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**AUTUMN NITROGEN (N) AND TRINEXAPAC-ETHYL (TE) APPLICATIONS INFLUENCE SPRING
VIGOR AND DENSITY OF C₃ PUTTING GREENS**

A Thesis in

Agronomy

by

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ABSTRACT

Putting greens across the northern United States and Canada are perennially affected by winter damage, which requires costly spring renovation. This damage ensues from any number of winter stresses, or a complex of combined stresses. Little is known about late autumn conditioning of putting green systems by application of soluble N and trinexapac-ethyl (TE) to achieve maximum plant hardiness, and prevent damage.

A field experiment was initiated in Sept. 2009 and 2010 to determine optimal timing of late season N and TE applications, as well as the interactive and/or main effects on tissue N concentrations, spring vigor, and density of an intensively-managed PG within the Pennsylvania State University Valentine Turfgrass Research Center (University Park, PA). The experimental design comprised treatments of N (30 or 60 kg ha⁻¹), trinexapac-ethyl (0, 0.088, or 0.044 + 0.044 kg ha⁻¹), and four autumn application timings; in a completely-randomized, 24-plot arrangement replicated six (2009-2010) or four (2010-2011) times. Resulting turfgrass clipping yield (CY; kg ha⁻¹), tissue N (g kg⁻¹), and canopy density (green normalized differential vegetative index; GNDVI) were measured in March and early-April of 2010 and 2011.

Regardless of N rate, N concentrations of post-application (late autumn) clippings were significantly higher on plots treated before the first hard frost, compared to plots treated after the first hard frost. Although plots treated later in the year contained less tissue N prior to winter, these differences were indistinguishable in spring, when tissue N concentrations were similar across all application dates. As one might expect, plots treated with the high N rate showed significantly greater tissue N in both autumn and spring, compared to plots treated with the low N rate. With regard to autumn tissue N, TE was not a significant influence. However, a difference was discerned

in spring analysis, as plots previously treated by TE showed significantly greater N concentration in spring tissue.

Spring CY decreased linearly over early to late-Fall application dates. Significant treatment interactions were observed for low vs. high N rate, as well as TE treatment. When N and TE were applied in autumn (particularly after the first hard frost), early spring growth was suppressed by residual activity of TE, even when tissue N concentrations were high. Conversely, when applications were discontinued earlier in autumn (before the first hard frost), spring growth was stimulated more so than by application of N alone. This result is likely a manifestation of post-regulation surge, commonly associated with the eventual metabolism of GA-inhibitors in TE.

Spring GNDVI readings were directly related to autumn N rate, but a positive effect of either TE application regimen was observed at $\alpha = 0.1$. Regardless of application date, the high N rate resulted in significantly higher GNDVI readings than the low N rate. As mentioned, application of autumn TE also improved density in spring. Furthermore, the greatest improvement of density is realized when N and TE applications are made prior to the first hard frost, but applications made after the first hard frost also prove worthwhile.

In summary, spring PG tissue N, vigor, and density appear positively influenced by Fall inputs of N & TE, particularly when applied up to 15-d prior to the first hard frost. While N applied after the first hard frost still proved beneficial, the 30 kg ha⁻¹ rate was as effective as the 60 kg ha⁻¹ rate. Late fall N fertilizer applications to internally-drained, sand-based putting greens should not exceed 30 kg ha⁻¹.

A similar study was initiated in Oct. 2010 at the Pennsylvania State University Joseph Valentine Turfgrass Research Center in University Park, PA. A randomized split-plot design of 90

plots was established on a mature, annual bluegrass putting green. The experimental design comprised paired main plots of Phosphorus treatment or none, each containing treatments of N (0, 30 or 60 kg ha⁻¹), trinexapac-ethyl (TE; 0 or 0.088 kg ha⁻¹), and three autumn application timings, in a completely-randomized, 15-split-plot arrangement. Resulting turfgrass canopy density (green normalized differential vegetative index; GNDVI), tissue N (g kg⁻¹), and clipping yield (CY; kg ha⁻¹) were measured in March and early-April of 2011.

The main plot effect of Phosphorus was not a significant source of variation for spring N offtake, tissue N, canopy density, or vigor. Autumn N offtake and tissue N were significantly affected by timing, treatment, and their interaction. Regarding the timing source, cumulative N offtake was greatest from plots treated earliest. The same general trend was observed of mean tissue N in autumn clippings, and was likely due to decreasing temperatures and associated decline in turfgrass assimilation rate. Spring GNDVI and vigor were significantly influenced by N rate, but not TE. While the effect of TE appears to have influenced spring vigor, the TE vs. No TE contrast was not significant at an alpha level of 0.05.

TABLE OF CONTENTS

List of Tables.....	vi
List of Figures.....	vii
Acknowledgements.....	ix
Chapter 1.....	1
Introduction.....	2
Winter Hardiness.....	2
Dehardening Processes.....	5
Winter Damage.....	6
Cultural Methods of Prevention.....	8
Trinexapac-ethyl Plant Growth Regulator.....	12
Socioeconomic Impact.....	14
Justification.....	15
Hypothesis.....	15
Objective.....	16
Chapter 2.....	19
Cohabited putting green study.....	20
Materials and Methods 2009-2010.....	20
Materials and Methods 2010-2011.....	22
Results and Discussion.....	25
Chapter 3.....	48
<i>Poa annua</i> putting green study.....	49
Materials and Methods.....	49
Results and Discussion.....	51
Chapter 4.....	64
Conclusions.....	65
References.....	68

LIST OF TABLES

Table 1-1: Types and symptoms of winter damage that most commonly occur on turfs (adapted from Beard, 1980).....	17
Table 1-2: Cool-season turfgrass resistance to freezing stress (adapted from DiPaola and Beard, 1992).....	18
Table 2-1: Soil chemical properties of 2009 cohabited putting green (0-10 cm), sampled prior to treatment initiation (Sept.).....	32
Table 2-2: Nutrient concentrations of 2009 cohabited putting green leaf tissue sampled prior to treatment initiation (Sept.).....	33
Table 2-3: Soil chemical properties of 2010 cohabited putting green (0-10 cm), sampled prior to treatment initiation (Sept.).....	34
Table 2-4: Nutrient concentrations of 2010 cohabited putting green leaf tissue sampled prior to treatment initiation (Sept.).....	35
Table 2-5: Analysis of variance for pooled (2009-2011) autumn tissue N and N offtake, and spring clipping yield (CY), tissue N, N offtake, and green normalized differential vegetative index (GNDVI).....	36
Table 3-1: Soil chemical properties of 2010 annual bluegrass putting green (0-10 cm), sampled prior to treatment initiation (Sept.).....	54
Table 3-2: Nutrient concentrations of 2010 annual bluegrass putting green leaf tissue sampled prior to treatment initiation (Sept.).....	55
Table 3-3: Analysis of variance for the dependent variable of spring N offtake, collected in spring 2011. Significance was determined at an alpha level of 0.05.....	56
Table 3-4: Analysis of variance for the dependent variable of GNDVI measurement, collected in spring 2011. Significance was determined at an alpha level of 0.05.....	57
Table 3-5: Analysis of variance for the dependent variable of clipping yield, collected in spring 2011. Significance was determined at an alpha level of 0.05.....	58

LIST OF FIGURES

Figure 2-1: Fall weather patterns for 2009 (A) and 2010 (B). Stars represent application dates...	37
Figure 2-2: Mean fall leaf tissue N concentration by treatment (pooled over application dates and years). Mean values having overlapping error bars are not significantly different (Fisher's LSD, $\alpha = 0.05$).....	38
Figure 2-3: Mean fall leaf tissue N concentration for all treatments by application date and year. Mean values having overlapping error bars are not significantly different (Fisher's LSD, $\alpha = 0.05$).....	39
Figure 2-4: Mean fall Nitrogen offtake (2009 and 2010) by treatment and timing after all treatments were applied and mowing was discontinued. For each day of year, the least significant difference between mean values is shown (Fisher's LSD, $\alpha = 0.05$).....	40
Figure 2-5: Mean spring tissue Nitrogen content of each experimental treatment (pooled across all application dates and years). Error bars indicate the least significant difference between mean values (Fisher's LSD, $\alpha = 0.05$).....	41
Figure 2-6: Mean spring tissue Nitrogen for all treatments by application date and year. Mean values having overlapping error bars are not significantly different (Fisher's LSD, $\alpha = 0.05$).....	42
Figure 2-7: Mean spring clipping yield (2010 and 2011) by treatment and timing. For each day of year, the least significant difference between mean values is shown (Fisher's least significant difference, $\alpha = 0.05$).....	43
Figure 2-8: Mean spring clipping yield for all treatments by application date and year. Mean values having overlapping error bars are not significantly different (Fisher's LSD, $\alpha = 0.05$).....	44
Figure 2-9: Mean spring Nitrogen offtake (2010 and 2011) by treatment and timing. For each day of year, the least significant difference between mean values is shown (Fisher's LSD, $\alpha = 0.05$).....	45
Figure 2-10: Mean spring Nitrogen offtake for all treatments by application date and year. Mean values having overlapping error bars are not significantly different (Fisher's least significant difference, $\alpha = 0.05$).....	46
Figure 2-11: Annual green normalized differential vegetative index (GNDVI) by treatment and timing in 2010 (A) and 2011 (B). For each day of year, the least significant difference between mean values is shown (Fisher's LSD, $\alpha = 0.05$).....	47
Figure 3-1: Autumn 2010 N off-take of all plots after all treatments were applied and mowing was discontinued for the year.....	59

Figure 3-2: Fall 2010 tissue N content analyzed after all treatments were applied and mowing was discontinued for the year.....	60
Figure 3-3: Spring 2011 N offtake of all experimental treatments averaged across application date.....	61
Figure 3-4: Spring 2011 GNDVI readings of each experimental treatment averaged across all application dates.....	62
Figure 3-5: Spring 2011 CYs of each experimental treatment averaged across all application dates.....	63

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The completion of this thesis shall signify the closing of a successful chapter, and the exciting beginning of a new.

CHAPTER 1

INTRODUCTION

Putting greens are the most valued and intensively managed areas comprising any golf course (Schlossberg, 2006; Schlossberg and Schmidt, 2007). Putting greens in the Northern United States and Canada are primarily composed of creeping bentgrass (*Agrostis stolonifera* L.) and annual bluegrass (*Poa annua* L.). These putting greens perennially suffer winter damage, which can require costly spring renovation. Winter damage ensues from a complex of related stresses: freezing temperatures, crown hydration, traffic, desiccation, frost-heaving and low temperature pathogenesis. Plant crown health is crucial to surviving cold stress (DiPaola and Beard, 1992), as prolonged exposure to sub-optimal temperatures may severely damage golf course putting greens. While numerous studies report freeze tolerance of C₄ grasses, research conducted on C₃ turfgrasses has been more limited, specifically in relation to green-height turfgrass systems and autumn cultural practices.

Winter Hardiness

Due to the intensive management imposed, putting greens have limited cold temperature tolerance and are most prone to winter damage (Levitt, 1980). Cold temperature tolerance is a function of plant hardiness, and the terms are synonymous. Therefore, a turfgrass plant possessing tolerance for cold temperature stress is also 'hardy.' Plant hardiness is a widely accepted turfgrass term, but is not a quantifiable measurement. Plant hardiness develops as autumn temperatures decline from the optimum shoot-growth temperature range of 15-24°C (DiPaola and Beard, 1992). As turfgrasses become acclimated to cold temperatures, several metabolic processes facilitate hardiness (Dionne et al., 2001ab).

During late autumn, turfgrass shoot growth subsides and photoassimilates (carbohydrates) accumulate. Enzymes convert these carbohydrates to soluble sugars in the cytosol, which is part of the cell's protoplasm. Concurrent synthesis of proteins enables a boosted capability to bind water within the protoplasm of the crown tissue cells. These soluble sugars then increase osmotic potential of the protoplasm. Generally, this transpires over a three to four week period (Beard, 1973; Carrow et al., 2001). Active growth negatively influences hardiness because growth processes reduce carbohydrate stores and hydrate the cytosol (Beard, 1973; Taiz and Zeiger, 2006). As turfgrass plants build carbohydrate reserves, cold temperature tolerance improves (Davis and Gilbert, 1970).

Patton et al. (2007), demonstrated the role of carbohydrates in freeze stress tolerance, specifically as it relates to warm season grasses like zoysiagrass (*Zoysia* spp.). Carbohydrates protect membranes through colligative action of low molecular-weight sugars (Santarius, 1982). In theory, increased carbohydrates should result in greater tolerance of low temperatures.

Because carbohydrates play such an important role in establishing plant hardiness, we must understand the composition of these carbohydrates. Polysaccharides, or high molecular weight (HMW) storage carbohydrates of monocot grasses are predominantly fructosans and starch (Carrow et al., 2001; Taiz and Zeiger, 2006). Disaccharides (sucrose) and monosaccharides (fructose and glucose) are mobile, low molecular weight (LMW) carbohydrates. Sucrose is the principal carbohydrate transported throughout the plant (Taiz and Zeiger, 2006).

Literature relating temperature and carbohydrate reserves of turfgrasses is variable and conflicting (Beard, 1973). Carbohydrates accumulate as decreasing temperatures depart from the

optimal growth range (Beard, 1973; Pollock and Cairns, 1991). Some literature cites excessive N fertilization as detrimental to plant hardiness (Beard, 1973; Carrow et al., 2001).

The low range of annual N requirement for cool-season turfgrasses is 88.2-147 kg ha⁻¹. The moderate range for cool-season grasses is 147-294 kg ha⁻¹. The high N range is 294-392 kg ha⁻¹ (Carrow et al., 2001). As N fertility increases from very low to moderate levels, carbohydrate reserves are stored in roots, stolons, rhizomes, stem bases, and leaf bases. Conversely, increasing N from moderate to higher levels, carbohydrate reserves decline in cool-season grasses as a shift in the allocation of photoassimilates (carbohydrates) turns to shoot growth and leaf elongation. This function results in diminishing levels of carbohydrate reserves in tissue (Carrow et al., 2001). However, if N is applied in late autumn when metabolic rates are low and temperatures are not within the optimal range for actively growing meristems, then N fertility may stimulate chloroplast synthesis, photosynthesis, and accumulation of photoassimilates.

