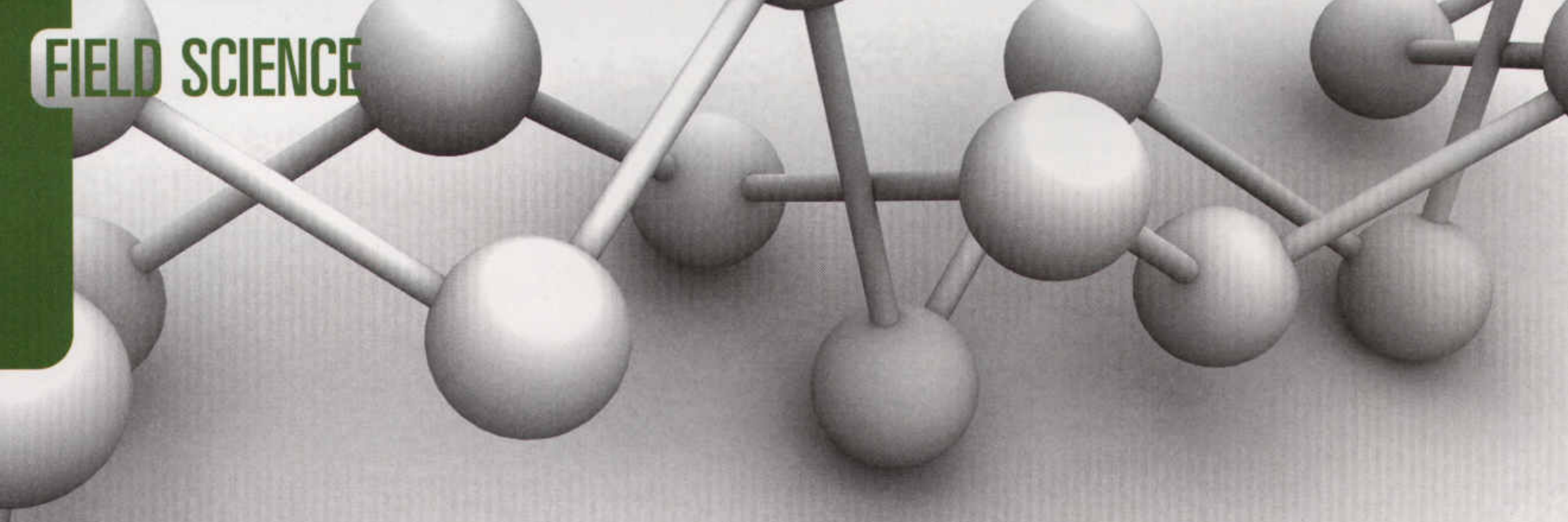

Playing defense vs. free radical damage

By Mike Winkenhofer and Jeff Haag

The goal of every sportsturf manager is to not only provide a safe playable surface, but to also provide aesthetically pleasing green turfgrass. To achieve the latter involves a reciprocal balance between soil, fertility, moisture, temperature, humidity, grass species used, mowing techniques, cultural practices, preventing reactive oxygen species (free radicals) damage from occurring, and cooperation from Mother Nature. This article discusses what free radicals are, factors that cause free radical damage, and mechanisms you can use to prevent development and alleviate their existence if they do develop.

What are free radicals?

Most turfgrass managers know that research has shown that free radicals, if not quickly converted to water and ground-state oxygen by antioxidants, can bleach chlorophyll, and can damage lipids, proteins, and DNA inside cells of the turfgrass plant.

So exactly what are free radicals? Typically, stable molecules contain pairs of electrons. When a chemical reaction breaks the bonds that hold the paired electrons together, free radicals are produced. Free radicals contain an odd number of electrons, which make them unstable, short-lived, and highly reactive. As they combine with other atoms that contain unpaired electrons, new radicals are created, and a chain reaction begins. This chain reaction or accumulation of reactive oxygen species in plants is generally ascribed to several possible sources, which can be attributed to environmental causes such as drought, heat, ultraviolet light, airborne photooxidants, or chemicals such as herbicides. Accumulation of reactive oxygen species is central to plant response to several pathogens. The free radicals (reactive oxygen species) are singlet, hydroxyl, superoxide, and hydrogen peroxide.

