SELECTIVE ABSORPTION OF IRON FROM IRON CHELATES BY SOYBEAN PLANTS[1]

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The earlier work of Wallace et al. (7, 8) indicated that iron and chelating agent were absorbed by plants in approximately equivalent quantities. Later studies (9) include data which suggest non-equivalent uptake of chelate components.

Several reports from this laboratory (1, 6) have placed emphasis on the capacity of plant roots to absorb iron from chelate and to exclude the chelating agent. Most of this work was done with the ferric chelate of ethylenediamine di(*o*-hydroxyphenylacetic acid) (FeEDDHA) and with plants that were chlorotic when treated. The present report includes studies with green plants and two additional chelates. The use of Fe^{55} and C^{14} labeled chelates provided a sensitive means of assay for the chelates and also permitted their application at relatively low rates in order to prevent root injury (3).

MATERIALS & METHODS

The standard nutrient was an iron-free modified Steinberg solution (5) adjusted to pH 6.5. A liter of the nutrient contained 150 mg $Ca(NO_3)_2 \cdot 4H_2O$; 35 mg $Mg(NO_3)_2 \cdot 6H_2O$; 9 mg NH_4NO_3; 12 mg K_2SO_4; 32 mg KNO_3; 2.8 mg K_2HPO_4; 0.7 mg $(NH_4)_2SO_4$; 0.234 mg $MnCl_3 \cdot 4H_2O$; 0.204 mg H_3BO_3; 0.088 mg $ZnSO_4 \cdot 7H_2O$; 0.02 mg $CuSO_4 \cdot 5H_2O$, and 0.012 mg $Na_2MoO_4 \cdot 2H_2O$.

Seeds of Hawkeye soybean (*Glycine max* (L.) Merr.) were germinated between layers of moist muslin on stainless steel wire frames in pyrex trays. The muslin extended over the edges of the frames into water maintained at one-fourth inch below the seeds. The trays were covered with aluminum foil and kept at 21 C for 3 days. Seedlings were then placed in perforated black lucite frames with their roots extending into standard nutrient. After 3 days of partial shading in these frames the hypocotyls were approximately 15 cm long. Uniform seedlings were then bound in groups of 30 and transferred, one group per jar, to 8 liters of standard nutrient. Illumination at 1,500 ft-c was provided 16 hours each day by fluorescent and incandescent lamps. The chlorosis of plants grown in the iron-deficient medium was first visible on the 12th day after germination and quite pronounced on the 18th day. Green plants were obtained by growth from the 6th to 18th day in standard nutrient containing 1.1×10^{-5} M FeEDDHA.

For collection of exudate the roots of the plants were rinsed and the groups placed individually in 1 liter of aerated nutrient containing chelates given in later treatment designations. The beakers of nutrient were covered with polyethylene film to prevent excessive evaporation and to hold the plant stems in place. The stems were cut off about one centimeter below the cotyledons, bent down, and fastened with the cut ends extending into centrifuge tubes for collecting exudate. Collecting assemblies were covered with bell jars and kept in darkness at 21 C.

Three chelating agents were used: ethylenediaminetetraacetic acid (EDTA); diethylenetriaminepentaacetic acid (DTPA); and ethylenediamine di(*o*-hydroxyphenylacetic acid) (EDDHA). These agents were combined with equivalent iron to give the following chelate treatments: Fe^{55}EDTA, Fe^{55}DTPA, Fe^{55}EDDHA, FeC^{14}EDTA, FeC^{14}-DTPA, and FeC^{14}EDDHA. Each chelate was used at a concentration of 2.2×10^{-5} M in the standard nutrient adjusted to pH 6.5. Assays of the nutrient solutions (before containing plants) gave 251 cps/μg Fe^{55}, 49 cps/μg C^{14}EDTA, 36 cps/μg C^{14}DTPA, and 40 cps/μg C^{14}EDDHA. All assays of nutrient solutions and stem exudate were made by drying aliquots directly on planchets and counting in a proportional counter.

Electrophoresis was carried out on Whatman 3MM paper, using 0.05 M barbital buffer, pH 8.6 (4). Distribution of radioactivity on electropherograms of nutrients and exudates was determined by exposures to no-screen x-ray film. Intact plants were pressed between sheets of botanical drying paper and dried in a forced draft oven at 70 C before exposures to x-ray film.

