Application of Trinexapac-ethyl and Propiconazole Enhances Superoxide Dismutase and Photochemical Activity in Creeping Bentgrass (*Agrostis stoloniferous* var. *palustris*)

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ABSTRACT. Superoxide dismutase (SOD) activity is closely associated with stress tolerance of creeping bentgrass [*Agrostis stoloniferous* L. var. *palustris* (Huds.) Farw (syn. *A. palustris* Huds.)]. This study was conducted to investigate the influence of two plant growth regulators (PGRs) on the endogenous antioxidant SOD level and photochemical activity in 'Pennncross' creeping bentgrass grown under two fertilizer regimes. Mature 'Pennncross' was treated monthly with TE at 0.44 g a.i./100 m² and PPC at 3.37 g a.i./100 m² from May through November at the Virginia Tech Turfgrass Research Center, Blacksburg, Va. Foliar application of TE and PPC increased SOD activity, photochemical activity, and Fm730/Fm690 ratio of creeping bentgrass under the two fertilization regimes as well as when the grass was exposed to a low soil moisture environment. TE reduced clipping weight consistently regardless of the fertilization regime. In contrast, PPC increased clipping weight slightly. Both TE and PPC significantly reduced Dollar spot disease (*Sclerotinia homoeocarpa* Bennett) under both high and low fertilization regimes. No significant fertilization × PGR interactions for SOD, photochemical activity of PS II, and Fm730/Fm690 were observed in well-watered or drought stressed bentgrass. Improvement in stress tolerance of creeping bentgrass by the PGRs appears to be associated partially with an increase of endogenous SOD activity. Chemical names used: trinexapac-ethyl (TE); propiconazole (PPC).

Creeping bentgrass [*Agrostis stoloniferous* L. var. *palustris* (syn. *A. palustris*)], a cool season turfgrass species used widely on golf course putting greens, bowling greens, and tennis courts in North America, is influenced frequently by environmental stresses and disease (Goodman and Burpee, 1991). Dollar spot, a serious disease caused by *Sclerotinia homoeocarpa*, severely reduces the quality of creeping bentgrass. Water deficits and high temperatures cause photoinhibition and growth reduction of this grass (Lawlor, 1995; Schmidt and Zhang, 1997).

Stress tolerance and quality of turfgrass is associated closely with endogenous antioxidant activity (Zhang and Schmidt, 1999). Environmental stress damages plant cells by production of reactive oxygen species (ROS) (superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen) (Bowler et al., 1992). Because the Calvin cycle activity for carbohydrate production and photosynthetic electron transport efficiency are reduced by environmental stresses, the excess energy may be diverted to reduce molecular oxygen, resulting in overproduction of ROS (Smirnoff, 1995). Plant antioxidant metabolites and enzymes (such as superoxide dismutase) protect plant cells by scavenging ROS. Price and Hendry (1989) reported that high antioxidant activity is associated with increased drought tolerance in grasses.

Reduction of photosynthetic electron transport stimulates production of chlorophyll fluorescence. Fluorescence emission by the chlorophyll of the photosystems makes it possible to conduct nondestructive assays to examine photochemical events of the photosynthetic process (Miles, 1990; Schmidt and Zhang, 1997). The ratio of variable fluorescence to maximum fluorescence (Fv/Fm), called maximum quantum efficiency of photosystem II, reflects the photochemical efficiency of photosystem

II (PSII) (Bolhar-Nordenkamp and Lechner, 1988; Miles, 1990). A plant with high Fv/Fm exhibits high photosynthetic activity under either normal or stress conditions. The value of the Fm690/Fm735 ratio increased with decreasing chlorophyll content of plant leaves (Hak et al., 1990; Rinderle and Lichtenthaler, 1988). In other words, the value of the Fm730/Fm690 ratio increases as chlorophyll content increases.

