A Selection Procedure for Iron-deficiency Stress Tolerance among Warm-season Turfgrasses Using Ferrihydrite-amended Growth Media

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Abstract. Iron-deficiency (Fe-deficiency) stress, characterized by chlorosis of leaf tissue, is a major limiting factor in turfgrass production on calcareous soils. The objectives of this study were to: 1) evaluate ferrihydrite-amended growth media and the threshold amount of Fe initially added for use in a whole-plant screening procedure for selecting cultivars that are tolerant to Fe-deficiency stress conditions; 2) measure and evaluate whole-plant growth characteristics that could be an index of Fe deficiency stress; and 3) assess the potential of using a synthetically produced Fe oxide, ferrihydrite, as a slow-release Fe fertilizer source. Iron-stress sensitive 'Raleigh' St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] and Fe-stress tolerant 'Tifway' bermudagrass [*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Davy] cultivars were grown under glasshouse conditions in a medium consisting of quartz sand, 5% (m/m) CaCO₃, and a ferrihydrite amendment providing Fe in concentrations of 0, 15, 30, 46, or 120 mg·kg⁻¹ media, (equivalent to 2, 3, 4, 5, or 10 mg DTPA-extractable Fe/kg media). There also was a nonlimiting iron control. St. Augustinegrass was first rated for iron chlorosis 83 days after planting (DAP) while bermudagrass was first rated at 294 DAP. Initial Fe levels equivalent to 5 mg DTPA-extractable Fe/kg media showed potential for screening genotypes. Visual estimates of iron chlorosis and chlorophyll contents of leaves were the best indicators of low soil Fe availability. A single ferrihydrite soil amendment at 10 mg DTPA-extractable Fe/kg media was adequate in preventing chlorosis for the duration of the study (174 and 509 days for St. Augustinegrass and bermudagrass, respectively). Chemical name used: Diethylenetriaminepentaacetic acid (DTPA).

Iron-deficiency (Fe-deficiency) stress in plants, characterized by leaf chlorosis, can be a major limiting factor in turfgrass growth and can result in aesthetic and economic losses. Although high amounts of total Fe are commonly found in soil, any plant growing in a calcareous soil (pH ≈ 7.8 to 8.4) is in a potential Fe-deficiency stress environment, since solution-phase Fe is at a minimum concentration within this pH range (Lindsay, 1979; Lindsay and Schwab, 1982).

Iron chelates and salts are the most commonly used forms of supplemental Fe and are applied either to the soil or sprayed directly onto the foliage, but may be phytotoxic to turfgrasses (Beard, 1984; Carrow et al., 1988; Deal and Engel, 1965). Another disadvantage is that they are effective for relatively short periods of time and consequently several applications are required per year (Ryan et al., 1975). Also, the Fe from chelates applied to the soil may not be immediately available for root absorption. The application of the chelate Fe-EDDHA [ethylenediamine di-(o-hydroxyphenylacetate)] to Fe-deficient annual ryegrass (*Lolium multiflorum* Lam.) was ineffective due to the high stability of this form and the inability of the plant to extract the Fe (Lucena, 1988).

Iron in the soil environment is most commonly in the form of Fe oxides and hydroxides. The dissolution of Fe oxide is largely influenced by its reactive surface area, which may be influenced by the species, crystal size, and/or crystal order (Schwertmann and Taylor, 1989). Application of hematite, magnetite, or limonite, which are all crystalline forms of Fe oxide and relatively unreactive, had no effect on sorghum [*Sorghum bicolor* (L.)

Moench] grown in three Fe-deficient soils (Olson, 1950); however, the addition of Fe as a poorly crystalline or amorphous Fe oxide, of very small size and large surface area (e.g., ferrihydrite), provided adequate Fe for the growth of sorghum grown on Fe-deficient calcareous soils (Vempati and Loeppert, 1986). Thus, ferrihydrite may show promise as a new source of slow-release Fe.

In turfgrass management schemes, the use of turfgrass species and cultivars that are tolerant to Fe-deficiency stress conditions is coupled with the application of Fe amendments to alleviate chlorosis. Turfgrass cultivars differ in Fe-deficiency stress tolerance (Harivandi and Butler, 1980; Kurtz, 1981; McCaslin et al., 1981). These differences may be expressed as differences among cultivars in growth, Fe accumulations, leaf chlorophyll contents, and/or the degree of chlorosis exhibited by the plant. The determination of turfgrass characteristics closely associated with Fe-deficiency stress tolerance would facilitate development and selection of tolerant cultivars.