A previous study provides information regarding amino acids, protein, and carbohydrate changes in annual bluegrass and creeping bentgrass during cold acclimation. Dionne et al. (2001b) investigated amino acid changes in relation to winter hardiness of three annual bluegrass ecotypes. They reported no significant results. However, another of their studies reported significant carbohydrate changes during cold acclimation of the same three annual bluegrass ecotypes. In this study (Dionne et al., 2001a), they initiated three temperature-conditioning treatments on each of the three ecotypes and reported LT₅₀ values (lethal temperature that kills 50% of plant population) ranging from -17.4 to -21.7 °C. Plants acclimated above freezing temperatures (2°C) in an unheated greenhouse for two weeks accumulated both more HMW and LMW carbohydrates than non-acclimated controls. These conditioning-treatments continued to compile HMW carbohydrates when incubated at 2°C for an additional two weeks; however, LMW

carbohydrates declined. Thus, maximum hardiness may have been attained, redirecting the plants energy to produce HMW carbohydrates for winter storage (Dionne et al., 2001a).

Several experiments explored the role of carbohydrates in winter hardiness of different grass species. Suzuki and Nass (1988) reported an association between HMW carbohydrates (fructans) and hardiness in winter cereal cultivars. Likewise, Kiyomoto and Bruehl (1977) linked snow mold resistance to carbohydrate accumulation in winter wheat varieties. Yet, Dionne et al. (2001a) observed that LMW carbohydrates provide better hardiness in annual bluegrass ecotypes under environmentally controlled conditions. However, they did not observe this same correlation in trials that simulated winter conditions (Dionne et al., 2001a). Thus, determining what role carbohydrates play in winter hardiness appears compromised by simulated conditions and may require field experimentation.

Dehardening Processes

As winter passes, plant hardiness declines. This decline causes problems in late February and early spring when temperatures fluctuate considerably. As temperatures increase, snow melts and crown tissue begins to hydrate. Therefore, the plant becomes more vulnerable to 'crown hydration' winter damage. Additionally, warm temperatures can stimulate an increase in metabolic activity, which also results in diminished hardiness (Beard, 1973).

'Dehardening' describes a process that takes place in spring when plant hardiness diminishes. Tompkins et al. (2000) observed the dehardening of creeping bentgrass and annual bluegrass as it relates to soil temperature and crown hydration, and reported plant hardiness declined as soil temperatures increased. Crown hydration level also increased with soil temperature. When snow cover persisted, plant hardiness lasted longer. Furthermore, recovery of

hardiness was limited following a period of increased soil temperature and lack of snow cover. Consequently, the plant becomes more susceptible to winter injury following a period of dehardening and/or warming soil temperatures. This accentuates the need for research that investigates ways of maximizing plant hardiness and maintaining that hardiness late into spring, even when temperatures pre-maturely warm.

Winter Damage

Desiccation

Plant desiccation, often referred to as 'wind burn,' follows extended winter drought, and is a form of winter stress not dissimilar of summer drought stress. Simple leaves transpire water at temperatures >0 °C. In this case, the plant becomes vulnerable when soil water is frozen and/or unavailable. Elevated turfgrass areas exposed to high winds are more susceptible to desiccation than turfgrass growing in swales and/or depressions (Beard, 1980). Likewise, annual bluegrass is more susceptible to desiccation than creeping bentgrass (Larsen and Horgan, 2010).

Crown Hydration

Crown hydration occurs when hydrated crown tissue cells freeze and rupture following a dramatic temperature decline. Crown hydration is responsible for significant winter turf loss. This form of winter damage continues to frustrate superintendents of temperate and colder climates (Larsen and Horgan, 2010). Crown hydration is difficult to prevent and is the most detrimental form of winter damage. Submerged turf crowns are increasingly susceptible to cell rupture during freezing and thawing cycles (Roberts, 1995; DiPaola and Beard, 1992). Intracellular freezing is a result of accelerated temperature decrease and is usually lethal (Levitt, 1980). Periods of snowmelt in spring are critical. Extreme temperature fluctuations cause rapid freezing of standing water on

putting surfaces, leaving plant crowns susceptible to crown hydration. Consequently, surface drainage remains an important component of minimizing crown hydration injury (Larsen and Horgan, 2010). Because annual bluegrass maintains higher moisture content than creeping bentgrass (Tompkins et al., 2000), it is more susceptible to this type of injury (Larsen and Horgan, 2010). Additionally, creeping bentgrass stores greater carbohydrate reserves, allowing for prolonged dormancy through late winter and early spring when temperature fluctuations are most dramatic (Tompkins et al, 2000; Larsen and Horgan, 2010).

Suffocation

Anoxia and/or toxic gas accumulation can result from prolonged ice cover on turfgrass areas (Levitt 1980; Beard, 1980). Most research states that turf loss is a concern after sixty to ninety days of ice cover (Beard, 1980; DiPaola and Beard, 1992). A sulfide odor characterizes suffocation, and manifests over prolonged anaerobic soil conditions (Beard, 1980). Research suggests that microbial activity and respiration play a role in the accumulation of toxic gases. Thus, turfgrasses grown on soils high in organic matter (push-up style greens) are more susceptible to anoxia when gas exchange is restricted (Rochette et al., 2006).

Frost Heaving

Heaving occurs when repeated freezing and thawing pushes plants out of normal position and disrupts the crown/root/soil continuum. Although well-established turfgrass systems are rarely subject to heaving, it remains a cause of winter damage (Beard, 1980).

Pathogens

Typhula blight, caused by gray snow mold (*Typhula incarnata* L.), and Microdochium patch, caused by pink snow mold (*Microdochium nivale* L.), are winter diseases of creeping bentgrass and *Poa annua* systems. Typhula blight is prevalent at temperatures between -1 and 12.7° C (Couch, 1973). This disease develops when snow begins to melt, and is exceptionally destructive to turfgrasses that have not hardened off (Vargas, 1994). Typhula blights develop when prolonged periods of snow cover prevent the soil from freezing or allow frozen soil to thaw through geothermal heat flux. This phenomenon creates an atmosphere is at or near freezing temperatures (Smiley et al., 2007). The freezing temperatures create an optimal environment for *Typhula* pathogenicity, which depletes plant vascular and cellular tissue of stored carbohydrates (Kiyomoto and Bruehl, 1977).

Microdochium patch is prevalent at high atmospheric humidity and air temperatures between 0 and 7° C. Incidence of Microdochium patch is not limited to snow cover. It may also occur under wet, cool conditions of autumn or spring (Tani and Beard, 1997). This disease spreads through spore (conidia) distribution by personnel, equipment, and other mechanical activity. In the presence of favorable weather conditions, germinating conidia and mycelium grow from previously colonized plant debris, which infects new turfgrass plants (Smiley et al., 2007). Table 1-1 provides a summarized physical description of the aforementioned winter injuries.

Cultural Methods of Prevention

Superintendents employ an array of cultural practices that help prepare putting greens for winter, but even using 'textbook' practices may not be enough to prevent injury. Preparing a golf

course putting green for winter is a combination of conventional cultural practices, which include fertility regimen, topdressing, and Integrated Pest Management (IPM).

Nutrition/Soil Fertility

Potassium fertilization in autumn is important, and effective autumn fertilization regimens are imperative to winter survival. Autumn potassium applications improve freeze tolerance (Roberts, 1995; Beard, 1980; Beard, 1973). Turfgrass fertilized with a 1:2 N:K ratio showed 30 percent less winter injury compared to turfgrass not receiving potassium (Roberts, 1995). Additionally, Razmjoo et al. (1996) reported nearly a 65% increase of K in bentgrass tissue when fertilized with N at 96 kg ha⁻¹ as compared to plots fertilized at 8 kg ha⁻¹.

Phosphorus plays an important role in the synthesis of starch and glucose in C3 plants. Starch is synthesized in the chloroplast, while glucose is synthesized in the cytosol (Taiz and Zeiger, 1991). Hence, glucose plays an important part in dehydrating the cytosol in the autumn hardening process. When cytosolic orthophosphate (Pi) concentration is high, glucose synthesis occurs. When the cytosolic Pi concentration is low, starch synthesis occurs in the chloroplast. In rapidly growing plants, glucose, not starch, is the prominent product of photosynthesis. When glucose is not needed for actively growing plant parts, starch may be the predominant product of photosynthesis (Taiz and Zeiger, 1991). Regardless, proper P fertility contributes to synthesis and transport of all photoassimilates.

Nitrogen fertilization is an integral part of putting green management (Schlossberg, 2006). Late season N applications can stimulate accumulation of carbohydrates (Carrow et al., 2001). Some have suggested that autumn N applications can increase susceptibility to winter damage. However, this has not been observed at appropriate rates (Carrow et al., 2001). Ammonium-based

N sources stimulate the uptake of phosphorus and manganese (Schlossberg, 2006), which is helpful when availability of these plant essential nutrients is limited.

An experiment conducted on lawn-height turfgrass systems showed better autumn color retention and earlier spring green up on plots that were N-fertilized in late autumn (Walker et al., 2007). The effects of eight different N rates, ranging from 0 to 196 kg ha⁻¹ yr⁻¹, were observed on this two-year field study, which evaluated the above-ground response of Kentucky bluegrass (*Poa pratensis* L.; KBG), perennial ryegrass (*Lolium perenne* L.; PRG), and turf-type tall fescue (*Festuca arundinacea* Schreb.; TTTF). This study indicated late-autumn N applications to KBG facilitated timely spring green-up. Additionally, Walker et al. (2007) reported that a single N application of 73 kg ha⁻¹ yr⁻¹ in November on TTTF resulted in better canopy greenness than treatments applied in September. The study reported summer pathogen incidence, but not winter pathogen incidence. Therefore, a need to investigate the influence of late-season N on winter disease incidence exists.

A study administered by Powell et al. (1967) reported the effects of autumn and winter N fertilization of Cohansey bentgrass (*Agrostis palustris* Huds.) and 'Kentucky 31' tall fescue in Virginia. They reported that soluble N applied during autumn and winter increased the metabolic activity and color of leaf tissue. Plots that were fertilized with soluble N throughout the winter maintained better foliage color than plots that received their final N application in October. They determined that late autumn and winter applications of soluble N did not deplete carbohydrate reserves. Conversely, reserves depleted when N was applied during times of active top growth. Plants that received a high N rate of 100 kg ha⁻¹ each month, October through February, produced more photosynthates than plants fertilized with a single N application of 50 kg ha⁻¹ in October. They also determined that temperatures restricted top growth, but did not restrict photosynthesis to

the same degree. In addition, snow mold incidence was discovered near the experimental turf; however, there was no snow mold present in the high N plots.

Integrated Pest Management

Species selection is critical at establishment. Different species show variable tolerance to winter damage (Table 1-2). Contrasting cultivars within a species may also reflect variable tolerances. Many older golf courses depend on older cultivars, which may have lower resistance. However, re-establishment is an intensive and costly process that is rarely a practical option.

Winter diseases cause problems regardless of Autumn N rates, but fungicides control diseases when no other options exist (Tani and Beard, 1997). Turf managers routinely apply fungicides as a preventive application on many golf course putting greens, fairways, and tees. Several different products on the market prevent snow molds. Applications are typically made late in the season before the first snow. Fungicide efficacy may vary substantially depending on application timing, weather conditions, and snow cover duration (Worf, 1988).

Pentachloronitrobenzene (PCNB) has been widely applied to control pink and gray snow molds on golf courses in the northern United States (Worf, 1988; Landschoot et al., 2001). Chlorosis of actively growing turf following PCNB application has been reported; however chlorosis of dormant turf following PCNB application is less common (Landschoot et al., 2001). Smith et al. (1989) reported that PCNB applied in autumn was effective in controlling microdochium patch. Surprisingly, they also reported that PCNB selectively suppresses antagonists of *M. nivale*, which is a causal agent of microdochium patch. Little research has evaluated injury and disease augmentation (Landschoot et al., 2001). Consequently, research that investigates methods for enhancing turfgrass plants' natural defense mechanisms to overwintering pathogens is needed.

Topdressing

Superintendents often apply a thick layer of sand topdressing to putting greens in late autumn to help insulate and protect crown plant tissues. Topdressing applications increase freeze tolerance. Topdressing applied in the autumn will often leave only the plant tip exposed. This heavy topdressing helps insulate and protect the integrity of the crown (DiPaola and Beard, 1992). Additionally, topdressing dilutes problematic thatch that can harbor pathogenic agents (Vargas, 1994). Topdressing applications are relatively more effective at protecting the crown from desiccation injury than crown hydration (Larsen and Horgan, 2010). A thick topdressing layer will not prevent surface water from freezing and causing winter damage.

Trinexapac-ethyl Plant Growth Regulator

Trinexapac-ethyl (TE) is a plant growth regulator (PGR) commonly used in turfgrass management, especially in regard to putting green surfaces. Trinexapac-ethyl is a sprayable, microemulsion concentrate that inhibits gibberellic acid production late in the biosynthetic pathway by disrupting the conversion of GA₂₀ to GA₁ (Syngenta Crop Protection, LLC, 2011; Arteca, 1996). Gibberellin is a plant growth substance containing an ent-gibberel-lane skeleton that fosters cell division and/or elongation by the same means as gibberellic acid (GA₃). Gibberellin is active in many physiological processes in the plant including stem growth, bolting/flowering, seed germination, dormancy, sex expression, senescence, parthencarpy, fruit set, and growth (Arteca, 1996).

When applied to turfgrass systems, TE can reduce vertical shoot growth with minimal or no phytotoxicity, and often results in improved color. Additionally, TE increases tiller density without a reduction in root growth (Syngenta Crop Protection, LLC, 2011; Lickfeldt et al., 2001). This

increase in tiller density is a result of re-directed energy; rather than producing new leaves, the plant produces more tillers (Branham and Beasley, 2007). Tiller production usually increases about four weeks after treatment. When TE begins to lose efficacy, the extra tillers contribute to more total leaf production and thus generate more clippings than the turf would normally. This phenomenon is known as the rebound effect or post-regulation surge (Branham and Beasley, 2007). The rebound effect may be avoided by continual application of TE, which will suppress leaf elongation on the new tillers (Branham and Beasley, 2007).

Trinexapac-ethyl is absorbed through the foliage and the recommended application rate on green-height creeping bentgrass and annual bluegrass turfgrass systems is 0.05 kg ha⁻¹. This rate should provide approximately 50% growth reduction for up to four weeks. However, weekly applications are permissible, as long as the user does not exceed an annual rate of 2.7 kg ha⁻¹ (Syngenta Crop Protection, LLC, 2011).

Syngenta Crop Protection is the manufacturer of TE, and its trade name is Primo Maxx. Primo Maxx came on the market in 2002 and is classified as a "Class A" PGR (Thorne, 2006). The label states that TE can be used as a pre-stress conditioner for summer heat. When applied to actively growing turf before the onset of stress, TE can improve survival of stressed turfgrass. Likewise, the application of TE can reduce water use and improve drought tolerance (McCann and Huang, 2007). However, little is known about TE as a pre-stress conditioner for winter stresses such as crown hydration and wind desiccation. A study by Syngenta's field biology team researched TE's relevant application for winter pre-stress conditioning, and found that additional applications in the autumn improved chlorophyll content and total root mass production, which may facilitate healthier turf in spring. However, more research is required to validate these findings (Thorne, 2006).

