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Trinexapac-ethyl Restricts Shoot Growth and Improves Quality of 'Diamond' Zoysiagrass Under Shade

Y.L. Qian[1]
Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, CO 80523-1173

M.C. Engelke[2], **M.J.V. Foster**[3], **and S. Reynolds**[3]
Texas A&M Research and Extension Center, Texas A&M University System, 17360 Coit Road, Dallas, TX 75252-6599

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***Abstract*. Turfgrass is grown under extremely variable light intensities. This presents difficult management problems, and methods are needed to improve turf performance under variable shade conditions. Two experiments were conducted to determine the influence of trinexapac-ethyl (TE) on turf performance and physiological responses of 'Diamond' zoysiagrass [*Zoysia matrella* (L.) Merr.] under several light intensities. In a polyethylene-roofed greenhouse, 'Diamond' was sodded in 12 wooden boxes (1.2 × 1.2 × 0.16 m) (Expt. 1) and 18 fiber containers (55 × 38 × 12 cm) (Expt. 2). Treatments applied to boxes or containers included three levels of shade (40%, 75%, and 88%) with and without multiple TE applications at 48 g·ha^{-1} of active ingredient. Without TE treatment, vertical shoot growth increased linearly with increasing shade levels. Excessive shoot growth under 75% and 88% shade exacerbated energy depletion, as evidenced by the 45% and 67% lower rhizome mass and the 37% and 65% lower total nonstructural carbohydrate content (TNC), respectively, compared with turf under 40% shade. Trinexapac-ethyl reduced excessive vertical shoot growth and increased rhizome mass and TNC. Mean turf quality was increased by 0.7 and 1.4 units for turf receiving multiple TE applications under 75% and 88% shade, respectively. Trinexapac-ethyl did not increase turf quality or TNC under 40% shade. Canopy photosynthetic rate (Pn) was not affected 4 weeks after the initial TE treatment under any shade level. However, 34 weeks after the initial TE treatment a 50% higher Pn was observed for turf treated with TE under 88% shade, possibly because of higher tiller density. Repeated TE application increased turf quality and provided more favorable physiological responses (such as TNC and Pn) under 75% and 88% shade, where conditions favored vertical shoot growth. However, little or no improvement in turf quality was observed under 40% shade, where conditions favored slow vertical shoot growth. Chemical name used: 4-(cyclopropyl-α-hydroxy-methylene)-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester (trinexapac-ethyl).**

Abiotic factors that affect turfgrass performance are water, temperature, and light. An estimated 20% to 25% of turfgrass is grown under low light conditions (Dudeck and Peacock, 1992). Reduced light intensity limits photosynthate availability and alters carbohydrate partitioning. Such physiological changes are closely associated with morphological changes in response to shade (Peacock and Dudeck, 1981). Grasses grown under shade generally exhibit increased shoot-to-root ratios, decreased specific leaf weight, thinner leaf blades, lower seedhead formation, and faster stem elongation (Dudeck and Peacock, 1992; Kephart et al., 1992; Wilkinson and Beard, 1974). In a preliminary study, vertical shoot growth of 'Diamond' zoysiagrass, a relatively slow-growing grass under full sun, increased 220% and 360% as light intensity decreased from natural sunlight to 47% and 19% of full sunlight, respectively (Qian and Engelke, unpublished data). 'Diamond' seedhead formation was also markedly reduced under low light conditions. Gibberellins (GAs) promote stem elongation in many plants, and are partially involved in the control system of seedhead formation (Salisbury and Ross, 1985). Shading may stimulate the biosynthesis of GAs or increase plant sensitivity to GAs, as excessive shoot growth occurs under low light conditions. Enhanced vertical shoot growth is a shade avoidance mechanism of plants, but it is ineffective and undesirable in turfgrass because mowing requirements are increased. Rapid vertical shoot growth necessitates more clippings and exacerbates carbohydrate depletion in turfgrasses growing under low light conditions.

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[1]Assistant Professor.
[2]Professor.
[3]Research Assistant.

