THE EFFECT OF GROWING DEGREE DAY SCHEDULED TRINEXAPAC-ETHYL APPLICATIONS ON THE GROWTH RATE AND FERTILITY REQUIREMENTS OF CREEPING BENTGRASS GOLF PUTTING GREENS

by

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This is to certify that I have examined this copy of a master's thesis by

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and have found that it is complete and satisfactory in all respects,

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THESIS ABSTRACT

The plant growth regulator trinexapac-ethyl (TE) is widely used on golf course putting greens across the US. Numerous researchers have shown TE applications reduce clipping yield and increase turfgrass color, quality, and tiller density on a variety of cool- and warm-season turfgrass species. However, there has been limited research on the duration and magnitude of growth suppression on creeping bentgrass (*Agrostis stonolifera* Hud.) golf putting greens. Duration of TE-induced growth suppression is affected by plant metabolism which is strongly related to air temperature. The objectives of this thesis were to 1) investigate the effect of TE on yield of creeping bentgrass golf putting greens, 2) develop a growing degree day (GDD) model to predict duration of TE efficacy, and 3) investigate the effect of season long growth inhibition on creeping bentgrass nitrogen and phosphorus requirements. A GDD model was calibrated by measuring daily clipping yield following multiple TE applications. Re-application intervals varied and were based on several different GDD thresholds, base 0°C. Re-application of TE every 200 GDD resulted in season-long clipping yield suppression and was in contrast to the label directions (four week re-application interval) which did not result in clipping yield reductions over the course of the season. Application rate had little effect on the duration of clipping suppression and suggests that 200 GDD interval is adequate for all practical TE application rates on golf putting greens. The 200 GDD re-application interval sustained color and quality enhancements. Repeated TE applications every 200 GDD reduced nitrogen fertility requirements by 30-50% compared non-treated creeping bentgrass based on color and quality measurements. Trinexapac-ethyl also reduced Mehlich-3 soil test phosphorus critical values on several rating days however, the average reduction was less than variation that occurs between sampling

days and therefore practically inconsequential. In conclusion, this research has thoroughly investigated the effect of various TE application rate and re-application frequencies on creeping bentgrass putting greens. Re-applying TE every 200 GDD provided season-long yield suppression and resulted in increased color and quality allowing for substantially decreased nitrogen fertilization.

Chapter One: Trinexapac-ethyl Literature Review

ABSTRACT

The plant growth regulator trinexapac-ethyl (TE) was originally developed to reduce turfgrass mowing requirements. Additionally, TE has been reported to increase turfgrass color, density, and visual quality. Trinexapac-ethyl influences these turfgrass attributes by inhibiting synthesis of the plant hormone gibberellic acid; which initiates cell elongation. Numerous research articles have demonstrated TE suppresses clipping yield of many turfgrass species by 50% for four weeks. A notable exception is the creeping bentgrass (*Agrostis stolonifera* Hud.) golf putting greens which are substantially less affected by TE.

Trinexapac-ethyl affects clipping yield in two phases, relative yield suppression followed by yield enhancement. Enhancement of color and visual quality occur during the suppression phase and dissipate during the yield enhancement phase. Therefore maintaining relative yield suppression is desirable. Metabolism of TE is directly related to air temperature. The half life of TE in creeping bentgrass was found to be 5.3 days in a growth chamber with air temperature at 18°C and 3.4 days at 30°C. Calendar-based TE application intervals are therefore inefficient because they do not reflect air temperature and plant metabolism. Development of a TE metabolism model, based on air temperature, would indicate when TE re-applications are necessary to maintain yield suppression and sustain color and quality enhancement.

