Trinexapac-ethyl, Propiconazole, Iron, and Biostimulant Effects on Shaded Creeping Bentgrass

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SUMMARY. Creeping bentgrass (*Agrostis stolonifera*) is used extensively on temperate zone golf course greens, tees, and fairways, but often performs poorly in shade. Previous research has indicated that sequential applications of gibberellic acid (GA) inhibiting plant growth regulators (PGRs) such as trinexapac-ethyl (TE) increase cool-season turfgrass performance in 70-90% shade. This research was conducted to: 1) confirm appropriate TE application rates and frequencies for maintaining 'Penncross' creeping bentgrass in dense shade in the mid-Atlantic region of the U.S.; 2) determine the efficacy of other PGRs, biostimulants, and iron (Fe); and 3) assess whether the addition of a biostimulant with TE would have additive, synergistic, or negative effects. The other compounds tested against TE and the control were: propiconazole (PPC), iron sulfate, CPR (a seaweed and iron containing biostimulant), and a generic seaweed extract (SWE) (*Ascophyllum nodosum*) plus humic acid (HA) combination. These treatments were applied to 88% shaded bentgrass every 14 days from May or June through October in 2001 and 2002, with turf quality, leaf color, root strength, photochemical efficiency, and antioxidant enzyme superoxide dismutase (SOD) activity being determined. While the quality of control plots fell below a commercially acceptable level by the second month of the trial, repeated foliar TE application provided 33% to 44% better quality throughout the experiment. Propiconazole resulted in 13% to 17% better quality through September of each year. Trinexapac-ethyl and PPC resulted in darker leaf color and increased mid-trial root strength by 27% and 29%, respectively. Canopy photochemical efficiency and leaf SOD activity were also increased due to TE in August of both years. Treatment with Fe, CPR, or SWE+HA did not have an effect on quality, root strength, SOD, or photochemical efficiency, but periodic increases in color were observed. The addition of CPR to TE in 2002 provided results that were not different from those of TE-alone. This and previous studies indicate that restricting leaf elongation with anti-GA PGRs is of primary importance for improving shade tolerance, while treatments that increase leaf color or chlorophyll levels without restricting leaf elongation are relatively ineffective.

Closely mown creeping bentgrass has moderate shade tolerance compared to other cool-season turfgrasses (Stienke and Stier, 2003; Turgeon, 2002). Poor creeping bentgrass performance on shaded tees, fairways, and greens is often exacerbated by traffic, divoting, restricted airflow (Koh et al., 2003), and increased disease pressure (Vargas and Beard, 1981). Turfgrasses grown under reduced irradiance have decreased net photosynthesis, limiting carbohydrate availability resulting in reduced root mass and depth and poor shoot density (Allard et al., 1991; Beard, 1997; Qian et al., 1998). These potential negatives, coupled with an undesirable increase in leaf elongation and succulence under shade due to increased GA production (Tan and Qian, 2003), can combine to reduce persistence and recovery under traffic as was reported for zoysiagrass (*Zoysia japonica*) (Ervin et al., 2002).

Plant growth regulators that inhibit GA biosynthesis such as TE and flurprimidol have been used to maintain shaded turfgrasses (Ervin et al., 2002; Goss et al., 2002; Qian et al., 1998; Stienke and Stier, 2003; Stier and Rogers, 2001; Stier et al., 1999). Flurprimidol improved shade tolerance, sustaining kentucky bluegrass (*Poa pratensis*) tiller density for a longer period under dense shade than the control (Stier et al., 1999). Sequential TE applications at 14 to 28 d intervals reduced clipping yield and provided greater creeping bentgrass percent cover, quality, color, tiller density, shoot:root ratios, and total carbohydrate content under 60 to 80% reduced irradiance conditions (Goss et al., 2002). Stienke and Stier (2003) also reported that repeated TE applications at 14- or 28-d intervals improved creeping bentgrass quality, density, and chlorophyll levels under 80% shade.

Propiconazole is a sterol-inhibiting turfgrass fungicide used for the control of dollar spot (*Sclerotinia homoeocarpa*) and rhizoctonia blight (*Rhizoctonia solani*). Plant growth regulating effects on cool-season turfgrasses such as reduced shoot growth, darker green leaves, and delayed senescence due to propiconazole have been reported (Goatley and Schmidt, 1990; Kane and Smiley, 1983). Triazoles such as propiconazole are thought to function as PGRs through their inhibition of early-step GA biosynthesis (Fletcher et al., 2000). Although the application of propiconazole on shaded turf to jointly exploit its fungicidal and GA-inhibiting properties appears promising, little research has been conducted to investigate its possible utility.

