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CHELATES FOR MICRONUTRIENTS

Properties of Chelates and Their Use in Crop Production

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Precipitated iron may serve as a reserve iron supply for plants but a mechanism is required for making it available to plants. Synthetic chelating agents have been used effectively to extract iron from soils or as iron chelate to keep iron in a soluble form in growth media. The apparent stability of iron chelates differs. The capacity of plants to absorb iron from iron chelate depends upon the kind and concentration of chelating agent, concentration of iron, plant species, and for some plants, whether the plant is green or chlorotic. Chlorotic Hawkeye soybeans differentially absorbed iron and chelating agent. Iron supplied to chlorotic Hawkeye soybeans at 2×10^{-6}M FeEDDHA appeared in the stem exudate as Fe malate. Roots and chelating agents compete for the iron in a nutrient solution. Roots which compete most effectively appear to have a reductive process, which affects the stability or availability of iron at the root. The factors which affect the availability of iron may or may not be a part of the actual absorption mechanism.

Among the important functions of metal ions in biological systems is their action as cofactors in enzyme systems. The microelements are particularly important and an adequate available supply is necessary for plant growth and development. Most microelements will hydrolyze and precipitate at pH 6, if they are not carried as chelate compounds. This is particularly true of iron. Agriculturally, there has long been a need for a soluble or available source of iron for plant growth.

Synthetic iron chelates have been used effectively to keep iron soluble and available for plant growth (*8, 13, 18, 21, 22, 30*). Four synthetic chelates are discussed — ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), cyclohexanediaminetetraacetic acid (CDTA), and ethylenediaminedi(*o*-hydroxyphenylacetic acid) (EDDHA).

Properties of Chelates

The apparent stability constants for FeEDTA, FeCDTA, FeDTPA, and FeEDDHA are 24.8, 29.3 (*1*), 27.9, and 30 (*11*), respectively. FeEDDHA is the most stable of the iron chelates. EDDHA, accordingly, would be expected to be the most competitive for iron in a growth medium. The capacity of EDDHA to chelate iron was determined in a nutrient solution containing variable concentrations of EDTA, DTPA, and CDTA as competitive chelating agents (*6*). The competitive chelating agents were equilibrated at pH 6.5 for 1 hour with a complete nutrient solution containing either $2 \times 10^{-5}M$ or $4 \times 10^{-5}M$ of Fe added as $FeCl_3$. EDTA, DTPA, and CDTA were supplied at 0.16, 0.5, 1, 2, 4, 6, 12, 18, or $36 \times 10^{-5}M$ concentrations. After equilibration, $2 \times 10^{-5}M$ EDDHA was added to each of the nutrient solutions, and the FeEDDHA (Figure 1) concentration was determined colorimetrically at varied intervals with final measurements made after 30 days (*6*).

The chelating capacity of EDDHA decreased sharply when the concentration of each competing chelating agent reached $2 \times 10^{-5}M$: EDTA < DTPA < CDTA. By increasing the Fe concentration to $4 \times 10^{-5}M$, $2 \times 10^{-5}M$ EDDHA competed for Fe successfully with $2 \times 10^{-5}M$ EDTA, DTPA, and CDTA. In this case, there was sufficient iron for both EDDHA and the competing agent. An increase in concentration of EDTA, DTPA, or CDTA to $4 \times 10^{-5}M$ sharply decreased the amount of Fe chelated as FeEDDHA. The effectiveness of the competitors was related to the stability of their iron chelates: FeEDTA < FeDTPA < FeCDTA. Thus, chelating capacity of EDDHA is dependent upon both the concentration of the Fe and the competitive chelating agent in solution. Absorption of iron by roots may likewise be dependent upon both the concentration of the Fe and the competing ligands in solution.

Use of Chelates

The above chelating agents can be used to extract iron from soils. The

iron extracted could be a good test for available iron in a soil, if the iron chelated and extracted in water is related to the iron absorbed by the plant. To test this relationship EDDHA was used to extract three calcareous soils: Quinlan soil from Oklahoma, Tripp soil from Kansas, and Millville soil from Utah (*3*). The chelatable iron in the soil formed water-soluble FeEDDHA (Figure 1), which gave a red color. The soils differed in the amount of extractable FeEDDHA they contained (Figure 2): Quinlan < Tripp < Millville (*3*). EDDHA extractable iron was not necessarily related to plant response (*5*). PI soybeans developed iron deficiency in all three soils. Wheatland milo developed iron deficiency in only the Quinlan and Tripp soils, and Hawkeye (HA) soybeans remained green in all three soils. This indicates a difference in causative factors among the soils producing iron deficiency and a difference in plant response to these causative factors. Chelatable iron in a soil is not necessarily a reliable index of plant response, but it does give some information as to the status of iron in a soil.

