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BOTANY

The Effect of Iron Deficiency on the Absorption and Translocation of Iron and Phosphorus by Soybean Plants [1]

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It has been recognized that part of the problem of "iron chlorosis" may be due to restricted absorption or abnormal transport of iron (1). This study is concerned with some problems associated with the uptake and translocation of iron and phosphorous in iron-deficient and in healthy soybean plantss, *Glycine max* (L.) Merr.

The factors causing iron chlorosis may be related to the concentration of the bicarbonate ion, the concentration and the balance of the micro- and macro-elements, environmental factors, and the chemical status of the iron. These factors may cause plant species and varieties to differ in their susceptibility to iron-induced chlorosis (2, 3, 4).

An increase in the bicarbonate ion concentration in the soil reduced the movement of iron into the leaves and stem and resulted in an accumulation of iron in or on the roots (5, 9). A reduced phosphorus content in the roots may also result from this condition (5). Wadleigh and Brown (12) reported that chlorotic plants seemed to accumulate large amounts of iron and phosphorus in the roots and conductive tissue of the plants, but a smaller amount of these elements in the leaf parenchyma. These observations suggest that ferric-phosphate precipitation may have occurred in the conductive tissue of the plants associated with the iron-induced chlorosis, caused in part by a high bicarbonate ion concentration in the soil. Lindner and Harley (8) found a higher iron content in chlorotic leaves than in non-chlorotic leaves. They proposed that the iron is deposited as ferric phosphate in or along the veins in the leaf. This chlorosis could be overcome by decreasing the supply of phosphate or increasing the calcium supply.

Sideris and Young (11) also found that the uptake and translocation of iron in pineapple was reduced by phosphate due to precipitation.

Rediske and Biddulph (10) showed that a ferric-phosphate precipitant would readily form in a nutrient solution if an excess of phosphorus was present. At an equimolar concentration of iron and phosphorus in the nutrient solution no precipitant was formed.

Kessler and Moscicki (7) found that triiodobenzoic acid (TIBA) increased the transport of iron. The effect of TIBA upon the transport of iron was followed by the synthesis of chlorophyll in chlorotic leaves of peach and plum trees growing in high lime soils. Only localized green spots of chlorophyll are produced when iron sulfate is sprayed on the foliage compared to a complete re-greening that was produced when the leaf was treated with TIBA prior to the iron application. A 100 ppm treatment of TIBA increased the chlorophyll content, presumably by freeing immobilized iron in the leaf. Iron added after the treatment with TIBA was more effective in promoting synthesis of chlorophyll than iron alone. Maleic hydrazide, another auxin inhibitor, did not promote iron transport.

To further our understanding of the nature of iron deficiency chlorosis in plants experiments were made with the following objectives:

1. To determine the effect of different levels of iron supplied to the roots on the uptake and translocation of chelated and non-chelated iron by soybeans.
2. To determine whether 2,3,5-triiodobenzoic acid effects the translocation of iron from leaves of healthy and iron-deficient soybeans.
3. To determine the effect of different levels of iron supplied to the roots on the uptake and translocation of phosphorus by the roots or leaves of soybeans.

MATERIALS AND METHOD: *Preparation of plant material.* Seeds of *Glycine max* var. Ottawa Mandarin were allowed to germinate between wet paper towels. After 5 days the seedlings were transferred to tanks containing nutrient solutions.

Three nutrient solution designated as -Fe, 1X, and 10X were prepared. Each contained 2 ml per L of 1 M KH_2PO_4, KNO_3, and $MgSO_4 \cdot 7\ H_2O$ stock solutions, 3 ml per L of a 1 M $Ca(NO_3)_2 \cdot 4\ H_2O$ stock solution and 1 ml per L of micronutrient stock solution containing 2.5 g H_3BO_3, 1.5 g $MnCl_2 \cdot 4\ H_2O$, 0.1 g $ZnCl_2$, 0.05 g $CuCl_2 \cdot 2\ H_2O$, and 0.05 g MoO_3 per L.

A stock solution containing 24 g per L of Versenol 120 Iron Chelate,[3] containing 9% elemental iron was also prepared. One ml of this Versenol Iron Chelate stock solution was added to the 1X nutrient solution and 10 ml of this stock solution was added to the 10X nutrient solution. None was added to the -Fe nutrient solution.

