Growth Responses of *Lolium perenne* L. to Trinexapac-ethyl

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Abstract. A growth chamber and a greenhouse study were conducted to determine if successive applications of trinexapac-ethyl (TE) to developing perennial ryegrass (*Lolium perenne* L.) plants would reduce leaf elongation rate (LER) while increasing tiller number and root mass. Growth parameters measured were LER, tiller number, and root mass. In the growth chamber, developing perennial ryegrass plants were sprayed twice with TE at 0.24 kg·ha⁻¹ a.i. at 20 and 40 days after emergence. Leaf elongation rate was reduced by ≈35% following two applications of TE in both growth chamber experiments. This treatment increased the number of tillers per plant in the growth chamber at 60 days after emergence and in the greenhouse at 150 days after emergence, but had no effect on root or shoot mass in either location. Multiple applications of TE to developing perennial ryegrass turfs may favor quicker establishment in terms of tillering, while substantially reducing mowing requirement. Chemical names used: 4-cyclopropyl- α -hydroxy-methylene-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester (trinexapac-ethyl).

A plant growth regulator (PGR) that significantly decreases mowing frequency and increases tillering and rooting, while not reducing quality, would be a welcome turfgrass management tool. Trinexapac-ethyl (Primo; Novartis Crop Protection Inc., Greensboro, N.C.) is a Type II PGR that was introduced to the U.S. turfgrass market in 1991 (Watschke and DiPaola, 1995). Type II PGRs reduce cell elongation by interfering with the production of gibberellins (GAs). Specifically, the free acid of TE inhibits the hydroxylation of GA₂₀ to form the physiologically active GA₁ by competitively inhibiting the regulatory enzyme 3- β -hydroxylase (Adams et al., 1992; Hedden, 1991). This site of action distinguishes TE from other Type II PGRs, such as flurprimidol [α -(1-methylethyl)- α -(4-(trifluoromethoxy) phenyl)-5-pyrimidine methanol] and paclobutrazol [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol], which inhibit the oxidation of *ent*-kaurene to *ent*-kaurenoic acid, an earlier step in the gibberellin biosynthetic pathway (Rademacher, 1989). Growth is retarded by these PGRs, while the developmental sequence of the plant continues; photosyn-

thesis is not negatively affected (Qian et al., 1998; Stier et al., 1997) and new plant organs develop but are reduced in size (Kaufmann, 1994). The manufacturer claims that this use of TE on intensively managed cool-season and warm-season turfgrass significantly reduces clipping production and canopy height and increases tiller density and root mass, without causing a marked decrease in turf quality (Ciba-Geigy, 1993).

Monthly field applications of 0.40 kg·ha⁻¹ a.i. TE to a mixed sward of *Agrostis* spp., *Festuca* spp., and perennial ryegrass reduced reduction in clipping fresh mass 41% over a 3-year period in the United Kingdom (Daniels and Sugden, 1996). Application of 0.19 kg·ha⁻¹ a.i. TE to *Agrostis palustris* Huds. 'Pennecross' putting greens reduced canopy height by 32% 30 d after treatment (Burpee et al., 1996).

Although the partial blockage of GA₁ biosynthesis, with its subsequent effects on increased cell wall plasticity and elongation, would reduce plant height, the mechanism of the effect on tillering rate or root mass is less obvious. One hypothesis is that photosynthate not being used for cell elongation may be employed for increased cell division in shoot axillary and root apical meristems, resulting in the production of new tillers and roots. In a greenhouse study, Breuninger (1984) reported that flurprimidol did not affect the photosynthetic rate or root mass of Kentucky bluegrass (*Poa pratensis* L.), but increased tiller density. He speculated that photosynthate is continually used by treated plants for enhanced production of small tillers. Flurprimidol and paclobutrazol significantly reduced the amount of photosynthate partitioned to the roots of Kentucky bluegrass, while slightly increasing partitioning to axillary shoots (Hanson and Branham, 1987). Root length density (cm·cm⁻³)

of tall fescue (*Festuca arundinacea* Schreb.) was reduced by TE treatment at the 10- to 20-cm depth in one greenhouse experiment, but not in a second (Marcum and Jiang, 1997). In another study with perennial ryegrass, root length density from 0 to 40 cm was unaffected by TE treatment (Jiang and Fry, 1998).