The Fe/Cu + Mn ratio differed in the soil extracts and in the plant material harvested from the Quinlan, Tripp, and Millville soils (Table I). Iron deficiency was not corrected in Wheatland milo until an iron salt, in addition to the chelating agent, was added to the Quinlan and Tripp soils (*3*). The addition of more iron increased the Fe/Cu + Mn ratio in both the soil extract and the plant material.

In Wheatland milo, the chelating agent competed with the plant root for iron. Wheatland milo developed iron chlorosis when grown in a nutrient solution containing $1 \times 10^{-5}M$ Fe and the following concentrations of DTPA: 0.16, 1, 2, 6, and $18 \times 10^{-5}M$ (*6*). When the iron concentration was increased to $6 \times 10^{-5}M$, iron deficiency was corrected in all treatments up to and including $2 \times 10^{-5}M$ chelating agent. Corn, wheat, and okra developed severe iron deficiency when the molar concentration of DTPA exceeded that of the iron (*2*). Under similar treatments, internode elongation in red kidney beans was stopped (*2*). DTPA in excess of iron caused the manganese concentration in red kidney beans to increase from 22 to 70 p.p.m. and the calcium to increase from 1.7 to 3.2% (*6*). PI soybeans remained green when the growth medium contained twice as much DTPA as Fe, and HA soybeans remained green with five times more DTPA than Fe (*7*).

The growth response was different for each of the four chelating agents EDTA, DTPA, CDTA, and EDDHA (*7*). In general, as the molar concentration of the chelating agent was increased to exceed that of iron, the amount of iron absorbed by the plant decreased. The competitive effect of chelating agent was overcome by adding more iron to the nutrient solution. Roots of the different plant species appear to react somewhat like different chelating agents. Wheatland milo is unable to absorb and utilize the Fe from FeEDDHA, unless the Fe concentration exceeds the EDDHA concentration. In contrast, HA soybeans have the capacity to absorb Fe from EDDHA when the EDDHA molar concentration is 17 times greater than the Fe concentration.

Chelation by plant roots or power of absorption may not be directly involved, but rather the root may in some way affect the valence of the ionic species being absorbed. HA soybeans are known to have the capacity to reduce Fe^{+3} to Fe^{+2} (*4*). Bond and Jones (*1*) showed that the stability of ferrochelates is less than that of the ferrichelates.

Storage and mobilization of iron in animals are apparently controlled by its oxidation and reduction (*12*, *23*). Like the Fe^{+3} in the animal, Fe^{+3} at the root may be a rather immobile form of Fe. Ferrous iron may be more mobile and less stable than ferric iron, and the roots may compete more effectively for it.

The use of synthetic chelating agents to keep iron soluble and available for plant growth has caused inquiry con-

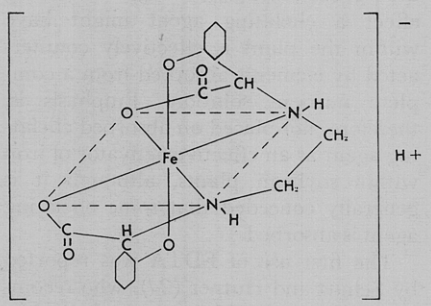

Figure 1. Iron chelated as FeEDDHA

Table I. Fe/Cu + Mn Ratio in Soil Extracts of Three DPTA-Treated Calcareous Soils and the Tops of Milo Grown in These Soils

	Soil Extracts			*Milo-Tops*		
	Millville	*Quinlan*	*Tripp*	*Millville*	*Quinlan*	*Tripp*
	Soils containing 97 p.p.m. DTPA chelate					
Fe/Cu + Mn ratio	2.00	0.68	1.41	0.41	0.24	0.21
Yield,[a] grams	...	...	...	2.74	0.37[b]	0.73[b]
	Soils containing 237 p.p.m. DTPA chelate					
Fe/Cu + Mn ratio	3.20	0.79	1.94	0.54	0.26	0.28
Yield,[a] grams	...	...	...	2.64	0.60[b]	1.00[b]

[a] 3 plants dry weight.
[b] Plants are chlorotic.

