

Chapter Four: The Influence of Trinexapac-ethyl on Creeping Bentgrass Golf Putting Green Critical Soil Test Phosphorus Level

ABSTRACT

Accurate determination of soil test phosphorus (STP) critical level has become important as many state and local regulations have banned turfgrass application to turfgrass unless soil tests indicate P application is required. The Mehlich-3 STP critical level for creeping bentgrass putting greens is not well established. The purpose of this study was to investigate how STP level and trinexapac-ethyl (TE) applications affect creeping bentgrass putting green performance. A research putting green was constructed with a range of STP levels with and without TE application. Monthly soil test values were paired with clipping yield, turfgrass visual quality, color, and tissue P to determine creeping bentgrass putting green STP critical during the 2009 and 2010 growing season. Turfgrass visual quality and color were the best turfgrass attributes used for determination of critical STP value because the slope of the linear-linear regression line changed markedly at the critical value. Visual quality and color STP critical values ranged from 4 to 12 mg P kg⁻¹ soil depending on time of sampling. Trinexapac-ethyl reduced STP critical levels on certain dates but differences were not larger than laboratory error and are therefore not of any practical significance. Trinexapac-ethyl increased turfgrass quality and color at STP values greater than the critical level.

INTRODUCTION

Creeping bentgrass putting greens are commonly constructed on sand-based media. In many regions of the United States, these sands are calcareous and have an alkaline pH. Such sands are infertile by nature and require regular fertilization. When soil P levels are not sufficient to sustain healthy growth, turfgrass stand density and visual quality becomes diminished and leaf margins exhibit a purple discoloration (Marchner, 1995; Carrow et al., 2001).

Chemical soil tests estimate plant available soil P but need to be calibrated to ensure accurate fertilizer recommendation. Numerous turfgrass attributes, including clipping yield, visual quality, and color, have been traditionally used for soil test calibration (All Soil Test P Refs). These values are plotted as a function of soil test phosphorus (STP) level. Various statistical models are used to estimate STP critical level; the point at which there is no response to additional STP level. Many early publications neglected STP level and found that many turfgrass sites were non-responsive P fertilization (Waddington et al., 1978; Dest and Guillard, 1987; Christians et al., 1979 and 1981). Christians et al., (1979) postured that STP levels were sufficient in these studies, preventing turfgrass response to phosphorus fertilization.

Dest and Guillard (2003) and Johnson et al. (2003) correlated various turfgrass attributes with STP level on established creeping bentgrass putting greens. Critical levels varied from 1.1 to 69.3 mg kg⁻¹ depending on soil extractant, turfgrass response, and statistical model used. Both reported STP level to be a good indicator of turfgrass quality. The Mehlich-3 soil extractant, which is commonly used throughout the US, was not evaluated in either of these studies.

Turfgrass growth rate is also known to affect STP critical level. Plant nutrient demand is controlled by growth rate (Adams, 1960). Therefore management practices that alter turfgrass

growth rate, i.e. N fertilization or plant growth regulator application, may alter STP critical levels. Colclough and Lawson (1989) reported that plots treated with 400 kg ha⁻¹ of N developed P deficiency symptoms when P fertilizer was withheld. Plant growth regulators reduce growth rate and therefore nutrient demand. Chapter three found TE reduced growth rate which led to reduced N demand in a creeping bentgrass putting green. However, it is unknown if growth regulation will also decrease critical STP level. To date, there has not been any published research which investigates the effect of plant growth regulators would have on STP critical level.

The objectives of this study were to correlate paired Mehlich-3 STP levels with visual quality, color, tissue P, and yield on a creeping bentgrass putting green, and investigate how applications of the plant growth regulator TE alter STP critical values within each rating day. Our hypothesis is that TE will reduce clipping yield (a driver of nutrient demand), and therefore reduce STP critical values.

METHODS

Green Construction and Experimental Design

In spring of 2008, a golf putting green research green was constructed at the O.J. Noer Turfgrass Research and Education Facility in Madison, WI. Construction methods and sand rooting medium met USGA specifications for golf course putting green construction (USGA Green Section Staff, 2004). Thirty cm of un-amended sand was placed over 10 cm of gravel subsurface. Plots measured 1.8 x 1.5 m and were arranged in a randomized complete block design with eight treatments replicated four blocks.

A range of STP levels were created during construction by incorporation of monopotassium phosphate (MKP; 0-23-28) at the rates of 0, 7.5, 15, and 30 mg P kg⁻¹ soil into the sand root zone with a roto-tiller at 10 cm increments. Fertilizer application rate was calculated based on a soil bulk density of 1.6 Mg ha⁻¹. Half the plots were treated with TE at the rate of 0.1 kg a.i. ha⁻¹ every 200 growing degree days to ensure season long yield reduction (Chapter 2). Liquid MKP applications were applied with a CO₂ powered backpack sprayer with TeeJet XR 8008 nozzles calibrated to deliver 7100 L ha⁻¹ at 276 kPa. Potassium sulfate (0-0-49) was added to MKP fertilizers provide a uniform 35 kg K ha⁻¹ for all P treatments. Following incorporation, irrigation was applied to 12-15% volumetric soil water content and the sand was compacted with sand pro (Deere and Co., Moline, Illinois). This process was repeated twice to achieve a final sand depth of 30 cm. To aid establishment, a final application of a complete fertilizer (10-8-18) was topdressed over the green surface at the rate of 20, 16, and 37 kg ha⁻¹ N, P, and K, respectively.

The green surface was seeded with creeping bentgrass (cv. 'Penn A4') at the rate of 98 kg of pure live seed ha⁻¹ on 3 June 2008. The green was fertilized weekly with 37 kg ha⁻¹ N with

urea (46-0-0) and was irrigated five times per day with two-minute irrigation cycles during the two month establishment. The first mowing at 10 mm height of cut occurred on June 2008 with a Toro Greensmaster 1000 (Toro Co., Bloomington, Mn). Height of cut was incrementally lowered to 3.2 mm during June and July 2008.

Six soil samples were taken from each plot with a 1.6 cm diameter soil core to a depth of 8 cm on 1 July, 1 August 2008, and 23 Apr 2009. Cores from each plot were combined, dried at 105°C for 24h, and sent to the University of Wisconsin Soil and Plant Analysis Laboratory (SPAL) in Madison, WI. All sample soil test P and K concentrations were quantified by Mehlich-3 extraction and inductively coupled plasma optical emission spectrometry (ICP-EOS) determination. Maintenance MPK were applied to specific plots on 11 July, 26 Aug 2008, and 24 Apr 2009 following soil test results to create a range in STP level. Fertilizer was applied with a CO₂ powered backpack sprayer with TeeJet AI 11004 nozzles calibrated to deliver 800 L ha⁻¹ at 276 kPa. Irrigation to a depth of 10 mm was applied immediately following fertilizer applications.