Various screening techniques have been applied to turfgrasses and other plants for differentiating Fe-deficiency stress tolerance. These have included the use of hydroponic solutions (Brown and Jones, 1976), uptake of ⁵⁹Fe (Kurtz, 1981), growing turfs in field plots known to produce Fe chlorosis (Harivandi and Butler, 1980), and growing turfgrasses and other plants in pots containing various mixtures of soil, sand, zeolite, and/or CaCO₃ (Bowen and Rodgers, 1987; Hernandez, 1987; Loeppert et al., 1984; McCaslin et al., 1981).

The objectives of this study were to: 1) evaluate ferrihydrite-amended growth media and the threshold amount of Fe initially added for use in a whole-plant screening procedure for selecting cultivars that are tolerant to Fe-deficiency stress conditions; 2) measure and evaluate whole-plant growth characteristics that could be indices of Fe-deficiency stress; and 3) assess the potential of using a synthetically produced Fe oxide, ferrihydrite, as a slow-release Fe fertilizer source.

Materials and Methods

'Raleigh' St. Augustinegrass, which is susceptible to Fe-deficiency stress, and 'Tifway' bermudagrass, which is tolerant to Fe-deficiency stress (McCaslin et al., 1981), were utilized in this study. Thirty-six plugs, 102 mm in diameter, of each species were collected from mature turf plots located at the Turfgrass Field Research Laboratory of Texas A&M Univ., College Station, in Nov. 1988. Roots were severed 50 mm (St. Augustinegrass) or 20 mm (bermudagrass) below the soil surface of each plug, and the remaining soil was removed by washing with water.

Six replicate plugs were individually transplanted onto one of five different soil-media treatments based on the amount of Fe initially added; a sixth treatment consisted of a nonlimiting Fe control. The base growth media for all six treatments, before the addition of Fe, consisted of 95% quartz sand, selected to be low in available Fe (<2 mg DTPA extract-

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able Fe/kg) as determined by the DTPA soil test (Lindsay and Norvell, 1978) and 5% (m/m) powdered reagent-grade CaCO_3 . The growth media were contained in polyvinyl chloride (PVC) cylinders (102 mm in diameter $\times$ 305 mm deep) with a PVC cap inserted on the bottom. To ensure adequate drainage, holes (1 mm in diameter) were drilled through the caps and a layer of pea gravel 37 mm deep was placed in the bottom of the capped cylinder to prevent the clogging of organandy cloth that was lining the caps.

Prior to this study, an incubation study was performed to determine DTPA-extractable Fe based on the amount of Fe added. Iron, as $\text{Fe}_2(\text{SO}_4)_3$, was added in concentrations ranging from 0 to 1000 mg Fe/kg to media composed of 142.5 g of sand and 7.5 g of CaCO_3 . Calcium sulfate is produced during the reaction of $\text{Fe}_2(\text{SO}_4)_3$ with CaCO_3 ; therefore, additional CaSO_4 was added in appropriate amounts to maintain 0.004 M CaSO_4 for all samples. The samples were incubated for 7 d, extracted with DTPA, and analyzed for Fe.

Based on the results of the equilibration study, Fe was added to give 0, 15, 30, 50, or 120 mg $\cdot$ kg $^{-1}$ media, which resulted in 2, 3, 4, 5, and 10 mg DTPA-extractable Fe/kg media. The medium contained in each PVC cylinder was individually prepared. The appropriate amount of 0.1 M $\text{Fe}_2(\text{SO}_4)_3$ was poured into a Waring blender, along with CaSO_4 at 0.004 M and CaCO_3 in a concentration equal to the Fe added. Deionized water was added to give a total volume of 340 mL, and the suspension was mixed for 30 s. Addition of CaCO_3 resulted in an increase in pH and the resulting precipitation of Fe oxide on the calcite particle surface according to the formula below:

X-ray diffraction spectrometry analysis of the dried suspension indicated the presence of 2-line ferrihydrite. The suspension was then uniformly sprinkled upon 3.47 kg of medium contained in a plastic pan, thoroughly mixed, and transferred to a PVC cylinder. The treated media were allowed to equilibrate for 1 week prior to planting. During this time, the average soil pH for all six treatments was 9.1.

The nonlimiting Fe treatment consisted of planting turf plugs on the base medium and applying a FeSO_4 solution at 0.61 g Fe/m 2 per week. Each FeSO_4 application to the control was equally split between a foliar spray and a soil drench; the soil drench was applied before the foliar spray.