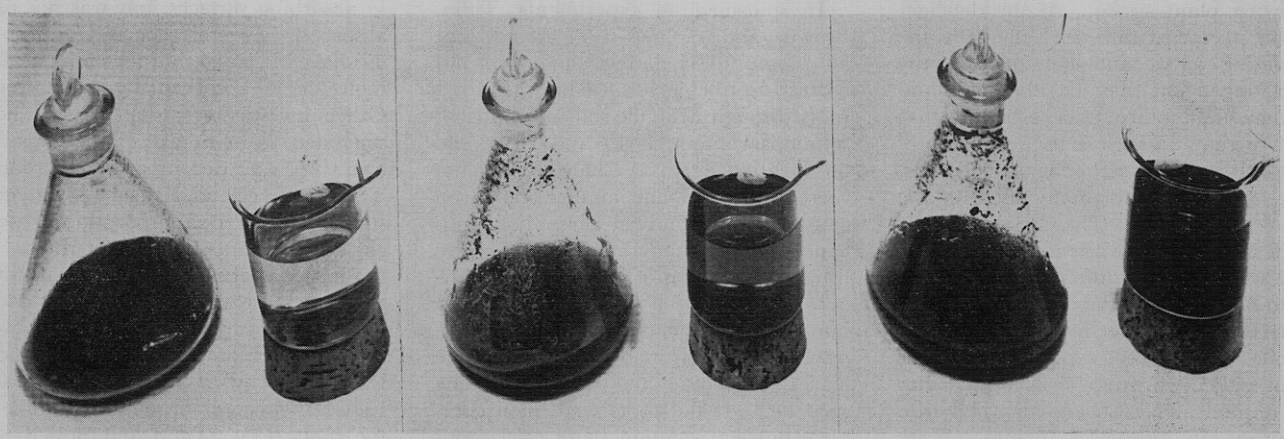

Figure 2. EDDHA (colorless) plus soil iron → FeEDDHA (red color)

EDDHA chelating agent used to extract iron from three soils Left to right, Quinlan, Tripp, and Millville soils, respectively

