3	38.2
A	2.3	7.5	A	44.3	91.4	A	208.6	70.4	A	108.6	45.8
Total	20.2	94.2	Total	107.0	186.0	Total	537.1	214.8	Total	266.3	155.7
43 - R	8.4	64.5	46 - R	19.6	26.6	49 - R	1.9	8.7	52 - R	159.5	50.6
P	3.7	2.1	P	0.0	2.7	P	2.9	0.9	P	4.0	0.0
L	41.8	184.7	L	46.0	55.6	L	89.9	58.8	L	73.5	232.9
A	31.8	36.3	A	31.2	42.6	A	7.8	9.4	A	120.2	38.3
Total	85.7	287.6	Total	96.8	127.5	Total	102.5	77.8	Total	357.2	321.8

Table 2. (Continued)

Culture solution pH 5.0						Culture solution pH 7.5					
Treatment and division number	Replication		Treatment and division number	Replication		Treatment and division number	Replication		Treatment and division number	Replication	
	A	B		A	B		A	B		A	B
54 - R	17.1	6.8	58 - R	39.6	59.3	62 - R	171.5	1.0	66 - R	229.9	228.4
P	1.0	0.7	P	1.2	0.1	P	0.5	1.0	P	0.0	25.0
L	29.9	6.6	L	39.0	61.8	L	78.0	24.0	L	87.0	74.5
A	31.2	5.3	A	97.7	41.3	A	171.1	0.0	A	140.0	165.9
Total	79.2	19.4	Total	177.5	162.5	Total	421.1	26.0	Total	456.9	493.8
55 - R	8.7	19.5	59 - R	86.8	39.8	63 - R	116.9	21.6	27 - R	213.5	91.4
P	21.7	1.2	P	5.7	3.5	P	1.9	1.3	P	4.1	28.0
L	7.2	11.1	L	23.5	174.0	L	35.1	4.7	L	81.7	79.4
A	1.7	26.2	A	81.7	89.1	A	55.9	3.9	A	335.0	134.9
Total	39.3	58.0	Total	197.7	306.4	Total	209.8	31.5	Total	634.3	333.7
56 - R	1.8	3.6	60 - R	16.4	61.7	64 - R	15.3	36.9	68 - R	133.5	115.1
P	1.2	0.6	P	1.0	9.3	P	0.9	0.8	P	2.3	74.7
L	22.5	11.1	L	58.5	78.1	L	72.9	7.1	L	119.4	112.7
A	26.3	9.2	A	123.7	65.8	A	101.8	12.9	A	243.0	153.6
Total	51.8	24.5	Total	199.6	214.9	Total	190.9	57.5	Total	498.2	456.1
69 - R	76.5	13.8	71 - R	144.2	52.6	73 - R	79.4	3.5	75 - R	3.0	4.2
P	0.4	0.5	P	3.4	1.0	P	0.0	1.1	P	1.2	1.5
L	56.3	17.6	L	51.8	15.9	L	139.8	5.0	L	21.9	25.3
A	185.9	9.2	A	172.5	26.4	A	45.1	1.5	A	1.6	8.0
Total	319.1	41.1	Total	271.9	95.9	Total	264.3	11.1	Total	27.7	39.0

Table 2. (Continued)

Culture solution pH 5.0						Culture solution pH 7.5					
Treatment and division number	Replication		Treatment and division number	Replication		Treatment and division number	Replication		Treatment and division number	Replication	
	A	B		A	B		A	B		A	B
77 - R	135.4	7.5	79 - R	22.4	123.5	81 - R	442.8	18.3	83 - R	28.1	80.0
P	1.0	1.4	P	6.9	3.0	P	8.4	0.5	P	6.4	3.7
L	148.7	15.5	L	762.2	118.4	L	247.3	25.8	L	72.2	163.1
A	311.9	8.7	A	54.3	85.3	A	255.3	7.8	A	63.5	26.5
Total	597.0	33.1	Total	845.8	330.2	Total	953.8	52.4	Total	170.2	273.3

* Plant divisions explained in text, page 16.

Figure 1. Radio-autographs of 12-day-old bean plants showing the effects of nutrient levels in nutrient solutions on the translocation of foliar applied iron. All received foliar applied $FeCl_3$ except c, e, and f which received DTPA-Fe instead. (a) Treat. 5; standard; pH 5.0, (b) Treat. 7; 5 mM KH_2PO_4; pH 5.0, (c) Treat. 8; pH 5.0, (d) Treat. 11; pH 7.5, (e) Treat. 13; pH 7.5; 5 mM KH_2PO_4, (f) Treat. 14; pH 7.5.

Figure 2. Radio-autographs of 12-day-old bean plants showing the effects of nutrient levels in nutrient solutions on the translocation of foliar applied iron. All received $FeCl_3$ as foliar applications. (a) Treat. 18; pH 5.0; 0.2 p.p.m. Cu, (b) Treat. 24; pH 7.5; 0.2 p.p.m. Cu, (c) Treat. 30; pH 5.0; 5.0 p.p.m. Mn, (d) Treat. 36; pH 7.5; 5.0 p.p.m. Mn, (e) Treat. 43; pH 5.0; 5.0 p.p.m. Zn, (f) Treat. 48; pH 7.5; 5.0 p.p.m. Zn.

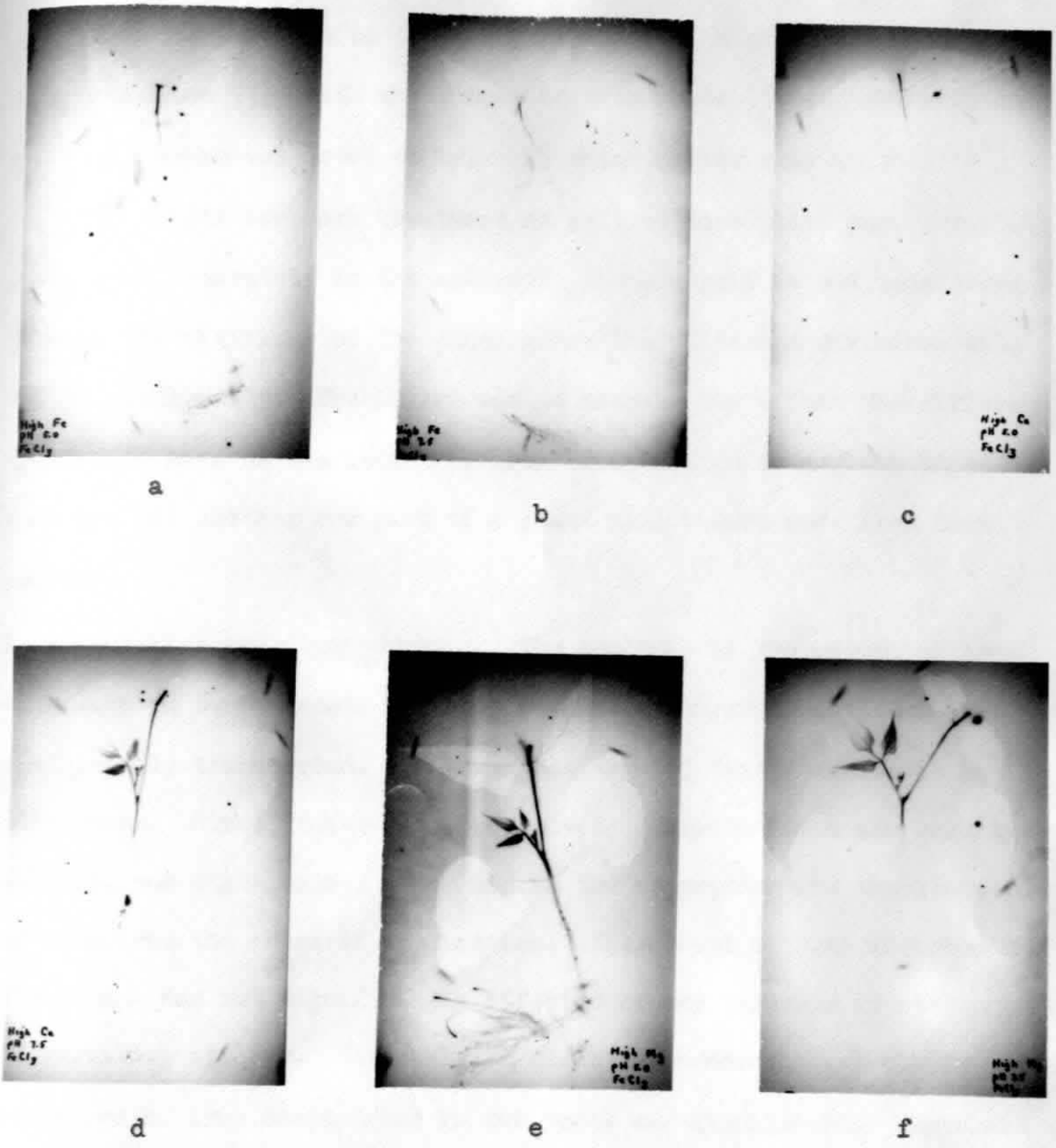

Figure 3. Radio-autographs of 12-day-old bean plants showing the effects of nutrient levels in nutrient solutions on the translocation of foliar applied iron. All received $FeCl_3$ as foliar applications. (a) Treat. 56; pH 5.0; 5 p.p.m. Fe, (b) Treat. 64; pH 7.5; 5 p.p.m. Fe, (c) Treat. 69; pH 5.0; 25 mM $CaNO_3$, (d) Treat. 73; pH 7.5; 25 mM $CaNO_3$, (e) Treat. 77; pH 5.0; 10 mM $MgSO_4$, (f) Treat. 81; pH 7.5; 10 mM $MgSO_4$.

manganese (30), high zinc (42), high iron (56), high calcium (69), and high magnesium (77) with the companion treatments (those appearing on the same horizontal level in table 2) which differ only in pH.