The PVC cylinders were supported by wooden racks located on a glasshouse bench. The temperature in the glasshouse ranged from 24 to 35 °C. The turfs were watered from the top with deionized water as necessary to prevent visual drought-stress symptoms. Fertilizer was applied biweekly using Fe-free Hoagland's nutrient solution (Hoagland and Arnon, 1938) to provide N at 3.7 g $\cdot$ m $^{-2}$ per month. Turfs were mowed with a hand-held clipper every 14 to 37 d, depending on rate of shoot growth. St. Augustinegrass and ber-

mudagrass were mowed at 50- and 25-mm cutting heights, respectively. To ensure a uniform cutting height, a guide, consisting of a PVC ring (102 mm in diameter $\times$ 50 or 25 mm deep), was placed on the upper end of each PVC cylinder. Clippings were collected from selected mowings, rinsed with deionized water, oven dried at 60 °C for 48 h, and weighed.

The Fe chlorosis of the St. Augustinegrass turf was visually rated about once every 2 weeks when the plants began to exhibit chlorotic symptoms 83 d after planting (DAP). The bermudagrass was rated for Fe chlorosis at 294, 321, and 509 DAP. The rating scale was 1 to 4, where 1 = 0 to 5%, 2 = 25%, 3 = 50%, and 4 >50% chlorosis.

Prior to the last mowing of St. Augustinegrass (174 DAP) and bermudagrass (509 DAP), the number of live shoots were counted from one-half of the surface area of each cylinder; counts were multiplied by 2 and reported on a cylinder basis (82 cm 2).

Leaf subsamples taken from the last mowing were digested (Parkinson and Allen, 1975) and the total Fe content in each of the digests was determined by atomic absorption spectrometry. Chlorophyll contents of the St. Augustinegrass leaf tissue were determined at 105 and 174 DAP by extracting fresh tissue subsamples with dimethylsulfoxide (DMSO), heating at 68 °C for 8 h, filtering, and measuring the filtrate spectrophotometrically at 645 and 663 nm (Hiscox and Israelstam, 1979). Because of changes in personnel, we were unable to determine chlorophyll contents of bermudagrass. Clipping yields were corrected for the subsamples used for Fe and chlorophyll content determinations.

St. Augustinegrass and bermudagrass were harvested 175 and 510 DAP, respectively. Turfs were removed from the cylinders and their crowns cut from the intact soil cores at the soil surface. Rhizomes (bermudagrass only) and roots were individually separated from the soil by washing. Crowns of each species and rhizomes and roots of bermudagrass were dried as previously described and weighed. Roots of St. Augustinegrass were immediately placed in individual cups containing a paraformaldehyde solution (40 g $\cdot$ L $^{-1}$), fixed for 24 h, and then dehydrated in the same cups by sequential submersions for 1 h each in 25%, 50%, and 70% (v/v) ethanol solutions. Roots were stored in a 70% (v/v) ethanol solution at 5 °C for subsequent root-length measurements, which were made with a Comair Root Scanner (Hawker De Havilland Limited, Systems Division, P.O. Box 580, Salisbury SA 5108, Australia) capable of detecting roots having diameters >0.1 mm. Following root-length measurements, roots were dried as previously described and weighed.

Samples (25 g) of the media from each cylinder were taken immediately before planting, midway through the studies, and after harvest. Plant-available Fe was determined in each sample as DTPA-extractable Fe (Lindsay and Norvell, 1978).

Analysis of variance (ANOVA) on all dependent variables was performed using a General Linear Model procedure for a completely

randomized design with six replications. Analysis of variance for the overall visual rating of Fe chlorosis was performed utilizing a General Linear Model procedure for a repeated-measures design, with soil-media treatments as main plots and rating dates as the repeated-measures factor. When ANOVA treatment effects were significant ($P \leq 0.05$), comparisons of treatment means were made by both a least significant difference procedure and a contrast procedure for procuring specified treatment comparisons. Pearson correlation coefficients were calculated on treatment means of selected variables on selected dates.

Results and Discussion

Iron-deficiency chlorosis in the St. Augustinegrass was initially rated at 83 DAP, whereas bermudagrass showed no Fe-deficiency symptoms at this time (Table 1). Visual chlorosis was not rated in the bermudagrass until 294 DAP, indicating it was considerably more Fe-stress tolerant than St. Augustinegrass.