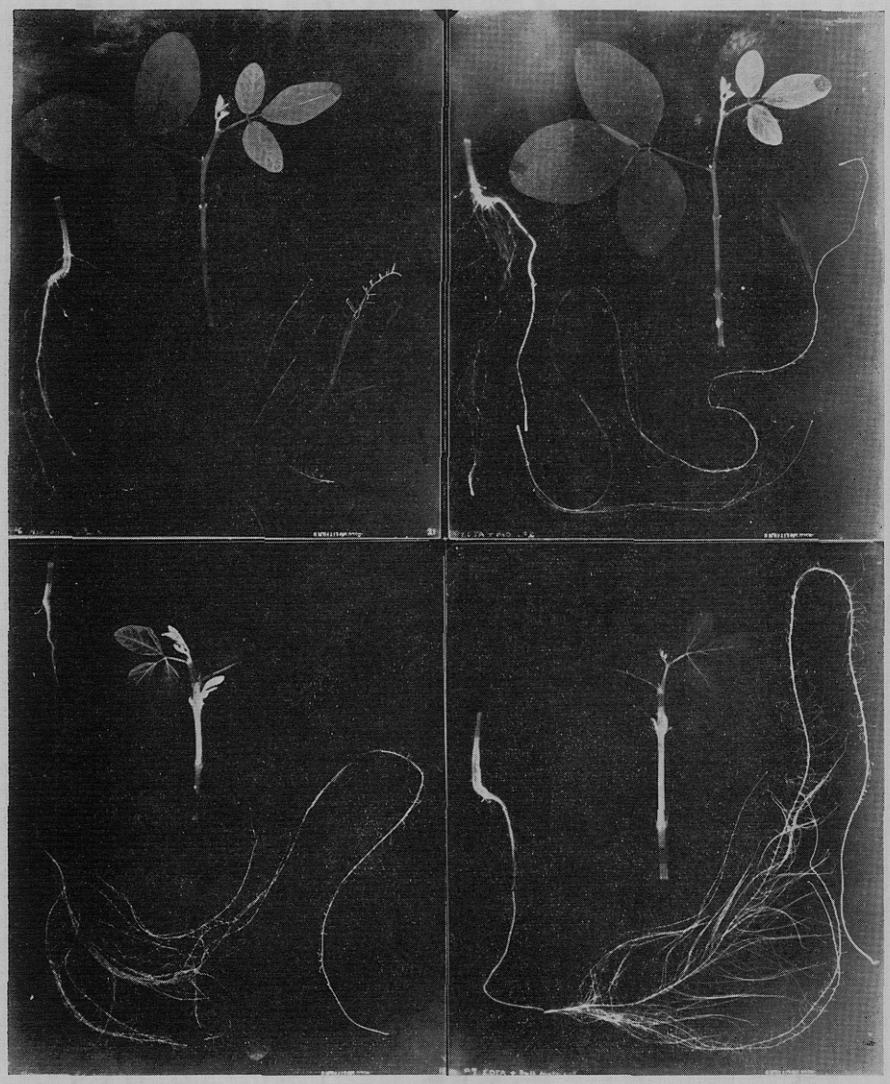

Figure 3. Effect of water (upper left), EDTA (upper right), nutrient solution (lower left), and nutrient solution plus EDTA (lower right) on the translocation of radioiron from the primary leaf of PI soybeans grown in a split medium

The Primary leaf was removed at the time of harvest

cerning the absorption of the chelating agents into the plant. As discussed by Chaberek and Martell (*8*), the introduction of a chelating agent into a plant may have serious and far-reaching implications with respect to the balance of essential trace metals maintained in the growing plant system. If the deficient metal is carried into the system by the chelating agent and metabolized, the chelating agent may be liberated, and it may bind other trace elements that are present. There is a possibility of not only creating secondary deficiencies, but also of fundamentally altering the plant metabolism. The chelating agent itself may be metabolized.

There are some differences of opinion as to how much of the chelating agent is absorbed and how effective it is in the plant. In split-root experiments, Weinstein and co-workers (*31*, *32*) found that 5.0 p.p.m. of Na_2EDTA corrected iron chlorosis in sunflower plants. These workers postulated that iron was absorbed by one portion of the root system, but was inactivated after entry into the plant. Na_2EDTA supplied through the other portion of the root system apparently chelated the inactivated iron and made it available for metabolic use. Similar work by DeKock (*10*) also tends to confirm this view.

In split-medium experiments, Tiffin, Brown, and Holmes (*27*) found that 20 p.p.m. of chelating agent did not correct iron chlorosis. They grew PI roots through Millville soil into nutrient solutions containing 10 and 20 p.p.m. of chelating agents. Chlorosis was not corrected and foliar applied radioiron caused greening only at the spot of application. Radioautograms showed very little translocation of radioiron throughout the leaves. If the roots extended into water or chelating agent alone (no nutrient), radioiron did move into the leaves (Figure 3) and there was some greening. If the roots were in a complete nutrient solution (with or without chelating agent), radioiron accumulated in the stem (Figure 3) at the point where the leaf containing the radioiron was attached to the stem (*27*).

Table II. Effect of Chelates and Varied P Concentration in the Nutrient Solution on Iron Content in PI Soybean Tops Grown with Split-Root

	Treatments		
P, P.P.M.	No Chelate	EDTA[a]	EDDHA[a]
	Fe Found, P.P.M.		
1	130	130	130
2	95	100	85
3	55[b]	75	46[b]
4	46[b]	60[b]	40[b]
5	47[b]	42[b]	33[b]
6	38[b]	27[b]	27[b]

[a] 45 P.P.M.
[b] Chlorosis developed.

In split-root experiments (*27*) where one set of roots was supplied 10 p.p.m. of Ca and 45 p.p.m. of EDDHA and the other set of roots was supplied with a complete nutrient solution containing 2 p.p.m. of Fe and increasing P concentrations of 1, 2, 3, 4, 5, and 6 p.p.m., the EDDHA treatments developed a more severe chlorosis than did the controls or the EDTA treatments. EDTA was slightly better than the controls (Table II). All of the plants were chlorotic in the 4-p.p.m. P treatments. Phosphate, roots, and chelating agent all appear to compete for the Fe supply (*27*) and thereby exert individual effects in the growth process. Any activating effect a chelating agent might have within the plant is effectively counteracted by elements absorbed from a complete nutrient solution. Emphasis is, therefore, not placed on absorbed chelating agent as an effective activator of iron within soybean plants, although it is generally conceded that some chelating agent is absorbed.