2009-2010 Experimental Protocol

The green was mowed 6 d wk⁻¹ at 3.2 mm height of cut with a Toro Greensmaster 1000. Nitrogen was applied biweekly as liquid urea fertilizer (46-0-0) at the rate of 10 kg N ha⁻¹. Overhead irrigation supplemented precipitation to 80% of estimated evapotranspiration daily from an on-site weather station. Sand topdressing was applied to a surface depth of approximately 1 mm monthly following soil sampling and data collection (fines free topdressing sand, Waupaca Sand & Solutions, Waupaca, WI). Both TE treatments and nitrogen fertilizers were applied with a CO₂ powered backpack sprayer with TeeJet AI 11004 nozzles calibrated to

deliver 800 L ha⁻¹ at 276 kPa. TE and N applications occurred from 11 May until 14 October 2009 and resumed from 1 April 2010 to 1 July 2010.

Data Collection

All data collection occurred in conjunction with monthly soil sampling; 22 May, 25 June, 14 July, 11 Aug, 11 Sept, 14 Oct 2009, and 1 April, 6 May, 3 June, and 1 July 2010. Soil sampling procedures were the same as described previously. All samples were sent to the University of Wisconsin Soil and Plant Analysis Lab (SPAL) in Verona, WI for Mehlich-3 soil P determination with ICP-OES detection. In 2010, a 12-cm diameter soil plug was taken to a depth of 12 cm to serve as a soil standard. Three samples from the soil standard were submitted with each set of monthly soil samples to determine laboratory analysis error.

Clippings were collected by mowing one 1.3 m pass down the center of each plot 24 h (\pm 2 h) following the previous mowing. Prior to clipping collection, 27 cm buffer alleys were mowed at the top and bottom of each plot to reduce variation caused by starting and stopping the mower. Clippings were then brushed from the mower collection bucket into paper bags before being placed in a drying oven for 24 h at 60°C. During 2010, the buffer alleys were mowed at 2 mm to obtain more accurate clipping yields by minimizing scalping that occurred between adjacent plots in 2009. Sand debris was removed for turfgrass clippings prior to measuring dry mass. Clippings were ground with a Wiley mill (Thomas Scientific, Swedesboro, NJ) and sent to SPAL for total mineral analysis via perchloric acid digestion and ICP-OES determination.

Visual turfgrass quality was rated on a 1 to 9 scale with 1 representing completely dead, 6 acceptable, and 9 perfect putting green quality. Turfgrass color index was quantified with the mean of 10 readings from a reflectometer (CM-1000, Spectrum Technologies Inc, Plainfield, IL)

on a 0-999 scale where greater readings indicate less reflectance of 700 nm light. The reflectometer was held one meter from the turfgrass surface with the incidental light meter pointed in the direction of the sun.

One 10-cm diameter by 10-cm deep plug was arbitrarily selected from six plots on 22 Nov 2009. Plugs were selected from plots below, near, and well-above the average visual quality STP critical level for both TE treatments. Plugs were then split in half, washed, and roots cut from the crowns. Twelve roots were selected from each plug half. Three roots were plated on a slide marked with four 3 mm gradations where each slide constituted one replicate. Mycorrhizae arbuscules were then counted by through-focusing in the four 3 mm sections of each root.

Statistical Analysis

Critical STP levels were determined with the non-linear regression procedure in SAS statistical software (SAS Institute, Cary, NC). Turfgrass visual quality and color index and clipping yield were subject to a linear-linear plateau regression model. Clipping tissue P contents were subject to logarithmic regression. The effect of TE on STP were compared via t-tests to determine the effect of TE across days, years, and during the whole study.

RESULTS AND DISCUSSION

Soil Test Values

The additional P fertilizer applications in 2008 and April 2009 created STP concentrations that were evenly distributed from 4 to 55 kg P ha⁻¹ on the 22 May 2009 sampling. Soil test P values declined significantly during the between 22 May 2009 until 1 July 2010 (Figure 4.1). The pH of the sand root zone decreased from 8.8 during construction to 7.8 at the start of data collection on 22 May 2009 and averaged 6.9 on 1 April 2010. High initial pH values suggest the sand was high in CaCO₃ and exchangeable sodium. Leaching of exchangeable sodium from the soil profile and carbonate neutralization likely caused the decrease in soil pH. The alkaline pH and calcareous nature of the sand root zone are conducive to rapid P immobilization which caused the drastic decrease in STP levels during the course of this study (Havlin et al., 2005). Maintenance P applications are there by justified for this soil type to sustain STP values. Analysis of 2010 soil standard data indicates that laboratory error can be substantial and caution must be exercised when comparing STP levels across rating days (Table 4.1).