An objective in this study was to evaluate the threshold amount of Fe initially added to the media that would prevent chlorosis. The nonlimiting Fe control treatment kept each cultivar essentially chlorosis-free throughout the study. Chlorosis in the highest ferrihydrite-amended media treatment (10 mg DTPA-extractable Fe/kg media) was not significantly greater than in the controls on three of five rating dates for the St. Augustinegrass and two of three rating dates for the bermudagrass. Conversely, plants in the next highest ferrihydrite-amended media treatment (5 mg DTPA-extractable Fe/kg media) were significantly more chlorotic than the controls on all rating dates for St. Augustinegrass and two of three rating dates for bermudagrass. Additionally, the 5-mg treatment produced significantly more chlorosis than the 10-mg treatment on four of five rating dates for St. Augustinegrass and two of three rating dates for bermudagrass. These data indicate that an initial ferrihydrite-amendment level equivalent to 5 mg DTPA-extractable Fe/kg media may be required to screen St. Augustinegrass and bermudagrass genotypes for Fe-stress tolerance. This Fe level is similar to those previously reported (McCaslin et al., 1981; McCaslin and Watson, 1976; Weiss, 1943). Additionally, our data indicate that initial Fe levels of 2 or 3 mg DTPA-extractable Fe/kg media cause even more chlorosis than the initial 5-mg level and may be too low for screening Fe stress-sensitive species because of how rapidly these species exhibit chlorosis.

Although there were significant differences in visual chlorosis between the 10- and 5-mg treatments, Fe concentration in the leaves did not differ. Also, there were large differences in the concentrations of Fe in the leaves between the nonlimiting control and the 10-mg treatments, yet the differences in chlorosis were considerably smaller. Pierson et al. (1986) also found very little association between the concentration of Fe in leaves of Kentucky bluegrass (*Poa pratensis* L.) and the degree of

Table 1. Visual chlorosis ratings, leaf Fe, and chlorophyll contents of St. Augustinegrass and visual chlorosis ratings and leaf Fe contents of bermudagrass.

Media treatments ^y (mg Fe/kg media)	St. Augustinegrass								Bermudagrass					
	Visual rating of Fe chlorosis ^z						Fe content of leaves (mg·g ⁻¹ dried tissue)	Chlorophyll content of leaves (µg·g ⁻¹ fresh tissue)		Visual rating of Fe chlorosis ^z				Fe content of leaves (mg·g ⁻¹ dried tissue)
	Days after planting								Days after planting					
	83	97	117	137	174	Overall	174	105	174	294	321	509	Overall	509
Control	1.2	1.0	1.1	1.0	1.0	1.1	467	1.57	1.25	1.0	1.0	1.0	1.0	337
10	1.5	1.9	1.5	1.4	1.7	1.6	146	1.12	1.34	1.0	1.4	2.0	1.5	85
5	2.7	2.7	2.2	2.5	1.9	2.4	195	0.72	0.95	1.1	2.1	3.3	2.2	94
4	2.6	2.6	1.8	2.6	2.1	2.3	162	0.79	1.04	1.4	1.9	2.6	2.0	76
3	3.4	3.2	3.1	2.6	3.0	3.1	218	0.69	0.77	2.2	3.4	3.3	3.0	136
2	3.1	2.9	3.0	2.9	2.6	2.9	171	0.61	0.74	2.0	3.2	3.7	2.9	130
LSD, <i>P</i> ≤ 0.05	0.5	0.5	0.6	0.6	0.6	0.4	87	0.22	0.28	0.4	0.5	1.0	0.6	111
Treatment comparisons														
Control vs. 10			***			*	***	***					*	***
Control vs. 5	***	***	***	***	**	***	***	***	*	***	***	***	***	***
10 vs. 5	***	**	*	**		***		***	**	**	**	*		

^z1 = 0 to 5%, 2 = 25%, 3 = 50%, and 4 >50% chlorosis.^yInitial DTPA-extractable Fe levels. Control treatment consisted of no Fe oxide initially added to media and applying FeSO₄ via foliar spray and soil drench.*, **, ***Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

Fe chlorosis; however, other studies involving turfgrasses have indicated that leaf tissue Fe may be indicative of Fe-stress conditions (Harivandi and Butler, 1982). The results, therefore, suggest that the concentration of Fe in the leaves of turfgrass may be an unreliable index of Fe-deficiency stress.