McCullough et al. (2007) administered a study that observed the influences of TE on growth, quality, and performance of bermudagrass (*Cynodon* spp.) and bentgrass putting greens through the summers of 2003 and 2004 in Clemson, SC. They reported discoloration of bermudagrass from several different treatment regimens; however, they did not observe bentgrass discoloration at any rate. As compared to the non-treated, bentgrass clipping yields were reduced in plots receiving weekly, semimonthly, and every third week treatments of TE at 0.017 kg ha⁻¹, 0.033 kg ha⁻¹, and 0.05 kg ha⁻¹, respectively. Additionally, they reported no negative rooting effects on plots treated with TE.

McCullough et al. (2006) further examined bermudagrass putting green growth, color, and nutrient partitioning as influenced by nitrogen and TE. Root masses of TE treated bermudagrass were similar to the untreated; however, they reported a 5% increase in stolon and rhizome mass, and 18% increase in chlorophyll concentration. Although leaf N, P, K, Mg, S, and Fe concentrations decreased on TE treated bermudagrass, rhizome concentrations increased 8-36%. Consequently, a net increase in bermudagrass nutrient retention was reported on TE treated plots.

Socioeconomic Impact

Turf killed by winter damage leads to soil erosion, reduced aesthetics, and expensive and stressful turf reestablishment (DiPaola and Beard, 1992). When snow recedes in spring, golfing members are ready to play golf. Golfer expectations are higher than ever. Winter damage that results in dead turf may force a superintendent to temporarily close an entire putting green complex. This scenario is stressful for golfers and superintendents alike. Renovation is never speedy and subject to the mercy of erratic spring weather patterns. Furthermore, the club can lose revenue generated by 'playing rounds,' all while spending thousands of dollars to renovate a

damaged putting green. Unfortunately, the golf course Superintendent can be fired over such a case.

Justification

The proposed experiment will investigate how winter damage may be prevented by timing and rate of N and TE applications in autumn. Because there are no definitive 'treatments' (chemical sprays) that protect putting greens from damage such as crown hydration and desiccation, the cultural practices employed must be useful and effective. Accurate application rates and application timing are imperative to winter preparation. The proposed experiment will evaluate different N rates, different TE rates, and different application timings. The resulting data will be useful for drawing conclusions on how to prevent winter damage using Nitrogen and TE fertility. In addition, the system of interest ('Penn G-2' creeping bentgrass and annual bluegrass mixture) is used commonly on putting greens, tees, and fairways across the Northern United States and Canada.

Hypothesis

Compared to the moderately low rates currently applied in autumn, increased but accurately timed applications of N and/or Trinexepac-ethyl (TE) may increase winter survival and spring density without stimulating early spring top-growth; thus affording autumn color retention, timely spring green up, and cold temperature stress tolerance.

Objective

To determine optimal timing of late season N and TE applications to cool season turfgrasses, as well as the interactive or main effects of N rate and TE treatments, on winter survival and spring vigor of intensively-managed putting green systems.

Table 1-1: Types and symptoms of winter damage that most commonly occur on turfs (adapted from Beard, 1980).

Type of winter damage	Symptoms
Desiccation (wind burn)	Leaves turn distinctly white but remain erect; occurs most commonly on higher locations that are more exposed to drying winds; can range from small, irregular patches to extensive kill of large areas
Direct Low Temperature Kill/Crown Hydration	Leaves initially appear water-soaked, turning whitish-brown and progressing to a dark brown; the leaves are limp and tend to lie as a mat over the soil; putrid odor is frequently evident; occurs most commonly in poorly drained areas such as soil depressions; frequently appear as large, irregular patches
Fusarium Patch (Pink Snow Mold)	Pink mycelium on leaves; 1 to 2 inch, tan, circular patches (in autumn); or white mycelia mass on leaves, white to pink circular patches up to 2 feet in diameter (in winter/spring)
Typhula blight (Gray Snow Mold)	Light gray mycelium on leaves, especially at the margins of the advancing ring; whitish-gray, slimy circular patches of up to 2 feet in diameter; brown sclerotia are ranging up to 1/8 inch in diameter.

Table 1-2: Cool-season turfgrass resistance to freezing stress (adapted from DiPaola and Beard, 1992).

Turfgrass species	Genus	Crown moisture %	Ranking	LT₅₀ °C
Rough bluegrass	Poa	72	Excellent	--
Creeping bentgrass	Agrostis	54-61	Excellent	-35
Bromegrass	Bromus	--	--	-30
Kentucky bluegrass	Poa	73-78	Good	-21 to -30
Canada bluegrass	Poa	--	Good	--
Colonial bentgrass	Agrostis	--	Good	--
Redtop	Agrostis	--	Good	--
Annual bluegrass	Poa	80	Medium	-17.4 to -21.7 (added from Dionne et al. 2001a)
Creeping red fescue	Festuca	78	Medium	-24
Tall fescue	Festuca	74-77	Medium	--
Alkaligrass	Puccinellia	--	--	-21 to -27
Hard fescue	Festuca	--	--	-21
Perennial ryegrass	Lolium	79-81	Poor	-5 to -15
Annual ryegrass	Lolium	80	Very poor	--

CHAPTER 2

COHABITED PUTTING GREEN STUDY

Materials and Methods 2009-2010

A study was initiated in Sept. 2009 at the Pennsylvania State University Joseph Valentine Turfgrass Research Center in University Park, PA. A randomized split plot design comprising 144 plots (91 x 183 cm) was established on a mature putting green cohabited by 'Penn G-2' creeping bentgrass and annual bluegrass. Plots were mowed at 3.2 mm 6 d week⁻¹, and clippings were not returned. The putting green received maintenance fertilizer applications in early Sept. 2009 using urea (46-0-0), and granular K₂SO₄ (0-0-50) and MgSO₄ at rates of 40, 300, and 110 kg ha⁻¹, respectively. Soil of plugs sampled from the 0-10 cm depth of four randomly-selected split-plots within each main-plot were composited on 28 Sept. for analysis of soil pH (1:1), cation exchange capacity (CEC), and Mehlich-3 extractable nutrient concentrations (Table 2-1) by the Pennsylvania State Univ. Agric. Analytical Services Lab (PSU-AASL). Leaf tissue samples were also collected on 28 Sept. and analyzed by PSU-AASL for baseline nutrient concentrations (Table 2-2).

On 30 Sept. 2009, six treatments were applied in a 1010 L ha⁻¹ spray volume to randomly-selected plots in each of six main plots (comprising three replicated blocks in total). A CO₂-pressurized, single-nozzle (Tee-Jet TP11008E, Spraying Systems Co., Wheaton, IL), wand-sprayer was used to apply N at 30 or 60 kg ha⁻¹ (1:1 urea-N:NH₄NO₃-N) in a tank-combination of trinexapac-ethyl (4-[Cyclopropyl- α -hydroxymethylene]-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester) growth regulator (Primo MAXX 11.3% a.i.) at 0, 0.044, or 0.088 kg ha⁻¹. Plots initially treated with trinexapac-ethyl (TE) at the 0.044 kg ha⁻¹ rate received a second TE application at the 0.044 kg ha⁻¹ rate, 8 days after initial treatment (DAIT). Thus, all plots treated with TE received a total of 0.088 kg ha⁻¹ in either a full- or split-application regimen. The identical procedure was repeated on

randomly-selected plots remaining in each main plot on 10 Oct., 21 Oct., and 1 Nov., 2009. Clipping yield (CY) was collected 21 Oct. from plots treated on the first two application dates (22 or 11 DAIT). On 12 Nov. 2009, CY was collected from the remaining plots (22 or 11 DAIT). Oven-dried (60 °C) clipping yields were weighed, ground to pass a 0.15-mm sieve, and analyzed for total C and N content by a medium-temperature, furnace combustion CHNS analyzer (EA-1110, CE Instruments, Milan, Italy). Autumn N-offtake (kg N ha⁻¹) was calculated as the product of CY and tissue N concentration on a per plot basis.

Putting green mowing was discontinued in late Nov. 2009. One main plot in each block was randomly selected for preventative treatment of snow mold by pentachloronitrobenzene (PCNB; 24% a.i.) at a rate of 10.6 kg ha⁻¹ on 3 Dec. 2009. Plots were not covered over the subsequent winter season.

The putting green was mowed at a height of 3.6 mm on 23 Mar. 2010. Multiple canopy reflectance measures were collected from all plots 25 Mar. and 1 Apr. 2010 using a Crop Circle ACS-210 radiometer (Holland Scientific, Lincoln, NE). The ACS-210 measures reflectance from light emitted by a modulated polychromatic light emitting diode (LED) array at 590– (VIS₅₉₀) and 880–nm (NIR₈₈₀), and is considered an *active* sensor. The sensor was held at waist height to provide a plot ‘field of vision’ approximately 64-cm wide. Reflectance measures were logged six to eight times each second while traveling lengthwise along each plot center, compiling 15-25 readings per experimental unit. A Trimble Pro XRS global positioning system (GPS) receiver and TSCe field computer (Trimble Navigation Limited, Sunnyvale, CA) simultaneously logged both reflectance and location of each reading. Reflectance values collected from inter-plot borders were subsequently purged from each geo-referenced data set. Paired reflectance measures were used to calculate green normalized vegetation indices (GNDVI; Eq. [1]) as shown (Schmidt et al., 2009):

$$\frac{NIR_{880} - VIS_{590}}{NIR_{880} + VIS_{590}} = GNDVI \quad \text{Eq. [1]}$$

Compiled GNDVI values, describing Spring turfgrass canopy density, were averaged on a per-plot basis. Clipping yield (CY), a measure of turfgrass growth rate and vigor, was collected from all plots on 6 Apr. 2010. Clippings were oven dried and weighed. Sub-samples were ground in preparation for analysis of C and N content (as previously described). Spring N-offtake (kg N ha⁻¹) was calculated as the product of CY and tissue N concentration on a per-plot basis. Effects of treatments, and their interactions, were statistically analyzed using PROC MIXED (SAS Institute, 2001).

Materials and Methods 2010-2011

A study was initiated in October 2010 at the Pennsylvania State University Joseph Valentine Turfgrass Research Center in University Park, PA. A randomized complete-block design of 96 plots (91 x 183 cm) was established on a mature putting green cohabited by 'Penn G-2' creeping bentgrass and annual bluegrass. Plots were mowed at 3.2 mm 6 d week⁻¹, and clippings were not returned. The putting green received maintenance fertilizer applications throughout the summer of 2010, and was fertilized on 28 Sept. 2010 with granular K₂SO₄ (0-0-50) at a rate of 122 kg ha⁻¹. As described, soil from plugs of four randomly-selected split-plots within each block were composited for fertility analysis (Table 2-3). Likewise, turfgrass leaf tissue was collected on 29 Sept. 2010 and submitted to PSU-AASL for analysis of baseline tissue nutrient concentrations (Table 2-4).

On 1 Oct. 2010, six treatments were applied in a 1010 L ha⁻¹ spray volume to randomly-selected plots in each of 4 blocks. A CO₂-pressurized, single-nozzle (Tee-Jet TP11008E, Spraying

Sytems Co., Wheaton, IL) wand-sprayer was used to apply N at 30 or 60 kg ha⁻¹ (1:1 urea–N:NH₄NO₃–N) in a tank-combination of trinexapac-ethyl (4-[Cyclopropyl-a-hydroxymethylene]-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester) growth regulator (Primo MAXX 11.3% a.i.) at 0, 0.044, or 0.088 kg ha⁻¹. Plots initially treated with trinexapac-ethyl (TE) at the 0.044 kg ha⁻¹ rate received a second TE application at the 0.044 kg ha⁻¹ rate, 6 days after initial treatment (DAIT). Thus, plots treated with TE received a total of 0.088 kg ha⁻¹ in either a full– or split–application regime. The identical procedure was repeated on randomly-selected plots remaining in each block on 10 Oct., 20 Oct., and 30 Oct., 2010. Clipping yields (CY) were collected 8 Oct. from all plots treated on the first application date. Clipping yields were collected on the 19 Oct. for all plots treated on both the first and second application date. Clipping yields were collected on 28 Oct. from all plots treated on the first, second, and third application dates. Likewise, CY were collected on 10 Nov. from all 96 plots. Oven-dried (60 °C) clipping yields were weighed, ground to pass a 0.15-mm sieve, and analyzed for total C and N content as described. Autumn N-offtake was calculated using CY and tissue N as described.

Putting green mowing was discontinued after the final cutting on 10 Nov. 2010. Plots were not covered over the subsequent winter season.

The putting green was mowed at a height of 2.8 mm on 18 Mar. and 3 Apr. 2011 to remove desiccated leaf tips. Multiple canopy reflectance measures were collected from all plots on 30 Mar. and 7 Apr. 2011 using the aforementioned Crop Circle ACS-210 radiometer.

Compiled GNDVI values, describing Spring turfgrass canopy density, were averaged on a per-plot basis. Clipping yield (CY), a measure of turfgrass growth rate and vigor, was collected from all plots on 14 Apr. 2011. Clippings were oven dried and weighed. Sub-samples were ground

in preparation for analysis of C and N content (as previously described). Spring N-offtake was calculated as previously described.

Results and Discussion

Autumn N Assimilation and Growth

Nitrogen is removed from a turfgrass system every time turfgrass is mowed and clippings are removed. Thus, it is important to understand how much N is assimilated and removed from the system due to subsequent clippings.

Autumn tissue N concentrations were affected by year, treatment, and timing; however an interaction of timing and year was highly significant. Nitrogen rate was the primary treatment influence of autumn tissue N concentrations following application (Table 2-5). Regardless of TE, timing, and/or year, plots receiving the 60 kg ha⁻¹ N applications showed greater tissue N concentration than plots treated at the lower N rate. While statistically significant, the observed increase was marginally representative of the associated two-fold increase in water-soluble N delivery (Figure 2-2). Yet the observed leaf tissue N concentrations of all treated plots reside within the N sufficiency range for putting greens cohabited by annual bluegrass and creeping bentgrass (Carrow et al., 2001).

Application timing significantly affected leaf tissue N by year. Data show plots treated on the first two 2009 application dates, prior to the first hard frost (DOY 287; Figure 2-1), maintained similar tissue N levels (ranging from 40-42 kg ha⁻¹). Plots treated by applications after the first hard frost in 2009 maintained lesser tissue N concentrations, ranging from 32-34 kg ha⁻¹ (Figure 2-3). The drastic drop in tissue N for the latter two application dates is attributed to colder temperatures (Figure 2-1), and a reduced rate of physiological assimilation by plant leaf and root tissue following the first hard frost.