Plants were allowed to grow in aerated nutrient solution in a controlled environment room at a temperature of 24° C ± 1° C and a relative humidity of 45% ± 3%

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[3] Obtained from Dow Chemical Co., Midland, Michigan.

during a 12-hour light cycle. The fluorescent and incandescent light provided about 1500 ft-c illumination at plant level. During the 12-hour dark cycle, a temperature of $18° C \pm 1° C$ and a relative humidity of $97\% \pm 3\%$ was maintained.

HARVEST TECHNIQUES: At 3 weeks of age the healthy plants had one more set of trifoliate leaves than did the chlorotic plants. Plant height measurements recorded in the results are given in centimeters (cm) from the cotyledonary node to the shoot apex. In the treatments where radioactive phosphorus or iron was applied to aerial parts of 3-week-old soybean plants, the plants were removed from the tanks containing the mineral solutions and placed four per cup in 6-oz. waxed cups containing 150 ml of the complete mineral solution per cup before treatment. When radioactive phosphorus or iron was added to the mineral solution, at harvest the plant roots were rinsed three times in water made acid with dilute HCl before being dried. Following the absorption of the radioisotope, the plants were cut into various parts as shown in Figure 1. These plant parts were then dried for 24 hours at 110° C, weighed, completely digested with nitric acid, and the thin dry residue counted with a Tracerlab Superscaler.

Additional details of individual experiments will be given with the experimental results.

EXPERIMENTAL RESULTS: *Plant growth in relation to iron supply.*

When plants were grown in the -Fe solution they became chlorotic and their growth was reduced. Plants grown in the 10X solution had sufficient iron and increased in height and dry weight 100% over the chlorotic -Fe plants by the end of 3 weeks (Table 1). Plants

TABLE 1. The effect of the Iron Supply in the Nutrient Solution On the Height and Dry Weight of 3-Week-Old Soybean Plants.

Iron status of the nutrient solution [a]	Plant height [b] (cm)	Dry weight per plant [c] (gm)
-Fe	15	0.2804
1X	25	0.3719
10X	35	0.5953

[a] See materials and methods for explanation of the symbols -Fe, 1X, and 10X.

[b] Figures are an average of two experiments with 16 plants per treatment.

[c] Figures are an average of two experiments with 8 plants per treatment.

grown in the 1X solution were slightly chlorotic and were of an intermediate height and dry weight (Table 1). When plants grown in the 10X solution for 3 weeks were transferred to the -Fe solution they became chlorotic, first at the shoot apex and later all of the leaves. Only plants supplied with iron throughout the 6-week growing period produced pods (Table 2). If 3-week-old chlorotic soybean plants were adequately supplied with iron for the subsequent 3-week period, growth was enhanced, but the new supply of iron did not compensate for the previous retardation of growth (Table 2).

TABLE 2. The effect of various iron levels on the height of Soybean Plants.

Nutrient solution								
-Fe			10X			-Fe	10X	
Initial Ht.[a] (cm)	Final Ht.[b] (cm)	% Increase	Initial Ht. (cm)	Final Ht. (cm)	% Increase	Initial Ht. (cm)	Final Ht. (cm)	% Increase
16[c]	16	0	33	72[d]	118	14	27[e]	93

[a] When the soybeans plants were 3 weeks old, the initial height measurements were taken and the nutrient solution was changed.

[b] Final height measurements were taken 3 weeks after the nutrient solution was changed.

[c] Figures given are averages of 18 plants.

[d] These plants averaged four pods per plant at this time; none of the plants in other treatments produced pods.

[e] Three-week-old soybean plants transferred from the -Fe nutrient solution to the 10X nutrient solution.

Root Absorption and Translocation of Iron. To each cup containing either four 3-week-old chlorotic soybean plants from the -Fe solution or containing four 3-week-old healthy soybean plants from the 10X solution, was added 25 lambda (λ) of Fe^{59} Cl_3 containing approximately 22 microcuries (uc). To two of the cups, one containing chlorotic plants and the other containing healthy plants, was added an additional 0.2 ml Versenol 120 chelate per cup. At the end of 3 days the plants were harvested according to Plan A, Figure 1, and the radioactivity of the different parts was determined.

