Laboratory and greenhouse evaluation of four iron fertilizer sources

R. Jay Goos

Dep. of Soil Science, School of Natural Resource Sciences, North Dakota State Univ., NDSU Department 7680, Box 6050, Fargo, ND 58108-6050, USA

Abstract

A relatively new iron fertilizer in the United States is FeHBED [Fe-N,N′-bis(2-hydroxybenzyl)ethylenediamine-N,N′-diacetate], a chelate with a stability constant for ferric iron greater than FeEDDHA [Fe-ethylenediamine-N,N′-bis(o-hydroxyphenylacetate)]. This study compared response of soybean [Glycine max (L.) Merr.] over two greenhouse cropping periods to FeHBED, FeEDDHA-1 (~80% ortho-ortho isomer), FeEDDHA-2 (~50% ortho-ortho isomer), and FeEDDHSA [Fe-ethylenediamine-N,N-di([2-hydroxy 5-sulfophenyl])acetate]. FeHBED and FeEDDHA-1 gave positive responses to all measured parameters (chlorophyll level, dry matter production, Fe uptake) that were not significantly different (.05 level) from each other for the two cropping periods. The two products were similar in performance. FeEDDHA-2 and FeEDDHSA generally gave lesser responses than FeHBED or FeEDDHA-1. The relative effectiveness of the four materials to increase dry matter or Fe uptake was predicted by a simple laboratory test, which measures the proportion of applied Fe remaining soluble after a 1-wk incubation with soil.

1 INTRODUCTION

Iron deficiency chlorosis (IDC) is a common and destructive disorder of soybean [Glycine max (L.) Merr.] grown on calcareous soils of the north-central and Great Plains regions of the United States (Hansen et al., 2004). Selection of a resistant cultivar is the most effective control measure (Goos & Johnson, 2000, 2001), but identification of resistant cultivars is complicated by the short life-span of cultivars in the marketplace, changing transgenic trait platforms, and inconsistent methods of rating and reporting chlorosis resistance scores by seed companies. Thus, additional control measures are needed. Application of an effective iron fertilizer, like FeEDDHA [Fe-ethylenediamine-N,N′-bis(o-hydroxyphenylacetate)], either on or near the seed at planting can reduce chlorosis severity (Kaiser et al., 2014; Lingenfelzer et al., 2005; Wiersma, 2007). While FeEDDHA is an effective Fe fertilizer, commercial samples vary with regard to the proportions of iron chelated with the effective ortho-ortho isomer and ineffective condensates and isomers (Schenkeveld et al., 2007, 2008). (The expression “ortho-ortho isomer” is an inclusive term, combining both meso and racemic forms; see Schenkeveld et al., 2007, Figure 1, for an illustration.) Recently, FeHBED [Fe-N,N′-bis(2-hydroxybenzyl)ethylenediamine-N,N′-diacetate] was introduced in the U.S. market. This chelate has a stability constant 10^4 greater than FeEDDHA (Nadal et al., 2012), and...
recent manufacturing improvements have reduced production costs (Olszewski et al., 2014). Evaluations of FeHBED have been made in hydroponic systems (Chaney, 1988; Nadal et al., 2008) and in greenhouse studies using calcareous soil (Martin-Fernandez et al., 2017). Two studies suggested that FeHBED may provide crops with a longer-lasting response than FeEDDHA (Bin et al., 2016; Nadal et al., 2012), but additional evaluation of this conclusion is needed. The objectives of this study were to compare the responses of soybean to four iron sources and to compare plant responses to a simple soil incubation test of chelate persistence in the soil solution.