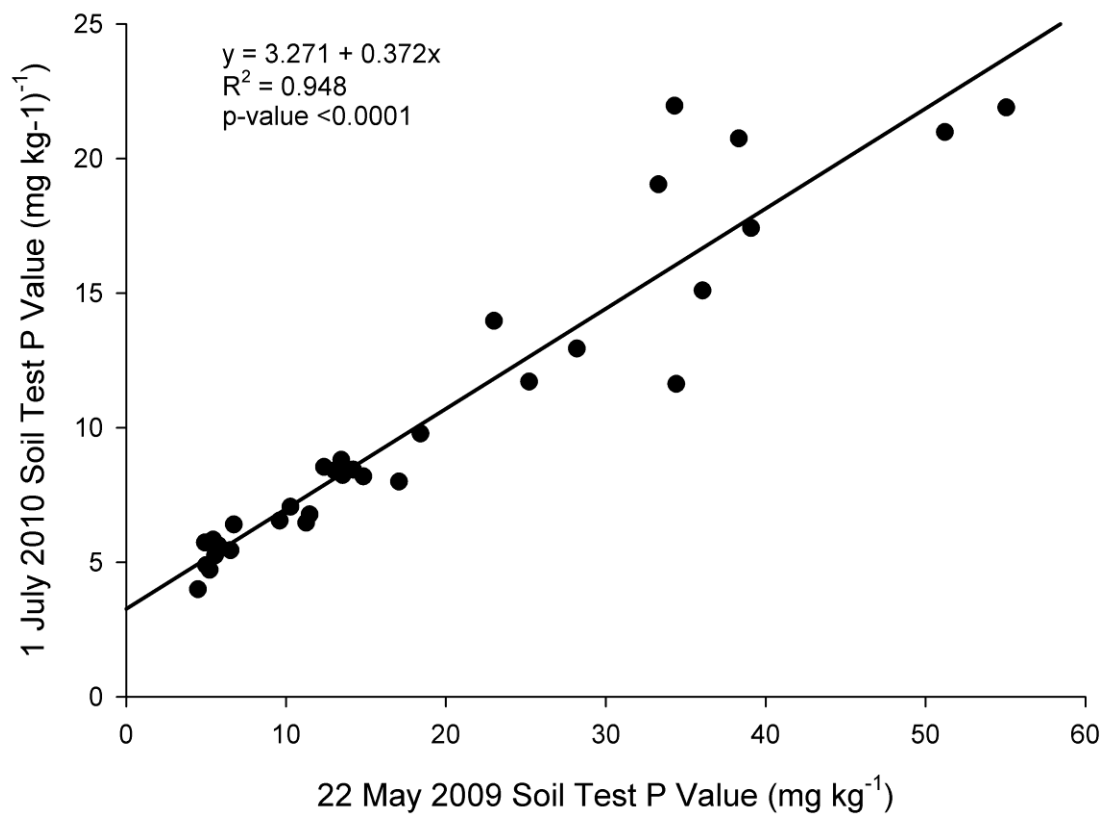


Figure 4.1. The change in plot soil test P level from the beginning of this study on 22 May 2009 to the end on 1 July 2009. The alkaline soil pH after construction and sand calcareous parent material likely caused increased P immobilization.

Table 4.1. The change in STP level of the soil standard during monthly sampling.

Soil Testing Day	Average STP Value	Standard Deviation
	mg P kg ⁻¹ soil	mg P kg ⁻¹ soil
1 April 2010	3.3c	0.29
5 May 2010	4.7b	0.21
3 June 2010	7.5a	0.47
1 July 2010	5.1b	0.38

Visual Turfgrass Quality and Color Index

Visual turfgrass quality responded dramatically to STP level and supports reports by Dest and Guillard (2003) and Johnson et al. (2003) that visual quality is the best response for STP critical value determination (Figure 4.2). September 2009 data were omitted because of laboratory STP levels were elevated compared to sampling in August and October 2009, likely due to laboratory error. Turfgrass visual quality responded linearly to STP level until the critical level had been surpassed and no further response to STP level occurred. Soil test P critical levels ranged from 5.3 to 10.4 kg P ha⁻¹ depending on rating date (Figure 4.3). Critical values were greatest in early in each year and diminished as each season progressed. Application of TE did not statistically reduce STP critical level (Figure 4.3). On two days TE significantly altered critical STP value, but the change was within soil sampling and analysis error.

Soil test P level affected color index similarly to visual quality (Figure 4.4). Critical values ranged from 4.9 to 7.7 mg kg⁻¹ depending on rating date. As with turfgrass visual quality, TE application did not significantly affect color index STP critical value. There was a TE x day interaction; however, differences in STP critical value were less than laboratory error (Figure 4.4). Distinction of color index on STP critical level was not as obvious as with visual quality. Kruse et al. (2005) reported that some reflectometers can be limited in their ability to discern P deficient leaves from healthy tissue. Therefore, decreased color index at low STP levels is most likely attributed to decreased stand density that resulted from extreme P deficiency. This further supports visual quality ratings is the most appropriated attribute used to determine STP critical P level since both turfgrass color and density are factors used to generate overall visual quality ratings.