There is a catch-22 to light. We know that light is necessary for photosynthesis to occur but it can also play a part in free radical formation. When photosynthetic organisms are exposed to ultraviolet radiation, significant, irreversible damage to important metabolic processes within the cell may occur (such as lesions in DNA and inhibition of photosynthesis). Through these reactions and others, radical forms of oxygen are often created. Many studies suggest that this damage is due to oxidative stress resulting from either UV-A or UV-B or both.

The major effect of ultraviolet-B light on the thylakoid proteins is the breakdown of the reaction centre D1 protein. One must question whether ultraviolet-B radiation will become an even more serious factor in the years to come. The depletion of the stratospheric ozone is causing renewed concern about the increased level of ultraviolet-B radiation reaching the Earth's surface, and it is known that exposure to environmental ozone can cause significant damage to turfgrass by imposing oxidative stress.

Photosynthetic light absorption and energy use must be kept in balance to prevent formation or reactive oxygen species in one important component of the turfgrass cell where chlorophyll is developed, the chloroplast. In the case of drought for example, it causes stomatal closure, which limits the diffusion of carbon dioxide to chloroplasts and thereby causes a decrease in CO? assimilation in favor of photorespiration that produces large amounts of hydrogen peroxide.

Under these conditions the probability of singlet oxygen production at Photosystem II and superoxide production of Photosystem I is increased. These may cause direct damage, or induce a cell suicide program. We suspect this to be the case as well since we are currently seeing a gradual increase in yearly temperatures across the world, and an increase in skin cancers in humans. How it affects turfgrass plants in the years to come remains to be seen.

Defense mechanisms: antioxidants

The antioxidants a-tocopherol (Vitamin E), ascorbic acid (Vitamin C), carotenoids (B-Carotene), vitamin B6, and mannitol contained in some biostimulants all play a vital role in scavenging free radicals. Applications of biostimulants, which supplement plant hormones, have been shown to enhance the presence of various antioxidants in the leaves, reducing stress and promoting faster recovery from injury. Applying this type of biostimulant product to sports fields several weeks before the beginning of play and following up with regular applications during the season can enhance the presence of antioxidants for stress reduction and recovery.

Carotenoids (B-Carotene). In terms of its antioxidant properties, carotenoids can protect Photosystem I and Photosystem II in one of four ways: by reacting with lipid peroxidation products to terminate chain reactions; by scavenging singlet oxygen and dissipating the energy as heat; by reacting with triplet or excited chlorophyll molecules to prevent formation

