PHOSPHORUS RESPONSE AND ORTHOPHOSPHATE LEACHING IN FLORATAM ST. AUGUSTINEGRASS AND EMPIRE ZOYSIAGRASS

By

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To my wife, Patricia

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TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

PHOSPHORUS RESPONSE AND ORTHOPHOSPHATE LEACHING IN FLORATAM ST. AUGUSTINEGRASS AND EMPIRE ZOYSIAGRASS

By

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Information on critical phosphorus (P) concentration in tissue of *Stenotaphrum secundatum* (Walt) Kuntze cultivar 'Floratam' (St. Augustinegrass) and *Zoysia japonica* cultivar 'Empire' (Zoysiagrass) is limited. Knowledge of critical leaf tissue P concentrations for these turfgrass species can help to avoid unnecessary P fertilization and reduce the risks of negative consequences to the environment. A hydroponic study was established to determine the critical P concentration in leaf tissue of 'Floratam' St. Augustinegrass and 'Empire' zoysiagrass. Six levels of P (0, 90, 135, 203, 304, and 456 mg P m^{-3}) were used. Plant growth rate, P concentration in leaf tissue, visual ratings of turfgrass quality, percent green turf cover and chlorophyll index (CI) were evaluated biweekly for 140 days. Turfgrass visual quality rating increased with increasing P supply. Maximum zoysiagrass growth rate and percent green cover were reached at 1.67 g P kg^{-1} and 1.35 g P kg^{-1}, respectively. Maximum St. Augustinegrass growth rate and percent green cover were reached at 1.73 g P kg^{-1} and 1.48 g P kg^{-1}, respectively. Consequently, a P concentration in leaf tissue of 1.35 g P kg^{-1} and 1.67 g P kg^{-1} for zoysiagrass and 1.48 g P kg^{-1} and 1.73 g P kg^{-1} for St. Augustinegrass could be used as the threshold concentrations for maintenance of maximum green turf density and

maximum growth and recovery rates, respectively. Phosphorus fertilization in low P retention soils can result in P leaching to ground water. Another study was conducted to evaluate the effect of P application rate on orthophosphate (P_i) leaching. St. Augustinegrass and zoysiagrass were grown in a “clean” sand with very low extractable P and negligible P soil storage capacity. Five rates of P were supplied (from 0 to 5 g m^{-2} $year^{-1}$). Phosphorus uptake, plant dry matter accumulation, and Mehlich I extractable P (M1-P) were determined biweekly for 140 days (May to September) during 2008 and 2009. Orthophosphate leaching rate and P_i concentration in leachate from zoysiagrass were greater than from St. Augustinegrass. Phosphorus uptake rate over time in St. Augustinegrass was greater than in zoysiagrass. The root system of St. Augustinegrass was more extensive and deeper than in zoysiagrass. Rate of P_i leaching was positively related to amount of rainfall plus irrigation received by the turf. Phosphorus fertilization over time increased M1-P, phosphorus saturation ratio (PSR) and reduced of the soil phosphorus storage capacity (SPSC). Greater volume-weighted P_i concentrations in leachates were measured in soils with greater M1-P and PSR values and lower SPSC values. Orthophosphate concentrations in compliance with the U.S. Environmental Protection Agency (USEPA) water quality criteria for Florida were measured in soils with a PSR as high as 0.6. Total estimated amount of P leached from fertilizer application was below 5% and 0.25% in zoysiagrass and St. Augustinegrass, respectively. The results of this research indicate that if P fertilization is required based on tissue analysis and the SPSC is positive, it would be environmentally safe to supply P at a maximum rate of 0.54 g P m^{-2} per application (1.07 g P m^{-2} per year) to St. Augustinegrass and 0.2 g P m^{-2} per application (0.8 g P m^{-2} per year) to zoysiagrass.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Florida is the fourth most populous state in the United States of America. The population in Florida increased 16% from the year 2000 to 2009, and during the same period the estimated housing units increased 21% (US Census Bureau, 2010). The population increase and related growth of the residential sector has favored an expansion of the total area under turfgrass in the state (Haydu et al., 2005).

Tufford et al. (2003) found greater concentrations of nitrate (NO_3) and total phosphorus (TP) in urban streams than in streams from non-developed sites. Urban run-off has been identified as an important nonpoint source of phosphorus (P) and nitrogen (N) to surface waters and a major cause of lake deterioration (Carpenter et al., 1998). Erickson et al. (2001) reported that concentrations of inorganic N in runoff from St. Augustinegrass [*Stenotaphrum secundatum* (Walt) Kuntze] grown in a sandy soil on a 10% slope were not different from those measured in rainfall. They also measured greater losses of N through leaching than runoff; however, the amount of leaching was low. Phosphorus losses through runoff and leaching increase when runoff or water percolation caused by heavy rainfall occurs shortly after a P fertilizer application (Soldat and Petrovic, 2008).

Shuman (2002) reported increased P leaching from ‘Tifdwarf’ bermudagrass (*Cynodon* spp.) supplied with high irrigation rates. Greater P leaching was measured from creeping bentgrass (*Agriostis stolonifera* L.) grown in a sand (80% of particles between 0.25 mm and 0.5 mm) than in a sandy loam or silt loam soil (Petrovic, 2004). In soils dominated by “clean” (uncoated) sands the risk of P leaching is greater than in soils with coated sands (Harris et al., 1996). Phosphorus enrichment of surface fresh

water bodies (lakes, reservoirs, streams, and headwaters of estuarine systems) has been recognized as the most frequent cause of eutrophication (Correll, 1998). Phosphorus is an essential plant nutrient (Raghothama, 2005). Adequate fertilization is required to maintain high quality home lawn turfgrasses (Trenholm and Unruh, 2005). Limited information is available on the relationship between P application rates to home lawn warm season turfgrasses and P losses through leaching, especially in soils with low P retention capacity. Therefore, it is imperative to develop improved fertilization practices to maintain high quality turfgrasses and reduce negative impacts on the environment associated with P loading of surface water bodies.

Phosphorus: A non-Renewable Resource

Marine sediments (840,400 x 10^{12} kg) represent the main reserve of P on earth, followed by terrestrial soils (96 to 160 x 10^{12} kg), dissolved inorganic P in the ocean (80 x 10^{12} kg), and the biota (2.6 x 10^{12} kg in terrestrial biota and 0.05-0.12 x 10^{12} kg in marine biota) (Stevenson and Cole, 1999). Inorganic P fertilizers are produced from phosphate bearing minerals such as flourapatite [$Ca_{10}(PO_4)_6F_2$] and hydroxyapatite [$Ca_{10}(PO_4)_6OH_2$]. Phosphate rock (PR) is a frequently used term that refers to mineral assemblages (rock) with high concentration of P-bearing minerals (Stewart et al., 2005). The most abundant phosphate mineral in soils and sediments is flourapatite (Harris, 2002). There are two types of phosphate rocks: sedimentary (also called *phosphorites*) and igneous. About 80% of the worlds PR production come from sedimentary PR deposits. Main reserves of phosphate rock in the world are located in Morocco and Western Sahara, United States, South Africa, and China (Stewart et al., 2005). Phosphorus is finite resource (Cordell e al., 2009) and it has been estimated that at

current mining rates PR reserves may be exhausted in a period as short as 90 years (Stewart et al., 2005).

Soil Phosphorus Reactions

In relatively unweathered soil the main P minerals are apatites, while in highly weather soils the dominant mineral forms of P are aluminum (Al) and iron (Fe) phosphates (Pierzynsky et al., 2005b). Total soil P concentration usually rages between 50 to 3000 mg P kg^{-1}. Commonly the concentration of P decreases with depth in the soil profile (Stevenson and Cole, 1999).The content of organic forms of P in the soil vary depending on the soil type (15-80%), and the main organic soil P forms are inositol phosphates (10-50%), phospholipids (1-5%) and nucleic acids (0.2-2.5%) (Stevenson and Cole, 1999).

In most soils, P availability is limited by the highly reactive nature of P in the soil environment. In soils, P is present in more than 170 minerals with different solubility (usually sparingly soluble) and which tend to become more insoluble with time (Holford, 1997). The most common form of P in the environment is the phosphate anion (PO_4^{3-}). Phosphate has the tendency to form stable minerals because the electronegativity of the oxygen (O) ions is much greater than the P ions, thus, electrons are spatially distributed towards the O ions creating a negative charge at the surface of the O tetrahedron (Harris, 2002). This results in a strong attraction between phosphate ions and cations in the crystal structure of phosphate minerals, and also explains the great affinity of the phosphate ion for positively charged surfaces of metal oxides and aluminosilicates (Harris, 2002). In acid soils, P is specifically adsorbed or chemisorbed (forms an inner sphere complex in which the phosphate ion is bonded directly to the metal at the mineral surface) to Fe and Al oxides and hydroxides and also can

precipitate as highly insoluble Fe and Al phosphates (wavellite, varascite, strengite) (McBride, 1994; Harris, 2002). In calcareous soils, the sparingly soluble di-calcium phosphate ($Ca_2(HPO_4)_2$) and tri-calcium phosphate ($Ca_3(PO_4)_2$) are formed which eventually becomes the highly insoluble carbonate hydroxyapatite [$3(Ca_3(PO_4)_2)\cdot CaCO_3$] (Stevenson and Cole, 1999). Phosphorus is most plant available at a soil pH near 6.5 (Havlin, 1999).

Desorption of P adsorbed to mineral surfaces (clays, Fe-Al oxide-hydroxides, carbonates) and dissolution of primary and secondary P-bearing minerals replenishes the concentration of inorganic soluble P as it is removed from the soil solution by plant uptake, leaching, or subsurface runoff (Havlin, 1999; Brady and Weil, 1999).

Soil organic P is decomposed to soluble organic P and latter converted to soluble inorganic P forms (H_2PO_4, HPO_4^{2-}) through mineralization (at C:P ratios <200:1) carried out by soil microorganisms. Soluble inorganic P may be incorporated into the soil microbial biomass and become immobilized (at C:P ratios >200:1) (Pierzynsky et al., 2005b). Figure 1-1 depicts the myriad of processes controlling soil P availability and possible pathways for P losses from the soil system.

Phosphorus from phosphate fertilizers undergo a series of reactions in soil upon solubilization of the fertilizer granule, that lead to increasing lower solubility of the resulting forms of P in the soil (Hedley and Mclaughlin, 2005). Three zones or bands that form around P fertilizer granules as it dissolves in the soil have been identified. The first zone consists of the fertilizer granule and immediate fertilizer-soil interface (0-2 mm from fertilizer granule). In this region, the metastable triple point solution of monocalcium phosphate (MCP) formed as the P fertilizer (single superphosphate or

concentrated superphosphate) granule dissolves in the soil solution is concentrated enough to exceed the maximum soil sorption capacity. Precipitates of dicalcium phosphate (CaH_2PO_4) and dicalcium phosphate dihydrate ($CaH_2PO_4 \cdot 2H_2O$) form at the granule as MCP starts to move away from it (Hedley and Mclaughlin, 2005). In the second region, a phosphorus saturated soil zone next to the fertilizer granule (~ between 2 mm and 10 mm from the fertilizer granule), the solubility product of Fe and Al phosphates is exceed and precipitation of these minerals proceeds (Hedley and Mclaughlin, 2005). In the third region, the soil P concentration does not exceed either soil P sorption maxima or the solubility product of metal phosphates (>10 mm from fertilizer granule), particularly amorphous Al and Fe phosphates. Hence, in this zone soil sorption reactions [non specific adsorption (electrostatic attraction to positively charged soil surfaces); ligand exchange or chemisorption, which involves a covalent bond between phosphate and metal ion at the mineral surface; and P occlusion by amorphous, organo-mineral coatings on soil particles surfaces] predominate and control the solubility and availability of P to plants (Hedley and Mclaughlin, 2005).

Phosphorus and Plant Nutrition

Phosphorus participates in nearly all metabolic processes in plants. Phosphorus is a component of enzymes, phospholipids, and nucleic acids (DNA, RNA), adenosine diphosphate (ADP), adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADP). Consequently, P plays a key role in storage and transfer of energy required in the plant's metabolism, it is involved in the transfer of genetic information, it participates in the process of photosynthesis (P is a constituent of ATP, 3-phosphogliceric acid), it is required to maintain membrane stability necessary for

uptake of water and nutrients, and it regulates the formation and translocation of sugars and starches (Bennett, 1993, Carrow et al., 2001).

Phosphorus Uptake and Use by Plants

Phosphorus is absorbed by plants as orthophosphate (P_i) ions, in either the monovalent ($H_2PO_4^{-1}$) or divalent form (HPO_4^{2-}) (Vance, 2003). The dominant P_i species is dependent on the soil solution pH. Below a pH of 7.2 the main species is $H_2PO_4^-$ whereas above that pH HPO_4^{2-} is the main P_i species in solution. Maximum P uptake rates in higher plants takes place at a solution pH between 5 and 6, at which $H_2PO_4^-$ is the dominant species (Schachtman et al., 1998). Orthophophate is completely available to plants, but the majority (>90%) of the soil P is in non labile forms such as phosphate minerals, humus P, insoluble Ca, Fe and Al phosphates and P specifically adsorbed by hydrous oxides and aluminosilicates minerals (Mengel and Kirkby, 2001). As P_i is removed from solution, it is transported primarily (~95%) by diffusion (P moves from an area of greater concentration to an area of lower concentration) from other sections of the profile towards the root surface. High P concentration in solution can be found in highly fertilized soils as well as in soils that have received manure additions over a prolonged period of time. Under these conditions mass flow may account for a greater portion of plant P_i uptake (Kovar and Claassen, 2005). Phosphorus diffusion coefficient is very slow (10^{-12} to 10^{-15} m^2 s^{-1}), hence, P_i depletion zones form around roots when plant uptake is rapid (Schachtman, 1998).

Since $H_2PO_4^-$ is negatively charged it passes readily through the spaces between the cellulose microfibrils and it is repelled in the pectin network by negatively charged surfaces (Miyasaka and Habte, 2001). The P_i acquired by roots is rapidly loaded into the xylem but before it has to enter the cytoplasm. The concentration of P_i in the soil

solution rarely exceeds 10 µM and in most soils it is about 2µM. In contrast, the concentration of P_i in plant tissues ranges between 5 to 20 mM (Raghothama, 1999). In order to overcome the step concentration gradient between the soil solution and the cytoplasm (~2000-fold) and the negative membrane potential, active transport of P across the plasmalemma is required (Vance, 2003).

It has been proposed that the active uptake of P_i into the cytoplasm is an energy-mediated cotransport process, which is driven by protons generated by a plasma membrane H^+-ATPase. A transient decrease of the cytoplasmic pH takes place as P_i is transported from the solution coupled with H^+. Phosphate uptake is accompanied by 2 to 4 H^+ ions per $H_2PO_4^-$ that is transported (Schachtman et al., 1998). As concentration of P_i in solution decreases the number of H^+ ions that accompany each $H_2PO_4^-$ increase. Furthermore, the H^+ ions required for the co-transport of $H_2PO_4^-$ are supplied either by the solution or by the activated proton pumps in the plasma membrane. In addition to the transient decrease in the cytoplasm pH (0.2 to 0.3 pH units) due to the $H^+/H_2PO_4^-$ co-transport, a temporary depolarization of the membrane takes place due to the uptake of excess positive charge (2 to 4 H^+ per $H_2PO_4^-$) (Ullrich and Novak, 1990). The plasmalemma membrane is repolarized as the H^+-ATPase pumps protons out to maintain the cytoplasm pH and generate the necessary electro-chemical potential gradient for continued P_i uptake (Raghothama, 1999).

Once P_i enters the cytoplasm it may be transported into the vacuole due to the action of H^+-ATPases pump at the tonoplast. The storage of P_i in vacuole under conditions of sufficient supply results in a fairly stable P_i concentration in the cytoplasm when external P_i supply decreases. The maintenance of a constant concentration of an

ion in the cytoplasm is defined as cytoplasmic homeostasis (Miyasaka and Habte, 2001). Lee et al. (1990) reported that P_i concentration in the cytoplasm remained fairly constant (5-10 mM) during changes in external P_i concentration, whereas, vacuolar P_i concentration changed widely. As the P_i concentration in solution decreased from 0.45 to 0.05 mM, the vacuolar P_i decreased in relation to the cytoplasmic P_i (Lauer et al., 1989).

Moreover, kinectic analysis of P_i uptake indicates that plants have a low and a high-affinity uptake system. The low-affinity system is operative when the concentration of P_i in solution is high. On the other hand, the high-affinity system operates under low P_i concentrations (Vance et al., 2003). The high-affinity transporters are membrane-associated proteins that translocate P_i from the solution, containing µM P_i concentrations, to the cytoplasm where the P_i concentration may be in the mM range. The expression of P_i transporters is favored under P_i starvation conditions. In addition, the expression of P_i transporters genes is not only a rapid response to P_i starvation but is also a reversible process when the P_i limiting conditions disappear (Raghothama, 1999).

Plants grown in a P limited environment utilize a variety of mechanisms to increase P_i acquisition capacity such as (i) release of low-molecular weight organic acids and phosphatase to the rhizosphere to solubilize inorganic and organic P_i sources, (ii) partition a greater amount of assimilates to root growth, (iii) modify the morphology (more root hair production) and architecture (greater root growth in P_i –rich sections of the root environment), (iv) accelerate P_i uptake rate from solution, and (v) establish symbiotic relationships with mycorrhizal fungi to explore a greater soil volumes and

solubilize inorganic and organic forms of P from distant sections of the soil profile (White and Hammond, 2008). Intracellular phosphatases are involved in the release of P_i from senescent tissue which is then remobilized to actively growing tissues (Marschner, 2005). On the other hand, if there is an adequate P_i supply and the uptake rate exceeds the plant demand several processes may take place in the plant to prevent the accumulation of P_i in toxic concentrations. For example, conversion of P_i to organic storage forms (e.g., phytic acid), reduction of P_i uptake rate, and P_i loss by efflux (Lee et al., 1990). Phosphorus is very mobile in plants and it can be translocated downwards or upwards in plant tissues. Hydrolysis of organic P originally accumulated in older leaves releases P_i that is then remobilized via phloem to actively growing young leaves. High concentration of P_i in the phloem sap, usually present under low shoot demand, and the associated remobilization of P_i from shoots to roots can act as a feedback signal to regulate P uptake. Similarly low concentrations of P_i in the phloem related to high shoot demand result in greater uptake (Mengel and Kirkby, 2001).

Nutrient use and acquisition efficiency concepts facilitate evaluating the ability of the plant to absorb and utilize nutrients for maximum yields (Baligar et al., 2001). Plan species can be classified into efficient and inefficient nutrient users based on the nutrient efficiency ratio, which is calculated as the units of yield per unit of nutrient element in tissue (i.e., g P kg^{-1} dry matter) (Baligar et al., 2001). Moreover, the ability of the plant to absorb the nutrients supplied with fertilizer could be evaluated using the apparent nutrient recovery (ANR) efficiency, which is defined as the ratio of additional nutrient uptake of fertilized plants over unfertilized plants to the quantity of nutrient applied (Baligar et al., 2001). Moreover, differences in phosphorus use efficiencies

(PUE) have been reported for corn (*Zea mays* L.) genotypes (Fageria and Baligar, 1997; Netto and Lopez de Souza, 2008).

Phosphorus Diagnostic Tools: Critical Concentrations in Soil and Tissue

The critical nutrient concentration range has been defined as the nutrient concentration in the plant below which a yield response to added nutrients occurs (Havlin, 1999). Alternatively, it has been defined as the nutrient concentration (also called 'level') in the diagnostic tissue (i.e., leaf tissue) just below the level that gives optimal growth (Epstein and Bloom, 2005). Typically, 90% of maximum dry matter (DM) yield is used as a reference point to identify the critical tissue level (Marschner, 1995; Epstein and Bloom, 2005). The curve that relates plant growth rate or yield to nutrient concentration in tissue or nutrient supply is called a growth response curve (Figure 1-2). The growth response curve has three distinct regions: first, the deficiency range where growth rate increases with increasing nutrient supply and tissue concentration, the second region is the adequate range (also called 'sufficiency range') in which growth rate reaches a maximum and does not change by increasing nutrient supply or nutrient concentration in tissue (i.e., luxury consumption), and the third one is the toxic range region where the growth rate decreases with increasing supply (Marshner, 1995).

In P deficient turfgrasses, reduced shoot growth is associated with decreased rate of leaf expansion. Photosynthesis can be reduced by P deficiency but decrease in shoot growth is observed prior to decrease in photosynthetic rate. Phosphorus deficient leaves become dark green due to an increase in chlorophyll concentration associated with reduced leaf expansion. Older, lower leaves may turn more dark green than upper younger leaves (Carrow et al., 2001). As the P deficiency becomes more severe, the dark green color turn into a purplish to reddish color, especially in the older leaves

(Bennett, 1993). Photosynthates produced after shoot growth is limited, are used to maintain root growth and also can accumulate in leaf tissue (Carrow et al., 2001). These purple and reddish colorations appear in leaves (particularly along the veins) with severe P deficiency due to accumulation of anthocyanin associated with increased sucrose concentration in leaves. It has been proposed that anthocyanin protects nucleic acids from UV damage and chloroplasts from photoinhibitory damage related to P-limited photosynthesis (White and Hammond, 2008). Phosphorus deficient leaves senesce prematurely (Westermann, 2005). Phosphorus deficiency is more likely to occur during turfgrass establishment due to limited root growth, particularly with seedlings (Carrow et al., 2001).

Phosphorus toxicity in plants is not common (Carrow et al., 2001; Westermann, 2005). When P toxicity is present, younger leaves show interveinal chlorosis, necrosis and tip die back, marginal leaf scorch and shedding of older leaves (Bennett, 1993; Westermann, 2005). Iron deficiency may be induced by high P concentration especially in low-Fe soils (Carrow et al., 2001). Excess P can cause reduce turfgrass quality and chlorophyll concentration, and decreased top and root growth rate (Bennett, 1993). Menn and McBee (1970) noted a growth suppression of bermudagrass (*Cynodon dactylon* x *C. transvalensis*) with a leaf tissue P concentration of 4.5 g P kg^{-1} DM. Cakmak and Marschner (1987) noted that high P concentrations in plant tissue caused a decrease of the physiological availability of zinc (Zn). Moreover, a feedback mechanism that controls the retranslocation of P_i in phloem from shoots to roots is impaired in Zn-deficient plants (leading to low P_i concentration in the root phloem sap);

hence, the transport of P_i from roots to shoot is not regulated and toxic concentrations of P accumulate in leaf tissues (Marschner and Cakmak, 1986).

The critical P concentrations in leaf tissue of turfgrasses range between 1 g P kg^{-1} DM and 4 g P kg^{-1} DM; however, it varies widely among species and cultivars within species (Bennett, 1993). Carrow et al. (2001) indicated that the critical concentration range of P in turfgrasses oscillates between 2 g P kg^{-1} DM and 5.5 g P kg^{-1} DM. Phosphorus is required during the establishment of zoysiagrass and St. Augustinegrass, particularly in low-P soils (Bennett, 1993). The work of Liu et al (2006) and Liu et al. (2008) indicated that the critical leaf tissue P concentration of St. Augustinegrass [*Stenotaphrum secundatum* (Walt) Kuntze] ranges between 1.6 g P kg^{-1} and 1.8 g P kg^{-1}. Liu et al. (2008) also indicated that the critical Mehlich 1 extractable soil P (M1-P) for 'Floratam' St. Augustinegrass grown in sandy soils was 10 mg P kg^{-1} soil. Reports on 'Empire' zoysiagrass (*Zoysia japonica*) critical P concentration in leaf tissue or soil were not found. Adequate bermudagrass (*Cynodon dactylon* x *C. transvalensis* L.) visual quality was obtained at a leaf tissue P concentration ≥ 2 g P kg^{-1} DM (Menn and McBee, 1970).

In the state of Florida the extractable soil P concentration is determined using the Mehlich 1 extracting solution (Mylavarapu, 2009). The Mehlich 1 extracting solution consists of 0.05 M HCl + 0.0125 M H_2SO_4 (Mehlich, 1953). The chemical extracting principle of this solution is acid dissolution (i.e., HCl, H_2SO_4) of insoluble P forms (i.e., Al-P, Fe-P, Ca-P) as well as SO_4^{2-} exchange for $H_2PO_4^-$ from outer sphere adsorption sites, which reduces the readsorption of solubilized P (Kamprath and Watson, 1980; Beegle, 2005). This extracting solution should be used in acid soils (pH<6.5) with low

CEC (< 10 cmol/kg), low clay content, low soil organic matter content and predominantly kaolinitic clay minerals (Sims, 2009). In addition, Mehlich 1 extracting solution should not be use in alkaline soils (pH>6.5) or calcareous soils with high base saturation, cation exchage capacity and clay content because these soils tend to neutralize the acids of the extracting solution (reducing the ability of the acid to extract P) and P may be precipitated during extraction (Kuo, 1996; Beegle, 2005). Moreover, Mehlich 1 extracting solution should not be used in soils where rock phosphate was recently applied because the acid in the extracting solution would dissolve plant unavailable P from the rock phosphate (Kuo, 1996; Beegle, 2005). In the state of Florida, a very low, low, medium, high, and very high Mehlich 1 extractable soil P concentration is considered to be < 10 mg P kg^{-1}, 10 to 15 mg P kg^{-1}, 16 to 30 mg P kg^{-1}, 31 to 60 mg P kg^{-1} and >60 mg P kg^{-1}, respectively (Mylavarapu et al., 2009).

Phosphorus Leaching from Turfgrasses and Environmental Implications

Leaching is a process that describes the elluviation of solutes through the soil profile in percolating water (Haygarth and Sharpley, 2000). Another pathway of P movement from the soil to water bodies is subsurface flow, which consists of lateral flow of water below the soil surface (Haygarth and Sharpley, 2000). Leaching of P into ground water bodies followed by the discharge of P-enriched ground water into surface water bodies can lead to eutrophication (Pierzynski et al., 2005). Eutrophication is the process of nutrient enrichment of surface water bodies (Foy, 2005). Surface water eutrophication causes an increase in primary production (algae, phytoplankton, macrophytes), oxygen depletion, fish kills and reduction of aquatic biodiversity, it also causes a reduction in water clarity, substitution of phytoplankton with blue green algae which produce toxins that threatens human and animal health, it increases water

treatment costs (due to taste and odor problems caused by algae and algae removal), it impairs waters for recreational activities and reduces the value of shoreline properties (Carpenter et al., 1998; Foy, 2005). The main cause of eutrophication of surface freshwater bodies (lakes, reservoir, rivers, streams, and head waters of estuarine systems) is excessive P concentrations (Correll, 1998; Foy, 2005).

Phosphorus is regarded to have low mobility in most soils especially those with high clay content and aluminum and iron oxides and hydroxides (Brady and Weil,1999; Sims et al., 1998). Nonetheless, P can leach from soils with high degree of P saturation (soils with low ability to retain P and that maintain high concentration of P in the soil solution), preferential flow (rapid downward water and solute movement through biopores, cracks, voids in the soil that bypasses most of the soil matrix) and soils with artificial drainage (Sims et al., 1998; Pierzynski et al.,2005b). Excessive P fertilization to turfgrass grown in sandy soils with low P retention capacities and an abundance of macropores promotes P leaching (Soldat and Petrovic, 2008).

The majority of sod farms in Florida are located in the south central portion of the state (Satterthwaite et al., 2009), where Histosols and Spodosols dominate (Erickson et al., 2010). Sod production is increasingly growing in areas of the state where sandy soils dominate (Satterthwaite et al., 2009). The risk of P leaching is greater in soils dominated by “clean” (uncoated) sands than soils with coated sands (Harris et al., 1996). Sand coatings impart soil P retention capacity because constituents like kaolinite, hydroxyl-interlayered vermiculite, gibbsite and Fe oxyhydroxides have greater affinity for P than uncoated quartz surfaces (Harris et al., 1996).

Increase in the soil P concentration as measured with different soil P tests has resulted from P accumulation in the soil over time due to continued P application and/or high application rates (Cope, 1981; Magdoff et al., 1999; Sims et al., 1998). Greater concentrations of P in the soil solution (i.e., water extractable P, 0.01 *M* $CaCl_2$ extractable soil P) has been related to increasing extractable soil P concentrations (Maguire and Sims, 2002a; Maguire and Sims, 2002b; Khiari et al., 2000; McDowell et al., 2001). Moreover, increasing P concentrations in leachates has been linked to increasing P concentrations in the soil solution (Heckrath et al., 1995, Pautler and Sims, 2000; McDowell and Sharply, 2001; Sims et al., 2002; Maguire and Sims, 2002b). However, it has been shown that a soil test by itself does not provide enough information to ultimately assess the risk of P losses from a soil (Hooda et al., 2000; Sims et al., 2002, Pautler and Sims, 2000).

The use of indices that reflect the ability of the soil to retain additional P have proofed to be more adequate to evaluate the risk of soil P losses to water bodies (Paulter and Sims, 2000; Hooda et al., 2000). The soil P saturation ratio (PSR), which is calculated as the ratio of the concentration of extractable soil P to the sum of the concentration of extractable Al and Fe in the soil is one of these indices. Nair et al. (2004) noted that in Florida sands the risk of P losses from the soil increased beyond a PSR of 0.15. In their work, they determined that the concentration of P in the soil solution (as indicated by the concentration of water extractable soil P) increased abruptly above a PSR of 0.15; hence, they recommended that no P additions should be conducted in a Florida sand with a PSR greater than 0.15 (i.e., the “threshold PSR”). Nair and Harris (2004) developed the soil P storage capacity (SPSC) concept, which

enables to estimate the amount of P that could be “safely” added to the soil before exceeding the threshold PSR (i.e., 0.15 for Florida sands). Consequently, the SPSC would be negative if the PSR >0.15 (i.e., the soil would act as a source of P) and it would be positive if the PSR<0.15 (i.e., the soil would act as a sink of P). In addition, greater P leaching from turfgrass systems has been observed in coarse textured soils than from soils with finer texture (Petrovic, 2004).

Heavy rainfall, especially soon after a fertilization event, has been reported to increase the amount of P leaching (Erickson et al., 2005; King et al., 2006). Several authors have reported an increase in leaching from turfgrasses in response to excessive irrigation rates (Snyder et al., 1984; Morton et al., 1988; Shuman, 2002; Barton and Colmer, 2006). Excessive P application rates can also lead to increased P leaching from turfgrass systems (Shuman, 2001; Shuman, 2002; Elliot et al., 2002; Guertal, 2006; King et al., 2006).

Turfgrass Fertilization and Water Quality Ordinances in Florida

The main turfgrass species cultivated in the state of Florida is St. Augusitnegrass (*Stenotaphrum secundatum* (Walt) Kuntze) cultivar ‘Floratam’. St. Augustinegrass is primarily used for home lawns. Another widely adapted turfgrass species in Florida also used in home lawns is Zoysiagrass (*Zoysia japonica*) cultivar ‘Empire’, which is the fourth most produced turfgrass species in the state (Satterthwaite, 2009).

Concern regarding nutrient enrichment (particularly N and P) of ground water, surface water bodies and its relationship with coastal eutrophication has increased in the last years in Florida (Hartman et al., 2008). Consequently, many municipal governments (St. Johns County, City of Naples, City of Sarasota, Lee County, Charlotte County, Marrion County, City of Jacksonville among others) have adopted or are

evaluating the possibility to adopt ordinances that regulate the use of fertilizer in home lawns within their respective districts (Hartman et al. 2008). One of the regulations contemplated in these ordinances that relate to P fertilizer imposition of a “blackout period”, by which N and P fertilizer application is prohibited during the summer months (June 1st to September 30th). The rationale behind this ordinance is that rainfall increases during the summer months and it could increase the risk of P losses to water bodies (FDEP, 2010). However, shoot and root growth as well as nutrient uptake of warm season turfgrasses is greater as the solar radiation, temperature and day length increase which coincides with the summer months (Sartain, 2002; Trenholm et al., 1998; Carrow et al., 2001; King et al., 2006, Christians, 2007). Some have expressed concern that these ordinances may have unintended negative effects on the environment (Hochmuth et al., 2009). One of the arguments related to these ordinances is that interruption of adequate fertilization to turfgrass during prolong periods of time (especially during the period of the year of highest growth and uptake rate), will result in a reduction of vigor, density (roots and shoots), and health of the turfgrass. Under these circumstances the turf would be less able to uptake nutrients and the risk of nutrient losses (through runoff and leaching) from these weaken turfgrass systems would increase (Hochmuth, 2009).

In addition, the “Labeling Requirements for Urban Turf Fertilizers Rule” (Rule 5E-1.003) establishes a maximum P application rate to urban turfgrasses in the state of Florida of 0.54 g P m^{-2} application^{-1} or 1.07 g P m^{-2} year^{-1} (State of Florida, 2007). Recently, the U.S. Environmental Protection Agency (USEPA) proposed the “Water Quality Standards for the State of Florida’s Lakes and Flowing Waters Rule” in which

maximum allowed total P concentration in surface waters bodies was established (Table A-2).

The relationship between P application rate and P losses from turfgrass systems is very complex. It is necessary to understand the P requirements of home lawn warm season turfgrass and their ability to uptake P from the soil depending upon their P status. Phosphorus application in excess of plant requirements may result in increased leaching. Hence, it is crucial to generate information about the effect of plant nutritional status on P depletion rate from solution by warm season turfgrasses. Field studies are necessary to incorporate the influence of climatic conditions (i.e., precipitation, solar radiation, temperature) on the growth and P uptake by the turfgrass as well as on the movement of P through the profile.

Hypotheses and Research Objectives

The following hypotheses were formulated:

- Leaf growth rate, turf visual quality and percent green turf cover will increase to a maximum with increasing leaf tissue P concentration beyond which no additional response to P supply and increasing leaf tissue P will be observed.
- Rate of P depletion from solution by ‘Empire’ Zoysiagrass and ‘Floratam’ St. Augustinegrass grown in hydroponic culture will be inversely related to P leaf tissue P concentration.
- Greater P supply and leaf tissue P concentration will result in greater dry matter and P partitioning to leaf tissue.
- Phosphorus use efficiency will be inversely related to P supply and leaf tissue P concentration.
- There is a maximum phosphorus application rate to ‘Floratam’ St. Augustinegrass and ‘Empire’ Zoysiagrass below which P leaching is minimized.
- Rate of P leaching will be inversely related to plant growth and uptake rate and will increase with increasing rainfall and soil PSR.

- Growth rate and turf visual quality will increase in response to increasing P application rate, Mehlich 1 extractable soil P and leaf tissue P.

The overall objective of this research was to determine the critical leaf tissue P concentration for maximum sustainable levels of growth and quality in 'Floratam' St. Augustinegrass and 'Empire' Zoysiagrass and to identify the threshold P application rate that minimizes P leaching from these turfgrass species grown under field conditions.

Specific objectives addressed in this dissertation are the following:

- To determine critical P concentrations in leaf tissue of 'Floratam' St. Augustinegrass and 'Empire' Zoysiagrass based on leaf growth rate, visual quality and percent green turf cover.
- To evaluate the influence of leaf tissue P concentration on the rate of P depletion from solution (P influx) by these turfgrass species grown in hydroponic culture.
- To study the effect of P supply and leaf tissue P concentration on dry matter and P partitioning as well as P-use efficiency in these turfgrass species under glasshouse and field conditions.
- To assess the relationship between P supply and P leaching rate in 'Empire' Zoysiagrass and 'Floratam' St. Augustinegrass grown under field conditions.
- To investigate the interaction between plant uptake, rainfall, irrigation, M1-P and PSR ratio with P leaching rate in these turfgrass systems.
- To study the effect P supply rate, M1-P and leaf tissue P concentration on growth rate and visual quality in 'Empire' Zoysiagrass and 'Floratam' St. Augustinegrass grown under field conditions.

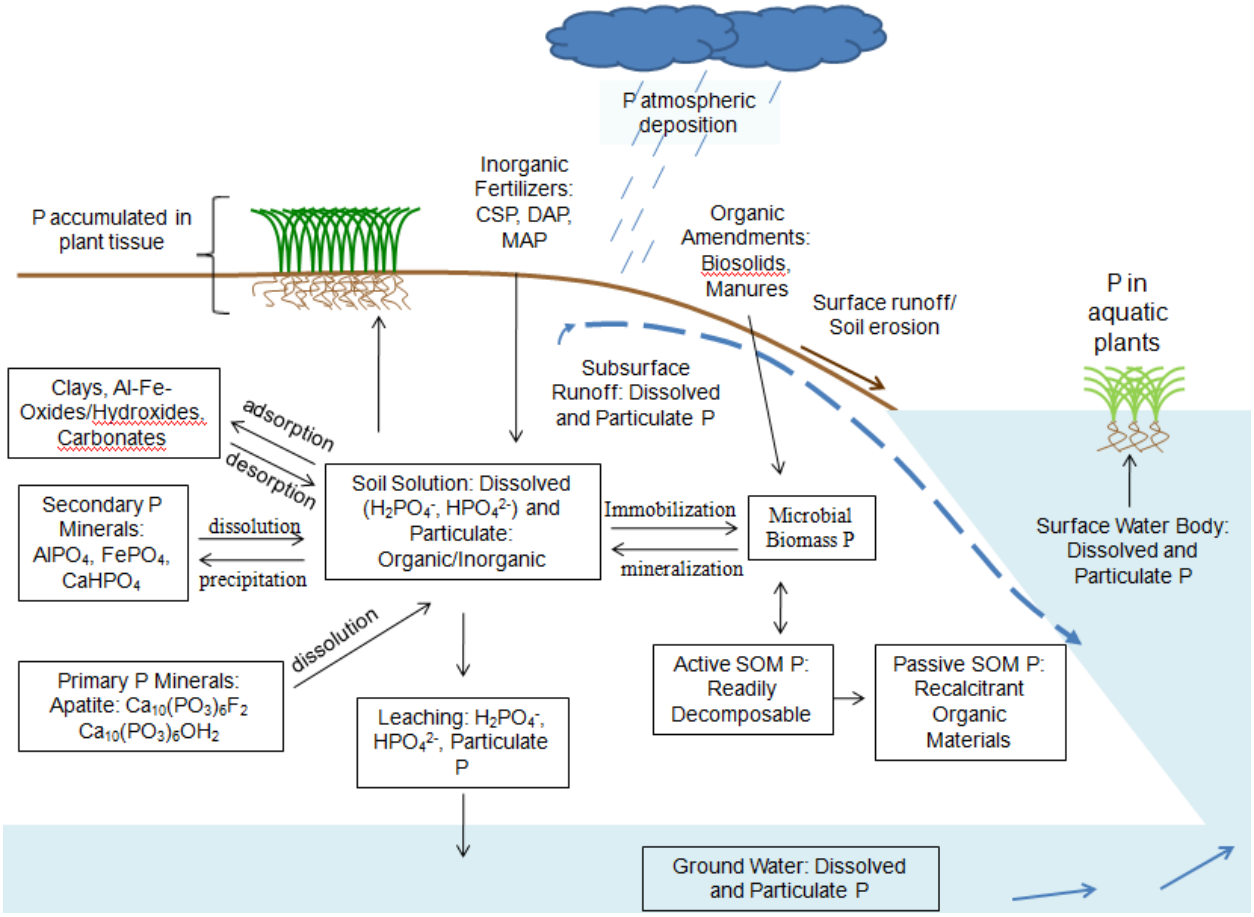


Figure 1-1. The soil P cycle (adapted from: Brady and Weil, 1999; Havlin et al., 1999; and Pierzynski et al., 2005b). SOM stands for soil organic matter.

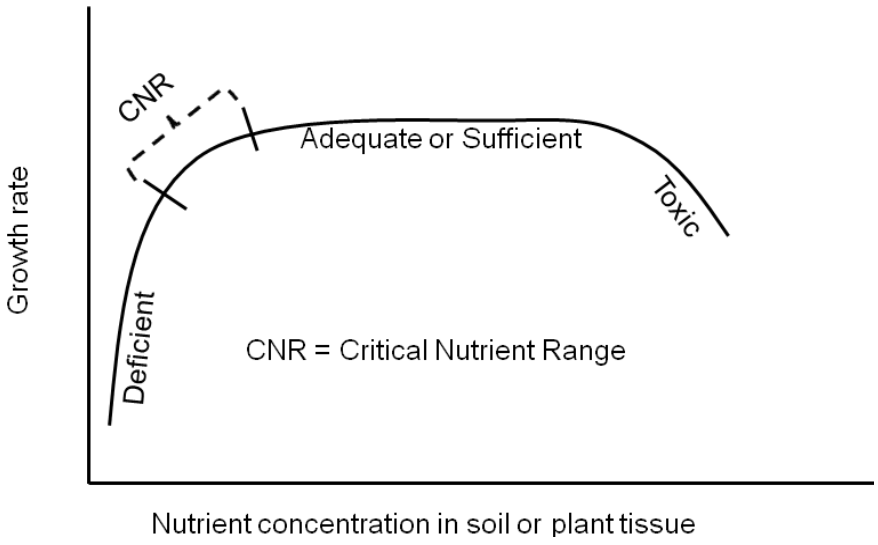


Figure 1-2. Plant growth rate as influenced by nutrient concentration in soil or leaf tissue (adapted from: Westermann, 2005).

