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Drought Responses of Perennial Ryegrass Treated with Plant Growth Regulators

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Abstract. **Plant growth regulators (PGRs) are used on golf course fairways, and may have positive or negative effects on turfgrass response to drought. Greenhouse and field studies in 1995 and 1996 evaluated perennial ryegrass (*Lolium perenne* L.) response to drought stress after PGR application. Turf was maintained under golf course fairway conditions, and received adequate water until PGR application; thereafter, irrigation was withheld. Trinexapac-ethyl was the only PGR evaluated that enhanced turfgrass quality during dry down in the greenhouse, had no deleterious effects on rooting, and suppressed canopy height for up to 2 weeks in the field. Ethephon enhanced quality during drought in the greenhouse, reduced root length density (RLD) in the field from 0- to 10-cm and 10- to 20-cm depths, but had no effect on canopy height. Mefluidide caused unacceptable chlorosis, reduced rooting at 0 to 40 cm, and suppressed canopy height. Paclobutrazol had negligible effects on ryegrass quality, rooting, and canopy height. Chemical names used: (2-chloroethyl) phosphonic acid (ethephon); *N*-{2,4-dimethyl-5-[[(trifluoromethyl)-sulfonyl] amino]phenyl} acetamide (mefluidide); (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-1,2,4,-triazol-l-yl) penta-n-3-ol (paclobutrazol); 4-(cyclopropyl--hydroxy-methylene)-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester (trinexapac-ethyl).**

Lack of water available for irrigation is one of the most important problems facing the turfgrass industry. Development of new management strategies to improve turfgrass drought resistance may include the use of plant growth regulators (PGRs), which have been used to suppress shoot growth and seed head formation and to reduce mowing expenses.

Plant growth regulators that contribute to the development of short, compact turf have reduced evapotranspiration (ET) by up to 29% in greenhouse studies with warm- and cool-season turfgrasses (Green et al., 1990; Marcum and Jiang, 1997). Flurprimidol {α-(1-methylethyl)-α-[4-(trifluoro-methoxy) phenyl] 5-pyrimidine-methanol} and mefluidide reduced ET of Kentucky bluegrass (*Poa pratensis* L.) for 35 to 42 d in the field, but turf then exhibited a growth flush and a higher ET rate than untreated plants (Doyle and Shearman, 1985).

Mefluidide applied in late spring improved annual bluegrass (*Poa annua* L.) drought resistance by suppressing shoot growth and enhancing rooting (Cooper et al., 1987). Root length, root mass, and root : shoot mass ratio of a red fescue (*Festuca rubra* L.)–Kentucky bluegrass turf were not affected by three consecutive annual applications of amidochlor {(*N*-[(acetylamino)methyl]-2-chloro-*N*-(2,6-diethyl phenyl)acetamide}, mefluidide, or ethephon (Bhowmik, 1987).

Perennial ryegrass is used widely on golf course fairways and tees throughout cool and transition zone regions of the United States. Identification of one or more PGRs with potential to enhance perennial ryegrass drought resistance would be valuable to golf course superintendents. The objective of this study was to evaluate the effects of four PGRs on drought response of perennial ryegrass maintained under golf course fairway conditions.

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Materials and Methods

Field studies in 1995 and 1996, and a greenhouse study were conducted to evaluate ryegrass response to ethephon, mefluidide, paclobutrazol, and trinexapac-ethyl. Applications were at the manufacturers' recommended rates (Table 1) with a backpack CO_2-pressurized (242 kPa) sprayer equipped with 8004 flat-fan nozzles and calibrated to deliver 560 L·ha^{-1}. Application rates were doubled inadvertently on the first application in the field in 1995 (Table 2).

Greenhouse study. Twenty polyvinyl chloride (PVC) columns measuring 92 cm deep × 30 cm in diameter were sealed at one end with a fiberglass cap perforated with five 2.5-cm-diameter holes. Each was filled with a field loam soil (30.5% sand, 48% silt, 21.5% clay) after a 3-cm-deep pea gravel layer had been placed at the bottom of each column to prevent soil loss through holes. Then, columns were watered frequently to allow settling prior to planting.