Trinexapac-ethyl (TE) is a plant growth regulator (PGR) used by turf managers to reduce mowing requirements. In a previous study, we examined the effects of TE with different application rates and intervals on 'Diamond' zoysiagrass grown under 87% shade (Qian and Engelke, 1998). Results indicated that repeated monthly applications of TE at a reduced manufacturer's recommended rate, or repeated bimonthly applications at the manufacturer's recommended rate, improved performance. We suggested that TE enhanced the shade tolerance of 'Diamond' by offsetting the excessive vertical shoot growth stimulated by heavy shade. However, no information is available concerning the effects of TE on the magnitude of shoot growth suppression or the degree of improvement in turf performance of 'Diamond' grown under various light intensities. Understanding the morphological and physiological changes induced by PGRs under different levels of shade is essential for proper use of TE in variable environments.

This study was undertaken to evaluate the influence of TE on 'Diamond' zoysiagrass grown under a range of shade conditions by 1) determining turf performance and shoot growth; 2) quantifying rhizome mass and total nonstructural carbohydrate content in rhizomes; and 3) measuring photosynthesis and respiration rates.

Materials and Methods

Two experiments were conducted in a double-layer polyethylene greenhouse at the Texas A&M Univ., Dallas Research Center. The maximum/minimum air temperature in the polyhouse ranged from 40 to 27 °C during the summer to 25 to 13 °C during the winter.

Experiment 1. Washed 'Diamond' zoysiagrass was sodded in May 1993 on a 85 sand : 15 peat (by volume) soil mix retained in 12 wooden boxes (1.2×1.2×0.16 m). Holes were drilled at the bottom for drainage; irrigation was applied daily or every other day to prevent drought stress. Turf was mowed weekly at a 1.4-cm height using a reel mower and the clippings were removed. Between 1993 and 1996, 146 kg·ha^{-1} of N from a 20N–4.4P–16.6K fertilizer was applied each year. During the study period in 1997, N was applied monthly at 25 kg·ha^{-1} using a 20N–4.4P–16.6K water-soluble fertilizer. Aeration, followed by topdressing, was performed twice in 1996.

This experiment was a two-factor, split-plot design with four replications. The main plot treatments, which were randomly applied to the 12 boxes, were three levels of shade, consisting of black nylon shade cloth with 47% or 73% light-screening properties, and a fully exposed control. Shade cloth was mounted on PVC frames and supported 10 cm above the turf surface. Light intensity was quantified on several cloud-free days by measuring photosynthetically active radiation (*PAR*) beneath the shade structures with a radiometer (model LI-170; LI-COR, Lincoln, Nebr.), and expressed relative to incident *PAR* outside the polyhouse. Since the plastic roof provided ≈60% sunlight transmission and no artificial light was applied throughout the study, the three light levels were 60% (control), 25%, and 12% of outside incident light (i.e., 40%, 75%, and 88% shade). Shade treatments were imposed on 14 Jan. 1997, and maintained during the study period until 4 Dec. 1997, when the experiment was terminated.

The subplot treatment was trinexapac-ethyl (TE) application. Each box was split along a central line into two subplots, to which the TE treatment (repeated TE application or non-treated control) was randomly assigned. The chemical was first applied on 14 Jan. 1997, and additional applications were made on 26 Mar., 30 Apr., 5 June, 17 July, 17 Aug., 17 Sept., and 25 Nov. 1997. Treatments were reapplied when previously TE-treated 'Diamond' under 88% or 75% shade exhibited significantly higher weekly vertical shoot growth than that of the control. Trinexapac-ethyl was applied at half the strength of the manufacturer's recommended rate (48 $g \cdot ha^{-1}$, a.i.) with a CO_2-pressurized sprayer that delivered 518 $L \cdot ha^{-1}$ at a pressure of 1.5 $kg \cdot cm^{-2}$.