Enhanced tillering and/or rooting of mature turfgrass may result in enhanced performance under biotic and abiotic stress. In addition, enhanced tillering and/or rooting of seedlings during turfgrass establishment may hasten the opening of an area for recreational use. However, information as to the effect of TE on tillering of turfgrass is very limited. A growth chamber and a greenhouse study therefore were conducted to determine if multiple applications of TE to developing perennial ryegrass plants would reduce LER while increasing tillering and root mass.

Materials and Methods

Growth chamber study. 'Prizm' perennial ryegrass was seeded in 10-cm-diameter × 10-cm-deep pots containing Isolite 1CG (Sundine Enterprises, Arvada, Colo.), a calcined diatomaceous medium with chemical composition of 77% SiO₂, 12% Al₂O₃, and 4% Fe₂O₃, a cation exchange capacity of 7.0 meq/100 g and a pH of 6.5. The pots were placed on a mist bench and seedling emergence occurred ≈4 d after planting. At this time, all but three evenly spaced seedlings were removed from each pot, and the pots were transferred for the remainder of the experiment to a controlled environment growth chamber. Plants were grown for 60 d in the growth chamber with 14-h photoperiod, 22 °C day/18 °C night temperature, and an average photosynthetic photon flux (PPF) at leaf level of 250 μ mol·m⁻²·s⁻¹, as measured with a LI-1000 datalogger with a LI-190SA quantum sensor (LI-COR, Inc., Lincoln, Nebr.) every 2 weeks and in three locations (left, middle, right) in the chamber. Pots were arranged in a completely randomized design with four replications and rotated every 5 d; experimental units were single pots containing three plants each (subsamples) for a total of eight pots (24 plants). Four pots (12 plants) were treated with TE and four were not. The experiment was conducted twice.

Each pot was well watered, receiving 100 mL of distilled water every other day. This saturated the medium to the point where drainage occurred. The plants were fertilized once a week with a solution that delivered the following quantities of elements (mg·m⁻²): 61.0 N, 8.0 P, 33.0 K, 0.2 Fe, 0.04 B, 0.11 Cu, 0.11 Mn, 0.11 Zn, and 0.002 Mo. A hand-held pump sprayer was used to apply TE at 0.24 kg·ha⁻¹ a.i. on 22 Nov. (20 d after emergence) and 12 Dec. 1996 (40 d after emergence) in Expt. 1 and 27 Jan. and 17 Feb. 1997 in Expt. 2.

The number of tillers per plant and the mean blade length of each unmowed plant was recorded every 5 d. The length of each individual blade was measured (in mm) by hand with a ruler from the collar to the tip of the blade. A tiller was defined as a shoot that had separated from the parent plant for most of the

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length of the leaf sheath (Lush, 1990). In the second cycle of each experiment, grass plants were clipped 2.0 cm above the soil surface and TE was reapplied. Clipping was performed to simulate the mowing that would normally occur on a newly seeded turfgrass area; clipping yield data were not taken. Shoot and root weight per pot were obtained at the end of both experiments by separating the roots from the shoots, washing all of the media from the roots, and drying them in a forced-air oven for 24 h at 70 °C. The data were converted to weight per area of pot surface (shoots) or volume of medium (roots).

Leaf elongation rate and tiller number per plant data were analyzed by analysis of variance (ANOVA) as a three-factor factorial with subsamples (TE, experiments, and TE application cycles). Root mass data were analyzed by ANOVA as a single-factor experiment. All statistical analyses were performed with SAS (SAS Institute, 1988).