To compare quantities of isotopes absorbed, exudate was collected from groups of soybean plants precultured without iron and subsequently treated with Fe^{55} or C^{14} labeled chelates. Assays of radioactivity in samples of nutrient were taken before and after the absorption period of 22 hours. Exudate collected after 22 hours was sampled for counting and electrophoresis. A similar experiment was carried out with

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TABLE I

COMPARATIVE ASSAYS OF NUTRIENT & OF EXUDATE FROM CHLOROTIC SOYBEAN PLANTS TREATED WITH LABELED CHELATES

TREATMENT	ASSAY OF NUTRIENT		ASSAY OF EXUDATE, cps/ml
	BEFORE*, cps/ml	AFTER**, cps/ml	
Fe^{55} EDTA	314	116	10,750
Fe^{55} DTPA	312	52	10,980
Fe^{55} EDDHA	322	99	8,370
FeC^{14} EDTA	318	321	9
FeC^{14} DTPA	307	311	5
FeC^{14} EDDHA	314	313	5

* Before plants were placed in the nutrient.
** After containing plants 22 hr.

green plants using the same nutrients and sampling procedures.

A related experiment was designed to compare the levels of isotopes accumulated by intact plants precultured with and without iron. For the period of isotope absorption plants were grown under 7 hours light, 8 dark, and 7 light, for a total of 22 hours in nutrient containing Fe^{55} or C^{14} labeled EDDHA. Four green plants were placed in 1 liter of Fe^{55} nutrient and four green plants were placed in a liter of C^{14} nutrient. Fe^{55} and C^{14} treatments were also used for chlorotic plants. The plants from each of the four treatments was harvested as replicates and exposed to X-ray film for 20 days.

RESULTS & DISCUSSION

A comparison between Fe^{55} and C^{14} absorbed by chlorotic soybean plants is given in table I. The absorption of large amounts of iron resulted in decreased nutrient counts and very high exudate counts. In sharp contrast to changes in Fe^{55}, the C^{14} counts for the nutrient were not significantly different after the absorption period, and very little C^{14} was absorbed by the root and translocated in the exudate.

Assays of nutrient and exudate following the chelate treatments of green soybean plants are given in table II. Although green plants absorbed much less iron than the chlorotic plants, the quantities of Fe^{55} and C^{14} in the exudate of the green plants were still very different.

The electrophoretic patterns of labeled nutrients are shown in figure 1. Paths 1, 2, and 3 show radioiron spotted initially at the origins as the Fe^{55} chelates of EDTA, DTPA, and EDDHA, respectively. The darkened areas of paths 4, 5, and 6 show the movement of C^{14} label for the same chelating agents. The iron chelate FeEDTA moved intact as an anion to 10.5 cm as shown by both labels. FeEDDHA also moved intact to 4.5 cm. Apparently the C^{14} DTPA moved to about 13 cm, with some tailing from 9 cm. The Fe^{55} pattern (path 2) shows that iron is separated from DTPA in the electrophoretic process.

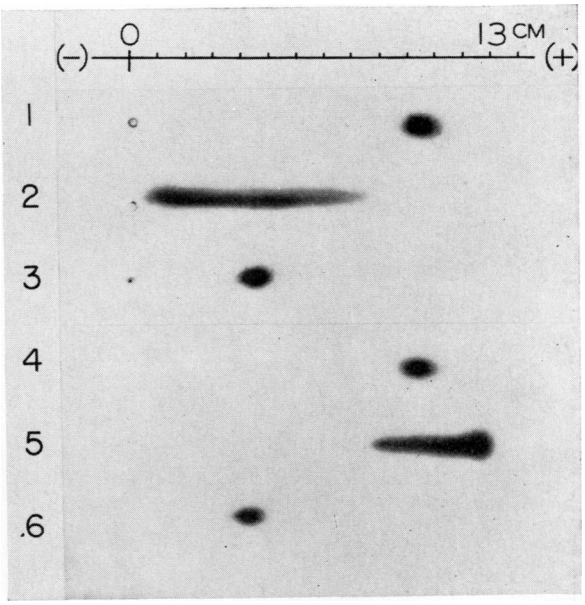

FIG. 1. Radioautograph showing electrophoretic patterns of Fe^{55} and C^{14} labeled chelates. Paths 1, 2, and 3 show Fe^{55} labels initially applied as the iron chelates of EDTA, DTPA, and EDDHA, respectively. Paths 4, 5, and 6 show the C^{14} label for the same chelates.