Extracts of the seaweed *Ascophyllum nodosum* and of leonardite to isolate HA are primary ingredients in commercial biostimulants (Schmidt et al., 2003). As opposed to PGRs that restrict leaf elongation, SWE and HA have been shown to stimulate or maintain vegetative growth and photosynthetic efficiency during drought and heat stress (Zhang and Schmidt, 1999; 2000). The commercial biostimulant CPR (Emerald Isle, Ltd., Ann Arbor, Mich.) is a 4N–0P–1.2K (1.1% nitrate nitrogen, 2.9% urea, 1.5% soluble potash) that contains 28.3% SWE and 28.3% nonionic surfactant.

Application of both SWE+HA, rather than either alone, have repeatedly provided improved color, chlorophyll retention, and photochemical efficiency of cool-season turfgrasses under drought (Schmidt et al., 2003). These improved responses to drought have recently been tied to the substantial cytokinin levels

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found in both materials (Zhang and Ervin, 2004). Chlorophyll content has been noted to decline in dense shade (Bell and Danneberger, 1999; Gaussoin et al., 1988) with such effect presumably contributing to decreased net photosynthesis, reduced rooting and loss of turf density (Beard, 1997). Cytokinins have been shown to stimulate chlorophyll biosynthesis and regulate senescence (Binns, 1994). However, because cytokinins are produced in roots and xylem translocated to shoots, shade-induced reductions in rooting and evapotranspiration imply reduced cytokinin shoot availability. Higher levels of chlorophyll and cytokinins due to SWE+HA or CPR applications may function to improve creeping bentgrass shade tolerance, but little information is available regarding such potential.

Iron plays an important role in chlorophyll formation and is used on fine turf to provide a greening response without increased shoot growth (Glinski et al., 1992; Zhang et al., 2002). Stier and Rogers (2001) investigated whether monthly iron sulfate treatment of bluegrasses (*Poa* spp.) under 85% to 90% shade would improve tolerance. They found that iron did not improve shade tolerance, having negligible effects on quality, color, chlorophyll content, and plant density.

While multiple trials have confirmed TE's utility for improving turfgrass shade tolerance, little information is available concerning the influence of propiconazole, biostimulants or Fe on maintaining shaded creeping bentgrass. Our objectives were to: 1) confirm an appropriate TE application rate and frequency for maintaining 'Penncross' creeping bentgrass in dense shade in the mid-Atlantic region of the U.S.; 2) determine the efficacy of other PGRs, biostimulants, and Fe; and 3) assess whether the addition of a biostimulant with TE would have additive, synergistic, or negative effects.

Materials and methods

A mature (>15 years old) 'Penncross' creeping bentgrass sward [with <30% annual bluegrass (*Poa annua*) invasion] on a Groseclose silt loam soil (a clayey, Kaolinitic, mesic Typic Hapludult, pH 6.0, OM 2.8%, P 46 lb/acre (51.6 kg·ha^{-1}), K 132 lb/acre (147.9 kg·ha^{-1})] at the Virginia Tech Turfgrass Research Center, Blacksburg, was used for this study. Nitrogen was applied as a liquid suspension to all plots at 0.5 lb/1000 ft^2 (24.41 kg·ha^{-1}) from stabilized urea (46N–0P–0K) monthly from May through October in 2001 and April, June, August, and October in 2002. Total N applied was 3 lb/1000 ft^2 (146.5 kg·ha^{-1}) in 2001 and 2 lb/1000 ft^2 (97.6 kg·ha^{-1}) in 2002. Total N applied to the experimental area was reduced in 2002 based on the senior author's agronomic judgment regarding shaded creeping bentgrass N needs for adequate color and clipping responses. Chlorothalonil and iprodione were applied on a preventive schedule to control leaf spots (*Bipolaris sorokiniana*, *Dreschlera catenaria*), dollar spot and rhizoctonia blight diseases. Halofenozide [2.9 oz/1000 ft^2 (9.23 L·ha^{-1})] was applied each June for preventive control of white grubs (*Popillia japonica*) and watered in. Irrigation was applied at the first sign of visual moisture stress on the full sun portion of the study area with a traveling water wheel with a radius of throw that covered the creeping bentgrass inside and outside of the shade covers. Approximately 1 inch of water was applied at each irrigation event. Irrigation was not applied based on estimated evapotranspirational (ET) demand. However, by irrigating the higher ET-demand non-shaded borders on an infrequent and deep schedule, moisture stress under the shade covers was never noted. Mowing was performed on a Monday, Wednesday, Friday schedule with a triplex reel mower (Toro Greensmaster 3100; Toro Co., Minneapolis, Minn.).