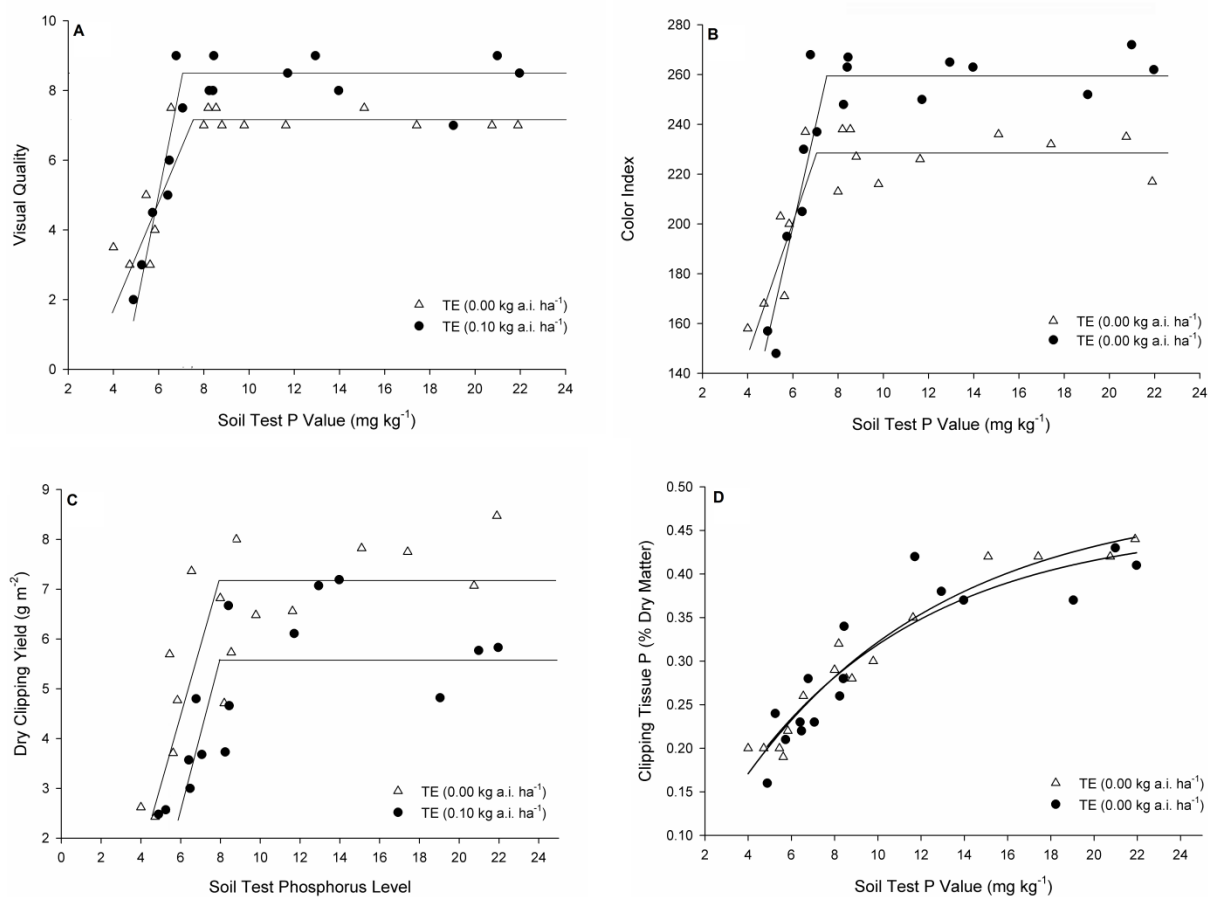


Figure 4.2. The effect of soil test P (STP) level on turfgrass visual quality (A), color index (B), clipping yield (C), and clipping tissue P level (D) on 1 July 2010. These figures are representative of all sampling dates described in this chapter.

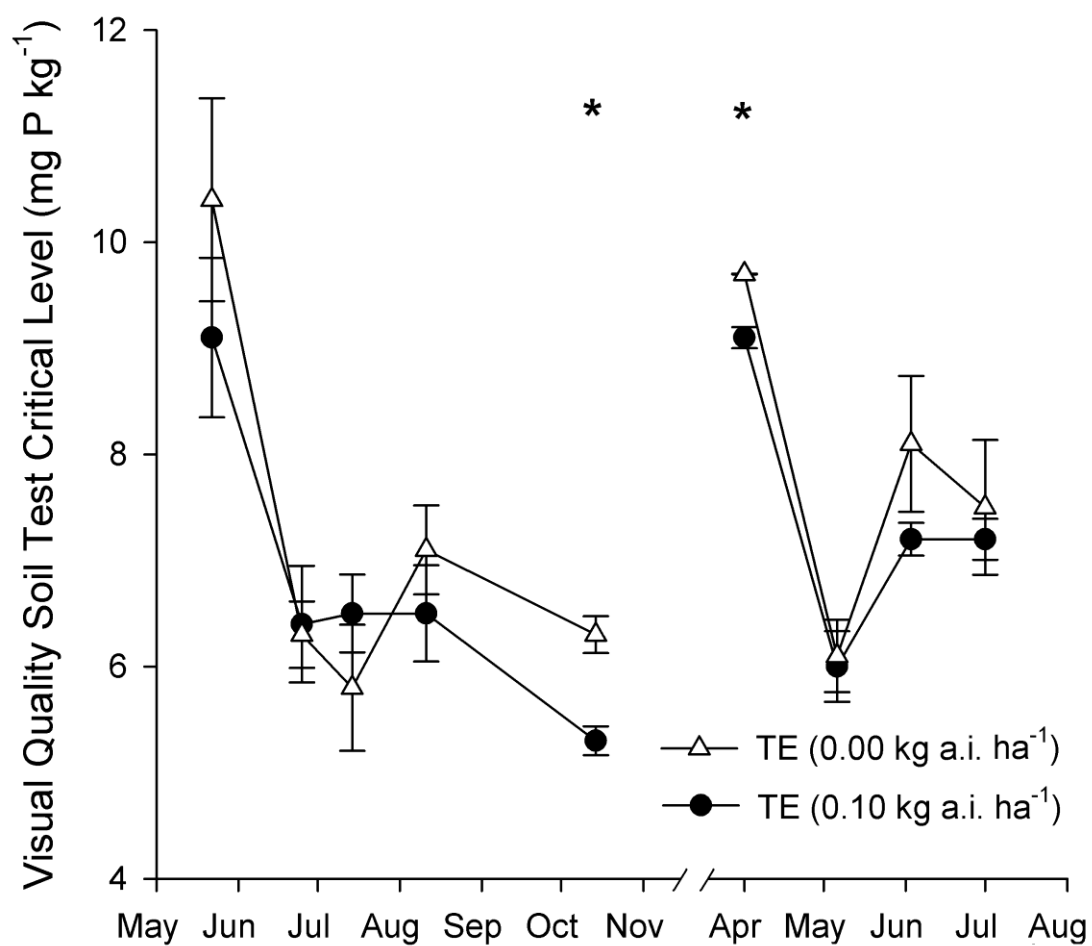


Figure 4.3. Visual quality soil test P (STP) critical value as a function of date and trinexapacetyl (TE). Rating days where TE significantly affected STP critical value are denoted with an asterisk. Error bars represent standard error of the mean with 16 degrees of freedom.