The first use of EDTA was reported by Schatz and Hutner (*24*), who recommended it as a stable, nonmetabolizable, and nontoxic reagent. Hutner and co-workers (*17*) considered the chelating agent as a carrier which delivered metals to absorbing surfaces, but was not itself absorbed. Several reports (*14*, *15*, *29*, *30*) have indicated that the entire chelate molecule is absorbed by plant roots. Other investigators (*16*, *19*, *20*) have suggested plant uptake of the chelating agent or a decomposition product. Tiffin, Brown, and Krauss (*28*) demonstrated a differential absorption of metal chelate components by plant roots. As the iron was absorbed from the nutrient solution, there was a sevenfold increase in its chelating capacity. Analyses showed that the increase in chelating capacity was caused by an increase in iron-free EDDHA concomitant with Fe uptake by the roots (*28*). Tiffin and Brown (*25*) further showed a selective absorption of Fe from FeEDTA, FeDTPA, and Fe-

Table III. Comparative Assays of Nutrient and of Exudate from Chlorotic and Green Soybean Plants Treated with Labeled Chelates

Treatment	Assay of Nutrient, C.P.S./Ml. Before[a]	After[b]	Assay of Exudate, C.P.S./Ml.
	Chlorotic Plants		
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
	Green Plants		
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

[a] Before plants were placed in nutrient. [b] After containing plants for 22 hours.

EDDHA by HA soybeans. Both Fe^{55}- and C^{14}-tagged chelates were used in these studies. Chlorotic HA soybeans absorbed more Fe^{55} from the nutrient solution than C^{14} (Table III). The stem exudate contained much more Fe^{55} than C^{14}. Green HA soybeans absorbed much less Fe^{55} (Table III) than chlorotic HA soybeans. The C^{14} absorbed was approximately the same for both green and chlorotic plants. Radioautograms showed that this same relationship was true for intact plants (*25*). Thus, the magnitude of differential absorption of Fe and chelating agents is dependent upon the iron stress or extent of the iron deficiency in the plant.

Tiffin, Brown, and Krauss (*28*) showed that xylem exudates increase both in Fe-EDDHA and in total iron as the Fe-EDDHA concentration is increased in the nutrient solution. But, the iron concentration as FeEDDHA was very low when compared to total iron in the exudate. For soybeans grown in a nutrient solution containing 660 p.p.m. of FeEDDHA, 0.84% of the FeEDDHA in the nutrient solution was found in the soybean exudate. This was reduced from 0.84 to 0.27% where 55 p.p.m. of FeEDDHA was in the nutrient solution. Thus, as the iron chelate concentration is increased in the nutrient solution, more iron chelate molecules appear in the stem exudate. On a percentage basis, this has always been below 1% (*28*). Cocking (*9*) has found that treating tomato roots with $10^{-3}M$ EDTA at pH 7.2 brings about separation of the cells of the tissues into single cells and chains of cells. Thus, in chelate studies, to minimize any effects of chelating agent on the structure of the root, minimum quantities of the chelating agent are used.

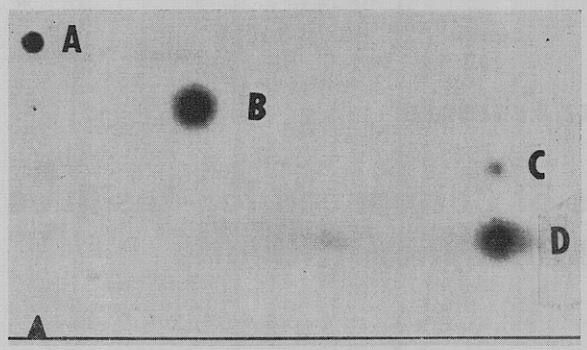

Figure 4. Electrophoregram (Fe^{59}) showing movement of iron from origin (left) to anode (right)

Top to bottom: (A) $FeCl_3$ spot (origin), (B) FeEDDHA spot, (C) stem exudate spot from green HA soybean (Fe malate), (D) stem exudate spot from chlorotic HA soybean (Fe malate)

Where minimum quantities of Fe^{59}-EDDHA were used to supply iron to HA soybean plants, the Fe^{59} did not appear as Fe^{59}EDDHA in the stem exudate (Figure 4) (*26*). Instead, most of the radioiron appeared as iron malate (Figure 4). Iron malate was determined electrophorectically and malate by chromatography (*26*). How iron is transferred from the metal chelate in the growth medium to the iron malate in the stem exudate remains as a challenge for further study. A reduction of Fe^{+3}, in the metal chelate, to Fe^{+2} appears to be involved. This change in valence may be related to the availability of iron, but may not be a part of the actual absorption mechanism.

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End of Symposium