Annual Bluegrass and Creeping Bentgrass Response to Varying Levels of Iron

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Abstract. Many biotypes of annual bluegrass (*Poa annua* L.) are found on golf course putting greens. Although normally considered an invasive weed, annual bluegrass can provide as good a putting surface as creeping bentgrass (*Agrostis palustris* Huds.). The most desirable biotypes of annual bluegrass are primarily vegetative and have a low flowering frequency. Whether the nutritional requirements of annual bluegrass biotypes differ from one another or from creeping bentgrass is unknown. The response of three flowering (FAB, high seedhead production) and three vegetative (VAB, low seedhead production) biotypes of annual bluegrass (AB), and the three parents of 'Pennncross' creeping bentgrass (CB) to varying levels of iron (Fe) in greenhouse sand culture was investigated. After establishment, clones were grown for 3 weeks and irrigated with a half-strength Hoagland's solution containing 0, 2, 4, 6, and 8 mg·L⁻¹ Fe in citrate-Fe. Shoot and root responses to Fe were similar for the VAB and FAB biotypes. However, VAB had higher color ratings (darker green leaf color) with Fe treatment level at 4 mg·L⁻¹ than did FAB or CB, which required 6 mg·L⁻¹ Fe for acceptable color. Growth of creeping bentgrass was greater than that of annual bluegrass at every Fe level tested. Shoot dry weights of CB increased significantly with Fe treatment level up to 6 mg·L⁻¹. Shoot dry weight of AB increased up to 4 mg·L⁻¹ Fe and then declined at ≥6 mg·L⁻¹. Root growth of CB increased up to 6 mg·L⁻¹ Fe, but then decreased significantly at 8 mg·L⁻¹ Fe. Root growth of AB increased slightly up to 4 mg·L⁻¹ Fe and then declined at 6 and 8 mg·L⁻¹. Shoot tissue concentrations of Fe were similar for AB and CB at each Fe rate tested except at 8 mg·L⁻¹ Fe, where Fe levels in CB were significantly lower. Based on this work, creeping bentgrass and annual bluegrass respond differently to Fe nutrition, but different biotypes of annual bluegrass appear to respond similarly.

Iron (Fe) is often considered to be the most widely used turfgrass micronutrient fertilizer (Turner and Hummel, 1992). It is directly associated with chlorophyll synthesis, a constituent of heme and non-heme enzymes, and may play a role in nucleic acid synthesis. Turf has shown enhanced color and growth with the use of Fe-containing fertilizers (Carrow et al., 1988; Christians, 1996; Minner and Butler, 1984; Wehner and Haley, 1990). Although Jones (1980) reported that 35 to 100 mg·kg⁻¹ Fe was sufficient in turfgrass shoot tissue, different turf species, cultivars, and genotypes can differ in their ability to acquire and use Fe (Harivandi and Butler, 1980).

Very little information is available on the Fe requirements of turf species used on golf

course putting greens. Creeping bentgrass (CB) and annual bluegrass (AB) are the major cool-season turfgrass species found on greens, with AB often considered to be an invasive weed (Mitich, 1998). Two biotypes of AB can exist on greens. *Poa annua* var. *annua* L. is a winter annual, upright-growing biotype having prolific seedhead production and light green color (Turgeon, 1999). These characteristics generally restrict its use as a turf species (Wu et al., 1992). The biotype *Poa annua* var. *reptans* Hausskn. is a perennial with a prostrate growth habit and a low frequency of flowering (Turgeon, 1999). This biotype can better survive summer conditions than flowering AB biotype (FAB), tolerate close mowing, and provide a putting surface that is nearly as good as CB.

Whether the two biotypes of AB respond differently to Fe, or whether they differ from CB is unknown. The objective of this research was to determine if color and growth of AB biotypes and CB species differ in response to different levels of Fe.

Materials and Methods

A greenhouse sand culture experiment was conducted with clones of CB and AB. The CB clones or genotypes were the three parents of the cultivar Pennncross maintained in the green-

house as foundation plant material. The clones of AB were three genotypes of vegetative AB biotype (VAB) and three genotypes of FAB (Table 1). These genotypes originated from AB selections made by Dr. David R. Huff from putting greens at the Oakmont Country Club (Oakmont, Pa.). The selections had been vegetatively increased in greenhouse pots and categorized morphologically according to flowering frequency, leaf texture, and leaf color.