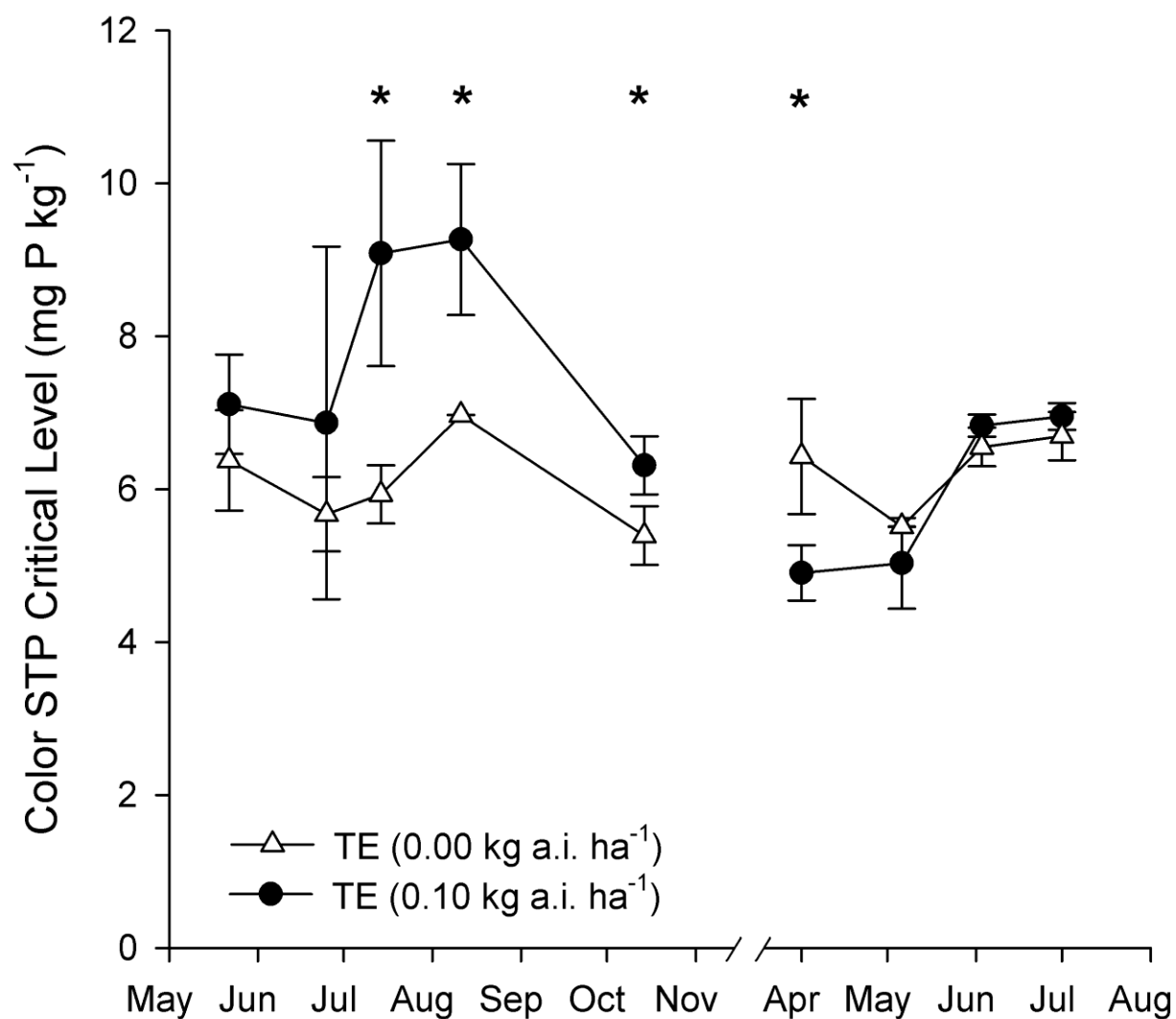


Figure 4.4. Color index soil test P (STP) critical value as a function of date and trinexapacetyl (TE). Rating days where TE significantly affected STP critical value are denoted with an asterisk. Error bars represent standard error of the mean with 16 degrees of freedom.

The visual quality and color index model plateaus were affected by TE application, date, and TE x date, and TE x year interactions (Figure 4.5 and 4.6). Trinexapac-ethyl enhanced visual quality and color index model plateaus on every date except for the first rating day, 22 May 2009, and is responsible for the TE x date interaction (Figure 4.5). Application of TE into October of 2009 sustained turfgrass quality enhancement into spring 2010. As a result, TE enhanced turfgrass quality and color index on all rating dates in 2010 and caused the TE x year interaction. Turfgrass visual quality enhancements increased in magnitude relative to the non-treated control as the each season progressed. This is consistent with the report by McCullough et al. (2006a) and has been further documented in chapters two and three of this thesis.

Clipping Yield

Clipping yield data from 2009 was omitted from this study because of substantial variability that resulted from scalping between plots during clipping collection. When the greens mower would stop at the end of P deficient plots, increased verdure from turfgrass in the adjacent plot caused the mower to scalp and interfered with yield measurements. This problem was resolved in 2010 by mowing buffer passes between plots at 2 mm and verified that TE sustained clipping yield suppression. Linear-linear plateau regression with the 2010 data found the STP critical level ranged from 4.2 to 12.2 mg kg⁻¹(Figures 4.7). Trinexapac-ethyl application significantly reduced the clipping yield STP critical level and plateau value by 29 and 14%, respectively in 2010. This level of growth suppression is consistent with reports by McCullough et al., (2006b) and chapters two and three in this thesis. Depression in the STP critical value in 2010 supports our hypothesis that reduced clipping yield results in lower STP critical level; however, the 2.2 mg kg⁻¹ decrease in critical level is still within laboratory error and therefore of little practical importance. There was TE x date interactions for both STP critical value and yield plateau. Critical level was not significantly reduced on the 6 May and 3 June 2010 rating

days and yield plateau was not statistically reduced on 1 April and 6 May 2010 (Figures 4.7 and 4.8).