CHAPTER 2
EMPIRE ZOYSIAGRASS AND FLORATAM ST. AUGUSTINEGRASS GROWTH, DENSITY AND QUALITY RELATIVE TO TISSUE PHOSPHORUS CONCENTRATION, CHLOROPHYLL INDEX AND PHOSPHORUS SUPPLY IN HYDROPONIC CULTURE

Florida population grew 16% from 2000 to 2009 (U.S. Census Bureau, 2009). An increase in the total area of turfgrass in the state has been linked to the accelerated population growth in Florida and most of this expansion has occurred in the residential sector (Haydu et al., 2005). *Stenotaphrum secundatum* (Walt) Kuntze (St. Augustinegrass) is the most widely cultivated turfgrass species in Florida and 'Floratam' is the most planted cultivar (Satterthwaite et al., 2007). Zoysiagrass (*Zoysia japonica)* occupies the fourth largest area of sod production in the state of Florida and 'Empire' is the zoysiagrass cultivar most widely cultivated (Satterthwaite et al., 2007). Adequate fertilization is required to obtain and maintain high quality turfgrass (Trenholm and Unruh, 2005). In low fertility, sandy soils, like those present in many regions in Florida, P fertilization may be required. Most of Florida's sod production is located in sandy soils (Satterthwaite et al., 2007).

In soils dominated by "clean" (uncoated) sands the risk of P leaching is greater than in soils with coated sands (Harris et al., 1996). Excessive application of P fertilizer to turfgrasses grown in sandy soils with low P retention capacities and large amounts of macropores can lead to P leaching (Soldat and Petrovic, 2008). In addition, eutrophication of P limited aquatic systems has been linked to P enrichment of surface water bodies (Correll, 1998, Foy, 2005). Adequate assessment of the plant P status to determine if P fertilization is required would reduce the risk of P losses from turfgrass systems while maintaining high turf quality.

An extensively used concept to evaluate the nutrient status of plants is the critical tissue concentration, the nutrient concentration in the diagnostic tissue that relates to 90% of maximum growth (Epstein and Bloom, 2005). Alternatively, it has been defined as the nutrient concentration in plants below which plant growth or yield response to increased nutrient concentration or nutrient supply is observed (Havlin et al., 1999). The determination of critical nutrient concentration in turf leaf tissue should incorporate measures of turf quality and turf density as response variables. Information regarding critical leaf tissue concentration in St. Augustinegrass and zoysiagrass is limited (Liu et al., 2006; Liu et al., 2008) and most is based on turf biomass accumulation rate.

Turfgrass cover is a major component of turfgrass aesthetics. Green turfgrass cover can be accurately and precisely measured with digital image analysis (Richardson et al., 2001).Vigorous turfgrass growth is desirable for faster recovery after periods of stress, but excessive biomass accumulation rate could result in greater maintenance requirements and expense. A balance between growth rate and turf density may lead to high quality turf without an intense management regime.

The following hypotheses were tested in this study: (i) leaf growth rate, visual quality and percent green turf cover will increase to a maximum with increasing leaf tissue P concentration beyond which no additional response to P supply will be observed, (ii) a leaf tissue P concentration that results in maximum growth rate will be sufficient to maintain maximum turf density and visual quality and (iii) differences in green turf cover associated with increasing leaf tissue P concentration can be precisely measured by digital image analysis. The objectives of this study were (i) to determine critical P concentrations in leaf tissue of Floratam St. Augustinegrass and Empire

zoysiagrass based on leaf growth rate, visual quality and percent green turf cover, and (ii) to evaluate the applicability of digital image analysis in turfgrass nutrient response studies.

Materials and Methods

Hydroponic System Description and Turfgrass Establishment

The study was conducted in the Turfgrass Envirotron facility on the University of Florida campus (29° 38' N, 82° 21' W). *Zoysia japonica* cultivar 'Empire' (zoysiagrass) and *Stenotaphrum secundatum* (Walt) Kuntze cultivar 'Floratam' (St. Augustinegrass) certified sod from the G.C. Horn Turfgrass Field Laboratory near Citra, Florida were selected as the test cultivars.

The sod was washed thoroughly to remove soil from the root system, cut into 20 cm x 33 cm rectangles and transferred to a hydroponics system. Polyvinyl chloride (PVC, 22 mm outer diameter) pipe was used to construct a 22 cm by 33 cm frame and covered with poly hardware cloth (13-mm square openings). The sod was placed on the plastic screen which was used as a grass bedding surface. Turf roots were carefully passed through the openings of the plastic screen to promote contact with the nutrient solution. Each experimental unit was placed in a 25 cm x 36 cm x 23 cm plastic tub (~20 L) used as the hydroponic container. The tubs corresponding to a given treatment were connected to a larger nutrient reservoir (120 L), and the nutrient solution was constantly circulating between the tubs and the nutrient reservoir. A submersible pump delivered nutrient solution to the corresponding tubs at a rate of 12 L per minute. A 25 mm outer diameter PVC threaded male adaptor placed at the bottom of each tub was connected to a 25 mm inner diameter, 25 cm long PVC pipe in the inner side of the tub. This pipe

was used to regulate the level of the solution as well as to drain and return the solution by gravity to the corresponding nutrient solution reservoir.

Light penetration through the outer surfaces of the tubs was restricted by black latex paint. During the first two weeks of the experiment, the nutrient solution level was maintained in contact with the plastic hardware net to stimulate root growth. Thereafter, the solution level was lowered to about 2.5 cm from the plastic screen to favor incorporation of oxygen as the continually circulating solution enter in contact with the solution present in the tub.

The turfgrass was maintained from December 19th 2008 until May 24th 2009 on a modified half strength Hoagland solution (Hoagland and Arnon, 1950) without P to reduce tissue P levels. Phosphorus treatments were imposed on May 24th, 2009 and the study was continued for 140 days thereafter.

Average relative humidity and temperature in the glasshouse were 70% and 28.4 °C, respectively. The relative humidity and the temperature oscillated between 20% and 89% and 21°C and 39°C, respectively. In addition, the average photosynthetic photon flux density between 0700 and 1800 h was 898 µmol quanta m^{-2} s^{-1} and ranged from 1.63 to a 2,459 µmol quanta m^{-2} s^{-1}.

Phosphorus Treatments and Nutrient Solution Description

Six levels of P (0, 90, 135, 203, 304 and 456 mg P m^{-3}) were supplied with reagent grade mono potassium phosphate (22.6 % P) in a modified full strength Hoagland (Hoagland and Arnon, 1950) nutrient solution (Table 2-1). The concentration of P in the soil solution rarely exceeds 10 µ*M* (310 mg P m^{-3}) and in most soils it is about 2 µ*M* (Raghothama, 1999). In a preliminary study (data not presented herein) a maximum concentration of P in solution of 456 mg P m^{-3} was estimated to be sufficient to increase

the P concentration of the turfgrass species of interest above the critical level; hence, P concentrations between 0 and 456 mg P m^{-3} were selected for this experiment. The P treatments were replicated five times and arranged in a split-plot randomized complete block design with turfgrass species as the main effect and P application rate as secondary effect. Chelated iron (Sequestrene 330) was supplied biweekly through foliar application at a rate of 0.5 g Fe m^{-2} (Carrow, 2007). The nutrient solution was replaced twice a week. Initial solution pH oscillated between 5.5 and 6 and the initial nutrient solution temperature ranged between 25°C and 30 °C.

Tissue Sampling and Phosphorus Analysis

Top growth was harvested every two weeks to a height of approximately 10 cm. Roots were clipped to about 10 cm when their length was about 15 cm (the depth of the water column in which the roots were grown was approximately 15 cm). All tissue samples were dried at 70 °C to constant weight, weighed, and then ground to pass a # 40 mesh sieve (425 µm openings size). The change in dry matter (DM) accumulation per unit area (m^2) and time (days) was monitored during the entire evaluation period. Dry tissue was ashed and digested with 6 *M* HCl according to the standard operation procedure WLB-SP-009 of the Wetland Biogeochemistry Laboratory at the University of Florida, “Total Phosphorus of Soil, Sediment and Plant Tissue by Ignition or Ashing Method”. Phosphorus concentration in the digestate was determined following USEPA method 365.1 “Determination of Phosphorus by Semi-automated Colorimetry” (O’Dell, 1993).

Turf Visual Quality and Chlorophyll Index

Turf visual quality was evaluated biweekly using a scale of 1 to 9, where 1 represents brown, dormant turf and 9 represents superior quality. A value of 5.5 was

considered the minimum rating for an acceptable turf visual quality (Skogley and Sawyer, 1992). Chlorophyll Index (CI) was measured biweekly with a CM 1000 Chlorophyll Meter (Spectrum Technologies Inc, Illinois, USA) just prior every leaf tissue harvest. Chlorophyll index is a measure of the relative greenness of the leaf. The CM 1000 chlorophyll meter measures the ambient and reflected light intensities at wavelengths of 700 nm and 840 nm to estimate the quantity of chlorophyll in leaves. Chlorophyll *a* absorbs 700 nm light; hence, reflection of 700 nm light is reduced relative to the reflected 840 nm light. The 840 nm light provides a measure of the reflectiveness of the leaf surface. Physical characteristics of the leaf such as leaf hairs and waxy surfaces can reduce light reflection. The CI is obtained by comparing the ratio of the 700 nm and 840 nm in available light (ambient light) to the ratio of the same wavelengths of reflected light. The CI is reported in a scale of 0 to 999 (Spectrum Technologies Inc, CM 1000 chlorophyll meter manual, 2009).

Digital Image Analysis

Green turf cover can be determined more accurately and precisely with digital image analysis than with subjective methods such as visual ratings of turf density (Richardson et al., 2001). Horst et al. (1984) assessed the reliability of visual evaluation of turf quality and density. They reported that common techniques utilized by researchers for turfgrass visual quality and density evaluations are inadequate. Achieving high turfgrass quality from the aesthetics standpoint is the main objective of turfgrass management; hence, in a healthy, dense and uniform turf, high biomass accumulation is not essentially an advantageous attribute (Christians et al., 1979). Therefore, digital image analysis was utilized in this experiment to incorporate a non-

subjective, reliable method to evaluate the percent green turf cover as a measure of turf density and uniformity.

Digital images were obtained with a Canon PowerShot A630 (Canon Inc., New York, USA) digital camera mounted on a light box constructed to fit exactly over the tubs utilized as hydroponic containers. The dimensions of the light box were 25 cm x 36 cm x 30 cm. The bottom side of the light box was open to allow placement over the turf, and a 38 mm diameter opening was drilled in the upper side of the light box to accommodate the camera lens. A 13 W compact fluorescent (day-light) bulb on one side of the light box provided uniform light intensity.

Leaf tips were trimmed to approximately the same height (about 15 cm) in all treatments prior collecting the digital images. The images obtained were saved in JPEG format with an image size of 5 mega pixels (2,592 by 1,944 pixels). Camera settings consisted of an exposure time of 1/13 seconds, an aperture of F8, and a focal length of 7 mm. All digital images were resized to 800 by 600 pixels using ACDSee Pro (v. 2.5, ACDSee Systems International Inc., Victoria, British Columbia, Canada).

The digital images were analyzed using SigmaScan Pro (v. 5.0, SPSS Inc., Chicago, IL) and the “turf analysis macro” (Karcher and Richardson, 2005) for batch analysis of turf digital images. The color threshold settings were a hue range from 50 to 107 and a saturation range from 0 to 100, which selectively identified green pixels in the images. Richardson et al. (2001) utilized a hue range from 57 to 107 and a saturation range from 0 to 100 to quantify bermudagrass (*Cynodon dactylon* L.) green turf cover using DIA. The turf analysis macro calculated the percent green turf cover by dividing the green pixels by the total number of pixels in each image.

Statistical Analysis

Non linear regression analyses (Proc Reg) in SAS Statistical Software v. 9.2 (SAS Institute, 2009) was used to relate response variables (leaf growth rate, turf visual quality, and percent green turf cover) to explanatory variables (leaf tissue P concentration, initial solution P concentration and CI). The general linear model procedure (Proc GLM) of SAS was used to conduct analysis of variance and mean separation was carried out using single degree of freedom contrast analysis.

Results and Discussion

Phosphorus Concentration in Leaf and Root Tissue

The average P concentration in leaf tissue immediately following turf establishment in the hydroponic system was 2.1 g P kg^{-1} for zoysiagrass and 3.4 g P kg^{-1} for St. Augustinegrass (Figure 2-1). Liu et al. (2006) evaluated the P requirement of Floratam St. Augustinegrass [*Stenotaphrum secundatum* (Walt) Kuntze] in solution culture and determined that maximum growth rate was associated with a leaf tissue P concentration of 1.6 g P kg^{-1}. Response to P supply requires a concentration of P in leaves below the critical level. Thus, the turf was maintained for 103 days on a modified full strength Hoagland solution (Table 2-1) without P until the P concentration in zoysiagrass and St. Augustinegrass leaf tissue decreased to an average across all experimental units of 0.64 g P kg^{-1} and 0.28 g kg^{-1}, respectively (Figure 2-1). Once the leaf tissue P had reached a sufficiently low level, P was supplied in a modified full strength Hoagland solution.

Leaf tissue P concentration in zoysiagrass exposed to an initial solution concentration of 135, 203, 304 and 456 mg P m^{-3} increased at a rate of 1, 3, 6, and 8 mg P kg^{-1} day^{-1}, respectively, during the 140 days post treatment application (Figure 2-

1). Leaf tissue P concentration increased at a rate of 3, 5, 6, 7, 10 and 12 mg kg^{-1} day^{-1} in St. Augustinegrass exposed to initial solution concentrations of 0, 90, 135, 203, 304 and 456 mg P m^{-3}, respectively (Figure 2-1).During the same time period, zoysiagrass leaf tissue P concentration decreased at a rate of 2 and 1 mg P kg^{-1} day^{-1} in turf exposed to initial solution P concentrations of 0 and 90 mg P m^{-3}, respectively (Figure 2-1). Increased growth rate in response to greater radiation flux as the growing season progressed as well as to a limited P supply and uptake could have resulted in P dilution and may explain the decrease in leaf tissue P concentration overtime in the 0 and 90 mg m^{-3} P treatments.

Phosphorus treatments positively affected zoysiagrass leaf tissue P concentration. Zoysiagrass leaf P concentration increased linearly with increasing P supply at a rate of 3 mg P kg^{-1} per mg P m^{-3} of solution (Figure 2-2).The average zoysiagrass leaf tissue P during the period of greatest growth rate was 0.38, 0.72, 0.90, 1.07, 1.36, and 1.78 g P kg^{-1} in turf exposed to an initial P concentration in solution of 0, 90, 135, 203, 304 and 456 mg m^{-3}, respectively. Release of P from the thatch layer into solution and the positive effect of adequate supply of macronutrients and micronutrients on root growth, may have favored P uptake, and increased the leaf tissue P concentration of St. Augustinegrass in the 0 mg P m^{-3} treatment (Figure 2-1). Translocation of P to slow growing young tissue, such as that observed in P deficient plants, may result in increased P concentration. Phosphorus may be translocated from older to younger plant tissues, especially when the P supply and uptake is insufficient to meet the demand of young tissues (Mengel and Kirkby, 2001). St. Augustinegrass leaf P concentration increased linearly with increasing P supply at a rate of 2.7 mg P kg^{-1} per every mg P m^{-3}

of solution (Figure 2-2). Average St. Augustinegrass leaf tissue P concentration during the period of greatest growth rate was 0.58, 0.84, 1.0, 1.30, 1.57 and 1.75 g P kg^{-1} in turf exposed to initial concentrations of 0, 90, 135, 203, 304 and 456 mg P m^{-3}, respectively.

Root tissue P concentrations in zoysiagrass and St. Augustinegrass across treatments immediately prior the beginning of the study were 0.49 g P kg^{-1} and 0.45 g kg^{-1}, respectively. Across sampling times, P concentrations in zoysiagrass and St. Augustinegrass root tissue of the control treatment were lower than in root tissue supplied with P. However, P concentrations in root tissue of turf supplied with P remained fairly similar regardless of the P supply rate (Figure 2-3). Zoysiagrass root tissue P concentration in the control treatment decreased by 35% during the evaluation period (140 days), while the P concentration of roots in the other treatments did not decrease. Root tissue P concentration in St. Augustinegrass grown in the control treatment decreased 22% during the evaluation period. Tissue P concentration increased over time at a rate (mg P kg^{-1} day^{-1}) up to 5 and 15 times greater in leaves than in roots of zoysiagrass and St. Augustinegrass, respectively. Liu et al. (2006) reported significant increase in St. Augustinegrass root tissue P concentration in response to P supply in solution raging from 0 to 775 mg P m^{-3}. In addition to differences in P application rates, maximum shoot growth rate in Liu et al. (2006) study was 1 g DM m^{-2} day^{-1} compared to 13 g DM m^{-2} day^{-1} measured in this study. Dilution of P due to high shoot growth and high shoot P demand may have prevented the P concentration in root tissue to increase in response to increasing P supply.

Leaf and Root Growth Rate

Leaf growth rates of zoysiagrass and St. Augustinegrass, increased with increasing leaf tissue P concentration to a maximum growth rate of about 13 g dry matter (DM) m^{-2} day^{-1} (Figure 2-4). Maximum growth rates were obtained at leaf tissue P concentrations of 1.67 g P kg^{-1} for zoysiagrass and 1.73 g P kg^{-1} for St. Augustinegrass. Andrew and Robins (1971) evaluated the growth response of nine tropical grasses to P supply to determine the critical tissue P concentrations. Critical tissue P concentrations ranged from 1.6 g P kg^{-1} to 2.5 g P kg^{-1}. The critical P level for maximum growth in leaf tissue of St. Augustinegrass grown in a pot study with four different soils ranged from 1.8 g P kg^{-1} to 1.9 g P kg^{-1} (Liu et al., 2008).

Greater P supply increased leaf tissue growth rate (Figure 2-5). Average maximum growth rates were 13.4 g DM m^{-2} day^{-1} at 382 mg P m^{-3} for zoysiagrass (Figure 2-5, Figure 2-6) and 13.45 g m^{-2} day^{-1} at 374 mg P m^{-3} for St. Augustinegrass (Figure 2-5, Figure 2-7).

Leaf growth rates and CI values were positively related (Figure 2-8). Zoysiagrass leaf growth rate increased with increasing CI, and reached a plateau at a CI value of 641, which corresponded to a maximum growth rate of 12.9 g DM m^{-2} day^{-1}. Above the critical CI, leaf tissue growth rate decreased (Figure 2-8). A positive linear relationship between CI and leaf tissue P concentration was observed in both turfgrass species (Figure 2-9). Chlorophyll index provides a measure of greenness of the turf foliage. St. Augustinegrass leaf growth rate increased linearly with increasing CI at a rate of 34 mg DM m^{-2} day^{-1} per unit increment in CI (Figure 2-8). Loss of green color and lower turfgrass growth is associated with reduced chlorophyll concentration in tissue (Carrow et al., 2001).

Zoysiagrass root growth rate did not show a clear response to P supply rate (Figure 2-3); however, an initial P concentration in solution of 203 and 304 mg P m^{-3} resulted in a root growth rate 24% and 43% greater than in the control treatment, respectively. Even though not significantly different from the control treatment, an initial P supply of 456 mg P m^{-3} produced the least zoysiagrass root growth (Figure 2-3). In contrast, the greatest zoysiagrass leaf tissue growth rate was attained in this treatment. The latter may reflect the tendency of the plant to partition greater amount of assimilates to leaf tissue to harvest more light energy instead of investing resources into root biomass when there is ample supply of nutrients and water in the growing medium.

Maximum zoysiagrass root growth rate corresponded to a root tissue P concentration of 0.62 g P kg^{-1}, which is only 37% of the leaf tissue P concentration (1.67 g P kg^{-1}) required for maximum leaf growth rate. St. Augustine root tissue growth rate increased with increasing P supply (Figure 2-3). A maximum root growth rate of 0.37 g m^{-2} day^{-1} was obtained in St. Augustinegrass turf exposed to an initial P concentration of 304 mg P m^{-3}; however, root growth rate did not increase beyond an initial P solution concentration of 203 mg P m^{-3} (Figure 2-3). Root growth rate in St. Augustinegrass turf exposed to initial P concentrations of 90, 135, 203, 304 and 456 mg P m^{-3} were 4.4, 6.9, 10.3, 11.2, 10.2 times higher than the root growth rate observed in the control treatment. St. Augustinegrass root growth rate was positively related to root tissue P concentration ($r = 0.75$, $p<0.01$) both of which increased quadratically with increasing P supply (Figure 2-3). Maximum St. Augustinegrass root growth rate corresponded to an average tissue P concentration of 0.97 g P kg^{-1} DM, which was 44% lower than the leaf tissue P concentration (1.73 g P kg^{-1} DM) required for maximum leaf growth rate.

Turf Quality

A positive quadratic response was obtained when zoysiagrass visual quality ratings were regressed against leaf tissue P concentrations (Figure 2-10). Turf visual quality reached a maximum at a leaf tissue P concentration of 1.7 g P kg^{-1}, which corresponded to a maximum visual quality rating of 8.7. Zoysiagrass turf visual quality attained in this experiment was excellent, as reflected by the plateau from the relationship between visual quality rating and leaf tissue P, which is only 3% below the maximum possible rating in the scale utilized in this study (Figure 2-10). St. Augustinegrass visual quality increased linearly with increasing leaf tissue P concentration (Figure 2-10). Liu et al. (2006) reported continuously increasing in St. Augustinegrass visual quality with increasing P supply from 0 mg P m^{-3} to 775 mg P m^{-3}. Leaf tissue P concentrations of 0.45 g P kg^{-1} for zoysiagrass and 1.15 g P kg^{-1} for St. Augustinegrass were required to attain visual quality ratings of 5.5, which is broadly used as the minimum rating for acceptable turf quality.

Turf visual quality increased with increasing P supply (Figure 2-11). Initial solution concentrations of 90 mg P m^{-3} and 203 mg P m^{-3} were required to increase zoysiagrass and St. Augustinegrass visual quality above a rating of 5.5, respectively. Maximum zoysiagrass visual quality was obtained at an initial P solution concentration of 370 mg P m^{-3} (Figure 2-11). Visual quality of St. Augustinegrass increased with increasing P supply from a minimum of 4.4 in the 0 mg P m^{-3} treatment to a maximum of 6.7 in turf exposed to an initial P concentration in solution of 456 mg P m^{-3} (Figure 2-11). St. Augustinegrass visual quality was not influenced by P supply above an initial P concentration in solution of 304 mg P m^{-3} (Figure 2-11).

As previously indicated, greater CI was related to greater leaf tissue P concentration (Figure 2-9). Zoysiagrass visual quality rating increased with increasing CI to a maximum rating of 8.7, which was attained at a CI of 654 (Figure 2-13). The difference between the critical CI for maximum zoysiagrass leaf growth rate and visual quality was less than 2%. Initial P concentration in solution required to maximize zoysiagrass leaf growth rate was only 3.2% higher than critical solution P for maximum Zoysiagrass turf visual quality. St. Augustinegrass turf visual quality increased linearly in response to CI (Figure 2-13). Maximum St. Augustinegrass visual quality corresponded to an average CI level of 450. Chlorophyll index in St. Augustinegrass turf exposed to an initial concentration of 456 mg P m^{-3} was 2.76 fold greater than in the control treatment. The condition of St. Augustinegrass turf in the control treatment was very poor and by the end of the evaluation period a substantial portion of leaf tissue was dead (Figure 2-12). The substantial amount of brown, dead turf in the control treatment may have caused a reduced CI reading from turfgrass in this treatment. Acceptable turf visual quality was attained at a CI value of 304 in Zoysiagrass and 319 in St. Augustinegrass (Figure 2-13).

Turf Green Cover

Digital image analysis (DIA) was utilized to evaluate the percent green turf cover (GC). Richardson et al. (2001) showed that DIA can accurately and precisely quantify green turf cover. Other authors have reported that DIA can accurately measure percent green leaf cover in soybeans (Purcell, 2000) and wheat (Lukina et al., 1999). The GC was obtained as the ratio of green pixels identified in each image to the total number of pixels in the digital image and this ratio was expressed as a percentage (Richardson et al., 2001). Percent green turf cover could be used as a measured of healthy turf density.

Quadratic relationships between GC and leaf tissue P concentration were found for both turfgrass species (Figure 2-14). A maximum GC of 92% was attained at a critical leaf tissue P concentration of 1.35 g P kg^{-1} in zoysiagrass and a maximum of 80% at 1.48 g P kg^{-1} in St. Augustinegrass (Figure 2-14). Increasing solution P concentration had a positive effect on GC (Figure 2-15). Percent green turf cover increased quadratically to a maximum of 93.5% at an initial solution P concentration of 303 mg P m^{-3} in Zoysiagrass and 82.4% at 335 mg P m^{-3} in St. Augustinegrass (Figure 2-15).

Greater GC was related to greater CI (Figure 2-16). Percent green turf cover peaked at a CI level of 479 and 363, which corresponded to a maximum GC of 90% and 77% in zoysiagrass and St. Augustinegrass, respectively (Figure 2-16). In addition, GC and leaf growth rate were positively related (Figure 2-17). A total of 84% of the variability on leaf growth rate in St. Augustinegrass and 70% of the variability on leaf growth rate in zoysiagrass was explained by GC. Maximum GC of 91% was attained at a leaf growth rate of 10.4 g DM m^{-2} day^{-1} in zoysiagrass and a maximum of 80% at 10.5 g DM m^{-2} day^{-1} in St. Augustinegrass (Figure 2-17).

The coefficient of variation (CV) for relationships between zoysiagrass leaf growth rate and leaf tissue P concentration, initial solution P concentration and CI were 13.1%, 13.5% and 21.1%, respectively, while the CV for the corresponding relationships established with GC as the explanatory variable did not surpass 5%. The CV for zoysiagrass leaf growth rate was 25% and for GC data was12 %. Relationships between St. Augustinegrass leaf growth rate as the response variable and leaf tissue P concentration, initial solution concentration and CI as explanatory variables had a CV of

12.7%, 11.6%, 16.9%, respectively, while the CV for the corresponding relationships using GC as the response were 6.9%, 6.9% and 8.5%, respectively. The CV for St. Augustinegrass leaf growth rate was 42.9% and 7.7% for the GC data. Leaf tissue P concentration explained 92% of the variability on zoysiagrass growth rate (Figure 2-4) while CI level explained 75% (Figure 2-8). Leaf tissue P concentration explained 81% of the variability on GC in zoysiagrass (Figure 2-14) and 86% was explained by CI level (Figure 2-16). Moreover, 92% and 85% of the variability in St. Augustinegrass leaf growth rate could be explained by leaf tissue P concentration (Figure 2-4) and CI (Figure 2-8), respectively. A total of 87% of the variability in St. Augustinegrass GC could be explained by leaf P concentration (Figure 2-14) while CI explained 81% (Figure 2-16). The strong relationship between leaf growth rate and GC (Figure 2-17), the lower variability of the GC data in comparison to the leaf growth rate data and the robust relationship between GC and leaf P concentration (Figure 2-14) as well as CI (Figure 2-16), indicate that GC as measured with DIA can be effectively utilized in turfgrass nutrient response experiments.

The critical values obtained for leaf tissue P concentration, initial solution P concentration and CI when zoysiagrass leaf growth rate was used as the response variable, were 19.2%, 20.7% and 25.3% lower, respectively, than the corresponding critical values for these independent variables when GC was used as the response. Critical leaf tissue P concentration, initial solution P concentration and CI as determined when St. Augustinegrass leaf growth rate was used as the response were 14.5%, 10.4% and 3.4% lower, respectively, than the corresponding values when GC was utilized as the response.

The critical leaf tissue P concentrations for zoysiagrass (1.67 g P kg^{-1}) and St. Augustinegrass (1.73 g P kg^{-1}) (Figure 2-4), corresponded to maximum leaf growth rate of about 13 g m^{-2} day^{-1}. This leaf growth rate was related to a maximum GC of 89% in zoysiagrass and 77% in St. Augustinegrass (Figure 2-17). Maximum GC of 92% was attained at 1.35 g P kg^{-1} in zoysiagrass. A maximum GC of 80% was reached at 1.48 g P kg^{-1} in St. Augustinegrass (Figure 2-14). Hence, critical leaf tissue P concentrations obtained using leaf growth rate and GC as the response variables were in agreement because they were related to basically the same maximum GC (i.e., 3 % difference in GC estimates). Leaf growth rate continued to increase beyond the critical leaf tissue P for maximum GC and reached a plateau at a leaf tissue P concentration that was 0.32 g P kg^{-1} and 0.25 g P kg^{-1} greater than that required for maximum GC in zoysiagrass and St. Augustinegrass, respectively (Figure 2-18). No additional increase in GC was obtained above a leaf growth rate of 10.5 g m^{-2} day^{-1} in either turfgrass species (Figure 2-18).

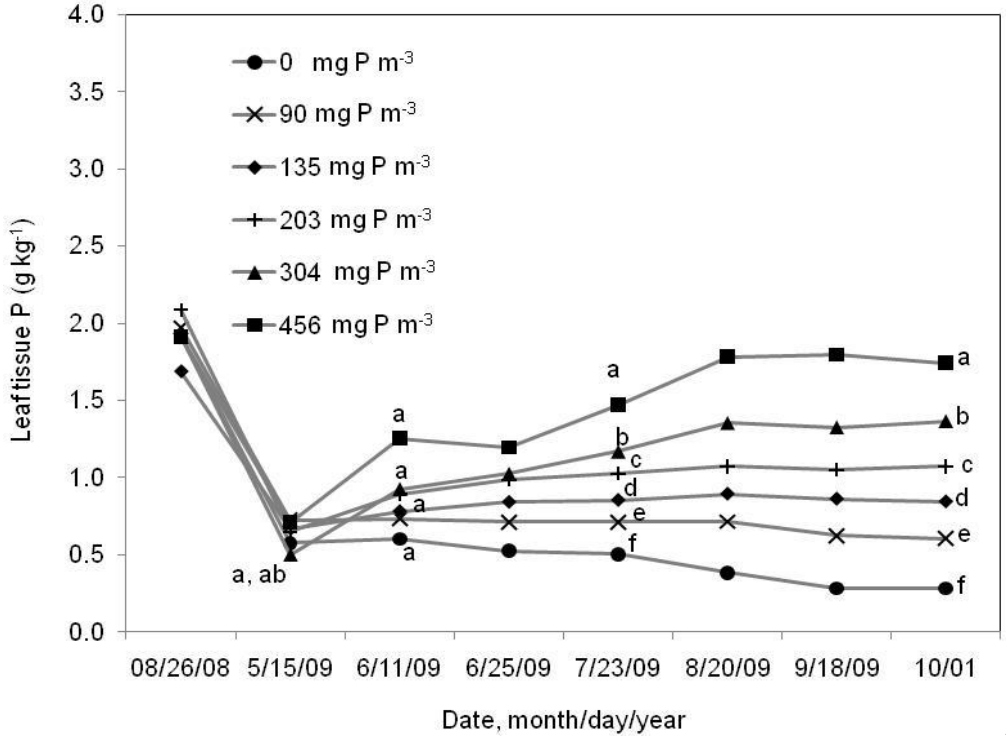
Therefore, in healthy, high quality turfgrass stands that are adequately supplied with water and other nutrients (specially nitrogen and potassium), leaf tissue P concentrations of 1.35 g P kg^{-1} in zoysiagrass and 1.48 g P kg^{-1} in St. Augustinegrass could be used to maintain high quality and maximum percent green cover, without the potential inconvenience of excessive growth and greater maintenance requirements (i.e., greater mowing frequency). According to non-linear regression analysis the estimated difference in zoysiagrass turf visual quality that relates to a leaf tissue P concentration of 1.35 g P kg^{-1} (i.e., critical leaf tissue P as determined with GC as the response) versus that obtained at 1.67 g P kg^{-1} (i.e., critical leaf tissue P as determined

using leaf growth rate as the explanatory variable) was only 0.21 turf visual quality rating units. The difference in St. Augustinegrass visual quality related to a critical leaf tissue P concentration of 1.48 g P kg^{-1} versus that attained at critical concentration of 1.73 g P kg^{-1} was 0.5 turf visual quality rating units. These differences in turf visual quality can be neither accurately nor precisely distinguished even by an experienced evaluator and most certainly among different evaluators and evaluation sites.

Alternatively, in low quality, low density stands that had been affected by pest or disease infections or that had received heavy loads of traffic, a leaf tissue P concentration of 1.67 g P kg^{-1} in zoysiagrass and 1.73 g P kg^{-1} in St. Augustinegrass could be used to maximize leaf growth rate and turf recovery rate. Phosphorus fertilization should be conducted only after the source of stress has been completely controlled (for example, after water stress or a disease outbreak has been completely controlled) and if the leaf tissue P concentration is less than the critical. Accordingly, the diagnosis of the P nutritional status of warm season turfgrass species should incorporate an assessment of the overall health and condition of the turf and also the quality and density that the turfgrass stand is expected to reach and maintain over time.

Table 2-1. Macronutrients and micronutrients concentrations and sources utilized in the nutrient solution.

Nutrient Solution	Concentration in Solution		Source Formula
	m*M*	µ*M*	
NH_4-N	7.5		NH_4NO_3
NO_3-N	7.5		NH_4NO_3
K	6.0		KCl
Ca	2.0		$CaCl_2 \cdot 2H_2O$
Mg	2.0		$MgSO_4 \cdot 7H_2O$
S	2.0		$MgSO_4 \cdot 7H_2O$
Mn		9.0	$MnCl_2 \cdot 4H2O$
Zn		1.5	$ZnCl_2$
Cu		1.5	$CuCl_2 \cdot 2H_2O$
B		45.0	H_3BO_3
Mo		0.1	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$



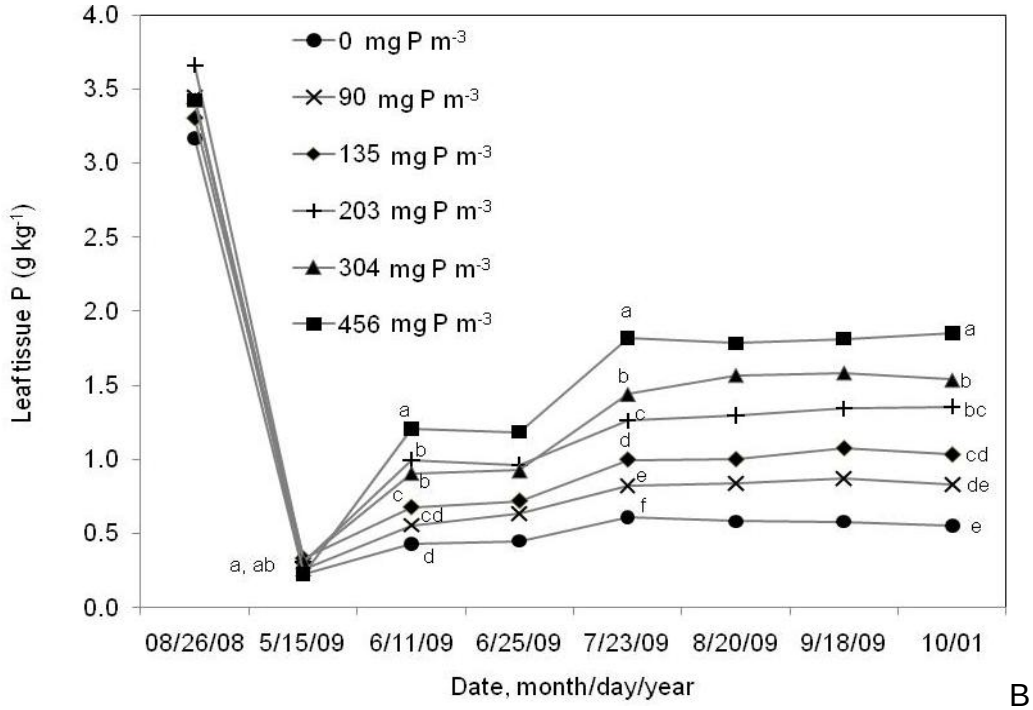


Figure 2-1. Phosphorus concentration in leaf tissue over time as influenced by phosphorus supply rate. A) Empire zoysiagrass and B) Floratam St. Augustinegrass. Symbols labeled with the same letter within a sampling date are not significantly different at p = 0.05 by single degree of freedom contrast analysis.