Greenhouse study. Twenty-five, 10-day-old seedlings of 'Prizm' perennial ryegrass were transplanted into polyvinyl chloride (PVC) tubes in the greenhouse on 20 Oct. 1996. The PVC tubes were 10 cm in diameter and 46 cm in depth, and were filled with washed sand. The experiment was a randomized complete-block design with one factor (two levels of TE: none or 0.24 kg·ha⁻¹ a.i.) and five replications. The plants were watered three times a week until drainage occurred, clipped weekly to 2.5 cm, and fertilized every 2 weeks with a complete nutrient solution (analysis as above) that supplied 61 mg·m⁻² of N for a total of 610 mg·m⁻² of N during the course of the experiment. Greenhouse temperatures averaged 21 °C ± 3 °C day/15.5 °C ± 3 °C night. Average PPF at 1300 HR was 830 μmol·m⁻²·s⁻¹ on the greenhouse bench. Trinexapac-ethyl was applied at four monthly intervals (20 Nov. 1996, 20 Dec. 1996, 20 Jan. 1997, and 20 Feb. 1997) with a hand-held pump sprayer to deliver a rate of 0.24 kg·ha⁻¹ a.i.

All plants were harvested following 5 months of growth. Roots and shoots were separated and the sand was washed from the roots through graduated screens. The total number of tillers for each experimental unit were counted and converted to tillers dm⁻². Shoots were dried for 24 h at 70 °C and weighed. Root mass was determined following ashing for 4 h in a muffle furnace set at 550 °C. In the greenhouse experiment a muffle furnace was used to separate the organic mass (roots) from the mineral mass (sand).

During the course of this experiment, one of the five replications was lost to aphid damage. Each experiment was, therefore, analyzed as a one-factor ANOVA with unequal replication. Statistical calculations for the greenhouse data were performed manually.

Results

Growth chamber study. Perennial ryegrass LER was not decreased significantly by TE during the first application cycle of the first experiment (Fig. 1A). However, LER was

Fig. 1. Effect of trinexapac-ethyl (TE) on leaf elongation rate (LER) of 'Prizm' perennial ryegrass over two consecutive 20-d periods in a growth chamber. Plants were clipped to a 2.0-cm height above the surface of the medium at 40 d after emergence and TE was reapplied. A—Expt. 1; B—Expt. 2. Effect of TE significant at $P \leq 0.05$ (*) or 0.01 (**) by ANOVA.

reduced by 31% ($P = 0.04$) from 40 to 60 d after emergence following the second application (Fig. 1A).

The same general effects on LER were observed in Expt 2. Leaf elongation rate was not significantly decreased by TE until 40 d after emergence (Fig. 1B), but the reduction (37%) was significant ($P \leq 0.01$) on all four sample dates following the second application (Fig. 1B).

Tillering data were combined for the two experiments because the data were similar. Tillering began ≈21 to 28 d after emergence for both treatments. Trinexapac-ethyl had little effect on tiller number per plant at 40 d after emergence (Table 1), but had increased tiller number 60 d after emergence. No significant differences in root mass, shoot mass, or root : shoot ratio were observed in either growth chamber experiment (Table 1).

Greenhouse study. At 150 d after emergence, following four monthly applications, TE had increased tiller density significantly (Table 1), but differences in root and shoot mass were nonsignificant. However, TE had reduced the root : shoot ratio (Table 1).

Discussion

Why LER was not reduced by the first application of TE is not clear. Johnson (1993) and King et al. (1997), working with mature

field-grown, cool-season turf reported significant leaf growth suppression 7 d following TE application. We know of no previous data concerning the growth suppressive effects of TE on developing turfgrass plants.