LITERATURE REVIEW

Mowing is the most labor and fuel intensive practice associated with turfgrass management. It has been estimated that 70 to 80% of budget of a low budget golf course is spent on mowing. Therefore, growth reducing chemicals have the potential to significantly reduce mowing costs. Research with various compounds to reduce turfgrass growth rate have been sought since the 1940s (Watschke and DiPaola, 1995). The first compounds, called plant growth regulators (PGR), caused growth inhibition by slowing plant cell division. However applications of these PGRs caused turfgrass phytotoxicity which limited their use to low maintenance turf like roadsides and other hard to mow areas (Murphy et al., 2005). Eventually, PGRs with lower phytotoxicity were developed. These products inhibit gibberellic acid (GA) production and can safely be applied to turfgrass of any maintenance standard with little detrimental effect on turfgrass color or quality (Watschke and DiPaola, 1995). Trinexapac-ethyl is a GA-inhibiting PGR that became commercially available in the US during the early 1990s. This product quickly became used widely on golf course putting greens, tee, fairways, and athletic fields. In addition to growth suppression, TE has been shown to enhance turfgrass color, tiller density, and quality, and alter root architecture, carbohydrate concentrations, and nutrient allocation (Ervin and Zhang, 2008).

The rate of plant cell expansion is typically controlled by the plant hormones called gibberellins or gibberellic acids (Taiz and Zeiger, 2006). There are many structural forms of compounds classified as gibberellins; however, only gibberellins with particular chemical structures increase cell expansion (Taiz and Zeiger, 2006). In cool season turfgrasses, GA_1 increases cell expansion and growth rate (Reid and Ross, 1991). GA_{20} is the inactive precursor of GA that undergoes dehydroxylation to form GA_1 via 3β-hydroxylase. This process is regulated by 2-oxoglutaric acid inhibition of 3β-hydroxylase (Rademacher, 2000). Trinexapac-ethyl (4-[cyclopropyl-α-hydroxy-methylene]-3,5-dioxo-cyclohexane-carbxylic acid ethyl ester) is a foliarly absorbed compound that is converted by the plant to trinexapac acid, a structural mimic of 2-oxoglutaric acid (Beasley and Branham, 2005). After conversion, trinexapac acid acts in conjunction with 2-oxoglutaric acid to inhibit 3β-hydroxylase conversion of the inactive GA_{20} to the active GA_1 form (Rademacher, 2000). Tan and Qian (2003) showed that TE applied at 0.1 kg ha^{-1} reduced GA_1 leaf concentrations by 46% while GA_{20} concentrations increased by 146% compared to the non-treated Kentucky bluegrass (*Poa pratensis*). The reduction in GA_1 corresponded to a 50% reduction in mean weekly clipping yield, providing solid evidence that TE inhibits growth rate by reducing GA_1 concentration.

Clipping Yield

Trinexapac-ethyl is currently labeled in the US for use on all commonly grown cool- and warm-season turfgrasses. The Primo Maxx label (Syngenta Co., Greensboro, NC) lists numerous application rates dependent upon turfgrass species and maintenance standards (i.e. golf course putting greens and fairways). These application rates are stated to suppress clipping yield by 50% for four weeks. However, the label also states that application rate and frequency may need adjusting depending on management practices and environmental conditions. Such adjustment of TE application rate is difficult because confirmation of actual yield reductions in the field is not easily accomplished. Instead, turfgrass managers rely on researchers across the country to investigate the magnitude and duration of growth suppression caused by TE on various grasses (Table 1.1). Johnson (1994) first demonstrated TE induced growth suppression on hybrid and common bermudagrass. He demonstrated that monthly TE applications reduced the number of mowing applications required during a growing season by 30%. For a majority of turfgrass species the labeled application rate suppresses yield by 50% for four weeks (Table 1.1).

The notable exception is creeping bentgrass maintained at putting green mowing height. Interestingly, golf course putting greens routinely receive multiple TE applications. McCullough et al. (2006b) showed that TE-treated creeping bentgrass grown in the south-eastern US experienced a 20% decrease in yield which lasted for two weeks at the labeled application rate. . In 2007, McCullough et al. reported the effects of TE application rate and re-application frequency on a creeping bentgrass putting green during spring in South Carolina. The TE treatments consisted of labeled rate (0.05 kg a.i. ha^{-1}) applied every three weeks, 2/3 labeled rate applied biweekly, and 1/3 labeled rate applied weekly. Clipping yield reductions ranged from 0 to 40% during the experiment. Application rate did not affect the magnitude of growth suppression, but more frequent application intervals reduced daily clipping yield fluctuations compared to the control. This is in contrast to a 'Tifway' bermudagrass putting green that maintained 55% yield reduction for four weeks in that same study. It is unfortunate that this experiment was terminated prior to warmer summer months. To date, no one has measured the effect TE application rate and interval has on creeping bentgrass yield clipping yield during an entire growing season.