2 | MATERIALS AND METHODS

A greenhouse study was performed with five treatments and four replicates in a randomized complete block design. Into closed-bottom, 15-cm-diam. plastic pots was placed a mixture of 1 kg of white sand (20/40 mesh) and 1 kg of a calcareous Glyndon sandy loam (coarse-silty, mixed, superactive, frigid Aeric Calciaquolls). The soil (0–15 cm) was collected in a production field from an area consistently producing chlorotic soybeans. The soil was spread thinly to air dry and crushed to pass a 2-mm sieve. The soil contained 31 g kg$^{-1}$ organic matter, 60 g kg$^{-1}$ CaCO$_3$ equivalent. The pH of a 1:1 soil/water suspension was 8.0, and the electrical conductivity was 0.3 dS m$^{-1}$. Before mixing with the soil, the sand was treated with five solutions and mixed. The sand was treated with 5 ml of the iron treatment solution, 5 ml containing 100 mg N as NH$_4$NO$_3$, 5 ml containing 100 mg P as K$_2$HPO$_4$, 5 ml containing 5 mg each of Zn, Cu, and Mn as sulfate salts, and 5 ml of a rhizobial inoculant suspension (2 g peat inoculant suspended in 200 ml of water). The fertilized sand was mixed with soil and placed in a pot lined with a plastic bag. The soil/sand mixture was moistened with 200 ml of water, covered with a filter paper, and allowed to equilibrate for a week. The iron treatment solution consisted of 5 ml of water (control) or 5 ml of an iron solution formulated to provide 0.5 mg of Fe, according to the percentage Fe on the label (Table 1). The iron sources were a higher-quality FeEDDHA-1, with ~80% of the iron chelated as the ortho-ortho isomer of EDDHA (Soygreen, CHS), a lower quality FeEDDHA-2, with ~50% of the iron chelated as the ortho-ortho isomer of FeEDDHA (FerroActive H32, Deretil), FeEDDHSA (Sequester S, Malpryser), and FeHBED (Epsilon, ADOB). After the period of incubation, 12 ‘Stine 0480’ soybean seeds were planted per pot. Before planting, dry seeds were placed in growth pouches (Mega-International), treated with 0.01 M Ca(NO$_3$)$_2$, and allowed to imbibe and swell overnight. After emergence, an additional 100 mg N was added as NH$_4$NO$_3$, and the stand thinned to 4 plants pot$^{-1}$. The pots were weighed daily, and water lost to evapotranspiration was replenished. Pots were rotated in position on the greenhouse bench daily. The water content of the pots was gradually increased over a 2-wk period to 300 ml pot$^{-1}$. After the third trifoliolate leaflets reached full size, the relative chlorophyll of the first, second, and third trifoliolate leaflets was measured with a Minolta SPAD meter, the plant stems were excised just above the cotyledon node, and the tops rinsed in deionized water, dried at 65 °C, and weighed. The tops were ground to pass a 0.1-mm sieve, and a sample digested in HNO$_3$, and Fe was determined by plasma ICP (Havlin & Soltanpour, 1980). The contents of each pot were passed through a 4-mm sieve, and the roots and stem bases returned to the bottom of the pot. The soil/sand mixture was treated with 100 mg N as ammonium nitrate, returned to the pot, and replanted as before, to grow a second crop. An additional 100 mg of N as NH$_4$NO$_3$ was added after emergence, the stand thinned to 4 plants pot$^{-1}$, and a second crop was grown and analyzed as for the first crop.

The fertilizers were analyzed for water-soluble and “soil-stable” Fe. A sample of fertilizer containing 120 mg of Fe according to the label (e.g., 2 g of a 6% product) was dissolved in water and brought to 1 L. Into six 50-ml centrifuge tubes was placed 1 ml of the diluted fertilizer and 1 ml of water. Three tubes were frozen without soil; the other three tubes received 10 g of the Glyndon soil. The tubes receiving soil were incubated uncapped at room temperature (22–23 °C) in a chamber with elevated humidity. After 7 d, all tubes received 28 ml of 0.01 M CaCl$_2$, followed by shaking (15 min), centrifugation, and filtration. Nine milliliters of filtrate or standard (0, 1, 2, 5 mg L$^{-1}$ Fe) was treated with 1 ml of a solution containing 1 M HCl and 10 g L$^{-1}$ hydroxylamine HCl, and the Fe concentration determined by atomic absorption spectrometry. The soluble Fe results from the tubes not receiving soil represented the water-soluble Fe in the fertilizer. The soluble Fe results from the tubes incubated with soil represented the “soil-stable” Fe, meaning, the chelate is stable, retaining the Fe in a soluble form in the presence of soil.

Core Ideas

- Soybean response to four iron fertilizers was compared in a greenhouse study.
- Crop response was similar for FeHBED and a higher-quality FeEDDHA product.
- A lower-quality FeEDDHA product and FeEDDHSA gave lesser crop response.
- The relative performance of the four sources was predicted by a simple soil incubation test.
**TABLE 1** Characteristics of the fertilizers evaluated

| Fe source | Fe rate | Water-soluble Fe (% Fe) | Rate of “Soil-stable” Fe | Rate of “Soil-stable” Fe |
|-----------|---------|--------------------------|--------------------------|--------------------------|
| Control   | 0       | –                        | 0                        | 0                        |
| FeEDDHA-1 | 0.5     | 6.4                      | 5.3                      | 8.33                     |
| FeEDDHA-2 | 0.5     | 6.3                      | 3.0                      | 8.33                     |
| FeEDDHSA  | 0.5     | 6.0                      | 2.1                      | 8.33                     |
| FeHBED    | 0.5     | 3.3                      | 2.8                      | 16.66                    |

### RESULTS AND DISCUSSION

The properties of the four fertilizers are shown in Table 1. The water-soluble Fe in each material equaled or slightly exceeded the percentage Fe on the label. The materials differed greatly in the percentage of soil-stable Fe. The rate of soil-stable Fe applied per pot ranged from 0.18 mg pot$^{-1}$ for FeEDDHSA, to 0.44–0.47 mg pot$^{-1}$ for FeEDDHA-1 and FeHBED, respectively. FeEDDHSA has a lower stability constant, the ratio of chelated to unchelated Fe under standard conditions, for ferri-c iron than FeEDDHA and has been shown to lose a larger proportion of its soluble Fe upon incubation with alkaline soils than FeEDDHA (Cantera et al., 2002; Goos & Germain, 2001). Yet, FeEDDHSA is widely sold in this region, as it is more easily solubilized and more conveniently made into a liquid fertilizer than FeEDDHA.