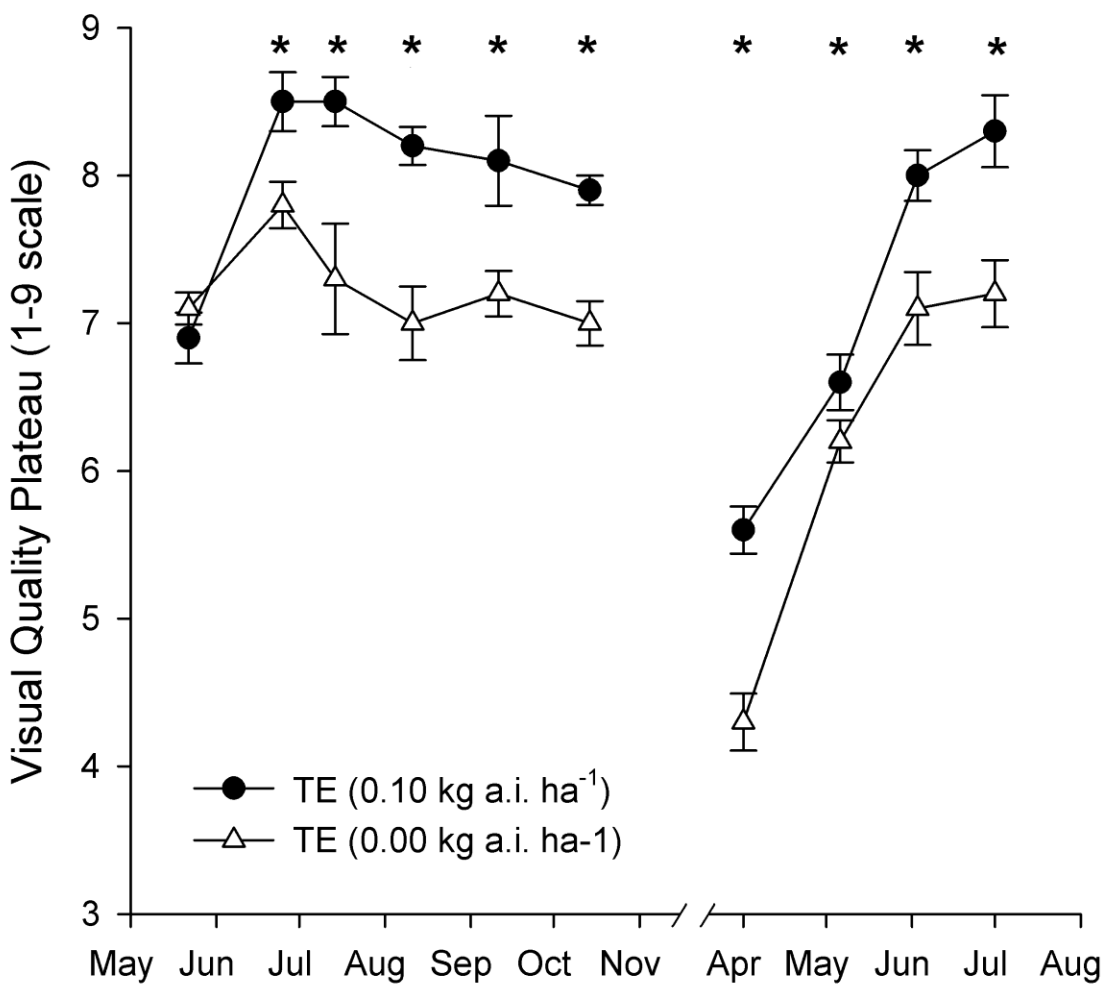


Figure 4.5. The effect of trinexapac-ethyl (TE) and date on visual quality plateau. Asterisks signify dates where TE significantly enhanced turfgrass visual quality. Application of TE into late fall 2009 enhanced early spring visual quality ratings on 1 April 2010. Error bars represent standard error of the estimate with 16 degrees of freedom.

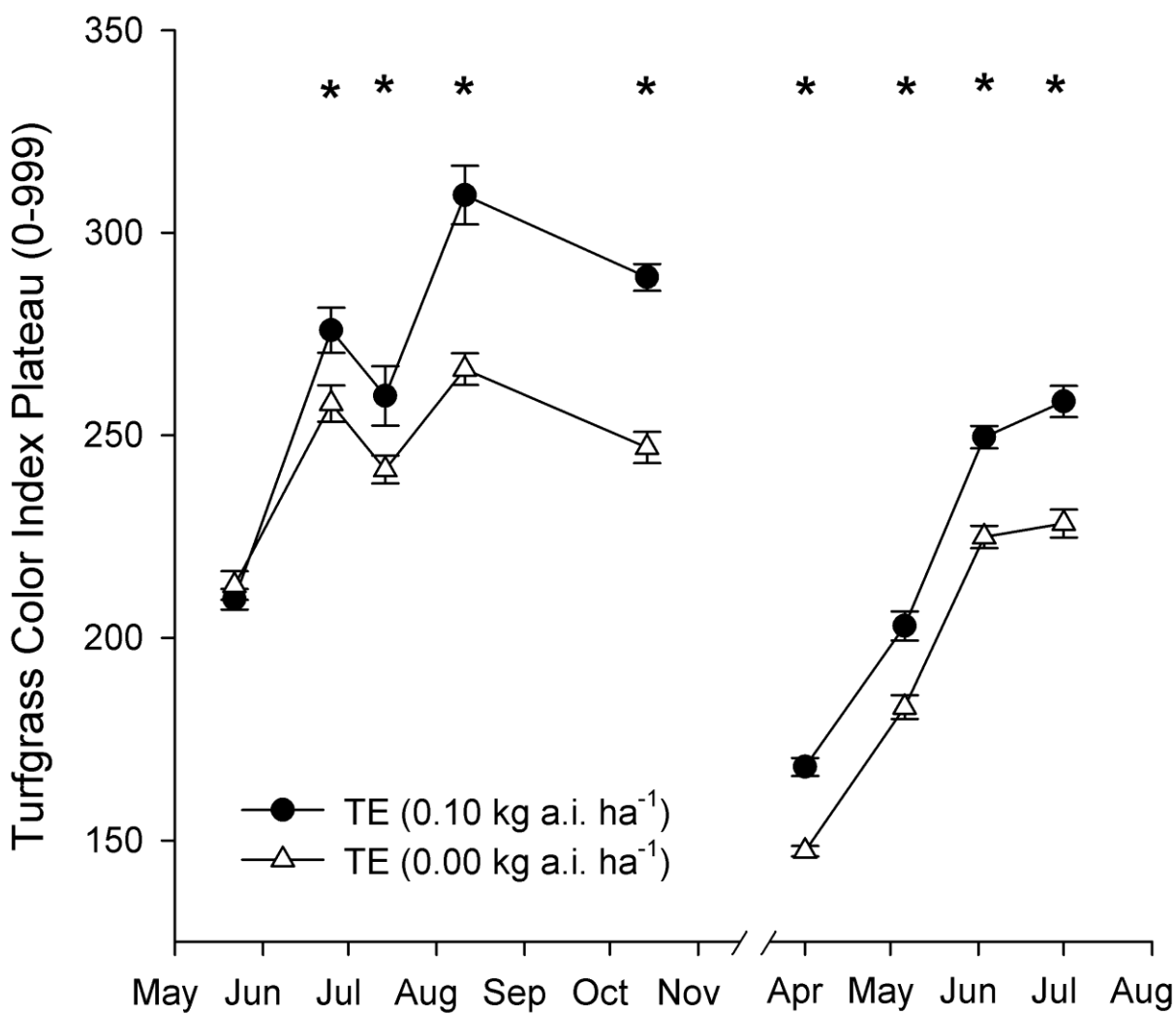


Figure 4.6. The effect of trinexapac-ethyl (TE) and date on color index plateau. Asterisks signify dates were TE significantly enhanced turfgrass color index. Application of TE into late fall 2009 enhanced early spring color index on 1 April 2010. Error bars represent standard error of the estimate with 16 degrees of freedom.