Four quonset-type frames [13 ft wide × 32 ft long × 6.5 ft high (4.0 × 9.8 × 1.98 m)] covered with black knit high-density polyethylene shade cloth specified to block 73% solar radiation (Wetzel, Inc., Harrisonburg, Va.) were installed over the mature field plots on 15 May 2001. The stationary shade frames were oriented lengthwise in an east to west direction, each separated by a 6-ft (1.8 m) alley. The shade cloth extended to 1 ft (0.3 m) above the turfgrass canopy on all sides. On the east and west sides the black fabric could be opened and pinned to the north and south sides to allow mowing. Shade cloth was installed and removed to coincide with deciduous tree leaf emergence and leaf senescence. In 2001, installation of the shade cloth was later than leaf emergence, due to delays in installation of the metal frames.

Air temperature and relative humidity inside of the block-three shade structure were logged hourly at 1 ft above the turf canopy with a Model 450 WatchDog data logger (Spectrum Technologies, Plainfield, Ill.) during the 2001 experimental period when the shade covers were in place. Data from 2002 are not available due to data logger malfunction. Ambient air temperatures were collected from a National Oceanic and Atmospheric Administration weather station located approximately 0.5 mile (0.80 km) south of the study area. Photosynthetically active radiation (*PAR*) levels were monitored with two quantum light sensors (Item 3688: Spectrum Technologies, Inc.), one inside and one outside of the third shade structure. The sensors logged one *PAR* reading (µmol·m^{-2}·s^{-1}) each hour. The sensors were mounted at two heights on the same pole. The inside sensor was at 1 ft, while the outside sensor was just above the top of the shade cover at 7 ft (2.1 m). Light intensity was monitored from 1 July through 31 Oct. 2001. For data presentation *PAR* readings were converted from µmol·m^{-2}·s^{-1} to mol·m^{-2}·d^{-1} based on the number of hours each day that light reached each sensor.

Treatments were arranged in a randomized complete-block design, with each shade frame serving as one replication. Each experimental unit had a plot size of 6 × 6 ft. There were six treatments in 2001 and an additional treatment was added in 2002. The following treatments were applied every 14 d from June through October in 2001 and May through October in 2002: 1) control; 2) 0.34 oz/acre (24 g·ha^{-1}) TE (Primo Maxx; Syngenta, Greensboro, N.C.); 3) 6 fl oz/1000 ft^2 (19.1 L·ha^{-1}) CPR; 4) 3.1 oz/acre (220 g·ha^{-1}) propiconazole (Banner Maxx; Syngenta, Greensboro, N.C.) 5) 5 g/1000 ft^2 [0.18 oz/1000 ft^2 (538 g·ha^{-1})] SWE (Acadian Seaplants, Dartmouth, N.S., Canada) + 15 g/1000 ft^2 [0.53 oz/1000 ft^2 (1.6 kg·ha^{-1})] HA (Plant Wise Biostimulants, Louisville, Ky.); 6) 0.11 lb/1000 ft^2 Fe (1.1 kg·ha^{-1} Fe) ($FeSO_4 \cdot 7H_2O$; Hi-Yield Chemical, Bonham, Texas). In 2002, a seventh treatment was added: TE + CPR as a tank-mix at their respective rates listed in treatments 2 and 3 above. All treatments were mixed in water and foliarly applied using a carbon dioxide (CO_2)-pressurized sprayer delivering

40 gal/acre (374.1 L·ha^{-1}) at 30 psi (206.8 kPa).

To evaluate rooting over the study period, a strip of sod was cut down the middle of each plot on 15 May 2001, 2 weeks prior to treatment initiation. The sod was temporarily removed and three successive (one for each sample date) 1 ft^2 (0.09 m^2) metal grates per plot were placed on the soil surface and spaced 10 inches (25.4 cm) apart. The metal grates [0.12 inch (3 mm) thick] have uniformly distributed rhombic openings [1.50 inch long × 0.63 inch wide at middle (3.8 × 1.6 cm)] for roots to grow past (Goatley and Schmidt, 1991; Zhang et al., 2003). The sod was then replaced and rolled with a hand-pushed metal drum of approximately 200 lb (90.7 kg). Each corner of the grate has a mesh opening, through which metal hooks on the end of a four-strand chain are connected. The maximum vertical force (kg) required to evenly pull the roots from the soil was measured with a hand-held push/pull scale (Model 80-D; Chatillon Scale Co., New York) connected to the chain. Each of the three grates per plot was used in succession to measure rooting strength at the end of the first season (30 Oct. 2001), the beginning of the second season (15 Apr. 2002) and the end of the trial (5 Nov. 2002).