Experiment 2. Sod of mature 'Diamond' zoysiagrass grown on a Houston black clay soil (fine, montmorillonitic thermic Udic Pellustert) was harvested on 22 Nov. 1996. Sod was transplanted to 18 fiber containers measuring 55 × 38 × 12 cm. Containers were filled with a 1 vermiculite : 1 peat : 1 soil mixture amended with 4.72 $kg \cdot m^{-3}$ 17N–2.6P–8.3K resin-coated fertilizer (Osmocote). A 20N–4.4P–16.6K soluble fertilizer was applied monthly to provide 25 $kg \cdot ha^{-1}$ of N.

The experiment was a split-plot design with six replications. As in Expt. 1, the main plot factor was shade, while the subplot factor was TE treatment. The components of treatments, procedures of application, and environmental conditions were identical to those in Expt. 1. The shade treatments were imposed on 30 Jan. 1997, and the initial TE treatment was applied on 1 Feb. 1997. Subsequent TE applications were made on 30 Apr., 12 June, 17 July, and 29 Aug. 1997. This experiment was terminated on 15 Sept. 1997. Throughout the study, turf was clipped weekly to 2.2 cm with clippings removed.

Data collection. Turf canopy height was measured prior to each mowing or clipping event. Turf quality was visually assessed on a 1 (worst) to 9 (best) scale; ratings of <6 were considered commercially unacceptable. The estimation of turf quality was based on primary components of color, density, and uniformity. Tiller density was determined using a core sampler (3.2-cm diameter × 10 cm deep) to remove two cores per plot at the end of the experiments and counting tillers. Seedhead production during the flowering season in October was estimated by randomly selecting a 62.5 cm^2 area in each plot using a plastic frame and counting seedhead number within the frame.

Rhizome mass was measured 30 weeks after exposure to low light treatments for both experiments by taking core samples (128 cm^2) to a 12-cm depth. Samples were washed and foreign matter, shoots, and roots were hand-separated from rhizomes. Rhizomes were placed in an oven at 100 °C for 1 h, and then at 70 °C for 24 h. After weighing, samples were ground in a Wiley mill to pass through a 1-mm screen. About 30 mg of the ground sample was placed in a test tube with 10 mL deionized water. The test tubes were autoclaved for 1 h to extract the hot water–soluble carbohydrates and gelatinize starch. After cooling, acetate buffer (pH 5.0) and 50 units of amyloglucosidase (A7420; Sigma Chemical Co., St. Louis) were added, and incubated for 1 h at 55 °C. The reaction was terminated by placing samples in a boiling water bath for 10 min. After centrifugation, 250 μL of the supernatant was used for TNC analysis. The analysis of TNC was performed using the procedures of Nelson (1944).

Canopy photosynthesis and respiration measurements were determined only in Expt. 1, using a portable infrared gas analyzer system (Model LCA-3; ADC Ltd., Hoddesdon, Herts, U.K.) equipped with a plexiglass canopy chamber. The bottom of the chamber (10 cm inside diameter, 8 cm height) was open so that it could be positioned over a 78-cm^2 area of turf and pressed 2 mm down into the soil. Tygon tubes and pumps allowed the entrance of reference air and exit of sample air. The chamber allowed measurement of apparent canopy photosynthesis (Pn) with minimum disturbance of the turf surface. Measurements were made 4 and 34 weeks after the initiation of treatments. Measurements of incident *PAR* were made within 10 s of the Pn measurements by placing a light quantum sensor (model LI-170; LI-COR) next to the canopy chamber. Dark respiration (Rn) of the canopy was measured immediately after the Pn measurement by completely covering the chamber with five layers of black cloth. The chamber was set and 2 min was allowed for each Pn and Rn reading. No attempt was made to exclude soil respiration in photosynthesis and respiration measurements.

Data analysis. Because of differences in the duration of study, mowing height, soil conditions, and treatment times, data from the two experiments were analyzed separately. Data on shoot growth (indicated by canopy height), turf quality, tiller number, rhizome mass, TNC, seedhead number, Pn, and Rn were analyzed using the GLM procedure, and treatment means were separated using Fisher's protected LSD (SAS Institute, 1989). Linear regression analysis was performed to determine the relationship between the measured parameters and level of shade, where shade level was the independent variable and each measured parameter was the dependent variable.