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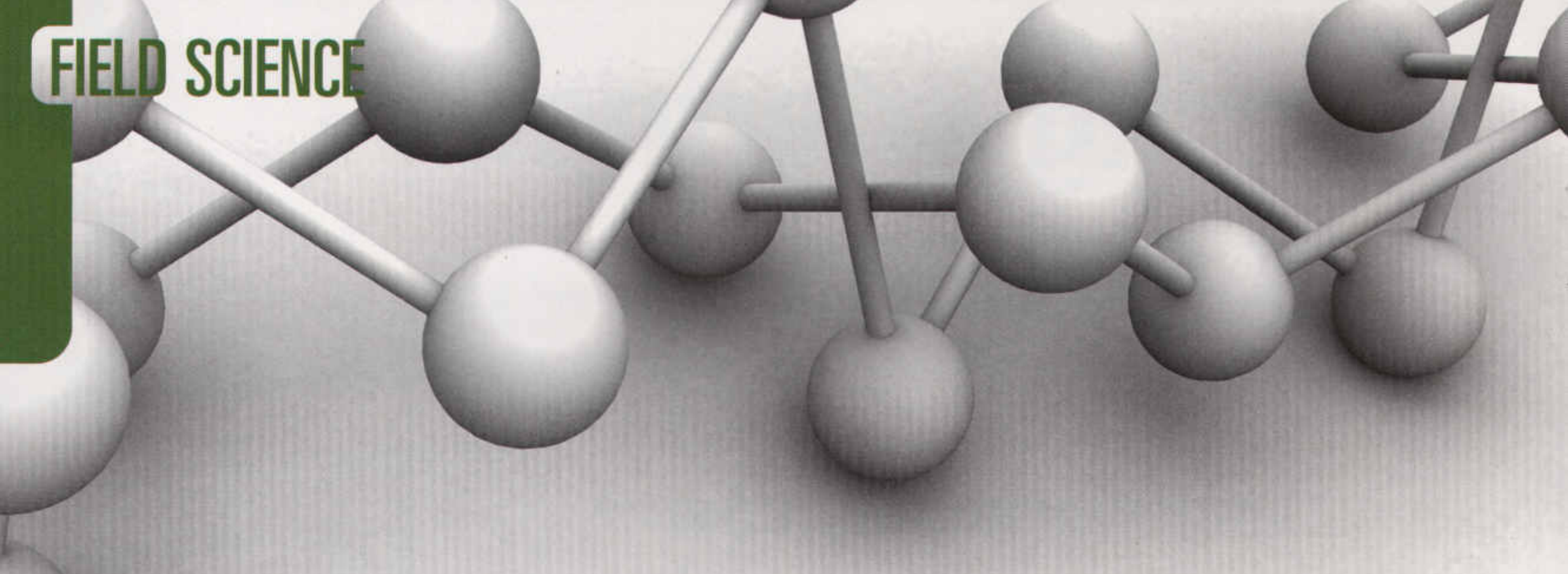

of singlet oxygen; or by dissipation of excess excitation energy through the xanthophyll cycle.

Xanthophylls function as accessory pigments for harvesting light at wavelengths that chlorophyll cannot, and transfer the light energy to chlorophyll, but they also absorb excess light energy and dissipate it in order to avoid damage in what is termed the xanthophyll cycle.

a-tocopherol (Vitamin E). This is considered a major antioxidant in chloroplasts in at least two different, but related roles: it protects Photosystem II from photoinhibition, and thylakoid membranes from photooxidative damage. The antioxidant properties of Vitamin E are the result of its ability to quench both singlet oxygen and peroxides, although Vitamin E is a less efficient scavenger of singlet oxygen than B-Carotene, it may function in the thylakoid membrane to break carbon radical reactions by trapping peroxyl radicals.

Vitamin E also has the ability to donate two electrons, which results in opening of the chromanol ring to form the corresponding tocoquinone derivative. These combined molecular characteristics allow Vitamin E to protect polyunsaturated fatty acids from lipid peroxidation by scavenging lipid peroxyl radicals that propagate lipid peroxidation chain reactions in membranes.

Ascorbic Acid(Vitamin C). It is generally believed that maintaining a high ratio of ascorbic acid is essential for the scavenging of free radicals, and is needed in high concentrations in the chloroplasts to be effective in defending the turfgrass against oxidative stress. Although ascorbic acid can directly scavenge the free radicals superoxide and singlet oxygen, probably the main benefit that ascorbic acid plays in the prevention of free radicals is that it is an excellent scavenger of the hydroxyl radical. The hydroxyl radical is dangerous to turfgrass because it can inhibit carbon dioxide assimilation by inhibiting several Calvin cycle enzymes.

Vitamin B6. Apart from its function as a cofactor, Vitamin B6 is also thought to act as a protective agent against reactive oxygen species (free radicals), such as singlet oxygen. Vitamin B6 is also the master vitamin in processing amino acids, and plays a very important role in developing proteins specifically designed to help chloroplasts, thylakoid membranes, Photosystem I, and Photosystem II to function properly.