Turfgrass quality and leaf color were rated monthly based on a visual scale of 1 to 9 with "9" indicating the best quality or the greenest leaf color and "6" representing minimum commercial acceptability for a shaded golf course fairway or tee.

Leaf samples were taken on 29 Aug. 2001 and 12 Aug. 2002 for antioxidant superoxide dismutase activity analysis according to the method of Giannopolitis and Ries (1977), with modifications as described in Ervin et al., (2004a). Approximately 0.07 oz (2 g) of fresh leaf tissue was randomly clipped from the middle 15 ft^2 (1.4 m^2) of each plot and immediately frozen in liquid nitrogen. The samples were then stored at –112.0 °F (–80 °C) until subsequent laboratory analysis.

Canopy photochemical efficiency readings were taken on the plots immediately prior to leaf tissue harvest for SOD analysis each August. The ratio of variable fluorescence (Fv) to maximum fluorescence (Fm) at 690 nm (Fv/Fm) is an indicator of the photochemical efficiency of photosystem II (Maxwell and Johnson, 2000; Zhang and Schmidt, 2000). Chlorophyll fluorescence was measured on the turfgrass canopy consisting of shoot material of various age and physiological status from newly emerged tillers to senescing tissue. An area of uniform turf density near the middle of each plot was selected randomly and covered for 15 min by a polyvinyl chloride (PVC) ring [4 inches diameter × 2 inches high (10.2 × 5.1 cm)] filled with Styrofoam [0.39 inch (10 mm) thick] for dark acclimation. An opening the size of the probe (0.39-inch diameter) is present in the styrofoam of each PVC ring and covered by a plastic plate. After the canopy is subjected to dark acclimation, the plastic plate is removed and the probe for the actinic light source inserted immediately into the opening. The ring is rotated 90° after each reading until three fluorescence measurements are logged. The values of Fv/Fm are calculated based on an average of three measurements per experimental unit.

Treatments were arranged in randomized complete blocks, with each of the four shade structures serving as one block. Data were subjected to analysis of variance to determine if significant treatment effects existed at each sampling date (SAS, 2001). Fisher's protected least significant difference values ($P = 0.05$) were calculated to separate treatment means when appropriate.

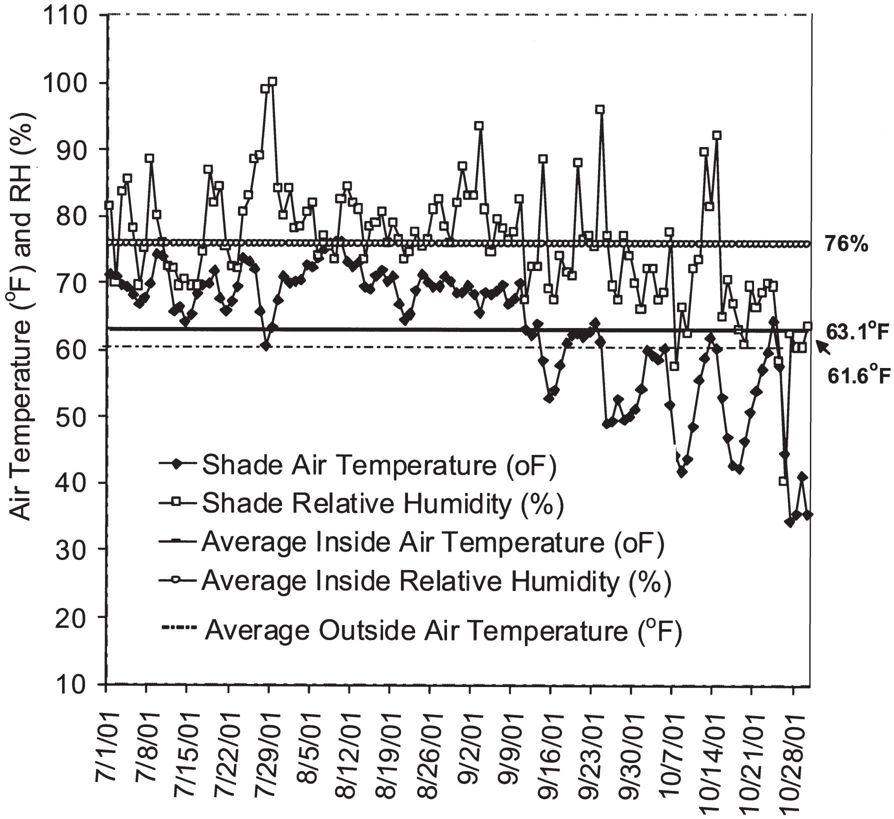

Fig.1. Average daily air temperature and relative humidity (RH) inside the shade covers at the Blacksburg, Va., site from 1 July through 28 Oct. 2001. The average air temperatures over this period were 63.1 °F (17.28 °C) inside the shade cover and 61.6 °F (16.44 °C) outside the shade cover. The average RH outside the shade cover was 76%. 5/9(°F – 32) = °C