Sods of a perennial ryegrass blend, composed of 39% 'Derby Supreme', 39% 'Regal', and 20% 'Gator', were planted in each column 2 months before PGR application. Turf received adequate water and was fertigated once weekly with a liquid fertilizer containing N at 250 mg·L^{-1}, P at 55 mg·L^{-1}, and K at 207 mg·L^{-1} plus micronutrients (B at 0.25 mg·L^{-1}, Fe at 0.12 mg·L^{-1}, Mg at 1.87 mg·L^{-1}, Mn at 0.75 mg·L^{-1}, Mo at 0.12 mg·L^{-1}, and Zn at 0.20 mg·L^{-1}) until PGR application. Grasses were clipped three times weekly at 2 cm. Greenhouse air temperature ranged from 16 to 26 °C, and high-pressure sodium vapor lamps provided 400 μmol·m^{-2}·s^{-1} supplemental lighting for 12 h daily.

Data were collected on turf quality, leaf relative water content (RWC), leaf water potential (Ψ_l), osmotic potential (Ψ_s), soil water content, and grass canopy height.

Visual turf quality was rated once weekly using a 0 to 9 scale, where 0 = brown, thin, nonuniform turf; 6 = minimum acceptable quality; and 9 = optimum color, density, and uniformity. Leaf relative water content, Ψ_l, Ψ_s, and osmotic adjustment were determined once weekly between 1200 and 1300 HR. Relative water content was calculated as:

$$RWC\ (\%) = \{(FM - DM)/(TM - DM)\} \times 100$$

where FM is leaf fresh mass; DM is leaf dry mass; and TM is leaf mass at full turgor. To obtain TM, fresh leaves were floated in water for 5 h at room temperature immediately after FM was measured.

Leaf water potential was measured using precalibrated thermocouple psychrometers (model 75-1 AC; J.R.D. Merrill Specialty Equipment Corp., Logan, Utah) and a dew point microvoltmeter (model HR-33T; Wescor, Logan, Utah). Leaf blades were excised and sealed immediately inside a small, watertight, stainless-steel psychrometer chamber. The leaves were equilibrated at 25 °C for 5 h before water potential was measured in the dew point mode with a cooling time of 15 s.

To determine Ψ_s, the thermocouple psychrometer chamber containing leaf tissue was frozen at –15 °C for 12 h. Chambers then were removed from the freezer, and the tissues thawed, and Ψ_s was measured as described for Ψ_l measurement. Leaf pressure potential (Ψ_p) was calculated as: $\Psi_p = \Psi_l - \Psi_s$.

To determine osmotic adjustment, leaf tissue was floated in water for 5 h at room temperature, patted dry, placed in a 1.5-mL micro test tube, and frozen. After thawing, leaf sap at 100% RWC then was obtained using a laboratory press (Fred S. Carver, Wabash, Ind.). Osmolality of sap was determined with a 5500 vapor pressure osmometer (Wescor).

Osmotic adjustment was calculated as:

$$\text{Osmotic adjustment} = \Psi_s(U) - \Psi_s(T)$$

Table 1. Effects of plant growth regulators (PGRs) on perennial ryegrass turf quality[z] in the greenhouse in 1996.

PGR	Application rate (g·ha^{-1} a.i.)	Weeks after treatment[y]								
		2	3	4	5	6	7	8	9	11
Trinexapac-ethyl	192	6.9 b[x]	7.4 a	7.4 ab	7.6 ab	7.5 a	7.5 a	7.1 a	6.6 a	4.6 a
Ethephon	3363	8.1 a	7.9 a	8.1 a	8.3 a	7.8 a	7.6 a	7.1 a	6.3 ab	4.4 ab
Mefluidide	134	3.6 c	3.9 b	4.3 b	3.5 c	3.1 c	3.0 c	3.3 b	3.3 c	2.5 c
Paclobutrazol	553	7.0 b	7.0 a	6.6 b	6.0 b	5.8 b	5.5 b	5.3 ab	4.3 c	2.8 bc
Control	---	7.0 b	7.0 a	6.8 ab	6.1 b	5.8 b	5.4 b	4.6 b	4.4 bc	2.6 c

[z]Turf quality was rated on a 0 to 9 scale, where 0 = brown, thin, nonuniform turf; 7 = minimum acceptable quality; and 9 = optimum color, density, and uniformity.
[y]Irrigation ceased 2 weeks after PGR application.
[x]Mean separation within columns by Fisher's protected LSD, $P < 0.05$.

where Ψ_s(U) is the osmotic potential of untreated leaves with 100% RWC, and Ψ_s(T) is the osmotic potential of treated leaves with 100% RWC.

