Plant and Soil **35**, 225–236 (1971) Ms. 1468

THE EFFECT OF CALCIUM BICARBONATE ON IRON ABSORPTION AND DISTRIBUTION BY *CHRYSANTHEMUM MORIFOLIUM*, (RAM.) *

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SUMMARY

Plants grown for two weeks in high-bicarbonate nutrient solution with iron became chlorotic, absorbed less iron, and translocated a lower percentage of absorbed iron than did green plants grown under low bicarbonate with iron. Chlorotic plants, pretreated in low-bicarbonate solutions lacking iron, absorbed more iron and translocated a higher percentage to leaves than the green plants.

Plants induced to chlorosis by high bicarbonate absorbed less iron after transfer to low-bicarbonate solution containing iron than did chlorotic plants pretreated with low-carbonate solution lacking iron.

Initial localization of iron occurred in the roots. A considerable amount of the iron initially found on the roots was translocated to developing shoots over a nine-week period unless the plants were grown in high bicarbonate solutions. More iron was translocated from roots of plants in minus-iron solutions following initial absorption than when iron was supplied in the nutrient solutions.

INTRODUCTION

Excess calcium carbonate often reduces iron uptake by plants as a result of reduced solubility of iron in the soil solution. Chlorotic plants, in such an environment, often do not respond immediately to the additions of soluble iron.

In some plant species which become chlorotic under calcareous conditions, the activity of catalase is reduced [5 6]. The respiration rate of root tips may be reduced by sodium bicarbonate [11]. The activity of particulate cytochrome oxidase from the roots is stimulated

* Journal Series Paper **736**. University of Georgia, College of Agriculture Experiment Stations, College Station, Athens, Ga. 30601.

by some salts of sodium, potassium, calcium, and magnesium, but is inhibited by sodium bicarbonate [10]. A reduction in the respiration rate of roots might be manifested by decreased absorption of iron.

Translocation of iron from the site of absorption in the root to the shoot could be reduced as a result of such changes. Translocation of radioiron has been reported to be impeded by bicarbonate [8 15].

Considerable localization of radioiron occurred external to the epidermis and within the cortex outside the endodermis of the pea root [3]. Furthermore, inhibiting the respiration rate of those roots with 2,4-dinitrophenol reduced the translocation of radioiron from the root to the shoot [2].

Little or no iron redistribution has generally been considered to occur from older tissues to the developing shoot tip, since chlorosis is first evident in the youngest leaves. However, the possibility of a significant iron reserve within some plants has been suggested by reports that young azalea plants have been grown in sand culture devoid of iron for 150 to 210 days before chlorosis developed [12 14]. By contrast, symptoms of nitrogen deficiency were evident after only 20 to 40 days. Translocation of radioiron from roots and old leaves to new growth in tobacco has been demonstrated [15], as has translocation of radioiron from the cotyledon to the tops in soybean[7].

The effect of calcium carbonate on the absorption and subsequent distribution of radioiron is the subject of this report. Both short-term and long-term distribution patterns are stressed.

METHODS

Experiment A

Effect of calcium bicarbonate on the short-term absorption and distribution of iron.

Rooted cuttings of *C. morifolium* cl. Legal Tender were grown in aerated solution culture[9] in a greenhouse for two weeks under the following treatments: (a) pH 5.5 (0.5 mmol ammonium sulfate in Hoagland #1 solution), low calcium bicarbonate, 2 ppm iron as ferric ammonium citrate; (b) pH 5.5, low calcium bicarbonate, no iron; (c) pH 7.0, high calcium bicarbonate, 2 ppm iron as ferric ammonium citrate.

High pH and high calcium bicarbonate were maintained by suspending finely ground calcium carbonate in the nutrient solution and aerating with CO_2-enriched air. A calcium bicarbonate concentration of approximately 3m*M* was obtained [1]. Adjustments in pH were made when daily check with pH meter indicated a change by either adding potassium carbonate or

adjusting carbon dioxide concentration in the aerating air. The low bicarbonate treatment received no additions of calcium carbonate or other forms of carbonate.

