Growth Retardant Effects on Visual Quality and Nonstructural Carbohydrates of Creeping Bentgrass

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Abstract. Plant growth regulators are becoming important tools for managing high-quality turf. However, long-term suppression of growth might affect the potential of the turf to recuperate from summer stresses. This study was initiated to determine the impact of popular commercial turfgrass growth regulators (TGR) on the accumulation of total nonstructural carbohydrates (TNC), and their effect on turf quality. Three experiments, one each year from 1994 through 1996, were conducted on creeping bentgrass (*Agrostis stolonifera* Huds. ‘Penncross’) in a greenhouse. Growth retardants evaluated included trinexapac-ethyl at 0.28 kg·ha⁻¹, flurprimidol at 0.56 kg·ha⁻¹, and paclobutrazol at 0.28 kg·ha⁻¹. Each retardant was applied at three different intervals: a single initial application or multiple applications every 2 or 4 weeks, for the first 8 weeks of each experiment. The verdure and roots were harvested and analyzed for TNC. Trinexapac-ethyl, flurprimidol, and paclobutrazol significantly increased the TNC of creeping bentgrass 2 weeks after their initial application, but TNC levels began to decrease at week 4. The TNC content was strongly influenced by TGR application rate but not application intervals. A single trinexapac-ethyl application at 0.28 kg·ha⁻¹ reduced TNC content more than did split applications, each at lower rates. Accordingly, TNC levels recovered more rapidly with lower, more frequent application rates; however, the retardation effect on vegetative growth diminished as the concentration was lowered. On the other hand, repeated low application rates of trinexapac-ethyl may have minimal effect on TNC accumulation. Chemical names used: [4(cyclopropyl- α -hydroxy-methylene)-3,5-dioxocyclohexanecarboxylic acid ethyl ester] (trinexapac-ethyl); $\{\alpha$ -[1-methylethyl]- α -[4-(trifluoromethoxy)phenyl]-5-pyrimidine-methanol} (flurprimidol); [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-1,2,4-triazole-1-yl]penta-n-3-ol] (paclobutrazol).

Growth regulators are becoming increasingly important tools for turfgrass managers. Mowing is a daily management practice and a major cost in most turf operations. Reduction in the mowing frequency through the use of turfgrass growth regulators (TGRs) can save time and money. Mowing can be reduced as much as 50% over a 5- to 8-week period following an application (Batten, 1983). While TGRs effectively reduce aboveground growth, their use may be accompanied by a reduction in turf quality (Billot and Hentgen, 1974; Watschke, 1976). This fact has restricted the widespread use of TGRs in intensively managed turfs where aesthetics are an important concern. The effect of TGRs on turf can vary depending upon the materials used, applica-

tion rate, species of grass, application timing, weather conditions, and management practices (Diesburg and Christians, 1989; Elkins et al., 1974; Parr et al., 1987; Wakefield and Dore, 1974).

The maintenance of healthy turf, particularly during stressful periods, is partially dependent on the level of carbohydrate reserves (Watschke et al., 1972), which are important for recovery from injury or stresses. The major total nonstructural carbohydrates (TNC) found in turfgrass shoots consist of the monosaccharides glucose and fructose, the disaccharide sucrose, various oligosaccharides of the β -(2-6)-linked polyfructosylsucrose type, starch, and the long-chain fructans (Hull, 1992). Creeping bentgrass fixes carbon through the C3 photosynthetic pathway and stores assimilated carbohydrates in the lower stems and crown regions. Levels of TNC in turfgrasses can be affected by growth retardants (Cooper et al., 1988; Watschke, 1976). However, long-term suppression of growth might jeopardize the turf's recuperative potential by lowering TNC levels.

Most research on TGRs has evaluated only single, annual applications. A single application of a TGR will suppress turf growth for a limited time, generally 3 to 8 weeks, which is

usually insufficient to modify the entire period of rapid vertical shoot extension. In high-maintenance areas, such as golf course fairways where turfgrass is regularly irrigated and fertilized, the required growth suppression period may be longer than the generally recommended application period (Kaufmann, 1990).

The impact of TGRs on turf quality and morphology has been well studied (Brueninger, 1984; Hawkins et al., 1985; Nelson et al., 1977; Spokas and Cooper, 1991), but their effects on carbohydrate metabolism and storage has received little attention. Additional research is needed to better understand the effect of growth retardants on TNC, especially in creeping bentgrass. The objectives of this research were to determine the effects of single and multiple applications of growth retardants on the TNC status and quality of creeping bentgrass.