The totals for each treatment as well as each plant part are also considered separately in the analysis. An analysis is not considered between the divisions of the plant since the different divisions will weight differently. The intent was to measure the effect the different treatments have on the amount of iron translocated to a given plant part and not whether one part of a plant will absorb more iron than another.

Interpretation and discussion. The analysis of the amount of iron translocated to the roots of the plant showed that the chelated iron was most readily transported. This was not true of translocation to the plant tops. Since DTPA-Fe is a relatively stable chelate and readily soluble, one might expect it to aid in the absorption and translocation of iron from the point of application. The amount of iron absorbed by division A was not significantly affected by any variable or combination of variables applied. The analysis did show, however, that the amount of translocated iron accumulated in the roots was significantly increased by the higher pH. Rediske (1950) found this to be true in his experiments. His explanation was that at lower pH's the plant was able to take up iron from its growing medium and redistribute it in the plant, thus making less need for iron by the plant. When the pH was at 7 the iron in the growing solution was not readily taken up by the plant and circulated. Thus there could be a "demand" for the iron applied to the plant leaf. Rediske (1950) also showed that when a bean plant was grown

in a culture solution at pH 4 it was very difficult to get it to go chlorotic.

Under the conditions of the experiment no combination of metalic elements used in the growing media had significant effects on the absorption and translocation of the applied iron. This is in direct disagreement with Rediske (1950) and Biddulph (1948, 1951), where they showed that nutrient cultures with pH's near 7 or with phosphorus concentration near 0.0001 M or more or with iron concentration of one p.p.m. or more or any combination of these factors inhibited the absorption and translocation of foliar applied iron. That iron is immobile within the plant is not new knowledge. Over 42 years ago Gile and Carrero (1916) painted the end of a chlorotic rice leaf with a solution of iron sulfate and found that only the painted area returned to a green color. Since approximately 50,000 millimicrograms of iron were applied to the plants in the present experiment, it is obvious from the data presented in table 2 that in many of the plants less than one percent of the iron was translocated from the center leaflet of the treated trifoliate to other parts of the plant.

Failure of these data to agree with results of Rediske (1950) and Biddulph (1951) might be attributed to the experimental conditions under which the work was conducted. The sunlight intensities, which varied from day to day and season to season, could not be controlled. Also, it was not possible to grow all of the plants of one replication at one time. An examination of the radio-autographs helps explain some of the discrepancies found. Some of the radio-autographs show pin point "hot" spots which are very probably contaminations. These may have been transported from the treated, very hot leaflet by tiny insects. When the

radio-iron was applied a bubble would occasionally occur which would later burst. A tiny droplet of the applied radio-active iron solution may have been flipped to some other plant surface. Such contaminations would be undetected when the plant is pelleted and counted.

Conclusions to Experiment I. The results of Experiment I proved to be erratic, possibly due to temperature and sunlight variations from day to day and month to month. Iljin (1951) noted that his plants responded differently in different seasons. This may have been the effects of changes in temperature and light. Lohnis (1951) noted that the temperature of the plant's environment influenced the toxic effects of manganese. The radio-autographs of Experiment I were made from a group of treatments in August 1956, while the data were collected over the previous eight months.

Even though results were erratic, the chelated iron still gave a significantly greater translocation of iron to limited parts of the plant. The high pH in the nutrient solution also made for a significantly greater translocation of iron from the treated trifoliate. The radio-autographs differ from the data, possibly due to different temperature and light conditions for their preparation and also due to their ability to differentiate between translocation and contamination.

It was apparent from this experiment that better control of the plant's environment was necessary to cut down variation. This has been done in Experiment IV.

Experiment II. Puddle vs. flap application: xylem vs. phloem transport

As was pointed out in Experiment I, facilities were not available in the greenhouse for accurate control of light, temperature, or relative humidity from season to season or day to day. During the summer of 1957

a plant growth room was developed in which these factors could be controlled.

Investigations by Biddulph (1941), Biddulph (1956), Oliver (1952), Silberstein and Wittwer (1951), Swanson and Whitney (1953), Koontz and Biddulph (1957), and Bukovac Wittwer (1957) have conclusively shown that foliar applications of phosphorus to the bean plant can be transported to other parts of the plant. Oliver (1952) and Silberstein and Wittwer (1951) have also produced evidence that foliar treatment of corn with phosphorus compounds results in an export of phosphorus by the plant to other parts of the plant.

The purpose of this investigation was twofold: (1) to determine whether phosphorus is exported from the leaf blade of King Croft (KY7) field corn and Red Kidney beans by way of the xylem or phloem and (2) to determine the effect of varying levels of nutrients in the nutrient solution on the export of phosphorus from the corn leaf.

Stout and Hoagland (1939) used a stripping method to show that phosphorus applied to the foliage of willow and geranium plants was exported from the foliage by way of the phloem. Biddulph and Markle (1944) later used an "inverse flap" method to show that application of phosphorus to the foliage of cotton was transported by way of the phloem. This inverse flap method is described below. Their theory was that the inverse flap method eliminated direct passage by way of the xylem. They checked the transport mechanism further by also using the stripping method of Stout and Hoagland (1939) and found the movement from the leaves to be by way of the phloem. Biddulph, Cory, and Biddulph (1956) steamed the petiole of bean plants with live steam and showed that the transportation of sulfur from the leaflet was essentially inhibited.

Method. The corn and beans were germinated in coarse sand. As soon as they were about three inches tall, they were selected for uniformity, wrapped with a small amount of cotton just above the roots, placed between the two halves of a one-hole cork, and placed in a hole in the lid of a wide mouth one-gallon milk jar. Three bean or four corn plants were used per jar. The nutrient solution used was the same as treatment number 5 in table 1 described in Experiment I except that it contained 0.25 instead of 0.005 millimolar of potassium acid phosphate, 0.01 p.p.m. of molybdenum, and a pH of approximately 6.0.

The plants were grown in a constant temperature room under warm white fluorescent lights which produced about 1,500 foot candles at the level of the plant. The light interval of 16 hours was held at approximately 85° F. and 45 percent relative humidity. During the night the temperature was dropped to about 70° F. and the relative humidity rose to about 75 percent. About two hours were required to change from one temperature and relative humidity level to another. These changes started at the moment the daylight interval started and ended.

Beans. On the fourth and eighth days following the placement of the beans into the nutrient culture the nutrient solution was replaced with new nutrient solution of the original nutrient strengths. On the eleventh day the leaf petiole of the oldest trifoliate leaf on one-half of the bean plants was steamed for thirty seconds with live steam. Immediately following the steaming, the middle leaflet of the oldest trifoliate leaf of all the plants was treated with 10 lambda of P^{32} labeled phosphoric acid which contained 0.877 microcuries of P^{32} on August 5, 1957. The pH of the P^{32} solution was approximately 2.5. Koontz and Biddulph (1957) recently found that monosodium acid phosphate

would have been more readily translocated. However, this was not known at the time of this investigation.

A second method of applying solution to the leaf was studied. This was the inverse flap method of Biddulph and Markle (1944). The flap was made by cutting across the midrib with a razor blade, while under distilled water, at a point about one-third the way up from the base of the leaflet. The flap was completed by cutting, under water, up along each side of the midrib toward the tip of the leaf for about one-half inch. This flap was immediately placed and held in a small vial filled with distilled water. As soon as about one-half of the water was gone the 10 lambda of P^{32} was added to the vial. The vials were empty in approximately four hours. These vials, produced by sealing off one end of a section of 5 millimeter outside diameter glass tubing, held about 0.10 milliliter. The puddle method was used in Experiment I. The leaf was laid out flat and a total of 10 lambda of the P^{32} was applied in small droplets to opposite sides of the midrib in a small area about one-third of the way up from the base of the leaf blade. This was covered with a beaker to retard evaporation and help prevent other leaves from moving into the treatment and becoming contaminated.

Twenty-four hours after treatment, the bean plants were harvested. The plants were separated into five parts, dried and pressed as described in Experiment I. The parts of three plants went into each sample.

There were at least three pots per replication.