An iron concentration was maintained at 1 ppm by adding ferric ammonium citrate at the beginning of the second week. Chemical analysis of the nutrient solution established that the concentration of iron in the high pH treatments was essentially zero after one week.

After two weeks, the plants were transferred to the laboratory, and absorption and distribution of Fe59 was followed while maintaining the same treatments during absorption periods of 24 and 26 hours. An ambient temperature of 28°C and continuous illumination at 800 ft–c (fluorescent lamps) was maintained. Iron was supplied at 2 ppm as ferric ammonium citrate labeled with Fe59 at 28mc/g Fe. Iron–59 remained in solution in that form in all treatments for the durations of the uptake periods.

A split-plot design was employed with calcium bicarbonate treatment as main plot, time as sub-plot, and five replications.

Plants were removed from solution at each sampling time and segmented into: (a) upper four leaves, (b) lower four leaves, (c) stems, and (d) roots. The stem with roots attached was washed by dipping repeatedly in acidified detergent solution, soaking 30 minutes in a solution of sodium ethylenediamine tetraacetate, flushing under running tap water, and blotting on absorbent tissues. The roots were then separated from the stem. Each tissue sample was dried at 70°C and weighed. After being crushed flat in the bottom of paper cups, the radioactivity in the tissue was determined with a Geiger-Mueller detector and standard scaler circuit. Iron concentration was calculated from the specific activity of the radiosotope.

Experiment B

Effect of pretreatment with calcium bicarbonate on the absorption and distribution of iron from solutions containing low calcium bicarbonate.

Young plants were similarly grown for two weeks in aerated solution cultures under the same treatments: (a) low bicarbonate with iron, (b) low bicarbonate without iron, or (c) high bicarbonate with iron. Plants from each pretreatment were brought to the laboratory and allowed to absorb Fe59 from a root environment of pH 5.5 and low calcium bicarbonate (identical to treatment 'a' of Experiment A) and with ambient conditions as described in Experiment A. A completely randomized design of five replications was used.

At the end of a 24-hour absorption period, the plants were segmented, radioactivity determined, and iron quantitated as before.

Experiment C

Effect of calcium bicarbonate on the long-term distribution and redistribution of iron.

Rooted cuttings were allowed to absorb Fe59 for 48 hours from aerated half-strength Hoagland's #1 solution at pH 5.5. The roots and basal stem portions were washed under running water.

The initial distribution of Fe59 was established by segmenting nine randomly selected plants into leaves, stems, and roots, and after drying, determining the radioactivity as described previously. A further indication of Fe^{59} distribution was obtained by preparing radioautographs of another group of similarly treated plants.

The effect of bicarbonate and iron on redistribution was followed by subjecting the Fe^{59}-labelled to low bicarbonate with iron, low bicarbonate without iron, and high bicarbonate with iron for a period of nine weeks. Treatments were identical to those of the earlier experiments. Weekly additions of ferric ammonium citrate were made to maintain an iron concentration close to 2 ppm in the appropriate solutions. Nutrient solutions were replaced with fresh solutions every two weeks. The plants were grown in the greenhouse under a minimum temperature of 16°C and a 16-hour photoperiod (artificial illumination) to assure vegetative growth. Six replications (glazed pot with 3 plants) were provided in a randomized complete block design.

The terminal bud and lower axillary buds were removed after two weeks, leaving the three uppermost axillary buds to develop. One plant from each pot was removed at this time and radioautographs prepared.

At the end of the ninth week, a second plant was taken for radioautography. The lateral shoots were removed from the main stem and mounted separately to facilitate preparation of the radioautograph. The last plant from each pot was segmented into: (a) leaves from lateral shoots, (b) stems of lateral shoots, (c) leaves on main shoot, (d) stem of main shoot, and (e) roots. After drying, the tissue samples were ashed at 500°C. The ash was dissolved in hydrochloric acid and plated. The radioactivity was determined for each plant, and the percentage of total radioactivity in each tissue segment was calculated.