After vegetative increase, washed tillers of each genotype were transferred to greenhouse "conetainers" (12.5 cm in length × 2.5 cm in diameter) filled with white silica sand (U.S. Silica, Mapleton, Pa., particle size 0.15–1.0 mm; 99.2% to 99.9% SiO₂) that had been prewashed with nitric acid. The acid prewash reduced the Fe content of the sand from 43.2 to 16.3 mg·kg⁻¹. Greenhouse temperature was 25 °C day/15 °C night with ± 2 °C fluctuation. No artificial light was used during either experiment.

Iron and genotype treatments were arranged in a two-factor split-plot design with Fe as the main-plot factor and genotype as the subplot factor. There were four Fe treatments in the first experiment and five in the second. The nine genotypes were used in both experiments. Plant material was established for 6 weeks in the conetainers using an Fe-deficient half-strength Hoagland's solution adjusted to pH 5.5–6.0 (Hoagland and Arnon, 1950). After establishment, the plants were clipped to a 1- to 2-cm height. Iron treatments were applied for 3 weeks by irrigating the turf every other day with half-strength Hoagland's nutrient solution containing 0, 2, 4, or 6 mg·L⁻¹ Fe as Fe-citrate (Fisher Scientific, Pittsburgh) in Expt. 1 and 0, 2, 4, 6, or 8 mg·L⁻¹ Fe in Expt. 2.

Data were collected for leaf color, shoot and root dry weights, and shoot tissue Fe content. Visual color ratings were taken when acute deficiency or toxicity symptoms appeared or 3 weeks after treatment initiation. Color was rated using a scale of 1 (yellow) to 9 (very dark green), with 6 being considered an acceptable green color for putting green turf. Shoot and root dry weights were measured at the end of each experiment. Dry weights were determined by oven-drying the plant tissue at 80 °C for 48 h. The dried shoot tissue was then ground to pass a 40-mesh screen using a Thomas-Wiley mill (model 3383-L10; Philadelphia). Shoot tissue Fe was determined by acid-ashing (Doty et al., 1982) and atomic absorption (AA) spectrophotometry (Video 22 Photometry Adsorption; Thermo Jarrell Ashe, Franklin, Mass.).

The data from both experiments were combined, as differences between experiments were nonsignificant. Further analysis was performed for main plot effect (Fe level) using replication × Fe level as the error term. The subplot effect (genotype) was tested using genotype × Fe as an error term. Fisher's protected least significant difference (LSD, *P* = 0.05) was used to determine if interspecific and intraspecific differences existed within and between CB, VAB, and FAB at each Fe

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Table 1. Turfgrass species and genotypes used to study the effect of varying levels of iron.

Genotype	Flowering frequency ^a	Texture	Color ^b
<i>Creeping bentgrass</i>			
Blue (BB)	NA	Medium	Green
Red (RB)	NA	Medium	Green
White (WB)	NA	Medium	Green
<i>Annual bluegrass</i>			
Oakmont 9G-1	Vegetative	Medium	Green
Oakmont 9G-6	Vegetative	Medium	Dark green
Oakmont 11G-6	Vegetative	Coarse	Light green
Oakmont 11G-2	Flowering	Coarse	Light green
Oakmont 18G-1	Flowering	Coarse	Light green
Oakmont 18G-2	Flowering	Fine	Light green

^aNA = not applicable; vegetative biotypes have ≤10% flowering tillers; flowering biotypes have ≥50% flowering tillers.

^bColor determined visually on clonal plant material grown under identical greenhouse conditions.

level tested. The linear, quadratic, or cubic main effects of Fe concentrations on CB, VAB, and FAB were tested using SAS general linear model (GLM) procedure (SAS Institute, 1996).

Results and Discussions

No intraspecific differences were found within CB, VAB, or FAB for any of the parameters measured in this study. Therefore, data within each group were combined for further statistical analysis to compare CB, VAB, and FAB responses to Fe.