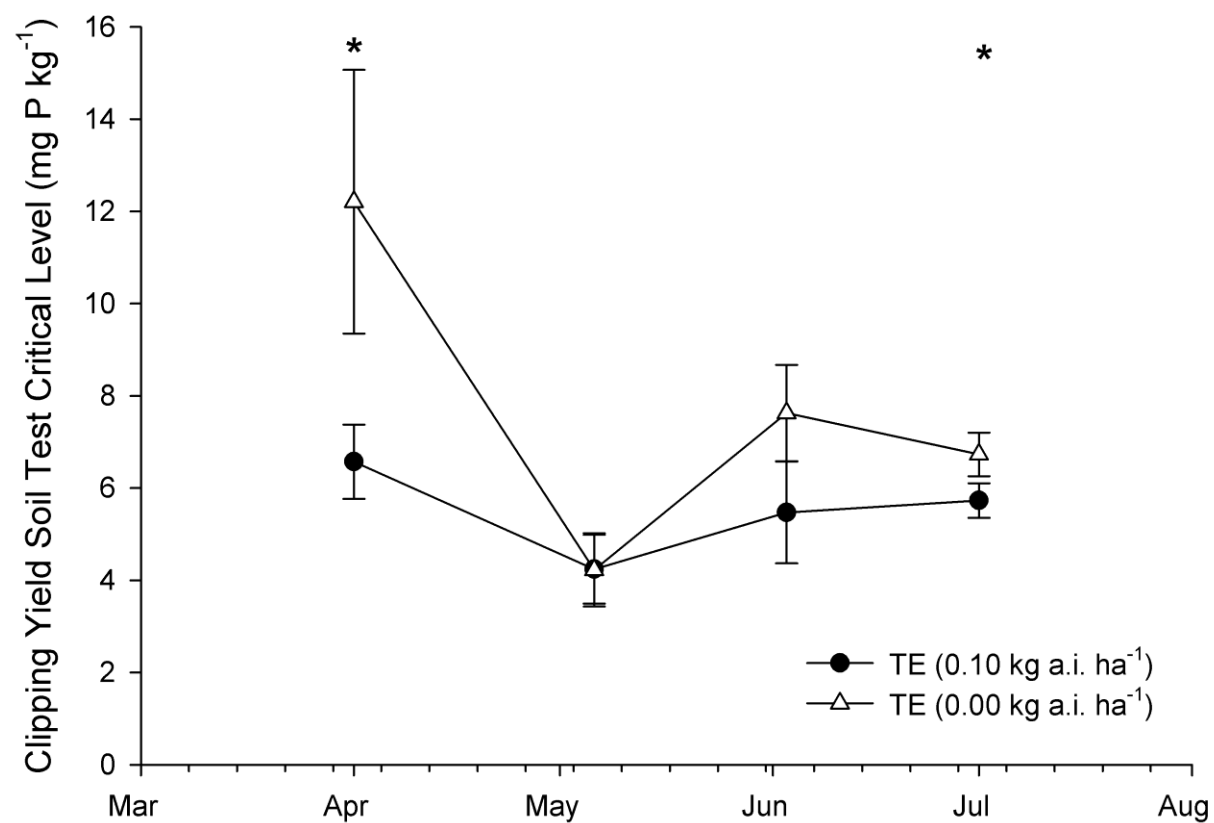


Figure 4.7. Clipping yield soil test P (STP) critical value as a function of date and trinexapacetyl (TE). Rating days where TE significantly affected STP critical value are denoted with an asterisk. Error bars represent standard error of the estimate with 16 degrees of freedom.

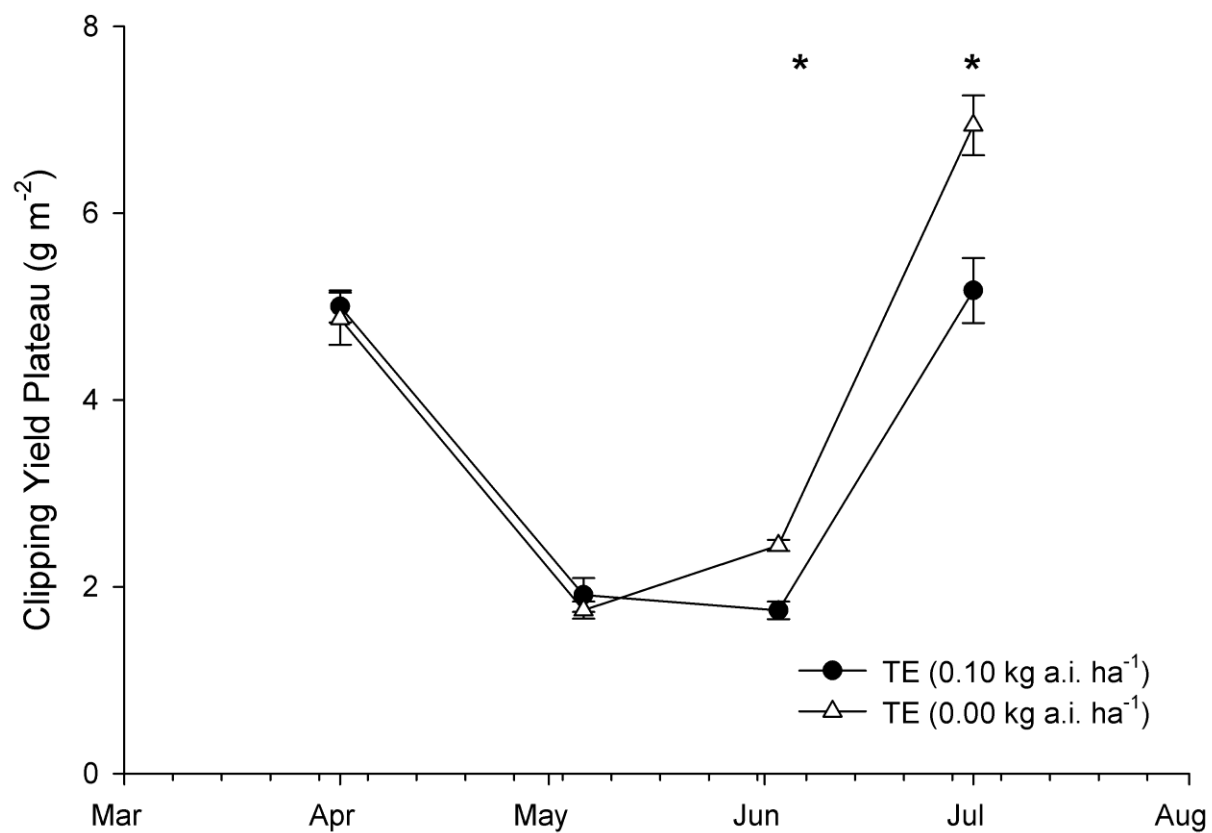


Figure 4.8. The effect of trinexapac-ethyl (TE) and date on clipping yield plateau. Asterisks signify dates were TE significantly suppressed turfgrass clipping yield. Error bars represent standard error of the estimate with 16 degrees of freedom.