Contrastingly, the same effect was not observed in the autumn of 2010 (Figure 2-3), when the first hard frost did not occur until after all treatments were applied (DOY 306; Figure 2-1). Thus,

the plants remained more physiologically active throughout the fall and showed only a slight linear decline in N assimilation over the application timings. Regardless, leaf tissue N concentration was greater in autumn 2009 than 2010, likely the residual effect of yearly variation in summer fertilization practices.

Autumn N offtake was affected by treatment, timing, and their interaction. Nitrogen and TE rates were the primary treatment influences of autumn N offtake following application, yet N and TE did not interact (Table 2-5). Contrast of 30 vs. 60 kg N ha⁻¹ treatments shows N rate to directly influence subsequent N offtake (Table 2-5), and is in agreement with recent literature describing similar turfgrass systems (Schlossberg and Schmidt, 2007). At any given N rate, plots not treated by TE showed greater N removed from the system than TE-treated plots (Table 2-5). This observation is the likely result of vegetative growth suppression by TE. Despite possessing similar N concentration in leaf tissue, TE treatment significantly limited autumn N removal from the system.

The influence of application timing on autumn N offtake, pooled over both years, is clearly shown (Figure 2-4). This observed decline in N offtake over successive autumn application dates is expected as more N removal opportunities were provided for earlier-applied treatments, compared to those receiving only a single cutting after treatment, i.e. application day of year (DOY) 304. However, increasing exposure to suboptimal growing conditions may also have been a contributing factor. Autumn N offtake, pooled over both years, was influenced by an interaction of timing and treatment. Essentially, the previously described treatment effects were more pronounced in early than late autumn (Figure 2-4). While autumn N offtake was significantly affected by an interaction of timing and year, year differences for any given timing mirrored leaf tissue N observations (Figure 2-3).

To summarize the results of late season N and TE treatment on autumn N assimilation and growth; climatic conditions associated with early application dates favored traditional treatment responses. However, compared to in-season response to varying N rates by similar putting green systems, a significant increase in late season N rate had minor influence on leaf tissue N and N offtake. For example, a two-fold increase in putting green annual N rate (146 to 293 kg ha⁻¹) has been shown to result in relative increases of 90% in N uptake and 17% greater leaf tissue N (Schlossberg and Schmidt, 2007). Pooled over both years of this study, autumn N offtake ranged from 2% (60 kg N ha⁻¹, Split TE, DOY 304) to 22.5% (30 kg N ha⁻¹, 0 TE, DOY 273) of the late season N applied.

Spring Vigor and Tissue N

Vigor is a measurement of growth in spring, and is an important component of a healthy turfgrass system. Vigor is measured by quantifying the clipping yield (CY) biomass of a standardized area (plot). Although early spring vigor may be perceived as beneficial, it can leave the plants more susceptible to damage by means of crown hydration (Beard, 1973; Taiz and Zeiger, 2006). Therefore, it may be beneficial to regulate early spring growth, especially if the turfgrass system already possesses acceptable canopy density and tissue N concentrations. Early spring growth can be suppressed by applying TE the previous autumn.

Table 2-5 shows that treatment, timing, and treatment by timing interaction all had a significant effect on spring CY and spring tissue N. As shown in Figure 2-8, the first two applications dates in fall 2009 produced significantly greater CY in spring 2010 compared to the final two application dates. As previously discussed, the last two applications of the year yielded less autumn N assimilation than the two earlier applications (Figure 2-3), and would therefore naturally produce less CY in spring. However, Figure 2-6 shows that all application dates had similar tissue N concentrations in early spring 2010, which means N concentration on any given

date was not solely responsible for the significantly suppressed growth (CY) of the final two application dates (linear timing contrast; Table 2-5).

The same trend regarding tissue N was observed in the fall of 2010 (Figure 2-3), when concentrations were significantly lower for the later application dates. However, once again tissue analysis in spring (Figure 2-6) showed higher N concentrations for the last two application dates compared to the first two application dates, indicating the applied N was taken up later in fall or early in spring 2011. Given this data and providing all other factors equal, it would seem spring CY would correlate directly with spring tissue N content - the greater the N concentration, the greater the growth. However when treatments are pooled, the spring CY trend actually shows a linear decline (Figure 2-8).

The linear decline of spring CY indicates tissue N concentration was not the primary influence on spring growth of the turfgrass system. Intuitively, the low vs. high N contrast was significant for both spring tissue N and CY (Table 2-5); however, suppressed spring growth from later N + TE application dates was consistent over both N rates. Therefore, the application of TE in late fall appears more responsible for suppressed growth the following spring (Figure 2-7). Application of TE significantly preserved N in leaf tissue over winter (Table 2-5; Figure 2-5).

Furthermore, Figure 2-7 illustrates the pooled timing by treatment interaction, or rebound effect, of late-fall applied TE the following spring. Compared to the N only treatments, which produced similar CYs across all application dates, the two latest applications of treatments containing TE (293 and 304 DOY) showed significantly lesser CY than the two earlier applications of the same treatments (Figure 2-7).

Moreover, it is possible that applications made earlier in the autumn were experiencing post-regulation surge when yields were collected in early April (Branham and Beasley, 2007). All

treatments containing TE showed greater spring vigor than the N only treatments on the first application date. Likewise, on the final application date, all treatments containing TE had suppressed vigor compared to N only treatments, meaning those later applications containing TE still had effective growth suppression in spring. Also, the split TE application suppressed growth more than did the full, single TE application, although these differences were not significant. This data supports late season application of TE in conjunction with N to limit early spring growth and preserve tissue N. As mentioned, overly-active spring growth diminishes hardiness in spring (Beard, 1973).

Spring N offtake

Spring N offtake mirrors spring CYs almost perfectly (Figures 2-9, 2-10). Table 2-5 shows that treatment was a significant source for variation of spring N offtake, and that it interacted with application timing. The 'low N vs. high N' and 'split TE vs. full TE' contrasts were significant (Table 2-5). While the direct relation of N rate and N offtake is expected, the significant difference between the split and full TE treatment is of interest.

The second application of the 'split TE' treatments was applied one week after the 'full TE' treatments were applied. This 'split TE' treatment suppressed spring N offtake more than the single 'full' application (Figure 2-9). Because the 'split TE' application produced slightly lesser CY than the counterpart 'full TE' application (although not significantly), it was enough to make the contrast of split vs. full TE significant at $\alpha = 0.1$ with regard to spring N offtake. Considering this contrast was not significant for any other dependent variable, data suggest that split applications at lower rates of TE may be preferred to a single, higher rate application for preserving tissue N and suppressing spring growth.

Canopy Density

Green Normalized Differential Vegetative Index (GNDVI) is a measurement of chlorophyll content and thus quantifies turfgrass canopy density. Bell et al. (2004) found a strong correlation between GNDVI and chlorophyll content ($r^2 = 0.75$) of mixed bermudagrass and creeping bentgrass stands. Likewise, Xiong et al. (2007) reported that GNDVI correlates better with turfgrass quality and N status than red light reflectance in relation to near infrared reflectance (R/NIR), and green light reflectance in relation to near infrared reflectance (G/NIR) in bermudagrass stands.

Canopy density is an important component of turfgrass systems, especially in the early weeks of spring when putting greens in northern climates can experience thinning from winter stresses. As shown in table 2-5, treatment had a significant effect on spring GNDVI readings. Furthermore, low vs. high N was significantly different, as was zero vs. full TE.

Figure 2-11 illustrates the differences in each treatment. The 60 kg ha⁻¹ N treatment had significantly better GNDVI than did the 30 kg ha⁻¹ treatment for both years. Low vs. high N was significantly different because N assimilation was greater in the high N treatments, thus producing more chlorophyll and contributing to better canopy density. As illustrated by Figure 2-11B, all 60 kg ha⁻¹ treatments resulted in higher GNDVI measurements than 30 kg ha⁻¹ treatments for every application date, regardless of TE. Warmer autumn temperatures in 2010 (Figure 2-1) throughout the timeframe of treatment application may have improved GNDVI going into winter 2010 compared to winter 2009. After all, GNDVI readings improved for the final two application dates (Figure 2-11B) as compared to 2009-2010 (Figure 2-11A) when GNDVI readings declined on the final two application dates due to colder weather.

The application of TE significantly enhanced Spring GNDVI readings when all treatment dates were pooled (Table 2-5). At any given N rate, the split or full application of TE significantly enhanced spring GNDVI readings, resulting in better turfgrass canopy density. This data supports

the claim made on the Syngenta label that TE improves canopy density (Syngenta Crop Protection, LLC, 2011). As shown by Figure 2-11AB, nearly every treatment containing TE yielded better Spring GNDVI on every application date compared to N alone.

Overall, Spring GNDVI measurements for the 2010-2011 study were drastically lower than those collected the previous year. Spring 2011 weather was much slower to warm and thus spring “green-up” was delayed considerably. However, readings were still collected on 30 Mar. and 7 Apr. in adherence to the original protocol. Similarly to 2009-2010, significant differences were observed for treatment, specifically low vs. high N and zero vs. full TE (Table 2-5). This supports the practice of using TE in conjunction with soluble N in late autumn to improve spring canopy density.

In summary, application of N and TE in late fall improves density, suppresses early spring growth, and preserves tissue N over winter – all favorable attributes of a healthy putting green.

Tables and Figures

Table 2-1: Soil chemical properties of 2009 cohabited putting green (0-10 cm), sampled prior to treatment initiation (Sept.).

Chemical Property	Mean	St. Dev.
Soil pH (1:1)	6.8	0.06
<u>Mehlich 3 Extractable</u>		
CEC (meq/100g)	5.4	0.32
P (ppm)	56.3	19.14
K (ppm)	70.7	2.31
Mg (ppm)	176.3	4.93
Ca (ppm)	771.7	60.39
S (ppm)	14.9	1.25

Table 2-2: Nutrient concentrations of 2009 cohabited putting green leaf tissue sampled prior to treatment initiation (Sept.).

Tissue Nutrient	Mean	St. Dev.
P (g kg⁻¹)	4.8	0.11
K (g kg⁻¹)	24.9	1.48
Ca (g kg⁻¹)	3.8	0.10
Mg (g kg⁻¹)	2.0	0.09
S (g kg⁻¹)	5.1	0.13
Mn (mg kg⁻¹)	134.9	4.62
Fe (mg kg⁻¹)	347.2	45.99
Cu (mg kg⁻¹)	10.9	1.25
B (mg kg⁻¹)	10.0	0.02
Zn (mg kg⁻¹)	40.3	1.32

Table 2-3: Soil chemical properties of 2010 cohabited putting green (0-10 cm), sampled prior to treatment initiation (Sept.).

Chemical Property	Mean	St. Dev.
Soil pH (1:1)	7.1	0.14
<u>Mehlich 3 Extractable</u>		
CEC (meq/100g)	5.9	0.42
P (ppm)	60.5	3.54
K (ppm)	87.5	12.02

Table 2-4: Nutrient concentrations of 2010 cohabited putting green leaf tissue sampled prior to treatment initiation (Sept.).

Tissue Nutrient	Mean	St. Dev.
P (g kg⁻¹)	4.85	0.07
K (g kg⁻¹)	24.0	0.71
Ca (g kg⁻¹)	3.95	0.35
Mg (g kg⁻¹)	2.2	0.14
S (g kg⁻¹)	-	-
Mn (mg kg⁻¹)	46.5	2.12
Fe (mg kg⁻¹)	162.5	27.58
Cu (mg kg⁻¹)	10.0	0.0
B (mg kg⁻¹)	22.0	1.41
Zn (mg kg⁻¹)	37.5	3.54

Table 2-5: Analysis of variance for pooled (2009-2011) autumn tissue N and N offtake, and spring clipping yield (CY), tissue N, N offtake, and green normalized differential vegetative index (GNDVI).

Source	df	Autumn		Spring				
		Tissue N	N offtake	CY	Tissue N	N offtake	GNDVI	
		<u>F Value</u>						
Year	1	0.0042	NS	NS	0.0080	0.0771	0.0002	
Block[Year]	8	-	-	-	-	-	-	
Treatment (TRT)	5	0.0032	0.0048	0.0621	0.0075	0.0438	0.0329	
Timing (TMG)	3	0.0040	0.0004	0.0939	NS	NS	NS	
TRT x TMG	15	NS	0.0161	0.0135	NS	0.0340	NS	
Year x TRT	5	NS	NS	NS	NS	NS	<0.0001	
Year x TMG	3	<0.0001	<0.0001	<0.0001		<0.0001	0.0707	
Year x TRT x TMG	15	NS	NS	NS	NS	NS	0.0002	
<u>TRT Contrasts</u>								
Low N vs. High N	1	0.0002	0.0156	0.0183	0.0032	0.0064	0.0036	
0 TE vs. TE	1	NS	0.0006	0.0756	0.0025	NS	0.0974	
TE: Split vs. Full	1	NS	NS	NS	NS	0.0859	NS	
N x 0 TE vs. TE	1	NS	NS	NS	NS	NS	NS	
N x TE: Split vs. Full	1	NS	NS	NS	NS	NS	NS	
<u>TMG Contrasts</u>								
Linear	1	0.0011	0.0001	0.0294	0.0894	0.0580	NS	
Quadratic	1	NS	NS	NS	NS	NS	NS	
Cubic	1	NS	NS	NS	NS	NS	NS	

Figure 2-1: Fall weather patterns for 2009 (A) and 2010 (B). Stars represent application dates.