The millimicrocuries of P^{32} present in each sample were determined with a model 183 Nuclear Scaler using a thin window GM tube type D34, containing a mica end window of 1.4 mg/cm^2. An operating voltage of 900 volts was used.

The findings were corrected for self-absorption, geometry, and decay, giving the P^{32} equivalent as of one day, August 5, 1957.

Corn. The corn plants were grown to only nine days of age. The culture solution was changed on the fifth day. On the eight day half the plants were steamed. The second leaf from the base received the steam treatment at about one inch out on the leaf blade from the leaf collar. The P^{32} was put on the leaf either by the puddle or leaf flap, about one inch above the steamed point.

Twenty-four hours after the treatment, the plants were harvested. The plants were disected and dried using two plants per sample. This number of plants per sample came about as a result of growing two replications per pot with two plants per replication. Replications one and two came out of one pot and three and four came out of the other pot.

Upon harvesting, the disected plant samples were put in paper bags and labeled R, P, L, T, and A to correspond roughly to the manner in which the bean plants were separated.

R = Roots of the corn plant

P = Lower leaf of each of the two plants

L = The part of the treated leaf from just above the collar down and including the node where the leaf was attached

T = The remainder of the treated leaf--too hot to be assayed

A = All remaining plant parts above the node of the treated leaf.

These samples were dried, crushed, pressed, weighed, assayed, and the assay results adjusted for decay in the same fashion as were the beans.

Results. The data for the bean and corn experiments are given in table 3. These results confirm the finding of Biddulph, Cory, and Biddulph (1956). The steaming of the leaf below the point of treatment

Table 3. The effect of steaming the leaf below the point of application on the number of millimicrocuries of P^{32} translocated from the point of application to other parts of the bean or corn plant

Application method	Plant part*	Beans Replication A	Beans Replication B	Beans Replication C	Corn Replication A	Corn Replication B	Corn Replication C	Corn Replication D
		muc	muc	muc	muc	muc	muc	muc
Puddle after steaming	R	0.04	0.07	0.28	1.57	0.60	0.27	0.10
	P	0.07	0.07	0.08	15.50	2.59	0.07	0.07
	L	1.80	3.24	1.54	0.71	9.22	0.07	0.06
	A	0.85	1.38	0.36	0.36	2.87	0.03	0.03
	Total	2.76	4.76	2.26	18.14	15.28	0.44	0.26
Flap after steaming	R	0.14	0.28	0.59	0.53	0.39	1.25	0.55
	P	0.07	0.11	0.15	0.62	0.52	0.43	0.49
	L	0.15	0.11	0.53	0.50	0.53	0.60	0.39
	A	0.29	0.14	0.36	0.43	0.77	0.41	0.36
	Total	0.65	0.64	1.63	2.08	2.21	2.69	1.79
Puddle without steaming	R	63.5	69.2	42.5	81.5	58.0	67.3	101.0
	P	2.0	2.4	1.2	12.2	8.9	14.8	11.0
	L	12.2	14.7	12.9	45.2	52.3	85.5	83.4
	A	34.0	52.4	51.0	129.0	114.0	149.0	147.0
	Total	109.7	138.7	107.6	267.9	233.2	316.6	342.4
Flap without steaming	R	186.0	127.0	108.0	77.0	57.0	91.0	87.3
	P	6.0	2.3	2.7	10.7	65.9	6.0	6.3
	L	44.2	26.0	34.3	44.5	27.4	3.0	33.5
	A	169.0	76.0	185.0	134.0	110.0	125.0	95.5
	Total	405.2	231.3	330.0	266.2	260.3	225.0	222.6

* See pages 16 and 31 of text for explanation.

strongly depressed the translocation of P^{32} both in corn and beans. This has been explained as being due to the blocking of the phloem by stabilizing organic complexes in the phloem. This is also evidence that the P^{32} movement is by way of the phloem and not by way of the xylem. The small amount of phosphorus that appeared in other parts of the plant where the petiole was steamed may have reached the xylem by diffusion from the phloem. It also appears that the phosphorus was a little more readily admitted to the xylem when the puddle method was used than when the flap method was used.

More phosphorus entered the bean plant by the flap method than by the puddle method. The flap method gives better contact between the leaf and the applied phosphorus by cutting through any cuticle and pubescence that might serve as leaf protectors. With the puddle method these surface factors were not mechanically disturbed. Apparently the leaves of the corn plant were still sufficiently young that their surface leaf protectors were not developed enough to hinder absorption, for with corn neither method appeared to be superior over the other.

Summary of Experiment II. A very large percentage of the foliar applied phosphorus that is absorbed by young bean or corn plants is translocated by way of the phloem. Only one or two percent got out by way of the xylem, probably by diffusion.

The leaf flap method of making foliar applications of phosphorus to eleven-day-old bean plants produced far greater absorption and translocation of phosphorus than did the puddle method. Yet, the same two methods applied to eight-day-old corn plants produced no major differences.

Experiment III. The influence of nutritional levels in nutrient solutions on foliar absorption and translocation of phosphorus by corn plants

Rediske (1950) used red kidney beans to demonstrate that absorption and translocation of foliar applications of iron were strongly depressed by high levels of iron in the plant. Based on this finding, the question was asked, "Is the absorption and translocation of phosphorus influenced by the nutritional level of the plant?" King Croft (KY7) field corn was used in this study.

Method. The corn plants were germinated and transferred to the one gallon milk jars using the same growing nutrient solution as used in Experiment II. Four plants were placed in each jar. Three plants from each jar were used as a single replication. The fourth plant was used in the preparation of a radio-autograph at harvest time.

On the fifth day the eight experimental variables described in table 4 were applied. On the eighth day the second leaf blade from the base of the corn plant was laid out flat and the P^{32} solution applied by the puddle method. Due to decay of the P^{32}, 15 lambda were used per plant instead of the 10 lambda used earlier.

On the ninth day the corn plants were harvested, disected, labeled, and dried as described in the earlier study. In the same fashion the plants were pressed, assayed, corrected for self absorption and geometry, and the final results adjusted to August 5, 1957.

Results. The data collected in this experiment are recorded in table 5. The radio-autographs, which represent each pot of all treatments applied, are reproduced in figures 4 and 5.

Statistical treatment. A statistical study of the data was made and

Table 4. Concentrations in nutrient solutions including a pH of 5.0 and 0.5 p.p.m. of boron*

Variable†	Treatment number	$Ca(NO_3)_2$	KNO_3	$MgSO_4$	KH_2PO_4	Cu	Mn	Zn	Fe
		mM	mM	mM	mM	p.p.m.	p.p.m.	p.p.m.	p.p.m.
L P	5	1.25	1.25	0.5	0.005	0.02	0.5	0.05	0.005
H P	7	1.25	1.25	0.5	5.000	0.02	0.5	0.05	0.005
H Cu	18	1.25	1.25	0.5	0.005	0.20	0.5	0.05	0.005
H Mn	30	1.25	1.25	0.5	0.005	0.02	5.0	0.05	0.005
H Zn	42	1.25	1.25	0.5	0.005	0.02	0.5	0.50	0.005
H Fe	56	1.25	1.25	0.5	0.005	0.02	0.5	0.05	5.000
H Ca	69	25.00	1.25	0.5	0.005	0.02	0.5	0.05	0.005
H Mg	77	1.25	1.25	10.0	0.005	0.02	0.5	0.05	0.005

* All solutions were continuously aerated with washed compressed air.
† L represents low. H represents high. The L P variable is the control treatment.

Table 5. The millimicrocuries (muc) of P^{32} absorbed by corn plants and appearing in different parts of the plants 24 hours after a foliar application of phosphorus

Treatment and plant division	Replication A	Replication B	Replication C	Totals
	muc	muc	muc	muc
5 - R	292	426	565	1283
P	19	14	8	41
L	742	347	330	1419
A	818	523	773	2114
Total	1871	1310	1676	4857
7 - R	350	251	430	1031
P	20	17	22	59
L	272	131	188	591**
A	386	619	425	1430
Total	1028	1018	1065	3111*
18 - R	123	377	245	745*
P	5	3	8	16
L	624	271	407	1302
A	311	264	371	946**
Total	1063	915	1031	3009*
30 - R	428	664	749	1841*
P	23	26	29	78**
L	590	378	522	1490
A	1012	650	907	2569
Total	2053	1718	2207	5978
42 - R	343	526	450	1319
P	11	22	19	52
L	460	395	365	1220
A	93	333	251	677**
Total	908	1276	1085	3269*
56 - R	419	589	419	1427
P	14	11	8	33
L	682	656	425	1763
A	801	1067	843	2711
Total	1916	2323	1695	5934
69 - R	599	799	806	2204**
P	20	24	25	69*
L	333	445	322	1100
A	864	1301	1077	3242**
Total	1816	2569	2230	6615*

Table 5. (Continued)

Treatment and plant division	Replication			Totals
	A	B	C	
	muc	muc	muc	muc
77 - R	401	632	557	1590
P	33	15	17	65
L	473	380	238	1091
A	1067	1027	877	2971*
Total	1974	2054	1689	5717

* Significant at .05 level from corresponding plant part in treatment 5.