Mannitol. The antioxidant mannitol has the ability to protect, and quench, two damaging free radicals: singlet oxygen, and hydroxyl. Singlet oxygen is damaging because it can react with proteins, pigments, and lipids, and is thought to be the most important species of light-induced loss of Photosystem II activity, as well as the degradation of the D1protein, which we will discuss later. It has further been demonstrated that when mannitol is present in the chloroplasts, it can protect turfgrass against oxidative damage by the hydroxyl radicals. The Senn, Senn, and Senn Company manufactures a biostimulant called N.O.G. (Natures Organic Growth) that contains and excellent source of mannitol, along with the other antioxidants mentioned.

Amino Acids. As we know, amino acids are the building block of proteins. Under optimal conditions proteins are able to perform the normal physiological function to synthesize amino acids, but intensively manicured turfgrass such as sportsturf are rarely operating under normal conditions due to low mowing heights and traffic stress placed upon them.

The topical application of amino acids plays an extremely important part in developing the proteins specifically designed to help chloroplasts, thylakoid membranes, Photosystem I, and Photosystem II to function properly. These proteins are known as D1, D2, CP43, CP47, and cytochrome b559. Of special importance is the D1 protein because it exhibits the highest turnover rate of all the thylakoid proteins, and is also highly vulnerable to the free radical *singlet oxygen*.

Humic acids are another compound that has shown to contain antioxidant properties that promote the scavenging of free radicals. The added benefits are that they also increase the availability of micronutrients, phosphate, and potassium to the plant, and enhance the chlorophyll content of turfgrass. Humic acid also has been shown to stimulate root initiation due to the auxin-like activity they contain, which is most likely due to their ability to inhibit indoleacetic acid (IAA) oxidase breakdown.

There is new evidence that carbon now plays a role in the overall development of the turfgrass plant leaf, that a reduction in carbon also reduces photosynthetic activity which reduces carbohydrate availability to the turfgrass plant, and there is also new evidence to suggest without proper amounts of carbon in the chloroplast that proper development of the turfgrass plant cannot occur.

There is also further evidence to suggest that, if there is an abundant source of carbon in the thylakoid membranes inside the chloroplasts, it can be mobilized for use as an energy source during senescence. As we discussed earlier, light absorption by the enzyme pheophorbide an oxygenase, the key enzyme in the pathway to chlorophyll degradation to senescence, can cause the development of the free radical singlet oxygen. ■

Mike Winkenhofer is sportsturf manager and Jeff Haag is assistant sportsturf manager for the University of Louisville. For a list of reference from this article, see www.sportsturfonline.com.

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Hybrid bluegrass vs. *Kentucky blue* and *tall fescue* in transition zone

By Dale J. Bremer, Kemin Su, Steven J. Keeley, and Jack D. Fry

Bill Snyder Family Stadium, courtesy Kansas State University Athletic Department

In some areas of the United States, Kentucky bluegrass and tall fescue are subjected to frequent drought, which results in heat and drought stress symptoms, and irrigation is required to maintain acceptable quality. Kentucky bluegrass (KBG) commonly goes dormant during periods of high temperature and drought. Tall fescue (TF) has good drought avoidance because of its relatively deep rooting system, but some turfgrass managers prefer the finer texture and recuperative capacity that KBG offers.

New hybrid bluegrass (HBG) cultivars, which are genetic crosses between KBG and native Texas bluegrass, have the appearance of KBG but may be able to withstand higher temperatures and extended drought without going dormant. In warm climates HBG may stay green all year long. Furthermore, HBG may use less water than other cool-season species while maintaining their green color. This is especially important given the increasing competition for water and the rising costs of irrigation.