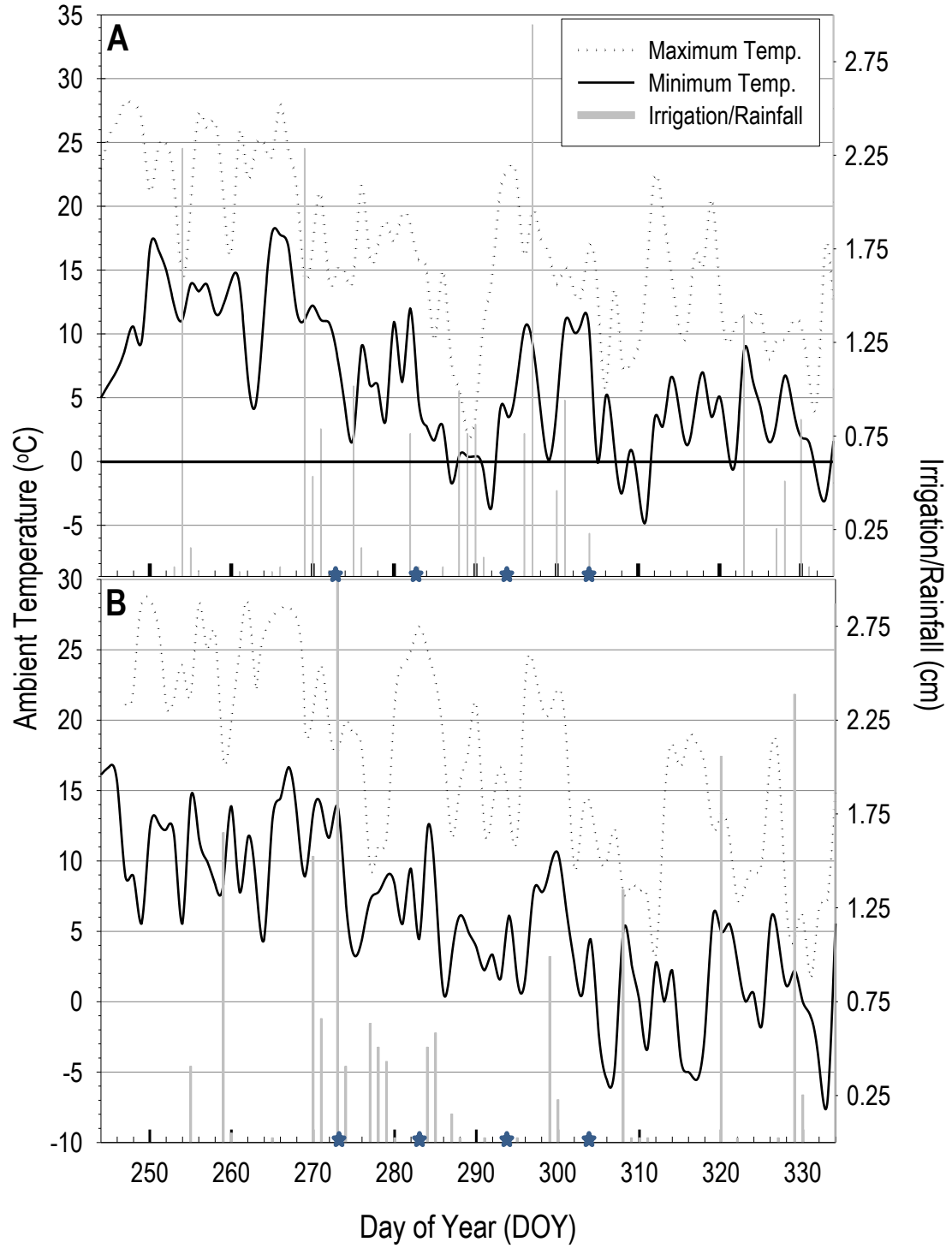


Figure 2-2: Mean fall leaf tissue N concentration by treatment (pooled over application dates and years). Mean values having overlapping error bars are not significantly different (Fisher's LSD, $\alpha = 0.05$).

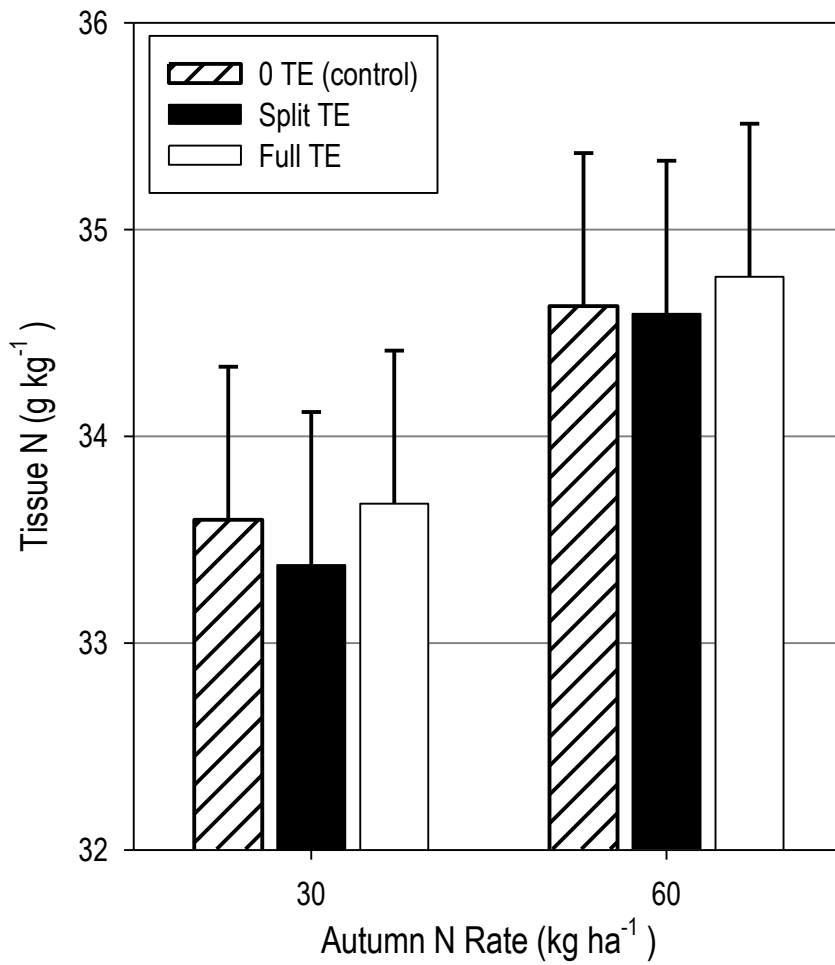


Figure 2-3: Mean fall leaf tissue N concentration for all treatments by application date and year. Mean values having overlapping error bars are not significantly different (Fisher's LSD, $\alpha = 0.05$).

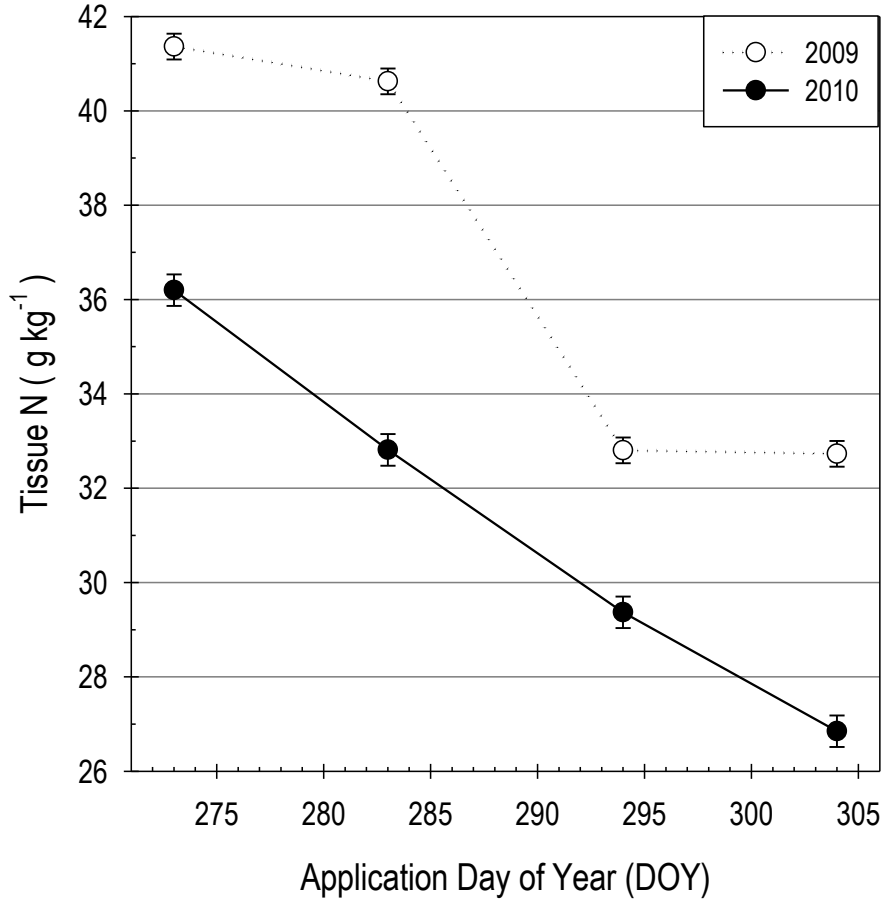


Figure 2-4: Mean fall Nitrogen offtake (2009 and 2010) by treatment and timing after all treatments were applied and mowing was discontinued. For each day of year, the least significant difference between mean values is shown (Fisher's LSD, $\alpha = 0.05$).

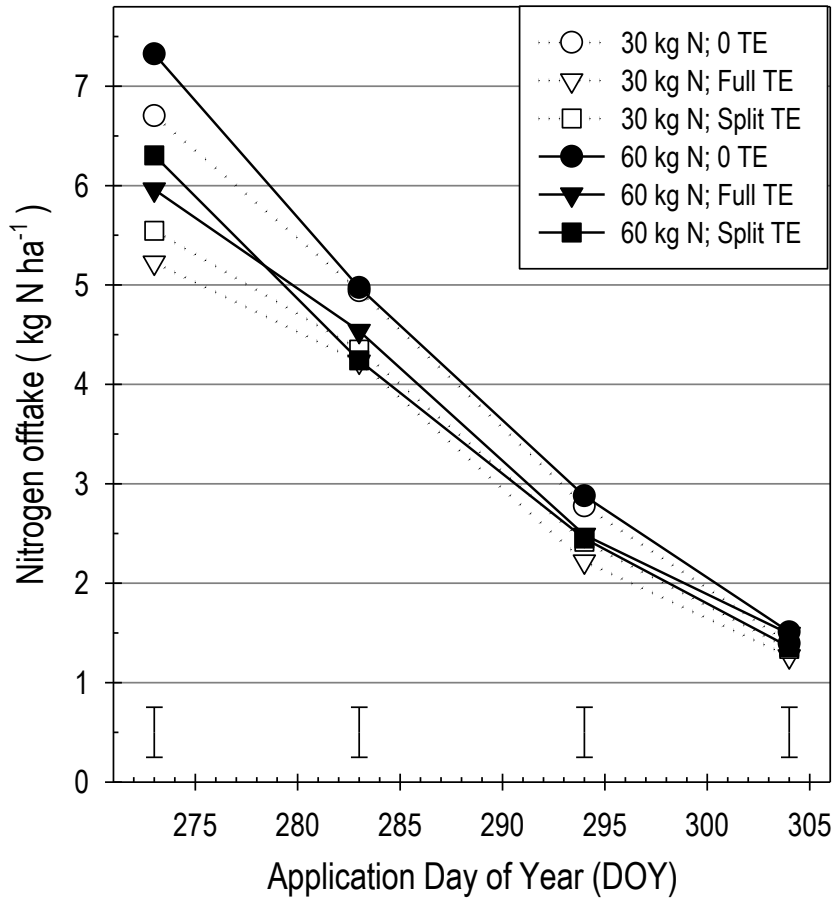


Figure 2-5: Mean spring tissue Nitrogen content of each experimental treatment (pooled across all application dates and years). Error bars indicate the least significant difference between mean values (Fisher's LSD, $\alpha = 0.05$).

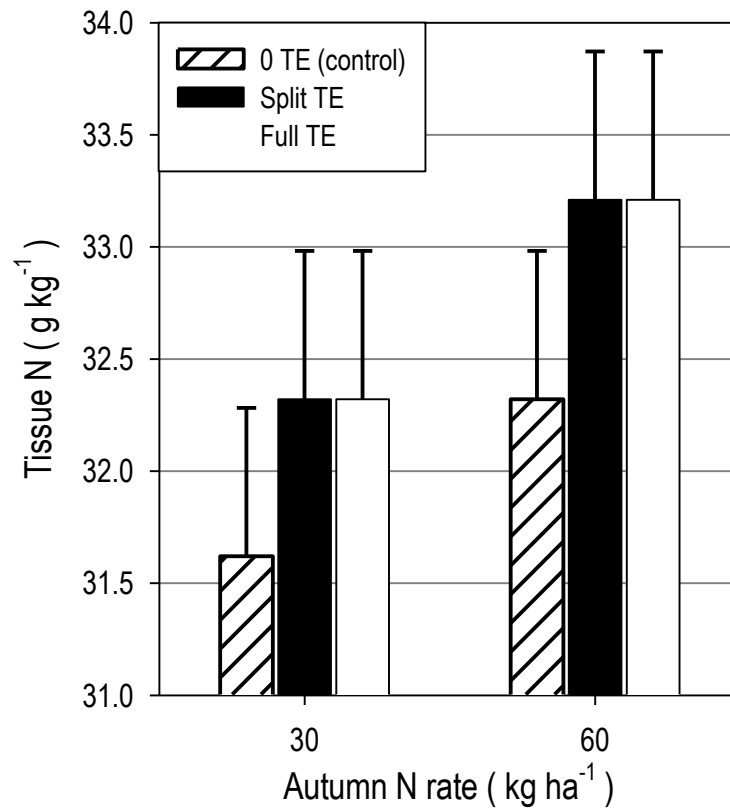


Figure 2-6: Mean spring tissue Nitrogen for all treatments by application date and year. Mean values having overlapping error bars are not significantly different (Fisher's LSD, $\alpha = 0.05$).

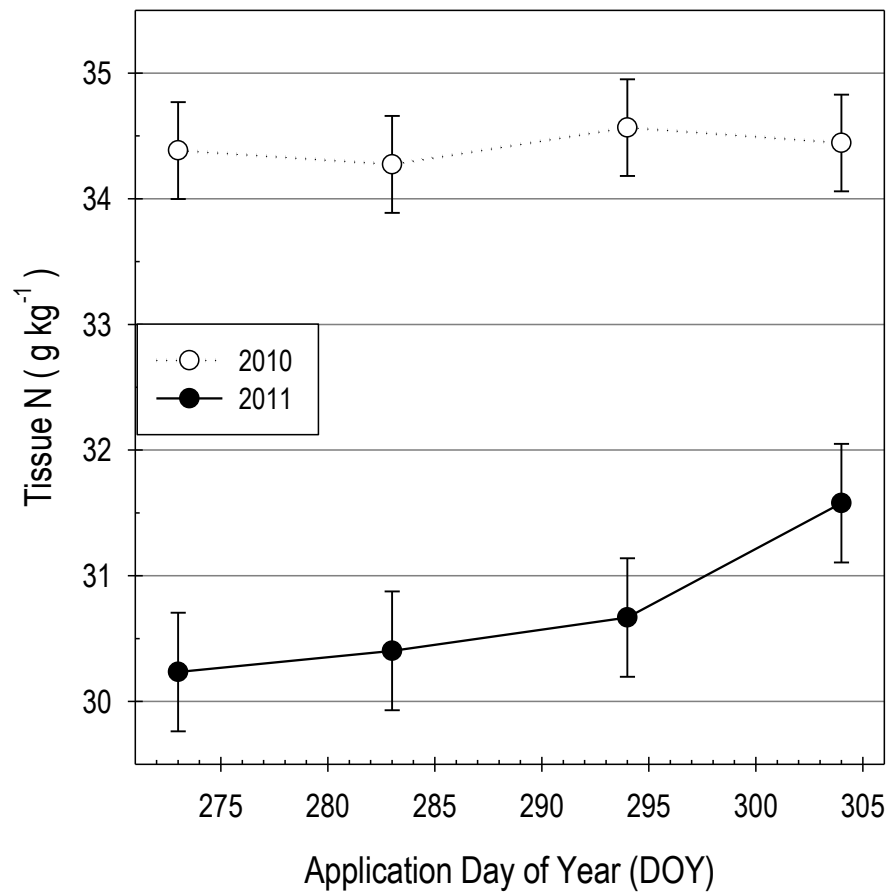


Figure 2-7: Mean spring clipping yield (2010 and 2011) by treatment and timing. For each day of year, the least significant difference between mean values is shown (Fisher's least significant difference, $\alpha = 0.05$).