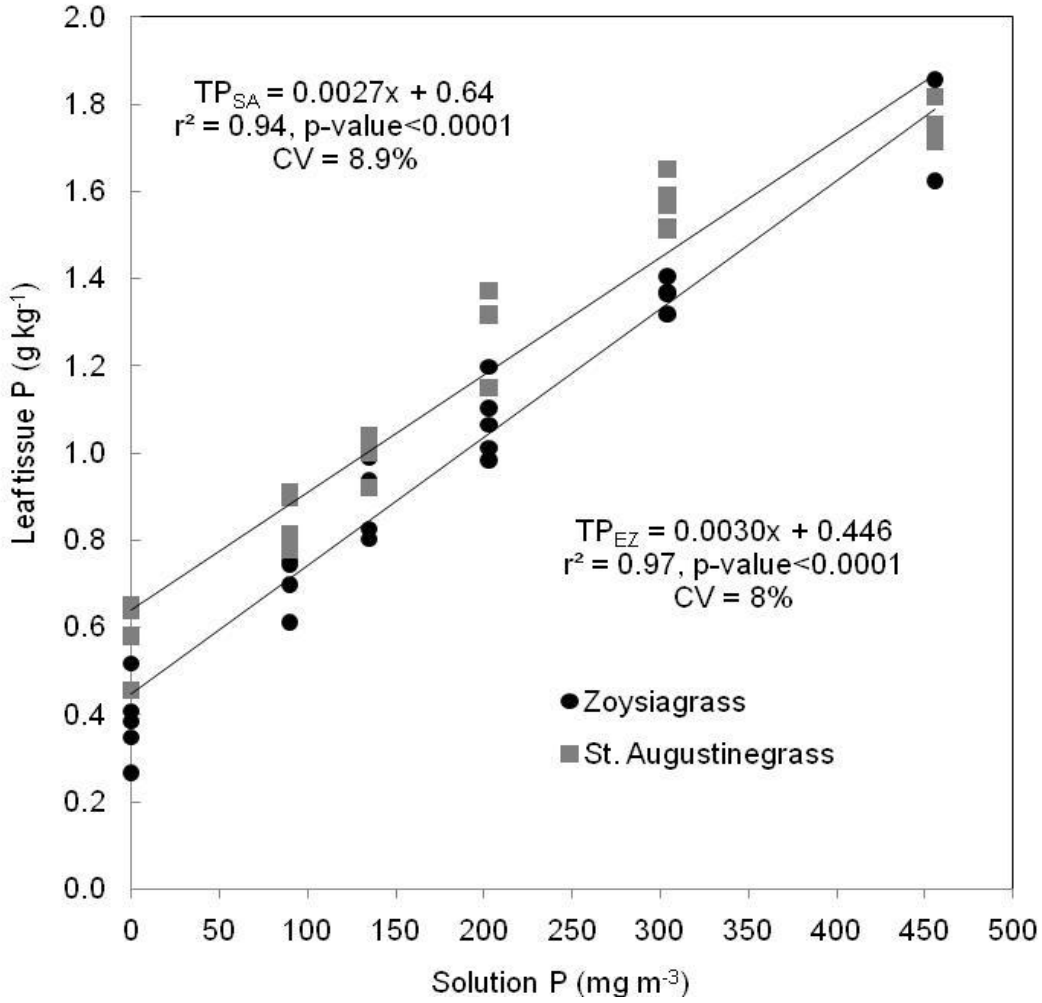
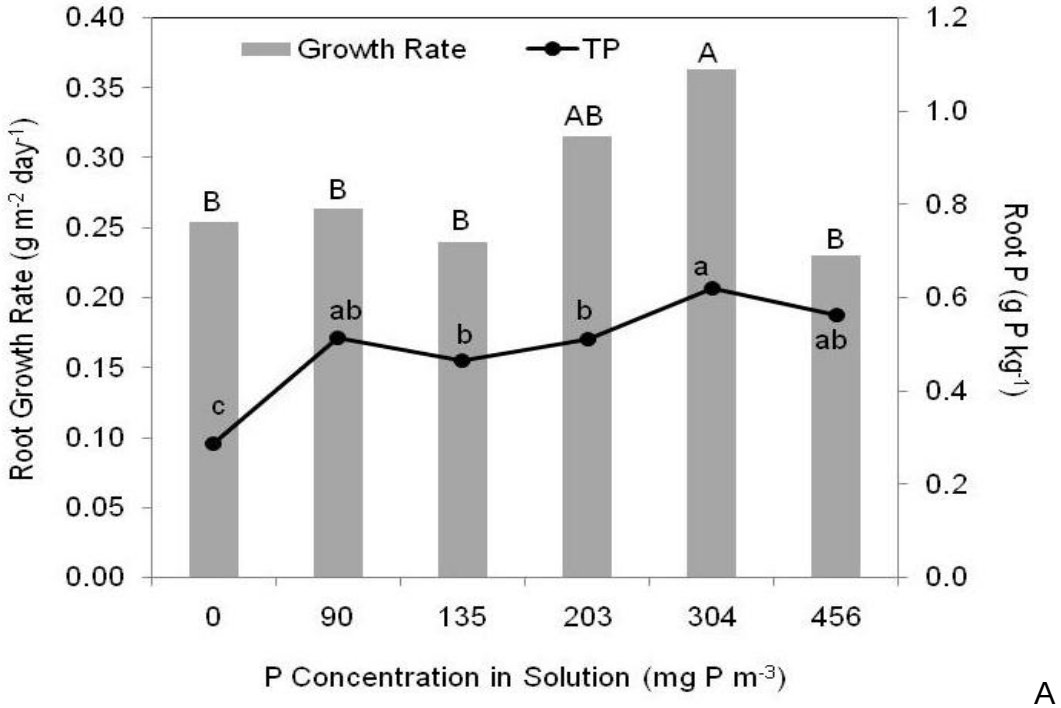
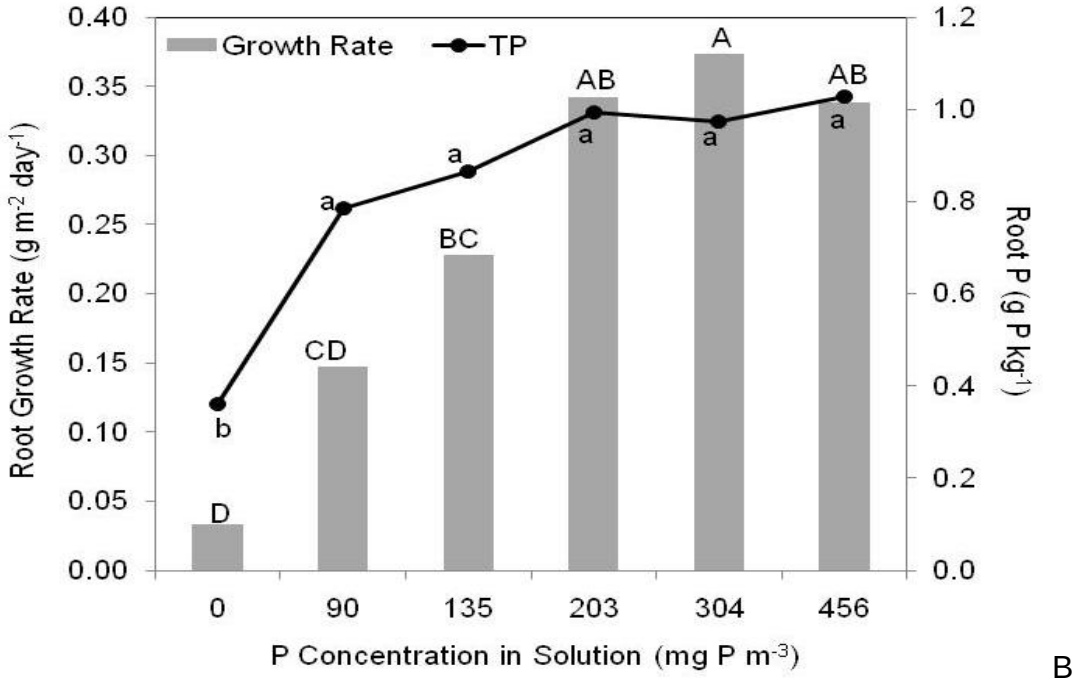


Figure 2-2. Phosphorus concentration in Empire zoysiagrass (EZ) and Floratam St. Augustinegrass (SA) leaf tissue in relation to phosphorus supply rate.

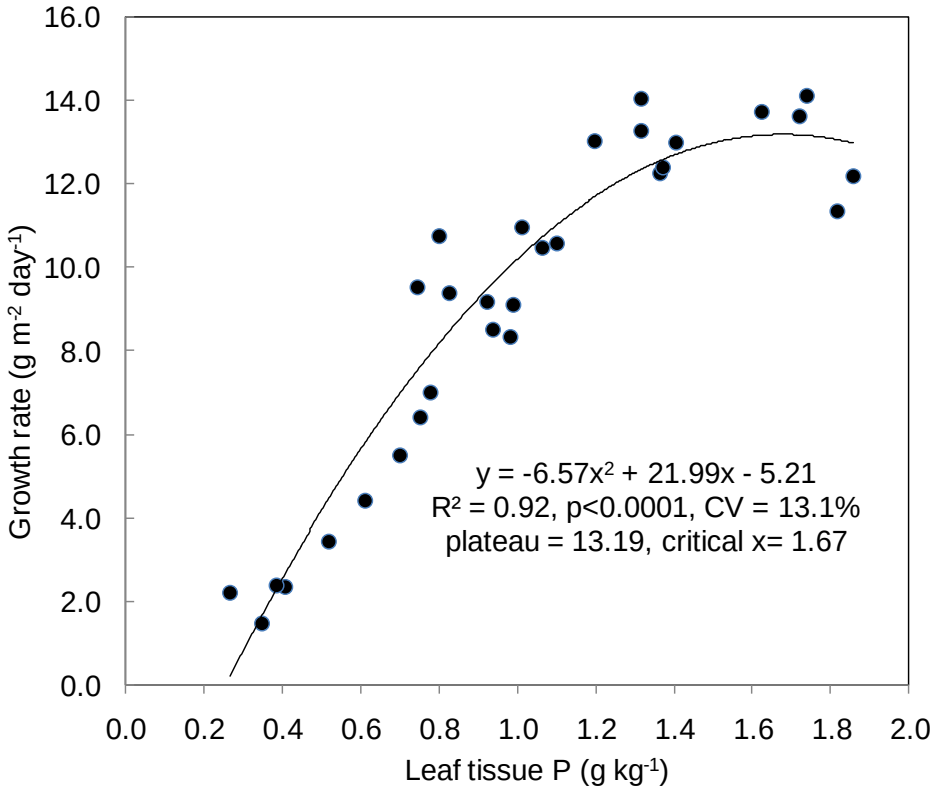


A

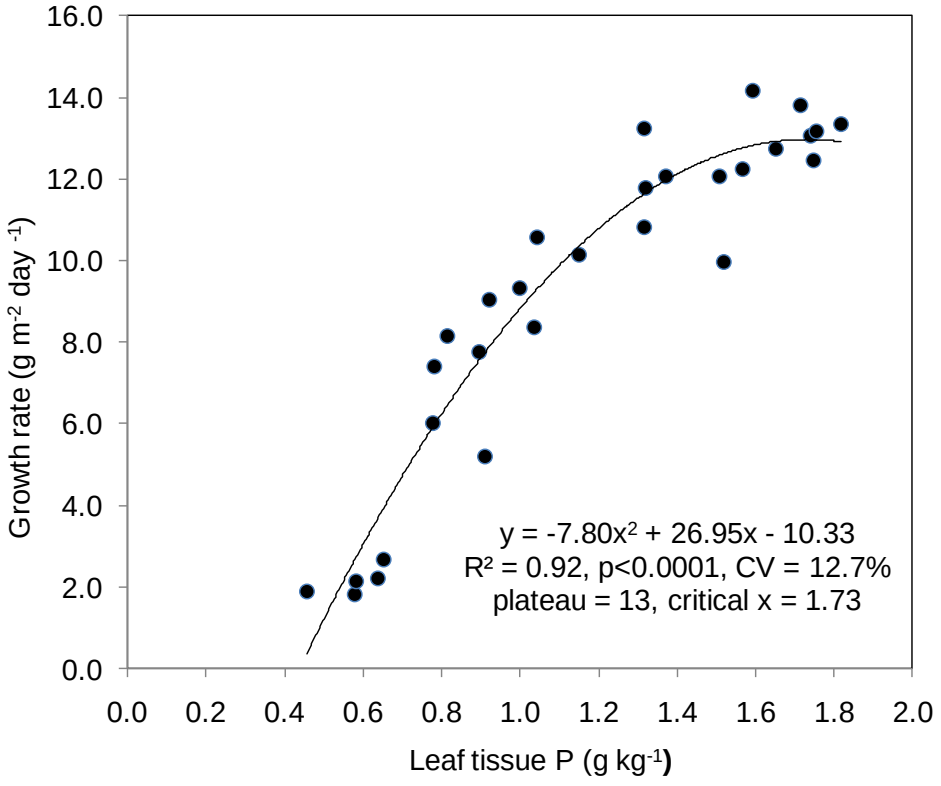


B

Figure 2-3. Root growth rate and P concentration in root tissue relative to solution phosphorus concentration. A) Empire zoysiagras and B) Floratam St. Augustinegrass. Columns or data points along a line labeled with the same letter are not significantly different at p = 0.05 by contrasts analysis. Capital letters indicate statistical differences in root growth rate among treatments. Lower case letters indicate statistical differences in root tissue P among treatments.

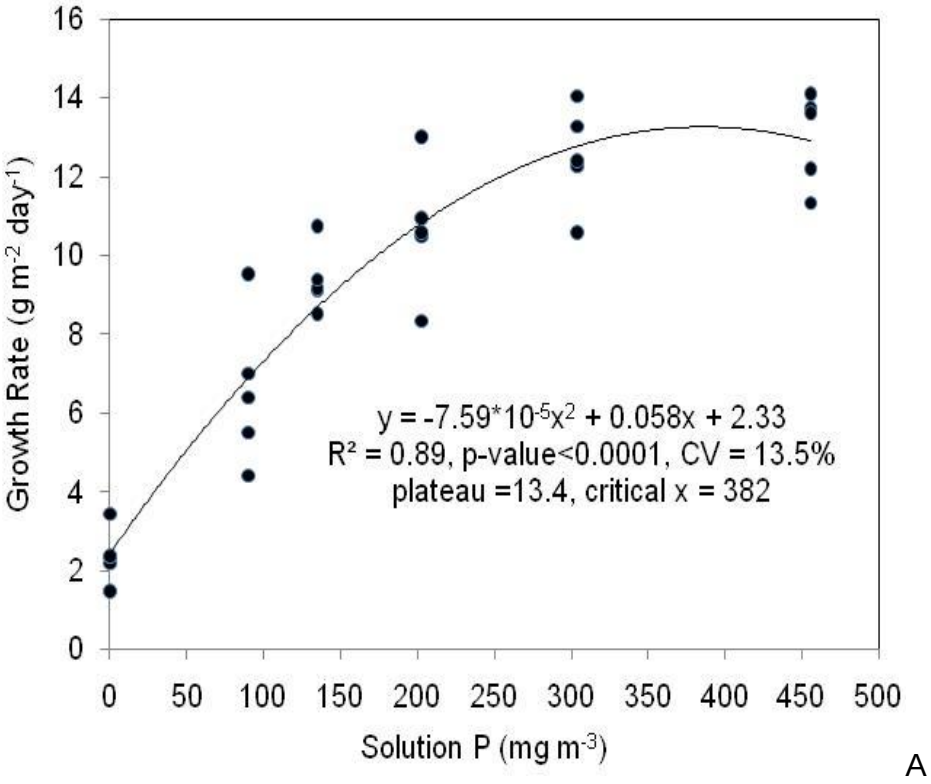


A

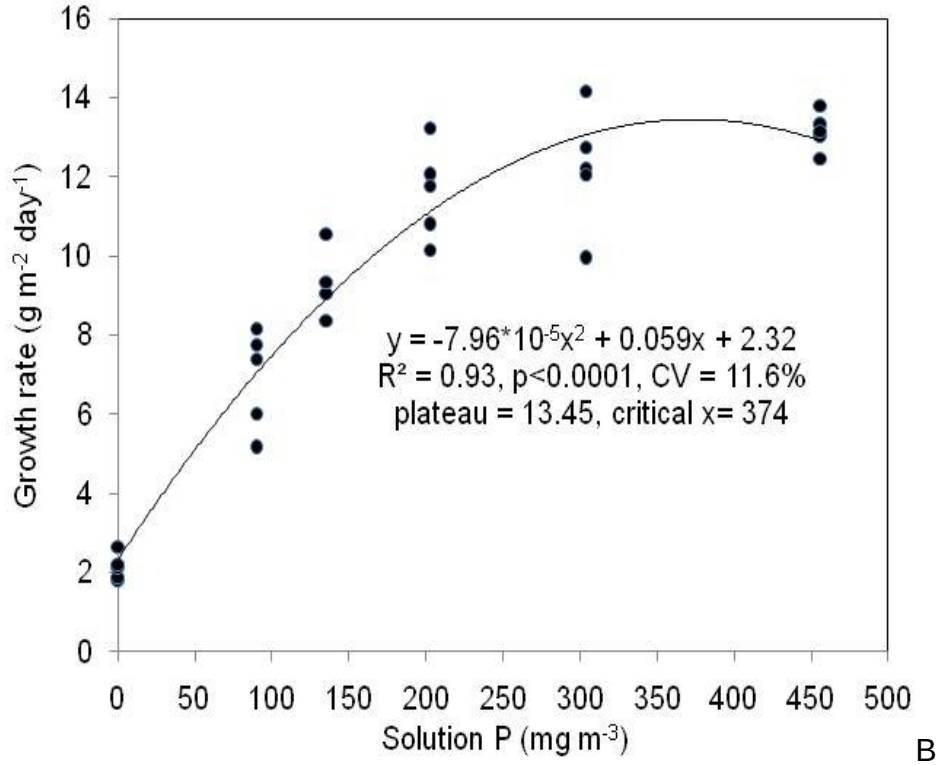


B

Figure 2-4. Aboveground tissue growth rate relative to leaf tissue P concentration. A) Empire zoysiagrass and B) Floratam St. Augustinegrass.



A



B

Figure 2-5. Aboveground tissue growth rate relative to phosphorus concentration in solution. A) Empire zoysiagrass and B) Floratam St. Augustinegrass.

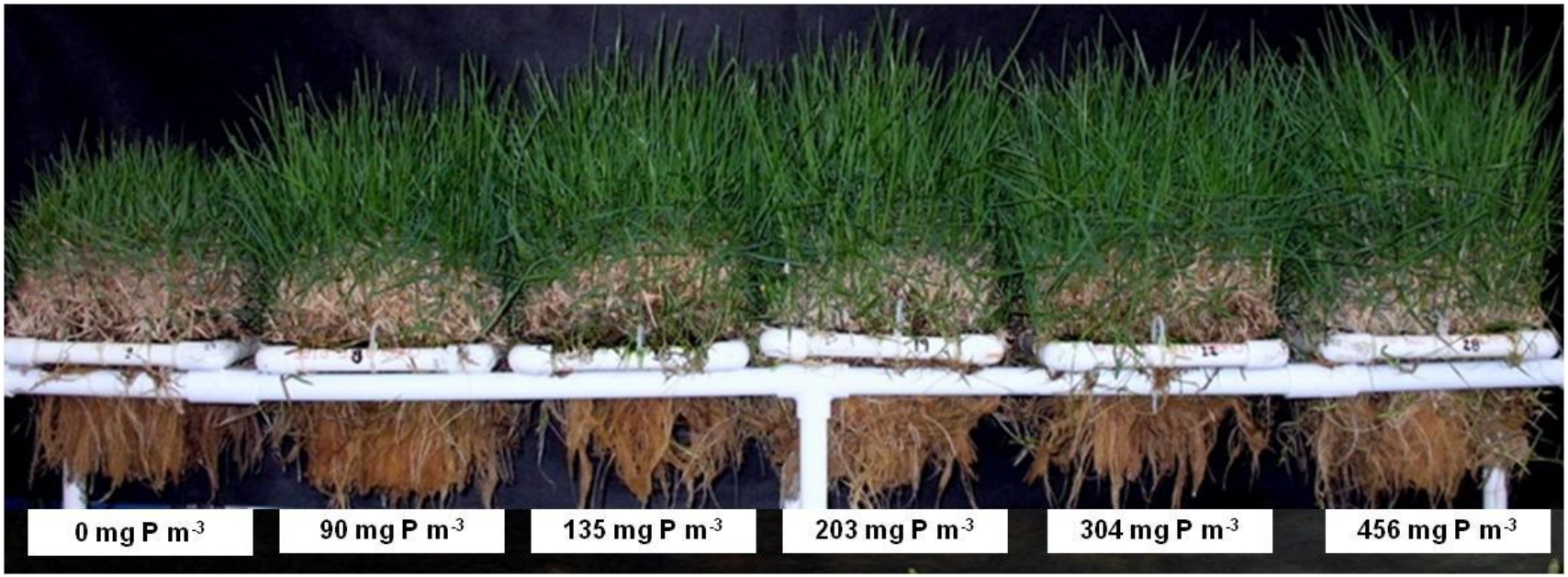


Figure 2-6. Empire zoysiagrass aboveground biomass accumulation relative to P concentration in solution.

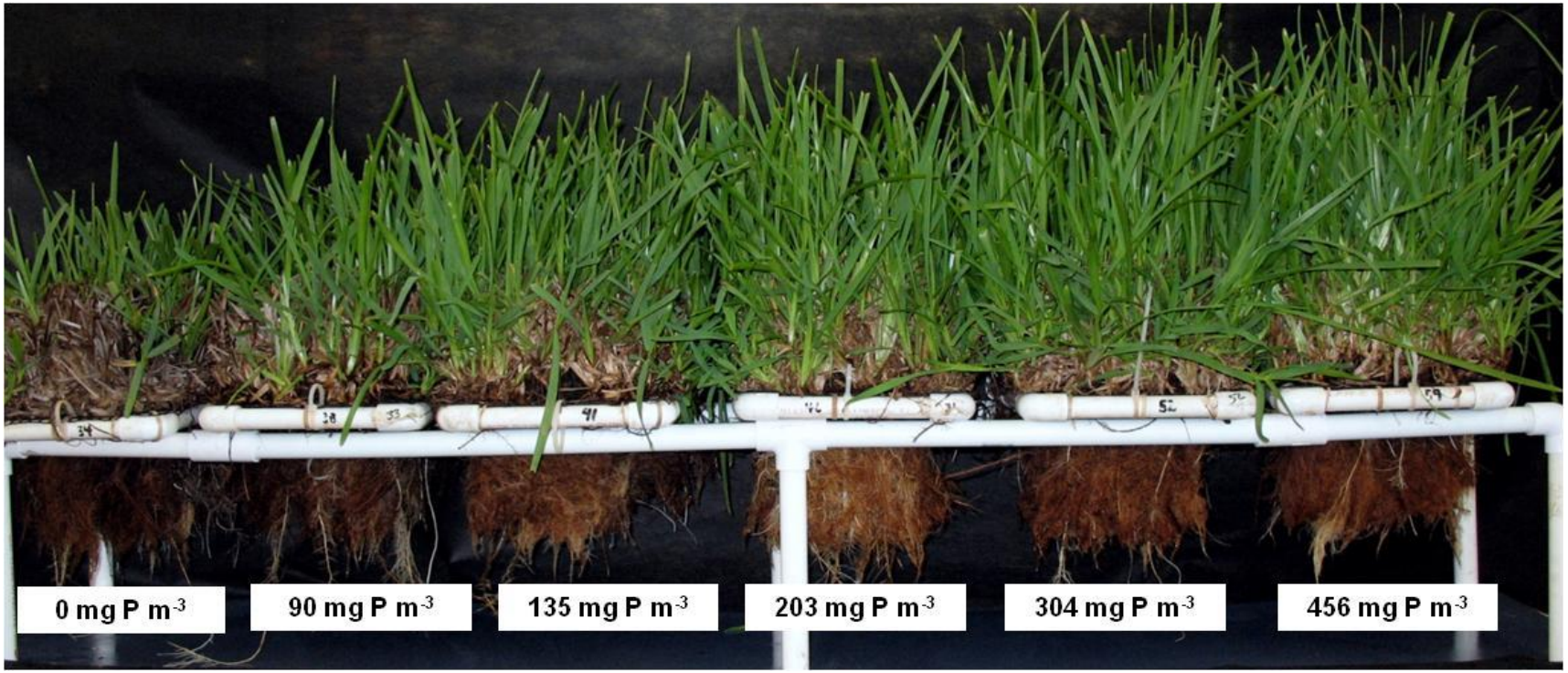


Figure 2-7. Floratam St. Augustinegrass aboveground biomass accumulation relative to P concentration in solution.

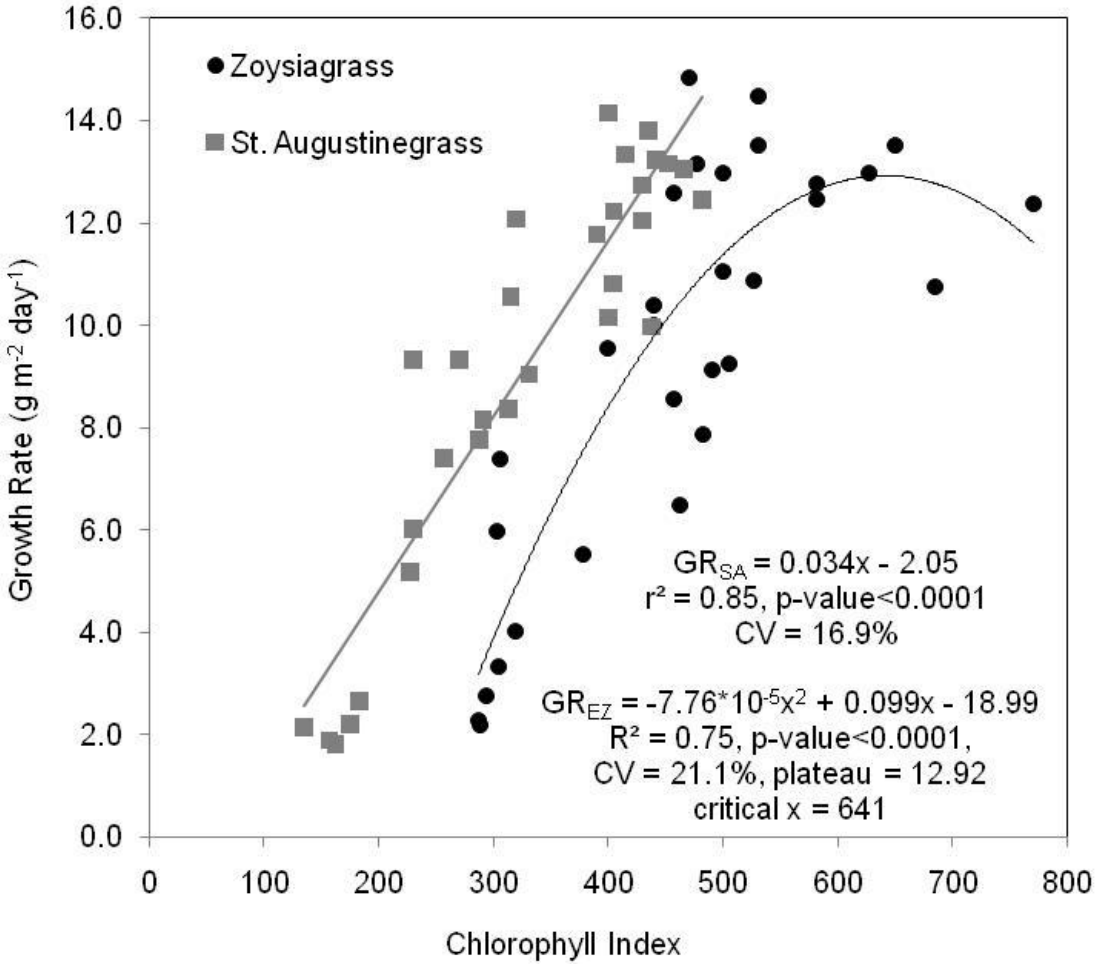


Figure 2-8. Empire zoysiagrass (EZ) and Floratam St. Augustinegrass (SA) aboveground tissue growth rate (GR) relative to chlorophyll index level.

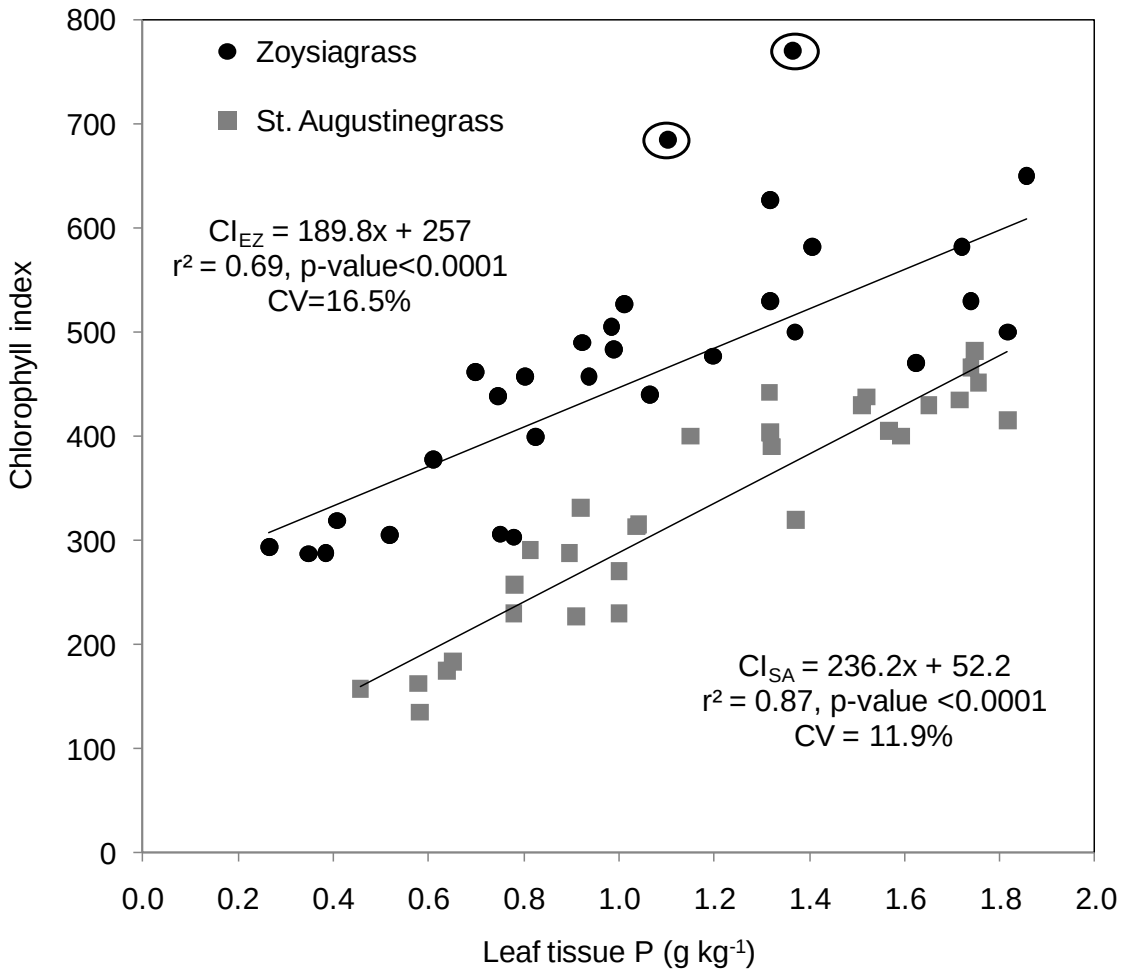


Figure 2-9. Empire zoysiagrass (EZ) and Floratam St. Augustinegrass (SA) chlorophyll index (CI) level in relation to P concentration in leaf tissue. Circled points were not included in the regression analysis for EZ.

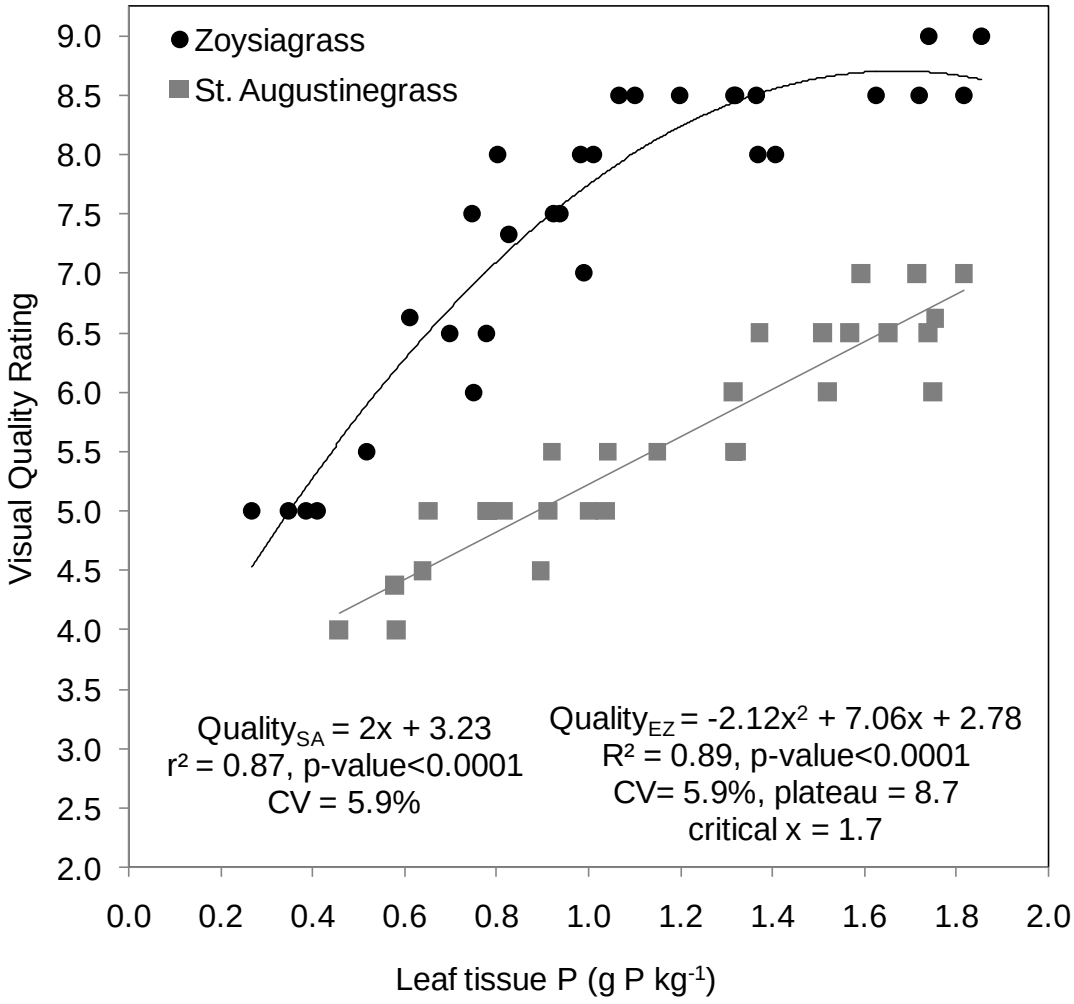
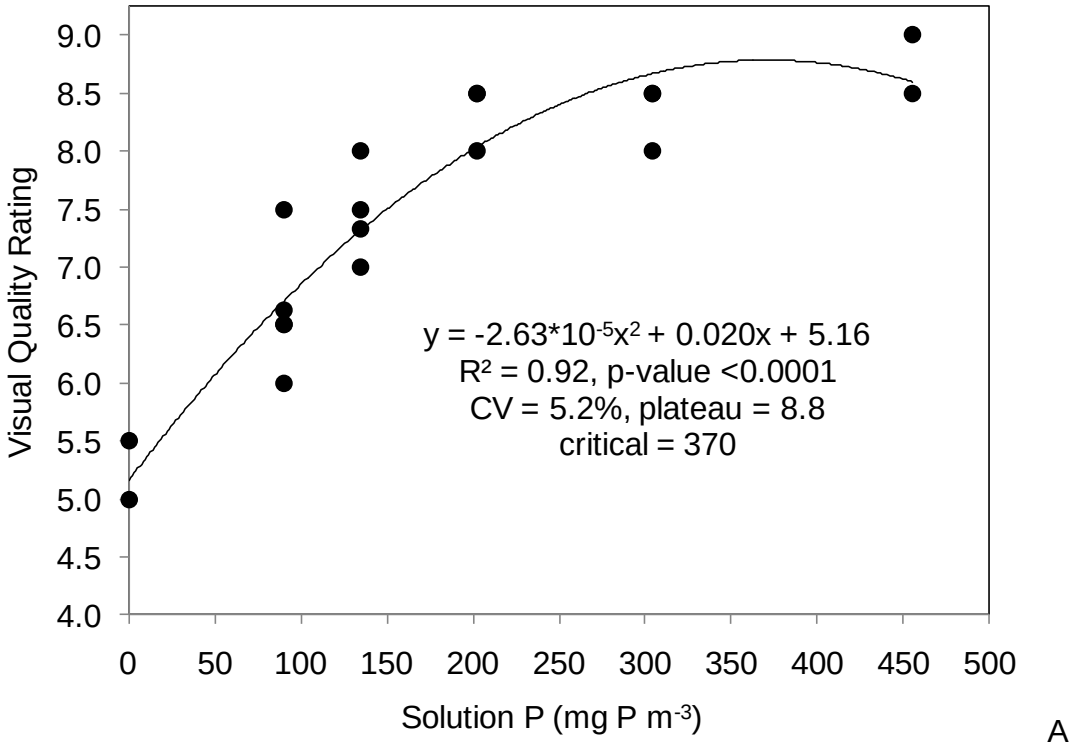
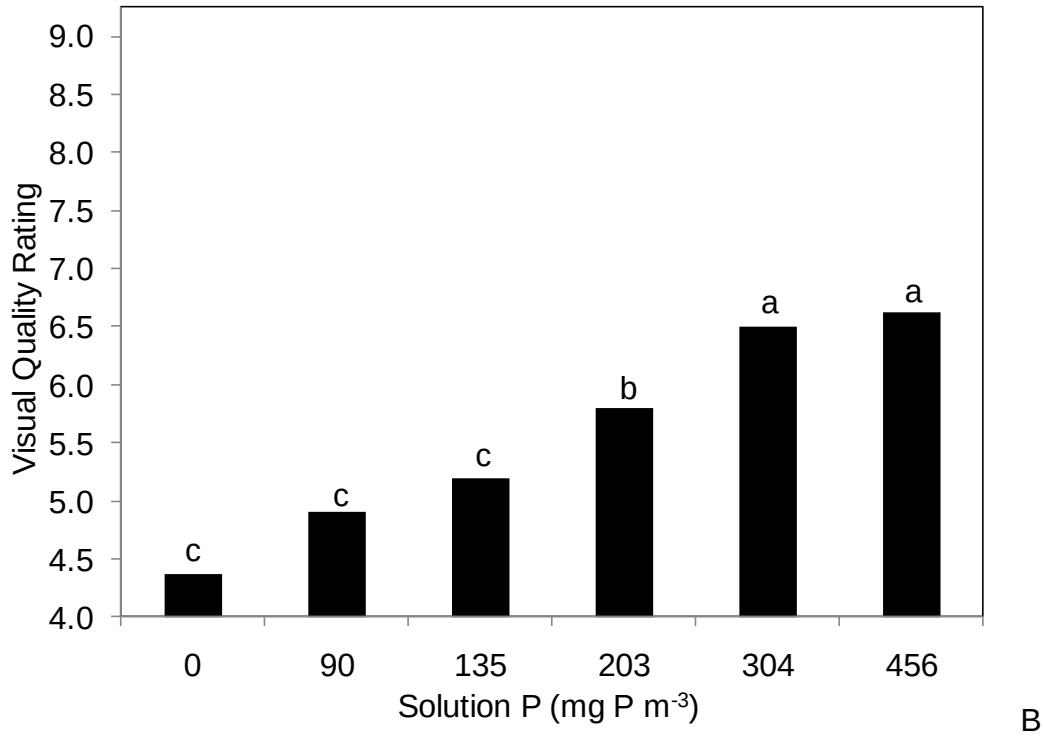


Figure 2-10. Empire zoysiagrass (EZ) and Floratam St. Augustinegrass (SA) visual quality rating relative to leaf tissue P concentration.



A



B

Figure 2-11. Visual quality rating relative to P concentration in solution. A) Empire zoysiagrass and B) Floratam St. Augustinegrass.

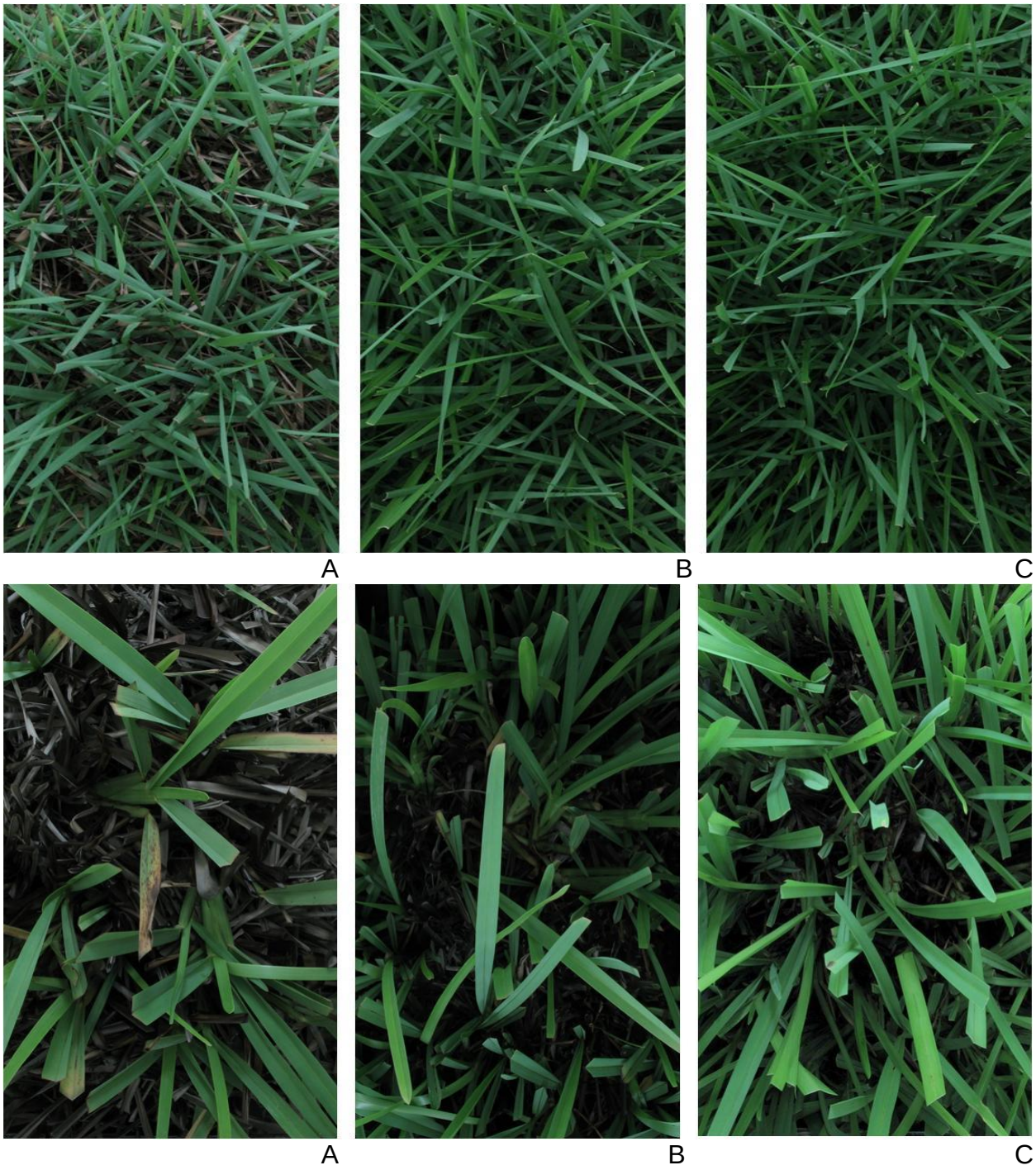


Figure 2-12. Visual quality relative to P concentration in solution in Empire zoysiagrass (top) and Floratam St. Augustinegrass (bottom). A) 0 mg P m^{-3}, B) 135 mg P m^{-3}, C) 456 mg P m^{-3}.