Results and discussion

ENVIRONMENTAL DATA. Average air temperature and relative humidity under shade from July through Oct. 2001 was 63.1 °F (17.28 °C) and 76%, respectively (Fig. 1). Slightly elevated relative humidity under shade most likely resulted in shaded air temperatures being slightly higher than the average non-shaded air temperature mean of 61.6 °F (16.44 °C) over the same period. These temperatures did not often deviate from the optimum [60 to 75 °F (15.6 to 23.9 °C)] for cool-season turfgrass shoot growth (Beard, 1973) and were most likely not a factor in observed quality decline in this trial.

Monthly *PAR* directly above the shade cover averaged 45 to 50

$mol \cdot m^{-2} \cdot d^{-1}$, while inside values averaged 88% less, ranging from 5.71 to 6.24 $mol \cdot m^{-2} \cdot d^{-1}$ from July through Oct. 2001 (Table 1). Shaded light intensity levels were lower than expected given that 73% shade fabric was utilized because of the decreased average daylength recorded at the inside *PAR* sensor (Table 1). Dense stands of deciduous trees bordering the east and west sides of the study area prevented incident radiation from reaching the inside sensor, which was 6 ft lower than the outside sensor, by approximately 1.5 h each morning and evening. Previous cool-season turfgrass shade research has indicated that non-PGR treated stands require a minimum irradiance level of approximately 5.0 $mol \cdot m^{-2} \cdot d^{-1}$ to maintain acceptable quality for periods of 2 months or longer when subjected to light traffic (Stier and Rogers, 2001; Stier et al., 1999). Therefore, irradiance levels in this experiment were sufficiently low to reduce quality and determine differences due to treatments as regards shade tolerance.

CONTROL. Creeping bentgrass untreated control quality fell below a commercially acceptable level in July 2001 and continued to be below acceptable throughout the experiment (Table 2). Quality decline was primarily due to extensive canopy thinning rather than a large loss in leaf color. The leaf color of control plots was first recorded at about 6.0 and remained there for the remainder of the trial (Table 3).

TRINEXAPAC-ETHYL. Twice-monthly applications of TE significantly improved creeping bentgrass quality relative to the control in every month of the study over both years (Table 2). Further, it was only on the last rating date of 2002 that the quality of TE-treated bentgrass fell below a commercially acceptable level of 6.0. Leaf color due to TE was also consistently darker than the control plots over every month of the trial (Table 3). Darker green TE-treated leaf tissue has been related to increased mesophyll cell density and chlorophyll concentration (Ervin and Koski, 2001; Stienke and Stier, 2003).

At the Aug. 2001 and 2002 measurement dates, canopy photochemical efficiency on TE plots was also greater than the control (Table 4). In a non-shaded study comparing eight kentucky bluegrass cultivars, comprising a range of genetic leaf colors, the darkest green cultivar had significantly greater chlorophyll concentration and canopy photochemical efficiency than did the lightest green cultivar (Ervin et al., 2004b). Although not measured in the present study, TE may have increased chlorophyll concentration resulting in darker green leaves and greater photochemical efficiency. Results from other researchers, however, indicate that greater chlorophyll or radiation use efficiency levels in reduced light conditions do not necessarily correspond to greater shade tolerance. Stienke and Stier (2003) reported that increased chlorophyll concentration in shaded TE-treated creeping bentgrass did not increase single leaf photochemical efficiency, but density and quality were improved nonetheless. Huylenbroek and Van Bockstaele (2001), reported that while perennial ryegrass (*Lolium perenne*) had increased chlorophyll content and net photosynthesis in 65% shade, leaf elongation rate was increased relative to full sunlight and percent cover declined faster than all other species compared. Such results support the supposition that reducing leaf elongation through proper use of anti-GA PGRs may be the primary means for improving cool-season shade tolerance rather than manipulation of leaf chlorophyll levels or photosynthetic capacity.

In August of both years SOD activity was also greater for TE relative to the control (Table 4). Greater relative SOD activity is most commonly associated with increased tolerance to

Table 1. Average monthly photosynthetically active radiation (*PAR*), percent *PAR* transmission through the shade cover, and daily light duration directly above (outside) and inside of the shade cover in 2001 at the Blacksburg, Va., creeping bentgrass site.[z]

	PAR ($mol \cdot m^{-2} \cdot d^{-1}$)[y]			Light ($h \cdot d^{-1}$)	
Month	Outside	Inside	Transmission (%)	Outside	Inside
July	50.4	5.71	11.3	13.6	10.7
Aug.	49.9	5.88	11.8	13.3	10.5
Sept.	49.7	6.24	12.6	11.9	8.7
Oct.	45.2	5.78	12.8	11.1	7.5

[z] Year 2002 data were lost due to datalogger malfunction.