When the plants were supplied radioactive iron to their roots, the chlorotic plants accumulated a greater total amount of activity than did the healthy plants (Table 3).

TABLE 3. The absorption and distribution of Radioactive iron supplied to the roots of 3-week-old Soybean plants.

Treatment	Cpm per plant part R [a]	St₁	St₂	Total cpm
Chlorotic plants				
Receiving $Fe^{59}Cl_3$	24240 [b]	530	495	25265
Receiving $Fe^{59}Cl_3$V120	6600	3840	3020	13460
Healthy plants				
Receiving $Fe^{59}Cl_3$	13220	23	30	13273
Receiving $Fe^{59}Cl_3$V120	3010	870	640	4520

[a] See harvest plan in Figure 1-A.

[b] Each value is the average of four plants.

When chelated radioactive iron was supplied to the plant roots the total amount of radioactive iron taken up by the plants was less than the amount taken up by the plants when non-chelated radioactive iron was supplied to the root. In both the chlorotic and healthy plants the translocation of radioactive iron out of the roots and into the different plant parts was enhanced if the radioactive iron was supplied in chelated form (Table 3).

The experiment was repeated, with similar results.

Leaf absorption and translocation of iron. In this study 3-week-old chlorotic soybean plants from the -Fe solution and 3-week-old healthy soybean plants from the 10X solution were placed in waxed cups containing a complete mineral solution. Two 15-drops of Fe^{59} Cl_3 containing a total of 12.1 uc of activity were applied to the center leaflet of the first trifoliate leaf. Forty-eight hours after the application of the radioisotope the plants were dissected according to Plan A, Figure 1, except that the center leaflet of the first trifoliate leaf was discarded and the remainder of this leaf was counted separately as (Lf_2).

When both chlorotic and healthy plants received radioactive iron via the leaves, the chlorotic plants accumulated a greater total amount of activity. The healthy plants, however, translocated 46 per cent of the iron away from the third trifoliate leaf compared to the chlorotic plants in which 22% of the iron was translocated (Table 4).

TABLE 4. The translocation of Fe^{59} applied to the leaves of Chlorotic or Healthy Soybean plants.

State of plant	Cpm per plant part R	St_1	Lf_2	St_2	Total cpm
Chlorotic	45[a]	630	5940	990	7605
Healthy	15	16	65	23	119

[a] Each value is the average of four plants.

THE INFLUENCE OF 2,3,5-TRIIODOBENZOIC ACID ON THE TRANSLOCATION OF IRON.

In these experiments either the primary leaf or the terminal leaflet of the first trifoliate leaf was sprayed to runoff with 100 ppm 2,3,5-triiodobenzoic acid 3-3½ hours before the radio-iron was supplied to the same organs. Both iron-deficient and healthy soybean plants were studied. The radio-iron was applied as drops to the TIBA-treated leaves and 12 or 17 uc of activity was delivered to each plant. The same amount of radio-iron was delivered to all of the plants in any one experiment.

After 41 hours the plants were cut into three parts according to Plan A, Figure 1, except that the first trifoliate leaf (minus the "supply" leaf) was included with the St_2 part.

In Table 5 are given the results of one experiment where the TIBA treatment and the supply of radio-iron was to one primary leaf of a series of chlorotic soybean plants. TIBA had no significant effect on the movement of iron into the plant. In general TIBA did not enhance the transport of iron from either the primary or trifoliate leaves of either healthy or chlorotic soybean plants.

THE ABSORPTION AND TRANSLOCATION OF P^{32} BY SOYBEAN ROOTS OR LEAVES AS INFLUENCED BY IRON STATUS.

In this study chlorotic plants from the -Fe solution, the 1X solution, and healthy plants from the 10X solution were used. These plants were placed (four per cup) in waxed cups containing 150 ml of -Fe solution. 10 of P^{32} containing about 32 uc of activity was applied to the primary leaf on the side of the first trifoliate leaf of eight plants from each solution. The other 24 plants, four per cup, received 10 of P^{32} per cup via the nutrient solution. After 5 hours the plants receiving leaf application of P^{32} were cut into three parts as shown in Plan B, Figure 1. The primary leaf which received the radioisotope was discarded. Those plants which received the P^{32} via the roots

TABLE 5. The effect of a foliar treatment of TIBA on the translocation of Radio-iron from a primary leaf of Chlorotic Soybean plants.