Volumetric soil water content was measured at 10-, 30-, 50-, and 70-cm depths once weekly. Soil moisture probes (30-cm long) were installed horizontally at each depth through holes that had been drilled through the wall of the container. Clear silicone glue was used to seal the holes and anchor the probes. Volumetric soil water content was measured using a time-domain reflectometer (TDR) (Soil Moisture Equipment Corp., Santa Barbara, Calif.).

Turf canopy height was measured once weekly on Monday, 3 d following the previous mowing. To measure height, a ruler was set on end on the soil surface, and a thin 10-cm-diameter cardboard disc with a hole in its center was dropped freely over the ruler onto the turf canopy in three random locations per container. Canopy height was calculated as the distance between the soil surface and the disk.

Field studies. A 'Gator' perennial ryegrass turf was maintained under golf course fairway conditions at the Rocky Ford Turfgrass Research Center at Manhattan, Kans. Plots measured 2 × 3 m and were arranged in a randomized complete-block design with four replications. Soil was a Chase silt loam (fine, montmorillonitic, mesic Aquic Arquidolls), pH 6.8. Mowing was done at 2 cm three days weekly with a reel mower, and clippings were returned. Turf received N at 150 kg·ha^{-1}/year in three equal applications in May, September, and November. Turf was well watered until the first PGR application on 2 June each year. Irrigation to wet the soil to 40 cm was applied 6 weeks after the first PGR application each year, and just prior to the second PGR application on 18 July. No supplemental irrigation was applied after the second PGR application. Weekly rainfall totals following the 2 June 1995 application were: 42, 0, 30, 5, 30, and 55 mm for weeks 1–6 after treatment, respectively. Weekly rainfall amounts following the 18 July 1995 application were 71, 5, 4, 0, 68, and 0 mm for the same periods.

The same totals following the 2 June 1996 PGR application were 25, 12, 2, 2, 0, and 61 mm, and 28, 21, 41, 8, 36, and 0 mm after the 18 July 1996 application.

Data were collected on turf quality, rooting, leaf RWC, Ψ_l, Ψ_s, soil water content, canopy minus air temperature, canopy height, and mass of clippings. Turf quality, leaf RWC, Ψ_l, Ψ_s, and canopy height were determined as described for the greenhouse study.

To determine PGR effects on rooting, four 40-cm-long × 2-cm-diameter cores were removed from each field plot on 14 Sept. 1995, 8 weeks after the second PGR application. In 1996, cores were sampled on 16 July and on 31 Aug., 6 weeks after the first and second PGR applications, respectively. Cores were divided into 10-cm-long sections, which were sealed in ziplock freezer bags and frozen at –15 °C for later analysis. After thawing, roots were washed free of soil and stained with methyl violet dye (Harris and Campbell, 1989). Root length density (RLD) in each section was determined with a digital image analyzer (Monochrome AgVision System, Decagon Devices, Pullman, Wash.) as described by Harris and Campbell (1989). Total root length was defined as the sum of the root length of all four 10-cm-long sections. Roots then were dried in an oven at 70 °C for 48 h and weighed.

Volumetric soil water content at 0- to 15-cm depths in each plot was measured once weekly using an IRAMS time domain reflectometer (Soil Moisture Equipment, Logan, Utah). Canopy minus air temperature was measured weekly on sunny days at 1300 HR using an infrared thermometer (Everest Interscience, Tustin, Calif.). The thermometer was held ≈1 m aboveground at a 45° angle, and four measurements were taken in each plot.

Data for all measured variables were subjected to analysis of variance, and means were separated using Fisher's protected least significant difference (LSD).

Table 2. Effects of plant growth regulators (PGRs) on perennial ryegrass turf quality[z] in the field in 1995 and 1996.