The results from the electrophoresis of labeled exudate (see assays, table I) are shown in figure 2. The radioautograph of the Fe^{55} (paths 1, 2, 3) represents an exposure of 8 hours. Although some trail-

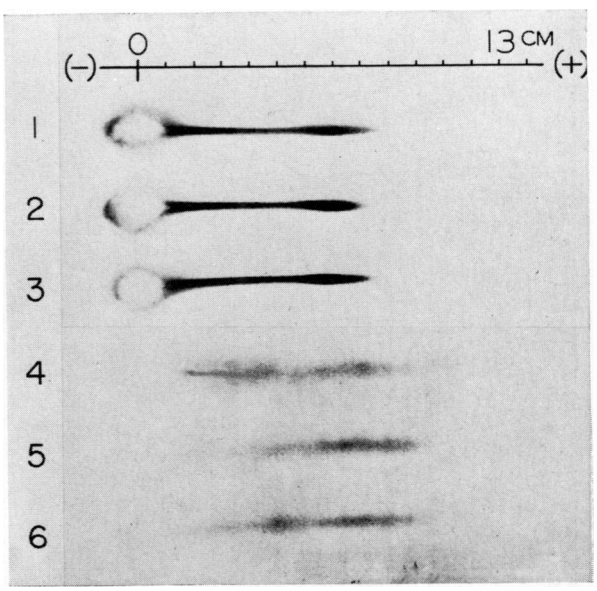

FIG. 2 Radioautograph showing electrophoretic patterns of Fe^{55} and C^{14} labeled soybean stem exudates. Paths 1, 2, and 3 show Fe^{55} in exudate from plants with roots in Fe^{55} EDTA, Fe^{55} DTPA, and Fe^{55} EDDHA, respectively. Paths 4, 5, and 6 show C^{14} in exudate from plants treated with the same agents labeled with C^{14}.