[y] Light intensity was monitored hourly with a quantum light sensor and calculated based on total light intensity per day and actual number of hours each sensor received light each day.

Table 2. Creeping bentgrass quality under 88% shade as influenced by 14-d sequential treatments of trinexapac-ethyl (TE), propiconazole (PPC), iron sulfate ($FeSO_4$), CPR (a seaweed and iron containing biostimulant), and seaweed extract (SWE) + humic acid (HA) from June through Oct. 2001 and 2002 in Blacksburg, Va.

	Turf quality (9 = best; 6 = acceptable; 1 = dead)				
	2001				
Treatment	20 June	24 July	21 Aug.	25 Sept.	30 Oct.
Control	6.9 a[z]	5.1 c	5.0 c	4.8 bc	4.3 c
TE	6.9 a	6.3 a	6.1 a	6.0 a	6.4 a
PPC	6.3 a	5.8 b	5.9 ab	5.8 a	5.0 bc
$FeSO_4$	7.0 a	5.5 bc	5.1 c	5.0 bc	4.4 bc
CPR	6.9 a	5.5 bc	5.3 c	5.4 ab	5.4 ab
SWE+HA	6.9 a	5.4 bc	5.4 bc	4.6 c	4.9 bc
	2002				
	18 June	1 July	12 Aug.	1 Sept.	9 Oct.
Control	4.8 c	4.8 cd	4.8 c	4.1 c	3.1 b
TE	7.8 a	6.4 ab	6.6 a	6.4 a	5.5 a
PPC	7.5 a	6.4 ab	5.9 ab	5.3 b	2.9 b
$FeSO_4$	5.8 c	4.9 cd	6.5 a	3.8 c	3.1 b
CPR	6.0 b	5.6 bc	5.1 bc	4.1 c	3.0 b
CPR+TE	7.8 a	6.8 a	6.0 ab	5.8 ab	4.4 a
SWE+HA	5.8 c	4.5 d	5.0 bc	4.4 c	3.3 b

[z] Values followed by same letters within same column each year are not different significantly at a least significant difference level of 0.05.

Table 3. Creeping bentgrass leaf color under 88% shade as influenced by 14-d sequential treatments of trinexapac-ethyl (TE), propiconazole (PPC), iron sulfate (FeSO4), CPR (a seaweed and iron containing biostimulant), and seaweed extract (SWE) + humic acid (HA) from June through Oct. 2001 and 2002 in Blacksburg, Va.

	Leaf color (9 = darkest; 1 = no green color)				
	2001				
Treatment	20 June[z]	24 July	21 Aug.	25 Sept.	30 Oct.
Control		6.3 b[y]	6.0 c	6.0 b	6.5 c
TE		7.1 a	7.0 a	8.0 a	8.0 a
PPC		7.0 a	6.0 c	7.6 a	7.5 ab
$FeSO_4$		6.6 ab	6.1 bc	6.3 b	6.8 bc
CPR		6.3 b	6.3 b	6.5 b	7.0 bc
SWE+HA		6.5 ab	6.0 c	6.5 b	6.3 c
	2002				
	18 June	1 July	12 Aug.	1 Sept.	9 Oct.
Control	5.8 c	6.0 d	6.0 d	6.0 c	6.0 b
TE	7.5 ab	7.9 a	7.8 a	7.9 a	7.9 a
PPC	6.8 abc	7.3 b	6.5 cd	7.0 b	7.5 a
$FeSO_4$	7.0 bc	6.8 c	6.3 cd	6.5 bc	6.0 b
CPR	6.0 c	7.0 bc	6.8 bc	6.8 b	6.6 b
CPR+TE	7.8 a	8.0 a	7.4 ab	8.0 a	7.8 a
SWE+HA	5.8 c	6.9 bc	6.0 d	6.8 b	6.5 b

[z] Data collection inadvertently missed on this date.

[y] Values followed by same letters within same column each year are not different signficantly at a least significant difference level of 0.05.

Table 4. Creeping bentgrass leaf superoxide dismutase (SOD) activity and canopy photochemical efficiency in August under 88% shade as influenced by 14-d sequential treatments of trinexapac-ethyl (TE), propiconazole (PPC), iron sulfate (FeSO4), CPR (a seaweed and iron containing biostimulant), and seaweed extract (SWE) + humic acid (HA) from June through Oct. 2001 and 2002 in Blacksburg, Va.