** Significant at .01 level from corresponding plant part in treatment 5.

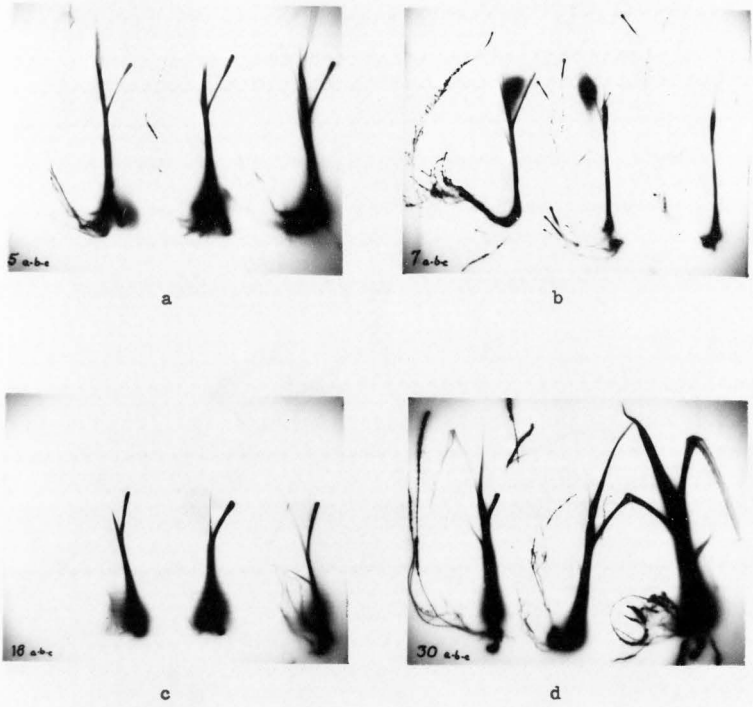

Figure 4. Radio-autographs of nine-day-old corn plants showing the effects of nutrient levels in nutrient solutions on the absorption and translocation of foliar applied phosphorus. (a) Treat. 5; standard, (b) Treat. 7; 5 mM KH_2PO_4, (c) Treat. 18; 0.2 p.p.m. Cu, (d) Treat. 30; 5.0 p.p.m. Mn.

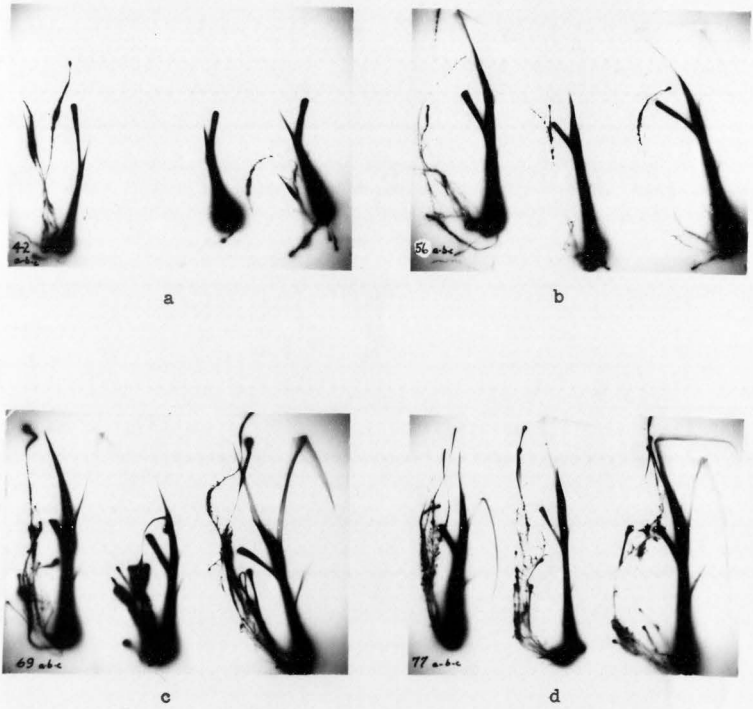

Figure 5. Radio-autographs of nine-day-old corn plants showing the effects of nutrient levels in nutrient solutions on the absorption and translocation of foliar applied phosphorus. (a) Treat. 42; 0.5 p.p.m. Zn, (b) Treat. 56; 5 p.p.m. Fe, (c) Treat. 69; 25 mM $CaNO_3$, (d) Treat. 77; 10 mM $MgSO_4$.

the results are summarized in table 6. Those values which are significantly different from the standard, treatment 5, are identified with asterisks. Any treatment might be compared with any other treatment using the L. S. D. values in table 6, and significant ones do occur, but this identification has not been made here.

Table 6. A comparison of the millimicrocuries of P^{32} absorbed by the divisions of the plant for the various treatments and their degree of significance

Variable	Treatment number	Plant division				
		R	P	L	A	Total
L P	5	1283	41	1419	2114	4857
H P	7	1031	59	591**	1430	3111*
H Cu	18	745*	16	1302	946**	3009*
H Mn	30	1841*	78**	1490	2569	5978
H Zn	42	1319	52	1220	677**	3269*
H Fe	56	1427	33	1763	2711	5934
H Ca	69	2204**	69*	1100	3242**	6615*
H Mg	77	1590	65	1091	2971*	2717
L. S. D. - .05		431	27	543	808	1333
L. S. D. - .01		598	37	753	1122	1849

* Significant at 5 percent level.
** Significant at 1 percent level.

Interpretation and discussion. The effect of the various treatments on the plants can be judged somewhat by observing the radio-autographs in figures 4 and 5.

When the plant parts receiving treatment 7 are compared to the standard, the plants receiving treatment 5, we find a significant reduction in the amount of P^{32} absorbed by division L of the plant specifically. Isotopic dilution would probably explain this since treatment 7 (table 4) is the one which received a high phosphorus application.

A study of radio-autographs a and b in figure 4 quickly confirms the reduced absorption indicated by the data. Another interesting observation during this study occurred in the plants receiving treatment 7. In these corn plants, as in the case of all other plants, the first step in harvest was to remove the P^{32} treated portion of the leaf about one inch below the point of treatment before the rest of the harvesting was performed. Within 20 minutes after this leaf end was removed, all plants receiving treatment 7 were extruding a little sap at the point where the leaf end was cut off. No plants in any other treatment exhibited this phenomenon. There is no explanation given here for this.

The plants receiving high copper applications in the nutrient solution, treatment 18 (c of figure 4), also showed a significant reduction in the absorption of the foliar applied phosphorus in the total plant and in divisions R and A specifically. This inhibition can probably be explained by the observed toxic effects of applied copper to the corn plants. The plants receiving treatment 18 exhibited greatly reduced vigor and produced a bronze to yellow color in the leaves. This reduced vigor and size can be detected in radio-autograph c of figure 4. The roots were not killed but were damaged somewhat. The toxic effects of the copper probably damaged the absorption and transport mechanism of the plant and thus accounts for the reduced translocation of the phosphorus.

The high rate of manganese received by the plants to which treatment 30 (d of figure 4) was applied statistically increased the amount of P^{32} absorbed by the roots, division R, and first leaf, division P, of the plant. This is not readily apparent when comparing radio-autograph

d with a of figure 4 but looks like a possibility. Manganeous phosphate, dibasic, is slightly soluble while manganeous phosphate, tribasic, is only very slightly soluble. The phosphorus may have moved into the roots by way of the phloem and part of it, a significant quantity, precipitated into one of these compounds before it could be moved to the upper part of the plant. Manganic phosphate is another possibility and it is a highly insoluble compound.

The plants receiving a high zinc application, treatment 42, also exhibited retarded phosphorus absorption. The plants also suffered from much reduced vigor and growth, visible in radio-autograph a in figure 5, and a bronze-yellow coloring as did the plants receiving the excess copper. Here again the induced reduction of phosphorus absorption was probably due to injury to the absorption and transport mechanism.

Treatment 56, one which provides a high level of iron in the nutrient solution, caused no significant inhibition or enhancement of P^{32} absorption over the standard. These plants (b of figure 5) exhibited extra good vigor and had by far the best color of all of the treatments. However, there apparently was not enough iron in the proper form in the plant to either inhibit phosphorus absorption from the treated leaf or concentrate it in the plant in some one part as some insoluble phosphorus compound. Since so much evidence has been acquired that extra phosphorus in a plant will precipitate iron in the plant, one would expect the extra iron in the plant would have a similar affect on the phosphorus. However this is not apparent here.

The statistical treatment of the data shows that the high calcium nitrate treatment, treatment 69, greatly enhanced the accumulation of P^{32} in all parts of the plant except the leaf receiving the applied P^{32}.

Whether this effect is due to the calcium or nitrate or both is not known but the efficiency of the plant for absorbing the P^{32} has been greatly improved over the standard. This large accumulation of P^{32} can be detected in radio-autograph c of figure 5. Since the plant would probably use the nitrate faster than the calcium, the plant sap would be expected to become less acid. The calcium could possibly combine with some of the phosphorus but as monocalcium phosphate it still would be readily soluble. Dicalcium phosphate would also probably be fairly readily soluble in the plant.