Materials and Methods

Three experiments, one each year from 1994 through 1996, were conducted on pot-grown creeping bentgrass in a greenhouse. For all experiments, creeping bentgrass was seeded at 49 kg·ha⁻¹ in 15-cm-diameter $\times$ 15-cm-deep plastic pots, filled with calcined clay (Profile; Aimcor, Buffalo Grove, Ill.). The calcined clay has a cation exchange capacity of 33 cmol/kg with 71% of the particles passing through a 320 mm-mesh screen. A square of cheesecloth was laid at the bottom of the plastic pots to hold the calcined clay while allowing drainage. The greenhouse was maintained at a minimum of 16 °C and a maximum of 30 °C. The pots were placed on north–south oriented benches in an east–west oriented glasshouse. From May to September, a whitewash shading compound was applied to the outside surface of the glass. Typical midmorning photosynthetic photon flux (PPF) levels at the turf canopy were $\approx$ 30% of incident outside radiation. An additional 300 μ mol·s⁻¹·m⁻² PPF (measured with a LI-COR 190SA quantum sensor; Lincoln, Nebr.) was supplied to the canopy by supplemental lighting with metal halide lamps (General Electric MH1000 BU) over a 14-h daily light period.

The seeded pots were irrigated as needed to prevent drying throughout the experiments. The turf was hand-clipped every 2 to 3 d to a 2.5-cm height and received N at 46, 60, and 52 kg·ha⁻¹ per month in experiments 1, 2, and 3, respectively, from a modified Hoagland's solution (Hoagland and Arnon, 1950). Foseryl-AL [aluminum tris (O-ethyl phosphonate)] was applied as needed for the control of Pythium blight disease. The first greenhouse study was initiated on 19 Apr. 1994 and continued through 20 Oct. 1994, the second from 18 Nov. 1994 through 16 Jun. 1995, and the third from 6 May to 18 Oct. 1996.

Growth retardants evaluated in the 1994 study included trinexapac-ethyl at 0.28 kg·ha⁻¹, flurprimidol at 0.56 kg·ha⁻¹, and paclobutrazol at 0.28 kg·ha⁻¹. Selected rates were the upper limits for label-recommended single applications for each material. Each retardant

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was applied at three different application intervals: a single application, an application every 2 weeks for 8 weeks, or an application every 4 weeks for 8 weeks. Each application was at the same rate as the single application. The experimental design was a factorial combination of retardant rate and application intervals in a randomized complete-block design with five replications. No applications were made after the first 8 weeks.

In the 1995 greenhouse experiment, only trinexapac-ethyl was investigated to obtain more detailed information on its effect on the TNC content of creeping bentgrass at a wider range of application frequencies and rates. In addition to a 0.28 kg·ha⁻¹ treatment at three different application intervals, as described for the 1994 experiment, trinexapac-ethyl was also applied every week at 0.03 kg·ha⁻¹, every 2 weeks at 0.06 kg·ha⁻¹, or every 4 weeks at 0.09 kg·ha⁻¹. Each application schedule delivered a total of 0.28 kg·ha⁻¹ trinexapac-ethyl, the same as the single application. No applications were made after 8 weeks.

In the 1996 study, a single application was made of trinexapac-ethyl and paclobutrazol at 0.28 kg·ha⁻¹ and flurprimidol at 0.56 kg·ha⁻¹. The 1995 and 1996 experiments were arranged in a RCB design with five replications. Each treatment in each replication had 10 to 12 pots to facilitate destructive sampling.

All TGR treatments were made with a compressed air boom sprayer installed inside a stationary spray chamber, delivering 468 L·ha⁻¹ at 179 kPa. Pots were watered to field capacity within 2 h of application to facilitate uptake of the root-absorbing retardants (flurprimidol and paclobutrazol). Treatments were initiated after a 9-, 12-, or 13-week turf establishment period to provide similarly sized

plants for the 1994, 1995, and 1996 experiments, respectively. Clipping yields were not measured in any of the greenhouse experiments.

Plant tissue was destructively harvested from one pot for each treatment in each replication 1 d before the initial TGR applications and thereafter every 2 weeks for 18 weeks in 1994 and 1995. In the 1996 study, tissues were sampled every week for the first 10 weeks of the experiment. The verdure and roots were harvested separately by cutting the extracted turf below the crown area from the pots. The verdure and roots were placed on a wire screen, and the calcined clay was washed from the tissue. Washed tissue was dried for 1 h at 100 °C and then for 48 h at 70 °C before dry masses were recorded. All tissue samples were ground to a powder in a Wiley-Mill to pass through a 425-mm mesh screen and stored at -15 °C until analyzed for total nonstructural carbohydrates.