In our 2-year study, two HBG were compared with KBG and TF in the stressful climate of the transition zone. The objectives were to evaluate KBG (Apollo), TF (Dynasty), and two cultivars of HBG (Thermal Blue (HBG1) and Dura Blue (HBG2)) for: 1) canopy establishment rates after fall seeding; 2) visual quality and growth characteristics of canopies; and 3) drought resistances under different irrigation regimes and deficits.

The study was conducted from September 2002 to October 2004 at the Rocky Ford Turfgrass Research Center near Manhattan, KS. The soil at the site was Chase silt loam (fine, smectitic, mesic-Aquertic, Argiudolls). Thirty-six subplots were established in a split-plot design. Two irrigation treatments and a control, replicated three times each, were applied to whole plots arranged in a Latin square design. Four species or cultivars of turfgrasses were established in the subplots. One species/cultivar of each turfgrass was planted once within each whole plot; species/cultivar was randomly assigned to subplots within each whole plot. Therefore, each irrigation-by-species/cultivar treatment combination was replicated three times in the entire study.

Irrigation treatments included the replacement of 100 and 60% of the water lost from plants and soil via evapotranspiration (ET), and control plots received only natural precipitation. Water was applied twice weekly through a fan spray nozzle attached to a hose; a meter was attached to ensure proper application rate. To determine irrigation requirements, evapotranspiration (ET) was calculated by using the Penman-Monteith equation, and climatological data were obtained at a weather station located at Rocky Ford.

In 2003, irrigation treatments were not applied because of a moderate billbug infestation that affected a number of plots of KBG, HBG1, and HBG2 despite insecticide applications. Plots in 2003 were irrigated every 3-4 days, providing 40 mm of water per week in the absence of rain, to minimize stress related to billbug damage. Therefore, irrigation treatments were applied for only one year (2004) of the study.

John Mascaro's Photo Quiz

Can you identify this sports turf problem?

Problem: Brown area

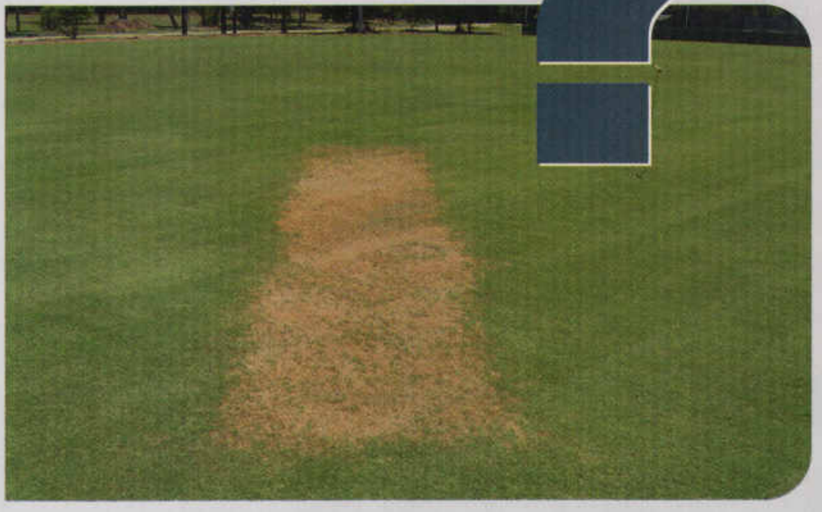
Turfgrass Area: Baseball outfield

Location: Dallas, TX

Grass Variety: 419 bermudagrass

Answer to John Mascaro's Photo Quiz on Page 43

John Mascaro is President of Turf-Tec International



Canopy evaluations

Turfgrass canopy establishment was evaluated visually after seeding, from 17 December 2002 to 20 July 2003. Establishment was estimated as percentage of the ground surface covered by turfgrass canopies within each plot (0 to 100%).