EXPERIMENTAL RESULTS

Experiment A

Plants grown for two weeks in solutions of low pH and devoid of iron were chlorotic and absorbed almost five times more iron during a 24-hour absorption period than did green plants grown at a low pH with ample iron (Table 1). Plants under high pH and high calcium bicarbonate were chlorotic, but absorbed less than a fifth as much as did the green plants. Both the green and chlorotic plants continued to absorb iron at an appreciable rate for 36 hours. In contrast, the plants under high pH and high calcium bicarbonate absorbed only a trace of iron.

Radioactivity determinations on aliquots of the nutrient solutions indicated that iron was still in solution in approximately the same concentration in all treatments after 36 hours. Therefore, failure to

TABLE 1

The effect of calcium bicarbonate, pH and previous iron supply on the absorption and distribution of iron in *C. morifolium*, cl. Legal Tender

Treatment				Iron distribution (μg Fe) *				
HCO_3 (mmol)	pH	Fe (ppm)	Duration (hrs)	Total	Upper leaves	Lower leaves	Stem	Root
0	5.5	2	24	5.51[a]	0.11[b]	0.15[b]	0.19[b]	5.06[b]
0	5.5	0	24	24.47[b]	4.58[c]	5.12[c]	2.57[c]	12.20[c]
3	7.0	2	24	0.92[a]	0.01[a]	0.01[a]	0.02[a]	0.88[a]
0	5.5	2	36	22.77[x]	1.31[x]	1.70[y]	0.81[y]	18.95[x]
0	5.5	0	36	36.09[x]	4.38[y]	3.84[z]	2.06[z]	25.81[x]
3	7.0	2	36	1.10[y]	0.01[x]	0.01[x]	0.02[x]	1.06[y]

* Means with the same superscript letter are not significantly different at the 95% level of confidence as determined by Duncan's Multiple Range Test. Comparisons were made among treatments within each time of observation.

absorb iron during these short-term observations was not due to precipitation of iron.

Very little iron was translocated to the leaves in 24 hours except in the chlorotic plants which were exposed to low calcium bicarbonate and deprived of iron (Table 1). These chlorotic plants absorbed and translocated 50 times more iron to leaves than did green plants which were grown under low bicarbonate, and 500 times more than did chlorotic plants grown under high bicarbonate. Essentially no iron was translocated to leaves under conditions of high calcium bicarbonate and high pH. Only in the green plants was more iron transported to leaves in 36 than in 24 hours.

Iron was not localized in stem tissue in any treatment. The least quantity of iron in stems was in plants under high-calcium bicarbonate treatment, where the lowest distribution from the roots occurred.

Most of the absorbed iron remained in the roots. Approximately 90 per cent of the iron absorbed during the first 24 hours remained in the roots of plants in low calcium bicarbonate and adequate iron. An even higher percentage (97 per cent) remained in roots of plants grown under high calcium bicarbonate. There was more radioiron in roots of the plants grown in solutions of low calcium bicarbonate and no iron, but this amount was only 50 per cent of the iron absorbed. Considerable iron continued to accumulate in roots under low bicarbonate treatments over the 36-hour period.

Experiment B

More iron was absorbed by chlorotic plants, pretreated with low bicarbonate and no iron, than by chlorotic plants pretreated with high bicarbonate and iron (Table 2). There was no difference in the amount of iron absorbed by green plants pretreated with low bicarbonate and by chlorotic plants pretreated with high bicarbonate.

High-bicarbonate pretreatment inhibited iron distribution similar to the effect of higher bicarbonate during iron uptake. Most of the iron remained in the roots in all treatments. Less iron was translocated from roots to leaves in the chlorotic plants pretreated with bicarbonate than in plants of the other two treatments. Much more of the iron that moved out of the roots in the high bicarbonate treatment remained in the stems than was observed in the low bicarbonate treatments.

More iron was distributed to the upper four leaves than to the lower four in all treatments, particularly when considerable iron was translocated from the roots.