Concentrations of TNC in the samples of root and verdure of creeping bentgrass were determined by the method described by Westhafer et al. (1982). Tissue samples of 60 mg were extracted by 0.1 M phosphate buffer (pH 5.5). Glucose, fructose, sucrose, and fructans were quantified with a single aqueous extraction using glucose oxidase and invertase. Reducing sugars (glucose and fructose) and sucrose were determined by absorbance at 540 nm using a Hitachi U-2000 Spectrophotometer (Chicago). Fructan absorbance was determined at 490 nm. Data are presented based on TNC, which was the total of all analyzed carbohydrate fractions.

Creeping bentgrass quality was rated for injury or phytotoxicity from growth-retardant treatments at 1- or 2-week intervals before

tissue sampling. Visual quality was subjectively rated on a scale based on 1 = completely brown and dead turf, ≥7 = commercially acceptable, and 9 = best quality. Visual quality ratings were initiated after 4 weeks in the 1995 experiment.

Separate analyses of variance (ANOVA) were performed on data collected for each experiment. Fisher's Protected least significant difference procedure was used to separate means when appropriate.

Results and Discussion

Analysis of the 1994 study showed significant differences in main effects for both TGR and application intervals. Differences in treatment application intervals were not consistent in subsequent studies and are not presented. No significant interactions were observed for any of the evaluations. Only the main effect of TGR will be discussed. In the 1994 study, differences in root TNC were also measured. Subsequent studies did not confirm the observed differences, however, so root data are not presented.

Observation of turf quality. In 1996, single applications of trinexapac-ethyl, flurprimidol, or paclobutrazol significantly reduced turf quality up to 3 weeks after application (Table 1). In the 1995 study, trinexapac-ethyl at 0.28 kg·ha⁻¹ lowered turf quality up to week 6. For every date of evaluation, the lowest observed turf quality was above the commercially acceptable level of 7.

Leaves of the treated plants were darker green than those of control plants, suggesting a possible increase in chlorophyll concentration. Spokas and Cooper (1991) reported that the chlorophyll content of Kentucky bluegrass

Table 1. Growth regulator effect on visual quality^a of creeping bentgrass turfgrass in 1995 and 1996 greenhouse studies.

Application		Week after treatment										
rate		1	2	3	4		6		8		10	
Treatments ^y	(kg·ha ⁻¹)	1996	1996	1996	1995	1996	1995	1996	1995	1996	1995	1996
Control	---	8.5	8.7	8.7	8.2	8.6	8.2	8.2	8.6	8.9	8.7	8.9
Trinexapac-ethyl	0.28	7.4	7.6	7.8	7.3	8.4	7.5	8.6	8.4	9.0	8.5	8.7
Flurprimidol	0.56	7.5	7.7	7.4	---	8.0	---	8.0	---	8.8	---	8.9
Paclobutrazol	0.28	7.8	7.6	7.4	---	8.1	---	7.9	---	8.7	---	9.0
cv	---	5.7	5.8	6.9	8.9	5.7	10.4	4.7	9.3	2.3	10.1	2.7
LSD _{0.05}		0.3	0.4	0.5	0.2	NS	0.3	NS	NS	NS	NS	NS

^aTurfgrass quality rating scale: 1 = completely brown, 6.5 = minimally acceptable, 9 = ideal turf with dark-green color.

^yInitial applications were made on 11 Feb. 1995 and 7 Aug. 1996.

^{ns}Nonsignificant.

Table 2. Growth regulator effect on total nonstructural carbohydrates (TNC) of creeping bentgrass verdure in 1994, 1995, and 1996 greenhouse studies.

TGR	Appl. rate ^y (kg·ha ⁻¹)	TNC content in verdure ^a (mg·g ⁻¹)																	
		Week 2			Week 4			Week 6			Week 8			Week 10			Week 12		
		1994	1995	1996	1994	1995	1996	1994	1995	1996	1994	1995	1996	1994	1995	1996	1994	1995	1996
Control	---	55	136	17	96	111	26	67	129	32	65	123	47	80	121	56	69	123	70
Trinexapac-ethyl	0.28 ^x	78	158	23	90	110	27	56	112	32	47	89	43	44	98	38	44	100	51
Flurprimidol	0.56	76	24	86			21	59		18	46		26	37		31	29		33
Paclobutrazol	0.28	69	15	72			20	50		19	43		27	35		24	26		29
cv	---	25.8	12.8	40.8	29.2	14.1	41.9	31.9	15.0	40.5	37.1	16.0	39.2	46.6	15.2	41.3	50.3	18.2	51.5
LSD	---	12.0		16.4			9.3	10.6		7.9	16.3	12.9	8.8	12.8	13.9	9.7	17.5	9.1	14.8
		**	NS	NS	*	NS	NS	**	NS	**	**	**	**	**	**	**	*	**	**

^a1994 TNC data are main effect means across all application intervals; 1995 and 1996 TNC data are treatment means only.