Results and Discussion

Shoot growth. Turf canopy height varied greatly with measurement date. However, graphs generated by SAS indicated that the rank among treatments for turf canopy height over time was generally the same. Therefore, data over time were pooled and means were calculated (Table 1). Turf canopy height increased linearly with shade level ($r = 0.88$). Trinexapac-ethyl application reduced the canopy height before mowing at all levels of shade. In Expt. 1, canopy heights of turf treated with TE were 0.3, 0.8, and 1.0 cm lower than those of the respective controls under 40%, 75%, and 88% shading. In Expt. 2, canopy heights of turf treated with TE were 0.6, 1.1, and 1.2 cm lower than those of the respective controls under 40%, 75%, and 88% shading. Hence, the effectiveness of TE in reducing vertical shoot growth generally increased with increasing shade level.

The duration of shoot growth suppression

Table 1. Effects of trinexapac-ethyl (TE) on mean turf canopy height, tiller density, rhizome mass, and total nonstructural carbohydrate content (TNC) of 'Diamond' zoysiagrass under three light intensities in two experiments.

	Expt. 1				Expt. 2			
	% shade			Shade	% shade			Shade
Treatment	40	75	88	effect	40	75	88	effect
	Mean turf canopy height (cm)[z]							
Control	1.5 a[y]	2.1 a	2.4 a	****	2.9 a	3.6 a	3.9 a	****
TE	1.2 b	1.3 b	1.4 b	**	2.3 b	2.5 b	2.7 b	**
	Tiller density (no./cm²)[x]							
Control	12.6[NS]	8.8[NS]	4.4 b	***	15.0[NS]	10.2[NS]	6.7 b	****
TE	11.7	9.2	6.6 a	*	15.5	10.0	9.5 a	**
	Rhizome dry mass (kg·m⁻²)[x]							
Control	0.71[NS]	0.39 b	0.26 b	****	0.77[NS]	0.41[NS]	0.20 b	****
TE	0.67	0.55 a	0.31 a	***	0.94	0.51	0.30 a	****
	TNC (mg·g⁻¹)[x]							
Control	13.4[NS]	6.8 b	3.2 b	****	20.1[NS]	15.0 b	9.4 b	***
TE	12.7	8.5 a	6.8 a	****	22.5	17.2 a	13.5 a	****
	Seedhead density(no./dm²)[w]							
Control	19 b	0	0	---	54 b	4[NS]	0	*
TE	29 a	0	0	---	106 a	12	0	***

[z]Mean of each measurement before each mowing event throughout the study period.
[y]Mean separation within parameters and columns by Fisher's LSD test, $P \le 0.05$.
[x]Determined 30 weeks after exposure to shade and TE treatments.
[w]Determined in Oct. 1997.
[NS, *, **, ***, ****]Nonsignificant or significant linear relationship between measured parameter and level of shade at $P = 0.05$, 0.01, 0.001, or 0.0001 level, respectively.

by TE was shorter when temperatures were higher. During early spring (January to March) and fall (September to November), TE suppressed vertical shoot growth for >70 d before a sequential TE application was needed. During April to August, when temperatures were higher, TE suppressed shoot growth for <45 d before an additional TE application was needed. Cooler temperatures plus shorter days after October undoubtedly postponed the need for application of TE, even though turf was maintained in the polyhouse. Diesburg and Christians (1989) also stated that the efficacy of plant growth regulators is dependent on environmental conditions.

Turf quality. A significant treatment × date interaction was observed for turf quality (Fig. 1). In Expt. 1, turf under 40% shade consistently produced quality ratings >7.5 (Fig.1, A). Turf under 88% and 75% shade, with or without TE treatment, declined in quality over time. However, as indicated from Fig. 1, quality declined more slowly in TE-treated turf. Under 88% shade, the time to a commercially unacceptable level was 281 d for TE-treated vs. 147 d for the nontreated 'Diamond'. Under 75% shade, the quality of turf in nontreated plots became unacceptable in 290 d, whereas that of TE-treated turf remained acceptable throughout the study period. The improvement of turf quality under 75% and 88% shade by repeated TE applications was due mainly to a darker green color (data not shown) and increased tiller density (Table 1). We observed a slight recovery in turf quality at 75% and 88% shade levels in late October to November, possibly because cooler temperatures and shorter days slowed shoot growth.