TE alters growth rate in two distinct phases. Fagerness and Yelverton (2000) described a period of enhanced clipping yield following growth suppression called 'post-inhibition growth enhancement' in comparison to non-treated bermudagrass. This growth response will be hereafter referred to as the rebound phase of growth regulation and has been observed with other turfgrass species including creeping bentgrass and Kentucky bluegrass (Beasley and Branham, 2005). Researchers have speculated that the rebound phase is caused by an accumulation of total non-structural carbohydrates during the suppression phase which enhances clipping yield during rebound (Ervin and Zhang, 2008). However, based on the findings of Tan and Qian (2003), another reasonable hypothesis is that the rebound phase is a result of GA_{20} accumulation during the suppression phases which is converted to GA_1 after TE has been metabolized.

Table 1.1. The influence of TE application rate and re-application frequency on magnitude and duration of growth suppression in various turfgrass species.

Turfgrass Species and Mowing Height	Application Rate	Re-application Frequency	Growth Suppression	Approximate Duration of Growth Suppression	Reference
Common name; mm	kg a.i. ha^{-1}	Weeks	% of control	Weeks	
Creeping bentgrass; 3.2	0.05	4	20%	2	McCullough et al., 2006b
Creeping bentgrass; 3.2	0.02, 0.03, 0.05	1, 2, 3	20-40%	3	McCullough et al., 2007
Kentucky bluegrass; 30	0.05	4-6	20%	4-6	Stier and Rodgers, 2001
Kentucky bluegrass, 35	0.05	4	50%	4	Tan and Qian, 2003
Kentucky bluegrass; 32	0.14, 0.29, 0.58	none	44-73%	4-5†	Beasley and Branahm., 2007
Rough bluegrass; 80	0.29	6	55-80%	6	Gardner and Wherley, 2005
Sheep fescue; 80	0.29	6	35-50%	6	Gardner and Wherley, 2005
St. Augustinegrass; 75	0.14, 0.29	2, 4	50%	4	McCarty et al., 2004
Supina bluegrass; 30	0.05	4-6	60%	4-6	Stier and Rodgers, 2001
Tall fescue; 38	0.29	none	44-77%	4	Richie et al., 2001
Tall fescue; 80	0.29	6	58-76%	6	Gardner and Wherley, 2005
'TifEagle' Bermudagrass; 3.2	0.05	4	60%	3	McCullough et al., 2007
'Tifway' Bermudagrass; 16	0.07, 0.11	4	60%	4	Fagerness and Yelverton, 2000
'Tifway' Bermudagrass; 25	0.11	4	50%	4	Fagerness et al., 2004
Zoysiagrass; 12	0.05, 0.10, 0.19	4, 8, 12	25, 27, 0%	4-6	Qian and Engelke, 1999

† Duration dependent on summer or fall season

Turfgrass Carbohydrates

Han et al. (1998, 2004) that total nonstructural carbohydrate concentrations (TNC) increased in the verdure of creeping bentgrass two weeks after initial TE application. Elevated TNC levels then declined 4 to 16 weeks after TE application. A similar phenomenon occurred in hybrid bermudagrass after sequential TE applications (Waltz and Whitwell, 2005). Richie et al. (2001) stated in the title of their publication that there was no increase in TNC in tall fescue 6 -7 weeks after a single TE application; this result is not surprising based on the findings of Han et al. (1998, 2004). Temporal variability of TNC concentrations are likely result of the suppression and rebound growth phases. Increased TNC concentration in the plant two weeks after TE application coincides with yield suppression and is supported by Table 1.1. As turfgrass growth rate increases during the rebound phase, TNC concentrations diminish as a result of increased growth rate.