The effect of the fertilization treatments on the chlorophyll level, growth, and Fe uptake by the two crops of soybean is shown in Table 2. For the first crop, the severity of IDC was extreme in the control treatment, with a relative chlorophyll level, by SPAD meter, of 7, averaged over the first, second, and third trifoliolate leaflets. Normal soybean leaflets typically have a SPAD reading of >30. The application of all four Fe sources dramatically increased chlorophyll levels, dry matter production, and Fe uptake over the control. For chlorophyll level, tissue Fe concentration, and Fe uptake, the greatest response was given by FeEDDHA-1. For dry matter production, the greatest response was from FeHBED, although the differences were not significantly different (.05 level) between these two sources for these three parameters. In general, the responses given by FeEDDHSA were less than for the other sources.

Crop response to iron fertilization was less for the second crop than for the first crop. In general, FeHBED gave a slightly greater response than FeEDDHA for chlorophyll level, dry matter, and Fe uptake, but the differences were not statistically significant. No significant differences between treatments were observed for tissue Fe concentration. It has been suggested that crop response to FeHBED may be longer lasting than crop response to FeEDDHA (Bin et al., 2016; Nadal et al., 2012). The results of this study suggest that this effect may be small. Response to all three parameters (chlorophyll, dry matter, and Fe uptake) was significantly less for FeEDDHA-1 and FeEDDHSA than for FeHBED or FeEDDHA-1 for the second crop. This suggests that multiple crops and limited rates of Fe may be necessary to fully

**TABLE 2** Effect of four iron fertilizers on leaf chlorophyll level, aboveground dry matter production, and Fe uptake. Each crop was grown until the third trifoliolate leaflets were fully developed

| Fe source | First crop | Second crop |
|-----------|------------|-------------|
|           | Rel. chl.$^{a}$ | Fe uptake | Rel. chl.$^{a}$ | Fe uptake |
|           | mg pot$^{-1}$ | μg g$^{-1}$ | μg pot$^{-1}$ | μg g$^{-1}$ | μg pot$^{-1}$ | μg g$^{-1}$ | μg pot$^{-1}$ | μg g$^{-1}$ | μg pot$^{-1}$ |
| Control   | 0          | 7.0d       | 2.01b       | 47a        | 98c       | 9.7c       | 1.65b       | 42          | 68c       |
| FeEDDHA-1 | 0.5        | 35.8a      | 4.35a       | 70b        | 302a      | 16.0ab     | 2.62a       | 40          | 106ab     |
| FeEDDHA-2 | 0.5        | 30.2b      | 4.45a       | 57a        | 255a      | 10.7c      | 1.97b       | 39          | 75c       |
| FeEDDHSA  | 0.5        | 25.6c      | 3.78a       | 52a        | 196b      | 12.0bc     | 2.07b       | 41          | 85bc      |
| FeHBED    | 0.5        | 31.5ab     | 4.47a       | 59ab       | 264a      | 18.7a      | 2.91a       | 42          | 123a      |
| $F$       | **         | **         | **          | **         | **        | **         | **          | **          | **        |
| LSD (0.05)| 4.5        | 0.72       | 12          | 57         | 4.5       | 0.50       | –           | 29         |

*Note. In each column, values followed by the same letter are not significantly different at the .05 level by Duncan’s Multiple Range Test. ns, nonsignificant.

$^a$Based on the % Fe on the label; see Table 1.

$^b$Relative chlorophyll content of the first, second, and third trifoliolate leaflets, using a Minolta SPAD meter.

*Significant at $p < .05$.

**Significant at $p < .01$. 
separate the relative effectiveness of Fe chelates in greenhouse studies.

Dry matter production and Fe uptake were summed across both crops and plotted versus the rate of soil-stable Fe applied per pot (Figure 1). In general, the simple soil test properly ranked the four fertilizers with regard to effectiveness, even though three different chelate chemistries were involved with the study.

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AUTHOR CONTRIBUTIONS
R. Jay Goos: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing-original draft; Writing-review & editing.

CONFLICT OF INTEREST
The author declares no conflict of interest.

ORCID
R. Jay Goos https://orcid.org/0000-0002-1437-8712

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FIGURE 1 Dry matter or iron uptake, summed over both crops, as related to the rate of “soil-stable” Fe applied.
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