Except for 88% shade without TE, 'Diamond' in all treatments maintained acceptable quality in Expt. 2. However, turf quality decreased with increasing shade (Fig. 1B). Repeated TE application increased turf quality of 'Diamond' by 0.6 and 1.3 units under 75% and 88% shade, respectively.

These results indicated that TE treatment improved turf quality under 75% and 88% shade levels, where conditions favored higher vertical growth rates, but not under 40% shade, where conditions favored slow vertical growth. Stier and Rogers (1996) reported that TE enhanced the turf quality of tall fescue under 75% to 95% shade.

Rhizome mass and TNC. Rhizome production per unit of turf area declined linearly with shading in both experiments (Table 1). Without TE application, 75% and 88% shade reduced rhizome mass by 45% and 63%, respectively, in Expt. 1 and 48% and 74%, respectively, in Expt. 2, in comparison with turf under 40% shade. Treatment with TE increased rhizome mass, the effect becoming significant as light level decreased.

In both experiments, the content of TNC in rhizomes decreased as shading increased. The higher TNC in Expt. 2 was possibly due to the higher cutting height compared with Expt. 1.

Trinexapac-ethyl treatment significantly improved TNC status of 'Diamond' grown under high shade levels (Table 1). Repeated TE application increased TNC by 113% and 25% under 88% and 75% shade, respectively, in Expt. 1, and 44% and 15%, respectively, in Expt. 2. Under 40% shade, the effect of TE was nonsignificant.

The rhizome is the major organ for carbohydrate storage in rhizomatous grasses (White, 1973). The higher rhizome mass and higher TNC of rhizome tissue in TE-treated turf may give 'Diamond' advantages for better survival and recovery under heavy shade.

Seedhead density. The formation of seedheads in October was markedly reduced by shade treatments (Table 1). In fact, no seedheads were observed under 88% shade. Treatment with TE induced seedhead production; however, the stimulation of flowering by TE was most pronounced at 40% shade. The reason for this difference in seedhead formation is not fully understood, and deserves further study. Further research could be beneficial in industry seed production for seeded zoysiagrasses. Hampton and Hebblethwaite (1985) also observed increased flower and seed yield for perennial ryegrass treated with the plant growth regulator paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-1,2,4,triazole-1-ethanol]. They suggested that the increases were associated with increased production of fertile tillers and seeds per spikelet. Increased seedhead density with TE in our study may have resulted from reduced competition between reproductive and vegetative sinks.

Photosynthesis and respiration. Four weeks after the initial treatment, canopy Pn and Rn decreased with decreasing light level (Table 2). There was no significant effect of TE treatment regardless of light level.

Thirty-four weeks after the initial treatment, canopy Pn decreased as shade level increased from 40% to 88%. Photosynthetic rates under 40% and 75% shade were not significantly influenced by TE treatment; however, under 88% shade, treatment with TE increased Pn by 50%. The higher Pn under low light intensity may partially account for the higher rhizome mass and higher TNC in TE-treated plants.

Canopy dark respiration of nontreated 'Diamond' decreased with increasing shade level, possibly because of the lower soil and air temperatures. Thermocouples installed under turf surfaces indicated that the mean temperatures were 26.7, 24.4, and 22.2 °C under 40%, 75%, and 88% shade, respectively. Ludlow et al. (1974) reported that the reduced root mass in shaded *Panicum maximum* Jacq. contributed to reduced respiration levels. Respiration