TE is a widely used growth retardant in some systems of turfgrass management. It suppresses shoot growth by inhibiting production of gibberellic acid (GA₁) from GA₂₀ (DiPaola and Shepard, 1996). PPC is used as a fungicide in controlling diseases, such as Dollar spot in some turfgrass species. Since PPC is a triazole compound, it serves as a biostimulant in regulating plant physiological processes (Fletcher et al., 1986).

Stier et al. (1997) noted that TE and nitrogen did not significantly impact photosynthesis of Kentucky bluegrass (*Poa pratensis* L.). Inhibition of TE on clipping weight, however, was observed in several studies (Bingaman and Christians, 1997; Buelow et al., 1997; Bush et al., 1997). DiPaola and Shepard (1996) reported that TE enhanced turfgrass quality by increasing turf density and root production. The beneficial influences of triazole compounds on plant growth have been reported in several studies (Fletcher et al., 1986; Jung et al., 1987). Although the two growth regulators are being used widely in turfgrass management, the underlying physiological mechanisms need further evaluation. The objective of this research was to investigate the influence of TE and PPC, along with fertilization, on endogenous superoxide dismutase (SOD) activity and photochemical activity of creeping bentgrass under stress.

Materials and Methods

FIELD STUDY. This study was conducted at the Virginia Tech Turfgrass Research Center, Blacksburg, Va. from April 1996 through November 1997. A mature 'Pennncross' creeping bentgrass area was mowed to a height of 0.625 cm three times weekly. Chipco 26019

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FLO (Iprodione: 3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidine carboxamide; a.i. 23.3%), a contact fungicide with relatively short efficacy duration, was applied at 120 mL/100 m² in July and August as a curative control for Dollar spot disease. The turfgrass was irrigated twice a week to supply a total of 5 cm of water to prevent drought stress.

A split plot experimental design was used. The main plots were as follows: 1) low fertility: 200 g N (from urea)/100 m²; 28 g P/100 m²; and 108 g K/100 m², and 2) high fertility: 500 g N (from urea)/100 m²; 56 g P/100 m²; and 216 g K/100 m². Urea (46% N) was dissolved in water and the solution sprayed over foliage monthly starting in late April and ending late November. Super triphosphate (20% P) and potash (50% K) were applied to each plot by hand in late May, July, and October according to the rates above. The turfgrass was irrigated immediately after fertilization.

Separate subplots, 2 m², were treated with TE (Primo WSB, Novartis, Greensboro, N.C.) at 0.44 g a.i./100 m² or PPC (Banner, Novartis, Greensboro, N.C.) at 3.37 g a.i./100 m² monthly beginning in late April and ending late November 1996 and 1997. The two PGRs were dissolved in water, and the solutions were applied evenly over foliage tissue at 35 mL·m⁻². All treatments were replicated four times. Data were subjected to analysis of variance and means separated by Duncan's multiple range test or LSD.

Leaves sampled from each plot in late May, July, September, and November were frozen immediately in liquid nitrogen and stored at -20 °C for superoxide dismutase (SOD) analysis. Each frozen sample (1.0 g) was homogenized in 10 mL of 0.05 M Na₂HPO₄/NaH₂PO₄ (pH 7.0) buffer. The homogenates were filtered through four layers of cheesecloth and then centrifuged at 4 °C for 20 min at 15,000 g_n. The supernatants were collected for SOD assay according to the procedure of Giannopolitis and Ries (1977). One enzyme unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of nitro blue tetrazolium (NBT) reduction measured at 560 nm on a spectrophotometer.

The function of the photosynthetic system was probed by measuring chlorophyll fluorescence with a dual wavelength fluorometer (OS-50, Opti-sciences, Inc. Tyngsboro, Mass.) monthly from March through December 1996 and 1997. Fm730/Fm690, which is related positively to chlorophyll content, and photochemical activity (Fv/Fm) were determined from the chlorophyll fluorescence signals (Hak et al., 1990; Rinderle and Lichtenthaler, 1988). The values of Fv/Fm and Fm730/Fm690 were calculated based on averages of three readings from each plot.