PGR	Application rate (g·ha^{-1} a.i.)	Weeks after treatment[y]					
		1	2	3	4	5	6
				Applied 2 June 1995			
Trinexapac-ethyl	384	7.3 b[x]	7.0 c	7.0 c	7.4 a	8.8 a	7.0 a
Ethephon	6726	8.8 a	8.8 a	9.0 a	7.6 a	8.3 a	6.5 a
Mefluidide	268	7.0 b	5.3 d	6.0 d	4.8 b	6.8 b	5.3 b
Paclobutrazol	1106	8.8 a	8.0 b	8.3 b	7.5 a	9.0 a	7.0 a
Control	---	9.0 a	8.0 b	8.3 b	7.5 a	8.8 a	6.8 a
				Applied 18 July 1995			
Trinexapac-ethyl	192	7.8 b	8.5 a	8.8 a	8.7 a	8.4 a	5.6 a
Ethephon	3363	9.0 a	8.3 a	7.8 b	8.0 b	6.9 c	4.5 c
Mefluidide	134	6.0 c	4.0 b	4.0 c	4.8 c	5.0 d	2.9 d
Paclobutrazol	553	8.5 a	8.3 a	8.3 ab	8.7 a	8.1 ab	5.3 a
Control	---	8.8 a	8.5 a	8.8 a	8.4 a	8.0 b	4.9 bc
				Applied 2 June 1996			
Trinexapac-ethyl	192	9.0 ax	9.0 a	8.6 a	7.4 b	7.4 a	8.1 a
Ethephon	3363	9.0 a	9.0 a	8.6 a	7.7 a	7.4 a	7.6 bc
Mefluidide	134	8.5 b	8.0 b	7.0 b	5.6 c	5.6 b	6.0 d
Paclobutrazol	553	9.0 a	9.0 a	8.5 a	7.4 b	7.3 a	7.5 c
Control	---	9.0 a	9.0 a	8.6 a	7.4 b	7.2 a	7.9 ab
				Applied 18 July 1996			
Trinexapac-ethyl	192	7.5 b	7.0 b	---	6.9 a	6.6 a	7.1 a
Ethephon	3363	7.9 a	7.5 a	---	7.2 a	6.8 a	7.3 a
Mefluidide	134	5.8 c	4.9 c	---	5.9 b	5.8 b	5.9 b
Paclobutrazol	553	7.5 b	7.3 ab	---	7.3 a	6.9 a	7.3 a
Control	---	7.8 ab	7.0 b	---	7.0 a	6.7 a	7.1 a

[z]Turf quality was rated on a 0 to 9 scale, where 0 = brown , thin, nonuniform turf; 7 = minimum acceptable quality; and 9 = optimum color, density, and uniformity.
[y]No irrigation was applied after PGR application in the field.
[x]Mean separation within columns and years by Fisher's protected LSD, $P < 0.05$.

Results

Turf quality. Ethephon and trinexapac-ethyl enhanced perennial ryegrass quality during desiccation in the greenhouse (Table 1). Trinexapac-ethyl enhanced turf quality from 6 to 11 weeks after treatment (WAT). Quality of ethephon-treated turf was superior to that of untreated turf on all dates except 3 and 9 WAT. Mefluidide reduced turf quality from 2 to 7

WAT. Paclobutrazol did not affect turf quality.

Ethephon enhanced turf quality in the field between 2 and 3 WAT on 2 June 1995 (Table 2). Despite the PGRs being applied at twice the recommended rate, only mefluidide-treated turf exhibited unacceptable quality 2 to 6 WAT.

The most severe drought period during field studies occurred following the 18 July PGR application in 1995. A significant decline in turf quality in all treatments was observed between 5 and 6 WAT (Table 2). Trinexapac-ethyl enhanced turf quality at 5 and 6 WAT, and paclobutrazol enhanced turf quality at 6 WAT.

Only moderate drought stress was observed in 1996, and quality of turf in untreated plots was acceptable (rating >7) on each rating date except at 5 WAT on 2 June (Table 2).