TABLE 2

The effect of prior exposure to high calcium bicarbonate on the absorption and distribution of Fe^{59} in *C. morifolium* cl. 'Legal Tender'. Fe^{59} was absorbed from solutions of low calcium bicarbonate over a 24-hour period

Pretreatment			Iron distribution (μg Fe)*				
HCO_3 (mmol)	pH	Fe (ppm)	Total absorbed	Upper leaves	Lower leaves	Stem	Root
0	5.5	2	6.92[a]	0.13[a]	0.06[a]	0.212[a]	6.52[b]
0	5.5	0	8.23[b]	0.21[a]	0.12[b]	0.400[c]	7.50[c]
3	7.0	2	5.81[a]	0.04[b]	0.03[a]	0.340[b]	5.40[a]

* Means with the same superscript letter are not significantly different at the 95% level of confidence as determined by Duncan's Multiple Range Test.

Experiment C

The distribution of iron after 24 hours of absorption was similar to that observed in the green plants of the preceding experiment.

A radioautograph obtained after the initial period of absorption and distribution (Fig. 1) illustrated the preferential transport of the absorbed radioiron to the young tissue. The prominence of the veins in the radioautograph of the lower leaves indicated iron did not readily move from the veins into the mesophyll tissue.

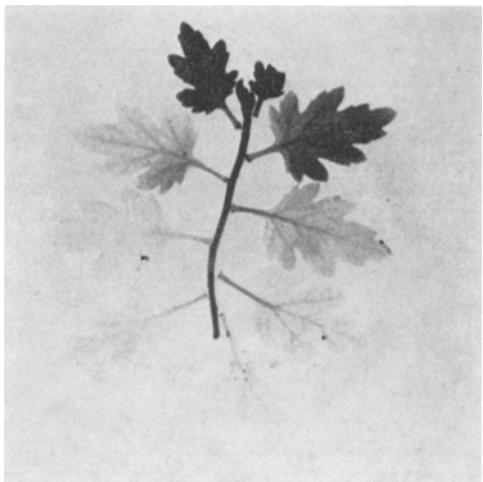

Fig. 1. Radiograph depicting the initial distribution of Fe^{59} in a young chrysanthemum plant prepared after absorbing $Fe^{59}Cl_3$ for 48 hours from Hoagland's #1 solution at pH 5.5.

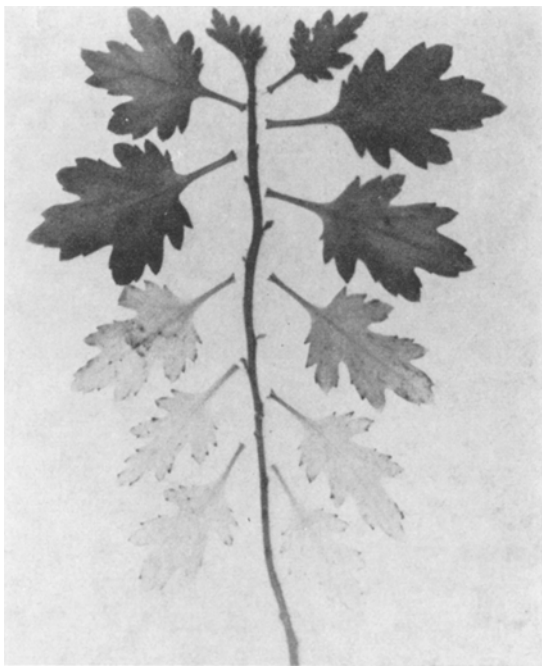

Fig. 2. Radioautograph depicting the distribution of Fe^{59} two weeks after absorbing $Fe^{59}Cl_3$. The plant had been grown in aerated nutrient solution in the greenhouse at 16°–26°C and 16-hour photoperiod.

TABLE 3

The effects of high calcium bicarbonate on the long-term distribution of root-absorbed iron in *C. morifolium*, cl. 'Legal Tender'

Treatment			Iron distribution* (per cent of total Fe^{59} in each segment)				
HCO_3 (mmol)	pH	Fe (ppm)	New leaf	New stem	Old leaf	Old stem	Root
0	5.5	2	2.78[a]	0.48[a]	3,25[b]	4.44[a]	89.00[a]
0	5.5	0	13.18[b]	1.71[b]	6.61[c]	6.04[a]	72.42[b]
3	7.0	2	0.78[a]	0.23[a]	1.67[a]	4.91[a]	92.72[a]

* Means with the same superscript letter are not significantly different at the 95% level of confidence as determined by Duncan's Multiple Range Test.