To confirm a correlation between Fm730/Fm690 and chlorophyll content of creeping bentgrass, a series of leaf samples with different chlorophyll content was generated by treating fresh leaves (2 g) with 0.4% paraquat dichloride (1,1'-dimethyl-4,4'-bipyridium dichloride; a.i. 37.0%) solution (6 mL). The treated leaves were shaken in a plastic bag to achieve a uniform distribution of paraquat solution on the leaves, then placed in a 15-cm diameter dish and incubated with an irradiance of 50 µmol·m⁻²·s⁻¹ produced by fluorescent lamps at room temperature for 24 h. Chlorophyll fluorescence (Fm730/Fm690) was measured 7 times over the 24 h. Leaf samples were collected and frozen every 3 h immediately after each chlorophyll fluorescence measurement. Total chlorophyll content was determined based a procedure described by Leegood (1993). Both chlorophyll content and Fm730/Fm690 measurements were repeated three times, and the average was used to establish a correlation. The relationship between chlorophyll content and Fm730/Fm690 for creeping bentgrass in this study was $Y = 3.694 - 13.529X + 15.146X^2$ ($R = 0.9962$), where Y = total chlorophyll content (µg/g fresh leaves), and X = Fm730/Fm690. Dollar spot incidence was

evaluated monthly during the summer based on a visual scale of 1 to 9 with 9 indicating the most severe disease incidence.

GREENHOUSE STUDY. To examine the influence of the PGRs on creeping bentgrass under low soil moisture, a 10 cm diameter plug was removed from each treated plot in July 1997. The soil was trimmed from the plug to 2 cm and then transplanted to a clear plastic container which contained 10 cm deep soil with a moisture of 5% (e.g., -0.5 MPa water potential) according to the technique described by Zhang and Schmidt (1999). There were 12 plugs for each fertilization regime, with six plugs in each container.

The containers were sealed with clear plastic film and grown under shade cloth (75% light reduction) which was used to reduce the temperature (temperature ranges from 21 to 26 °C) inside the container. The light intensity in the greenhouse, supplied by natural sunlight, was 4500 µmol·m⁻²·s⁻¹ at 2 p.m. The containers were opened once a week to check disease and increase gas exchange. Based on a separate experiment, the plastic cover permits air exchange between inside the container and outside while preventing water vapor inside the container from escaping. The soil moisture level was checked once a week by weighing the containers which remained the same during the experiment.

Six weeks after the plugs were initially placed in the terrariums, chlorophyll fluorescence was measured. On this date, fresh leaf samples were collected, frozen with liquid N, and stored at -20 °C for SOD analysis. Also on this date, soil was washed from the roots, which were then dried at 60 °C for 24 h, and weighed.

Fig. 1. Superoxide dismutase (SOD) activity (average of two fertility regimes because F × PGR interaction was not significant) of 'Penncross' creeping bentgrass as influenced by plant growth regulator treatments in 1996 and 1997. Vertical bars represent LSD at $P = 0.05$.

Fig. 2. Photochemical activity (Fv/Fm)(average of two fertility regimes because F $\times$ PGR interaction was not significant) of 'Pennncross' creeping bentgrass as influenced by plant growth regulator treatments and fertility regime in 1996 and 1997. Vertical bars represent LSD at $P = 0.05$.

Results

FIELD STUDY. Superoxide dismutase activity of creeping bentgrass exhibited seasonal fluctuation, with a peak in September regardless of fertilization (Fig. 1). There was no significant fertilization $\times$ PGR interaction for SOD activity (data not presented). Foliar application of TE and PPC increased endogenous antioxidant superoxide

dismutase (SOD) activity of creeping bentgrass based on the average of two fertilization regimes. Greatest effects of the growth regulators on SOD activity were observed during Summer 1996.

Photochemical activity (Fv/Fm) peaked in the fall regardless of fertilization in 1996 or 1997 (Fig. 2). The interaction between fertilization and PGR for photochemical activity was not significant. Application of TE or PPC increased the photochemical activity under both fertility regimes.