	SOD activity (unit/g fresh wt)[z]		Photochemical efficiency	
Treatment	29 Aug. 2001	12 Aug. 2002	29 Aug. 2001	12 Aug. 2002
Control	2172 b[y]	4700 b	0.60 c	0.52 c
TE	3781 a	7078 a	0.65 a	0.60 a
PPC	3879 a	5415 ab	0.63 ab	0.56 b
FeSO4	4049 a	5297 ab	0.61 bc	0.54 bc
CPR	3018 ab	6267 ab	0.63 ab	0.57 ab
CPR+TE[x]	---	6622 a	---	0.57 ab
SWE+HA	4434 a	5650 ab	0.62 bc	0.56 b

[z] One unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of the rate of nitro blue tetrazolium reduction.

[y] Values followed by same letters within each column are not different significantly at a least significant difference level of 0.05.

[x] This treatment was evaluated in 2002 only.

oxidative stress caused by high light intensity, heat, and drought (Smirnoff, 1995). In reduced light, greater SOD may reflect an increased ability of the TE treated plants to absorb and assimilate inorganic nitrogen for biosynthesis of amino acids and proteins such as SOD. A measure of the N content in the sampled leaf tissue would have provided some evidence for or against this statement, but N levels were not measured in this study. The increased density of the TE-treated creeping bentgrass, as evidenced by greater quality ratings, may have also contributed to greater surface root density and subsequent increased nitrogen assimilation. More detailed future research should be conducted to investigate these possibilities.

Greater quality, color, photochemical efficiency and SOD did not result in greater root strength for TE relative to the control when measured in Oct. 2001 (Table 5). However, a relatively mild Nov. 2001 through Feb. 2002 period, where soil temperature at a 4-inch depth averaged 42 °F (5.6 °C) (data not shown), appears to have allowed continued non-shaded root development. Thus, greater shoot density going into the winter in the TE-treated plots appears to have resulted in more root development over the non-treated plots, because a significant increase in root strength was measured in TE-treated plots in April just prior to the beginning of the 2002 shade period. By the end of the trial (Nov. 2002), the negative effects of continuous 90% shade were readily seen by the large decrease in rooting on all plots. However, the TE treatment still resulted in the greatest root strength at this final measurement date (Table 5).

PROPICONAZOLE (PPC). Quality was greater than the control due to PPC on seven of 10 rating dates over both years (Table 2). On five of 10 dates, PPC quality was equivalent to that of TE. However, at the end of both years, PPC quality had dropped significantly below the commercially acceptable level of 6.0, while that only occurred for TE in Oct. 2002. Higher leaf color on six of eight rating dates (Table 3) was partially responsible for higher quality ratings, while greater density contributed the rest. Greater density and color most likely contributed to improved canopy photochemical efficiency on one of the two measurement dates relative to the control (Table 4). Greater canopy health was also reflected in significantly higher SOD activity in Aug. 2001 (Table 4), and greater root strength at the end of 2001 and the beginning of 2002 (Table 5).

Trinexapac-ethyl primarily, and PPC secondarily (Fletcher et al., 2000), act by inhibiting GA production and limiting leaf elongation. Limiting cell elongation also functions to increase chlorophyll concentration (and leaf color) by increasing mesophyll cell density (Ervin and Koski, 2001). Reducing energy-wasting leaf elongation and improving chlorophyll concentration due to anti-GA PGR applications has repeatedly been shown to result in improved shade tolerance of cool and warm season turfgrasses (Ervin et al., 2002; Qian et al., 1998; Stienke and Stier, 2003; Stier and Rogers, 2001; Stier et al., 1999). While TE has continually been shown to improve shade tolerance, this is the first report of which we are aware indicating the utility of PPC for improved turfgrass shade tolerance. An added benefit of

Table 5. Creeping bentgrass root strength under 88% shade at three sample dates as influenced by 14-d sequential treatments of trinexapac-ethyl (TE), propiconazole (PPC), iron sulfate (FeSO4), CPR (a seaweed and iron containing biostimulant), and seaweed extract (SWE) + humic acid (HA) from June through Oct. 2001 and 2002 in Blacksburg, Va.

Treatment	Root strength (kg)		
	30 Oct. 2001	18 Apr. 2002	7 Nov. 2002
Control	26.0 b[z]	59.8 b	8.5 b
TE	23.3 b	81.8 a	16.3 a
PPC	37.5 a	84.3 a	11.3 ab
$FeSO_4$	23.8 b	71.8 b	10.5 b
CPR	23.0 b	73.3 ab	8.0 b
CPR+TE	---	80.3 a	9.3 b
SWE+HA	26.3 b	70.0 b	9.0 b

[z] Values followed by same letters within same column are not different significantly at a least significant difference level of 0.05.

using PPC in a disease-conducive shade environment (Vargas and Beard, 1981) is its fungicidal properties.