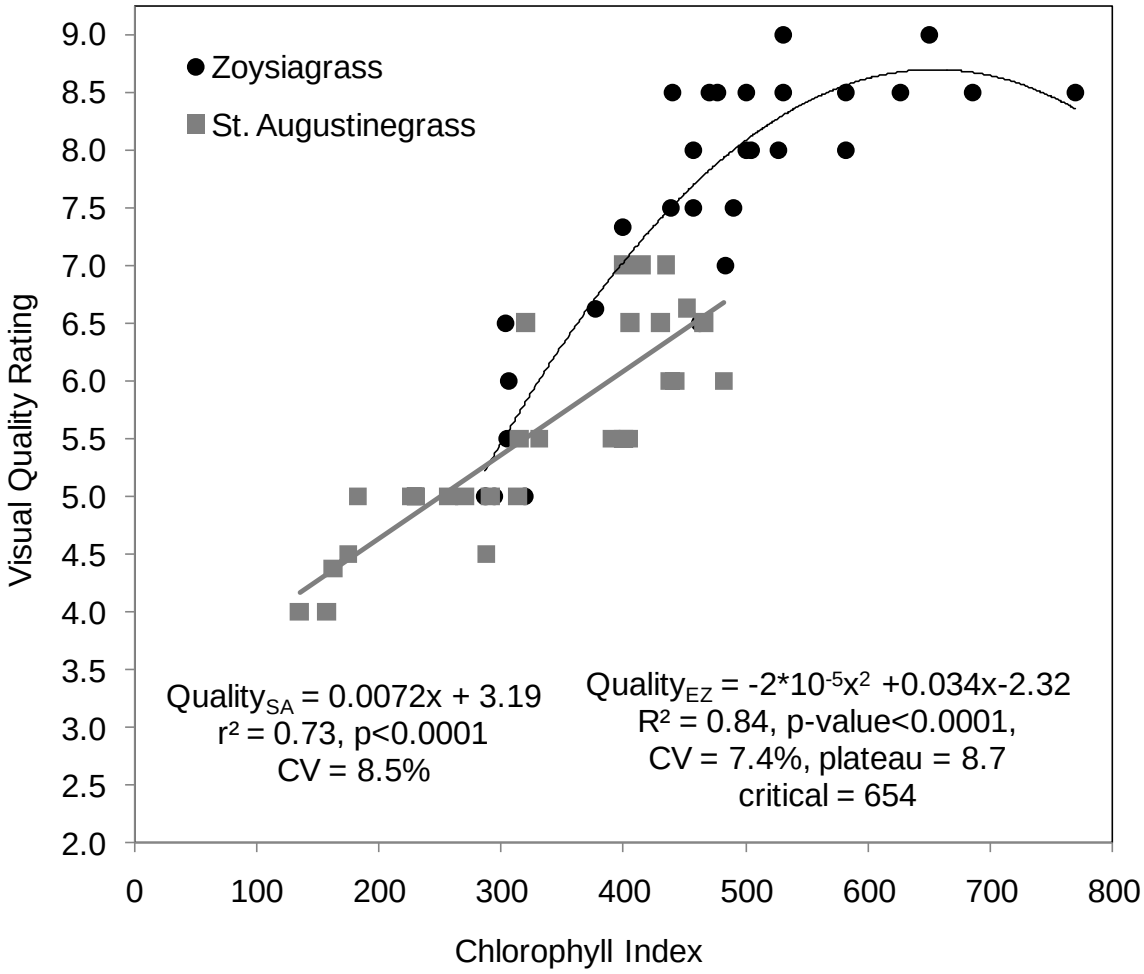


Figure 2-13. Empire zoysiagrass (EZ) and Floratam St. Augustinegrass (SA) visual quality rating relative to chlorophyll index level.

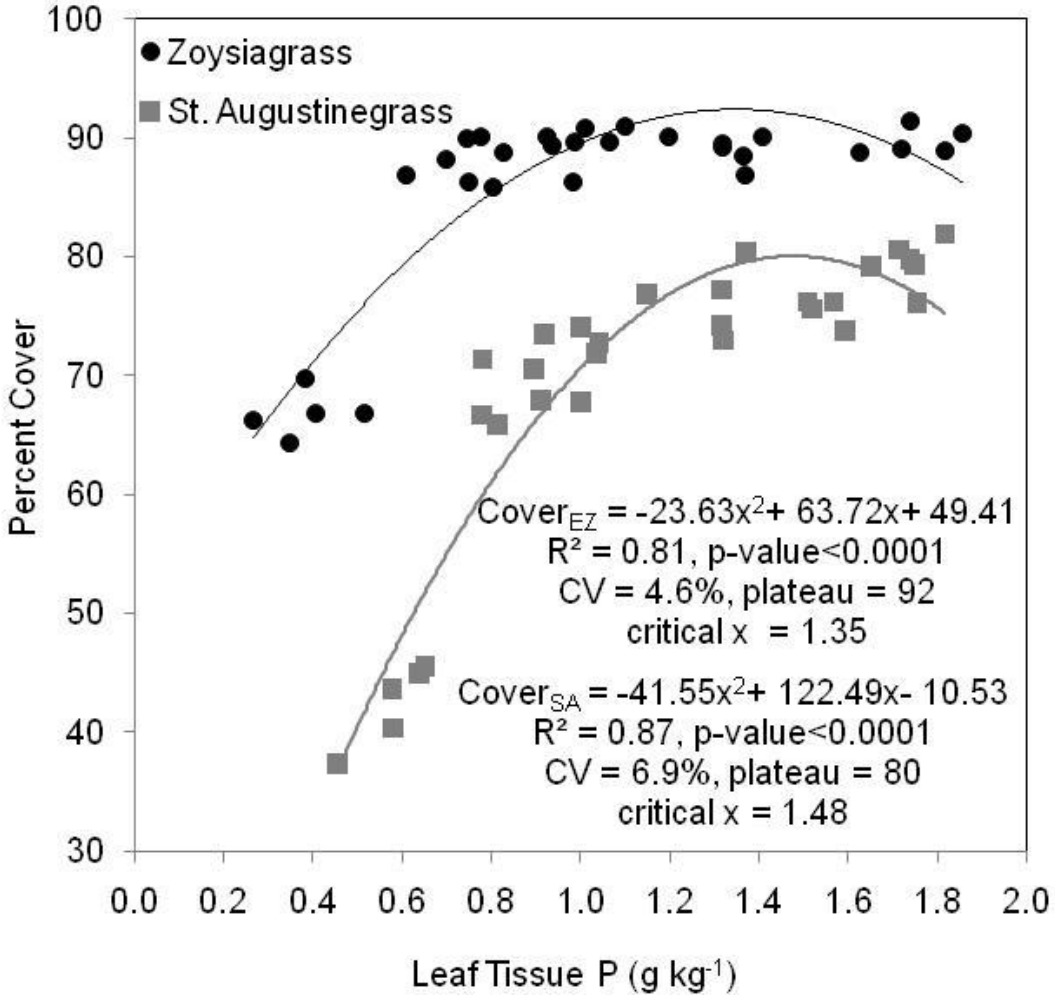


Figure 2-14. Empire zoysiagrass (EZ) and Floratam St. Augustinegrass (SA) percent cover relative to leaf tissue P concentration.

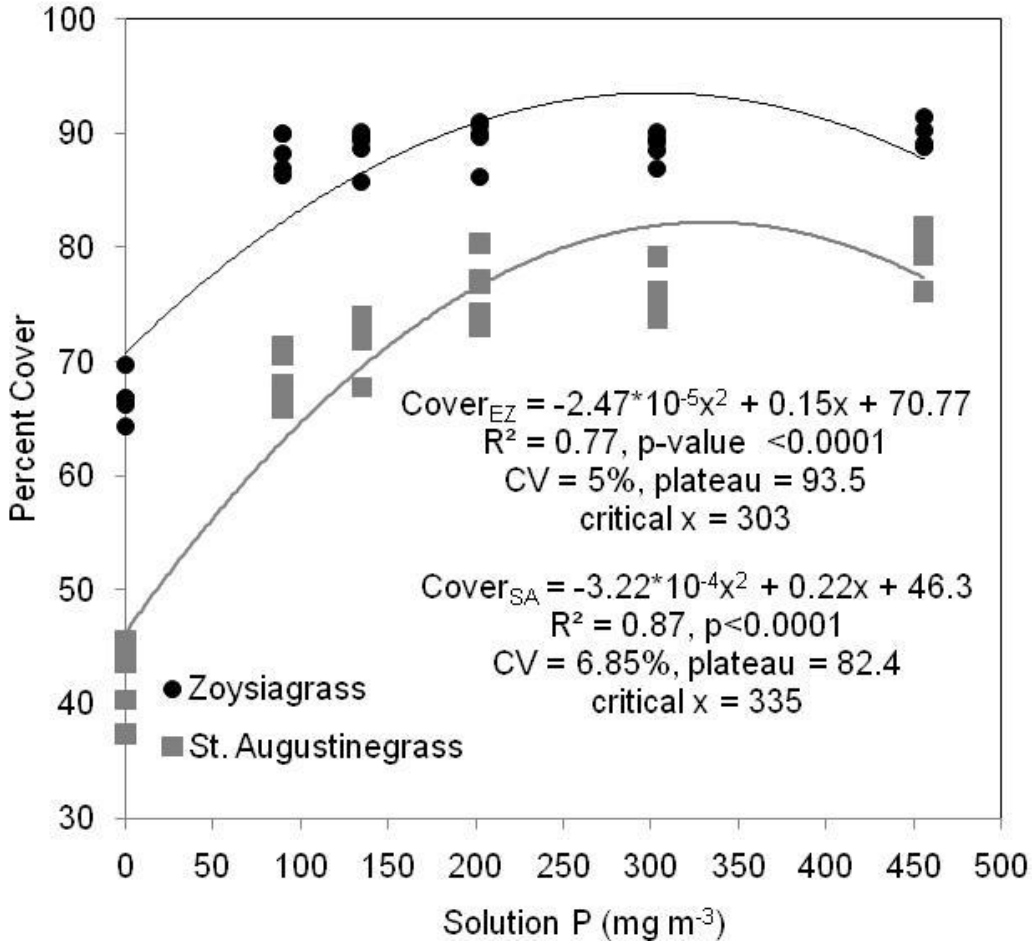


Figure 2-15. Empire zoysiagrass (EZ) and Floratam St. Augustinegrass (SA) percent cover relative to solution P concentration.

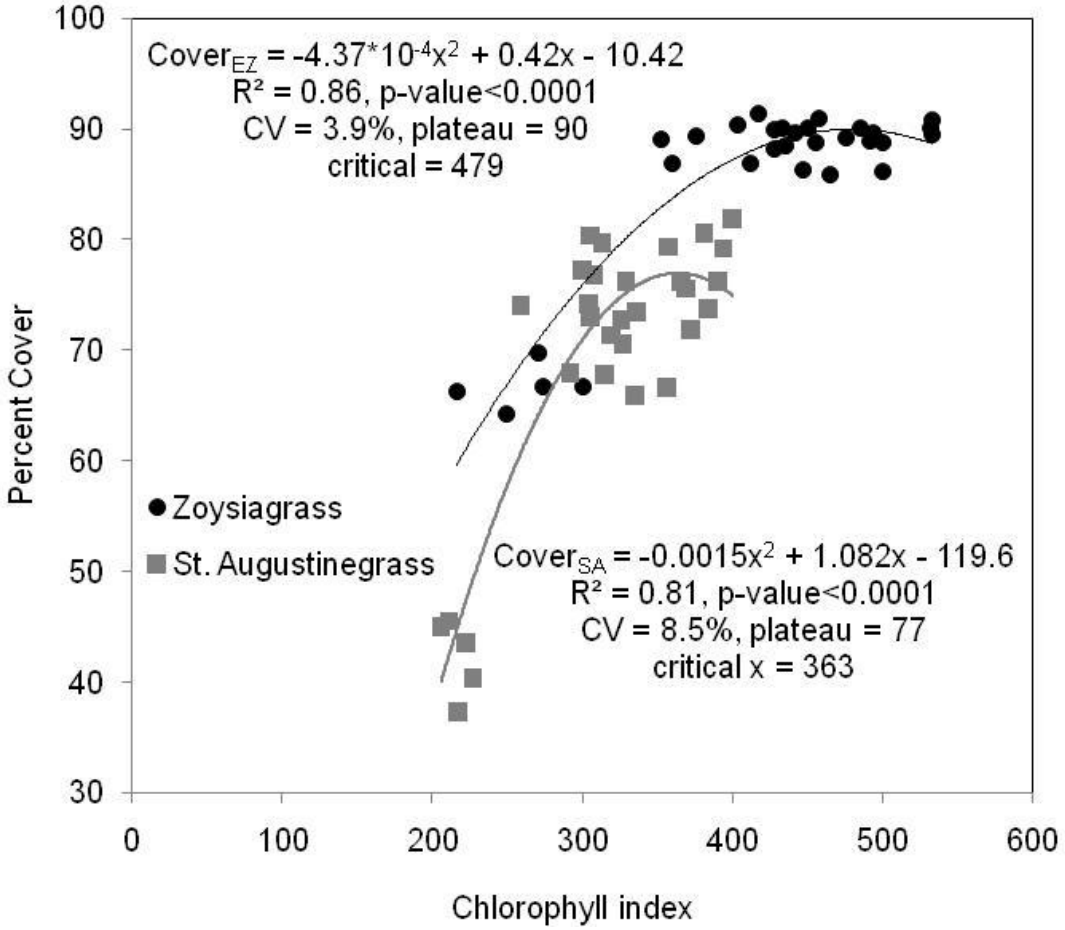


Figure 2-16. Empire zoysiagrass (EZ) and Floratam St. Augustinegrass (SA) percent cover relative to chlorophyll index level.

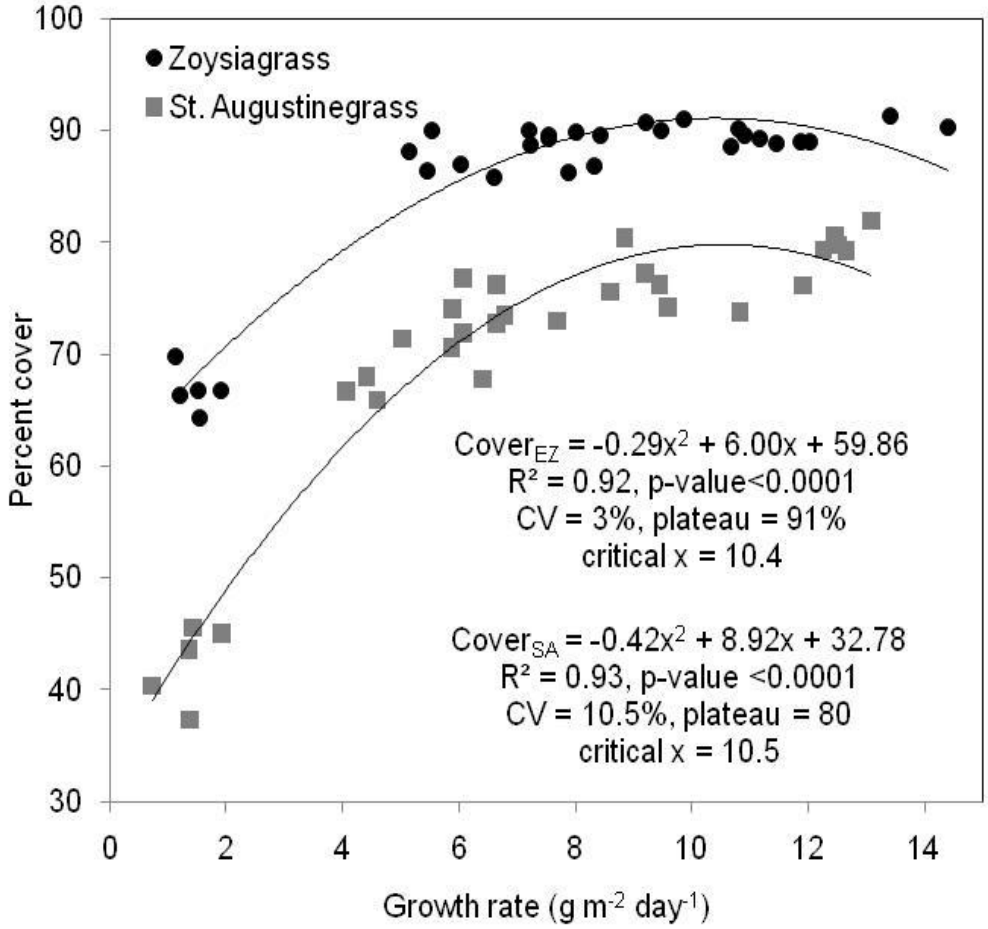
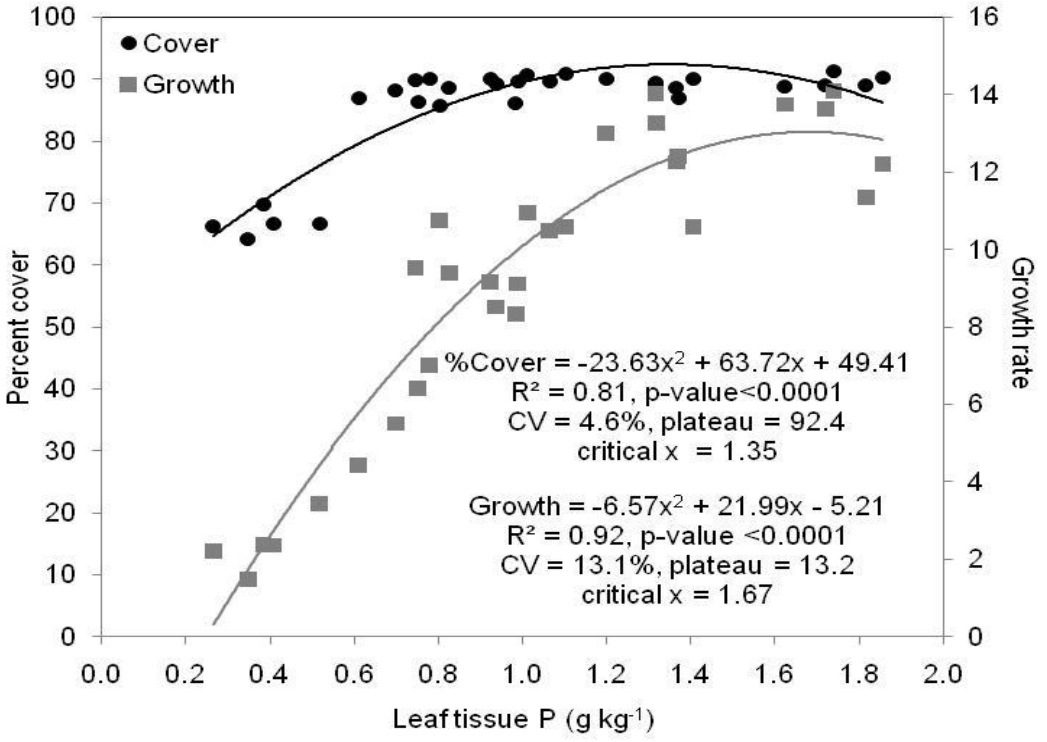
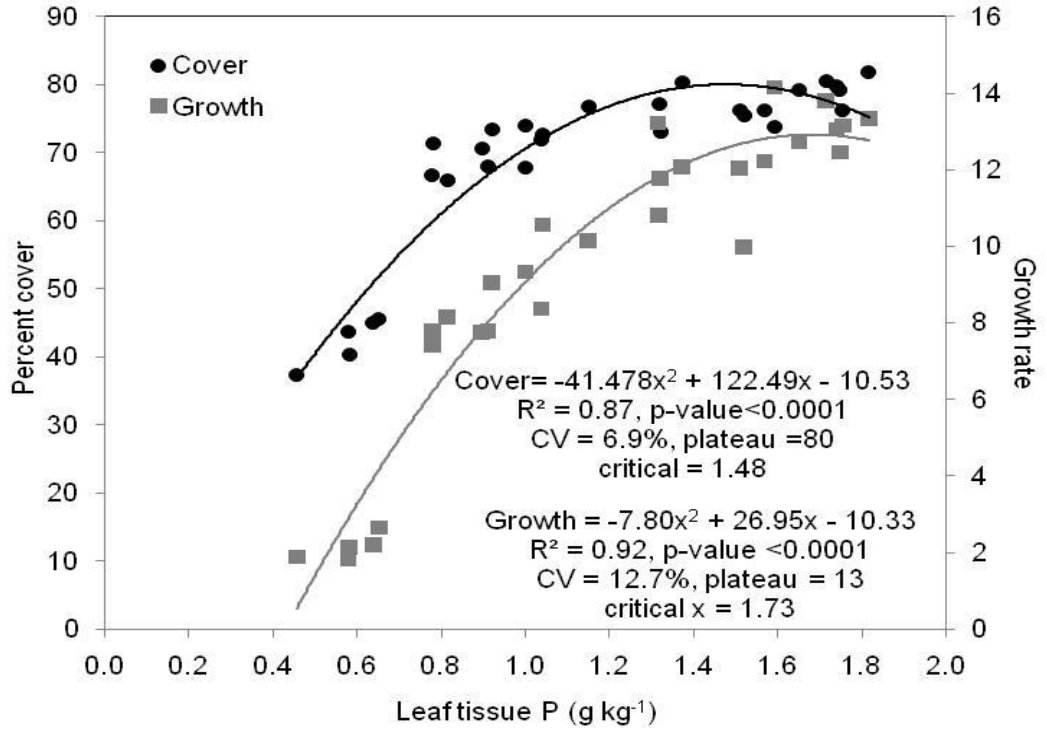


Figure 2-17. Relationship between percent cover and aboveground tissue growth rate in Empire zoysiagrass (EZ) and Floratam St. Augustinegrass (SA).



A



B

Figure 2-18. Percent cover and aboveground tissue growth rate as related to P concentration in leaf tissue. A) Empire zoysiagrass and B) Floratam St. Augustinegrass.

CHAPTER 3
EMPIRE ZOYSIAGRASS AND FLORATAM ST. AUGUSTINEGRASS PHOSPHORUS UPTAKE RATE, USE EFICIENCY AND PARTITIONING RELATIVE TO TISSUE PHOSPHORUS CONCENTRATION AND PHOSPHORUS SUPPLY IN HYDROPONIC CULTURE

Phosphorus is an essential plant nutrient (Raghothama, 1999). It is involved in many key processes in plants such as energy transfer and generation, synthesis of nucleic acids, photosynthesis, glycolysis, respiration, synthesis and stability of plant membranes, activation and inactivation of enzymes, redox reactions, metabolism of carbohydrates and biological nitrogen fixation (Vance et al. 2003). Phosphorus speciation in solution depends on the soil solution pH, below a pH of 6 most of the inorganic P in solution is in the monovalent ($H_2PO_4^-$) form (Schachtman et al., 1998). Plants absorb P against a steep gradient because in most soils the concentration of available inorganic P in solution is about 2 µ*M* while in plants the concentration of P oscillates between 5 and 20 m*M* (Raghothama, 2005). Active transport of P across the plasma membrane is required to overcome this concentration gradient (Vance et al., 2003). Maximum P influx increases under P deprivation (Clarkson, 1984). Leaf expansion, leaf surface area and number of leaves decreases in P deficient plants. Correspondingly, P deficient plants allocate greater amount of assimilates to roots (Marschner, 1995). Furthermore, in P starved plants, P is translocated from older tissues to young actively growing tissues. The latter may require depletion of P storage pools and the breakdown of organic P forms present in older tissues (Schachtman et al., 1998). Plant P acquisition potential is relevant from the agronomic and environmental point of view. High P-use efficiency is another desirable attribute in plants. As indicated by White and Hammond (2008), P use efficiency may be defined as the ratio of crop yield to the amount of P accumulated in the plant. Akhtar et al. (2007)

reported that P use efficiency of P deficient plants was significantly higher than that measured in P sufficient plants. Efficient use of P fertilizer in turfgrass systems is crucial to attain adequate turf quality and growth while conserving a non renewable resource and avoiding the negative implications of P losses to the environment.

The following hypotheses were tested: (i) phosphorus use efficiency and rate of P depletion from solution by ‘Empire’ zoysiagrass and ‘Floratam’ St. Augustinegrass will be inversely related to P supply and leaf tissue P concentration, and (ii) greater P supply and leaf tissue P concentration will result in greater dry matter and P partitioning to leaf tissue followed by thatch and the smaller fraction will be allocated to roots.

The objectives of this study were (i) to evaluate the influence of P supply rate and leaf tissue P concentration on the rate of P depletion from solution and P-use efficiency in St. Augustinegrass and zoysiagrass, and (ii) to study the effect of P supply rate and leaf tissue P concentration on dry matter and P partitioning in these turfgrass species.

Materials and Methods

Hydroponic System Description and Turfgrass Establishment

This study was conducted at the Turfgrass Envirotron facility on the University of Florida campus (29° 38’ N, 82° 21’ W). *Zoysia japonica* cultivar ‘Empire’ (zoysiagrass) and St. *Stenotaphrum secundatum* (Walt) Kuntze cultivar ‘Floratam’ (St. Augustinegrass) certified sod from the G.C. Horn Turfgrass Field Laboratory near Citra, Florida was selected as the test cultivars. The sod was washed thoroughly to remove soil from the root system, cut into 20 cm x 33 cm rectangles and transferred to a hydroponics system. Polyvinyl chloride (PVC, 22 mm outer diameter) pipe was used to construct a 22 cm by 33 cm frame and covered with poly hardware cloth (13-mm square openings). The sod was placed on the plastic screen which was used as a grass

bedding surface. Turf roots were carefully passed through the openings of the plastic screen to promote greater contact with the nutrient solution.

Each experimental unit was placed in a 25 cm x 36 cm x 23 cm plastic tub (~20 L) which was used as the hydroponic container. The tubs corresponding to a given treatment were connected to a larger nutrient reservoir (~120 L), and the nutrient solution was constantly circulating between the tubs and the nutrient reservoir. A submersible pump delivered nutrient solution to the corresponding tubs at a rate of 12 L per minute. A 25 mm outer diameter PVC threaded male adaptor placed at the bottom of each tub was connected to a 25 mm inner diameter, 25 cm long PVC pipe in the inner side of the tub. This pipe was used to regulate the level of the solution as well as to drain and return the solution by gravity to the corresponding nutrient solution reservoir. Light penetration through the outer surfaces of the tubs was restricted by black latex paint. During the first two weeks of the experiment, the nutrient solution level was maintained in contact with the plastic hardware net to stimulate root growth. Thereafter, the solution level was lowered to about 2.5 cm from the plastic screen to favor incorporation of oxygen as the continually circulating solution enter in contact with the solution present in the tub. The turfgrass was maintained from December 19th 2008 until May 24th 2009 on a modified half strength Hoagland solution (Hoagland and Arnon, 1950) without P to reduce tissue P levels. Phosphorus treatments were imposed on May 24th, 2009 and the study was continued for 140 days thereafter.

Average relative humidity and temperature in the glasshouse were 70% and 28.4 °C, respectively. The relative humidity and the temperature oscillated between 20% and 89% and 21°C and 39°C, respectively. In addition, the average solar radiation intensity

between 700 and 1800 h was 898 µmol quanta m^{-2} s^{-1} and ranged from 1.63 to a 2,459 µmol quanta m^{-2} s^{-1}.

Phosphorus Treatments and Nutrient Solution Description

Six levels of P (0, 90, 135, 203, 304 and 456 mg P m^{-3}) were supplied with reagent grade mono potassium phosphate (22.6 % P) in a modified full strength Hoagland (Hoagland and Arnon, 1950) nutrient solution (Table 2-1). The concentration of P in the soil solution rarely exceeds 10 µ*M* (310 mg P m^{-3}) and in most soils it is about 2 µ*M* (Raghothama, 1999). In a preliminary study (data not presented herein) a maximum concentration of P in solution of 456 mg P m^{-3} was estimated to be sufficient to increase the P concentration of the turfgrass species of interest above the critical level; hence, P concentrations between 0 and 456 mg P m^{-3} were selected for this experiment. The P treatments were replicated five times and arranged in a split-plot randomized complete block design with turfgrass species as the main effect and P application rate as secondary effect. Chelated iron (Sequestrene 330) was supplied biweekly through foliar application at a rate of 0.5 g Fe m^{-2} (Carrow, 2007). The nutrient solution was replaced twice a week. Initial solution pH oscillated between 5.5 and 6 and the initial nutrient solution temperature ranged between 25°C and 30 °C.

Tissue Sampling and Analysis

Top growth was harvested every two weeks to a height of approximately 10 cm. Roots were clipped to approximately 10 cm when their length was about 15 cm (the depth of the water column in which the roots were grown was approximately 15 cm). All tissue samples were dried at 70 °C to constant weight, weighed, and then ground to pass a # 40 mesh sieve (425 µm openings size). The change in dry matter (DM) accumulation per unit area (m^2) and time (day) was monitored during the entire

evaluation period. Dry tissue was ashed and digested with 6 *M* HCl according to the standard operation procedure WLB-SP-009 of the Wetland Biogeochemistry Laboratory at the University of Florida, “Total Phosphorus of Soil, Sediment and Plant Tissue by Ignition or Ashing Method”. Phosphorus concentration in the digestate was determined following USEPA method 365.1 “Determination of Phosphorus by Semi-automated Colorimetry” (O’Dell, 1993).

Turf Visual Quality and Chlorophyll Index

Turf visual quality was evaluated biweekly using a scale of 1 to 9, where 1 represents brown, dormant turf and 9 represents superior quality. A value of 5.5 was considered the minimum rating for an acceptable turf visual quality (Skogley and Sawyer, 1992). Chlorophyll Index was measured biweekly with a CM 1000 Chlorophyll Meter (Spectrum Technologies Inc, Illinois, USA) just prior every leaf tissue harvest.

Rate of Phosphorus Depletion from Nutrient Solution

The experimental units corresponding to treatments supplied with 203 mg P m^{-3}, 304 mg P m^{-3} and 456 mg P m^{-3}, were utilized in this part of the study. The turf was exposed to an initial solution P concentration of 409 mg P m^{-3}. The initial nutrient solution weight and its corresponding initial P concentration were determined prior to placing the turf in the solution. In addition, initial solution pH, electrical conductivity (EC) and temperature were also recorded. On average, the initial solution volume per experimental unit (assuming a solution density of 1 g cm^{-3}), initial soluble reactive P concentration, initial pH, initial EC and initial temperature were 13.7 L, 409 mg P m^{-3}, 5.8, 26 mS m^{-1} and 25.9 °C, respectively. The change in these variables was determined approximately every 90 minutes for a period of ten hours starting between the 800 and 1800 h during 5 consecutive days. However, only the data collected during

the first 4 hours is presented herein. At the end of the evaluation period all the roots from each experimental unit were harvested and scanned with an Epson Perfection V700 Photo dual lens scanner (Epson Corporation, Japan). The digital images obtained were then analyzed with WinRhizo Software Pro v. 2007d (Reagent Instruments Canada Inc., Ottawa, ON, Canada) to determine the total root length in each experimental unit. The rate of P depletion was calculated as the change in the solution P content over time per meter of root.

Statistical Analysis

Non linear regression analysis (Proc Reg) in SAS Statistical Software v. 9.2 (SAS Institute, 2009) was conducted to relate response variables such as rate of P accumulation in leaf tissue, rate of P depletion from nutrient solution and P use efficiency to explanatory variables like leaf tissue P concentration, initial solution P concentration and thatch tissue P content. Furthermore, mean separation was carried out using single degree of freedom contrast analysis (Proc GLM) in SAS Statistical Software.

Results and Discussion

Dry Matter and Phosphorus Partitioning

There was no treatment effect on thatch or root dry matter accumulation in zoysiagrass (Figure 3-1). Total zoysiagrass dry matter accumulation in leaves increased with increasing P supply; however, no treatment effect on the fraction of total DM accumulated in leaf tissue was observed above an initial P concentration in solution of 90 mg P m^{-3} (Figure 3-1). The percent of St. Augustinegrass total dry matter accumulated per unit area of leaves increased in response to increasing P supply. In contrast, as the P supply increased a lower fraction of the total DM accumulated per unit

area of St. Augustinegrass turfgrass was partitioned to thatch tissue (Figure 3-1). Dry matter accumulation in leaves of St. Augustinegrass exposed to an initial P solution concentration of 456 mg P m^{-3} was 3.3 times greater than in the control treatment. Thatch dry matter accumulation was 13% greater in the P starved St. Augustinegrass turf in comparison to the turf growing in the highest P supply treatment. No treatment effect on the percent of total dry matter accumulated in roots of St. Augustinegrass was observed (Figure 3-1). St. Augustinegrass leaf:root ratio increased with increasing P supply (Table 3-1). There was no significant treatment effect on the St. Augustinegrass leaf plus thatch to root ratio (Table 3-1). Phosphorus concentration in leaf and thatch tissue of St. Augustinegrass increased with increasing supply, while root tissue P concentration did not increase significantly above 90 mg P m^{-3} (Table 3-1). Leaf:root ratio of zoysiagrass increased with increasing P supply but no increase in leaf+thatch:root ratio was observed above 90 mg P m^{-3} (Table 3-2). Only in the zoysiagrass exposed to an initial solution P concentration of 456 mg P m^{-3} the leaf plus thatch to root ratio was greater than in the control treatment. The concentration of P in zoysiagrass leaf, thatch and root tissue increased with increasing P supply (Table 3-2). On average across treatments 65.6%, 19.8% and 14.6% of the total P content per unit area of zoysiagrass turf was allocated to thatch, leaf and root tissue, respectively (Figure 3-2). In the case of St. Augustinegrass the fraction of total P accumulated per unit area that was allocated to thatch was 58%, to leaf was 26% and to root tissue was 16% (Figure 3-2). The percentage of total P content (PC) in leaf tissue per unit area of zoysiagrass and St. Augustinegrass turf increased with increasing P supply (Figure 3-2). An initial P concentration in solution of 90 mg P m^{-3} was required to increase the PC

in zoysiagrass leaves with respect to the control treatment (Figure 3-2). The PC per unit area allocated to zoysiagrass leaves did not increase above an initial solution P concentration of 203 mg P m^{-3} (Figure 3-2). The fraction of P stored in the thatch layer of zoysiagrass decreased with increasing P supply, while no treatment effect on zoysiagrass root P storage was established (Figure 3-2). The combined effect of greater dry matter allocation to leaves and greater leaf tissue P concentration with increasing P supply explains the greater PC in leaves as a result of increasing initial solution P concentration. Despite the decrease of St. Augustinegrass dry matter accumulated in the thatch layer associated with greater P supply (Figure 3-1), the total amount of P stored per unit area in the thatch layer of St. Augustinegrass increased. Phosphorus concentration of St. Augustinegrass thatch tissue supplied with high P levels was greater and it may account for the positive effect of increasing P supply on thatch P content. However, the fraction of the total P accumulated per unit area of St. Augustinegrass that was partitioned to thatch decreased with increasing P rate, possibly due to the parallel increase in P partitioning to leaf tissue (Figure 3-2). Phosphorus supply level did not have a clear effect on the fraction of total P content per unit area of St. Augustinegrass allocated to roots (Figure 3-2). Phosphorus is a phloem mobile nutrient element and thus it can be remobilized from older plant tissues to actively growing points (Marschner, 1995). Once older leaves and stems die, they may become part of the thatch layer. As the thatch layer decomposes it could release P to the solution from where it could be absorbed and translocated back to actively growing tissues, such as leaf and root tips. Phosphorus concentration and total P storage in leaf tissue were positively related to the size of the P pool in the thatch layer (Figure 3-3).

Average P accumulation rate in leaf tissue over the entire growth season (May through September) in turf with a leaf P concentration near the critical level was 18.5 mg P m^{-2} day^{-1} for zoysiagrass and 17.5 mg P m^{-2} day^{-1} for St. Augustinegrass. Leaf tissue P concentration required for maximum leaf growth rate was determined to be 1.67 g P kg^{-1} for zoysiagrass and 1.73 g P kg^{-1} for St. Augustinegrass (Figure 2-4). According to non-linear regression analysis, P content in the thatch layer of turfgrass with a leaf tissue P concentration near the critical for maximum leaf growth would be 2 g P m^{-2} for zoysiagrass and 2.03 g P m^{-2} for St. Augustinegrass (Figure 3-3). Under the conditions of this experiment, turfgrass growing in a medium with very low P concentration or very low P bioavailability could use the P storage pool in the thatch layer to meet the P demand required for maximum leaf growth during a period of approximately 108 days (2000 mg P m^{-2}/ 18.5 mg P m^{-2} day^{-1}) in the case of zoysiagrass and 116 days (2030 mg P m^{-2}/ 17.5 mg P m^{-2} day^{-1}) for St. Augustinegrass. If the clippings are returned to the turf and eventually become part of the thatch layer, only about 40% and 36% of the P stored in leaf clippings of zoysiagrass and St. Augustinegrass, respectively, would have to be mineralized and absorbed by the plant to meet the P demand for maximum leaf growth during a period of 180 days. Under the climatic conditions where this experiment was conducted (North Central Florida) the length of the period that promotes high leaf growth rate is shorter than 180 days. Since the rate of P accumulation in leaf is a function of leaf growth rate, lower P demand would result from a decrease in leaf growth. Accordingly, the amount of P required to be absorbed from the thatch layer to support leaf growth would be lower and the thatch P reserve would last longer. After 243 days of P starvation the fraction of the turf surface covered by

green leaves was on average 67% in zoysiagrass and 43% in St. Augustinegrass, which corresponded to 74% of the maximum green leaf cover attained in zoysiagrass and 53% in St. Augustinegrass turf growing with adequate P supply. These results emphasize the importance of the thatch P storage pool and P remobilization from older tissues to meet the P demand of actively growing parts of the plant.

Phosphorus Uptake Rate

Phosphorus uptake into leaf tissue was calculated as the product of leaf tissue growth rate (i.e., g DM m^{-2} day^{-1}) and phosphorus concentration in leaf tissue (g P kg^{-1} DM). Increasing P supply resulted in both greater leaf tissue P concentration (Figure 2-2) as well as greater leaf tissue growth rate (Figure 2-5). Leaf phosphorus accumulation rate increased linearly with increasing initial solution P concentration (Figure 3-4). This response is the result of the cumulative effect over time (i.e., days or weeks) of an increased biomass accumulation rate and increased tissue P concentration in response to greater P supply. During the period of highest growth rate about 23 mg P m^{-2} day^{-1} were taken up and allocated to leaves by turf supplied with an initial P concentration of 456 mg P m^{-3} (Figure 3-4).

In contrast to the positive relationship established between P accumulation rate in zoysiagrass leaf tissue and solution P concentration, increasing the concentration of P in solution (Figure 3-5) and concomitantly the P concentration in zoysiagrass leaf tissue (Figure 3-6), decreased the rate of P depletion from the nutrient solution by zoysiagrass. The rate of P depletion by zoysiagrass from the nutrient solution was lower at a solution concentration of 304 and 456 mg P m^{-3} than in a solution concentration of 203 mg P m^{-3} (Figure 3-5). A minimum P depletion rate from solution of 0.99 µg hr^{-1} m^{-1} of root was estimated to occur at a zoysiagrass leaf tissue P concentration of 1.65 g P kg^{-1} (Figure

3-6). Phosphorus may be retranslocated from shoots to roots to send a feedback signal that regulates the uptake of P by roots based on shoot phosphorus demand (Marschner, 1995). The rate of P depletion from solution by St. Augustinegrass was not influenced by P supply level (Figure 3-5). Phosphorus depletion rate from solution and P concentration in St. Augustinegrass leaf tissues were not related (Figure 3-6).