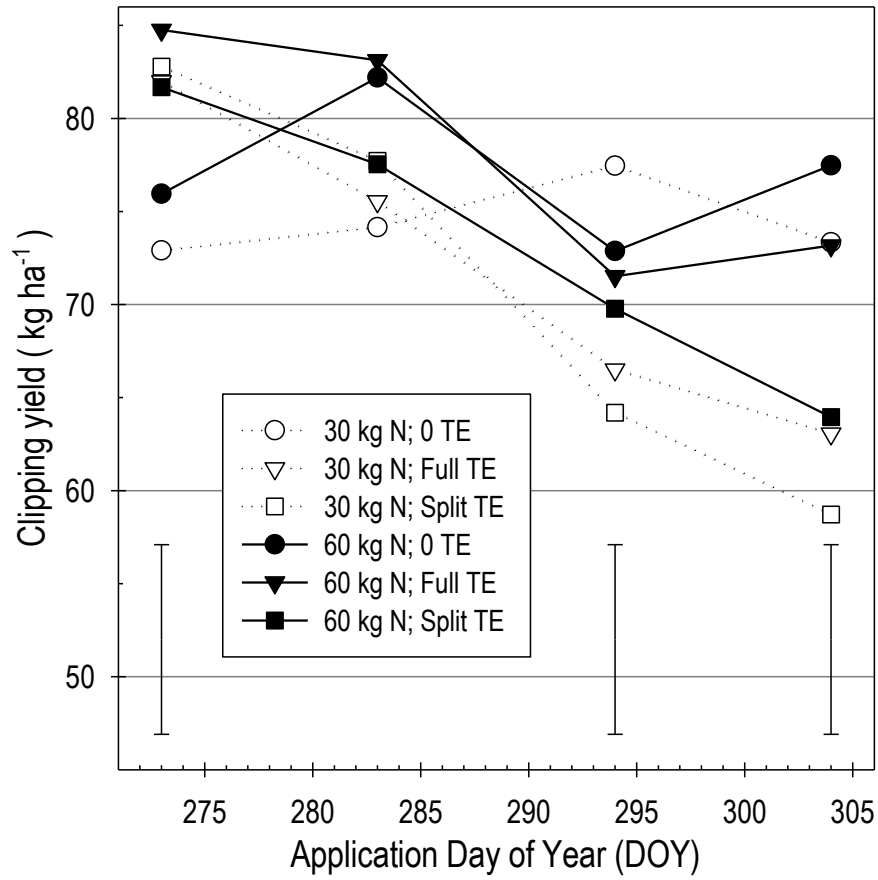


Figure 2-8: Mean spring clipping yield for all treatments by application date and year. Mean values having overlapping error bars are not significantly different (Fisher's LSD, $\alpha = 0.05$).

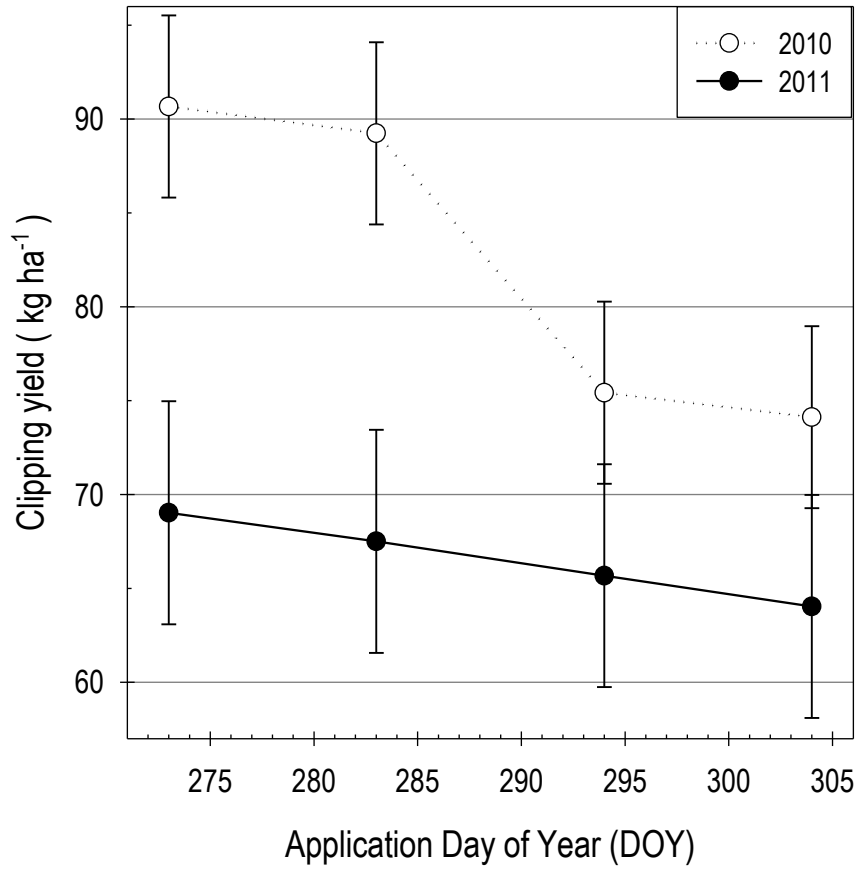


Figure 2-9: Mean spring Nitrogen offtake (2010 and 2011) by treatment and timing. For each day of year, the least significant difference between mean values is shown (Fisher's LSD, $\alpha = 0.05$).

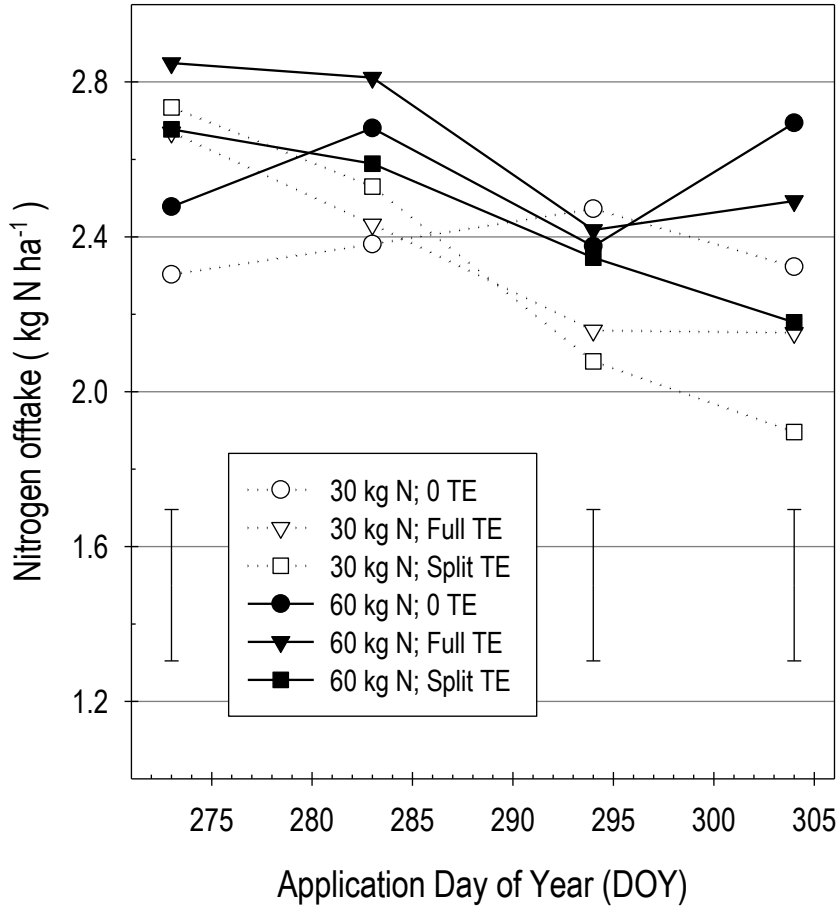


Figure 2-10: Mean spring Nitrogen offtake for all treatments by application date and year. Mean values having overlapping error bars are not significantly different (Fisher's least significant difference, $\alpha = 0.05$).

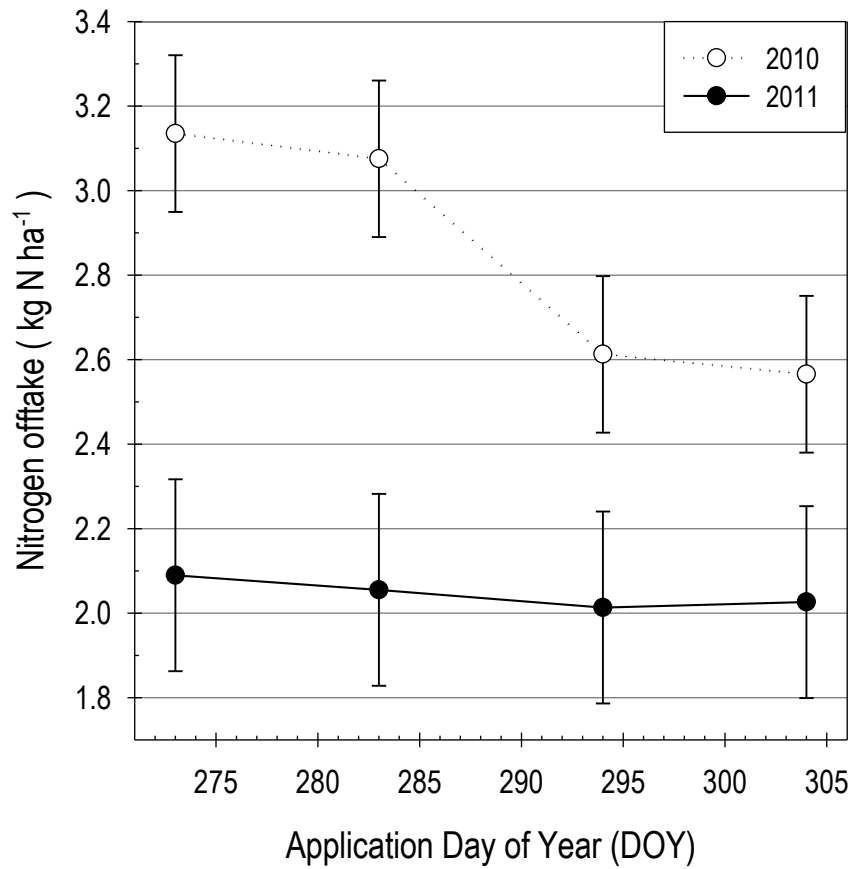
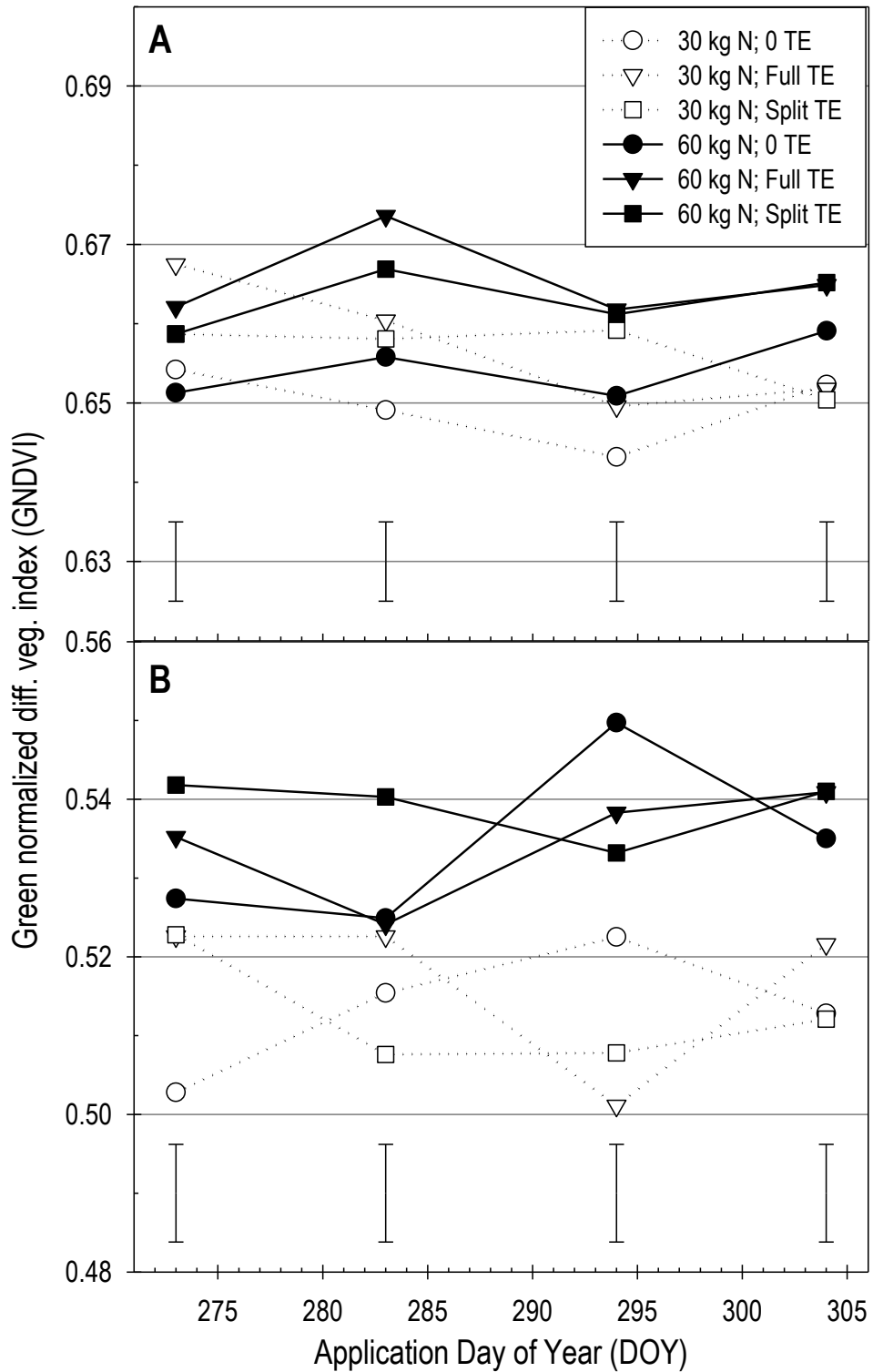


Figure 2-11: Annual green normalized differential vegetative index (GNDVI) by treatment and timing in 2010 (A) and 2011 (B). For each day of year, the least significant difference between mean values is shown (Fisher's LSD, $\alpha = 0.05$).