All plots were evaluated biweekly for visual turfgrass quality, which was rated on a scale from 1 (dead, brown turf) to 9 (optimum uniformity, density, and color) and 6 was considered acceptable quality for a home lawn; all evaluations in each year were conducted by the same person. Turfgrass density and color were also evaluated visually in each plot independent of, albeit less frequently than, quality during the same periods in 2003 and 2004.

Clippings were collected every 3 weeks, or 4 times from 5 August to 10 October in 2003, with a walk-behind rotary mower equipped with a modified collection bag that allowed for complete capture of clippings from each plot. Clipped biomass was determined gravimetrically after samples had been dried in a forced-air oven for 48 hours at 65 degrees C.


Kentucky bluegrass


Tall fescue turfgrass

Vertical growth rates were measured biweekly to monthly from 14 July to 21 October in 2003 and biweekly from 14 June to 12 August in 2004. Daily vertical growth rates were calculated as the increase in canopy heights between consecutive mowing events, divided by the number of days between mowing. Canopy heights were measured immediately after mowing at two randomly selected locations within each plot; each location was marked with a flag that remained until the next mowing date. Before the next mowing, canopy heights were measured a second time at the same (marked) locations. Canopy heights were measured by placing a circular piece of lightweight cardboard over the canopy and centered on the flag; the cardboard was rigid enough to hold its shape but lightweight enough to minimize the bending of the canopy by its weight. The height of the canopy was then measured at four perpendicular spots around the circumference of the cardboard. The flags were then removed before mowing and then reinserted at new, random locations for the next measurements.

Tests of differences in canopy establishment, visual quality, canopy color and density, clipping biomass, vertical growth rates, and irrigation effects among treatments were conducted with the mixed linear model procedure of SAS.

Plot maintenance

All plots were mowed with a walk-behind rotary mower at a height of 2.5 inches once or twice weekly as needed to prevent removing more than 1/3 of the canopy height. All plots were fertilized with approximately 225 kg urea N ha?1 yr?1 in 2003 and 2004, in split applications in September, November, May, and July. Because bluegrass billbug had infested some bluegrass plots at the research center in previous years, plots were treated in 2003 with 0.36 kg a.i. ha?1 of imidacloprid (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine, 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) on 7 June and 0.09 kg a.i. ha?1 of bifenthrin (2-methyl-1,1-biphenyl-3-y1)-methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl cyclopropanecarboxylate) on 1 August, and in 2004 with 0.44 kg a.i. ha?1 of imidacloprid on 19 April, 0.125 kg a.i. ha?1 of bifenthrin on 27 May, and 1.69 kg a.i. ha?1 of halofenozide (Benzoic acid, 4-chloro-, 2-benzoyl-2-(1,1-dimethylethyl)hydrazide) on 9 July. Preemergence herbicide applications included 0.56 kg a.i. ha?1 of dithiopyr (/S, S/?-dimethyl 2-difluoromethyl-4-isobutyl-6-trifluoromethylpyridine-3,5-dicarbothioate) on 17 May 2003 and 27 May 2004. Broadleaf pests were treated as needed, and no fungicides were applied during the study.

Establishment was most rapid in TF, which was at 90% cover by 17 December 2002 and reached 100% by 7 May 2003. Both KBG and HBG1 established more slowly than TF and were similar to each other; HBG1 reached 99% cover by 13 June and KBG by 28 June. The HBG2 was slowest to establish, reaching 96% cover by 19 July. Percentage cover for HBG2 was lower than for TF, KBG, and HBG1 during the entire period of establishment evaluations. Establishment in TF was 99% complete 37, 52, and more than 73 days faster than in HBG1, KBG, and HBG2, respectively.

Visual quality was generally lower in the bluegrasses than in TF during 2003 and 2004. Mean quality, when averaged over the season, ranked TF>KBG>HBG1>HBG2 both years; all differences in mean quality among species or cultivars during the season were significant in both years, except between KBG and HBG1 in 2003.