Rooting. Only trinexapac-ethyl did not reduce RLD in the field at one or more sampling depths in 1995 or 1996 (Table 3). In 1995, mefluidide reduced RLD by 37% at a 20- to 30-cm depth and by 61% at a 30- to 40-cm depth.

Following the 2 June 1996 application, ethephon reduced ryegrass RLD by 28% and 25% at 0- to 10- and 10- to 20-cm depths, respectively; mefluidide reduced RLD by an average of 45% across all depths; and paclobutrazol reduced RLD by 25% at 0 to 10 cm, but increased it by 38% at 30 to 40 cm (Table 3).

Ethephon reduced ryegrass RLD by 32% at 10 to 20 cm following the 18 July 1996 application (Table 3). Mefluidide reduced RLD by an average of 50% across all depths.

Canopy minus air temperature. Mefluidide-treated ryegrass generally exhibited a canopy minus air temperature up to 4 °C higher than that of untreated turf (data not shown). Other PGRs had little effect on canopy minus air temperature.

Leaf water status and soil water content. Plant growth regulators had no effect on leaf RWC, Ψ_l or Ψ_s, or soil water content during desiccation in the greenhouse or in the field (data not shown).

Turf canopy height. As soil dried in the greenhouse, canopy height decreased, indicating that growth was inhibited (data not shown). Trinexapac-ethyl reduced canopy height by up to 24% through 4 WAT, ethephon and paclobutrazol had no effect, and mefluidide reduced canopy height by up to 26% through 2 WAT. Plant growth regulators had no effect on canopy height after 4 WAT.

Trinexapac-ethyl and mefluidide reduced canopy height by up to 27% in the field at 1 and 2 WAT on 2 June 1995 (data not shown). Paclobutrazol reduced canopy height for 1 WAT, whereas ethephon did not. Following PGR application on 18 July 1995, mefluidide suppressed canopy height by up to 28% through 5 WAT. No other PGRs reduced canopy height.

No PGR provided >2 weeks of canopy height suppression following either field application in 1996 (data not shown). All PGRs except ethephon provided some degree of canopy height suppression at 1 or 2 WAT.

Discussion

Trinexapac-ethyl was the only PGR evaluated that enhanced perennial ryegrass quality during drought in the greenhouse, had no deleterious effect on RLD or TRL, and suppressed canopy height following one or more applications.

Trinexapac-ethyl interferes with gibberellin biosynthesis, reducing cell elongation and subsequent plant leaf expansion (Kaufmann, 1986). It is absorbed primarily by the crown and leaf blade (Fagerness and Penner, 1996). Enhanced quality during drought was observed 6 to 11 weeks after trinexapac-ethyl application in the greenhouse, but on only two of 23 rating dates in the field. The mechanisms responsible for enhanced quality are not known.

The failure of trinexapac-ethyl to inhibit rooting of ryegrass suggests that trinexapac-ethyl did not affect drought avoidance. Drought resistance has been positively correlated with maximum rooting depth and RLD deep in the soil profile in cool- and warm-season grasses (Hays et al., 1991; Marcum et al., 1995; Qian et al., 1997).

Trinexapac-ethyl reduced canopy height through 4 WAT in the greenhouse, but suppression did not extend beyond 2 WAT in the field. Lack of irrigation probably reduced the impact of all PGRs on leaf elongation, for drought stress alone will slow leaf elongation (Taiz and Zeiger, 1991). A longer period of canopy height suppression may have been observed if irrigation had not been restricted. Trinexapac-ethyl has provided up to 6 weeks of growth suppression in bermudagrass (Wiecko, 1997).

Ethephon, which is hydrolyzed to ethylene in the plant, inhibits shoot and root cell division and elongation (Abeles, 1973). Ethephon enhanced turf quality during dessication in the greenhouse and in the field at 2 and 3 WAT on 2 June 1995, and 2 WAT on 18 July 1996. It reduced rooting at 0 to 20 cm in 1996, and has been reported to inhibit tall fescue rooting (Marcum and Jiang, 1997). Ethephon did not reduce perennial ryegrass canopy height.