Clipping Tissue Phosphorus Content

Clipping tissue P content increased with increasing STP level. Logarithmic regression models were highly significant on all rating days, $p < 0.0001$, regardless of TE treatment (Figure 4.2). Application of TE did not affect clipping tissue P content on any day, p-values ranged from 1.000 to 0.388. This is in contrast to reports of decreased leaf P content in creeping bentgrass and hybrid bermudagrass (Fagerness et al., 2004; McCullough et al., 2006a).

Arbuscular Mycorrhizal Infections

There was an interaction between STP level and TE application when arbuscules were quantified from November 2009 soil plugs (Table 4.2). Plots not treated with TE had more arbuscular mycorrhizal fungi (AMF) associations at the higher STP level. This effect is contrary to the literature that suggests plants suppress AMF associations as soil fertility increases (Johnson et al., 1997). Plots treated with TE had the same number of AMF associations regardless of soil test phosphorus level. Feng et al. (2002) reported trinexapac-ethyl applications increased root associations with symbiotic arbuscular mycorrhizal fungi in a creeping bentgrass research putting green during summer in Alabama. It is unclear how increased AMF associations at low STP levels with TE helped turfgrass health because clipping tissue contents were unchanged with TE application.

Table 4.2. The effect of soil test P (STP) and trinexapac-ethyl (TE) on arbuscular mycorrhizal fungal associations. Application of TE sustained AMF associations regardless of STP level. Arbuscule counts back-transformed from a logarithmic transformation.

STP Level mg P kg ⁻¹ Soil	TE Application Rate kg a.i. ha ⁻¹	Arbuscules Count # 3mm Root Section ⁻¹
3	0.0	7.8D
8	0.0	7.0CD
20	0.0	12.8A
3	0.1	9.9ABC
5	0.1	9.9BC
30	0.1	11.6AB

CONCLUSIONS

The Mehlich-3 STP critical value for creeping bentgrass grown on calcareous, sand-based putting greens ranged from 4 to 12 mg P kg⁻¹ depending on turfgrass response parameter.

Turfgrass quality was to be the most effective response for determination of STP critical levels because of high responsiveness to STP level prior to the critical value. Quality response stops abruptly after STP level exceeded the critical level. Trinexapac-ethyl suppressed clipping yield by 14% in 2010 and is consistent with findings by McCullough et al. (2006b) and as observed in Chapters two and three of this thesis. Trinexapac-ethyl applications statistically reduced STP critical values on select rating day, but not by a practical level. Turfgrass color, visual quality, and AMF associations were enhanced with TE application after SPT values were above the critical soil value. It is unclear what effect increased AMF associations had on STP critical value determination because tissue P content was not altered with TE application.

REFERENCES

- Adams, W.A. 1960. Effects of nitrogen fertilization and cutting height on the shoot growth, nutrient, removal, and turfgrass composition of an initially perennial ryegrass dominant sports turf. *Proceedings of the Third International Turfgrass Research*. 1:343-359.
- Carrow, D.N., D.V. Waddington, and P.E. Rieke. 2001. *Turfgrass soil fertility and chemical problems: Assessment and management*. Ann Arbor Press, Chelsea, MI.
- Christians, N. E., D. P. Martin, and J. F. Wilkinson. 1979. Nitrogen, phosphorus, and potassium effects on quality and growth of Kentucky bluegrass and creeping bentgrass. *Agron. J.* 71:564-567.
- Christians, N.E., D.P. Martin, and J.F. Karnok. 1981. The interrelationship among nutrient elements applied to calcareous sand greens. *Agron. J.* 73:929-933.
- Colclough, T., and D.M. Lawson. 1989. Fertilizer nutrition of sand golf greens V. Rootzone nutrient analysis. *J. Sports Turf Res. Inst.* 65:73-79.
- Dest, W.M., and K. Guillard. 1987. Nitrogen and phosphorus nutritional influence on bentgrass-annual bluegrass community composition. *J. Am. Soc. Hortic. Sci* 112:769-773.
- Fagerness, M. J., D. C. Bowman, F. H. Yelverton, and T. W. Jr. Rufty. 2004. Nitrogen use in Tifway bermudagrass, as affected by trinexapac-ethyl. *Crop Sci.* 44:595-599.
- Feng, Y., D.M. Stoeckel, E. van Santan, R.H. Walker. 2002. Effects of subsurface aeration and trinexapac-ethyl application on soil microbial communities in a creeping bentgrass putting green. *Biol. Fertil. Soils.* 36:456-460.
- Guillard, K., and W.M. Dest. 2003. Extractable soil phosphorus concentration and creeping bentgrass response on sand greens. *Crop Sci.* 43:272-281.
- Hamel, S.C., and J.R. Heckman. 2006. Predicting need for phosphorus fertilizer by soil testing during seeding of cool season grasses. *HortScience.* 41:1690-1697.
- Johnson, N.C., J.H. Graham, and F.A. Smith. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist.* 4:575-586.
- Johnson, P.G., R.T. Koenig, and K.L. Kopp. 2003. Nitrogen, phosphorus, potassium requirements in calcareous sand greens. *Agron. J.* 95:697-702.
- Kruse, J.K., N.E. Christians, and M.H. Chaplin. 2005. Remote sensing of phosphorus deficiencies in *Agrostis stolonifera*. *Int. Turfgrass Soc. Res. J.* 10(2):923-928.
- Marchner, H. 1995. *Mineral nutrition of higher plants*. 2nd ed. Academic Press, New York.

- McCullough, P. E., H. Liu, L. B. McCarty, T. Whitwell, and J. E. Toler. 2006a. Growth and nutrient partitioning of 'TifEagle' bermudagrass as influenced by nitrogen and trinexapacetyl. *HortScience*. 41:453-458.
- McCullough, P. E., H. Liu, L. B. McCarty, and J. E. Toler. 2006b. Ethephon and trinexapacetyl influence creeping bentgrass growth, quality, and putting green performance. [Online] *Appl. Turfgrass Sci.* p. [1-7].
- USGA Green Section Staff. 2004. USGA recommendations for a method of putting green construction: The 2004 revision. *USGA Green Section Record* 31:1-3.
- Waddington, D.V., T.R. Turner, J.M. Duich, and E.L. Moberg. 1978. Effect of fertilization on 'Penncross' creeping bentgrass. *Agron. J.* 70:713-718.