The same distribution pattern to upper leaves was apparent after two weeks under treatment (Fig. 2). Three of the four uppermost leaves had been present and fully developed, but not completely expanded on the young plant at the time of initial absorption and distribution. Consequently, there were no apparent differences among the treatments and only one radioautograph is shown to illustrate the pattern of distribution. There was no continued movement of radioiron into the lower leaves.

It is evident from the percentages of Fe 59 calculated for each plant segment at the termination of the experiment that iron continued to be translocated from the roots to the developing leaves under conditions of low calcium bicarbonate, but not in the presence of high calcium bicarbonate (Table 3). The greatest amount of transport from the root occurred when no external source of iron was supplied.

These differences can be seen by comparing the radioautographs in Figure 3. The lateral shoot of the plant grown in low calcium bicarbonate without iron (Fig. 3B) apparently had more radioiron available to it during the development of the 4th, 5th, and 6th leaves than did equivalent leaves on either the low calcium bicarbonate, plus iron (Fig. 3A) or the high calcium bicarbonate, plus iron (Fig. 3C) treatments. The radioactivity in the terminal bud of the low-calcium bicarbonate chlorotic plants indicated that there continued to be a supply of iron from the initial absorption nine weeks earlier. The transport of iron to the terminal leaves did not prevent chlorosis, but did reduce the degree of chlorosis as compared to plants under