Under the conditions present in this experiment, the rate of P depletion from the nutrient solution is equivalent to the whole plant P uptake rate. Moreover, under these conditions P concentration in tissue is the main controlling factor of P depletion rate. On the contrary to the rate of P accumulation in leaf tissue over time, P depletion rate from solution is not affected by differences in biomass accumulation rate resulting from a gradient of tissue P concentration. The later premise is justifiable because the duration of the P depletion rate evaluation period was not long enough (i.e., less than 240 minutes) to yield different plant biomass accumulation among experimental units with different tissue P concentrations. Consequently, the rate of P depletion from solution as a function of solution P concentration and tissue P concentration provide different information than that revealed by the relationship between the rate of P accumulation in leaf tissue and the P supply rate. The former indicates the “instantaneous” P uptake rate as a function of tissue or solution P concentration and the later represents the long term cumulative P accumulation rate as a function of growth rate and leaf tissue P.

Furthermore, the inverse relationship between zoysiagrass P depletion rate and leaf tissue P suggests that there is a feedback mechanism in zoysiagrass that limits P uptake as the P concentration in tissue approaches the critical (1.67 g P kg^{-1}) for maximum growth rate. The rate of P depletion from solution by St. Augustinegrass did

not change over a wide range of leaf tissue P concentration (Figure 3-6). Phosphorus deficient St. Augustinegrass depleted P from solution at the same rate of turf with a P concentration in leaf tissue above the critical (Figure 3-6). The tendency for luxury consumption of St. Augustinegrass in addition to its fairly constant P uptake rate despite P supply level or leaf tissue P concentration, suggest that this turfgrass species has great potential to uptake excess P from the soil solution from where it could be lost to the surrounding environment (i.e., P leaching).

Despite the fact that P uptake dynamics from soil solution may be entirely different from those in hydroponic culture (i.e., P in the nutrient solution is in a soluble form, fully available and in direct contact with the root surface), it is necessary to emphasize the importance of the genetic control on P uptake rate depending upon the P nutritional status of the plant. Greater P uptake efficiency will depend on the relative amount of P that the turf can absorb and how fast it can uptake the P from the soil solution as long as it remains available.

Phosphorus Use Efficiency

Phosphorus use efficiency (PUE) was defined as the mass of leaf dry matter accumulated per unit mass of P allocated to leaf tissue (i.e., kg leaf DM g^{-1} of P). Zoysiagrass and St. Augustinegrass PUE decreased with increasing initial P concentration in the nutrient solution (Figure 3-7). A minimum PUE of 0.5 kg leaf DM g^{-1} P was estimated for zoysiagrass exposed to an initial P concentration in solution of 357 mg P m^{-3}. St. Augustinegrass PUE reached a minimum of 0.54 kg leaf DM g^{-1} P at an initial P concentration in solution of 377 mg P m^{-3} (Figure 3-7).

Phosphorus use efficiency was also inversely related to P concentration in leaf tissue (Figure 3-7). A minimum zoysiagrass PUE of 0.56 kg leaf DM g^{-1} P corresponded

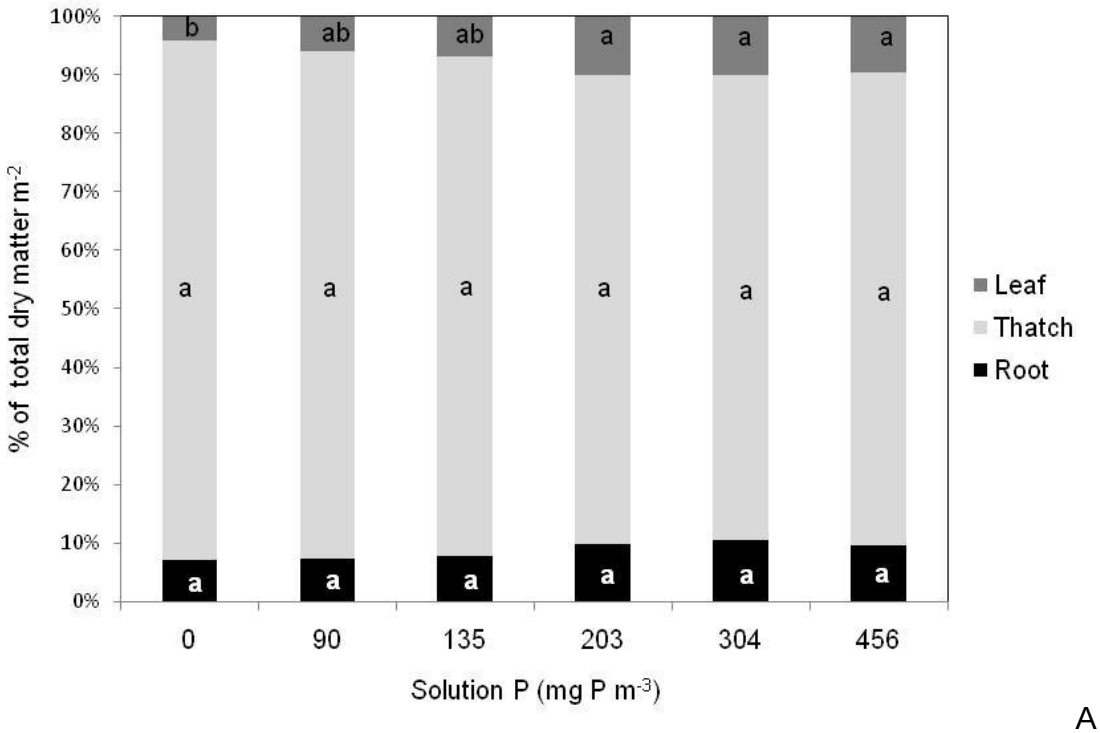
to a leaf tissue P concentration of 1.58 g P kg^{-1}. Minimum St. Augustinegrass PUE (0.60 kg leaf DM g^{-1} P) was attained at a P concentration in leaf tissue of 1.65 g P kg^{-1} (Figure 3-7).

Relative P use efficiency (RPUE) is equivalent to PUE but expressed in a scale between 0 and 1. Since PUE is influenced by the leaf tissue P concentration, comparisons of PUE between plants with different tissue P concentrations would not be adequate. By expressing PUE in a relative scale, the confounding effect of differences in leaf tissue P concentration is removed. Zoysiagrass RPUE decreased by 81% when the P solution concentration was increased from 0 mg P m^{-3} (i.e., control treatment) to 357 mg P m^{-3}. Relative P use efficiency of St. Augustinegrass exposed to a P concentration in solution of 377 mg P m^{-3} was 76% lower than when growing in a solution without P (Figure 3-8).

Minimum rate of P depletion from the nutrient solution by zoysiagrass corresponded to a leaf tissue P concentration of 1.65 g kg^{-1} (Figure 3-6). As previously indicated, maximum leaf growth rate of zoysiagrass was attained at a concentration of P in solution of 382 mg P m^{-3} (Figure 2-5) and leaf tissue P concentration of 1.67 g P kg^{-1} DM (Figure 2-4). Both of these values are very similar to the P concentration in solution (357 mg P m^{-3}) and leaf tissue P concentration (between 1.58 g P kg^{-1} DM and 1.65 g P kg^{-1} DM) at which the PUE and P depletion rate of zoysiagrass reached a minimum. This evidence supports that the change in zoysiagrass leaf growth rate per additional unit of P absorbed approaches a minimum at the leaf tissue P concentration related to a minimum “instantaneous” P uptake rate (i.e., influx P rate) from solution and to a minimum rate of P assimilation into biomass of any additional P absorbed.

In addition, maximum leaf growth rate of St. Augustinegrass was reached at concentration of P in leaf tissue of 1.73 g P kg^{-1} (Figure 2-4) and a nutrient solution concentration of 374 mg P m^{-3} (Figure 2-5). These values are in close agreement with the P concentration in solution (377 mg P m^{-3}) and the leaf tissue P concentration (1.65 g P kg^{-1}) at which the PUE of St. Augustinegrass reached a minimum. Tissue P concentration is equivalent to the ratio of P uptake rate to growth rate. Thus, the critical leaf tissue P concentration would be a function of the plant's ability to withstand a greater tissue P concentration without minimizing its instantaneous capacity to uptake P and transform it into biomass.

Relative P use efficiency of St. Augustinegrass over a wide range of P supply and leaf tissue P concentrations was greater than in zoysiagrass (Figure 3-8). The latter may be related to the greater fraction of total dry matter and P content per unit area that is partitioned to leaf tissue in St. Augustinegrass as the P supply increases. St. Augustinegrass showed great ability to maintain high P uptake rates even at high P concentrations in leaf tissue. Consequently, the factor that appears to limit leaf growth rate of St. Augustinegrass as the leaf tissue P concentration increases is the associated decrease in the PUE.



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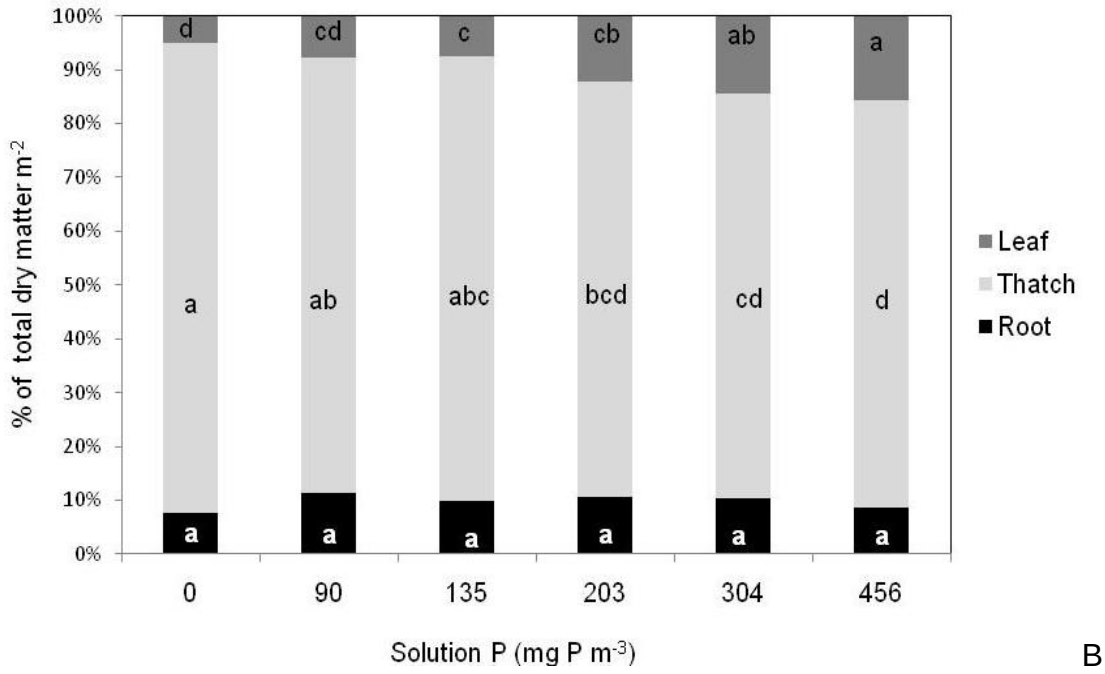


B

Figure 3-1. Partitioning of dry matter into leaves, thatch and roots relative to solution P concentration. A)Empire zoysiagrass and B) Floratam St. Augustinegrass. Columns labeled with the same letter within a tissue type (i.e., leaf, thatch or roots) across treatments are not significantly different at p = 0.05 by contrasts analysis.

Table 3-1. Empire zoysiagrass phosphorus concentration in leaf, thatch and root tissue relative to solution phosphorus concentration.

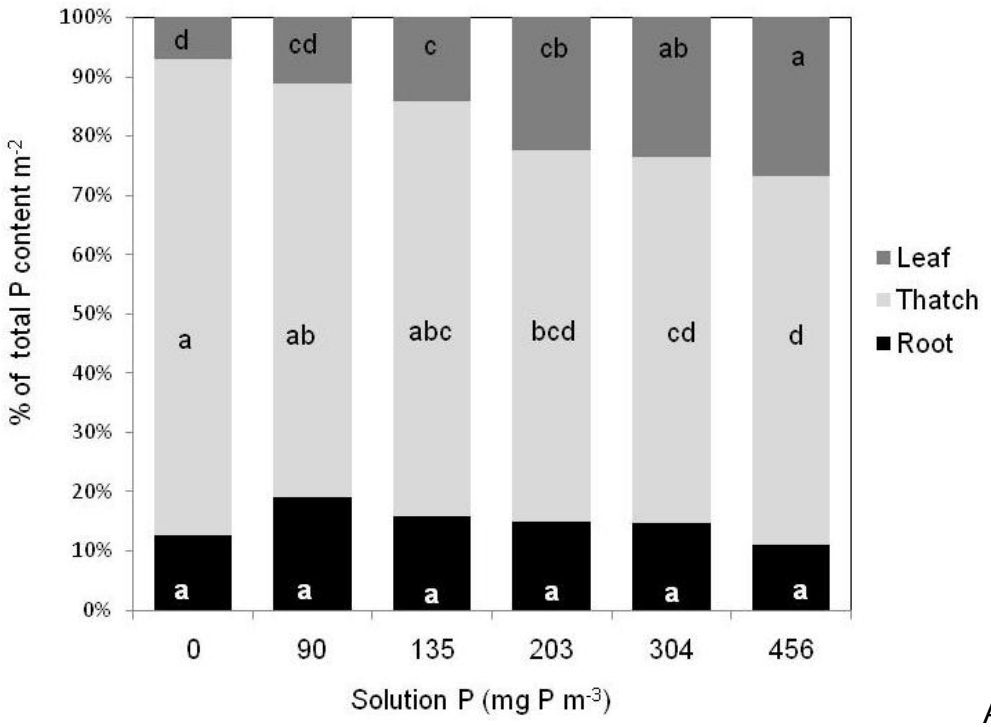
Solution P Concentration mg m^{-3}	Leaf	Thatch	Root	Leaf:Root Ratio	Leaf+Thatch: Root Ratio
		g P kg^{-1}			
0	0.23^{e}	0.13^{c}	0.25^{b}	0.62^{b}	10.86^{a}
90	0.43^{e}	0.25^{bc}	0.64^{a}	0.83^{ab}	10.87^{a}
135	0.68^{d}	0.29^{bc}	0.67^{a}	0.89^{a}	12.22^{a}
203	0.97^{c}	0.37^{ab}	0.66^{a}	1.03^{a}	9.38^{a}
304	1.18^{b}	0.45^{ab}	0.71^{a}	1.00^{a}	9.38^{a}
456	1.85^{a}	0.60^{a}	0.72^{a}	1.03^{a}	10.90^{a}

Values labeled with the same letter within a given column are not significantly different at p=0.05 according to single degree of freedom contrast analysis.

Table 3-2. Floratam St. Augustinegrass phosphorus concentration in leaf, thatch and root tissue relative to solution phosphorus concentration.

Solution P Concentration mg m^{-3}	Leaf	Thatch	Root	Leaf:Root Ratio	Leaf+Thatch: Root Ratio
		g P kg^{-1}			
0	0.50^{d}	0.21^{c}	0.35^{d}	0.57^{d}	7.80^{b}
90	0.86^{c}	0.30^{bc}	0.63^{c}	0.69^{d}	7.93^{ab}
135	1.00^{c}	0.35^{ab}	0.56^{c}	0.77^{d}	9.28^{ab}
203	0.91^{c}	0.34^{ab}	1.02^{b}	1.16^{c}	8.55^{ab}
304	1.32^{b}	0.41^{a}	1.17^{a}	1.44^{b}	9.06^{ab}
456	1.79^{a}	0.44^{a}	0.83^{a}	1.86^{a}	10.76^{a}

Values labeled with the same letter within a given column are not significantly different at p=0.05 according to single degree of freedom contrast analysis.



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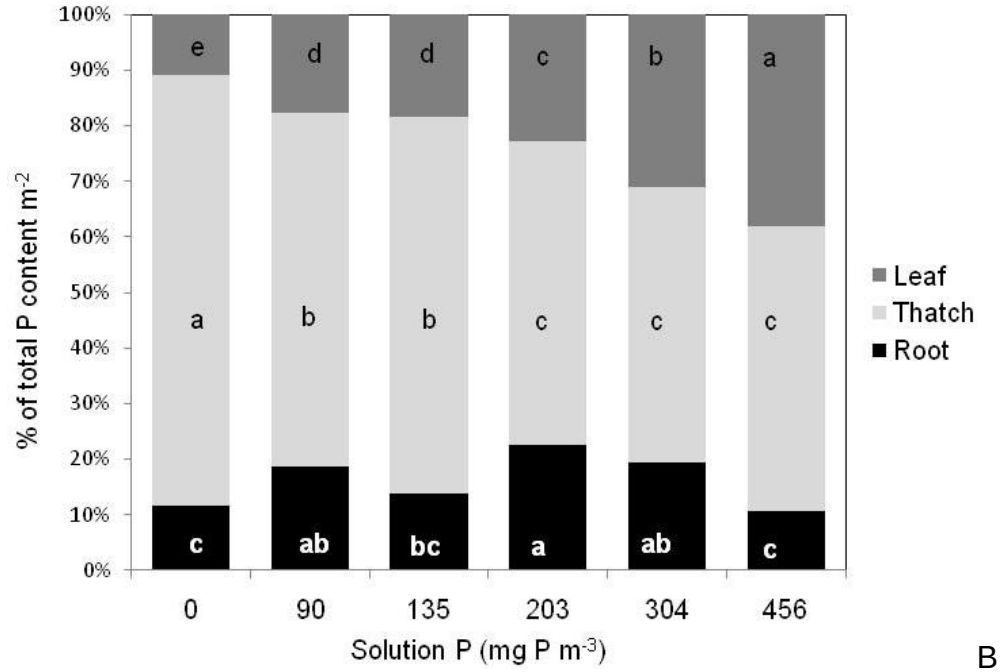
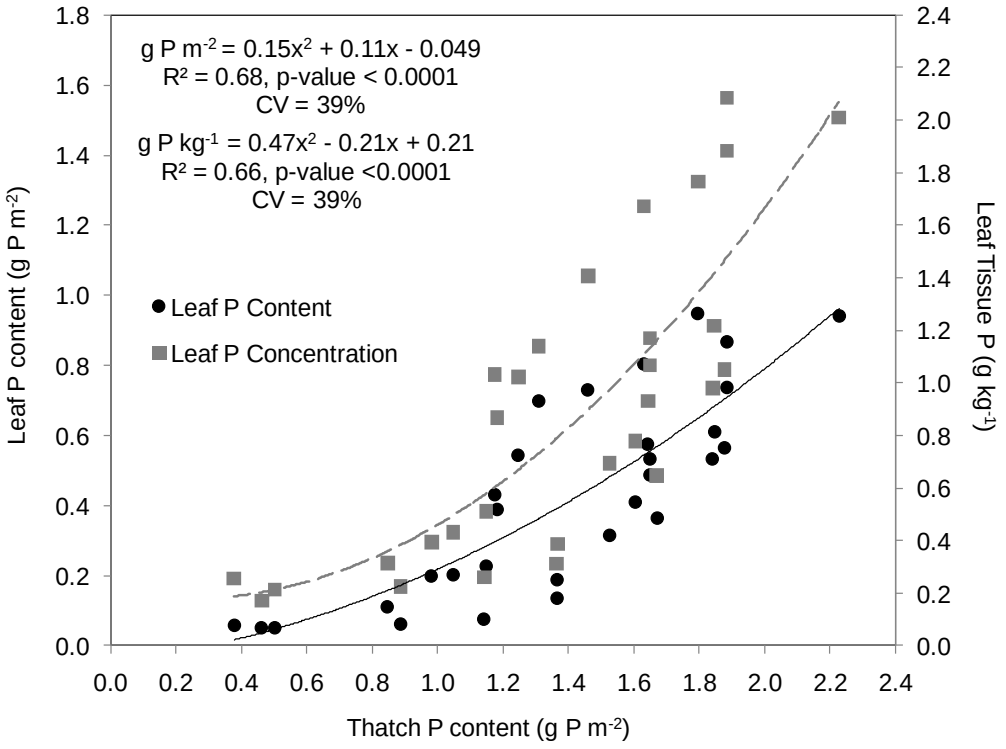


B

Figure 3-2. Distribution of total phosphorus content per unit area into leaves, thatch and roots relative to solution phosphorus concentration. A) Empire zoysiagrass and B) Floratam St. Augustinegrass. Columns labeled with the same letter within a tissue type (i.e., leaf, thatch or roots) across treatments are not significantly different at p = 0.05 by single degree of freedom contrasts analysis.



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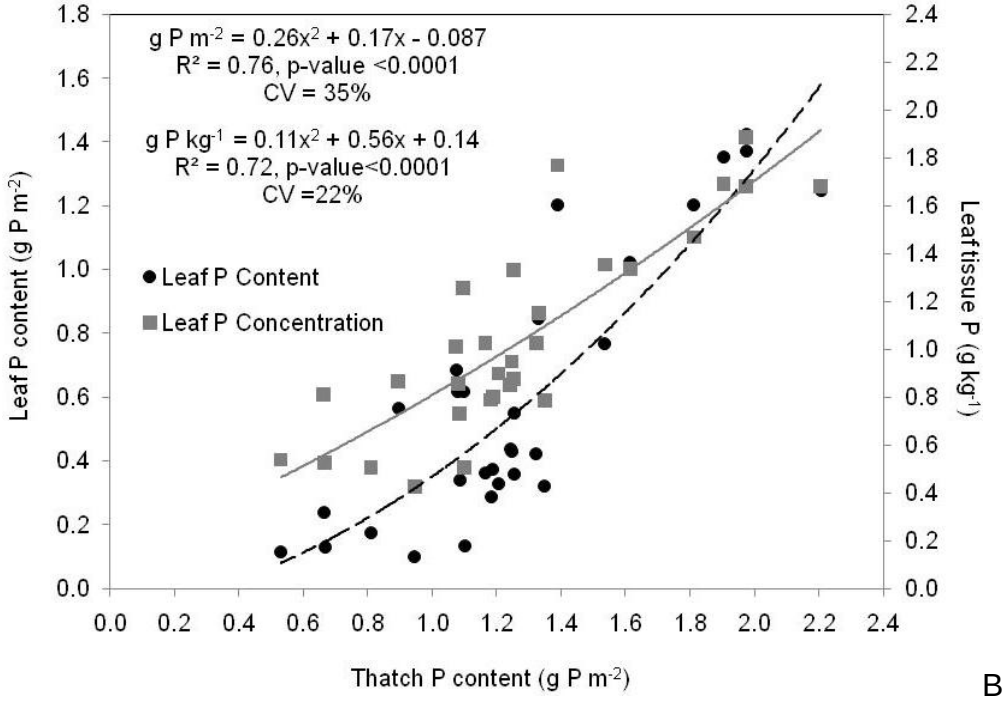


B

Figure 3-3. Relationship between P content and P concentration in leaf tissue and phosphorus storage in the thatch layer. A) Empire zoysiagrass and B) Floratam St. Augustinegrass.

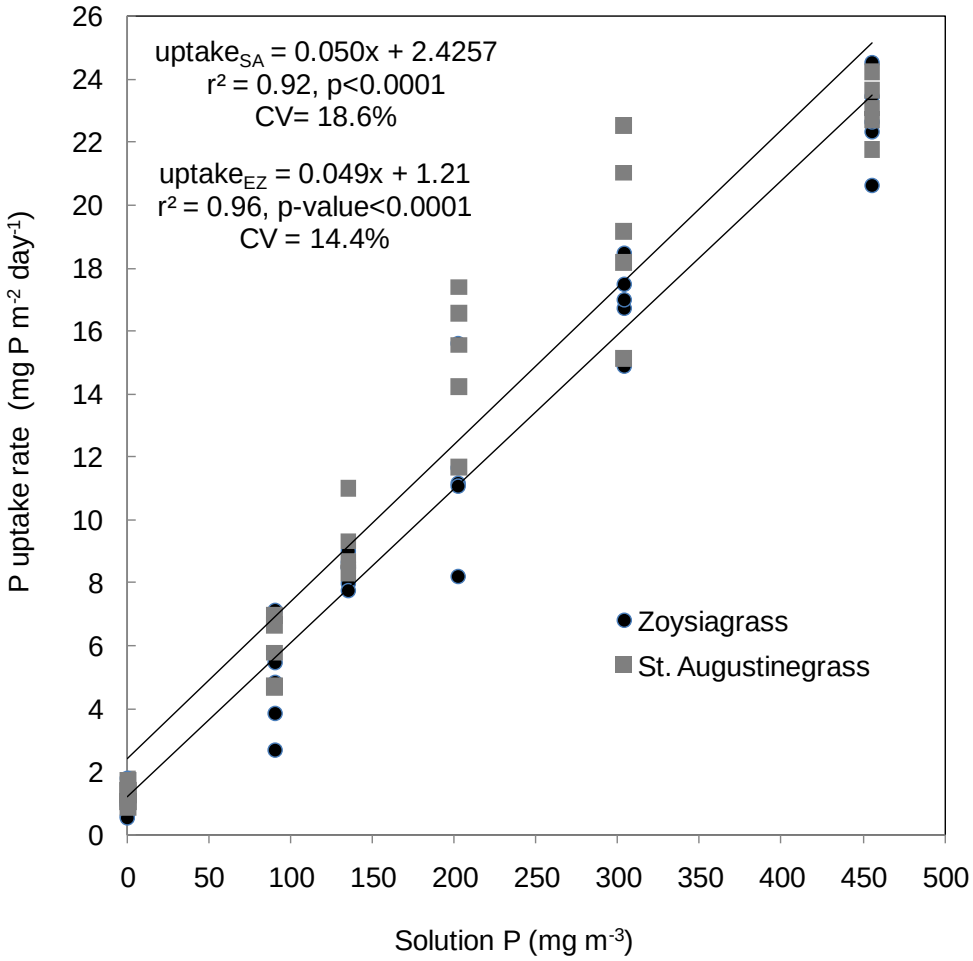


Figure 3-4. Empire zoysiagrass (EZ) and Floratam St. Augustinegrass (SA) phosphorus uptake rate into leaf tissue relative to solution P concentration.

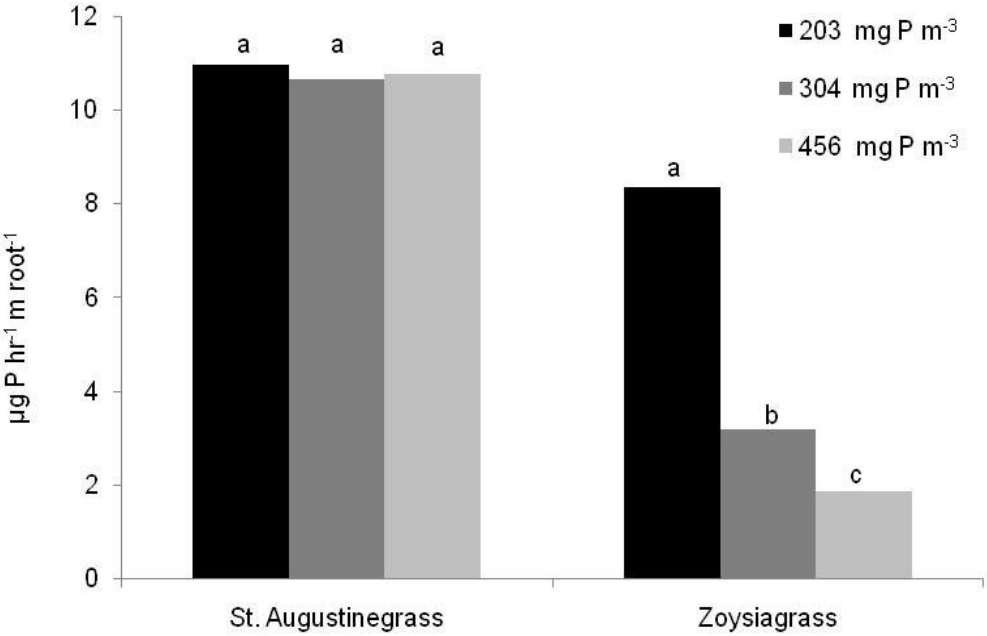


Figure 3-5. Phosphorus depletion rate from the nutrient solution by Empire zoysiagrass and Floratam St. Augustinegrass in relation to phosphorus supply. Columns labeled with the same letter within a given turfgrass species are not significantly different at p=0.05 according to single degree of freedom contrast analysis.

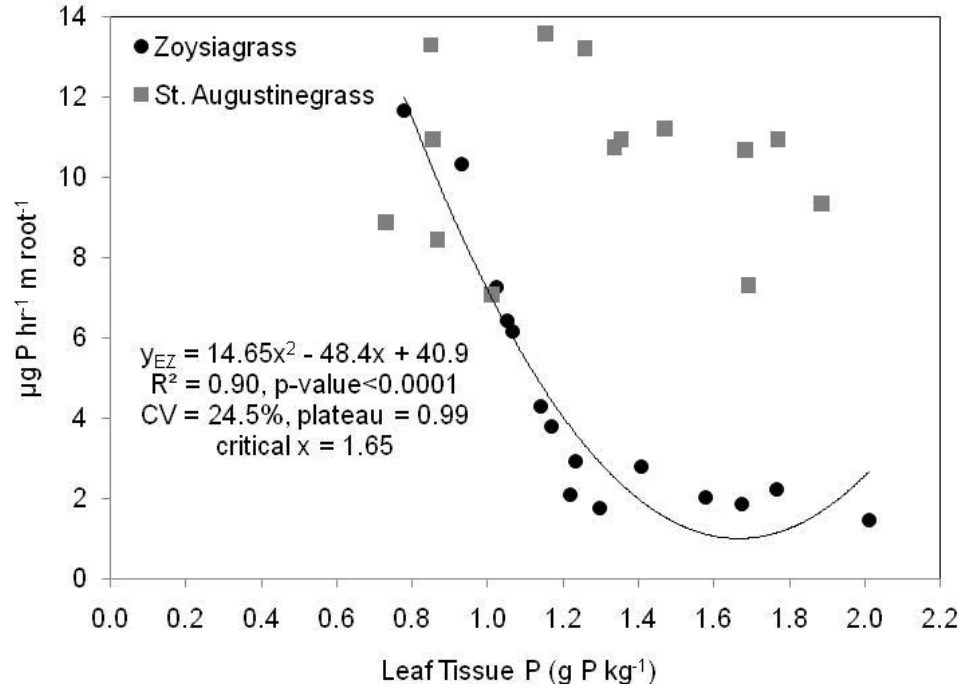
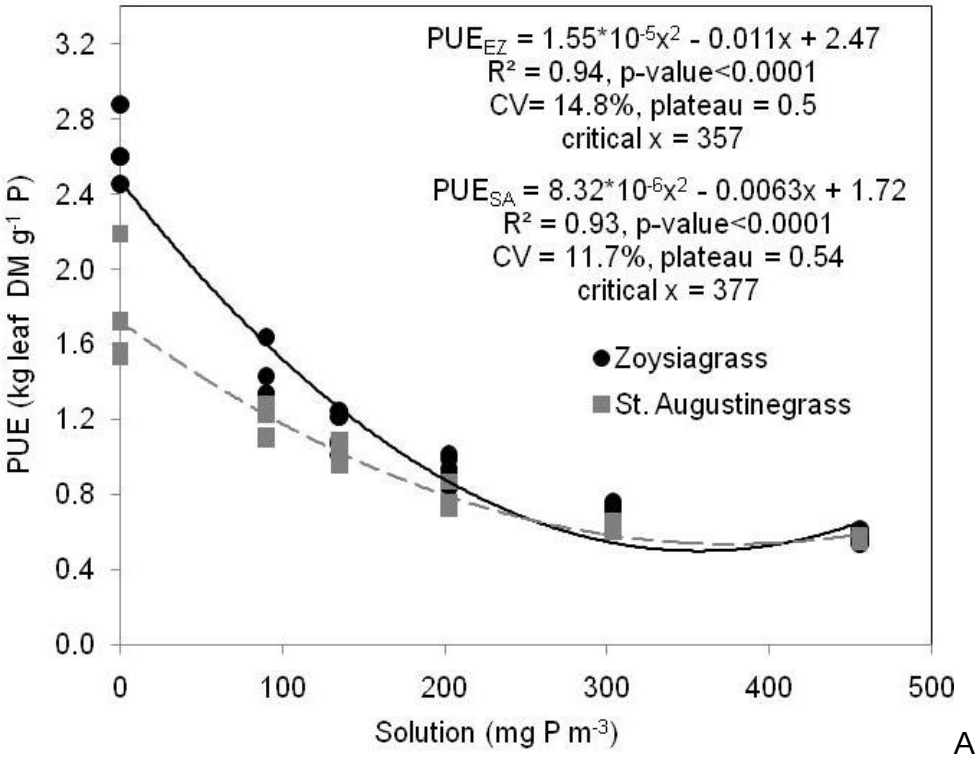


Figure 3-6. Phosphorus depletion rate from the nutrient solution by Empire zoysiagrass (EZ) and Floratam St. Augustinegrass (SA) as related to phosphorus concentration in leaf tissue.



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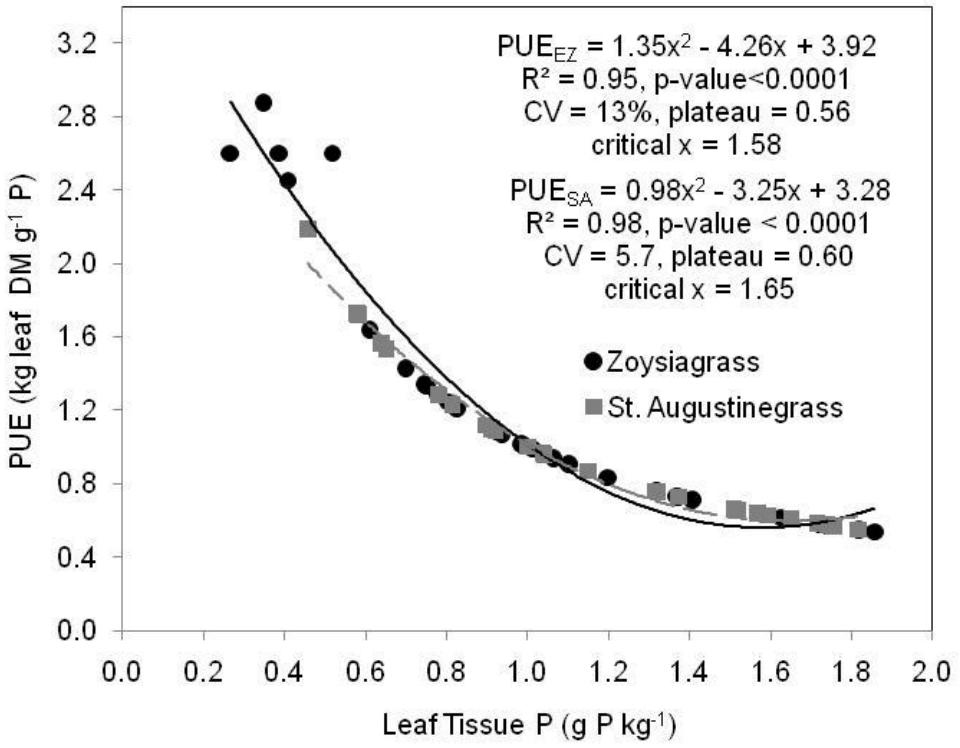
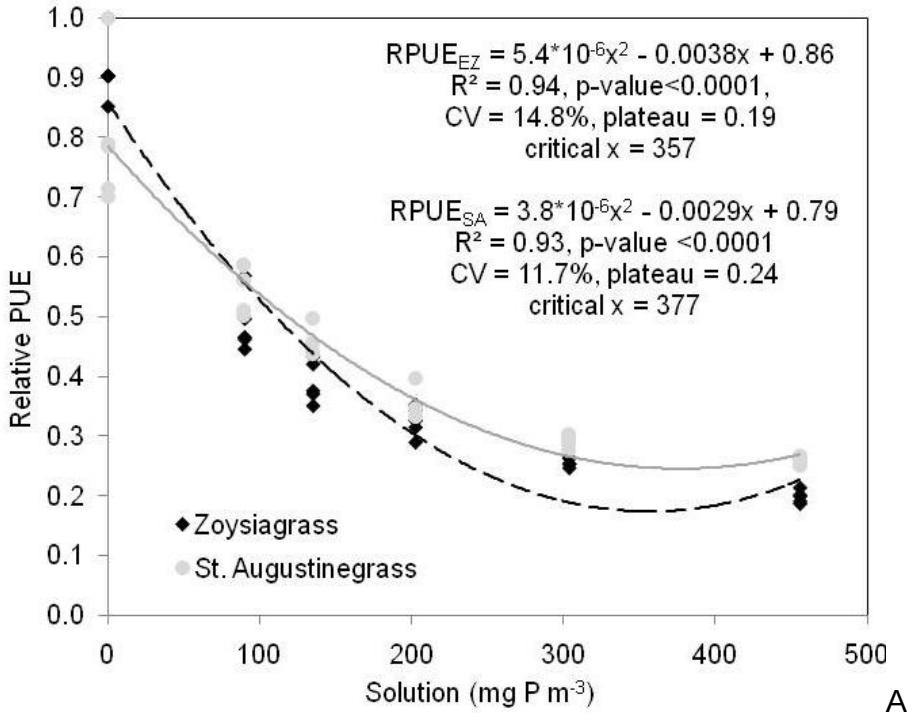


B

Figure 3-7. Phosphorus use efficiency (PUE) of Empire zoysiagrass (EZ) and Floratam St Augustinegrass (SA). A) PUE as influenced by phosphorus concentration in the nutrient solution and B) PUE in relation to leaf tissue P concentration.



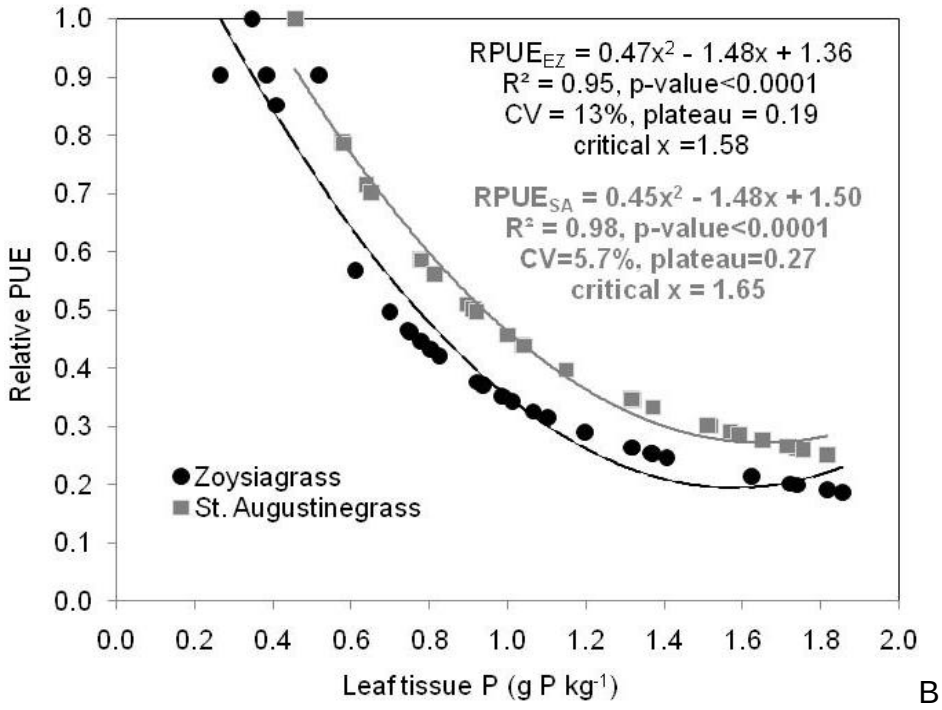


Figure 3-8. Relative P use efficiency (RPUE) of Empire zoysiagrass (EZ) and Floratam St. Augustinegrass (SA). A) RPUE in relation phosphorus concentration in solution and B) RPUE as influenced by leaf tissue P concentration.