With St. Augustinegrass, the control and 10-mg treatments had greater chlorophyll concentrations than did the 5-mg treatment. The correlations between chlorophyll content and visual rating of Fe chlorosis among the six treatments were significant [chlorophyll content (105 DAP) vs. visual rating of Fe chlorosis (117 DAP) ($r = -0.85$); chlorophyll content (105 DAP) vs. visual rating of Fe chlorosis (97 DAP) ($r = -0.98$); chlorophyll content (174 DAP) vs. visual rating of Fe chlorosis (174 DAP) ($r = -0.85$)]. These data suggest that chlorophyll concentrations may provide a reasonable index of Fe-deficiency stress, at least for St. Augustinegrass. White and Schmidt (1989, 1990) found no effect of added Fe on the chlorophyll concentration in bermudagrass subjected to chilling conditions; however, they grew the turf in a low pH soil (pH 5.7), presumably resulting in adequate Fe prior to the study.

Initial Fe levels of the ferrihydrite-amended media treatments (10, 5, 4, and 3 mg DTPA-extractable Fe/kg media) decreased during the

duration of the study, especially between the first and second sampling dates (Table 2). These decreases probably reflect leaching of the colloidal Fe oxides, although care was taken to minimize excess watering. Mineralogical transformations of the Fe from a poorly crystalline Fe oxide (ferrihydrite) to a more crystalline and consequently less DTPA-extractable form (e.g., goethite) may also have occurred. The 10 mg DTPA-extractable Fe/kg media treatment had a greater Fe concentration than the 5-mg treatment for the duration of the study, whereas there were no differences between the 5- and 4-mg treatments. These data are consistent with visual chlorosis ratings (Table 1).

Generally, clipping yields of both species were unaffected by Fe treatments. In prior studies, the application of Fe had no effect on clipping yields of bermudagrass (White and Schmidt, 1990), Kentucky bluegrass (Yust et al., 1984), or centipedegrass [*Eremochloa ophiuroides* (Munro.) Hack.] (Carrow et al., 1988). Alleviating Fe-deficiency chlorosis along with increasing visual turfgrass quality by Fe applications and amendments without increasing clipping yields is a valuable practice in the turfgrass industry. In addition, Fe treatment did not affect St. Augustinegrass root mass and length, crown mass, and shoot

number, and bermudagrass root, rhizome, and crown mass, and shoot number.

The synthetic growth media, consisting of 95% quartz sand, 5% CaCO₃ (m/m), and an initial ferrihydrite-amendment level equivalent to 5 mg DTPA-extractable Fe/kg media, has potential for screening St. Augustinegrass and bermudagrass genotypes for Fe-deficiency stress tolerance. An advantage of this media compared to native calcareous soils is that the former can be consistently reproduced at different locations over an unlimited number of years.

The best criteria for determining Fe-deficiency stress tolerance in either St. Augustinegrass or bermudagrass is the degree of visual chlorosis and, in the case of St. Augustinegrass, the concentration of chlorophyll in the leaves. Tissue Fe concentration and various measurements of above- and below-ground growth generally did not reflect Fe availability in the soil.

The results of this study indicate that a ferrihydrite soil amendment at the rate of 10 mg DTPA-extractable Fe/kg media was adequate as an Fe source for St. Augustinegrass and bermudagrass cultivars used within the time period of the study (175 and 509 d for the St. Augustinegrass and bermudagrass, respectively). Though these glasshouse results suggest the potential use of ferrihydrite as a slow-release Fe fertilizer source, tests under field conditions are required for additional assessment.

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Table 2. DTPA-extractable Fe (mg Fe/kg media) after three different sampling dates for St. Augustinegrass and bermudagrass.

Media treatments ^z (mg Fe/kg media)	St. Augustinegrass			Bermudagrass		
	Days after planting					
	7	115	175	7	133	510
Control	1.9	2.0	1.9	1.8	2.5	2.5
10	8.5	3.2	4.8	8.3	3.9	3.0
5	5.4	2.3	2.9	4.8	2.5	2.4
4	4.6	2.8	2.5	4.9	2.3	2.0
3	2.4	1.4	1.5	2.2	1.2	1.2
2	1.6	1.0	1.0	1.5	0.9	0.7
LSD, $P \leq 0.05$	0.9	0.7	0.3	1.3	0.5	0.6
Treatment comparisons						
10 vs. 5	***	**	***	***	***	*
5 vs. 4			**			

^zInitial DTPA-extractable Fe levels. Control treatment consisted of no Fe oxide initially added to media and applying FeSO₄ via foliar spray and soil drench.*, **, ***Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

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