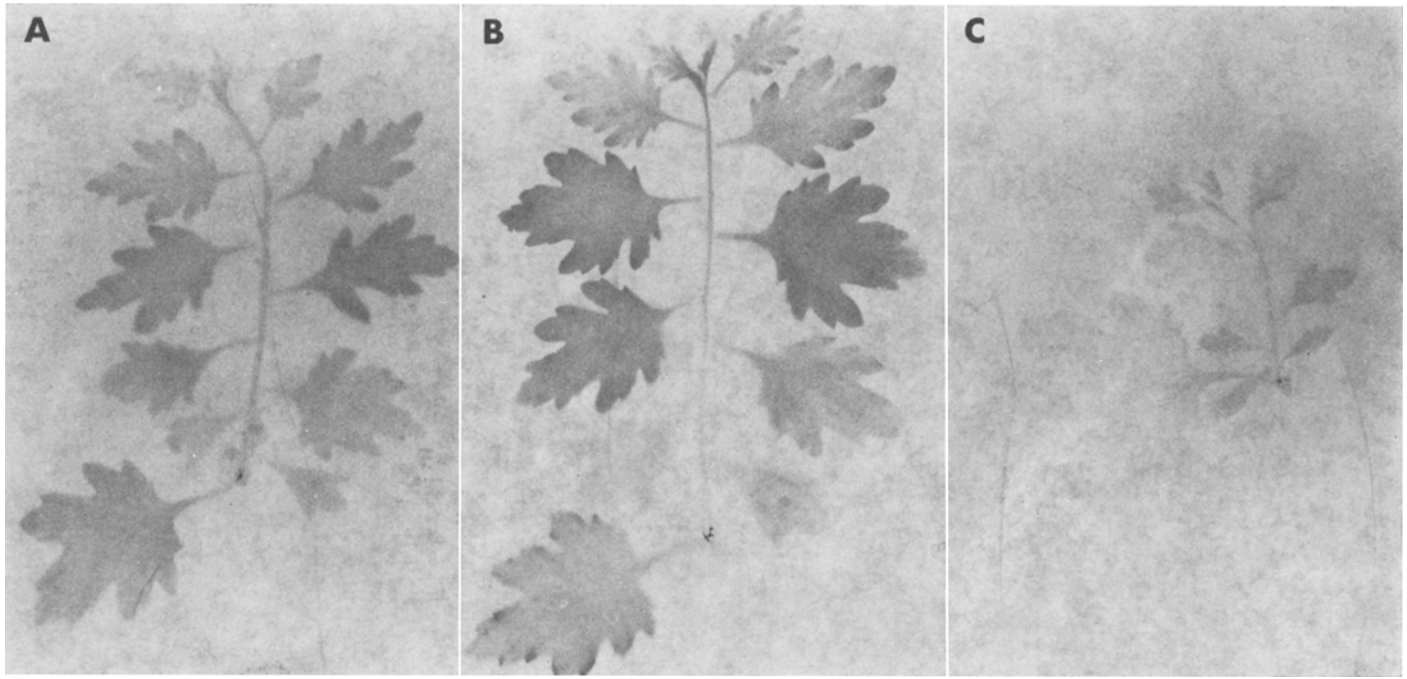

Fig. 3. Radioautographs of lateral shoots and subtending leaves of chrysanthemum plants which developed while the plants were being grown for nine weeks at: **A** pH 5.5, low bicarbonate and with iron (2 ppm) supplied to roots, **B** pH 5.5, low bicarbonate and no iron in the root medium and **C** pH 7.0, high bicarbonate, and with iron (2 ppm) supplied to the root medium. All plants initially were permitted to absorb Fe^{59} ($Fe^{59}Cl_3$) for 48 hours at pH 5.5.

high-calcium bicarbonate treatment. Consequently, differences in growth between the slightly chlorotic and green plants of the two low-calcium bicarbonate treatments was not as great as that between the extremely chlorotic plants in high calcium bicarbonate and those in low calcium bicarbonate.

DISCUSSION

Chrysanthemum plants grown in a nutrient solution lacking in iron absorbed more iron later when iron was added than those which had been grown in an iron-rich medium. Internal changes associated with chlorosis caused by withholding iron were not inhibitory on absorption when iron was later made available. This was in sharp contrast to the reduced capacity for iron absorption by chlorotic plants induced with high calcium bicarbonate. Apparently chlorosis caused by iron deficiency predisposed the plant to increased absorptive capacity, but that caused by high bicarbonate did not. The increased capacity for iron absorption by iron-depleted plants was particularly evident from the contrast between Experiments A and B. The plants in Experiment A had been under intermittent mist as cuttings during propagation. They were immediately subjected to treatment after rooting without iron being supplied in one treatment. When iron was made available, the iron-deficient, zero-bicarbonate plants absorbed 24 μg iron in 24 hours. The plants in Experiment B had been grown on nutrient solution containing iron from the time of rooting until beginning the experiment (2 weeks). After two weeks of pretreatment, the iron-deficient plants absorbed 8 μg iron. That amount was significantly higher than that absorbed by iron-sufficient plants but was much less than the amount absorbed by the plants in experiment A which had not received iron since the cuttings were removed from the stock plants. The statistically significant contrasts between iron-deficient and iron-sufficient plants in each experiment established the increased capacity for iron absorption by iron-deficient plants. The sharp contrast between amounts absorbed by the extremely deficient, iron-deprived plants of Experiment A and the slightly deficient plants of Experiment B adds further credibility to the argument for increased absorptive capacity.

A high percentage of the iron absorbed by minus-iron chlorotic

chrysanthemum was translocated to the leaves. The concentration in the leaves after 24 hours was 50 ppm, which probably is adequate for re-greening. Normal appearing green leaves of this cultivar contained 70 ppm iron [13]. In fact, recovery by absorption of iron in distilled water added to nutrient solution was a problem encountered in maintaining chlorosis under low calcium bicarbonate.

Translocation of iron from roots to leaves was inhibited by high bicarbonate. Not only was less iron found in the leaves of high-bicarbonate plants than observed in minus-iron plants, but a lower percentage of that quantity absorbed was eventually distributed to the leaves. This was obviously a separate phenomenon, not just an expression of reduced absorption, as has been reported for tobacco [15].

The effect of chlorosis induced by calcium bicarbonate was shown to be due at least partly to internal changes and not limited to the immediate effect on the external root medium. The capacity for both absorption and translocation of iron continued at a reduced level after transfer to a low bicarbonate medium.