CHAPTER 4
ORTHOPHOSPHATE LEACHING IN EMPIRE ZOYSIAGRASS AND FLORATAM ST. AUGUSTINEGRASS GROWN IN A SANDY SOIL UNDER FIELD CONDITIONS

The risk of P leaching is greater in soils dominated by “clean” (uncoated) sands than soils with coated sands (Harris et al., 1996). Sand coatings impart soil P retention capacity because constituents like kaolinite, hydroxyl-interlayered vermiculite, gibbsite and Fe oxyhydroxides have greater affinity for P than uncoated quartz surfaces (Harris et al., 1996). The ability of the soil to retain P has been studied with the application of indices that account for the soil P concentration as well as the concentration of soil components that participate in P retention (Nair et al., 2004; Nair and Harris, 2004; Chrysostome et al., 2007). One of these indices is the soil P saturation ratio (PSR) which is the molar ratio of extractable P to the sum of extractable aluminum (Al) and iron (Fe) (Maguire and Sims, 2002a; Maguire and Sims, 2002b; Sims et al., 2002). Nair et al. (2004) reported that in Florida sands, the concentration of water extractable P (WEP) in the soil solution increases abruptly above a PSR of 0.15; hence, increasing the risk of P losses from the soil to the environment. Most of Florida’s sod production is located in sandy soils (Satterthwaite et al., 2007). Excessive P fertilization to turfgrass grown in sandy soils with low P retention capacities and an abundance of macropores promotes P leaching (Soldat and Petrovic, 2008). Guertal (2007) reported that P leaching from ‘Tifdwarf’ hybrid bermudagrass (*Cynodon spp.*) established on a sand-based putting green increased with greater P application rate. In addition, overwatering of home lawns can increase nutrient leaching (Morton et al., 1988; Snyder et al., 1984). In a study conducted in south Florida, greater P leaching was measured from a mixed-species arrangement of ornamentals, woody shrubs and trees than from ‘Floratam’ St. Augustinegrass [*Stenotaphrum secundatum* (Walt) Kuntze] monoculture (Erickson et

al., 2005). Bowman et al. (2002) compared nitrogen (N) leaching from six warm season turfgrass species, nitrate leaching was lowest from 'Raleigh' St. Augustinegrass [*Stenotaphrum secundatum* (Walt) Kuntze] and highest from 'Meyer' zoysiagrass (*Zoysia japonica*). 'Meyer' zoysiagrass recovered 63% of the N supplied while 'Raleigh' St. Augustinegrass recovered 84%. Nitrate leaching was inversely related to root length density at depths >30 cm (Bowman et al., 2002). Eutrophication of P limited surface aquatic systems has been linked to enrichment of the water column with P (Correll, 1998; Carpenter, 1998; Foy, 2005). The Florida Everglades is an oligotrophic, P limited wetland ecosystem with mean water-column total phosphorus (TP) concentrations in oligotrophic areas of about 10 µg L^{-1} (Noe et al., 2001). Water enrichment with P can modify the structure and function of the Everglades ecosystem (Noe et al., 2001). The main concentration of sod production in Florida (49%) is located in south central Florida (Satterthwaite et al., 2007) and may have an impact on the Everglades National Park.

Several local governments have established fertilizer ordinances aiming to reduce P enrichment of ground water and surface water bodies from urban turfgrass landscapes (Hartman et al., 2008). Among these ordinances is the so called "blackout fertilization period", which consists in banning P fertilizer application to urban turfgrasses between June 1st and September 30th (Hartman et.al, 2008). The rationale behind this ordinance is that rainfall increases during the summer months and it could increase the risk of P losses to water bodies (FDEP, 2010). However, shoot and root growth as well as nutrient uptake of warm season turfgrasses is greater as the solar radiation, temperature and day length increase which coincides with the summer months (Sartain, 2002; Trenholm et al., 1998; Carrow et al., 2001; Christians, 2007). In

addition, the “Labeling Requirements for Urban Turf Fertilizers rule” (Rule 5E-1.003) establishes a maximum P rate in the state of Florida of 0.54 g P m^{-2} $application^{-1}$ or 1.07 g P m^{-2} $year^{-1}$ (State of Florida, 2007). Recently, the U.S. Environmental Protection Agency (USEPA) proposed the Water Quality Standards for the State of Florida’s Lakes and Flowing Waters rule in which maximum allowed total P concentration in surface waters bodies is established (Table A-2). In the state of Florida, *Stenotaphrum secundatum* (Walt) Kuntze (St. Augustinegrass) is the most widely used turfgrass and the cultivar ‘Floratam’ occupies most of the area planted with this species in the state. In addition, *Zoysia japonica* (zoysiagrass) occupies the fourth largest area of sod production in the state of Florida and ‘Empire’ is the cultivar most widely cultivated (Satterthwaite et al., 2007). Research has shown that P leaching from turfgrass systems is influenced by a wide variety of factors and their complex interactions. Limited information on the relationship between P application rate and P leaching from St. Augustinegrass and zoysiagrass under highly favorable conditions for P leaching is available; hence, additional research on this topic is required.

The following hypotheses were tested in this study: (i) there is a maximum phosphorus application rate to St. Augustinegrass and zoysiagrass below which orthophosphate (P_i) leaching is minimized, (ii) the rate of P_i leaching will be inversely related to plant growth and uptake rate and will increase with increasing rainfall and soil phosphorus saturation ratio (PSR), (iii) the concentration of P_i in leachates will not increase if the rate that minimizes leaching is applied and (iv) there will be a species specific effect on P_i leaching rate in response to a giving P application rate.

The objectives of this experiment were (i) to evaluate the relationship between P supply and P_i leaching rate in zoysiagrass and St. Augustinegrass, (ii) to study the interaction between plant uptake, rainfall, irrigation, and soil PSR with P_i leaching rate in these turfgrass systems and (iii) to assess the relationship between P fertilizer rate and P_i concentration in leachates from St. Augustinegrass and zoysiagrass.

Materials and Methods

Experimental Site and Treatments Description

The study was conducted at the G. C. Horn Turfgrass Laboratory of the Plant Science Research and Education Unit of the University of Florida near Citra, FL (29°24' N, 82°10' W). Climatic conditions during 2008 and 2009 growth seasons (May to September) were as follows. Average temperature was 26°C and it oscillated between 13.7°C and 38.6°C. Cumulative precipitation (not including irrigation) during 2008 and 2009 growth seasons was 383 mm and 723 mm, respectively. Cumulative evapotranspiration during 2008 was 462 mm and 540 mm in 2009.

A total of 40 plots (3 m by 4.25 m) were established. The native soil was Candler sand (Hyperthermic, uncoated Lamellic Quartzipsamments) and tested medium Mehlich 1 extractable P (M1-P) (16 - 30 mg P kg^{-1}). A rectangular area (1.5 by 4.25 m) of native soil was excavated in the center of each plot to a depth of 45 cm and then back filled with low P sand. An additional amount of soil was excavated to place high-density polyethylene (HDPE) lysimeters in the center of each plot. The lysimeters were 57 cm in diameter and 88 cm height with a conical base (~168 L). Lysimeters were placed on a galvanized steel base 25.4 cm in height. Washed gravel was used to fill the bottom of the lysimeter which served as a leachate reservoir. Fitted non-woven polyolefin cloth was used to cover the gravel and was secured with a hoop of 13 mm HDPE tubing to

reduce soil intrusion into the leachate collection basin. Low density polyethylene (LDPE) tubing (9.5 mm outer diameter and 6.35 mm inner diameter) was connected to the base of each lysimeter and run underground to aboveground leachate collection towers. Each tube was number coded and attached to the corresponding outlet in a box placed on the collection tower.

Once in place, the lysimeter and the excavated area were filled with low P sand (less than 1% clay size fraction with traces of kaolinite and gibbsite) testing less than 10 mg P kg^{-1} of M1- P (Figure A-2). After back filling the excavated area, the top of the lysimeter was approximately 10 cm below the soil surface (Figure A-1). The experiment was established in a split-plot randomized complete block design with turfgrass species as the main effect and P application rate as secondary effect. The experiment consisted of two turfgrass species: *Stenotaphrum secundatum* (Walt) Kuntze 'Floratam' (St. Augustinegrass) and *Zoysia japonica* 'Empire' (zoysiagrass); P application rates were 0, 0.08, 0.2, 0.5, and 1.25 g P m^{-2} every 4 weeks during the first growing season (2008) and 0, 0.04, 0.1, 0.25, and 0.625 g P m^{-2} every 8 weeks during the second year of evaluation (2009). The source of P utilized was triple super phosphate (45% P_2O_5). Phosphorus fertilizer was broadcasted uniformly over the turf surface and application rates were replicated four times. This study included the maximum P application rates (0.54 g P m^{-2} $application^{-1}$ and 1.07 g P m^{-2} $year^{-1}$) allowed in Florida as stated in the "Labeling Requirements for Urban Turf Fertilizers rule" (State of Florida, 2007). Turfgrasses were established with soil free certified sod from the G.C. Horn Turfgrass Field Laboratory of the University of Florida. Nitrogen (N) and potassium (K) were supplied at a rate of 4.9 g m^{-2} and 4.06 g m^{-2}, respectively in combination with the P

fertilizer (Sartain, 2010; Trenholm and Unruh, 2005). Approximately 50 mm of water were applied weekly through irrigation (5 irrigation cycles per week) during the evaluation period. Irrigation was conducted despite the occurrence of rainfall. In the state of Florida, an irrigation rate of 12.7 mm to 19 mm of water per irrigation cycle, twice or thrice per week is recommended for home lawns during summer days without rainfall. Once rainfall has resumed, then it is recommended to stop the irrigation until the turf shows signs of drought (Trenholm and Unruh, 2005).

Soil Sampling and Analysis

Soil samples were collected prior to treatment application and every two weeks thereafter for the duration of each growing season. Composite samples consisting of two soil cores per plot were collected with a stainless steel 2-cm diameter soil probe from 0 to 7.5 cm, 7.5 to 15 cm and 15 to 30 cm. The holes created while sampling were refilled with uncoated sand immediately after collecting the sample. The top 0.5 cm of each soil core was removed to avoid potential contamination with P from the fertilizer applied on the turf surface. Soil samples were air dried and then passed through a 2-mm sieve. Mehlich I extractable soil P was determined following the extraction procedure described by Sims (2009). Phosphorus concentrations in sample extracts were determined with a Bran Leubbe Technicon Autoanalizer II (Seal Analytical, Mequon, WI, USA) as described in EPA method 365.1 “Determination of Phosphorus by Semi-automated Colorimetry” (O’Dell, 1993). Mehlich 1 extractable soil iron (M1-Fe) and aluminum (M1-Al) concentrations were measured using atomic absorption spectrophotometry (Varian Inc., Santa Clara, CA, USA). Soil carbon and nitrogen contents were measured with a Thermo Electron Flash (EA1112) Nitrogen and Carbon Analyzer (Thermo Electron Corporation, Milan, Italy). Soil pH and electrical conductivity

were determined in 2:1(v/v) water:soil ratio with a PC 700 pH/EC meter (Oakton Instruments, Vernon Hills, IL, USA).

Soil P saturation ratio (PSR) was calculated according to the following equation (Nair et al., 2004):

PSR = M1-P/(M1-Fe+M1-Al)

where M1-P, M1-Fe and M1-Al are expressed in moles. In addition, the soil P storage capacity was calculated as described by Nair and Harris (2004):

SPSC = [(0.15-PSR)*(M1-Fe+M1-AL)]*31= mg P kg^{-1} soil

The relative P adsorption capacity (RPA) was determined according to the procedure described by Harris et al., (1996) and was calculated as the ratio of total P adsorbed and maximum possible P that could be adsorbed from solution.

Tissue Sampling and Analysis

Tissue samples were collected immediately prior to treatment application and biweekly post treatment application for the duration of the growing season. Samples were collected by harvesting the leaf tissue over the low P sand area along the length of the plots. Clippings were collected with a walk-behind mower with a back bag-collector. The width of the mower swath was 54 cm and the turf area harvested per plot was 1.29 m^2. The mowing height was approximately 10.2 cm for St. Augustinegrass and 7.62 cm for zoysiagrass.

At the end of the 2008 growing season, composite root samples consisting of two 383-cm^3 soil cores per plot were taken from the top 15 cm of the soil profile. During 2009, composite root samples were collected prior to imposing P treatments at the beginning of the season and every four weeks thereafter. The root sampling depth

during 2009 was 0-15 cm and 15-30 cm. All root samples were washed free of soil and then scanned with an Epson Perfection V700 Photo dual lens scanner (Epson Corporation, Japan). The digital images obtained were then analyzed with WinRhizo Software Pro v. 2007d (Reagent Instruments Canada Inc., Ottawa, ON, Canada) to determine the total root length, root surface area, root volume, and average root diameter. Thatch tissue was separated from roots and washed free of soil. Leaf, thatch and root samples were oven dried at 70 °C to constant weight and dry matter content was recorded. Tissue samples were ground in a stainless steel Wiley mill to pass a # 40 mesh sieve (425 µm openings size). Ground samples were thoroughly mixed and 0.2 g of dried plant tissue was ashed and digested with 6 *M* HCL according to the standard operation procedure WLB-SP-009 of the Wetland Biogeochemistry Laboratory at the University of Florida, “Total Phosphorus of Soil, Sediment and Plant Tissue by Ignition or Ashing Method”. Phosphorus concentration was determined following USEPA method 365.1 “Determination of Phosphorus by Semi-automated Colorimetry” (O’Dell, 1993) using a Bran Leubbe Technicon Autoanalizer II (Seal Analytical, Mequon, WI, USA).

Leachate Collection, Sampling and Analysis

Leachate samples were collected prior to treatment application (i.e., baseline sampling) and every 7 days thereafter during each growth season. Leachate volume per lysimeter across turfgrass species and growing seasons oscillated between 3.1 and 46.6 liters with an average of 17 liters. Leachates were sampled according to a protocol approved by Florida Department of Environmental Protection (FDEP, 2008). Leachates were collected from the lysimeter by creating a vacuum (~0.85 bars of tension) in the leachate collection line with the aid of a vacuum pump. The entire volume of leachates

was collected in 20-liter HDPE containers and the leachate volume was determined by weight. Leachate samples were collected 1 minute after continuous leachate flow had started. A 30 ml polyethylene syringe was used to collect the leachate sample that was passed through a disposable 0.45 µm pore size filter and dispensed into a 20 ml scintillation vial. Immediately after collection, each sample was placed in a cooler with ice water and kept between 0 and 4° C (no acid added).Upon arrival to the laboratory it was corroborated that the samples were within the adequate temperature range according to FDEP approved protocol. The concentration of P_i in leachate samples was determined within 24 hours post sampling and all analytical results were certified by the QA-QC officer of the Wetland Biogeochemistry Laboratory of the University of Florida. Every leachate sampling event was documented according to FDEP documentation requirements (FDEP, 2008). A chain of custody form accompanied the samples from the field to the laboratory. A Bran Leubbe Technicon Autoanalizer II (Seal Analytical, Mequon, WI, USA) was used to determine P_i concentration in leachates as described in the USEPA method 365.1 “Determination of Phosphorus by Semi-automated Colorimetry” (O’Dell, 1993). In order to account for differences in leachate volume among treatments and experimental units, the volume-weighted P_i concentration was calculated according to the following equation:

$$\text{Volume-weighted } P_i \text{ concentration} = [\Sigma (V_{kj} * (P_i)_{kj})] / \Sigma V_{kj}$$

Where for any given treatment:
V_{kj} = leachate volume from the j^{th} experimental unit at the k^{th} sampling event
$(P_i)_{kj}$ = P_i concentration from the j^{th} experimental unit at the k^{th} sampling event

Statistical Data Analysis

Normal distribution of the data was tested graphically using normal probability plots and numerically with the Shapiro-Wilk W test. Equal variance was also checked using residual plots and the Levene's test for homogeneity of variance. Natural Log transformation of P_i leaching rate and P_i concentration in leachate was required to meet ANOVA assumptions. Due to significant interactions between turfgrass species, years and treatments, the data were analyzed separately for each species within each year. Analysis of variance was conducted with the general linear model procedure (Proc GLM) of SAS system v.9.2 (SAS Institute, 2009) and mean separation was carried out according to single degree of freedom contrast analysis.

Results and Discussion

Influence of Phosphorus Rate on Selected Soil Chemical Properties

In the state of Florida, soil P concentration is routinely determined with the Mehlich 1 extracting solution. A M1-P concentration below 10 mg P kg^{-1} is considered very low (Mylavarapu et al., 2009). The prior P application M1-P value within the top 15 cm of the soil profile was 3.47 mg P kg^{-1} (Table 4-1).

Soil test P by itself may not provide sufficient information to ultimately assess the risk of P losses from the soil to water bodies (Paulter and Sims, 2000; Hooda et al., 2000). The ability of the soil to retain P can be evaluated through indices that account for the P concentration in the soil and also the capacity of the soil to retain additional P (Paulter and Sims, 2000; Hooda et al., 2000). One of these indices is the relative P adsorption capacity (RPA) which relates the total amount of P adsorbed by the soil to the maximum potential P adsorption from solution. The RPA is expressed in a scale from 0 to 1(Harris et al., 1996). Average RPA across turfgrass species prior to P

application was 0.01 which indicated a negligible capability of the soil to retain P. In acid sandy soils with low organic matter content, P retention is controlled by Al and Fe (Sims et al., 1998; Sims et al., 2002).

The PSR, defined as the molar ratio of extractable P to extractable Al+Fe, is similar to the degree of P saturation (DPS) concept (Breeuwsma and Silva, 1992) with the difference that PSR does not include the corrective constant “α” (factor included in the denominator of the DPS formula, P/α(Al+Fe), to account for the fraction of Al and Fe responsible for P sorption for a given soil) associated with the calculation of DPS (Maquire and Sims, 2002; Nair and Harris, 2004; Chrysostome et al., 2007) and it has been shown that PSR and DPS are linearly related (Sims et al., 2002; Khiari et al., 2000).The concentration of water-soluble soil P (WSP) increases slowly with increasing PSR until it reaches a “change point” above which the WSP concentration increases abruptly and the risk of P losses from the soil to the environment increases (Sims et al., 2002; Maguire and Sims, 2002). The risk of P losses to the environment through runoff and subsurface drainage from sandy soils in Florida increases above a PSR of 0.15 (Nair et al., 2004). The PSR determined in this study prior to treatment application was 0.33, which is twice as large as the threshold PSR identified for Florida sands (Nair et al., 2004). The PSR is a useful concept to evaluate the risk of P losses from the soil, but it does not allow estimating how much P could be retained by the soil before it becomes a source of P or how much P could be readily released from a P impacted soil (Nair and Harris, 2004).

The soil P storage capacity (SPSC) concept determines the “available” soil P storage capacity by comparing the soil PSR to the threshold PSR value (Nair and

Harris, 2004). In Florida sands, if the soil PSR is greater than the threshold PSR then the SPSC would be negative (the soil would be a source of P) and if the soil PSR is <0.15 then the SPSC would be positive (the soil would be a sink of P). The SPSC of the sand utilized in this study prior to P treatment application was negative (Table 4-1). Based on the PSR and the SPSC concepts, this soil would act as a source of P instead of a sink. The risk of P leaching in a soil like this receiving P fertilizer would be high. Prior to treatment application and assuming a bulk density of 1.5 Mg m^{-3}, up to 3.71 kg P ha^{-1} could be readily released from the top 15 cm of the soil profile to the soil solution. During the second growing season, on average across sampling dates and treatments, up to 10.87 kg P ha^{-1} could be readily released from the top 15 cm of the soil profile under zoysiagrass and 13.21 kg P ha-1 from soil under St. Augustinegrass (Table 4-3).

Total soil carbon content (TC) was negligible (Table 4-1). Additions of P to water percolating through the soil from mineralization of soil organic P would be minimal. The silt plus clay size particles accounted for less than 2% by mass (Table 4-1). Petrovic (2004) evaluated the influence of soil texture on the fate of N and P. The amount of P leached from Penncross creeping bentgrass (*Agrostis stolonifera ssp. palustris* Hud.) grown in sand was 3.5 fold greater than from turf grown in a silt loam and a sandy loam. Soil pH in the top 15 cm of the soil profile prior to treatment application was slightly acidic (Table 4-1). In this soil the most abundant orthophosphate species would be $H_2PO_4^{-1}$ (Lindsay, 1979).

There was no significant treatment effect on M1-P, PSR and SPSC in either turfgrass species during the first growing season; however, an increasing trend for M1-P in response to P supply rate was observed in both species (Table 4-2). Average M1-P

across sampling dates during the 2009 growth season increased with increasing P rate (Table 4-3). An increase in M1-P was positively related to PSR and inversely related to SPSC (Figure 4-1). Soil M1-P, PSR, and SPSC values in the P fertilized treatments of St. Augustinegrass were not significantly affected during 2009 by P supply up to 0.25 g P m^{-2} every 8 weeks (Table 4-3). The SPSC in zoysiagrass fertilized treatments significantly decreased with respect to the control in response to a P application of 0.1 g P m^{-2} every 8 weeks, while M1-P and PSR increased with a P application of 0.04 g P m^{-2} every 8 weeks (Table 4-3). Soil under zoysiagrass had greater levels of M1-P and PSR and lower SPSC values than soil under St. Augustinegrass (Table 4-2, Table 4-3), which was likely related to greater P uptake rate by St. Augustinegrass (Figure 4-2) than by zoysiagrass. Several authors have reported increased dissolved reactive P concentrations in leachates and runoff with increasing soil test P (Maguire and Sims, 2002b; Heckrath et al., 1995, Pote et al., 1999, Hesketh and Brookes, 2000). Lower SPSC values in soil under zoysiagrass could favor greater P leaching from this turfgrass species under the same growing conditions and P supply levels used in St. Augustinegrass. Overall, continuous P application over time increased M1-P and PSR and reduced SPSC values in both turfgrass species.

Orthophosphate Leaching Rate

Average P_i leaching rate (mg $H_2PO_4^-$ m^{-2} day^{-1}) across P fertilized treatments was 10.85 and 6.3 times greater in zoysiagrass than in St. Augustinegrass during the 2008 and 2009 growing seasons, respectively. Phosphorus concentrations in leaf tissue across P fertilized treatments were greater in St. Augustinegrass than zoysiagrass in both growing seasons (Table 5-1). Leaf growth rates were significantly greater in St. Augustinegrass than in zoysiagrass during both years (Table 5-1). As a result, P uptake

rates in St. Augustinegrass were greater than in zoysiagrass (Figure 4-2). Mehlich 1 extractable soil P was significantly lower in St. Augustinegrass than in Zoysiagrass treatments in both evaluation years (Table 4-2).Greater P uptake rate by St. Augustinegrass may have resulted in lower soil P test, lower P concentration in the soil solution and concomitantly lower leaching. McDowell et al., (2001) reported an increase in 0.01 M $CaCl_2$ extractable P in soil solution with increasing Olsen extractable soil P and Mehlich 3 extractable soil P. Phosphorus is absorbed by plants through active uptake due to the high concentration gradient between the soil solution P and P concentrations plants (Schachtman, 1998; Raghothama, 1999; Vance, 2003). Average leaf P concentration across treatments and evaluation years was 4.43 g P kg^{-1} in St. Augustinegrass and 2.95 g P kg^{-1} in Zoysiagrass (Table 5-1). These leaf tissue P concentrations were 2.6-fold greater that the critical leaf tissue P for maximum growth rate in St. Augustinegrass (1.73 g P kg^{-1}) and 1.8-fold greater than the critical in Zoysiagrass (1.67 g P kg^{-1}). As a result, these turfgrasses would have to spend a substantial amount of energy to absorb P and as depicted in Figure 3-6, the rate of P depletion from solution would be inversely related to leaf tissue P concentration (especially in the case of zoysiagrass). Reduced removal of P from solution could favor greater P_i leaching under these experimental conditions than would be observed from turfgrass with a leaf tissue P concentration slightly below or near the critical level.

The evaluation of root samples collected at the end of the 2008 growing season did not show a clear difference between species in terms of root biomass and root surface area. However, root volume and average root diameter values were greater in St. Augustinegrass than in zoysiagrass (Table 4-4, Table 4-5). During the 2009 growing

season, root samples collected from the top 15 cm of the soil profile revealed that root biomass, RLD, root surface area, root volume and average root diameter values of St. Augustinegrass were significantly greater than in zoysiagrass (Table 4-4). The same trend was observed in root samples collected during the second growing season from 15 to 30 cm depth (Table 4-5).

In addition to differences in size, the root systems of these species seem to have a different architecture. In the case of St. Augustinegrass, during the second growing season, an average of 35%, 40%, 39%, and 20% of the total root biomass, RLD, root surface area, and root volume contained in the top 30 cm of the soil profile, respectively, were allocated in the 15-30 cm depth (Table 4-4, Table 4-5). Similarly, during 2009, on average 19%, 31%, 24% and 18% of the total root biomass, RLD, root surface area, and root volume of zoysiagrass contained in the top 30 cm of the soil profile, respectively, were allocated to the soil layer between 15 and 30 cm (Table 4-4, Table 4-5). A larger and deeper root system could allow St. Augustinegrass to recover greater amounts of P from solution as it is carried downward by percolating water. In the event of high rainfall or excessive irrigation following fertilization, a more extensive and deeper root system would favor greater uptake efficiency (ratio of mass of P absorbed per unit area to mass of P supplied per unit area) and less leaching of applied P fertilizer. The rate of P depletion from solution was greater in St. Augustinegrass than in zoysiagrass (Figure 3-5 and Figure 3-6). Faster P depletion from solution in combination with deeper root system may have a synergistic effect on recovery of P from solution and reducing P leaching.

Orthophosphate leaching from the control treatments of both zoysiagrass (Figures 4-3 and 4-5) and St. Augustinegrass (Figures 4-4 and 4-6) was observed in both years. Phosphorus release from decomposition of the thatch layer in addition to P released by the soil would be potential sources of P_i leaching in unfertilized turf. The rate of P_i leaching from unfertilized St. Augustinegrass was 5.5 and 2 fold lower than in the control treatment of zoysiagrass during 2008 and 2009, respectively. On average across treatments and evaluation years, the amount of thatch dry matter accumulated per m^2 was 435 g greater in zoysiagrass than in St. Augustinegrass (Table 5-7, Table 5-8). Greater release of inorganic P from mineralization of organic P stored in the thatch layer of zoysiagrass may help explain the greater P_i leaching measured from the control treatments of this species.

During the 2008 growing season, an application of 0.8 g P m^{-2} $year^{-1}$ (i.e., 0.2 g P m^{-2} every 4 weeks) to zoysiagrass did not increase the rate of P_i leaching with respect to the control (Figure 4-3). Until the second P fertilization period (56 days after initiation), an application of 1.25 g P m^{-2} every 4 weeks to zoysiagrass did not increase P_i leaching in comparison to the control (Figure 4-3). The rate of P_i leaching from St. Augustinegrass during 2008 was not different in the fertilized treatments than in the control (Figure 4-4).

Average P_i leaching rate across treatments in zoysiagrass decreased by 31% from 2008 (Figure 4-3) to 2009 (Figure 4-5). Phosphorus application rate was reduced 4-fold from 2008 to 2009 and may explain the reduction in P_i leaching rate from zoysiagrass. During 2009, a P application rate of 0.1 g P m^{-2} and 0.25 g P m^{-2} every 8 weeks did not increase P_i leaching with respect to the control in zoysiagrass (Figure 4-5) and St.

Augustinegrass (Figure 4-6), respectively. The substantial reduction in the maximum P application rate that could be supplied to zoysiagrass and St. Augustinegrass without increasing leaching may be related to the residual effect of continuous fertilizer applications over time. As previously indicated, M1-P significantly increased over time (Table 4-2, Table 4-3) and the SPSC decreased concomitantly (Figure 4-1). On average across treatments M1-P and PSR increased 2.7 and 2.3-fold, respectively, from 2008 to 2009 in St. Augustinegrass (Table 4-2, Table 4-3). Similarly, M1-P and PSR values increased 2.3 and 2.2-fold, respectively, between 2008 and 2009 in zoysiagrass (Table 4-2, Table 4-3). The SPSC decreased 5.4-fold in St. Augustinegrass and 3.1-fold in zoysiagrass between 2008 and 2009 (Table 4-2, Table 4-3). During the 2009 growing season, overall health and condition of the turf was affected by disease and water stress. The latter may explain the lower leaf growth rate observed during 2009 (Table 5-5, Table 5-6). Since P uptake rate is the product of growth rate and P concentration in tissue, lower plant P uptake would be related to lower turf growth rate. Therefore, P application to turfgrass that is growing under suboptimal conditions may reduce the P uptake efficiency and increase the risk of P losses from the system.

The rate of P depletion from solution by zoysiagrass was inversely related to P concentration in leaf tissue (Figure 3-6). As the P concentration in tissue increased the PUE (mass of leaf biomass produced per unit of P allocated to leaf tissue) decreased (Figure 3-7). Average P concentration across treatments in leaf tissue of zoysiagrass and St. Augustinegrass during the 2009 growing season was 3.2 g P kg^{-1} and 4.4 g P kg^{-1}, respectively. These leaf tissue P concentrations are significantly greater than the critical P concentrations in leaf tissue for maximum growth rate identified for

zoysiagrass (1.67 g P kg^{-1}) and St. Augustinegrass (1.73 g P kg^{-1} DM). St. Augustinegrass has a tendency for luxury consumption (it continued to absorb P from solution at a fast rate even above the critical leaf tissue P for maximum growth), which in combination with high P supply, may have favored the great tissue P accumulation observed in this experiment. Under these experimental conditions the rate of P depletion from the soil solution by zoysiagrass would be limited in comparison to St. Augustinegrass resulting in lower recovery of the P carried downward by percolating water before it is removed from the section of the profile with greater root density.

Lower PUE would result in lower growth rate and indirectly in reduced P uptake. Zoysiagrass and St. Augustinegrass P uptake rates decreased significantly from 2008 to 2009 in both species (Figure 4-2). Lower plant P accumulation rates over time could have favored greater P concentration in the soil solution from where it is prompt to leaching.

As depicted in Figure 4-2, plant P uptake rate changed over the growth season. During the 2009 growing season, P fertilizer was applied at the beginning and at mid season. St. Augustinegrass uptake rate was significantly lower during the first half of the 2009 growing season. The later could have substantially reduced St. Augustinegrass P uptake efficiency. As a result, a greater fraction of the P supplied in the first fertilization cycle could have moved below the section in the soil profile with greatest root density. This scenario would favor greater P_i leaching over time. Zoysiagrass P uptake rate decreased in the second half of the 2009 growing season, but the change in uptake rate between fertilizer application periods was not as abrupt as that observed in St. Augustinegrass (Figure 4-2). Root biomass accumulation changed over the course of

the 2009 growing season (Table 4-3). Root biomass, root length density (RLD), root surface area, and root volume from the top 15 cm of the soil profile were less at the beginning and at the end of the growing season than near mid season (Table 4-4).Phosphorus application should be synchronized with the period of greater plant growth and plant P uptake rate. Adequate P fertilization timing could reduce leaching by increasing P uptake and accumulation in the turfgrass.

In addition, the rate of P_i leaching rate is calculated as the product of volume of leachate per unit area and time (i.e. liters m^{-2} day^{-1}) and the concentration of P_i in the leachate (i.e., mg P $liter^{-1}$). Changes in the rate of P_i leaching over time were associated to the fluctuations in the total amount of water (i.e., sum of rainfall plus irrigation) that the turf received (Figure 4-7). Fluctuations in the total amount of water received by the turf could explain a total of 73% of the variability on leaching rate from zoysiagrass and 50% of the variability on leaching rate from St. Augustinegrass, respectively (Figure 4-7). The sum of rainfall and irrigation in 2009 was about 45% greater than in 2008. Leachate volume across years, species and treatments increased linearly (r^2=0.63, $p<0.0001$) with increasing rainfall plus irrigation (Figure 4-8). The substantially greater amount of water that fell on the turf during 2009 may have promoted greater water percolation favoring an increase in P leaching. The ratio of water that enters the system and the amount of water that is lost through ET could influence the leachate volume. During 2008, ET was 21% greater than the rainfall, whereas during the 2009 growing season, ET was 25% less than the rainfall.

In the state of Florida, P fertilization of urban turfgrasses is limited to a maximum of 0.54 g P m^{-2} per application and 1.07 g P m^{-2} per year (State of Florida, 2007). In the

first growing season, a maximum P rate of 0.54 g P m^{-2} per application and 1.07 g P m^{-2} per year did not increase P_i leaching in areas planted with St. Augustinegrass (Figure 3-4). However, a P application rate to zoysiagrass greater than 0.2 g P m^{-2} per application or 0.8 g P m^{-2} per year increased P_i leaching rate over the control treatment (Figure 4-3). During the second growing season a P application rate of 0.1 g P m^{-2} per application (0.2 g P m^{-2} $year^{-1}$) for zoysiagrass (Figure 4-5) and 0.25 g P m^{-2} per application (0.5 g P m^{-2} $year^{-1}$) for St. Augustinegrass (Figure 4-6) did not increase P_i leaching rate.

The determination of a threshold P application rate to minimize P leaching from zoysiagrass and St. Augustinegrass should incorporate the assessment of an array of variables namely, SPSC, plant nutritional status, overall condition and health of the turf, application timing, precipitation distribution and intensity, rate of irrigation among others. The combination of soil, plant, weather, and management conditions (i.e., rate of irrigation) present in this study were highly conducive to P leaching. Therefore, the results from this experiment must be analyzed, interpreted and applied considering the conditions of the turfgrass system where P fertilization is intended.

In a P deficient turfgrass stand (i.e., P concentration in leaf tissue is below the critical level for maximum growth) grown in a soil with positive SPSC and that is not over irrigated, the risk of increased P leaching as a result of P fertilization would be much lower than that in a turfgrass system like the one utilized in this study (excessive P concentration in tissue and negative SPSC). Phosphorus fertilization should not be conducted if the leaf tissue P concentration is above the critical level or if the SPSC is negative. It is evident from the results of this study, that the soil P test by itself provides insufficient evidence to assess the risk of P loss through leaching in turfgrass systems.

On average the M1-P level did not surpass 10 mg P kg^{-1} soil, which is considered to be a very low M1-P level in Florida soils (Mylavarapu et al., 2009); however, P_i leaching from some of the fertilized treatments was significantly greater than from the control treatment. Therefore, the use of concepts that incorporate the ability of the soil to retain P, such as the PSR and SPSC, would aid substantially to assess the risk of P leaching from turfgrasses.

The P status of turfgrass grown in a soil that acts as a P source instead of a P sink should be adequate; hence, no P fertilization would be required. An example of this condition is the zoysiagrass and St. Augustinegrass grown in the no P added control treatment. The P concentration in leaf tissue of turf grown in these treatments remained above 2.5 g P kg^{-1} in zoysiagrass and 4 g P kg^{-1} in St. Augustinegrass for the entire evaluation period (i.e., more than three years since sod establishment). In addition, if P fertilization is determined to be required per tissue analysis, the application should be conducted during the period of greater growth rate. Fertilizer application should be avoided when there is high risk of heavy rainfall.

Based on the results of this research, if P fertilization is required based on tissue analysis and the SPSC is positive, it would be environmentally safe to supply a maximum P rate of 0.54 g P m^{-2} per application and 1.07 g P m^{-2} per year to St. Augustinegrass. Under the same conditions maximum P application rate in the case of Zoysiagrass should not exceed 0.2 g P m^{-2} per application and 0.8 g P m^{-2} per year. Therefore, the maximum permitted P application rate as currently stated in the “Labeling Requirements for Urban Turf Fertilizers Rule” should be modified to account for turfgrass species influence on the risk of P_i leaching and soil P status.

Volume-Weighted Orthophosphate Concentration in Leachate

During the 2008 growing season, an application of 0.2 g P m^{-2} every 4 weeks (i.e., 0.8 g P m^{-2} $year^{-1}$) to zoysiagrass did not increase P_i concentrations in leachates from fertilized treatments relative to the control (Figure 4-9). Volume-weighted P_i concentrations in leachates from St. Augustinegrass were not influenced by P supply rate during 2008 (Figure 4-10). The concentrations of P_i in leachates during 2008 from zoysiagrass fertilized with a rate ≥ 0.5 g P m^{-2} every 4 weeks increased over time (Figure 4-9). Residual effects of continuous P fertilization over time increased PSR values and decreased SPSC values (Table 4-2, Table 4-3), which likely increased P_i concentrations in the soil solution and leachates. Average P_i concentration in leachate during 2008 from zoysiagrass supplied with 0.2 g P m^{-2} every 4 weeks was 39 µg P L^{-1} (Figure 4-9), whereas average P_i concentration in leachate from St. Augustinegrass recorded during 2008 was less than 10 µg P L^{-1} (Figure 4-10).

During the 2009 growing season, a maximum of 0.1 g P m^{-2} every 8 weeks (0.2 g P m^{-2} $year^{-1}$) in zoysiagrass (Figure 4-11) and 0.25 g P m^{-2} every 8 weeks (0.5 g P m^{-2} $year^{-1}$) in St. Augustinegrass (Figure 4-12) did not increase P_i concentrations in leachates relative to the control. The average P_i concentration in leachate during 2009 from zoysiagrass supplied with 0.1 g P m^{-2} every 8 weeks was 26 µg P L^{-1} (Figure 4-11) and from St. Augustinegrass fertilized with 0.25 g P m^{-2} every 8 weeks was 15 µg P L^{-1} (Figure 4-12). Average P_i concentration in P fertilized treatments was 13.7-fold greater in zoysiagrass than in St. Augustinegrass during the first year of evaluation and 7-fold greater during the second evaluation year. The latter may be explained by the greater P

accumulation rate over time, greater rate of P depletion from solution and more extensive and deeper root system of St. Augustinegrass.

Total P concentration in leachate was not measured in this experiment. Main P sources that would influence the total P concentration of leachates in this system would be mineralization of organic P from thatch layer, release of P from soil solid phase and dissolution of inorganic P fertilizer. Phosphorus from all these sources would be inorganic soluble P. Particulate organic P from the thatch layer may be carried by percolating water, but it would likely represent a small fraction of the total P content in leachates. Hence, it may be reasonable to hypothesize that the P_i concentration measured in the leachates collected would be representative of the total P concentration. Elliot et al. (2002) applied concentrated super phosphate (56 kg P ha^{-1} and 224 kg P ha^{-1}) to bahiagrass grown in soil columns consisting of 15 cm of A horizon from either a Candler sand (M1-P of 5.7 mg P $kg^{-}1$) or an Immokalee sand (M1-P of 1.7 mg P $kg^{-}1$) overlying 28 cm of an E horizon from Myakka series (sandy, siliceous, hyperthermic Aeric Alaquods). Inorganic soluble reactive P measured in leachates from either soil combination accounted for over 95% of the total P leached. Pierzynsky et al. (2005), reported that total P concentrations in the order of 0.035 – 0.10 mg P L^{-1} and dissolved P concentrations of 0.01 – 0.03 mg P L^{-1} were associated to freshwater bodies eutrophication.

During 2008, an application as high as 5 g P m^{-2} $year^{-1}$ to St. Augustinegrass did not increase the P_i concentration in leachate above 10 µg P L^{-1} (Figure 4-10) which would comply with the numeric water quality criteria proposed by USEPA for P for Florida lakes and flowing waters (Table A-2). During the second year of evaluation, an

application rate to St. Augustinegrass of 0.5 g P m^{-2} $year^{-1}$ resulted in an average P_i concentration in leachate of 15 µg P L^{-1} (Figure 4-12). The later would be within the permissible concentration range (10 to 30 µg P L^{-1}) for clear acidic lakes (Table A-2). In the case of zoysiagrass, an application of 0.8 g P m^{-2} $year^{-1}$ during the 2008 growing season resulted in an P_i concentration of 39 µg P L^{-1} (Figure 4-11), which would be adequate for clear alkaline lakes (30 to 87 µg P L^{-1}) and slightly high for clear acidic lakes (Table A-2).