Leaf color. Leaf color ratings for all genotypes increased linearly as Fe rate increased (Table 2). Nontreated controls exhibited Fe deficiency symptoms on the younger leaves [yellow green to light green color (a rating of ≈4), and interveinal chlorosis]. The CB had higher color ratings than did VAB and FAB when no Fe was applied. All plant material receiving Fe at 2 mg·L⁻¹ showed no interveinal chlorosis, but had color ratings of less than acceptable (a rating of <6). When supplied with Fe at 4.0 mg·L⁻¹, the VAB had a color rating of 6.7, while CB had a rating of 6.0 and FAB had slightly less than acceptable color. When Fe was applied at 6 and 8 mg·L⁻¹, color ratings were consistently higher for VAB than for FAB and CB. No visible Fe phytotoxicity symptoms were observed in any of genotypes at any Fe level tested.

Color improvement following application of Fe is often attributed to increased chlorophyll content of leaves (Minner and Butler, 1984). The enhanced turf color ratings measured in our study are consistent with previous reports (Cooper and Spokas, 1991). However, no previous studies have determined if CB and AB biotypes respond differently to Fe. This study shows that VAB exhibits higher color rating scores in response to Fe than do CB and FAB at each concentration tested. Color of CB and FAB responds similarly to Fe application.

Shoot growth. Creeping bentgrass produced greater shoot dry weights in response to Fe than did either biotype of AB. No intraspecific differences in shoot growth were observed between the two biotypes of AB at any level of Fe tested.

Shoot dry weight of CB increased up to 6 mg·L⁻¹ Fe and then declined slightly at the 8 mg·L⁻¹ Fe level. In contrast, shoot dry weight of VAB and FAB increased only slightly between 0 and 4 mg·L⁻¹ Fe and maximum growth occurred at 4 mg·L⁻¹. Shoot growth of both AB biotypes receiving 8 mg·L⁻¹ Fe was less than or similar to that of the nontreated controls. Although no visible signs of Fe phytotoxicity were observed on VAB or FAB shoot tissue, shoot dry weight data indicated that AB biotypes were negatively affected by the higher concentrations of Fe used in this study. Deal and Engel (1965) reported that higher rates of Fe reduced Kentucky bluegrass (*Poa pratensis* L.) color, shoot and rhizome growth, and sod density. Yust et al. (1984) found that Fe rates of 4.5 to 17.7 kg·ha⁻¹ injured the foliage of Kentucky bluegrass. In our study, the total amounts of Fe that reduced shoot growth were 7.6 kg·ha⁻¹ for AB and 10.1 kg·ha⁻¹ for CB.

Root growth. Like shoot growth, root dry weight was significantly higher in CB than in VAB and FAB at each level of Fe tested, but differences between the VAB and FAB biotypes were nonsignificant. Root production of all genotypes responded quadratically to increasing Fe levels (Table 2). Inclusion of 6 mg·L⁻¹ Fe increased CB root dry weight by 91%, but the 8 mg·L⁻¹ Fe treatment was no more effective than the 2 mg·L⁻¹ Fe treatment. For the AB biotype, root dry weight was the highest with the 4 mg·L⁻¹ Fe treatment. Inclusion of 8 mg·L⁻¹ Fe reduced AB root dry weights relative to controls, indicating that AB biotypes are more sensitive to higher Fe treatment concentrations than are CB biotypes.

Root growth was also reduced by Fe treatments that delivered a total of 7.6 kg·ha⁻¹ Fe to AB and 10.1 kg·ha⁻¹ Fe to CB, which agrees with the data of Deal and Engel (1965) and Yust et al. (1984). The literature offers little explanation as to why Fe can affect turfgrass root development, but response may be indirectly related to its effects on shoot growth, photosynthesis, or carbohydrate status (Glinski et al., 1992). It could also be attributed to nutritional imbalances or the overproduction of free radicals during photosynthesis (Marschner, 1995).

Tissue Fe content. Shoot tissue Fe content of AB, but none of CB biotypes, increased linearly with level of Fe (Table 2). The Fe concentration of CB shoot tissue increased as Fe was applied up to 6 mg·L⁻¹ and then decreased at 8 mg·L⁻¹, resulting in a significant quadratic response.