The primary site of localization was in the root, which agrees with the reports of Branton and Jacobson [3] regarding iron localization in pea plants in a low-bicarbonate medium. Much of the iron initially fixed in the root was available for transport to developing leaves and shoot terminals. Redistribution occurred to a considerable extent if an external source of iron was not available to the growing plants and if calcium bicarbonate was low.

In the first studies no statistical differences were noted between amounts of iron distributed to upper leaves in contrast to lower leaves. However, with slightly larger and older plants in Experiment B, approximately twice as much iron was found to be translocated to the upper leaves as to lower in a 24-hour period, similar to reports for cotton [4]. Radioautographs showed much of the iron present in the lower leaves to be localized in and adjacent to major veins. In other words, mobile iron, likely to be available for physiological processes, was only distributed to youngest leaves and terminal meristems. Subsequent development of chlorosis, whether as a result of iron deficiency or as a result of excessive bicarbonate, did not appear to affect intraleaf mobility in the youngest leaves as growth occurred.

These findings may explain the difficulties often encountered in attempting to correct chlorosis resulting from high concentrations of

calcium bicarbonate in the soil solution. Even if iron is supplied in a soluble form as it was in these experiments, it may not be absorbed or translocated to the leaves in sufficient quantities to correct the chlorotic disorder.

Received March 2, 1970. Revised February 1971

LITERATURE CITED

1 Bradfield, R., Calcium in the soil: physio-chemical relations. Soil Sci. Soc. Am. Proc. **6**, 8–15 (1941).

2 Branton, C. and Jacobson, L., Iron transport in pea plants. Plant Physiol. **37**, 539–545 (1962).

3 Branton, D. and Jacobson, L., Iron localization in pea plants. Plant Physiol. **37**, 546–551 (1962).

4 Brown, A. L., Yamaguchi, S. and Leal-Diaz, L., Evidence for translocation of iron in plants. Plant Physiol. **40**, 35–38 (1965).

5 Brown, J. C., The effect of dominance of a metabolic system requiring iron or copper on the development of lime-induced chlorosis. Plant Physiol. **28**, 495–502 (1953).

6 Brown, J. C. and Hendricks, S. B., Enzymatic activities as indications of copper and iron deficiencies in plants. Plant Physiol. **27**, 651–660 (1952).

7 Brown, J. C. and Holmes, R. S., Iron, the limiting element in a chlorosis: Part I. Availability and utilization of iron dependent upon nutrition and plant species. Plant Physiol. **30**, 451–457 (1955).

8 Goss, J. A. and Romney, E. M., Effect of bicarbonate and some other ions on the shoot content of P^{32}, Ca^{45}, Fe^{59}, Rb^{86}, Sr,^{90} Ru^{106}, Cs^{137}, and Ce^{144} in bean and barley plants. Plant and Soil **10**, 233–241 (1959).

9 Hoagland, D. R. and Arnon, D. I., The water culture method for growing plants without soil. California Univ. Agr. Exp. Sta. Circ. **347** (1938).

10 Miller, G. W. and Evans, H. J., The influence of salts on the activity of particulate cytochrome oxidase from roots of higher plants. Plant Physiol. **31**, 357–364 (1956).

11 Miller, G. W. and Thorne, D. W., The effect of bicarbonate on the respiration of excised roots. Plant Physiol. **31**, 151–155 (1956).

12 Orr, H. P., Furuta, Tokuji and Bell, C. W., Azalea fertilization. Auburn Univ. Afr. Exp. Sta. Circ. **118** (1957).

13 Rutland, R. B., Some aspects of the effects of high concentrations of calcium carbonate and various orthophosphates on the absorption, translocation, and utilization of iron by *Chrysanthemum morifolium*. Ph.D. Thesis. Michigan State Univ., East Lansing (1965).

14 Twigg, M. C. and Link, C. B., Nutrient deficient symptoms and leaf analysis of azaleas grown in sand culture. Proc. Am. Soc. Hort. Sci. **60**, 369–375 (1951).

15 Wallace, A. and DeKock, P. C., Translocation of iron in plants, p. 323–331 *in* Proc. Sympos. on Use of Isotopes and Radiation in Soil-Plant Nutrition Studies. Intern. Atomic Energy Agency, Vienna, Austria (1965).