Greater P_i concentrations in leachate seemed to be associated to lower SPSC values and greater M1-P and PSR values (Table 4-6). In the case of St. Augustinegrass P_i concentrations between 10 µg P L^{-1} and 30 µg P L^{-1} were measured in soils with a PSR between 0.6 and 0.8 (Table 4-6). Leachate P_i concentrations remained between 10 µg P L^{-1} and 30 µg P L^{-1} in soils under zoysiagrass with a PSR ranging from 0.6 to 0.86. Leachate P_i concentrations as high as 359 µg P L^{-1} were observed in soils under zoysiagrass with M1-P levels lower than 10 mg P kg^{-1} (Table 4-4). It has been reported that in Florida sands the concentration of water soluble P increases rapidly above a PSR of 0.15 and thus this PSR has been proposed as a threshold to minimize risk of P losses from the soil (Nair et al., 2004; Nair and Harris, 2004). In this experiment a PSR as high as 0.6 was not related to a P_i concentration in leachates greater than 30 µg P L^{-1}. These results indicate that these turfgrass species have a great ability to uptake P from solution and maintain it cycling in the system with minimal negative impact to the environment. The threshold P application rates determined based on P_i leaching rate and volume-weighted P_i concentration in leachate as the response variables were in agreement for both turfgrass species and evaluation years.

Orthophosphate Leaching from Fertilizer Application

The maximum percent cumulative P leached from fertilizer application to St. Augustinegrass was estimated to be ≤ 0.22% (Table 4-7). Maximum amount of fertilizer P that leached from St. Augustinegrass between May 2008 and June 2010 was 0.14 kg P ha^{-1} and corresponded to the highest P application rate treatment (1.25 g P m^{-2} every 4 weeks during 2008 and 0.625 g P m^{-2} every 8 weeks during 2009) (Table 4-7). In the case of zoysiagrass, the maximum leaching of P from fertilizer application was estimated to be 4.71%. The latter corresponded to a total P leaching from fertilizer between May 2008 and June 2010 of 2.95 kg P ha^{-1} out of 62.5 kg P ha^{-1} applied during the same period (Table 4-7). No fertilizer P was applied during 2010; however, the P_i leached up until June 2010 is taken into account in this analysis. The amount of P leached from fertilized St. Augustinegrass treatments over that leached in the control treatment was negligible (Table 4-7).

A cumulative P application to zoysiagrass of 2.5 g P m^{-2} (0.5 g P m^{-2} every 4 weeks during 2008 and 0.25 g P m^{-2} every 8 weeks during 2009) between May 2008 and June 2010 resulted in an amount of P leached over that measured in the control treatment of 0.85 kg P ha^{-1} (Table 4-8). This cumulative application would represent an average P application per year of about 1.25 g P m^{-2} (a cumulative application of 2.5 g P m^{-2} over two years),which is greater than the maximum P application rate per year (1.07 g P m^{-2} $year^{-1}$) currently permitted in the state of Florida.

As previously indicated based on the P_i leaching rate results, if P fertilization is required per tissue analysis and the SPSC is positive, a maximum application of 0.8 g P $year^{-1}$ (0.2 g P m^{-2} $application^{-1}$) to zoysiagrass and 1.07 g P m^{-2} $year^{-1}$ (0.54 g P m^{-2} $application^{-1}$) to St. Augustinegrass would be environmentally safe. According to the

results presented in Table 4-7 and Table 4-8, these recommended P application rates would result in negligible leaching of P from fertilizer in the case of St. Augustinegrass (on average 10 g P ha^{-1} $year^{-1}$ leached over the control) and a reduction in P leaching from applied fertilizer in the case of zoysiagrass (on average the control treatment leached 10 g P ha^{-1} more than fertilized treatment).

Table 4-1. Baseline soil chemical characterization (n=20).

Variable	Units	Value
Mehlich 1 extractable P	mg kg^{-1}	3.47
Mehlich 1 extractable Al	mg kg^{-1}	11.0
Mehlich 1 extractable Fe	mg kg^{-1}	1.46
Soil P storage capacity	mg kg^{-1}	-1.65
Total carbon	g kg^{-1}	0.40
Total nitrogen	g kg^{-1}	0.10
pH		6.0
Soil electrical conductivity	µS cm^{-1}	28.3
Relative P adsorption capacity		0.01
Phosphorus saturation ratio		0.33
Silt+Clay	%	<2

Table 4-2. Effect of phosphorus application rate on Mehlich 1 extractable soil phosphorus (M1-P), soil phosphorus storage capacity (SPSC) and phosphorus saturation ratio (PSR) in the top 15 cm of the soil profile during the first growth season (2008).

Fertilizer Application Rate	Floratam St. Augustinegrass			Empire zoysiagrass		
	M1-P	SPSC	PSR	M1-P	SPSC	PSR
	mg P kg^{-1}			mg P kg^{-1}		
0 g P m^{-2}	2.27	-0.88	0.38	2.52	-1.29	0.38
0.08 g P m^{-2}	1.61	-0.20	0.27	2.92	-1.70	0.46
0.2 g P m^{-2}	1.90	-0.51	0.28	3.15	-1.93	0.51
0.5 g P m^{-2}	2.70	-1.32	0.44	3.55	-2.34	0.57
1.25 g P m^{-2}	2.91	-1.53	0.42	3.40	-2.18	0.56
p-value	0.1784	0.1789	0.0526	0.4037	0.4050	0.2393

Table 4-3. Effect of phosphorus application rate on Mehlich 1 extractable soil phosphorus (M1-P), soil phosphorus storage capacity (SPSC) and phosphorus saturation ratio (PSR) in the top 15 cm of the soil profile during the second growth season (2009).

Fertilizer Application Rate	Floratam St. Augustinegrass			Empire zoysiagrass		
	M1-P	SPSC	PSR	M1-P	SPSC	PSR
	mg P kg^{-1}			mg P kg^{-1}		
0 g P m^{-2}	4.56^{b}	-3.23^{a}	0.75^{b}	3.79^{c}	-2.58^{a}	0.62^{c}
0.04 g P m^{-2}	5.00^{b}	-3.67^{b}	0.76^{b}	5.76^{b}	-4.56^{ab}	0.92^{b}
0.1 g P m^{-2}	5.48^{b}	-4.17^{b}	0.76^{b}	6.54^{b}	-5.35^{b}	1.05^{b}
0.25 g P m^{-2}	5.84^{b}	-4.54^{b}	0.92^{b}	9.66^{a}	-8.50^{c}	1.31^{a}
0.625 g P m^{-2}	9.75^{a}	-8.54^{b}	1.07^{a}	9.53^{a}	-8.37^{c}	1.50^{a}
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Values within a given column with the same letter are not statistically different at p=0.05 according to single degree of freedom contrast analysis.

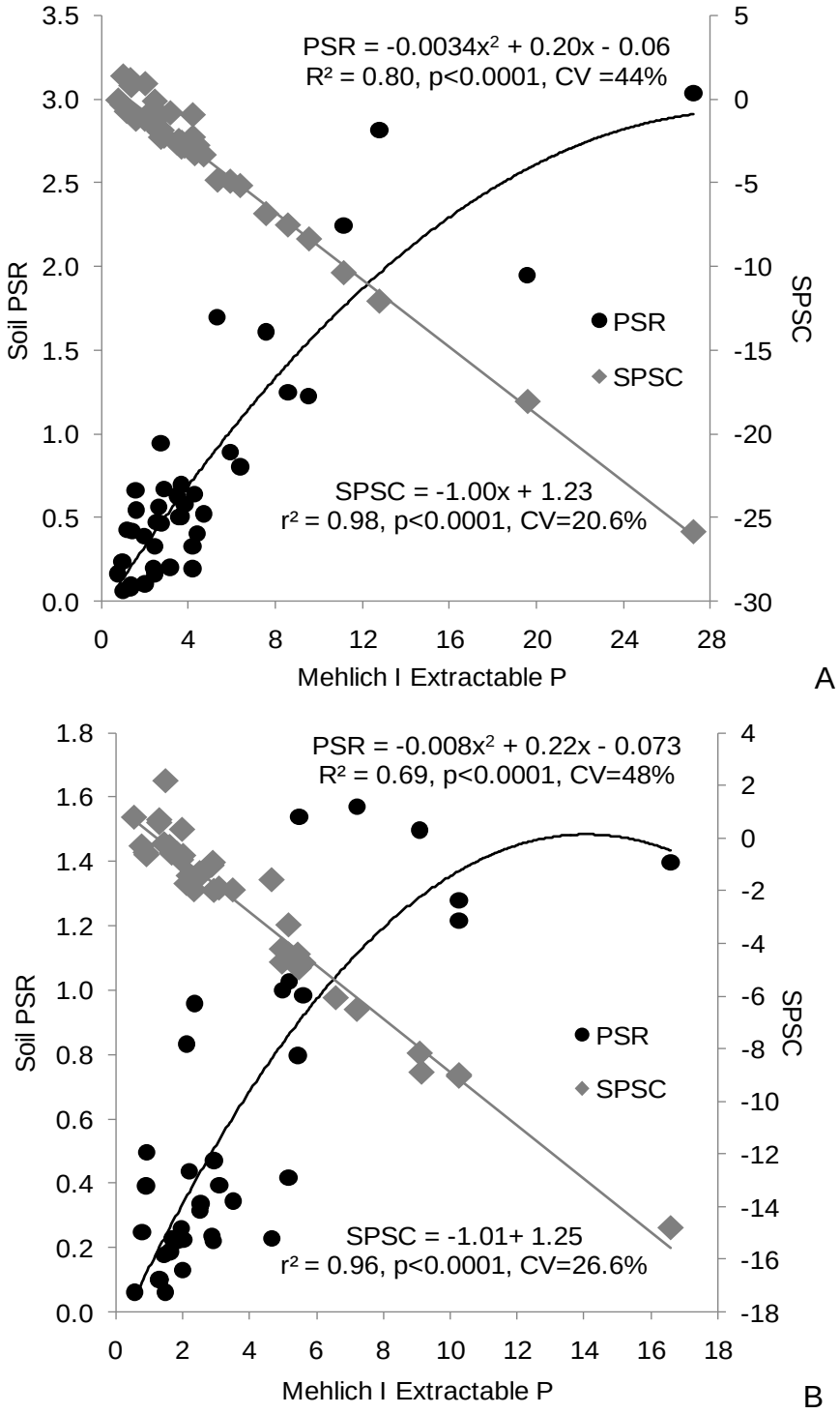


Figure 4-1. Soil phosphorus saturation ratio (PSR) and soil phosphorus storage capacity (SPSC) relative to Mehlich 1 extractable soil phosphorus concentration (M1P) within the top 15 cm of the soil profile during 2009. A) Empire zoysiagrass and B) Floratam St. Augustinegrass.

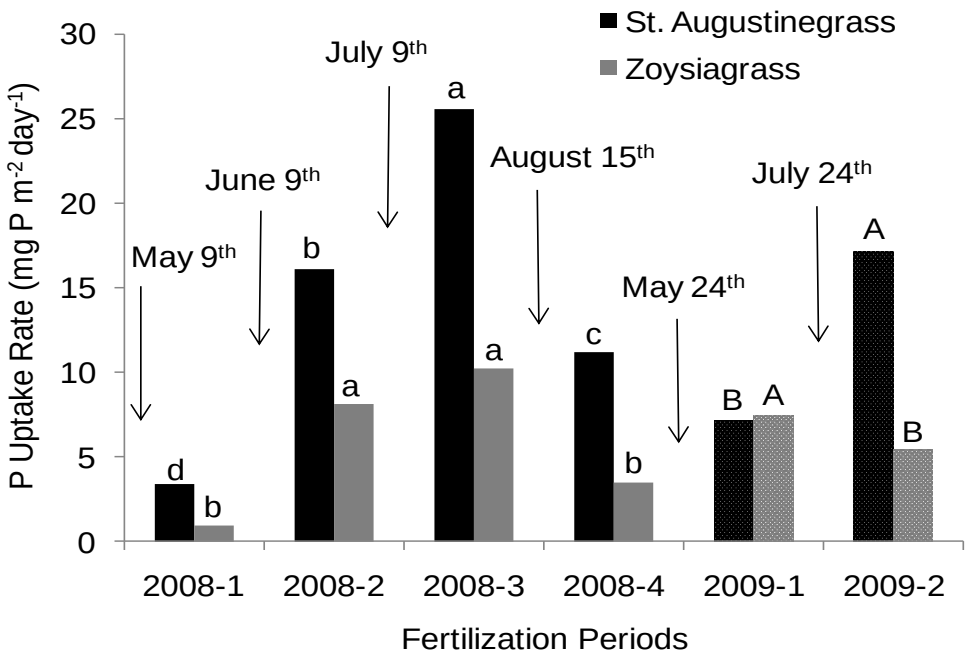


Figure 4-2. Phosphorus uptake rate in Empire zoysiagrass and Floratam St. Augustinegrass during each fertilization period. A fertilization period starts when a fertilizer application is conducted and ends when the following fertilization takes place. The date when each fertilizer application was carried out is indicated next to an arrow. Columns labeled with the same letter within a turfgrass species and year are not significantly different at p = 0.05 by single degree of freedom contrast analysis. Lower case letters indicate differences among fertilization periods during 2008 and upper case letters indicate differences among fertilizing periods during 2009.

Table 4-4. Change over time of Floratam St. Augustinegrass and Empire zoysiagrass root biomass, root length density, root surface area, root volume and average root diameter within the top 15 cm of the soil profile.

Date	Floratam St. Augustinegrass					Empire zoysiagrass				
	Root Dry Matter	Root Length Density	Root Surface Area	Root Volume	Root Diameter	Root Dry Matter	Root Length Density	Root Surface Area	Root Volume	Root Diameter
	kg DM m^{-3}	cm cm^{-3}	cm^2 cm^{-3}	mm^3 cm^{-3}	mm	kg DM m^{-3}	cm cm^{-3}	cm^2 cm^{-3}	mm^3 cm^{-3}	mm
9/8/2008	2.98	2.5	0.38	4.65	0.49	3.05	2.84	0.34	3.40	0.38
5/15/2009	1.77c	1.37ab	0.19b	2.11c	0.44d	1.52b	1.17bc	0.15c	1.64c	0.40d
6/10/2009	2.40b	1.41ab	0.23a	3.05b	0.52c	2.18a	1.53a	0.22a	2.52ab	0.45bc
7/8/2009	2.80a	1.28b	0.22ab	3.19b	0.56bc	1.65b	1.08c	0.16c	2.02c	0.47bc
8/5/2009	2.88a	1.34ab	0.25a	4.78a	0.77a	2.29a	1.22bc	0.22a	3.30ab	0.59a
9/3/2009	1.69c	1.25b	0.20b	2.96b	0.60bc	1.55b	1.11bc	0.17bc	2.07bc	0.48bc
9/30/2009	1.80c	1.28ab	0.21ab	2.82b	0.52c	1.51b	1.15bc	0.19bc	2.00bc	0.41cd
Overall	2.22	1.32	0.22	3.15	0.57	1.79	1.21	0.19	2.26	0.47
p-value	<0.0001	0.0100	0.0163	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Values within a given column and year with the same letter are not statistically different at p=0.05 according to single degree of freedom contrast analysis.

Table 4-5. Change over time of Floratam St. Augustinegrass and Empire zoysiagrass root biomass, root length density, root surface area, root volume and average root diameter within the 15 to 30 cm depth.

Date	Floratam St. Augustinegrass					Empire zoysiagrass				
	Root Dry Matter	Root Length Density	Root Surface Area	Root Volume	Root Diameter	Root Dry Matter	Root Length Density	Root Surface Area	Root Volume	Root Diameter
	kg DM m^{-3}	cm cm^{-3}	cm^2 cm^{-3}	mm^3 cm^{-3}	mm	kg DM m^{-3}	cm cm^{-3}	cm^2 cm^{-3}	mm^3 cm^{-3}	mm
6/10/2009	1.23	0.84b	0.13c	1.51b	0.47b	0.43	0.53	0.05	0.38	0.30
7/8/2009	1.24	0.73b	0.12c	1.55b	0.50b	0.48	0.50	0.05	0.50	0.34
8/5/2009	1.14	0.81b	0.13bc	1.56b	0.49b	0.40	0.48	0.05	0.47	0.34
9/3/2009	1.19	0.91ab	0.16a	2.40a	0.57a	0.41	0.71	0.08	0.66	0.32
9/30/2009	1.05	0.78b	0.14b	1.82b	0.50b	0.35	0.62	0.06	0.51	0.34
Overall	1.17	0.81	0.13	1.77	0.51	0.41	0.56	0.06	0.50	0.33
p-value	0.7035	0.0247	<0.0001	0.0003	0.0113	0.5643	0.0689	0.1719	0.2265	0.0732

Values within a given column with the same letter are not statistically different at p=0.05 according to single degree of freedom contrast analysis.

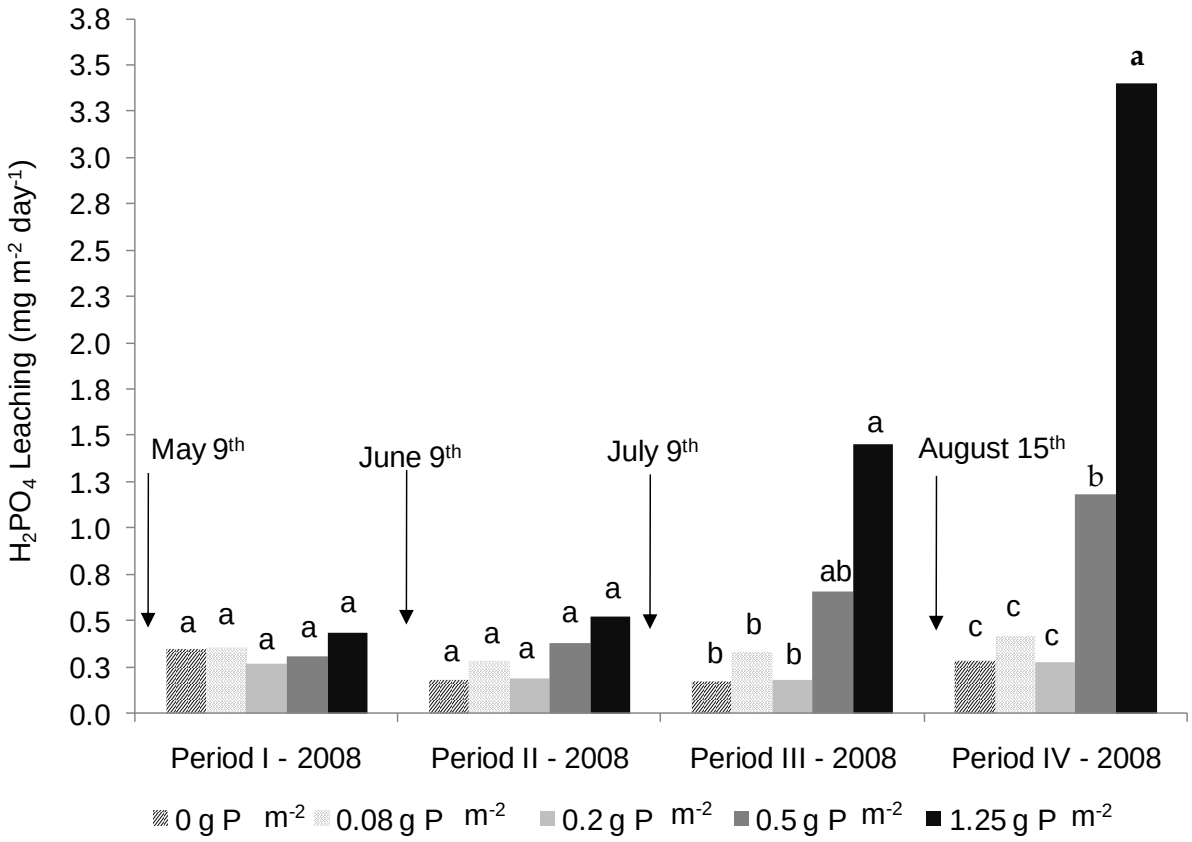


Figure 4-3. Orthophosphate leaching rate in Empire zoysiagrass as influenced by phosphorus application rate within each fertilizer application period during the first growing season (2008). The date when each fertilizer application was carried out is indicated next to an arrow. Columns with the same letter within an application period are not significantly different at p=0.05 according to single degree of freedom contrast analysis.

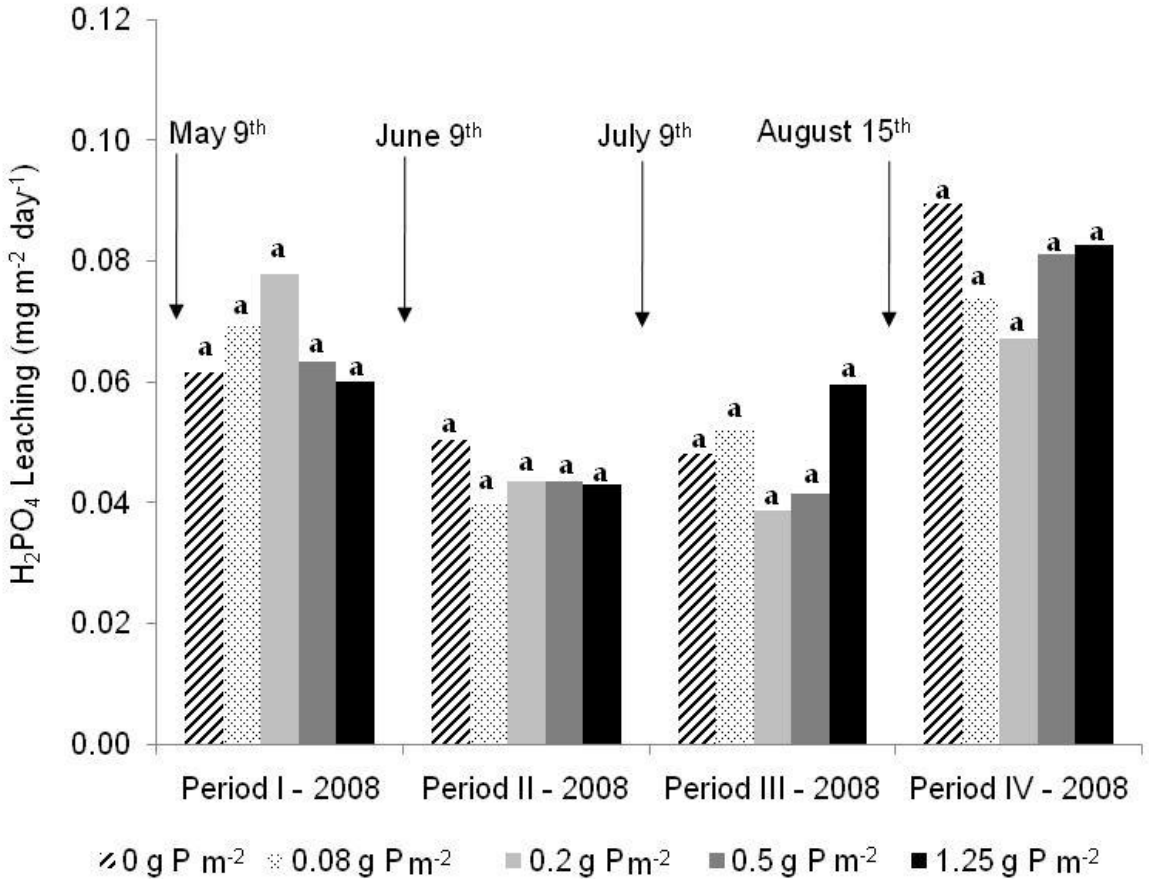


Figure 4-4. Orthophosphate leaching rate in Floratam St. Augustinegrass as influenced by phosphorus application rate within each fertilizer application period during the first growing season (2008). The date when each fertilizer application was carried out is indicated next to an arrow. Columns with the same letter within an application period are not significantly different at p=0.05 according to single degree of freedom contrast analysis.

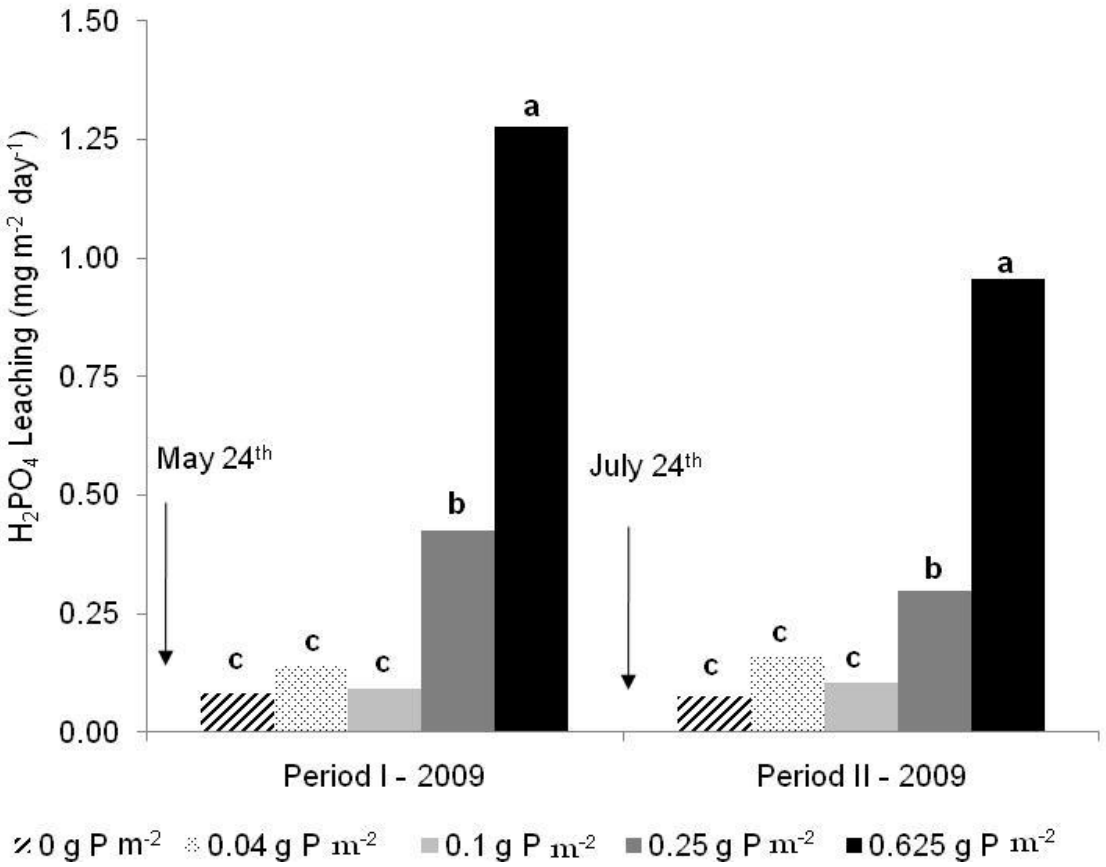


Figure 4-5. Orthophosphate leaching rate in Empire zoysiagrass as influenced by phosphorus application rate within each fertilizer application period during the second growing season (2009). The date when each fertilizer application was carried out is indicated next to an arrow. Columns with the same letter within an application period are not significantly different at p=0.05 according to single degree of freedom contrast analysis.

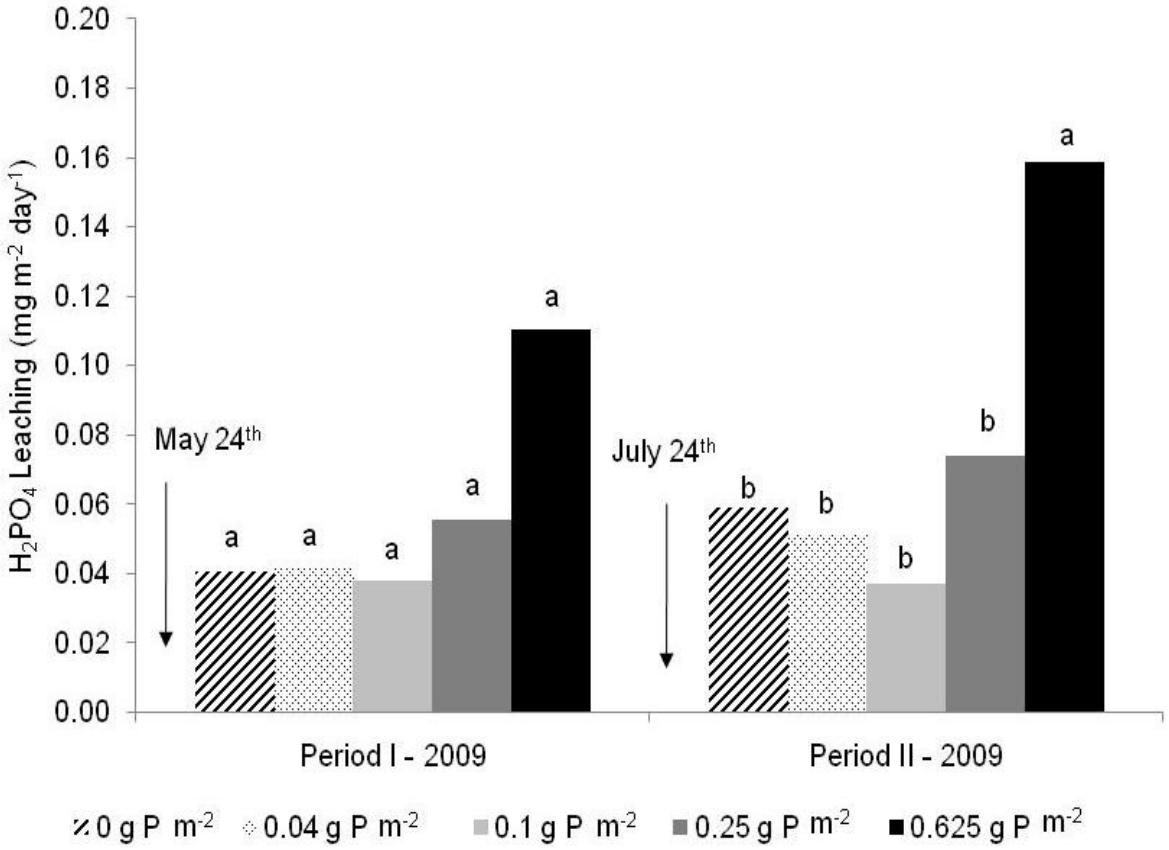
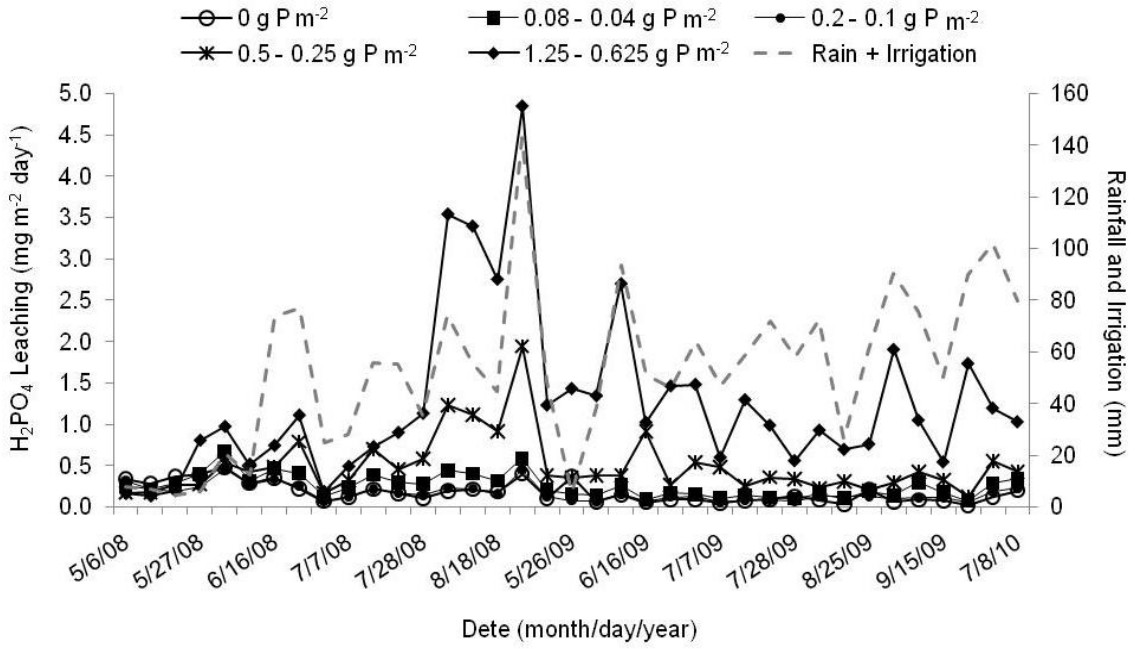
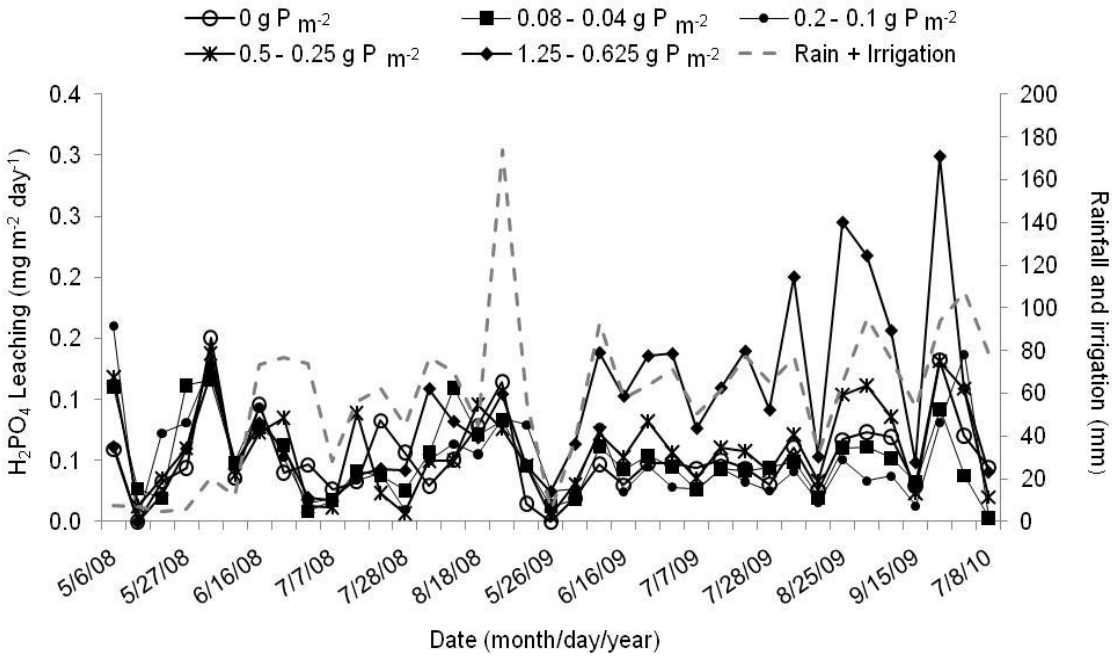


Figure 4-6. Orthophosphate leaching rate in Floratam St. Augustinegrass as influenced by phosphorus application rate within each fertilizer application period during the second growing season (2009). The date when each fertilizer application was carried out is indicated next to an arrow. Columns with the same letter within an application period are not significantly different at p=0.05 according to single degree of freedom contrast analysis.

A



A



B

Figure 4-7. Fluctuation of orthophosphate leaching rate and rainfall plus irrigation over time. A) Empire zoysiagrass and B) Floratam St. Augustinegrass.

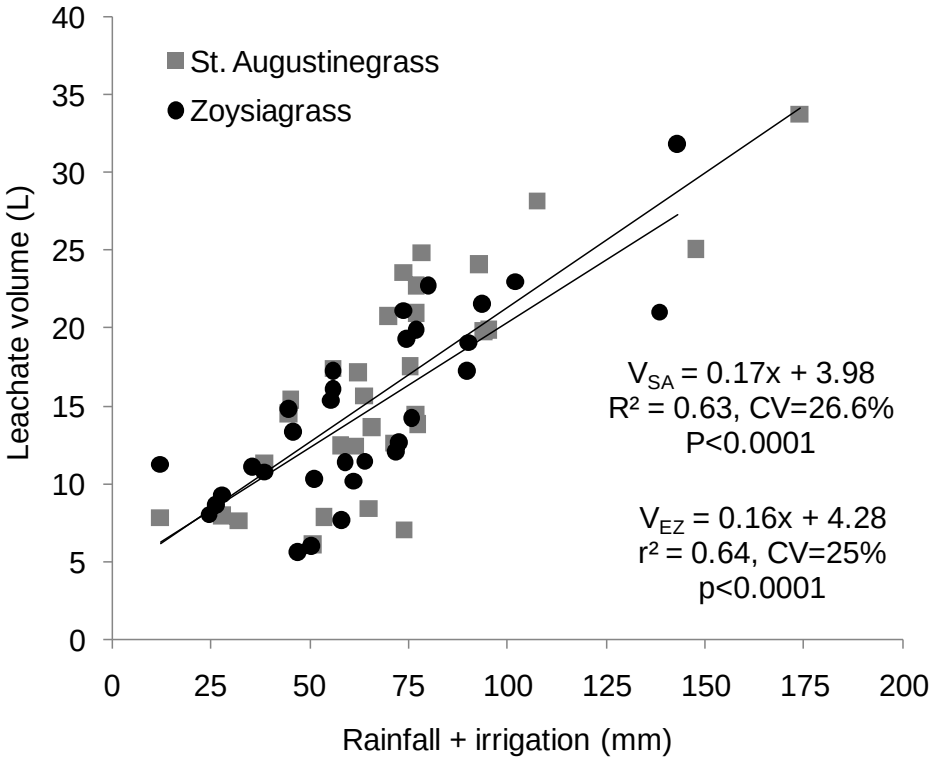


Figure 4-8. Weekly leachate volume from Empire zoysiagrass (EZ) and Floratam St. Augustinegrass (SA) relative to cumulative rainfall plus irrigation per week across years and treatments.

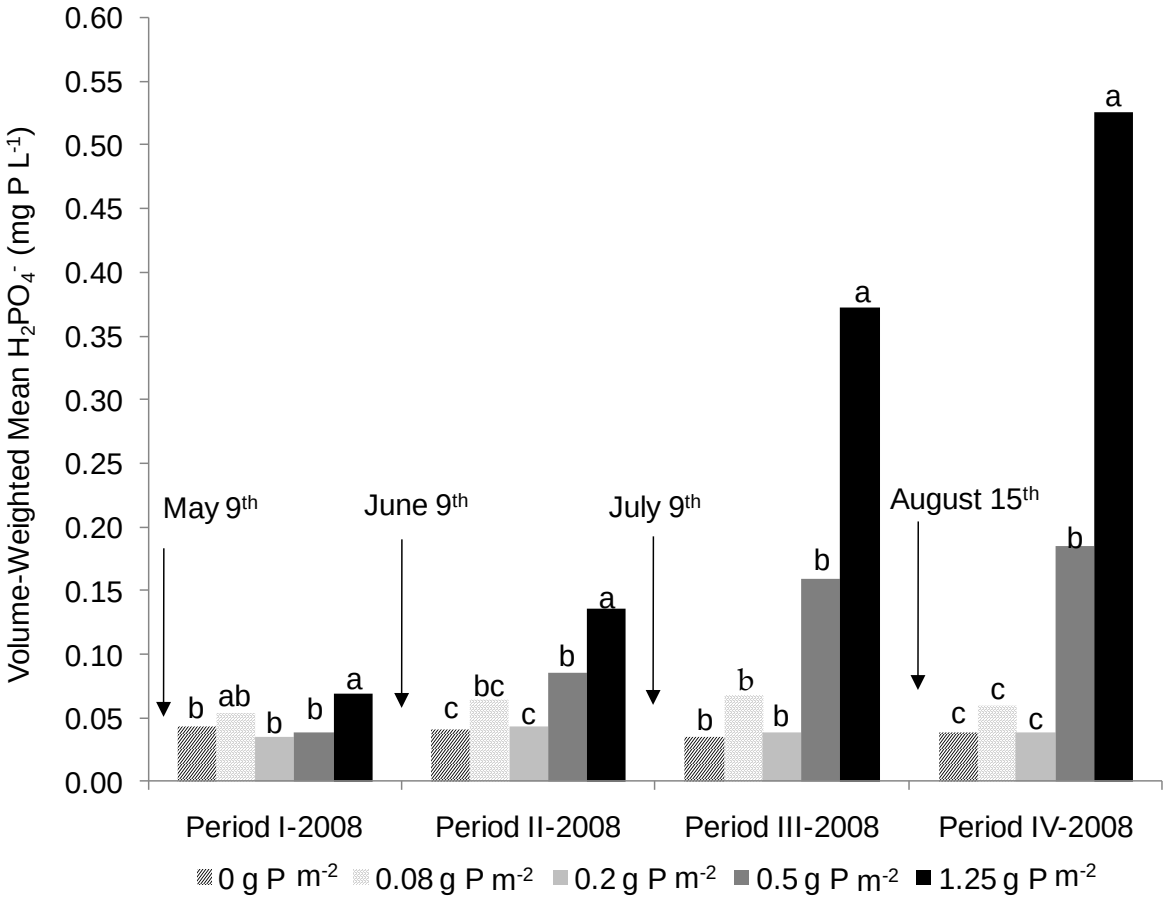


Figure 4-9. Volume-weighted orthophosphate concentration in leachate from Empire zoysiagrass as influenced by phosphorus application rate within each fertilizer application period during the first growing season (2008). The date when each fertilizer application was carried out is indicated next to an arrow. Columns with the same letter within an application period are not significantly different at p=0.05 according to single degree of freedom contrast analysis.