Despite color and growth differences between biotypes due to Fe treatments, Fe content did not differ among species or biotypes except at the 8 mg·L⁻¹ Fe level, where CB shoots contained significantly less Fe than VAB and FAB shoots.

Table 2. Average color and growth responses of creeping bentgrass (CB), vegetative annual bluegrass (VAB), and flowering annual bluegrass (FAB) to five rates of Fe.

	Fe treatment (mg·L ⁻¹)					
Biotype	0	2.0	4.0	6.0	8.0	Significance
	<i>Color</i>					
CB	4.1	5.0	6.0	7.0	8.0	Q**
VAB	3.6	5.3	6.7	7.8	8.7	Q***
FAB	3.5	4.7	5.9	6.8	7.3	Q***
LSD _{0.05}	0.2	0.2	0.3	0.3	0.6	
	<i>Shoot dry weight (g)</i>					
CB	1.31	1.94	2.35	2.63	2.62	Q***
VAB	0.55	0.72	0.85	0.73	0.42	Q***
FAB	0.58	0.73	0.90	0.80	0.35	Q***
LSD _{0.05}	0.16	0.20	0.20	0.22	0.12	
	<i>Root dry weight (g)</i>					
CB	1.42	1.99	2.42	2.72	1.98	Q***
VAB	0.57	0.73	0.81	0.65	0.25	Q***
FAB	0.59	0.74	0.87	0.74	0.30	Q***
LSD _{0.05}	0.19	0.22	0.22	0.25	0.13	
	<i>Shoot tissue Fe concentration (mg·kg⁻¹)</i>					
CB	81.4	89.6	102.2	111.6	98.8	Q*
VAB	80.0	92.3	101.2	112.9	115.6	L***
FAB	79.5	88.0	97.5	108.2	110.8	L***
LSD _{0.05}	6.4	6.8	7.4	9.9	10.7	

*, **, ***Significant linear (L) or quadratic (Q) Fe concentration effect at $P \leq 0.05$, 0.01, or 0.001, respectively.

Based on color ratings and growth data, shoot Fe concentrations ≤ 92 mg·kg⁻¹ might be considered deficient for CB and AB. Highest color ratings in VAB and FAB occurred when shoot Fe concentrations were the highest. However, this was not true for CB. Turf color and Fe content may not always be well-correlated (Glinski et al., 1992). The Fe content of the chlorophyll lamellae might have been a better indicator of the influence of Fe on color (Marschner, 1995).

Maximum shoot and root production for VAB and FAB did not coincide with the highest shoot Fe concentrations (Table 2). Although VAB and FAB had the highest color ratings at >110 mg·kg⁻¹ Fe, root and shoot dry weights declined at shoot Fe concentrations >100 mg·kg⁻¹. Similarly, shoot and root dry weight production in CB was not associated with the highest shoot Fe concentrations.

There are many genetic and environmental factors that can influence tissue Fe concentrations (Marschner, 1995). Jones (1980) reported a general sufficiency range of Fe for turfgrass to be 35 to 100 mg·kg⁻¹ shoot tissue, which is consistent with the concentrations reported in this study. However, Waddington and Zimmerman (1972) reported shoot Fe concentrations of 135 and 170 mg·kg⁻¹ for field-grown AB and CB, respectively, and that Fe concentrations could differ significantly among species and times of sampling. Therefore, the shoot Fe concentrations presented in this paper, as well as the concentrations presented by other researchers, may not be wholly reliable for field diagnostic purposes. However, this research does show that VAB, FAB, and CB respond differently to Fe in terms of color and shoot and root growth,

even when shoot tissue Fe concentrations are similar.

Conclusion

The AB and CB genotypes responded differently to Fe. The VAB had higher color ratings in response to Fe than did CB and FAB, and thus required lower levels of Fe for very good color. Growth response to Fe was much less in AB than in CB. The VAB and FAB biotypes, selected from putting greens, responded similarly to Fe in shoot and root production. The results of this study indicate that growth of AB biotypes is inhibited at lower Fe levels than is that of CB biotype. In both species, Fe levels that produced the higher color ratings did not result in higher shoot and root production.

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