Trinexapac-ethyl does not alter photosynthetic production in both warm- and cool-season grasses (Qian et al., 1998; Steinke and Stier, 2003). Heckman et al. (2001) showed that TE may suppress mitochondrial respiration. Additionally, TE applications increased cell cytokinin content which is also known to suppress respiration (Ervin and Zhang, 2008; Mok and Mok, 1994). Decreased respiration rate following TE applications reduced the sod roll temperature because heat accumulation is directly attributed to plant respiration (Heckman et al., 2001). Suppression of respiration in conjunction with maintained photosynthetic rate would cause increased net photosynthesis (Ervin and Zhang, 2008). Therefore, sustaining yield suppression with more frequent TE applications may sustain increased net photosynthesis and result in higher TNC concentration.

Turfgrass Color, Quality, and Density

In addition to decreased clipping yield, TE applications have been shown to increase turfgrass visual quality, color, and shoot density (Ervin and Zhang, 2008). Decreased GA_1 concentration causes reduced leaf cell length, increased mesophyll cell density, and increased chlorophyll concentration which causes increased turfgrass color (Ervin and Koski, 2001b; Stier and Rodgers, 2001; Bunnell et al., 2005) . Multiple TE applications have also increased turfgrass tiller density and leaf area (Ervin and Koski, 1998; Beasley and Branham, 2007). Turfgrass quality enhancements have been positively correlated with TE applications on many grass species (Ervin and Koski, 2001b; Goss et al., 2002; Steinke and Stier, 2003). Greater increases in turfgrass color are usually associated with increased plant growth regulator application rates and re-application frequency (Stier et al., 1999; Qian and Engelke, 1999). Visual turfgrass quality is a rating frequently used by turfgrass researchers that integrates numerous turfgrass characteristics including color, uniformity, texture, and density (Skogley & Sawyer, 1992). Increased turfgrass quality following TE applications is most likely a function of increased color and tiller density. Repeated TE applications typically enhanced turfgrass color, quality, and tiller density four to eight weeks after initial TE application (McCullough et al., 2006b).

Root Architecture and Nutrient Uptake

The effect of TE on rooting is not as evident as enhancements in color or quality. Generally, few significant differences in root mass or length have been reported during field experiments with TE for both warm and cool season grasses (Ervin and Koski, 2001a; Fagerness and Yelverton, 2001; Goss et al., 2002; Fagerness et al., 2004; Wherley and Sinclair, 2009).

Two notable exceptions include Bingaman et al. (2001) and Qian and Engelke (1999) who found TE increased rooting strength in transplanted Kentucky bluegrass sod, and turfgrass root mass was increased by 50-60% in shade. More commonly, TE is found to decrease root to shoot ratio because tiller density is increased with no effect on turfgrass rooting (Goss et al., 2002; Beasley et al., 2005). Greenhouse experiments with both warm- and cool-season turfgrass have observed changes in root architecture following TE application (Beasley et al.; 2005; McCullough et al., 2005; McCullough et al., 2006a). Kentucky bluegrass grown hydroponically had increased root diameter and root surface area following TE application (Beasley et al., 2005). Increased tiller density in that study resulted in no change in Kentucky bluegrass root surface area to shoot ratio, and there was no effect on root length (Beasley et al., 2005). Hybrid bermudagrass root mass was found to increase 23-43% after sequential TE applications in a greenhouse (McCullough et al., 2005; McCullough et al., 2006a). However, increased hybrid bermudagrass root mass has not yet been observed under field conditions.

Trinexapac-ethyl Absorption, Translocations, and Metabolism

Fagerness and Penner (1998a) used ^{14}C labeled TE to investigate absorption and translocation within Kentucky bluegrass. The plant base, crown and leave sheaths, absorbed 80% total applied product within the first hour with maximum absorption (90%) after eight hours. Leaf blades absorbed TE 60% of applied TE after 24 hours with 55% absorbed in the first eight hours. Roots absorbed a negligible amount of TE during the experiment. They concluded that TE is both xylem and phloem mobile although a majority of the TE was translocated to turf foliage.