CHAPTER 3

POA ANNUA PUTTING GREEN STUDY 2010-2011 SEASON

Materials and Methods

A study was initiated in October 2010 at the Pennsylvania State University Joseph Valentine Turfgrass Research Center in University Park, PA. A randomized split-plot design of 90 plots (91 x 183 cm) was established on a mature, annual bluegrass putting green. Plots were mowed 2 d week⁻¹ at 2.8 mm, and clippings were not returned. The putting green received maintenance fertilizer applications throughout the summer of 2010. Prior to treatment initiation, (28 Sep. 2010), the green was fertilized using granular K₂SO₄ (0-0-50) at a rate of 122 kg ha⁻¹. Soil from plugs of four randomly-selected split-plots within each main-plot was composited for analysis of soil pH (1:1), cation exchange capacity (CEC), and Mehlich-3 extractable nutrient concentrations by PSU-AASL (Table 3-1). Likewise, turfgrass tissue samples were collected to determine baseline conditions of tissue nutrient concentrations (Table 3-2).

On 3 Oct. 2010, four treatments were applied in a 1010 L ha⁻¹ spray volume to randomly-selected plots in each of 6 main plots (comprising 3 replicated blocks in total). A CO₂-pressurized, single-nozzle (Tee-Jet TP11008E, Spraying Systems Co., Wheaton, IL), wand-sprayer was used to apply N at 30 or 60 kg ha⁻¹ (1:1 urea-N:NH₄NO₃-N) in a tank-combination of trinexapac-ethyl (4-[Cyclopropyl-*a*-hydroxymethylene]-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester) growth regulator (Primo MAXX 11.3% a.i.) at 0 or 0.088 kg ha⁻¹. The identical procedure was repeated on remaining plots 15 and 27 Oct., 2010, and a control plot (0 N and TE) was maintained within each main-plot on each date. On 15 Oct., phosphoric acid (0-55-0) was applied at 155 kg ha⁻¹ (delivering 85.3 kg P₂O₅ ha⁻¹) to one main plot in each of 3 blocks. Clipping yields (CY) were collected on the 11 Oct. for all plots treated on the first application date. Clipping yields were

collected on the 27 Oct. for all plots treated on both the first and second application date. Clipping yields were collected on the 10 Nov. for all plots treated on the first, second, and third application dates. Oven-dried (60 °C) clipping yields were weighed, ground to pass a 0.15-mm sieve, and then analyzed for total C and N content as previously described. Autumn N-offtake was calculated as previously described.

Putting green mowing was discontinued after the final cutting on 10 Nov. 2010. Plots were not covered over the subsequent winter season.

The putting green was mowed at a height of 2.8 mm on 21 Mar. and 6 Apr. 2011. Multiple canopy reflectance measures were collected from all plots 30 Mar., and 7 Apr. 2011, using the aforementioned Crop Circle ACS-210 radiometer (Holland Scientific, Lincoln, NE).

Compiled GNDVI values, describing spring turfgrass canopy density, were averaged on a per-plot basis. Clipping yield (CY), a measure of turfgrass growth rate and vigor, was collected from all plots on 14 Apr. 2010. Clippings were oven dried and weighed. Sub-samples were ground in preparation for analysis of C and N content (as previously described). Spring N-offtake (kg N ha⁻¹) was calculated on a per-plot basis as previously described. Significance of the main plot effect (Phosphorus) was tested using its block interaction term.

Results and Discussion

Autumn N offtake and Tissue N

In autumn 2010, neither N offtake nor putting green tissue N was significantly influenced by the main-plot phosphoric acid application (data not shown). Autumn N offtake and tissue N were significantly affected by timing, treatment, and their interaction. Regarding the timing source, cumulative N offtake was greatest from plots treated earliest, and diminished noticeably over subsequent application dates (Figure 3-1). The same general trend was observed of mean tissue N in autumn clippings (Figure 3-2), and was likely due to decreasing temperatures and associated decline in turfgrass assimilation rate.

Treatment effect on autumn N offtake was primarily influenced by TE, as growth regulation significantly limited N offtake at each N rate (Figure 3-1). Moreover, cumulative Autumn N offtake from plots treated with both TE and 30 or 60 kg N ha⁻¹ was lesser than N offtake from the control plots. However, autumn mean tissue N was primarily influenced by N rate (Figure 3-2). Despite the ostensible influence of N application rate on tissue N, levels remained below typical recommended N sufficiency levels for annual bluegrass (Carrow et al., 2001). Significant interactive effects of timing and treatment on mean autumn tissue N are less easily explained (Figure 3-2). Regardless of N rate, TE limited tissue N on the first application date. Application of TE limited tissue N of plots receiving 60 kg N ha⁻¹ on the last application date in 2010 (Figure 3-2).

Spring N offtake and Tissue N

In spring 2011, N offtake was not significantly influenced by the main-plot phosphoric acid application (data not shown). Tissue N ranged from 30.8 to 35.1 g kg⁻¹, but was not significantly affected by any ANOVA source (data not shown). Nitrogen rate was responsible for the significant treatment effect observed on N offtake, (Table 3-3), clearly illustrated in Figure 3-3. While the effect of TE appears to have influenced spring N offtake, the TE vs. No TE contrast was not significant at an alpha level of 0.05 (Figure 3-3). The insignificance of treatment timing on spring N offtake is surprising (Table 3-3), particularly considering its influence on autumn N offtake months previous.

Canopy Density

Spring canopy density was not significantly influenced by the main-plot phosphoric acid application (data not shown). Likewise, autumn timing was not a significant source of canopy density variance in spring, and showed no interaction with treatment level or P application. Nitrogen rate was responsible for the significant treatment effect observed on canopy density (Table 3-4), as illustrated in Figure 3-4. Autumn application of TE had no meaningful effect on spring canopy density of the annual bluegrass putting green.

Spring Vigor

Spring vigor, or clipping yield, was not significantly influenced by the main-plot phosphoric acid application (data not shown). Likewise, autumn timing was not a significant source of canopy density variance in spring, and showed no interaction with treatment level or P application. Nitrogen rate was responsible for the significant treatment effect observed on clipping yield (Table

3-5), as illustrated in Figure 3-5. While the effect of TE appears to have influenced spring vigor, the TE vs. No TE contrast was not significant at an alpha level of 0.05 (Figure 3-5).

Tables and Figures

Table 3-1: Soil chemical properties of 2010 annual bluegrass putting green (0-10 cm), sampled prior to treatment initiation (Sept.).

Chemical Property	Mean	St. Dev.
Soil pH (1:1)	7.3	-
<u>Mehlich 3 Extractable</u>		
CEC (meq/100g)	7.9	-
P (ppm)	88.0	-
K (ppm)	65.0	-

Table 3-2: Nutrient concentrations of 2010 annual bluegrass putting green leaf tissue sampled prior to treatment initiation (Sept.).

Tissue Nutrient	Mean	St. Dev.
P (g kg⁻¹)	3.45	0.21
K (g kg⁻¹)	22.55	0.78
Ca (g kg⁻¹)	5.4	0.28
Mg (g kg⁻¹)	2.1	0.0
S (g kg⁻¹)	-	-
Mn (mg kg⁻¹)	39.5	2.12
Fe (mg kg⁻¹)	1958.5	133.64
Cu (mg kg⁻¹)	89.0	32.53
B (mg kg⁻¹)	32.0	1.41
Zn (mg kg⁻¹)	90.5	17.68

Table 3-3: Analysis of variance for the dependent variable of spring N-offtake, collected in spring 2011. Significance was determined at an alpha level of 0.05.

Source	Contrast	df	Estimate	Pr > t	Pr > F
Block		2			NS
Phosphorus (P)		1			NS
Timing (TMG)		2			NS
Treatment (TRT)		4			0.049
	Linear N rate (LN)		0.130	0.009	
	Quadratic N rate (QN)		-0.026	NS	
	N: 0TE vs. TE		-0.064	NS	
	LN vs. 0TE vs. TE		-0.024	NS	
TMG*TRT		8			NS
P*TMG		2			NS
P*TRT		4			NS
P*TMG*TRT		8			NS

Table 3-4: Analysis of variance for the dependent variable of GNDVI measurement, collected in spring 2011. Significance was determined at an alpha level of 0.05.

Source	Contrast	df	Estimate	Pr > t	Pr > F
Block		2			0.048
Phosphorus (P)		1			NS
Timing (TMG)		2			NS
Treatment (TRT)		4			0.002
	Linear N rate (LN)		0.484	<0.001	
	Quadratic N rate (QN)		0.009	NS	
	N: 0TE vs. TE		-0.005	NS	
	LN vs. 0TE vs. TE		<-0.001	NS	
TMG*TRT		8			NS
P*TMG		2			NS
P*TRT		4			NS
P*TMG*TRT		8			NS

Table 3-5: Analysis of variance for the dependent variable of clipping yield, collected in spring 2011. Significance was determined at an alpha level of 0.05.

Source	Contrast	df	Estimate	Pr > t	Pr > F
Block		2			NS
Phosphorus (P)		1			NS
Timing (TMG)		2			NS
Treatment (TRT)		4			0.005
	Linear N rate (LN)		4.358	<0.001	
	Quadratic N rate (QN)		-0.920	NS	
	N: 0TE vs. TE		-1.826	NS	
	LN vs. 0TE vs. TE		-0.709	NS	
TMG*TRT		8			NS
P*TMG		2			NS
P*TRT		4			NS
P*TMG*TRT		8			NS

Figure 3-1: Autumn 2010 N off-take of all plots after all treatments were applied and mowing was discontinued for the year.

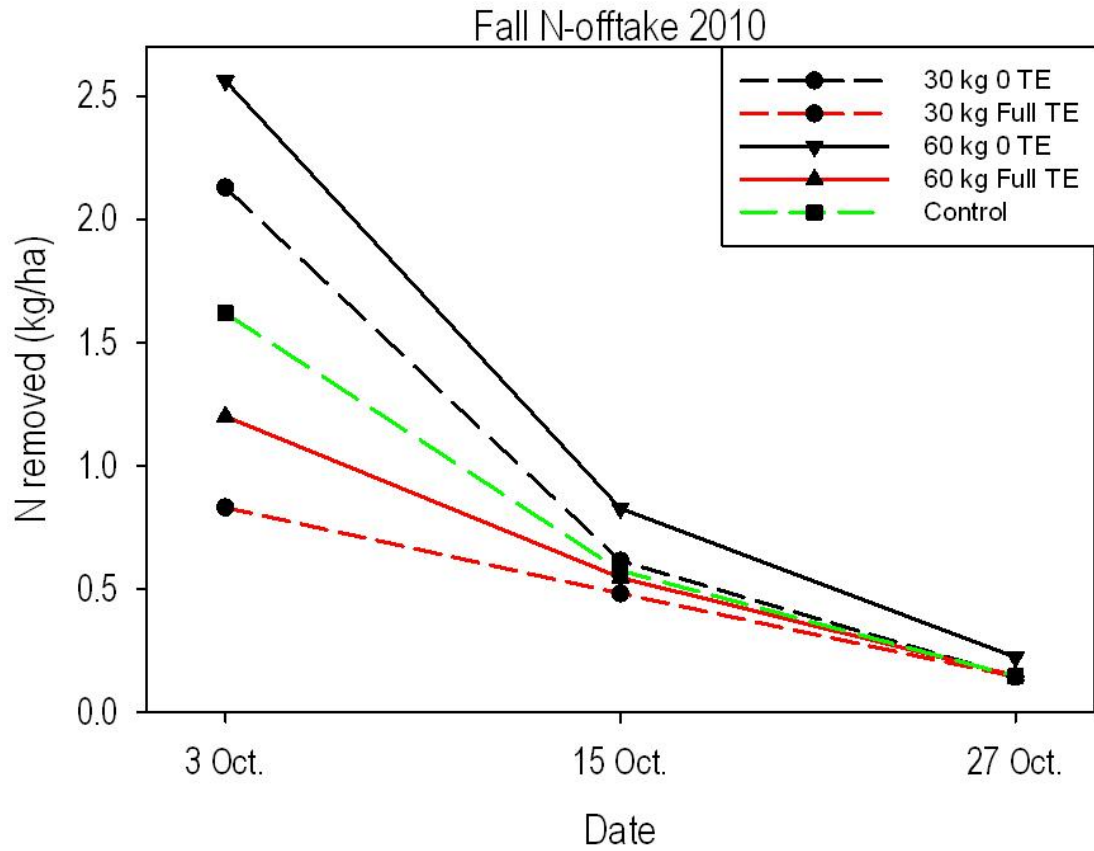


Figure 3-2: Autumn 2010 tissue N content analyzed after all treatments were applied and mowing was discontinued for the year.