^yTGR applications were made from week 0 to week 8. The first application dates were 30 June 1994, 11 Feb. 1995, and 7 Aug. 1996.

^xRate for each application.

^{ns}, *, ** Nonsignificant or significant at $P \leq 0.05$, 0.01, respectively.

treated with mefluidide {[N-[2,4-dimethyl-5-[[[bufluoromethyl]-sulfonyl]amino]phenyl]acetamide} and amidochlor {[N-(acetylamino)methyl]-2-chloro-N-(2,6-diethyl-phenyl)acetamide} was $\approx 35\%$ greater than that of nontreated turf 7 d after application. They suggested that the increase in chlorophyll content was due to a greater concentration of chlorophyll within the slower-growing leaves, since chlorophyll production may not be affected by growth retardants. Although verdure dry masses remained unchanged following trinexapac-ethyl treatments, leaf and stem tissue appeared thicker than those of untreated controls (data not shown).

TGR effects on nonstructural carbohydrates. In the 1994 experiment, trinexapac-ethyl, flurprimidol, and paclobutrazol significantly increased the TNC content of creeping bentgrass 2 weeks after their initial application, but TNC levels began to decrease at week 4 relative to the control (Table 2). The reduction of TNC in the verdure was significant from week 6 to the end of the experiment (week 16). Treated plants had significantly lower TNC than the control plants even 8 weeks after the last application. Plants treated with trinexapac-ethyl at $0.28 \text{ kg} \cdot \text{ha}^{-1}$ had a higher TNC content than plants treated with flurprimidol at $0.56 \text{ kg} \cdot \text{ha}^{-1}$ or paclobutrazol at $0.28 \text{ kg} \cdot \text{ha}^{-1}$ from week 10 to the end of experiment. In plants treated with trinexapac-ethyl, TNC levels appeared to recover earlier than in plants treated with the other retardants.

When trinexapac-ethyl was applied to 3-month-old creeping bentgrass in 1995, the overall effects were similar to those in 1994. The TNC content of the turf increased during the 2-week period after application, then declined (Table 2). This agrees with an earlier study (Pennucci, 1986), in which growth retardants induced increases in TNC that were transient and observed only at high application rates. In our study, trinexapac-ethyl treatments caused significant decreases in TNC content of the verdure 8 weeks after the initial application.

In the 1996 study, single applications of flurprimidol or paclobutrazol significantly reduced verdure TNC content 6 weeks after application (Table 2), whereas trinexapac-ethyl did not.

The accumulation of TNC in the verdure during the first 2 to 3 weeks after application of a growth retardant may indicate suppression of growth but not of photosynthetic activity. An initial rise in TNC levels was followed by a significant decline, probably due to a postinhibition growth response (Bruening,

1984). The TNC content of treated turfs remained low for up to 16 weeks. Photosynthetic activity might not be sufficient to replenish carbohydrate reserves even after the growth retardation period. Thus, the turf may not be vigorous enough to maintain a quality stand during a period of high stress. Available carbohydrates may have been utilized for something other than vertical growth. Plants treated with growth retardants seem to have a greater number of tillers with thicker stems and roots compared to untreated turfs. Therefore, the reduction in TNC content of treated plants may be attributed to continuous use of photosynthate for supporting the production and growth of tillers. The treatment effect on TNC shown in these greenhouse experiments may have been somewhat exaggerated compared with the effects in the field.

The TNC status of turf would probably be influenced more by morphological changes or other metabolic activities rather than by direct effects on plant respiration and photosynthetic rates. Individual leaf photosynthetic rate decreases with age (Morgan and Brown, 1983; Woledge and Leafe, 1976).

Some of the turf treated with growth regulators had unacceptable discoloration during the 1994 experiment, which indicates that inappropriate use of growth regulators may cause damage (Symington et al., 1986). Applications of a growth retardant at high rates might jeopardize the recuperative potential of a turfgrass for an extended period of time.

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