IRON. In general, iron sulfate applications failed to have any effect on quality, color, photochemical efficiency, or root strength compared to the control (Tables 2, 3, 4, and 5). In Aug. 2001, SOD activity was greater due to Fe, but not in 2002. Using an equivalent iron sulfate rate, but on a less frequent application schedule, Stier and Rogers (2001) also report that Fe failed to increase total chlorophyll levels and turf density of shaded kentucky bluegrass and supina bluegrass (*Poa supina*). Although they reported some increases in quality and color, they were infrequent and of slight magnitude. These authors speculated that under shaded conditions Fe may not have been taken up by the foliage or utilized for chlorophyll production. Even though the amount of foliar Fe applied per month in the current experiment was double that of Stier and Rogers (2001), no color response was noted. Under full-sun conditions the Fe rates used in these studies have consistently resulted in increased leaf color and chlorophyll levels (Turner and Hummel, 1992). Further research is warranted to investigate differences in Fe uptake and utilization of shaded turfgrasses relative to non-shaded to begin to determine physiological reasons for these observed differences.

CPR. Sequential applications of the SWE-containing formulated product-CPR, resulted in greater quality compared to the control on only two of 10 rating dates, but in general did not result in commercially acceptable quality (Table 2). Leaf color was greater due to CPR on three of nine rating dates (Table 3), as was photochemical efficiency in August of both years (Table 4). However, neither SOD activity nor root strength was affected (Tables 4 and 5).

SWE+HA. Quality on the SWE+HA plots fell in an equivalent manner to the control due to dense shade over both years (Table 2). Color was improved on only two of eight rating dates (Table 3), while canopy photochemical efficiency was slightly improved in Aug. 2002 (Table 4). In Aug. 2001, SOD activity was increased relative to the control, but not in Aug. 2002 (Table 4). As expected, given the limited positive responses detailed above, SWE+HA failed to improve root strength at all measurement dates (Table 6).

Biologically significant levels of cytokinins have recently been reported to be present in SWE and HA, with enhancement of leaf cytokinin levels due to SWE+HA application prior to drought resulting in improved tolerance (Zhang and Ervin, 2004). Cytokinins have been shown to stimulate chlorophyll biosynthesis and regulate senescence (Binns, 1994). Shade-induced shallow rooting may limit cytokinin availability to the shoots. Although neither endogenous cytokinin levels nor chlorophyll were measured in this experiment, it was presumed that exogenous applications of cytokinin-containing SWE and HA may increase these hormone and pigment levels enough to improve shade tolerance. However, our data indicate that the slight improvements in color and photochemical efficiency measured due to SWE+HA application to shaded creeping bentgrass were not sufficient to sustain commercially acceptable quality.

CPR + TE. In 2002, a tank-mix treatment of CPR + TE was added to investigate whether the positive effects of TE could be enhanced. The results for all parameters indicate equivalent responses whether TE was applied alone or in combination with CPR. These results serve to further support the view that reduced leaf elongation (or conserved photosynthate) due to anti-GA PGRs is of primary importance for improved shade tolerance.

In summary, application of TE every 14 d from May through October resulted in 33% better creeping bentgrass quality than the untreated control in 88% shade at the end of the first year and 44% better quality at the end of the second. These results were consistent with those in Michigan where comparable 2- or 4-week rates of TE also provided superior creeping bentgrass quality (Goss et al., 2002; Stienke and Stier, 2003). Propiconazole was also effective at improving shade tolerance, providing 17% and 13% better quality than the control through September of 2001 and 2002, respectively. Trinexapac-ethyl and PPC provided darker leaf color and increased Apr. 2002 root strength by 27% and 29%, respectively. Future research should investigate possible additive or synergistic TE + PPC rate and frequency combinations for greater improvements of creeping bentgrass shade tolerance. Slight, but inconsistent, increases in color due to Fe, CPR, or SWE+HA did not result in improved turfgrass quality or root strength. The addition of CPR to TE in 2002 provided results that were not different from those of TE-alone. These results support concluding that suppression of leaf elongation due to the anti-GA action of PGRs such as TE, and to a lesser extent PPC, are the primary factor in improved shade tolerance of creeping bentgrass. Increased leaf color or chlorophyll levels due to TE, PPC, Fe, or SWE appear to be of secondary and insufficient effect, by themselves, for improved shade tolerance.

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