Iron (Fe) is essential to plants and is directly involved in chloroplast development and important reactions of photosynthesis (Sharma, 2006). Although it’s the fourth most abundant element in the earth’s crust (Tisdale et al., 1993), its availability to plants in alkaline soils is very low because it is rapidly oxidized and immobilized. The effect of soil pH on the solubility of Fe is so pronounced that the solubility of Fe minerals decreases exponentially with each pH unit increase in the soil (Hansen et al., 2006).

Despite the low availability of Fe in alkaline soils, many plants have been able to adapt and grow well in these soil environments (Hansen et al., 2006). The responses of some grasses to Fe-deficiency stress has been shown to involve the increased production and exudation of organic chelates (phytosiderophores) from roots (Hansen et al., 2006). These phytosiderophores chelate Fe3+ in the soil, thus increasing its solubility and availability for plant uptake.

In the past, many Kentucky bluegrass (KBG; Poa pratensis L.) cultivars have been susceptible to Fe-deficiency chlorosis when grown on alkaline soils and consequently were routinely treated with Fe (Christians, 1998). It is widely reported that Fe is applied when turf is chlorotic with the result being a greening response.

Shallow rooting depth is a common problem in shortly mowed golf course and sports turf management venues and, as such, Fe is often used as a pseudo substitute for high rates of nitrogen (N), with Fe application providing a bright green color even when N availability is kept minimal to favor root development over shoot growth (Yust et al., 1984). The eyesore of chlorosis and the expense to treat it could be avoided if cultivars resistant to Fe deficiency were identified.

Quantifying phytosiderophore production over time while under Fe-deficiency stress has been used to identify Fe-deficiency-resistant genotypes in other monocots such as corn, oat and wheat (Lytle et al., 1990; Hansen and Jolley, 1995; Hansen et al., 1996) and could potentially be used as a screening technique for KBG (Cesco et al., 2006).
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Study methods
A hydroponic study was conducted with the objective of comparing the phytosiderophore release of four cultivars (Award, Baron, Limousine, and Rugby II) known to differ in their susceptibility to Fe-deficiency chlorosis. Seeds were germinated on a layer of cheesecloth (for wicking of moisture) atop a layer of plastic mesh (support) assembled between the cap and base of ABS DWV fittings.

The fittings holding seeds were placed in plastic trays filled with a complete nutrient solution (Barben et al., 2009). After 36 days of germination and growth in an environmental chamber, the groups of KBG were put into deficient (0.1 micromolar or μM) or adequate (10 μM) treatments of Fe with a solution pH of 7.4.

Based on previous work with other species, we expected susceptibility to Fe-deficiency chlorosis in KBG to be related to a cultivar’s inability to produce adequate phytosiderophore.

After treatment initiation, chlorosis scores were made daily. On days five through 13 of the treatments, phytosiderophore release was measured once daily using an indirect Fe-binding assay (Hansen et al., 1996). Plant tissue was dried, ground to pass a 1 millimeter (mm) screen, digested in nitric-perchloric acid, and analyzed for nutrient content by inductively coupled plasma (ICP, Thermo Electron Corporation, Franklin, Maryland) spectroscopy.

Physiological response
As expected, all cultivars showed much greater chlorosis at 1 than at 10 μM Fe. However, the Baron cultivar developed more severe chlorosis than Award, Rugby II and Limousine at both the high and low levels of Fe (Figure 1). In addition, Baron re-greened after day nine at the higher level of Fe while all other cultivars’ chlorosis worsened as the treatment progressed.

For all four cultivars grown at the low level of Fe, chlorosis increased during the course of the treatment.

Expectedly, the low Fe treatments had 25 percent less shoot Fe concentration than the adequate Fe treatments (Figure 2). Similarly, the low Fe treatments had 67 percent less root Fe concentration than the adequate Fe treat-
The unexpected result of our experiment was, however, that the cultivar developing adequate phytosiderophore was, however, that the cultivar developing adequate phytosiderophore. Based on previous work with other species (Hansen et al., 2006), we expected susceptibility to Fe-deficiency chlorosis in KBG to be related to a cultivar's inability to produce adequate phytosiderophore.

All four cultivars produced significant amounts of phytosiderophore in response to Fe deficiency at the low level compared to the high level of Fe. Baron, however, surprisingly produced 12 percent more phytosiderophore than the other cultivars.

**Physiology implications**

Based on previous work with other species (Hansen et al., 2006), we expected susceptibility to Fe-deficiency chlorosis in KBG to be related to a cultivar's inability to produce adequate phytosiderophore.

The unexpected result of our experiment was, however, that the cultivar developing the most chlorosis during the course of the treatment (Baron) also produced the most phytosiderophore and at a significantly higher level than the other cultivars. This finding implies that Fe-deficiency susceptibility in KBG may be related to inefficient uptake, transport or utilization physiology rather than phytosiderophore production and release.

This information is valuable for geneticists seeking the development of new cultivars that have high greenness scores in Fe-limiting soils.

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