Fagerness and Penner (1998b) also investigated TE efficacy under different spray parameters on Kentucky bluegrass and creeping bentgrass in a greenhouse. They found the growth suppression responded linearly with application rate and that species responded differently with maximum regulation occurring 2-3 weeks after TE application regardless of application rate. Sprayer application volume and ultraviolet degradation had a minimal effect on growth inhibition. Mowing height had an effect on clipping suppression as the high height of cut experienced greater relative growth inhibition.

Once in the plant, TE, is metabolized by the series of enzymatic processes (Eerd et al., 2003). Organic pesticides are metabolized in three phases (Hatzios, 1991; Shimabukuro, 1985). During Phase I metabolism, pesticides are transformed from parent compound to primary metabolites through processes including oxidation, reduction, and hydrolysis. During Phase II, the pesticides are conjugated with sugars, amino acids, or glutathione and are stored in cell organelles before further metabolism to secondary conjugates in Phase III. Toxicity and efficacy are reduced from one phase to the next (Eerd et al., 2003). These processes are enzyme mediated reactions and are therefore subject to Michaelis-Menten kinetics (Taiz and Zeiger, 2006).

Researchers have shown rate of metabolism is related to organism mass and increases exponentially with temperature in all living organisms (Hemmingsen, 1960; Kleiber, 1932). Traditionally this relationship is generally described as the Q_{10} and is only valid across a small temperature range at which a majority of biological organisms functions; $[Q_{10}]^{T/10}$(Gillooly et al., 2001). More recently the idea of universal temperature dependence is used to describe rate of biological processes (Gillooly et al., 2001). This formula relates the Boltzmann factor and body mass to more accurately predict metabolic rate; $B \sim M^{3/4}e^{-E_i/kT}$ where E_i is activation energy, T is temperature (°K) and k is the Boltzmann constant. This formula can remove 15% of the error in the Q_{10} across the biologically relevant temperature range of 0-40°C and is universal for all living organisms (Gillooly et al., 2001).

With knowledge of how temperature affects organism metabolism it's illogical to think that calendar based re-application intervals are efficient. For example, the Primo Maxx label (trade name for a commonly used TE product) states that the 0.05 kg a.i. ha^{-1} application rate will suppress bentgrass putting green yield for four weeks. However, data in Table 1.1 indicates that the duration of suppression is typically much shorter. Lickfeldt et al. (2001) found decreased TE efficacy as the temperatures increased into summer. A similar effect was seen in hybrid bermudagrass during fall (Fagerness et al., 2002). As the daily average air temperature decreased the duration and magnitude of the suppression period increased. McCullough et al. (2007) indicated that weekly application of TE provided more consistent growth suppression compared to bi- and tri-weekly applications when the total annual amount was constant across all application intervals on a creeping bentgrass putting green.

Beasley and Branham (2005) quantified TE half lives in Kentucky bluegrass and creeping bentgrass. Each species was treated with TE and placed in growth chambers set to constant air temperatures of 18 or 30°C. Plants were then harvested after different amounts of time for each specific temperature for trinexapac acid quantification with HPLC-UV. They found the half live of TE in creeping bentgrass to be 6.4 and 3.1 days for the 18 and 30°C growth chambers, respectively. The half lives at 18 and 30°C were 5.3 and 3.4 days for Kentucky bluegrass, respectively. A two year field study was also conducted with similar findings in Kentucky bluegrass during the summer (Beasley et al., 2007). The authors found that increased application rate had little effect on magnitude or duration of the suppression phase.

Rate of TE metabolism is controlled to a greater extent by temperature and not UV degradation (Beasley and Branham, 2005; Fagerness and Penner 1998b). A logical step forward with this research was to develop a model that used air temperature to optimize TE re-applications. The creation of such as model could have profound impacts on turfgrass growth and development. It would provide turfgrass managers a tool that could be used to predict both magnitude of growth suppression and when TE would need to be re-applied to maintain the clipping suppression phase. Implications of sustained yield suppression possibly include increased TNC content, color, quality and tiller density. Additionally, sustained yield inhibition would reduce nutrient removal and nutrient demand and may reduce fertility requirements.

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