Treatment 77, high magnesium sulfate, improved the efficiency of the plant for the absorption of phosphorus in the upper parts. This is not apparent in the radio-autographs in figure 5. No other part was significantly affected.

Summary of Experiment III. High levels of phosphorus in the nutrient solution inhibited phosphorus absorption by the corn plants. This was probably due to isotopic dilution.

The high applications of either copper and zinc proved toxic to the plants by probably injuring the absorption and translocation mechanism of the plants, therefore accounting for the reduced phosphorus absorption.

The high manganese treatment caused an accumulation of phosphorus in the roots and first leaf, possibly by precipitation, before it could move to the upper parts of the plant in significantly large amounts.

The high calcium nitrate treatment had the greatest total effect of any of the treatments in increasing phosphorus absorption in the plant.

The high magnesium sulfate treatment significantly increased the amount of phosphorus absorbed in the top part of the plant.

Experiment IV. The effects of varying nutrient levels in nutrient solutions on the translocation of foliar applied iron

This is a continuation of Experiment I except for modifications. The number of treatments applied was cut to eight. A plant growth room was used in which temperature, relative humidity, light interval, and light intensity could be controlled.

Method. In July 1958 a new series of bean plants was started under the light, temperature, and relative humidity conditions used in Experiment III.

The bean seeds were dusted lightly with Semasan dust and germinated on paper towels. As soon as they had germinated they were selected for size and uniformity and placed in sterile sand to grow. When the plants were about four inches tall, which was about five days after they were started on the paper towels, they were selected for uniformity and placed in sterile one gallon milk jars just as in Experiment I except the number of plants per jar was reduced from three to one.

The nutrient solution used for growing medium was changed from that used in Experiment I to the following concentrations: one, six, four, and two millimolar ammonium acid phosphate, potassium nitrate, calcium nitrate and magnesium sulfate, respectively, along with 0.5, 0.05, 0.02, 0.01, and 1.0 p.p.m. boron, manganese, zinc, copper, molybdenum, and iron, respectively.

Four days after the plants were placed in the gallon jars, the solution was replaced. On the eighth day the eight variables were applied that were used on the corn in Experiment III (table 4). On the eleventh day after transplanting into the nutrient solution and three days after the experimental variables were applied, the Fe^{56} labeled

iron was applied to the middle leaflet of the oldest trifoliate leaf as ferric chloride using the flap method described in Experiment II. This method was used because of the greater translocation it offered. Twenty-four hours later the plants were harvested, sectioned, dried, and pressed as described in Experiment I. The assay was made using a well type thallium activated sodium iodide crystal instead of the flat faced crystal used in Experiment I.

Nine different replications were grown over a two-week period, the last four being grown together. One of these replications was used for radio-autographs.

Results. The data collected in this experiment are recorded in table 7. The radio-autographs which represent each of the treatments applied are reproduced in figures 6 and 7.

Statistical treatment. A statistical survey of table 7 gave no significant differences between the standard, treatment 5, and any other treatment applied. Since the plants were not all grown at the same time and since considerable variation occurred between the replications, it was considered desirable to convert all replications to the same relative level of iron; one-hundred was selected for the relative amount. Thus all values became a percentage of the total iron absorbed by that particular replication. Table 8 results from this conversion.

A statistical treatment of table 8 gave a great many significant differences between the standard, which was the low phosphorus treatment number 5, and other treatments summarized in table 9. It is to be noted that no statistical treatment of division P of the plant, which is made up of the primary leaves, was made. These played such a very small part in absorbing iron that it was considered unimportant to consider them as such.

Table 7. The millimicrograms of iron absorbed and translocated to different parts of the plants 24 hours after a foliar application of ferric chloride solution

Treatment and division number	Replications								Totals
	A	B	C	D	E	F	G	H	
5 - R	27.3	6.7	4.7	23.2	368.2	183.4	38.3	111.7	763.5
P	4.0	1.5	0.9	6.5	34.6	23.9	2.5	16.0	89.9
L	9.4	8.2	3.6	10.8	3043.1	1190.1	15.9	1930.0	6211.1
A	35.0	7.5	3.8	20.8	231.8	161.2	23.0	134.3	617.4
Total	75.7	23.9	13.0	61.3	3677.7	1558.6	74.7	2192.0	7681.9
7 - R	6.2	18.9	10.9	11.2	16.4	19.8	59.7	67.5	210.6
P	2.7	2.1	2.9	1.1	1.2	3.2	3.0	3.7	19.9
L	5.4	11.5	5.7	3.7	7.4	10.5	18.5	62.3	125.0
A	8.6	11.0	13.0	8.3	8.2	11.7	26.7	44.3	131.8
Total	22.9	43.5	32.5	24.3	33.2	45.2	107.9	177.8	487.3
18 - R	29.3	26.6	1.2	36.9	571.0	52.1	649.5	235.7	1602.3
P	6.0	2.3	0.7	0.4	31.1	1.4	15.6	9.1	66.6
L	11.1	26.8	73.0	10.8	1938.0	20.6	2968.0	60.8	5109.1
A	23.5	73.8	14.8	14.9	553.5	29.0	553.0	102.4	1364.9
Total	69.9	129.5	89.7	63.0	3093.6	103.1	4186.1	408.0	8142.9
30 - R	22.4	0.8	23.0	8.5	57.0	54.1	68.5	23.0	257.3
P	4.2	0.2	0.9	1.2	2.8	2.5	6.1	1.3	19.2
L	9.9	0.01	8.0	15.3	24.2	30.7	3875.1	16.4	3979.6
A	25.3	0.2	8.9	20.4	62.2	59.1	161.9	26.1	364.1
Total	61.8	1.21	40.8	45.4	146.2	146.4	4111.6	66.8	4620.2

Table 7. (Continued)

Treatment and division number	Replications								Totals
	A	B	C	D	E	F	G	H	
42 - R	20.3	5.7	2.2	7.4	1.1	2.3	24.6	13.1	76.7
P	4.0	0.5	0.1	0.5	0.4	0.8	3.4	0.3	10.0
L	59.3	33.5	12.7	47.1	3.5	77.0	37.8	245.9	516.8
A	29.2	27.8	0.2	45.6	0.1	2.8	28.3	18.0	152.0
Total	112.8	67.5	15.2	100.6	5.1	82.9	94.1	277.3	755.5
56 - R	23.9	81.5	14.1	10.0	36.7	28.3	7.1	22.2	223.8
P	5.1	19.9	4.5	1.2	4.6	4.4	1.0	5.1	45.8
L	9.0	470.1	59.5	2.8	15.1	13.0	1.8	68.3	639.6
A	10.1	30.6	14.0	2.4	22.4	15.7	2.7	13.2	111.1
Total	48.1	602.1	92.1	16.4	78.8	61.4	12.6	108.8	1020.3
69 - R	17.7	15.6	6.5	11.1	95.3	150.4	59.3	47.2	403.1
P	3.4	4.5	2.2	3.8	18.6	7.1	3.4	3.6	46.8
L	10.1	281.6	4.6	7.5	499.4	50.4	42.8	19.9	916.3
A	20.8	17.6	3.0	9.4	97.6	104.5	32.3	42.1	327.3
Total	52.0	319.5	16.3	31.8	710.9	312.4	137.8	112.8	1693.5
77 - R	26.8	15.0	5.6	16.7	74.9	27.0	465.9	4.9	636.8
P	2.9	2.6	1.4	5.0	7.9	2.3	46.2	1.6	69.9
L	18.6	7.1	4.2	1.0	63.3	11.0	9580.3	2.7	9688.2
A	23.9	7.7	3.6	13.0	63.3	25.4	423.9	5.6	566.4
Total	72.2	32.4	14.8	35.7	209.4	65.7	10516.3	14.8	10961.3

a b c d

Figure 6. Radio-autographs of 12-day-old bean plants showing the effects of nutrient levels in the nutrient solution on the translocation of foliar applied iron. (a) Treat. 5; low phosphorus; standard, (b) Treat. 7; high phosphorus, (c) Treat. 18; high copper, (d) Treat. 30; high manganese.

Figure 7. Radio-autographs of 12-day-old bean plants showing the effects of nutrient levels in the nutrient solution on the translocation of foliar applied iron. (a) Treat. 42; high zinc, (b) Treat. 56; high iron, (c) Treat. 69; high calcium, (d) Treat. 77; high magnesium.