When measured after 6 weeks growth at a soil moisture of -0.5 MPa, the application of TE or PPC caused an increase of the Fm730/Fm690 of creeping bentgrass under either high or low fertility regimes (Table 1). No significant fertilization $\times$ PGR interaction for Fm730/Fm690 was obtained either in 1996 or 1997. This indicated that TE and PPC enhanced chlorophyll content of bentgrass as determined by the Fm730/Fm690 ratio (Rinderle and Lichtenthaler, 1988).

Application of TE reduced fresh clipping weight (20%) after 4 weeks of initial application under the high fertility regime in 1997 (Fig. 3). Under low fertility, clipping weight difference between the TE-treated and nontreated bentgrass was less pronounced. Application of PPC enhanced foliage growth under the low fertility regime during May through September, and under high fertility only in September (Fig. 3).

As expected, PPC significantly suppressed Dollar spot disease under the two fertility regimes (Fig. 4). Application of TE or PPC was most effective in reducing Dollar spot disease incidence during the summer months and under the low fertility regime (Fig. 4).

GREENHOUSE STUDY. Fertilization did not significantly influence SOD activity or photochemical activity of creeping bentgrass grown under a low soil moisture regime. Application of TE and PPC, however, promoted SOD activity and photochemical activity of creeping bentgrass under low soil moisture conditions and grown under the two fertility regimes (Table 2).

Foliar application of PPC enhanced root weight of bentgrass grown under low soil moisture (Table 3). However, no effect of TE on root development was found in this phase of the study. Although root weight was greater under the low fertilization regime, there was no significant fertilization $\times$ PGR interaction.

Discussion

Results of this study demonstrated that TE and PPC increased SOD activity of creeping bentgrass under both low and high levels

Table 1. Fm730/Fm690² ratio of 'Pennncross' creeping bentgrass as influenced by plant growth regulator (PGR) treatment and fertilization regime in July 1996 and 1997.

PGR ^y	Fm730/Fm690 ratio					
	Fertilization					
	1996			1997		
	Low	High	Mean	Low	High	Mean
TE	0.971	0.874	0.923 a ^x	0.588	0.615	0.602 ab
PPC	0.801	0.839	0.820 b	0.632	0.632	0.632 a
Control	0.717	0.816	0.767 c	0.567	0.561	0.564 b
Sources of variation						
Fertilization (F)	NS			NS		
PGR	*			*		
F $\times$ PGR	NS			NS		

²Fm730/Fm690 was determined by maximum chlorophyll fluorescence at 730 and 690 nm measured with a dual wavelength fluorometer (OS-50 model, Opti-Sciences Inc., Mass.) at an irradiance of $500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

^yTE = trinexapac-ethyl, PPC = propiconazole.

^xMean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

NS,*Nonsignificant or significant at $P \leq 0.05$, respectively.

Fig. 3. Fresh clipping weight of 'Penncross' creeping bentgrass as influenced by plant growth regulator treatments under two fertility regimes in 1997. Vertical bars represent LSD at $P = 0.05$.

Fig. 4. Dollar spot disease incidence of 'Penncross' creeping bentgrass as influenced by plant growth regulator treatments under two fertility regimes in 1997. Vertical bars represent LSD at $P = 0.05$. Dollar spot incidence was rated based on a visual scale of 1 to 9 with 9 indicating the most disease incidence.