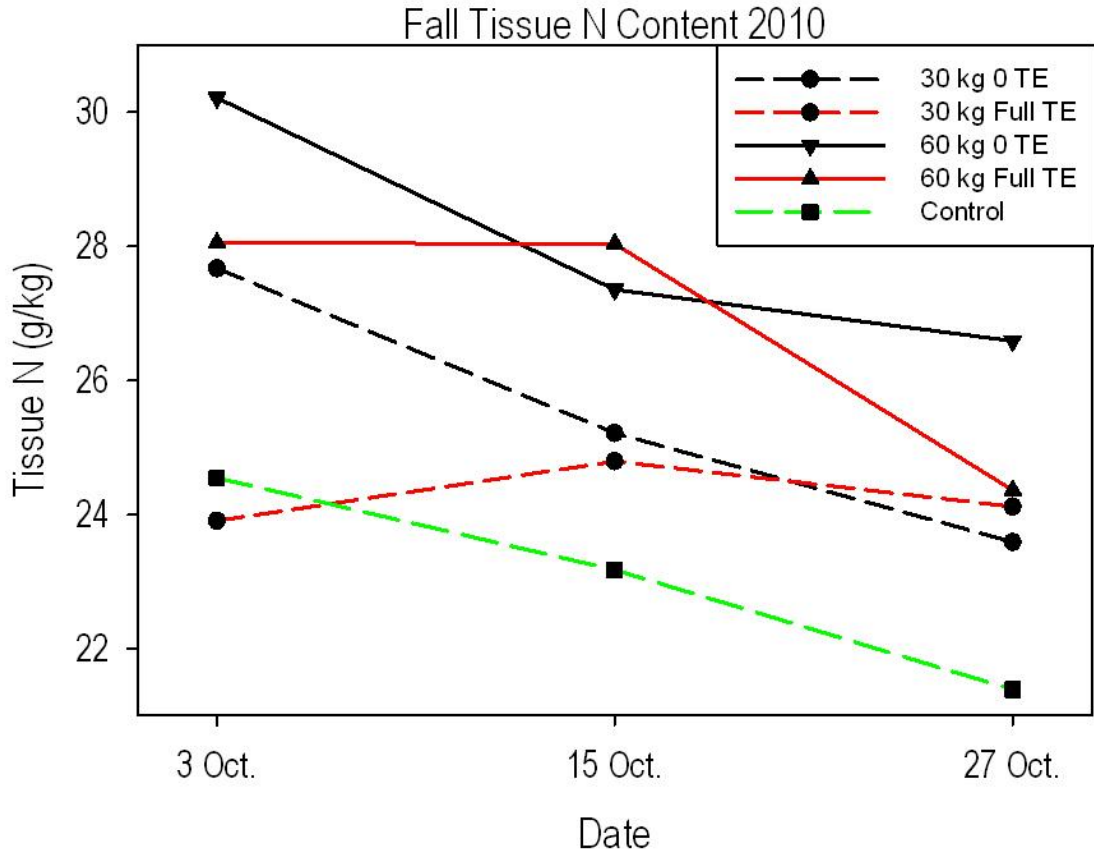


Figure 3-3: Spring 2011 N-offtake of all experimental treatments averaged across application date.

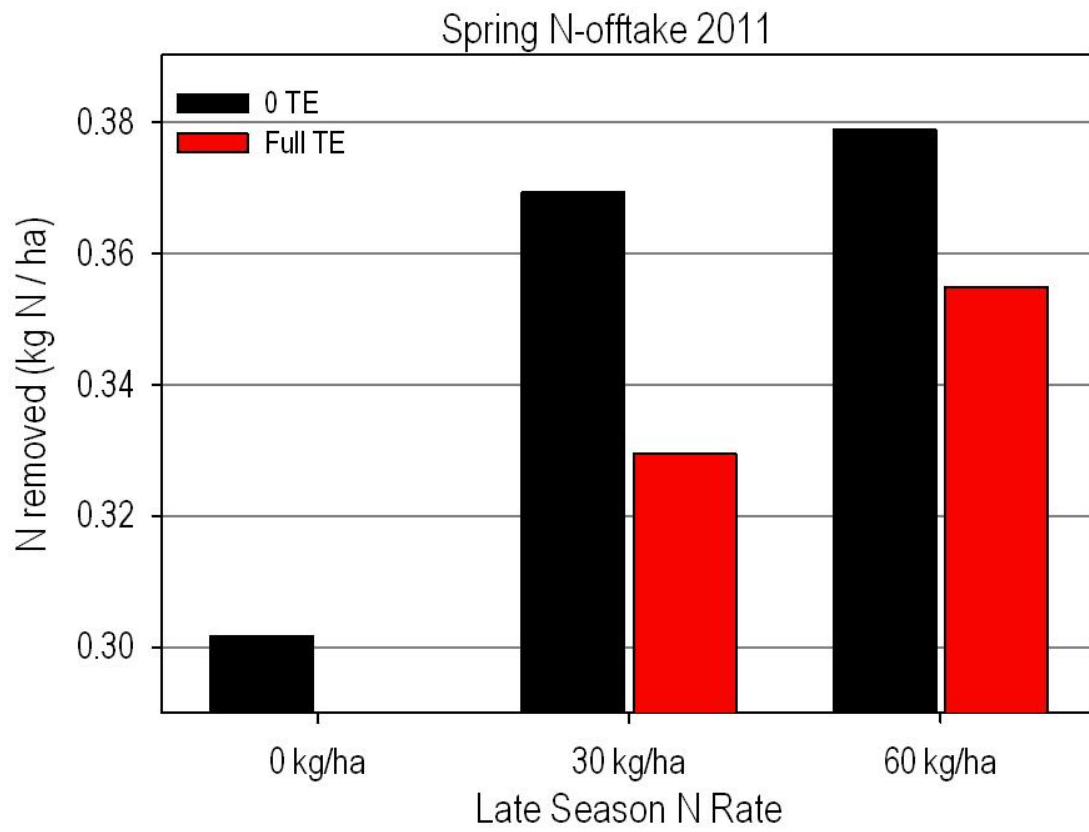


Figure 3-4: Spring 2011 GNDVI readings of each experimental treatment averaged across all application dates.

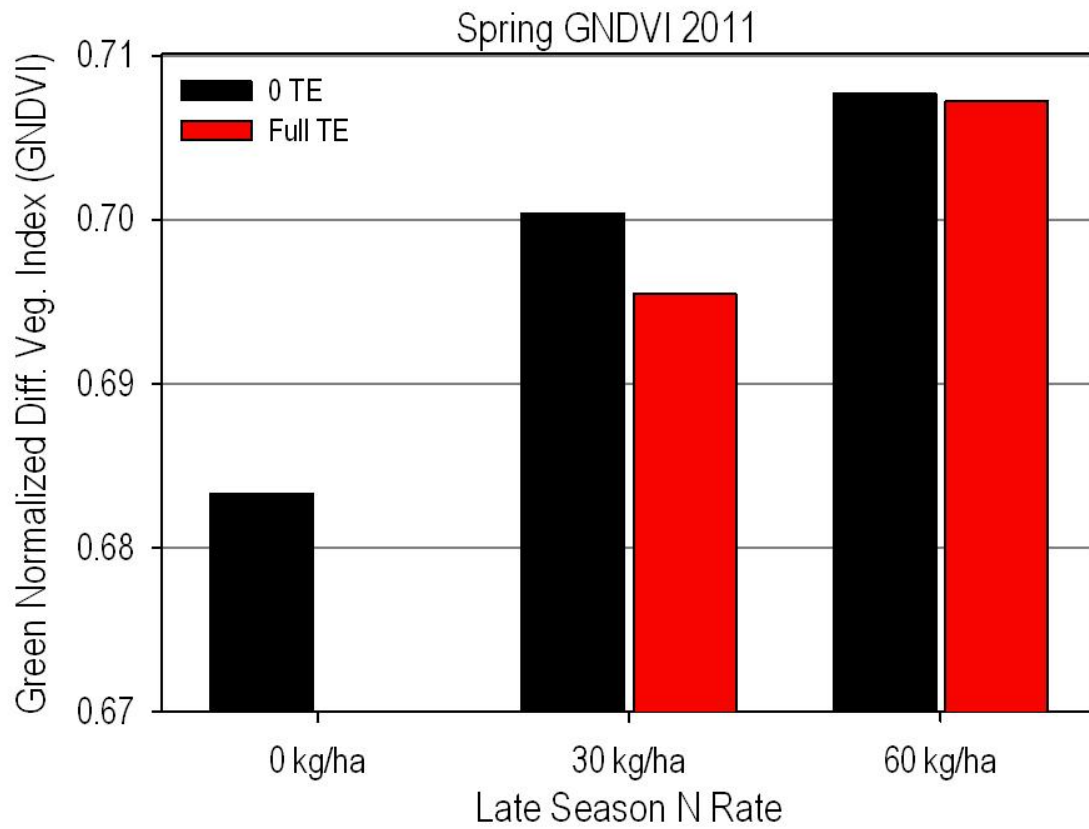
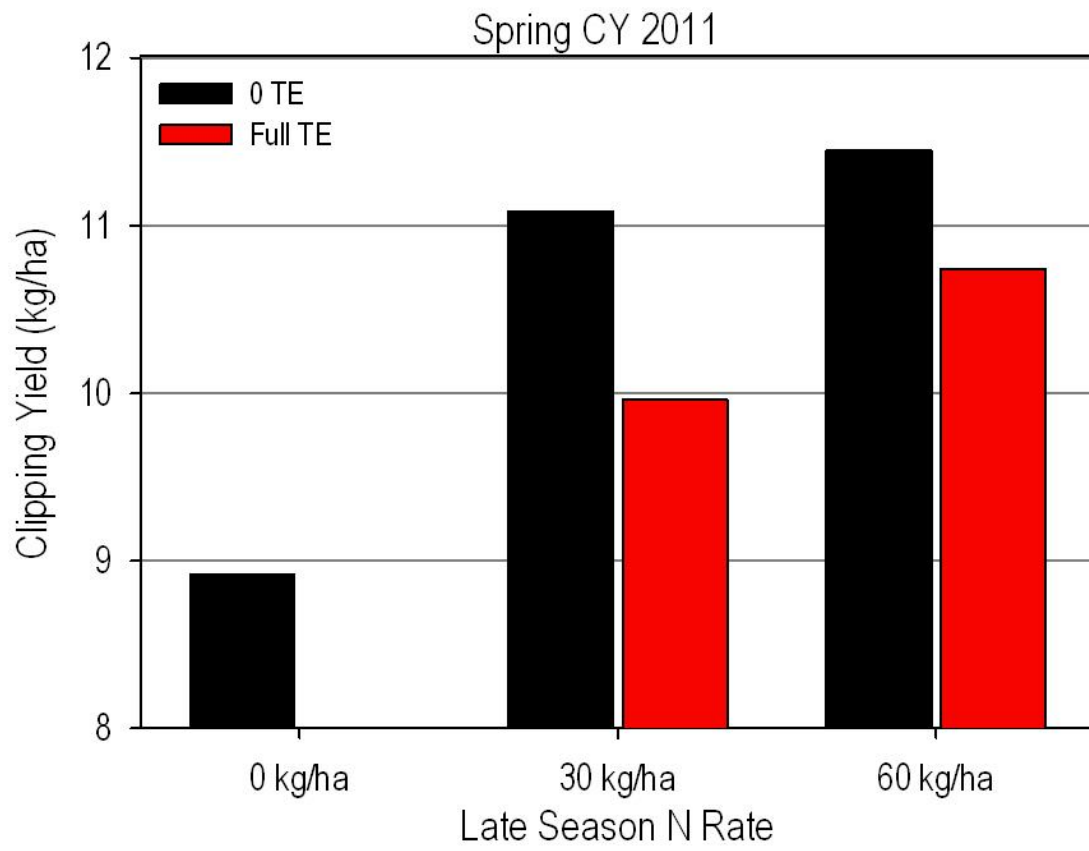


Figure 3-5: Spring 2011 CYs of each experimental treatment averaged across all application dates.



CHAPTER 4

CONCLUSIONS

Application of N and TE in late autumn can beneficially influence the spring vigor, density, and tissue N concentration of green-height turfgrass systems. While spring-time growth may be perceived as beneficial, suppressing early growth and maintaining hardiness may ultimately protect plants from late-winter damage that occurs when temperatures fluctuate drastically. As presented by the data in this study, repeated application of TE in late autumn will suppress growth in early spring, even when tissue N is high. In fact, TE significantly preserves tissue N, which can be beneficial for spring growth after TE is no longer active and weather patterns stabilize. Conversely, TE can be used to stimulate growth in early spring when applied up to 2 weeks prior to the first hard frost, but is not recommended because early growth diminishes hardiness.

Spring density was improved significantly when TE was applied with N. The high N rate (60 kg ha⁻¹) improved spring density marginally compared to the low N rate. While the difference was significant, application of 30 kg N ha⁻¹ resulted in a greater percent N recovery in spring. Although more of the high N rate applied may ultimately be recovered, the threat of N leaching does not comply with responsible IPM practices and is avoidable. Still, applying up to 60 kg N ha⁻¹ *prior* to the first hard frost in conjunction with TE can prove beneficial, particularly if putting greens possess an N deficiency in autumn.

The data also shows that tissue N levels can enter dormancy at a high concentration (40-42 g kg⁻¹) without any detrimental effect in early spring. When striving for such a high concentration late in the season (September or later), N should always be applied with a PGR such as TE. This will restrict top growth and improve density going into winter. In theory, a plant that is photosynthetically active but not growing will synthesize carbohydrates, contributing to winter hardiness and photoassimilate storage. Where winter damage threatens the health of a turfgrass

system, maintaining a dense turfgrass canopy may ultimately net less turf loss and aid in accelerated spring recovery.

Regarding application timing and autumn weather patterns, each year is different. While the first hard frost occurred on the exact date of historical prediction in 2009, the first frost of 2010 occurred more than two weeks later. Although the first hard frost cannot be accurately predicted, waiting until at least early October to apply $\sim 60 \text{ kg N ha}^{-1}$ is recommended so as to avoid stimulation of vegetative growth. If weather forecasters predict a frost on October 5, the high rate of N should be applied several days prior. After the first hard frost, a smaller amount of N ($\sim 5 \text{ kg ha}^{-1}$) should be applied in conjunction with TE ($\sim 0.044 \text{ kg ha}^{-1}$) every 7 to 10 days until early- to mid-November.

Although the *Poa annua* putting green experiment did not generate many significant findings, there is still much to be learned from this study. Upon visual observation, any of these plots treated with TE exhibited exceptional color and density in spring, even though these qualities were not significantly detectable. It is possible that TE was not as effective on these plots because the existing *Poa annua* stand had never been treated with TE, or any PGR. Although not well documented in the literature, conversation states TE may not be as effective until the second application. If this experiment were to be conducted again, several applications should be made to the turfgrass stand at the 'split' TE rate approximately 10 days apart, with the last application being 10 days before treatment initiation.

An interesting derivative of this study would start by applying a small N rate ($\sim 5 \text{ kg ha}^{-1}$) in conjunction with 0.044 kg ha^{-1} of TE over all plots in late September. Ten days later, the same application should be made to all plots, with the exception of one "treatment" in each block. That "treatment" would not receive any more N or TE for the remainder of the year. Ten days later, make the same application to all plots except one other "treatment" that would not receive anything

for the remainder of the year. This pattern should be repeated into mid-November. The idea is to follow the same (or similar) spoon-feeding regimen followed throughout the season, and observe how late in the year these applications may produce the best conditions in spring. Perhaps then there would be no need for a high N application in early October.

As far as other improvements or changes, this experiment could be expanded by collecting a core from each plot (after all treatments were applied) and simulating winter conditions in a controlled environment. Come spring, drastic temperature fluctuations could be simulated to invoke crown hydration stress. Furthermore, autumn GNDVI readings could be compared and contrasted to spring GNDVI readings to provide a better baseline of density measurements.

Upon reflection, I will remember this experiment as fun, interesting, informational, and (hopefully) a welcomed influence to improved putting green management. Ultimately, a putting green that exhibits exceptional density, acceptable tissue N concentrations, good color, and prolonged hardiness in early spring is a functional putting green, indicative of good management practices.

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