Treatment prior to application of Fe^{59}	Cpm per plant part[a] R	St_1	St_2	Total cpm
None	385[b]	3610	7660	13655
TIBA[c]	160	4610	11710	16480

[a] Plants had been grown in a -Fe solution and were chlorotic.
[b] Figures are averages of seven plants.
[c] The TIBA (100 ppm) was applied to the primary leaves 3 hours before the application of Fe^{59}.

were dissected into the following parts: (Lf_1) included the primary leaves, (Lf_2) included the first and second trifoliate leaves and the shoot apex, (St) included the stem between the cotyledonary node and the second trifoliate leaf. The presence of radioactivity was determined as described previously.

When P^{32} was supplied to the roots of the soybean plants it was noted that the more iron-deficient the plants, the less total P^{32} was accumulated by the entire plant (Table 6). Furthermore the more chlorotic the plants, the greater the percentage of P^{32} that remained in the primary leaves (Lf_1) and the smaller the percentage that moved upward into the trifoliate leaves (Lf_2).

TABLE 6. Translocation of Radioactive Phosphorus Supplied to the Roots of Soybean plants.

Solution in which plants were grown	Cpm per plant part Lf_1[a]	Lf_2[a]	St[a]	Total cpm
-Fe	950[b]	200	790	1940
1X	1070	1040	860	2970
10X	570	1990	1530	4190

[a] Lf_1 included the primary leaves. Lf_2 included the first and second trifoliate leaves and the plant apex. St included the stem between the cotyledonary node and the second trifoliate leaf.
[b] Each figure is the average of eight plants.

When P^{32} was applied to the primary leaf of the soybean plant, the more iron deficient the plant the smaller the total amount of P^{32} found in the plant (Table 7) The more chlorotic plants translocated a smaller percentage of the P^{32} to the roots (R) and a greater percentage of the P^{32} moved to the plant parts (St_1) (Plan B, Figure 1) compared to the healthy plants.

TABLE 7. Translocation of Radioactive Phosphorus Applied to the Primary Leaf of Soybean.

Solution in which plants were grown	Cpm per plant part R[a]	St_1	St_2	Total cpm
-Fe	27[b]	15	7	49
1X	560	45	270	875
10X	1150	35	340	1525

[a] See harvest plan in Figure 1B.
[b] Each value is the average of eight plants.

Similar results were obtained when the experiment was repeated.

DISCUSSION: Wadleigh and Brown (12) showed that chlorotic plants seemed to accumulate large amounts of iron and phosphorus in the roots, possibly due to precipitation as ferric phosphate.

The present study showed that when non-chelated radioactive iron was supplied to the roots of chlorotic soybean plants, these plants took up a larger total amount of radioactive iron than did healthy plants. Both the healthy and the chlorotic plants accumulated less of the chelated radioactive iron than of the non-chelated radioactive iron. But of the chelated radioactive iron taken up not only did a greater proportion of it move out of the roots into the plant leaves, but a greater total amount of radioactive iron accumulated in the leaves.

Thus if precipitation as ferric phosphate actually occurs in roots of chlorotic soybeans in the absence of the chelate, the addition of the chelate may reduce the precipitation and permit more iron to be translocated to the leaves.

Similarly if the roots of both chlorotic and healthy plants were supplied non-chelated radioactive iron the chlorotic plants accumulated more radioactive iron, but accumulated comparatively less of the iron in the leaves and other plant parts than did the healthy plants. Thus, in healthy plants the iron was more readily translocated and possibly less subject to precipitation as ferric phosphate.

Of considerable importance then are the factors affecting the translocation of iron. Kessler and Moscicki (7) studied the effect of triiodobenzoic acid (TIBA) and maleic hydrazide upon the translocation of iron. They reported that they could increase the amount of iron translocated from the leaves of peach and plum trees by first treating the leaves with a 100 ppm solution of TIBA. They measured chlorophyll formation as an index of the iron translocation. Although the causes for the increased transport of iron from leaves following TIBA treatment are not known, they suggested that TIBA may have freed the immobilized iron already in the plant leaf and the possible relationship of the suspending effect of TIBA upon polarity, its interference with the polar movement of auxin, and its destroying effect upon apical dominance on the downward translocation of nutrients in the plants.