Tillering was not increased by TE-treatment in the growth chamber experiments until 20 d after the second application, while tiller density was greatly promoted by TE-treatment in the greenhouse experiment. This may reflect differences in the time for growth and development (60 vs. 150 d) and the number of TE treatments (two vs. four). This, and previous research concerned with cool-season grass growth and development, indicate that tiller initiation occurs between 21 and 28 d after emergence (Krans and Beard, 1980; Skinner and Nelson, 1994), suggesting that a specific maturity level is a prerequisite for tiller development. Further, work by Krans and Beard (1980) indicated that photosynthate partitioning in Kentucky bluegrass and red fescue (*Festuca rubra* L.) seedlings follows a distinct developmental pattern. Their study indicated that leaves were the strongest sinks until ≈21 d after emergence, with stems becoming the dominant sink once the requisite amount of leaf area has been produced at ≈28 d after emergence. These results imply that once adequate leaf area is present, photosynthate not required for leaf growth and maintenance may be used for enhanced stem and root growth. In

Table 1. Effects of trinexapac-ethyl (TE) on perennial ryegrass tillering, root and shoot mass, and root : shoot ratio in a growth chamber and in a greenhouse.

Treatment	Application rate (kg·ha ⁻¹)	Growth Chamber ^a					Greenhouse ^b			
		Tillers/plant		Root mass (mg·cm ⁻³)	Shoot mass (mg·cm ⁻²)	Root: shoot ratio ^c	Tiller density (no. dm ⁻²)	Root mass ^a (mg·cm ⁻³)	Shoot mass (mg·cm ⁻²)	Root: shoot ratio
		40	60							
TE	0.24	9	29	2.07	13.70	0.60	341	1.02	25.93	1.84
Control	---	9	23	2.72	14.94	0.73	203	1.16	22.47	2.41
Significance (<i>P</i> = 0.05)		NS	*	NS	NS	NS	*	NS	NS	*

^aTE applied at 20 and 40 d after emergence; tiller per plant data are averages combined over Expt. 1 and 2; root mass, shoot mass and root : shoot ratio values are from Expt. 2 only.

^bTE was applied at 30, 60, 90, and 120 d after emergence; all reported values were taken at the end of the experiment (150 d after emergence).

^cAfter ashing.

^dBased on the actual measured root and shoot masses and not on the converted numbers reported in the table because of the volume/area incongruity.

^eNonsignificant.

our growth chamber study, tillering began at ≈20 d after emergence and LER was significantly reduced by TE at 40 to 50 d after emergence. This increased the number of tillers per plant at 60 d after emergence. It is not surprising, then, that after four applications of TE and 150 d of growth, the TE treated greenhouse plants had higher tiller densities than the untreated plants. A prolonged reduction in the amount of photosynthate needed for leaf growth may have resulted in more available energy for tiller production.

As previously reported for TE-treated perennial ryegrass and tall fescue (Jiang and Fry, 1998; Marcum and Jiang, 1997), rooting of perennial ryegrass was not increased by TE-treatment in either the growth chamber or the greenhouse study. Krans and Beard (1980) reported that during the first 35 d of seedling development, Kentucky bluegrass roots constituted a strong sink for photosynthate, requiring almost 20% of the assimilated carbon. After 35 d of growth, photosynthate allocated to the roots fell to ≈10%, while that allocated to stems increased to ≈50%. Hull (1976) reported that actively growing 4-year-old Kentucky bluegrass turf allocated ≈5% to 10% of current photosynthate to its roots, while 25% to 50% was allocated to stems. These data indicate that stems constitute a much stronger sink for photosynthate than roots following a 14- to 35-d period of seedling development. Thus, root mass was probably not increased by TE-treatment in our experiments because perennial ryegrass stem tissue was a stronger sink than the root tissue. This statement is supported by the fact that tillering of the TE-treated turf increased while shoot mass did not.

Our results indicate that successive applications of TE to perennial ryegrass reduces LER with no effect on root mass. In the greenhouse experiment, four applications of TE significantly increased tiller density, but in the

growth chamber experiments, two applications promoted tillering only on the last sample date. These findings suggest that further research is warranted to investigate whether multiple applications of TE on developing perennial ryegrass turfs may favor quicker establishment in the field because of enhanced tillering. Further research concerning mechanisms of TE effects on tillering and photosynthate partitioning is needed in conjunction with field studies.

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