The ranking of visual quality among species was, in part, a function of differences in canopy density and color. For example, canopy density was highest in TF and generally similar between KBG and HBG1 in both years, but a lighter color in HBG1 resulted in its lower quality rating in a number of instances. In 2003, quality in HBG2 was consistently lower among plots early in the growing season because of its slower establishment. Slower establishment in HBG2 resulted in a lower canopy density than in TF, KBG, and HBG1 up to 11 July 2003. Canopy density also was consistently lowest in HBG2 during 2004. Higher seeding rates may be required for HBG2 to obtain an adequate stand.

Vertical growth rates of HBG1 were similar to TF in 2003 and 2004,

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and were higher than KBG on three of four measurement dates in 2003 and on the last two dates in 2004. Averaged across the season, vertical growth rates of HBG1 were higher than those of KBG in 2003 and higher than those of KBG and HBG2 in 2004. It is clear that HBG1 shows little promise of reduced mowing frequency, compared with conventional cool-season species. The vertical growth rate of HBG2, by contrast, was more similar to KBG in both years. Averaged across the season, the vertical growth rate of HBG2 was lower than that of TF in 2003 and 2004 (albeit not significantly in 2004). Thus, there is potential for reduced mowing frequencies with HBG2 (and perhaps future cultivars of HBG), compared with TF.

Effects of water deficit

In 2004, frequent, above-average rainfall from May through August minimized the impact of water-deficit treatments; precipitation between June and August 2004 was 18.42 inches, which was 5.82 inches above normal. Drier (albeit cooler) weather during a 52-day period in September and October of 2004 allowed for a stronger comparison among species and cultivars under drought conditions; precipitation was 1.02 inches during September, which was 2.63 inches below normal, and only 0.27 inches occurred in early October before the study ended.

Visual quality ratings were similarly high among species and cultivars in well-watered plots at the end of the dry period in September and October 2004. Visual quality in the 60% ET treatment was significantly lower in HBG2 than in KBG, and quality in HBG1 and HBG2 was below the rating of 6 determined as acceptable for home lawns. In the control, which received only natural precipitation during this period (i.e., no irrigation), the quality of all bluegrasses was below 6 and ratings of KBG and HBG1 were significantly lower than those of TF. Visual quality in TF declined only from 8 to about 6.5 to 7 in the reduced-irrigation and control plots, indicating that TF was not substantially affected by drought in this study.

The lack of response of TF to water deficit may have been caused by a combination of deep soils at the research site and deep roots typical of TF that may extract soil water from lower in the profile. Higher quality in KBG than in HBG2 at 60% ET, and the lack of differences between KBG and HBG1 and HBG2 in the control, are in contrast to results from a study that reported higher quality in a HBG (Reveille) than in KBG (Bensun [A-34]) during prolonged dry down. The latter study also determined that the HBG had greater root length density and root mass in the 0- to 60-cm profile than the KBG had, and that greater dehydration avoidance was observed in the HBG than in KBG. Significant variation in drought resistance has been observed among cultivars of KBG and HBG however, and such differences among species and cultivars may have contributed to the contrasting results observed in our study. ■

Dale J. Bremer, Kemin Su, Steven J. Keeley, and Jack D. Fry, are with the Department of Horticulture, Forestry & Recreation Resources, Kansas State University. This research was first reported in Applied Turfgrass Science in June 2006. Acknowledgements and Literature Cited are available at www.sportsturfonline.com.

What it means to you

Our results indicate that tall fescue (TF) may be better suited than hybrid bluegrass (HBG) in areas of the transition zone where soils are deep, especially if drought resistance is a priority. Susceptibility of HBG to bluegrass billbug in this study also suggests that its maintenance costs may be higher than in TF, although other HBG cultivars not tested in this study may be more resistant to billbug damage. Because some cultivars of HBG also may exhibit higher drought resistance than others, further research is needed using new or different cultivars of HBG and in areas with different soils to more completely determine the potential for the use of HBG in the transition zone.