Table 8. The percentage of the total iron absorbed by a complete replication contained in each individual sample as calculated from table 7

Treatment and division number	Replications								Totals
	A	B	C	D	E	F	G	H	
5 - R	5.30	0.55	1.49	6.13	4.63	7.72	0.20	3.33	29.35
P	0.78	0.12	0.29	1.72	0.43	1.01	0.01	0.48	4.84
L	1.82	0.67	1.15	2.85	38.25	50.09	0.08	57.47	152.38
A	6.79	0.61	1.21	5.50	2.91	6.79	0.12	4.00	27.93
Total	14.69	1.96	4.13	16.20	46.23	65.61	0.41	65.27	214.50
7 - R	1.20	1.55	3.47	2.96	0.21	0.83	0.31	2.01	12.54
P	0.52	0.17	0.92	0.29	0.02	0.12	0.02	0.11	2.19
L	1.05	0.94	1.81	0.98	0.09	0.44	0.10	1.86	7.27
A	1.67	0.90	4.13	2.19	0.10	0.49	0.14	1.32	10.94
Total	4.44	3.57	10.34	6.42	0.42	1.90	0.56	5.29	32.94
18 - R	5.68	2.18	0.38	9.75	7.18	2.19	3.37	7.02	37.75
P	1.16	0.19	0.22	0.11	0.39	0.06	0.08	0.27	2.49
L	2.15	2.20	23.22	2.85	24.36	0.87	15.42	1.81	72.88
A	4.56	6.05	4.71	3.94	6.96	1.22	2.87	3.05	33.36
Total	13.56	10.62	28.53	16.64	38.89	4.34	21.75	12.15	146.48
30 - R	4.35	0.07	7.32	2.25	0.72	2.28	0.36	0.68	18.03
P	0.81	0.02	0.29	0.32	0.04	0.11	0.03	0.04	1.63
L	1.92	0.00	2.54	4.04	0.30	1.29	20.13	0.49	30.71
A	4.91	0.02	2.83	5.39	0.78	2.49	0.84	0.78	18.04
Total	11.99	0.10	12.98	11.99	1.84	6.16	21.36	1.99	68.41

Table 8. (Continued)

Treatment and division number	Replications								Totals
	A	B	C	D	E	F	G	H	
42 - R	3.94	0.47	0.70	1.96	0.01	0.10	0.13	0.39	7.70
P	0.78	0.04	0.03	0.13	0.01	0.03	0.02	0.01	1.02
L	11.51	2.75	4.04	12.44	0.04	3.24	0.20	7.32	41.54
A	5.67	2.28	0.06	12.05	0.00	0.12	0.15	0.54	20.87
Total	21.89	5.53	4.83	26.58	0.06	3.49	0.49	8.26	71.13
56 - R	4.64	6.68	4.48	2.64	0.46	1.19	0.04	0.66	20.79
P	0.99	1.63	1.43	0.32	0.06	0.19	0.01	0.15	4.78
L	1.75	38.55	18.92	0.74	0.19	0.55	0.01	2.03	62.74
A	1.96	2.51	4.45	0.63	0.28	0.66	0.01	0.39	10.89
Total	9.33	49.37	29.29	4.33	0.99	2.58	0.07	3.24	99.20
69 - R	3.43	1.28	2.07	2.93	1.20	6.33	0.31	1.41	18.96
P	0.66	0.39	0.70	1.00	0.23	0.30	0.02	0.11	3.42
L	1.96	23.09	1.46	1.98	6.28	2.12	0.22	0.59	37.70
A	4.04	1.44	0.95	2.48	1.23	4.40	0.17	1.25	15.96
Total	10.09	26.20	5.18	8.40	8.94	13.15	0.72	3.36	76.04
77 - R	5.20	1.23	1.78	4.41	0.94	1.14	2.42	0.15	17.27
P	0.56	0.21	0.45	1.32	0.10	0.10	0.24	0.05	3.03
L	3.61	0.58	1.34	0.26	0.80	0.46	49.78	0.08	56.91
A	4.64	0.63	1.15	3.43	0.80	1.07	2.20	0.17	14.09
Total	14.01	2.66	4.71	9.43	2.64	2.77	54.64	0.44	91.30

Table 9. A comparison of the relative amounts of iron absorbed by the divisions of the plant for the various treatments and their degree of significance

Variable	Treatment number	Plant division				
		R	P	L	A	Total
Low P	5	29.35	4.84	152.38	27.93	214.50
High P	7	12.54*	2.19	7.27*	10.94*	32.94**
High Cu	18	37.75	2.49	72.88	33.36	146.48
High Mn	30	18.03	1.63	30.71*	18.04	68.41*
High Zn	42	7.70*	1.02	41.54*	20.87	71.13*
High Fe	56	20.79	4.78	62.74	10.89*	99.20
High Ca	69	18.96	3.42	37.70*	15.96	76.04*
High Mg	77	17.27	3.03	56.91	14.09	91.30
L. S. D. - .05		16.31		104.7	16.06	127.40
L. S. D. - .01		21.76		139.43	21.43	169.93

* Significant at 5 percent level.
** Significant at 1 percent level.

Interpretation and discussion. A study of the values developed in table 9 show that the high phosphorus treatment significantly decreased the amount of iron absorbed by all parts of the plant. This is explained by the phosphorus having precipitated the iron at the site of absorption or soon thereafter and thus the labeled iron not appearing in other parts of the plant. This is in agreement with the work of Rediske and Biddulph (1953). These investigators also found that high levels of iron in the nutrient solution retarded the absorption of foliar applied iron. The data in table 9 show a similar tendency; however, it is not significant at the five percent level, except in the top portions of the plant.

The high level of copper applied here had no significant effects on the absorption of foliar applied iron, nor did it show its toxic effects as strongly as it did in the corn of Experiment III. Some reduced vigor was noticed, however. The high levels of manganese and zinc also

retarded the growth rate of the plant and showed some symptoms of toxicity. However, the growth rate of the plants was retarded little more than that of the standards which were suffering only from phosphorus and iron deficiencies. The absorption of foliar applied iron by those plants receiving high levels of manganese and zinc was significantly retarded in the plants as a whole. The inhibited absorption and translocation of foliar applied iron by the plants receiving the high levels of manganese and zinc might be due to the reduced vigor of the translocation mechanism of the plant. Koontz and Biddulph (1957) have found that reduced plant vigor by phosphorus deficiency seemed to retard absorption by the plant of foliar applied phosphorus.

The summary in table 9 also points out another significant retarded movement of foliar applied iron in the plant. High calcium has been found in excess in chlorotic plants in which carbohydrates were accumulating due to a failure of the plant to transport the carbohydrates away. This suggests a breakdown of the transport mechanism. However, Iljin (1952) had attributed the high calcium level to being as a result and not a cause of the chlorosis. High nitrogen levels were also associated with his findings. The high calcium levels used in Experiment IV were acquired through using calcium nitrate. Thus, the high calcium or high nitrate levels may each be aiding in inhibiting the movement of iron by somehow blocking the transport processes. Cain (1954) observed that calcium nitrate as the only source of nitrogen for blueberries produced chlorosis not unlike iron deficiency. However, when he supplied some ammonia nitrogen as a part of the nitrogen the chlorosis was prevented or, where the chlorosis had occurred, cured.

High magnesium levels have caused definite tendencies toward

retarded iron movement. While none of these are significant at the five percent level they come very close. Iljin (1952) has found that the same relationships exist for magnesium as for calcium.

The radio-autographs show conflicting differences when compared with the data. However, it must be remembered that the interpretation of the data comes as a result of the consideration of eight different replications with considerable variability one from another. The radio-autographs represent only one replication, a ninth one, which was not included in the statistically treated data and may be one that in parts varied considerably from the analyzed data.

It would seem that to make radio-autographs agree with data as variable as this has been, it would be necessary to produce a radio-autograph of every plant, followed by an assay of the various plant parts.

A possible cause for some of the variation in the data might be that the plants did not all receive the foliar-applied iron the same time of day. Work by Hanson and Biddulph (1953) suggests that the time of treating during the day might be of very great concern. They have found that the bean plant absorbs its maximum quantities of phosphorus from solution culture during mid-day. This would suggest that the morning foliar applications might have been more efficiently absorbed than the afternoon applications. Koontz and Biddulph (1957) also found that the rate of drying of the foliar application was important. The leaf flap used in Experiments II, III, and IV was stuck into a tiny vial in which extra distilled water was placed with the applied phosphorus or iron to make a total of approximately 0.1 milliliters. Drying in these instances would not be rapid. However, if the plant received its foliar

application during the late afternoon the treated site would be dry before the plant reached another period of rapid absorption. Hanson and Biddulph (1953) found that the absorption rate of the bean plant was low of nights even if the light was not cut off.

Summary of Experiment IV. The high levels of phosphorus, manganese, zinc, iron, and calcium nitrate in the nutrient solution have individually significantly decreased the absorption and translocation of foliar applied iron.

The reduced translocation by the phosphorus was probably due to a precipitation of the iron at the site of absorption. The manganese and zinc probably inhibited translocation due to their toxic effects. The high level of iron probably inhibited translocation as a result of isotopic dilution. The high level of calcium nitrate apparently created some type of block in the plants' transport mechanism, thus inhibiting the translocation of iron from the leaflet receiving the foliar application. The nitrate of the calcium nitrate treatment may have been entirely responsible for this block.