Table 2. Superoxide dismutase (SOD) activity and photochemical activity (Fv/Fm) as influenced by plant growth regulator (PGR) treatments and fertilization regimes in 'Penncross' creeping bentgrass grown under low soil moisture (-0.5 MPa) for 6 weeks

		Fertilization					
		Low	High	Mean	Low	High	Mean
Year	PGR ^z	SOD activity ($\times 10^3$ unit/g dry wt)			Fv/Fm ^y ratio		
1996	TE	13.7	10.5	12.1 b ^x	0.668	0.651	0.660 ab
	PPC	18.7	13.5	16.1 a	0.686	0.708	0.697 a
	Control	7.0	11.5	9.3 c	0.638	0.536	0.587 b
1997	TE	23.3	22.4	22.9 a	0.573	0.425	0.499 a
	PPC	19.4	18.8	19.1 b	0.463	0.415	0.439 a
	Control	14.2	15.4	14.8 c	0.328	0.390	0.359 b
Sources of variation (1996)							
Fertilization (F)			NS			NS	
PGR			*			**	
F $\times$ PGR			NS			NS	
Sources of variation (1997)							
F			NS			NS	
PGR			*			*	
F $\times$ PGR			NS			NS	

^zTE = trinexapac-ethyl; PPC = propiconazole.

^yPhotochemical activity was determined by variable and maximum chlorophyll fluorescence ratio at 690 nm (Fv/Fm) measured with a dual wavelength fluorometer (OS-50 model, Opti-Sciences Inc., Mass.) at an irradiance of $500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

^xMean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

NS,* Nonsignificant or significant at $P \leq 0.05$ or 0.01 , respectively.

Table 3. Root dry weight of 'Penncross' creeping bentgrass as influenced by plant growth regulator (PGR) treatments and fertilizer regimes under low soil moisture (−0.5 MPa) (July 1997) for 6 weeks.

PGR ^z	Root dry wt (mg/plug)		
	Fertilization		
	Low	High	Mean
TE	23	16	20 b ^y
PPC	77	47	62 a
Control	35	19	27 b
Mean	45	27	
Sources of variation			
Fertilization (F)		*	
PGR		*	
F × PGR		NS	

^zTE = trinexapac-ethyl, PPC = propiconazole.

^yMean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

NS,*,** Nonsignificant or significant at $P \leq 0.05$, respectively.

of fertility (Table 2). This is consistent with the results of Sankhla et al. (1992) who reported that triazole growth regulators enhanced hydrogen peroxide-scavenging enzymes and other antioxidants in Japanese barnyard millet (*Echinochloa frumentacea* L.). Some variations between 1996 and 1997 in SOD content and photochemical activity were observed. The significance of the yearly difference could not be evaluated in these studies because experimental environments (irradiance, etc.) may not have been exactly the same in both years.

Both growth regulators influenced photochemical activity (Fv/Fm), and chlorophyll content in terms of F730/Fm690 under high or low fertility regimes (Fig. 2). This supports results of Fletcher et al. (1986) who showed that triazole derivatives increased chlorophyll content of bean (*Phaseolus coccineus* L.) plants. Goatley and Schmidt (1990a) noted that PPC enhanced growth and the CO₂ exchange rate (CER) of Kentucky bluegrass. In another study, triazole-treated Kentucky bluegrass tended to have CER values similar to benzyladenine-treated Kentucky bluegrass, suggesting cytokinin-like activity of the triazoles (Goatley and Schmidt, 1990b). Enhancement of photochemical activity and chlorophyll content by PGRs may be due to their regulation of endogenous hormone activity.

Reduction of turfgrass clipping weight by TE treatment supports previous results by DiPaola and Shepard (1996), Fletcher et al. (1986), and Johnson (1997). The influence of PPC on clipping production, however, was not as significant as it was on antioxidant activity. This suggests the two growth regulators have greater impact on physiological activity than the morphological status of creeping bentgrass.

Under a low soil moisture environment, TE and PPC promoted SOD activity and photosynthetic capacity regardless of fertility (Table 2). Under biotic stress (Dollar spot disease), bentgrass treated with the two growth regulators had greater disease resistance than the control. Zhang and Schmidt (1999) noted that Kentucky bluegrass treated with hormone-containing products produced greater growth under low soil moisture. Results herein indicate that the use of TE and PPC increased resistance of creeping bentgrass to both drought and disease stress. The increased resistance may be due partially to increased level of SOD activity, an antioxidant enzyme.

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