Mefluidide is absorbed by leaves and exhibits little translocation to roots or lateral meristems (Watschke et al., 1992). It rapidly inhibits cell division and differentiation in meristematic areas (Kaufmann, 1986). Mefluidide can cause unacceptable phytotoxicity on tall fescue and some fine-textured species (Bhowmik, 1987; Chappell et al., 1977; Schott et al., 1977; Watschke, 1976, 1981). In this study, mefluidide application resulted in unacceptable perennial ryegrass quality throughout greenhouse and field evaluation periods, primarily due to turf chlorosis, followed by stand thinning. Mefluidide also reduced ryegrass RLD at a 30- to 40-cm depth in 1995, and at all sampling depths in 1996. Chlorosis probably inhibited photosynthesis, resulting in less assimilate distribution to roots, thereby reducing root growth. Paclobutrazol is root-absorbed (Barrett and Bartuska, 1982), and suppresses vegetative growth by inhibiting gibberellin biosynthesis (Johnston and Faulkner, 1985).

Paclobutrazol had no effect on ryegrass quality in the greenhouse, and enhanced quality on only one of 23 rating dates in the field. Root length density was reduced by paclobutrazol at 0 to 10 cm following the first application in 1996, but was enhanced at 30 to 40 cm. This suggests that paclobutrazol may have encouraged deeper root penetration. This would be beneficial in terms of promoting drought avoidance. However, deeper rooting was not observed following the 1995 paclobutrazol applications, or the 18 July 1996 application. Paclobutrazol had negligible effects on ryegrass quality and canopy height.

Only mefluidide reduced perennial ryegrass canopy height in the field for >3 weeks; it suppressed canopy height for 5 WAT on 18

Table 3. Perennial ryegrass root length density (RLD) and total root length (TRL) as affected by plant growth regulators (PGRs) in 1995 and 1996.[z]

PGR	Application rate ($g \cdot ha^{-1}$ a.i.)	RLD ($cm \cdot cm^{-3}$) Soil depth (cm) 0–10	10–20	20–30	30–40	TRL (cm)
		14 Sept. 1995				
Trinexapac-ethyl	192	10.4 a[y]	3.8 a	2.3 ab	1.9 a	2316 a
Ethephon	3363	10.6 a	3.5 a	3.1 a	2.3 a	2449 a
Mefluidide	134	10.2 a	2.8 a	1.5 b	0.7 b	1912 b
Paclobutrazol	553	8.3 a	4.4 a	2.5 a	1.7 a	2127 ab
Control	---	11.4 a	3.0 a	2.4 ab	1.8 a	2339 a
		16 July 1996				
Trinexapac-ethyl	192	14.0 ab[y]	6.0 a	4.0 a	2.6 ab	3346 a
Ethephon	3363	11.5 b	4.3 b	3.7 a	2.2 ab	2713 b
Mefluidide	134	11.7 b	2.9 c	1.7 b	1.1 c	2193 c
Paclobutrazol	553	12.0 b	5.6 a	4.8 a	2.9 a	3184 ab
Control	---	16.0 a	5.7 a	3.5 a	2.1 b	3419 a
		31 Aug. 1996				
Trinexapac-ethyl	192	16.5 ab	4.8 ab	2.9 ab	2.5 a	3350 ab
Ethephon	3363	16.4 ab	3.4 bc	2.0 b	1.7 ab	2947 bc
Mefluidide	134	15.0 b	2.4 c	1.3 c	0.6 b	2418 c
Paclobutrazol	553	19.2 a	4.7 ab	3.5 a	2.3 a	3715 a
Control	---	20.2 a	5.0 a	2.5 ab	1.9 a	3724 a

[z]PGRs were applied on 2 June and 18 July in both 1995 and 1996. Application rate on 2 June 1995 was twice the rate indicated.

[y]Mean separation within columns by Fisher's protected LSD, $P < 0.05$.

July 1995. Therefore, the value of PGRs in reducing ryegrass mowing requirements is unknown and should be evaluated thoroughly under conditions where turf receives adequate irrigation. Of the PGRs evaluated, trinexapac-ethyl had no negative effects on ryegrass response to drought and seemed to enhance quality during drought in the greenhouse and on selected dates in the field. Most importantly, trinexapac-ethyl had no deleterious effects on rooting of perennial ryegrass.

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