DISCUSSION OF EXPERIMENTAL RESULTS

The objective of these investigations was to determine the effects of varying levels of nutrients in nutrient solution on the absorption and translocation of foliar applied Fe^{59} labeled iron and P^{32} labeled phosphorus in red kidney beans and corn respectively.

Greenhouse work was initiated during the winter, spring, and summer of 1956. Numerous nutrient treatments were applied in nutrient cultures along with two forms of iron, DTPA-Fe and $FeCl_3$, applied to the middle leaflet of the oldest trifoliate leaf of eleven-day-old plants using the puddle method. At the termination of the greenhouse work during the summer of 1956 radio-autographs were made for a number of the treatments.

A statistical treatment of the data indicated that the chelated iron was absorbed in significantly greater amounts by the bean plants than the ferric chloride. This was attributed to the greater solubility of the chelated iron in the plant. Thus it would seem that if iron-chlorosis in a plant, especially in beans, were to be corrected by some sort of a spray application, a chelated iron compound might well be worthy of consideration for maximum results. However, there are many chelated and non-chelated iron compounds which could be considered.

It was also found that at pH 7.5 in the nutrient solution the plant was able to absorb and translocate more iron from foliar applications than at pH 5.0. This has been explained by the inability of the plant to get iron out of the nutrient solution due to its reduced solubility at pH 7.5, thus leaving the plant under a stress for iron. This condition allowed for maximum use of iron applied to a leaf. However,

the pH's above 7 in calcareous soils where lime-induced iron chlorosis occurs have not necessarily inhibited iron uptake by the plant from the soil. Many such chlorotic plants have been found to contain sufficient iron for normal plant development but in an inactive form. Thus the situation in lime-induced chlorosis seems to be a physiological phenomenon while in nutrient solutions it is apparently a cultural phenomenon; the first involves the inactivation of the iron after it enters the plant while the latter involves inactivation before it enters the plant.

High and low levels of phosphorus, copper, zinc, manganese, iron, calcium nitrate, and magnesium sulfate were compared to determine their influence on the mobility of iron from the point of foliar application. None of these proved to significantly alter the quantity of iron absorbed from the treated leaflet and transported to other parts of the plant. Since previous work by other investigators indicated that the concentrations of both phosphorus and iron influence the mobility of foliar applied iron some experimental technique was suspected. Other investigators found that temperature influenced a plant's reactions. Also they found that plants were seasonal in their responses. Thus it was desirable to move the experiments to an environment where temperature, light intensity, light duration, and relative humidity could be controlled.

During the summer of 1957 a plant growth room was established in which the light, temperature, relative humidity, and day length could be controlled. A short experiment was conducted to determine the merits of the leaf flap and puddle methods of foliar applications. The results very much favored the leaf flap method of application to eleven-day-old beans. However, neither seemed to be superior to the other in efficiency of utilization of foliar applied phosphorus when used on

eight-day-old corn plants. The puddle method was used in the greenhouse work because it lent itself to fair protection against insects which were in the greenhouse, especially during nights when the lights were on.

At the same time it seemed desirable to demonstrate whether the foliar applied elements were transported by way of the xylem or phloem. Phosphorus was used on corn and beans. The steam treatment at a point below the site of application of the phosphorus of both the corn and beans greatly inhibited the movement of the phosphorus. This suggests that any plant disease or disorder which might cause an inactivation of the phloem would practically stop movement of materials out of the leaves into the roots. Since the leaf parts beyond the point of steaming remained turgid, it was evident that the xylem remained open to the flow of water and its solutes.

During the summer of 1958 an experiment was initiated under controlled light, relative humidity, temperature, and day length to check out part of the greenhouse work of two years before. A pH of 5.0 was the only hydrogen ion level used. Ferric chloride labeled Fe^{59} was the only foliar application used. The treatment which contained low phosphorus and iron levels and otherwise normal levels of nutrients was used as the standard. Seven other treatments applied utilized high levels of phosphorus, copper, manganese, zinc, iron, calcium nitrate, and magnesium sulfate. The data proved to be erratic. However, high levels of phosphorus, manganese, zinc, iron, and calcium in the nutrient solution did significantly retard the absorption and translocation of foliar applied ferric chloride. High magnesium levels showed tendencies toward retarding iron mobility but it was not significant at the five percent level.

No treatment significantly enhanced the movement of the iron over the standard.

The inhibiting effect of the phosphorus has been attributed to its precipitating the iron as ferric phosphate at the site of absorption and preventing its translocation. The high levels of manganese and zinc have probably inhibited the translocation as a result of their toxicity, probably by destroying or disrupting the plant's transporting mechanism. It is not likely that an excess of available manganese or zinc will occur in the calcareous soils of the world due to the higher pH values. But excess does occur in acid soil where reduced iron translocation or iron deficiency has been reported. The high iron level in the plants probably had aleviated the plant's need for iron; thus its absorption from the treated leaflet was inhibited.

The inhibited absorption and translocation of foliar applied iron as a result of the high calcium nitrate treatment was probably due as much to the excess nitrate as due to the calcium. Both have been found associated with iron chlorosis in a number of plants. Magnesium has also been found in associations similar to those of calcium. Calcareous soils could supply the plant with an abundance of calcium and perhaps magnesium but not necessarily an excess of nitrates. However, this does not rule out the possibility of the failure of the plant to assimilate the nitrates taken up and thus accumulate them in the plant sap. Over fertilization with nitrate fertilizers might aid in building up the nitrate level in the plant.

The radio-autographs made of this experiment do not agree with the experimental data. This might be explained by observing that one replication of radio-autographs was produced while eight replications of data

were produced. Had all of the plants for the radio-autographs and the data been grown all at one time they would have more nearly received exactly the same treatments and would have possibly been in greater agreement. Since recent investigations have indicated that the plant may be at its maximum efficiency in its absorption and translocation of nutrients at mid-day, those plants receiving their foliar applications during the morning could be expected to absorb and translocate larger quantities. However, this would not be the full cause since one replication of a group together may show unusually low or high absorption and translocation when compared to the rest. Should the radio-autographs be made of one of these unusual replications, it certainly could not agree with the average of the experimental data. It seems that to properly use radio-autographs for illustrating purposes, it would be essential to radio-autograph all plants. This could then be followed with the plant assay. Under that procedure the radio-autographs of the plants most nearly representing the average results could then be selected and used for illustration.

An experiment similar to the above one was performed using nine-day-old corn plants instead of beans and using foliar application of phosphoric acid. Here the high phosphorus treatment inhibited the absorption of foliar applied phosphorus when compared to the standard, probably due to isotopic dilution. The high applications of copper and zinc also inhibited the translocation of phosphorus, probably due to the toxic effects of these metals though they only occurred in the nutrient solution at concentrations of 0.20 and 5.0 parts per million respectively. Though toxic effects were observed at 0.5 parts per million, manganese aided translocation of phosphorus. This would indicate that should manganese be readily available to plants growing in a calcareous soil, it

might aid in the absorption of phosphorus from the leaf. This phosphorus could, along with the extra manganese and its high oxidation potential, thus aid in making iron absorbed by the plant from the soil inactive and unavailable to plant functions.

The high treatments of calcium nitrate and magnesium sulfate enhanced the translocation of the foliar applied phosphorus with the calcium nitrate producing the strongest effect. This is just opposite to its effects on the foliar applied iron. Thus it would suggest that the transport mechanism of the plant is not fully blocked. The phosphorus was applied as an anion while the iron was applied as a cation. This difference appears to be significant.

The radio-autographs of this experiment represent one plant of each replication and are in fairly good agreement. All plants for this experiment were grown in one group.

SUMMARY OF ALL EXPERIMENTS

Greenhouse work was performed in 1956 using individual variables of pH, phosphorus, copper, manganese, zinc, iron, calcium nitrate, and magnesium sulfate in the nutrient solution in which red kidney beans were grown to determine their effects on the translocation of foliar applied ferric chloride and DTPA-Fe. The pH of 7.5 enhanced the translocation of foliar applied iron over pH 5.0. The foliar applied DTPA-Fe enhanced the translocation of iron over the foliar applied ferric chloride when compared to the standard which received low phosphorus and iron in the nutrient solution. No interactions nor metal treatments gave significant effects on translocation. Data was quite variable, possibly due to the many uncontrolled environmental factors.

During the summer of 1957 a plant growth room was completed. Initial experiments proved leaf flap method of making foliar applications of phosphorus to beans was superior to the puddle method. Little difference was observed when used on corn. Steam girdling experiments also demonstrated that movement out of the leaf was by way of the phloem. Experiments were also conducted to determine the effect two different levels of phosphorus, copper, manganese, zinc, iron, calcium nitrate, and magnesium sulfate in nutrient cultures had on the absorption and translocation of the foliar applied phosphoric acid in corn. High levels of phosphorus, copper, and zinc inhibited the translocation of the foliar applied phosphorus while high levels of manganese, calcium nitrate, and magnesium sulfate enhanced it.

During the summer of 1958 work was performed in a plant growth room to study element effects of the greenhouse work of 1956. Only pH 5.0 was used with ferric chloride as the foliar application. High levels of phosphorus, manganese, zinc, iron, and calcium nitrate significantly inhibited the translocation of iron over the standard which received low levels of phosphorus and iron in the nutrient solution.