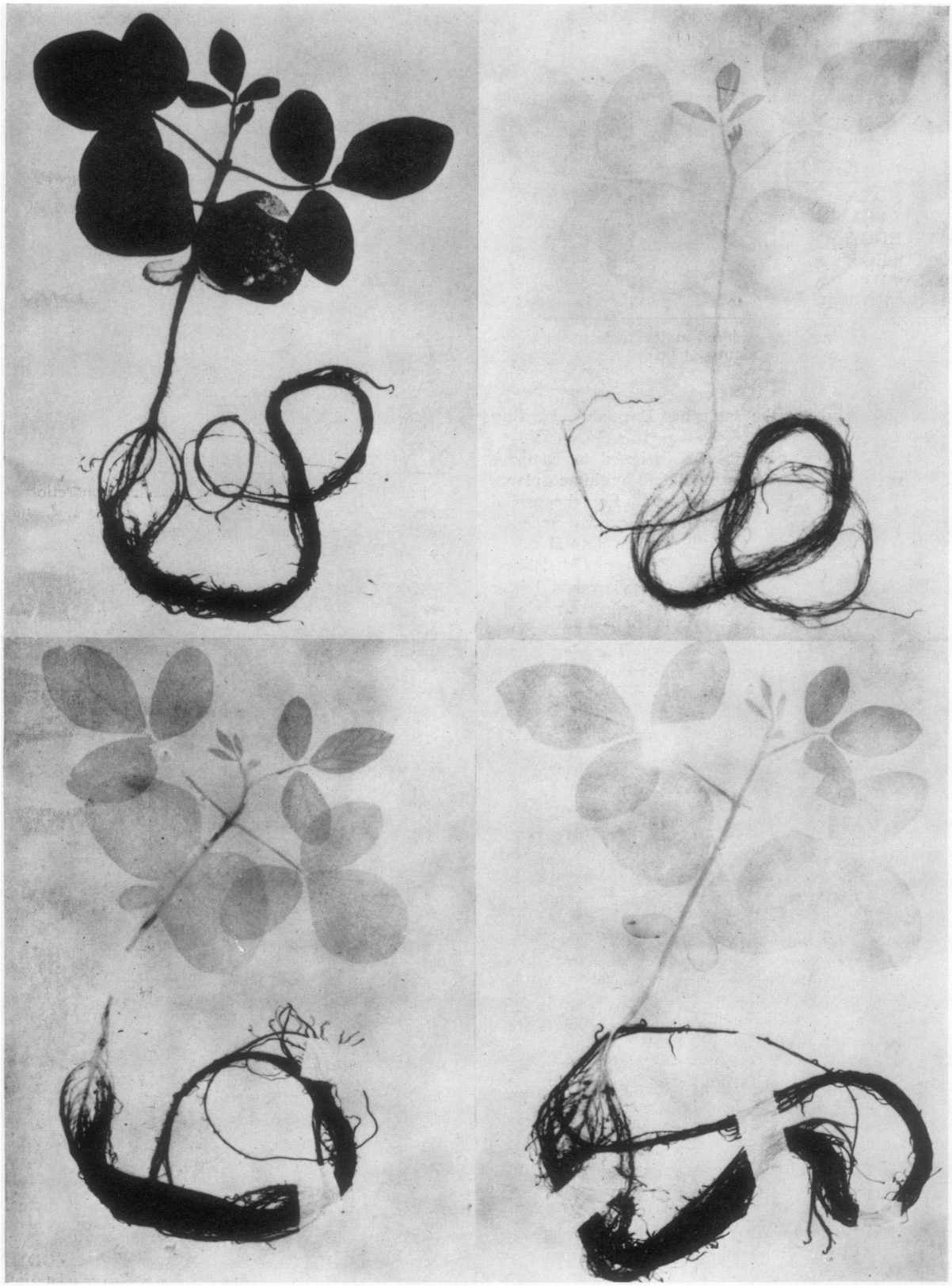

FIG. 3 Radioautographs of intact soybean plants showing distribution of isotopes from labeled chelate. The chlorotic plant (*upper, left*) and green plant (*upper, right*) show Fe^{55}. The chlorotic plant (*lower, left*) and green plant (*lower, right*) show C^{14}. The isotopes were supplied as Fe^{55} or C^{14} labeled EDDHA.

ing is evident, a considerable amount of iron is bound to a component(s) which moves between 6 and 9 cm. The C^{14} patterns (paths 4, 5, 6) represent an exposure of 90 days. The very small amounts of C^{14} do not appear to move electrophoretically as the intact chelating agents but stream out to about ten centimeters.

Radioautographs of green and chlorotic plants placed 22 hours in labeled FeEDDHA are shown in figure 3. The intact chlorotic plant (*upper, left*) shows accumulation of much more iron than the green plant (*top, right*). The chlorotic plant (*lower, left*) and the green plant (*lower, right*) accumulated about the same amount of C^{14}. These results confirm the exudate data for chlorotic plants, showing a great difference in quantity of isotopes absorbed. It is further shown that most of the Fe^{55} can be absorbed independently of C^{14}.

The comparison of C^{14} counts in exudate (tables I & II) shows that decapitated green and chlorotic plants absorb about the same amount of C^{14} from solutions containing EDTA, DTPA, and EDDHA. The variable supply of iron in the preculture period appears to have little effect on the subsequent absorption of the C^{14} label.

In regard to the absorption of iron the plant response was very different. Iron in the exudate of chlorotic plants (table I) ranged from 128 to 244 times higher than iron in the exudate of green plants (table II). In contrast to the data for C^{14}, the preculture iron supply had a very pronounced effect on the subsequent absorption of iron by the soybean plant.

TABLE II

COMPARATIVE ASSAYS OF NUTRIENT & OF EXUDATE FROM GREEN SOYBEAN PLANTS TREATED WITH LABELED CHELATES

TREATMENT	ASSAY OF NUTRIENT		ASSAY OF EXUDATE, cps/ml
	BEFORE*, cps/ml	AFTER**, cps/ml	
Fe^{55} EDTA	295	254	46
Fe^{55} DTPA	308	276	82
Fe^{55} EDDHA	299	237	65
FeC^{14} EDTA	321	317	7
FeC^{14} DTPA	316	308	4
FeC^{14} EDDHA	320	305	8

* Before plants were placed in the nutrient.
** After containing plants 22 hr.

From these observations it is clear that the ratio of Fe^{55}/C^{14} taken up by the plants was influenced by the availability of iron in the preculture period. Taken as an average for the three chelating agents the Fe^{55}/C^{14} ratio in the exudate gave a value of 10 for the green plants (table II) and 1,590 for the chlorotic plants (table I). These ratios emphasize the importance of careful control of factors relating to iron supply previous to iron-chelate applications. Especially would this be necessary if valid comparisons were to be made with other experiments.