The effect of TIBA on iron translocation from leaves of healthy or chlorotic soybean plants was studied. Radioactive iron was supplied to the TIBA-treated leaves, but did not increase the translocation of the radioactive iron in the plants in this experiment.

DeKock and Hall (6) found chlorotic leaves of *Spiraea japonica* to have a phosphorus to iron ratio of 45:1 while the healthy green leaves had a ratio of 20.6:1. They suggested that the ratio apparently decreased due to the movement of phosphorus out of the healthy leaf. Wadleigh and Brown (12) found that chlorotic plants of *Phaseolus vulgaris* accumulated large amounts of phosphorus as well as iron in the roots and conductive tissues. They attributed this to a possible ferric phosphate precipitation. In this regard the effect of an iron deficiency on the translocation of phosphorus may be important. We found that chlorotic plants absorbed less phosphorus by both roots and leaves and translocated less of what was absorbed when compared to healthy plants. This agrees with the data of Biddulph and Woodbridge (1) in their study of the uptake of phosphorus by roots with different levels of available iron. However, our study further shows that this was true whether the phosphorus was applied to the primary leaf or to the plant roots. Thus, an iron-induced chlorosis not only inhibits or reduces iron translocation but reduces phosphorus translocation, as well. The chlorotic plants absorbed larger amounts of iron but smaller amounts of phosphorus than did the healthy plants.

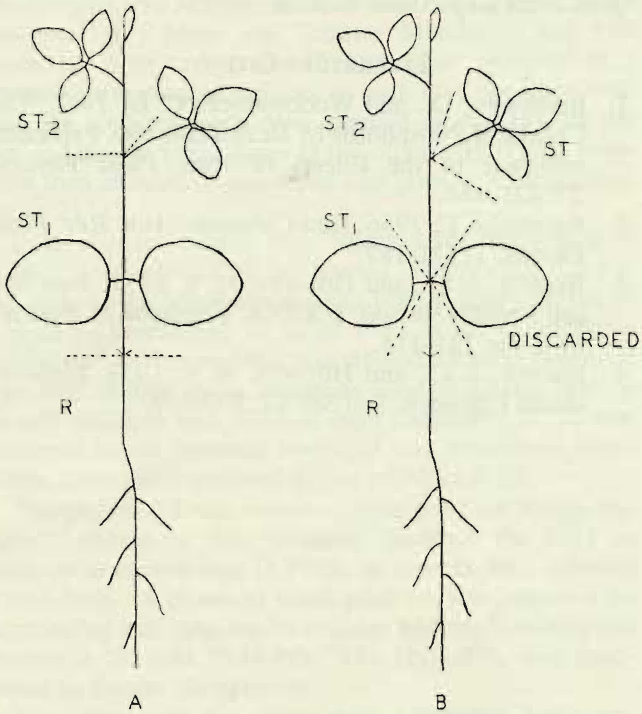

FIGURE 1. Dissection plans for treated plants. Plan A was used if the radioisotope was applied to the plant roots. Plan B was used if the radioisotope was applied to the primary leaf.

SUMMARY: The effects of different levels of available iron on the growth of *Glycine max* var. Ottawa Mandarin was studied under controlled environmental conditions.

Soybean seedlings which were supplied with sufficient amounts of iron chelate increased over 100% in plant dry weight and plant height over plants receiving no iron during the first 3 weeks of growth.

When radioactive iron was supplied to plant roots the chlorotic plants accumulated a greater total amount of the radioactive iron than did the healthy plants of equal age. When chelated radioactive iron was used, the total amount of radioactive iron taken up by both chlorotic and healthy plants via the plant roots was less compared with the absorption of inorganic iron, but the amount of radioactive iron translocated out of the roots into the different plant parts was increased.

When radioactive iron was applied via the leaves, the chlorotic plants accumulated a greater total amount of radioactive iron, but translocated a smaller percentage of the absorbed iron away from the site of absorption than did healthy plants.

A prior treatment of TIBA failed to enhance the translocation of iron applied to either the leaves or roots of chlorotic plants.

Chlorotic plants absorbed less total radioactive phosphorus and translocated a smaller percentage of the amount absorbed than did healthy plants regardless of whether the radioactive phosphorus was applied to the plant leaves or to the plant roots.

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