LITERATURE CITED

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

Biddulph, O. 1941. Diurnal migration of injected radiophosphorus from bean leaves. Am. Jour. Bot. 28:348-352.

Biddulph, O. 1948. Interrelations between iron and phosphorus in plant nutrition. Ore. St. Col., Corvallis, Biol. Colloquim, Nutrition, 24-27.

Biddulph, O. 1951. The translocation of minerals in plants. In: Mineral nutrition of plants. E. Truog, Ed., Madison: Univ. of Wisconsin Press. 261-275.

Biddulph, O., R. Cory, and S. Biddulph. 1956. The absorption and translocation of sulfur in red kidney bean. Plant Physiol. 31: 28-33.

Biddulph, O., and Jane Markle. 1944. Translocation of radiophosphorus in the phloem of the cotton plant. Am. Jour. Bot. 31:65-70.

Biddulph, S. F. 1956. Visual indications of S^{35} and P^{32} translocation in the phloem. Am. Jour. Bot. 43:143-148.

Bukovac, M. J., and S. H. Wittwer. 1957. Absorption and mobility of foliar applied nutrients. Plant Physiol. 32:428-435.

Cain, J. C. 1954. Blueberry chlorosis in relation to leaf pH and mineral composition. Proc. Am. Soc. Hort. Sci. 64:61-70.

Camp, A. F., B. R. Fudge, V. C. Jamison, John R. Sites, Charles R. Stearns, Jr., H. O. Sterling, W. L. Thompson, and R. F. Suit. 1945. Physiological studies. Fla. Agr. Exp. Sta. Ann. Rept. 164-165.

Chandler, W. V., and G. D. Scarseth. 1941. Iron starvation as affected by overphosphating and sulfur treatment on Houston and Sumter clay soils. Jour. Am. Soc. Agron. 33:93-104.

Chapman, H. D., G. F. Liebig, and A. P. Vaneslow. 1939. Some nutritional relationships as revealed by a study of mineral deficiency and excess symptoms on citrus. Soil Sci. Soc. Am. Proc. 4:196-200.

Christensen, Paul D. 1948. Crop response to application of copper sulphate on salty soils. (M. S. Thesis, Dept. of Agronomy) Utah State Agr. Col.

Church, A. H. 1879. A chemical study of vegetable albinism. Jour. Chem. Soc., Transaction 35:33-41.

Epstein, E., and P. R. Stout. 1951. The micronutrient cations iron, manganese, zinc, and copper: their uptake by plants from the absorbed state. Soil Sci. 72:47-65.

Franco, C. M., and W. E. Loomis. 1947. The absorption of phosphorus and iron from nutrient solutions. Plant Physiol. 22:627-634.

Gauch, H. G., and C. H. Wadleigh. 1945. Effect of high concentration of sodium, calcium chloride, and sulfate on ionic absorption by bean plants. Soil Sci. 59:139-153.

Gerretsen, F. C. 1950. Manganese in relation to photosynthesis . II. Reduction-oxidation potentials of illuminated crude chloroplast suspensions. Plant and Soil 2:159-1953.

Gile, P. L. 1916. Chlorosis of pineapples induced by manganese and carbonate of lime. Sci. 44(N.S.):855-857.

Gile, P. L., and J. O. Carrero. 1916. Immobility of iron in the plant. U. S. Dept. Agr. Jour. Agr. Research 7:83-87.

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

Haas, A. R. C. 1932. Injurious effects of manganese and iron deficiencies on the growth of citrus. Hilgardia 7:181-206.

Haas, A. R. C. 1942. Lime-induced chlorosis of citrus in relation to soil factors. Plant Physiol. 17:27-51.

Hanson, John B., and O. Biddulph. 1953. The diurnal variation in the translocation of minerals across bean roots. Plant Physiol. 28: 356-369.

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

Holmes, R. S., and J. C. Brown. 1957. Iron and soil fertility. Soil, U. S. D. A. Yearbook 111-115.

Iljin, W. S. 1944. Metabolism of the grapevine during lime chlorosis. Chem. Abs. 38:6332.

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

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

Johnson, M. O. 1917. Manganese as a cause of the depression of the assimilation of iron by pineapple plants. Ind. and Eng. Chem. 9: 47-49.

Koontz, H., and O. Biddulph. 1957. Factors affecting absorption and translocation of foliar applied phosphorus. Plant Physiol. 32: 463-470.

Lindner, R. C., and C. P. Harley. 1944. Nutrient inter-relations in lime-induced chlorosis. Plant Physiol. 19:420-439.

Lindsay, Willard L. 1953. Effect of bicarbonate ion and root aeration on lime-induced chlorosis. (M. S. Thesis, Dept. of Agronomy) Utah State Agr. Col.

Lohnis, Marie P. 1951. Manganese toxicity in field and market garden crops. Plant and Soil 3:193-222.

McGeorge, W. T. 1946. Soil properties contributing to citrus chlorosis as revealed by seedling tests. Ariz. Agr. Exp. Sta. Tech. Bul. 112.

McGeorge, W. T. 1948. Nutrient interrelations in lime-induced chlorosis as revealed by seedling tests and field experiments. Ariz. Agr. Exp. Sta. Tech. Bul. 116.

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

McGeorge, W. T. 1951. Lime-induced chlorosis on western crops. Better Crops with Plant Food 35(5):17.

Milad, Youssef. 1939. Physiological studies in lime-induced chlorosis. Ministry Agr., Egypt, Tech. Sci. Serv. Bul. 211.

Morris, H. D., and W. H. Pierre. 1947. The effect of calcium, phosphorus, and iron on the tolerance of lespedeza to manganese toxicity in culture solutions. Soil Sci. Soc. Am. Proc. 12:382-386.

Olver, W. F. 1952. Absorption and translocation of phosphorus by foliage. Sci. Agr. 32:427-432.

Olsen, Carsten. 1935. Iron absorption and chlorosis in green plants. Compt. Rend. Lab. Carlsberg Ser. Chem. 21:15-63.

Olson, R. V., and C. W. Carlson. 1949. Iron chlorosis of sorghums and trees as related to extractable soil iron and manganese. Soil Sci. Soc. Am. Proc. 14:109-112.

Oserkowsky, J. 1933. Quantitative relations between chlorophyll and iron in green and chlorotic pear leaves. Plant Physiol. 8:449-468.

Rediske, John H. 1950. The translocation of radioiron in the bean plant. (Ph. D. Thesis, The State College of Washington)

Rediske, John H., and O. Biddulph. 1953. The absorption and translocation of iron. Plant Physiol. 28:576-593.

Rogers, C. H., and J. W. Shive. 1932. Factors affecting the distribution of iron in plants. Plant Physiol. 7:227-252.

Sideris, C. P. 1950. Manganese interference in the absorption and translocation of radioactive iron (Fe59) in Ananas comosus. Plant Physiol. 25:307-321.

Silberstein, O., and S. H. Wittwer. 1951. Foliar application of phosphatic nutrients to certain vegetable crops. Proc. Am. Soc. Hort. Sci. 58:179-190.

Somers, I. I., and J. W. Shive. 1942. The iron-manganese relation in plant metabolism. Plant Physiol. 17:582-602.

Stout, P. R., and D. R. Hoagland. 1939. Upward and lateral movement of salt in certain plants as indicated by radioactive isotopes of potassium, sodium, and phosphorus absorbed by roots. Am. Jour. Bot. 26:320-324.

Swanson, C. A., and J. B. Whitney, Jr. 1953. Studies on the translocation of foliar-applied P^{32} and other radioisotopes in bean plants. Am. Jour. Bot. 40:816-823.

Thorne, D. W. 1941. Factors influencing the solubility of iron and phosphorus in chlorotic and non-chlorotic areas of Hyrum clay loam. Iowa State Col. Jour. Sci. 15:433-445.

Thorne, D. W., and F. B. Wann. 1950. Nutrient deficiencies in Utah orchards. Utah Agr. Exp. Sta. Bul. 338.

Thorne, D. W., and F. B. Wann. 1953. Chlorosis resistant shrubs. Farm and Home Sci. 14:16-17.

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

Vyunov, S. 1937. Chlorosis of fruit plants. Proc. Conf. Soil Sci., Saratov 2:343-344.

Wallace, T. 1928. Investigations on chlorosis of fruit trees; II. The composition of leaves, bark and wood of current season's shoots in cases of lime-induced chlorosis. Jour. Pomol. and Hort. Sci. 7: 172-183.

Wallace, T., and C. E. T. Mann. 1926. Investigations on chlorosis of fruit trees. I. The composition of apple leaves in cases of lime-induced chlorosis. Jour. Pomol. and Hort. Sci. 5:115-123.

Warnock, Robert E. 1952. The iron content of some plants as influenced by conditions associated with lime-induced chlorosis. (M. S. Thesis, Dept. of Agronomy) Utah State Agr. Col.