The reference to the uptake of C^{14} label has been deliberate, since it is not known that the C^{14} in the radioautographs or plant exudate represents intact molecules of the chelating agents. In previous experiments (6) a faint red color was observed in the stem exudates of sunflower and soybean plants when their roots were placed in FeEDDHA concentrations ranging above 200 ppm. The red color was also observed when Fe was added in vitro to exudate from plants with roots in high concentrations of the chelating agent, EDDHA. From this it was concluded that the experimental plants absorbed small amounts of EDDHA or some metal chelate of EDDHA.

In experiments reported here the FeEDDHA was used at low levels, approximately eight parts per million. Thus it was not possible to see the chelate color or to measure spectrophotometrically the quantity of chelate in the plant exudate.

From the fact that small amounts of chelate can be found in soybean exudate following high FeEDDHA treatments (6), it is possible that the darkened area barely visible at about 5 cm (path 6, fig. 2) represents intact EDDHA. It is also possible that EDTA is autographed at about ten centimeters (path 4, fig 2). However, this is not certain, and it is perhaps more significant that a greater part of the C^{14} (fig 2) moved at rates different from those of the intact chelating agents shown in figure 1.

There is the possibility that the C^{14} patterns in figure 2 represent new components which contain the label of the original chelating agents. If such is the case, then the breakdown of the chelators would be taking place at or in the roots. This would also suggest that the C^{14} in the intact plants (fig 3) may represent at least in part the breakdown products of EDDHA.

It was shown in a previous study (6) that radioiron added in vitro was bound by exudate from plants which were grown at zero levels of the chelate. This binding of iron was attributed to natural chelators present in the conductive tissue of the plants. The agents responsible for the binding and transport of iron have not been identified.

Recent unpublished data indicate that the quantities of iron bound in vitro by exudate from chlorotic and green plants are about the same, while the quantities of iron absorbed through the roots are very different. From this it appears that the low levels of root-absorbed iron in the exudate of green plants should not be attributed to a shortage of transporting agents in the exudate but to the control of absorption at the root.

The conditioning that takes place in soybean roots grown under deficient iron conditions is not known. It is known, however, that as the soybean plant becomes more chlorotic it has a greater capacity to absorb iron (1). It has also been shown that the chlorotic plant has a greater capacity than the green

plant to reduce ferric iron at the root (2). This was demonstrated by the formation of Prussian blue on roots placed in ferricyanide-ferrichloride solutions. Associated with the greater reductive capacity of the roots was a greatly increased rate of transport and accumulation of iron in the leaves of the chlorotic plants (2).

How closely a mechanism of absorption may be coupled to iron translocation in the soybean plant is not known. For example, it may be asked whether iron is separated from chelate at some specific locus in the root cells where it is immediately transferred to carriers in the exudate or whether iron passes through several intermediate forms before it is bound to the exudate components. A further problem has to do with the specific effects of a plant's preconditioning or nutrient history on the particular agents and mechanisms involved in the absorption and translocation of iron. These are but a few of the many questions that remain to be answered concerning the metabolism of iron in the soybean plant.

Summary

The relative uptake of metal chelate components was tested by adding labeled chelates to nutrients containing chlorotic or green soybean plants. Exudate containing either C^{14} or Fe^{55} label was collected from the cut stems for counting and electrophoresis; radioautographs were made of intact, treated plants. Results and conclusions are as follows:

I. The exudate of chlorotic plants was very high in Fe^{55} and very low in C^{14}; the average assay per milliliter gave the ratio cps Fe^{55}: cps C^{14} = 10,033: 6.

II. The exudate of green plants was relatively low in Fe^{55} and very low in C^{14}; the average assay per milliliter gave the ratio cps Fe^{55}: cps C^{14} = 64: 6.

III. The ratio of Fe^{55}/C^{14} found in the exudate was greatly influenced by the availability of Fe in the preculture period.

IV. Fe^{55} is bound to exudate components which move toward the anode. Electrophoretic evidence suggests that most of the C^{14} in the exudate is not present as the intact chelating agents supplied to the roots.

V. Chlorotic intact plants accumulated much more Fe^{55} than the green plants, but chlorotic and green plants accumulated about the same amounts of C^{14}, suggesting the non-dependence of the absorption-translocation rates for these isotopes.

VI. The synthetic chelating agents delivered Fe to the roots; very little emphasis is placed on their role in the translocation of iron within the soybean plants.

Acknowledgments

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