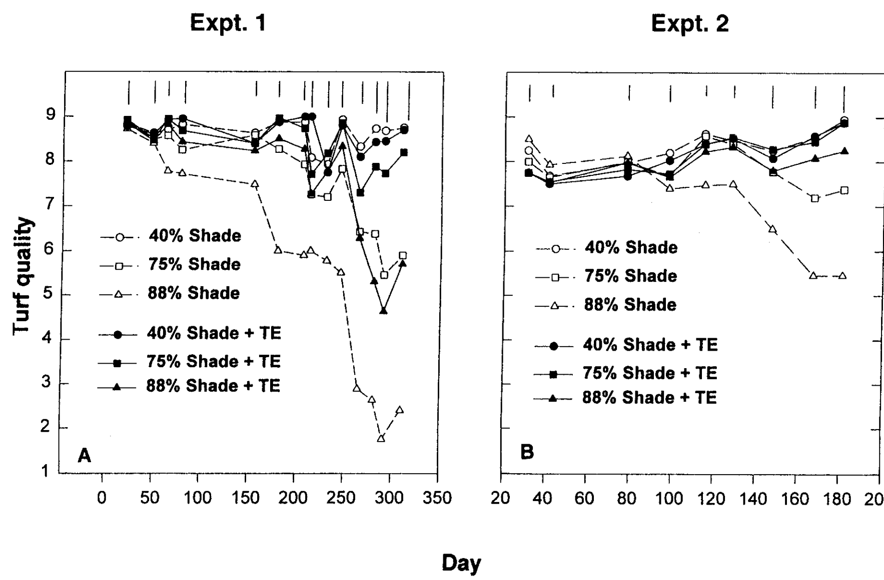

Fig. 1. Influence of trinexapac-ethyl (TE) treatment on visual turf quality of 'Diamond' zoysiagrass under 40%, 75%, and 88% shade in Expt. 1 (**A**) and Expt. 2 (**B**). Vertical lines are LSDS (0.05) for comparison of treatment means. Day 0 = day when shading began.

Table 2. Effects of trinexapac-ethyl (TE) on canopy photosynthetic rate (Pn) and dark respiration rate (Rn) of 'Diamond' zoysiagrass under three light intensities in Expt. 1.

	Week 4				Week 34			
	% shade			Shade	% shade			Shade
Treatment	40	75	88	effect	40	75	88	effect
			Pn ($\mu mol \cdot m^{-2} \cdot sec^{-1}$)					
Control	8.6^{NS}	4.8^{NS}	2.0^{NS}	***	6.4^{NS}	4.0^{NS}	1.6 b^z	***
TE	8.5	4.6	2.3	***	6.9	5.0	2.4 a	***
			Rn ($\mu mol \cdot m^{-2} \cdot sec^{-1}$)					
Control	6.2^{NS}	5.4^{NS}	5.4^{NS}	*	5.4^{NS}	4.3^{NS}	2.7 b	***
TE	6.1	5.1	4.7	NS	5.8	4.5	4.6 a	NS

^{z}Mean separation within parameters and columns by Fisher's LSD test, $P \leq 0.05$.

NS,*,**,***,****Nonsignificant or significant linear relationship between measured parameter and level of shade at P = 0.05, 0.01, 0.001, or 0.0001 level, respectively.

rates of turf grown under 40% and 75% shade were not influenced by TE treatment. However, TE-treated turf had a 70% higher respiration rate when grown under 88% shade. This result is probably related to the fact that the TE-treated plot had more living tissue, a higher tiller density, and higher metabolic activity and viability.

With increasing shade, 'Diamond' zoysiagrass displayed a linear increase in vertical shoot growth and a decrease in photosynthesis. Excessive vertical shoot growth and reduced photosynthesis under shade resulted in the eventual depletion of energy reserves. We demonstrate with this work that repeated TE application offsets the excessive shoot growth induced by shade, and results in growth conditions less conducive to the depletion of carbohydrates. Repeated TE application also provides more favorable photosynthesis and respiration responses. Turf quality of 'Diamond' zoysiagrass grown under 88% and 75% shade was significantly increased by repeated TE treatment 3 to 8 months after the initial treatment. The increases were proportionately higher as the level of shading increased.

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