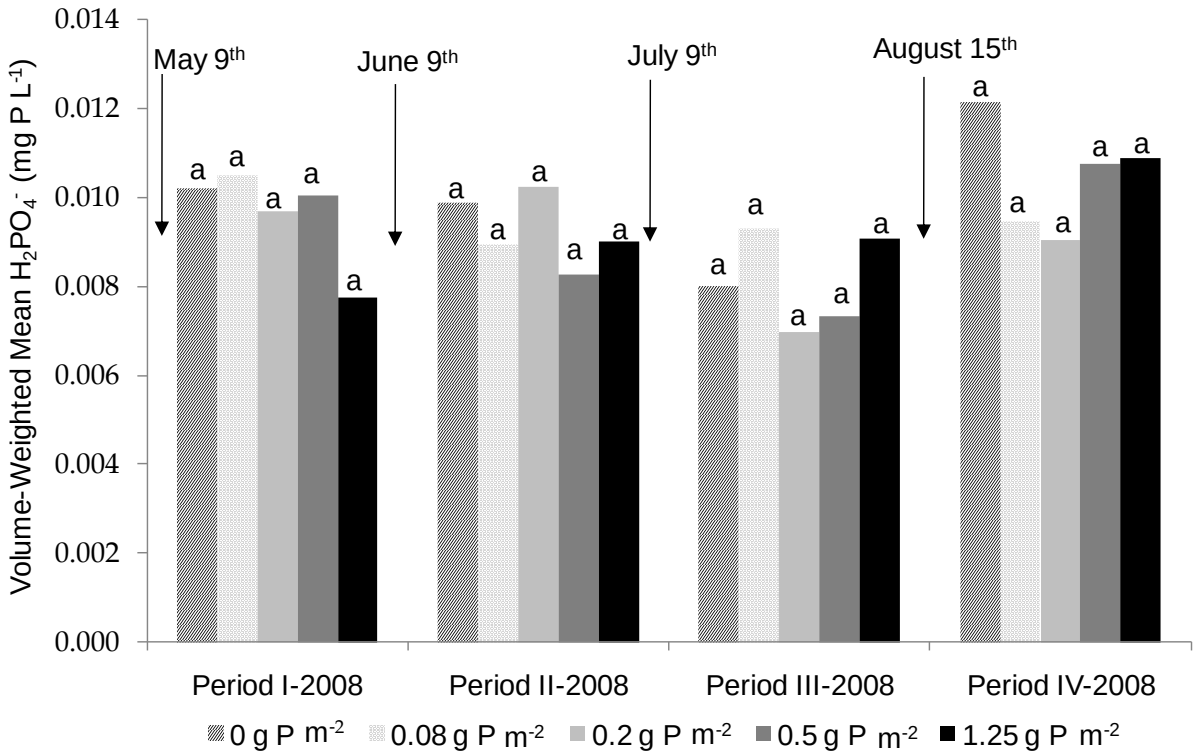


Figure 4-10. Volume-weighted orthophosphate concentration in leachate from Floratam St. Augustinegrass as influenced by phosphorus application rate within each fertilizer application period during the first growing season (2008). The date when each fertilizer application was carried out is indicated next to an arrow. Columns with the same letter within an application period are not significantly different at p=0.05 according to single degree of freedom contrast analysis.

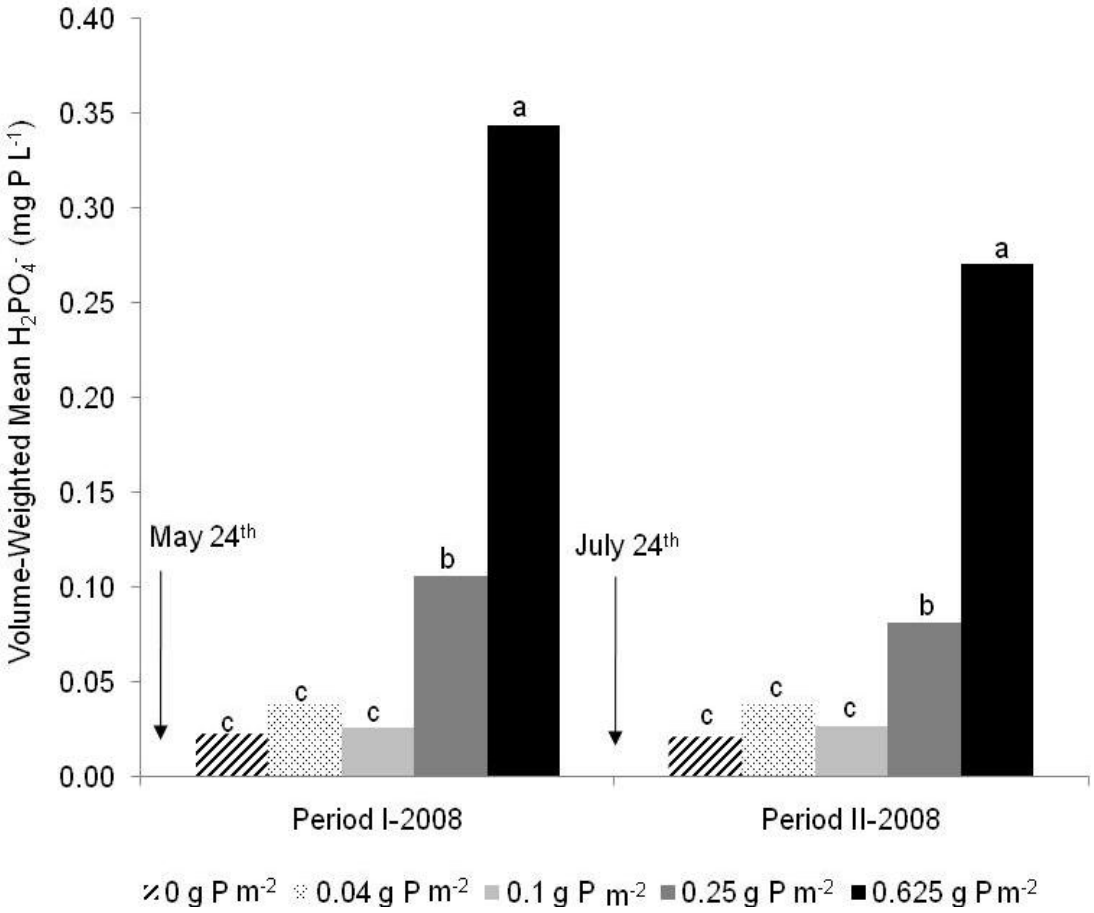


Figure 4-11. Volume-weighted orthophosphate concentration in leachate from Empire zoysiagrass as influenced by phosphorus application rate within each fertilizer application period during the second growing season (2009). The date when each fertilizer application was carried out is indicated next to an arrow. Columns with the same letter within an application period are not significantly different at p=0.05 according to single degree of freedom contrast analysis.

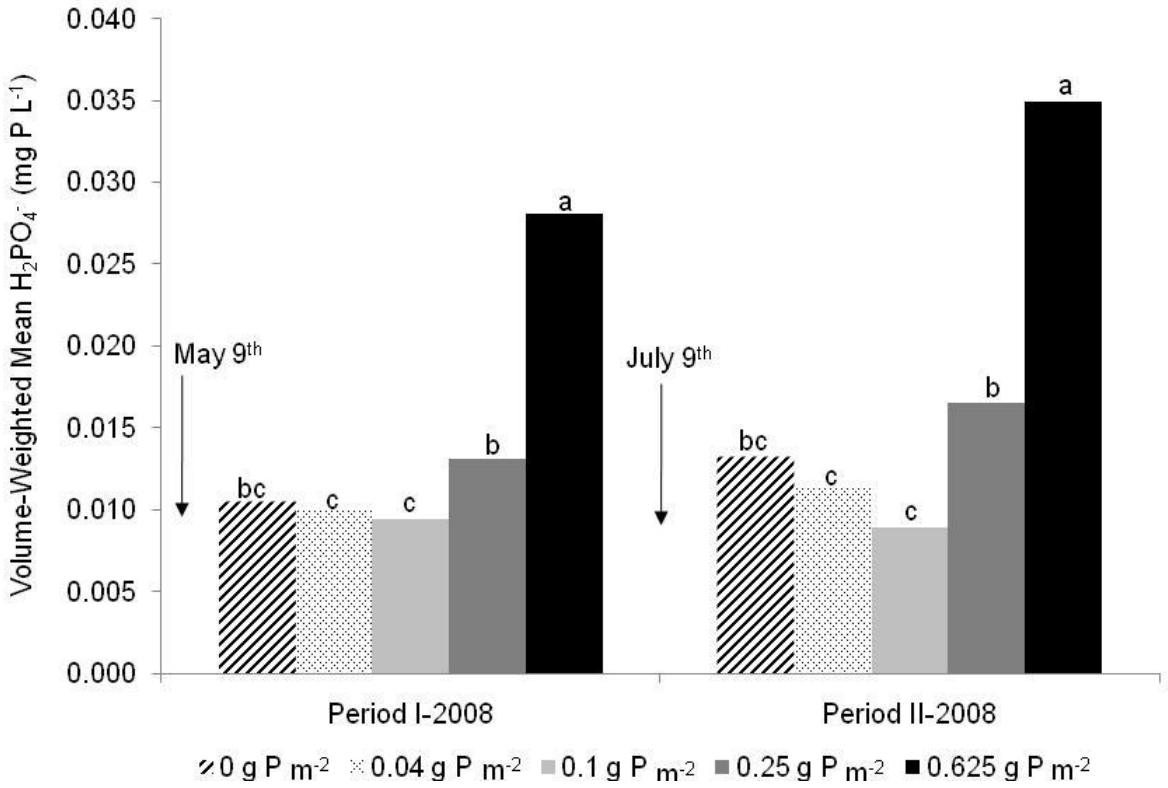


Figure 4-12. Volume-weighted orthophosphate concentration in leachate from Floratam St. Augustinegrass as influenced by phosphorus application rate within each fertilizer application period during the second growing season (2009). The date when each fertilizer application was carried out is indicated next to an arrow. Columns with the same letter within an application period are not significantly different at p=0.05 according to single degree of freedom contrast analysis.

Table 4-6. Othophosphate concentration in leachates as influenced by Mehlich 1 extractable soil P (M1-P), soil phosphorus storage capacity (SPSC), and soil phosphorus saturation ratio (PSR).

Orthophosphate Concentration	Floratam St. Augustinegrass			Empire Zoysiagrass		
	M1-P	SPSC	PSR	M1-P	SPSC	PSR
mg P L^{-1}	mg P kg^{-1}	mg P kg^{-1}		mg P kg^{-1}	mg P kg^{-1}	
<0.01	3.55 ± 0.73	-1.88 ± 0.71	0.53 ± 0.09	nd	nd	nd
0.01 – 0.03	4.90 ± 0.83	-2.84 ± 0.83	0.70 ± 0.10	4.51 ± 0.82	-3.38 ± 0.84	0.73 ± 0.13
0.03 – 0.087	11.28 ± 3.91	-10.20 ± 4.93	1.09 ± 0.22	4.89 ± 0.92	-3.82 ± 0.84	0.74 ± 0.13
0.05 – 0.157	nd	nd	nd	6.27 1.63	-4.33 1.82	0.87 0.31
0.157 – 0.359	nd	nd	nd	8.10 2.04	-6.16 2.21	1.27
>0.359	nd	nd	nd	nd	nd	nd

Values reported as 95% confidence interval (mean ± 1.96 ∗ standard error)
nd = no data

Table 4-7. Percent cumulative phosphorus leached from fertilizer application between May 2008 and June 2010 in Floratam St. Augustinegrass.

Fertilizer Application Period	Phosphorus Application Rate (g m^{-2})				
	0(0)	0.08(0.04)	0.2(0.1)	0.5(0.25)	1.25(0.625)*
2008-1	0.00	0.48	0.22	0.03	-0.01
2008-2	0.00	0.05	0.06	0.00	-0.01
2008-3	0.00	0.09	-0.02	-0.02	0.00
2008-4	0.00	-0.02	-0.06	-0.02	0.00
2009-1	0.00	0.04	-0.04	0.02	0.08
2009-2	0.00	-0.11	-0.14	0.06	0.19
2010	0.00	-0.17	-0.04	0.11	0.22
**kg P ha^{-1}	0.00	-0.01	0.00	0.03	0.14

*Values outside parentheses correspond to P application rates during 2008 (g P m-2 every 4 weeks). Values within parenthesis correspond to the P application rates during 2009 (g P m^{-2} every 8 weeks).

** kilograms of P leached over the control treatment per hectare between May 2008 and June 2010.

Table 4-8. Percent cumulative phosphorus leached from fertilizer application between May 2008 and June 2010 in Empire Zoysiagrass.

Fertilizer Application Period	Phosphorus Application Rate (g m^{-2})				
	0(0)	0.08(0.04)	0.2(0.1)	0.5(0.25)	1.25(0.625)*
2008-1	0.00	0.56	-1.48	-0.24	0.41
2008-2	0.00	2.31	-0.72	0.58	0.70
2003-1	0.00	4.19	-0.38	1.88	2.11
2008-4	0.00	3.84	-0.27	2.21	2.56
2009-1	0.00	3.59	-0.61	2.86	3.81
2009-2	0.00	4.46	-0.43	3.18	4.37
2010	0.00	5.88	-0.29	3.39	4.71
**kg P ha^{-1}	0.00	0.24	-0.03	0.85	2.95

*Values outside parentheses correspond to P application rates during 2008 (g P m-2 every 4 weeks). Values within parenthesis correspond to the P application rates during 2009 (g P m^{-2} every 8 weeks).

** kilograms of P leached over the control treatment per hectare between May 2008 and June 2010.

CHAPTER 5
QUALITY, GROWTH, PHOSPHORUS USE EFFICIENCY AND DRY MATTER PARTITIONING OF EMPIRE ZOYSIAGRASS AND FLORATAM ST. AUGUSTINEGRASS IN RESPONSE TO PHOSPHORUS FERTILIZATION UNDER FIELD CONDITIONS

Phosphorus is an essential plant nutrient (Raghothama, 1999). It is involved in many key processes in plants such as energy transfer and generation, synthesis of nucleic acids, photosynthesis, glycolysis, respiration, synthesis and stability of plant membranes, activation and inactivation of enzymes, redox reactions, metabolism of carbohydrates and biological nitrogen fixation (Vance et al. 2003).

Adequate fertilization is required to obtain and maintain high quality turfgrass (Trenholm and Unruh, 2005).Inorganic P fertilizers are obtained from treating rock phosphate (flourapatite) with sulfuric acid and phosphoric acid (CPHA, 2002). Phosphorus is a finite resource and known world reserves of phosphate rock may be exhausted in a period as short as 90 years (Stewart et al., 2005). Plants absorb P through active uptake (Vance et al., 2003) against a steep gradient because of the great difference between the P concentration in solution of most soils (~2 µ*M*) and the P concentration in plants (~5 and 20 m*M*) (Raghothama, 2005). Maximum P influx is associated to P depravation (Clarkson, 1984). Leaf expansion, leaf surface area and number of leaves decreases in P deficient plants (Marschner, 1995). Correspondingly, in P deficient plants greater amount of assimilates are allocated to roots (Marschner, 1995) and P is translocated from older tissues to young actively growing tissues (Schachtman et al., 1998).

Plant P acquisition potential is relevant from the agronomic and environmental stand point. High P use efficiency is another desirable attribute in plants. As indicated by White and Hammond (2008), P use efficiency may be defined as the ratio of crop

yield to the amount of P accumulated in the plant. Akhtar et al. (2007) reported that P-use efficiency of P deficient plants was significantly higher than that measured in P sufficient plants.

Diagnosis of the plant nutritional status is based on the critical nutrient concentration in tissue, the nutrient concentration in plants below which plant growth or yield response to increased nutrient concentration or nutrient supply is observed (Havlin et al., 1999). Several authors have reported positive response in terms of density, growth and quality of turfgrass to P fertilization (Kuo, 1993; Rodriguez et al., 2000; Guillard and Dest, 2003; Hull and Martin, 2004; Liu et al., 2008).In contrast, reduction in growth and turf quality has been related to excessive P supply (Menn and McBee, 1970, Rodriguez et al., 2000; Petrovic et al., 2005)

Stenotaphrum secundatum (Walt) Kuntze (St. Augustinegrass) cultivar 'Floratam' and *Zoysia japonica* (zoysiagrass) cultivar 'Empire' are widely cultivated turfgrass species in Florida (Satterthwaite et al., 2007). There is limited information about the response of these species in terms of quality and growth to P fertilization under field conditions. Phosphorus supply in excess of the turf demand may result in P losses and detrimental effects to the environment. It is imperative to understand how P rate influences P partitioning in these turfgrasses and the importance of different P pools within the plant on the P concentration in diagnostic tissues over time.

The following hypotheses were tested in this study: (i) growth rate and turf visual quality will increase in response to increasing P application rate, M1-P and leaf tissue P, (ii) partitioning of dry matter and P to leaves will increase with increasing leaf tissue P

concentration, and (iii) PUE will be inversely related to P supply and leaf tissue P concentration.

The objectives of this experiment were (i) to study the effect P supply rate, M1-P and leaf tissue P concentration on growth rate, visual quality, dry matter and P partitioning in zoysiagrass and St. Augustinegrass, and (ii) to evaluate the influence of P supply rate and leaf tissue P concentration under field conditions on P and dry matter partitioning as well as on PUE.

Materials and Methods

Experimental Site and Treatments Description

The study was conducted at the G. C. Horn Turfgrass Laboratory of the Plant Science Research and Education Unit of the University of Florida near Citra, FL (29°24' N, 82°10' W). Climatic conditions during 2008 and 2009 growth seasons (May to September) were as follows. Average temperature was 26°C and it oscillated between 13.7°C and 38.6°C. Cumulative precipitation (not including irrigation) during 2008 and 2009 growth seasons was 383 mm and 723 mm, respectively. Cumulative evapotranspiration during 2008 was 462 mm and 540 mm in 2009.

A total of 40 plots (3 m by 4.25 m) were established. A rectangular area (1.5 by 4.25 m) was excavated in the center of each plot to a depth of 45 cm and then back filled with low P sand (< 10 mg P kg^{-1} of M1-P), with less than 1% clay size fraction and traces of kaolinite and gibbsite (Figure A-2). The native soil was a Candler sand (Hyperthermic, uncoated Lamellic Quartzipsamments) and tested medium M1-P (16 - 30 mg P kg^{-1}). The experiment was established in a split-plot randomized complete block design with turfgrass species as the main effect and P application rate as secondary effect. The experiment consisted of two turfgrass species: *Stenotaphrum*

secundatum (Walt) Kuntze 'Floratam' (St. Augustinegrass) and *Zoysia japonica* 'Empire' (Zoysiagrass). Phosphorus application rates were 0, 0.08, 0.2, 0.5, and 1.25 g P m^{-2} every 4 weeks during the first growing season (2008) and 0, 0.04, 0.1, 0.25, and 0.625 g P m^{-2} every 8 weeks during the second year of evaluation (2009). The source of P utilized was triple super phosphate (45% P_2O_5). Phosphorus fertilizer was broadcasted uniformly over the turf surface and application rates were replicated four times. This study included the maximum P application rates allowed in Florida (0.54 g P m^{-2} $application^{-1}$ and 1.07 g P m^{-2} $year^{-1}$) as stated in the "Labeling Requirements for Urban Turf Fertilizers rule" (State of Florida, 2007). Turfgrasses were established with soil free certified sod from the G.C. Horn Turfgrass Field Laboratory of the University of Florida. Nitrogen (N) and potassium (K) were supplied at a rate of 4.9 g m^{-2} and 4.06 g m^{-2}, respectively, in combination with the P fertilizer (Sartain, 2010; Trenholm and Unruh, 2005). Approximately 50 mm of water were applied weekly through irrigation (5 irrigation cycles per week) during the evaluation period. Irrigation was conducted despite the occurrence of rainfall. In the state of Florida, an irrigation rate of 12.7 mm to 19 mm of water per irrigation cycle, twice or thrice per week is recommended for home lawns during summer days without rainfall. Once rainfall has resumed, then it is recommended to stop the irrigation until the turf shows signs of drought (Trenholm and Unruh, 2005).

Soil and Tissue Sampling and Analysis

Soil samples were collected prior to treatment application and every two weeks thereafter for the duration of each growing season. Composite samples consisting of two soil cores per plot were collected with a stainless steel 2-cm diameter soil probe from 0 to 7.5 cm, 7.5 to 15 cm and 15 to 30 cm. The holes created while sampling were refilled with uncoated sand immediately after collecting the sample. The top 0.5 cm of

each soil core was removed to avoid potential contamination with P from the fertilizer applied on the turf surface. Soil samples were air dried and then passed through a 2-mm sieve. Mehlich I extractable soil P (M1-P) was determined following the extraction procedure described by Sims (2009). Phosphorus concentrations in sample extracts were determined with a Bran Leubbe Technicon Autoanalizer II (Seal Analytical, Mequon, WI, USA) as described in EPA method 365.1 ”Determination of Phosphorus by Semi-automated Colorimetry” (O’Dell, 1993). Soil pH and electrical conductivity were determined in 2:1(v/v) water:soil ratio with a PC 700 pH/EC meter (Oakton Instruments, Vernon Hills, IL, USA).

Tissue Sampling and Analysis

Tissue samples were collected immediately prior to treatment application and biweekly post treatment application for the duration of the growing season. Samples were collected by harvesting the leaf tissue over the low P sand area along the length of the plots. Clippings were collected with a walk-behind mower with a back bag-collector. The width of the mower swath was 54 cm and the turf area harvested per plot was 1.29 m^2. The mowing height was approximately 10.2 cm for St. Augustinegrass and 7.62 cm for zoysiagrass.

At the end of the 2008 growing season, composite root samples consisting of two 383-cm^3 soil cores per plot were taken from the top 15 cm of the soil profile. During 2009, composite root samples were collected prior to imposing P treatments at the beginning of the season and every four weeks thereafter. The root sampling depth during 2009 was 0-15 cm and 15-30 cm. All root samples were washed free of soil and then scanned with an Epson Perfection V700 Photo dual lens scanner (Epson Corporation, Japan). The digital images obtained were then analyzed with WinRhizo

Software Pro v. 2007d (Reagent Instruments Canada Inc., Ottawa, ON, Canada) to determine the total root length, root surface area, root volume, and average root diameter. Thatch tissue was separated from roots and washed free of soil. Leaf, thatch and root samples were oven dried at 70 °C to constant weight and dry matter content was recorded. Tissue samples were ground in a stainless steel Wiley mill to pass a # 40 mesh sieve (425 µm openings size). Ground samples were thoroughly mixed and 0.2 g of dried plant tissue was ashed and digested with 6 *M* HCL according to the standard operation procedure WLB-SP-009 of the Wetland Biogeochemistry Laboratory at the University of Florida, “Total Phosphorus of Soil, Sediment and Plant Tissue by Ignition or Ashing Method”. Phosphorus concentration was determined following USEPA method 365.1 “Determination of Phosphorus by Semi-automated Colorimetry” (O’Dell, 1993) using a Bran Leubbe Technicon Autoanalizer II (Seal Analytical, Mequon, WI, USA).

Turf Visual Quality and Chlorophyll Index

Turf visual quality was evaluated biweekly using a scale of 1 to 9, where 1 represents brown, dormant turf and 9 represents superior quality. A value of 5.5 was considered the minimum rating for an acceptable turf visual quality (Skogley and Sawyer, 1992). Chlorophyll Index (CI) was measured biweekly with a CM 1000 Chlorophyll Meter (Spectrum Technologies Inc, Illinois, USA) just prior every leaf tissue harvest. Chlorophyll index is a measure of the relative greenness of the leaf. The CM 1000 chlorophyll meter measures the ambient and reflected light intensities at wavelengths of 700 nm and 840 nm to estimate the quantity of chlorophyll in leaves. Chlorophyll-*a* absorbs 700 nm light; hence, reflection of 700 nm light is reduced relative to the reflected 840 nm light. The 840 nm light provides a measure of the reflectiveness

of the leaf surface. Physical characteristics of the leaf such as leaf hairs and waxy surfaces can reduce light reflection. The CI is obtained by comparing the ratio of the 700 nm and 840 nm in available light (ambient light) to the ratio of the same wavelengths of reflected light. The CI is reported in a scale of 0 to 999 (Spectrum Technologies Inc, CM 1000 chlorophyll meter manual, 2009)

Digital Image Analysis

Green turf cover can be determined more accurately and precisely with digital image analysis than with subjective methods such as visual ratings of turf density (Richardson et al., 2001). Horst et al. (1984) assessed the reliability of visual evaluation of turf quality and density. They reported that common techniques utilized by researchers for turfgrass visual quality and density evaluations are inadequate. Achieving high turfgrass quality from the aesthetics standpoint is the main objective of turfgrass management; hence, in a healthy, dense and uniform turf, high biomass accumulation is not essentially an advantageous attribute (Christians et al., 1979). Therefore, digital image analysis was utilized in this experiment to incorporate a non-subjective, reliable method to evaluate the percent green turf cover as a measure of turf density and uniformity.

Digital images were obtained with a Canon PowerShot A630 (Canon Inc., New York, USA) digital camera mounted on a light box. The dimensions of the light box were 53 cm (width), 51 (length), and 61 cm (height). The bottom side of the light box was open to allow placing it over the turf. The bottom side of the light box was open to allow placement over the turf, and a 38 mm diameter opening was drilled in the upper side of the light box to accommodate the camera lens. Four 10-W compact florescent (day-light 6500 Kelvin) bulbs were placed inside the light box on the upper side to provide uniform

light intensity. Turfgrass was mowed to the proper height (10.2 cm for St. Augustinegrass and 7.62 cm for zoysiagrass) prior collecting the digital images.

The images obtained were saved in JPEG format with an image size of 5 mega pixels (2,592 by 1,944 pixels). Camera settings consisted of an exposure time of 1/13 seconds, an aperture of F8, and a focal length of 7 mm. All digital images were resized to 800 by 600 pixels using ACDSee Pro (v. 2.5, ACDSee Systems International Inc., Victoria, British Columbia, Canada).

The digital images were analyzed using SigmaScan Pro (v. 5.0, SPSS Inc., Chicago, IL) and the Turf Analysis macro (Karcher and Richardson, 2005) for batch analysis of turf digital images. The color threshold settings were a hue range from 50 to 107 and a saturation range from 0 to 100, which selectively identified green pixels in the images. Richardson et al. (2001) utilized a hue range from 57 to 107 and a saturation range from 0 to 100 to quantify bermudagrass [*C. dactylon x C. traansvalensis* (L.)] green turf cover using DIA. The Turf Analysis macro calculated the percent green turf cover by dividing the green pixels by the total number of pixels in each image. In addition the dark green color index (DGCI), a measure of the greenness of the turf, was automatically calculated by the software according to the following equation:

DGCI = [(Hue-60)/60 + (1-Saturation) + (1-Brightness)]/3 (Karcher and Richardson, 2003)

Statistical Data Analysis

Normal distribution of the data was tested graphically using normal probability plots and numerically with the Shapiro-Wilk W test. Equal variance was also checked using residual plots and the Levene's test for homogeneity of variance. Analysis of variance was conducted with the general linear model procedure (Proc GLM) of SAS

system v.9.2 (SAS Institute, 2009) and mean separation was carried out according to single degree of freedom contrast analysis.

Results and Discussion

Turf Visual Quality

Zoysiagrass visual quality was not influenced by P application rate during 2008 and in most of the evaluation times during the 2009 growing season (Figure 5-1). Visual quality of St. Augustinegrass fertilized with the highest P application (1.25 g P m^{-2} every 4 weeks during 2008 and 0.625 g P m^{-2} every 8 weeks during 2009) decreased over time, and by the end of the 2008 growing season it was lower than the turf visual quality in the other treatments (Figure 5-2). During 2009 St. Augustinegrass visual quality in the highest P application rate treatment was 18% lower than the average visual quality across sampling times in the control treatment (Figure 5-2). Average leaf P concentration across sampling times in St. Augustinegrass fertilized with the highest P application rate was 5.35 g P kg^{-1} in 2008 and 4.65 g P kg^{-1} in 2009 (Table 5-1, Table 5-2). These leaf tissue concentrations are about 3 fold greater than the critical leaf tissue P concentration for maximum St. Augustinegrass leaf growth rate (1.73 g P kg^{-1} DM). Leaf tissue P concentration in zoysiagrass remained below 3.8 g P kg^{-1} (Table 5-1, Table 5-2). Visual quality of zoysiagrass increased from 2008 to 2009 while visual quality of St. Augustinegrass during 2009 was lower than during the first growing season (Table 5-1, Table 5-2).

There was an inverse relationship between visual quality and leaf tissue P concentration in St. Augustinegrass during the second growing season (Table 5-2). Visual quality of zoysiagrass did not follow a clear trend in response to leaf tissue P concentration (Table 5-1, Table 5-2). As depicted in Figure 3-6 there seems to be a

feedback mechanism in zoysiagrass that reduces the rate of P depletion from solution as the P concentration in leaf tissue increases, reaching a minimum near the critical for maximum leaf growth rate. This feedback mechanism may have allowed zoysiagrass to reduce the amount of P accumulation in tissue under the conditions of high P supply present in this experiment. Excessive P accumulation in tissue appears to have negative effects on turf quality and possibly the tendency of St. Augustinegrass for P luxury consumption was responsible for the decrease in visual quality observed in this species over time. Evidence from research conducted in warm season turfgrasses (Menn and McBee, 1970; Rodriguez et al., 2000) as cool season turfgrass (Petrovic et al., 2005) demonstrate that turfgrass growth and quality are impaired by excessive P supply rate and P concentration in tissue.

Chlorophyll Index, Percent Green Turf Cover and Dark Green Color Index

Chlorophyll index did not follow a clear trend in response to P application rate in zoysiagrass or in St. Augustinegrass (Table 5-3, Table 5-4). Percent green turf cover in zoysiagrass and St. Augustinegrass was inversely related to P application rate during the first half of the 2009 growing season; however, no treatment effect on GC was observed thereafter (Table 5-3, Table 5-4). Dark green color index in zoysiagrass was inversely related to P application rate whereas no treatment effect on DGCI was observed for St. Augustinegrass (Table 5-4). Chlorophyll index in St. Augustinegrass was greater than in zoysiagrass (Table 5-3).

In order to observe a turfgrass response to P fertilization, the leaf tissue P concentration should be sufficiently lower than the critical leaf P concentration. Evidently that was not the case in this study. The lack of response to P treatments as evaluated by CI, GC and DGCI is likely explained by the excessively high P concentration in leaf

tissue measured in this experiment. Mineralization of organic P stored in the thatch layer may have supplied the P required to maintain high P concentration in leaf tissue even in the control treatment. If sprigs had been used instead of sod to establish the turf, the P concentration in tissue at the beginning of the experiment could have been much lower, increasing the probability of response to P supply. Growth and quality of Kentucky bluegrass (*Poa pratensis* L.) were inversely related to leaf tissue P concentration when leaf tissue P was greater than 3 g P kg^{-1} DM, the critical level for this turfgrass species. In contrast, yield was positively related to leaf tissue P concentration in a site where the leaf tissue P was <3 g P kg^{-1} (Petrovic et al., 2005).

Growth Rate

There was no treatment effect on zoysiagrass leaf growth rate during either evaluation year (Table 5-6). Leaf growth rate in the control treatment of zoysiagrass was 23% greater during 2008 and 14% greater during 2009 than in zoysiagrass supplied with the highest P application rate (Table 5-6). Zoysiagrass leaf growth rate was inversely related to leaf tissue P concentration (Table 5-1, Table 5-2). St. Augustinegrass leaf growth rate showed a slight response to P application rate during the 2008 growing season; however, during the second evaluation year St. Augustinegrass leaf growth rate was inversely related to P supply (Table 5-1, Table 5-2). Mehlich 1 extractable P increased in response to increasing P application rate (Table 5-1, Table 5-2). Leaf tissue P concentration and M1-P were positively related (Table 5-1, Table 5-2). Johnson et al. (2003) applied increasing rates of P (5.5 kg P ha^{-1} $year^{-1}$ to 110 kg P ha^{-1} $year^{-1}$) to Creeping bentgrass (*Agriostis stolonifera* Huds.) during a period of three years to evaluate the turfgrass response in terms of quality and growth to soil and tissue P concentration. They reported an increase in 0.5 *M* $NaHCO_3$ extractable soil

P as well as in leaf tissue P (ranged from about 1 g P kg^{-1} DM to over 7 g P kg^{-1} DM) with increasing P application rate. In that study maximum turfgrass quality was attained at 4 g P kg^{-1} P DM (Johnson et al., 2003).

The Leaf growth rate of St. Augustinegrass during the second growing season was inversely related to leaf tissue P concentration (Table 5-2). The inverse relationship observed between leaf growth rate and leaf tissue P concentration can be explained by the exceedingly high leaf tissue P concentration in both St. Augustinegrass and zoysiagrass (Table 5-1, Table5-2). The work of Liu et al. (2006) and Liu et al. (2008) indicated that the critical leaf tissue P for maximum growth of 'Floratam' St. Augustinegrass ranged between 1.6 g P kg DM and 1.8 g P kg DM. As depicted in Figure 2-4, the critical leaf tissue P for maximum leaf growth rate of 'Floratam' St. Augustinegrass in hydroponic culture was 1.73 g P kg^{-1}. The concentration in leaf tissue of St. Augustinegrass was between 2.2 and 3-fold greater than the critical leaf tissue P (1.73 g P kg^{-1} DM) for St. Augustinegrass (Table 5-1, Table 5-2). Cisar et al. (1992) observed increase in yield and quality of 'Floratam' St. Augustinegrass grown in a histosol with increasing P application rate (from 0 kg P ha^{-1} to 68 kg P ha^{-1} at planting), only when the WSP was less than 7.4 mg P kg^{-1} and the leaf tissue P was less than 2.4 g P kg^{-1}. This leaf tissue P concentration (2.4 g P kg^{-1}) was much lower that the P concentration in leaf tissue of the control treatment in both years (Table 5-1, Table 5-2). The P uptake feedback mechanism previously suggested for zoysiagrass, which operates in response to high leaf tissue P concentration (Figure 3-6), may give zoysiagrass an advantage over St. Augustinegrass when grown under high P supply conditions.

As previously shown excessive P concentration in leaf tissue have a detrimental effect on turf visual quality and growth rate (Table 5-1, Table 5-2). We observed chlorotic areas in St. Augustinegrass, particularly in the highest application rate treatment plots. Laboratory analysis of chlorotic tissue revealed an inverse relationship (r^2= 0.69, p<0.001) between zinc (Zn) and P concentration in tissue. However, when the tissue from the whole plot was analyzed (green and chlorotic tissue combined) no relationship between Zn and P concentration in tissue was found (r^2= 0.02, p<0.01). Cakmak and Marschner (1987) noted that high P concentrations in plant tissue caused a decrease of the physiological availability of zinc (Zn). Moreover, a feedback mechanism that controls the retranslocation of P_i in phloem from shoots to roots is impaired in Zn-deficient plants (leading to low P_i concentration in the root phloem sap); hence, the transport of P_i from roots to shoot is not regulated and toxic concentration of P accumulate in leaf tissues (Marschner and Cakmak, 1986). In soils with high or very high P concentration, zoysiagrass would be better able to regulate its rate of P accumulation in tissue, hence, avoiding excessive tissue P concentrations and the possible negative implications on quality and growth that it may have.

It was not possible to obtain a positive response of turfgrass growth rate to increasing M1-P concentration, because the leaf tissue P concentrations (Table 5-1, Table 5-2) were much greater than the critical leaf tissue P concentrations for maximum growth in both St. Augustinegrass and zoysiagrass (Figure 2-4). When the P concentration in leaf tissue is below the critical, the plant may rely upon reserves of available inorganic P present in the soil to meet its P requirements. The plant's growth rate increases with increasing P concentration until it reaches a maximum growth rate at

the critical leaf tissue P level. The results of this study suggest that in turfgrass with adequate P concentration in leaf tissue as well as large P reserves (i.e., P stored in thatch layer), growth and quality of the turf would be regulated by the depletion of P storage and concomitant decrease in leaf tissue P concentration over time but not by the size of the pool of plant available soil P. Waddington et al.(1978) evaluated the effect of P application (0 to 195 kg P ha^{-1}) on color and yield of 'Penncross' creeping bentgrass (*Agriostis palustris* Huds.) The P concentration in leaf tissue of the control treatment (no P added) was 5 g P kg^{-1} DM and in the 195 kg P ha^{-1} it was 8.4 g P kg^{-1} DM. They noted that increasing P application did not result in a significant difference in yield with respect to the control treatment, and concluded that greater response to P would have to result from P depletion of turfgrass in the control plots instead of greater P additions to the treated plots.

During 2008 St. Augustinegrass leaf growth rate was greater during the third fertilization period (July 9th to August 15th) and lowest at the beginning and end of the season (Table 5-5). A similar trend was observed for zoysiagrass leaf growth rate during 2008 (Table 5-6). Increase in solar radiation and temperature as the season progresses may likely explain the leaf growth rate pattern observed during the season. During the second growing season (2009) St. Augustinegrass leaf growth rate was significantly lower in the first half and it increased thereafter (Table 5-5). This trend may be related to a disease outbreak that occurred during the first half of 2009 growing season. Once the disease was controlled the turf growth and quality improved. Zoysiagrass leaf growth rate decreased during the second half of 2009 growing season (Table 5-6). The latter may have been related to water stress caused by malfunctioning

of the irrigation system. As the turf becomes water stressed, growth and uptake of nutrients could be impaired. On average across treatments and sampling times, St. Augustinegrass leaf growth rate was greater than in zoysiagrass in both evaluation years (Table 5-5, Table 5-6). Bowman et al. (2002) reported significantly greater total biomass of clippings in 'Raleigh' St. Augustinegrass than in 'Meyer' and 'Emerald' zoysiagrass.

Dry Matter and Phosphorus Partitioning

Overall dry matter (DM) partitioning was not affected by treatment in either turf species or evaluation years. During 2008, on average across treatments a total of 3.71 kg DM in St. Augustinegrass and 4.13 kg DM in zoysiagrass were accumulated per m^2 down to a depth of 15 cm (Table 5-7). The fraction of the total DM allocated to thatch tissue during 2008 was 85% in St. Augustinegrass and 87% in zoysiagrass (Table 5-7). During 2009, a total of 4.32 kg DM in St. Augustinegrass and 4.70 kg DM in zoysiagrass were accumulated per m^2 within the top 15 cm of the profile (Table 5-8).

Dry matter allocated to thatch tissue during the second growing season was 92% and 94% of the total DM accumulated per m^2 in St. Augustinegrass and zoysiagrass, respectively (Table 5-8). The slight increase in DM partitioning to thatch tissue between 2008 and 2009 (i.e. about 7% in both species), reflects the lower overall health and poorer condition of the turf during 2009. Root biomass measured during 2009 in the 15-30 cm layer of the profile was 45% lower in St. Augustinegrass and 78% lower in Zoysiagrass than in the top 15 cm layer (Table 5-8). No significant differences in root length density within the top 30 cm of the soil profile were found between 'Raleigh' St. Augustinegrass and two zoysiagrass cultivars (Bowman et al., 2002). However, at soil

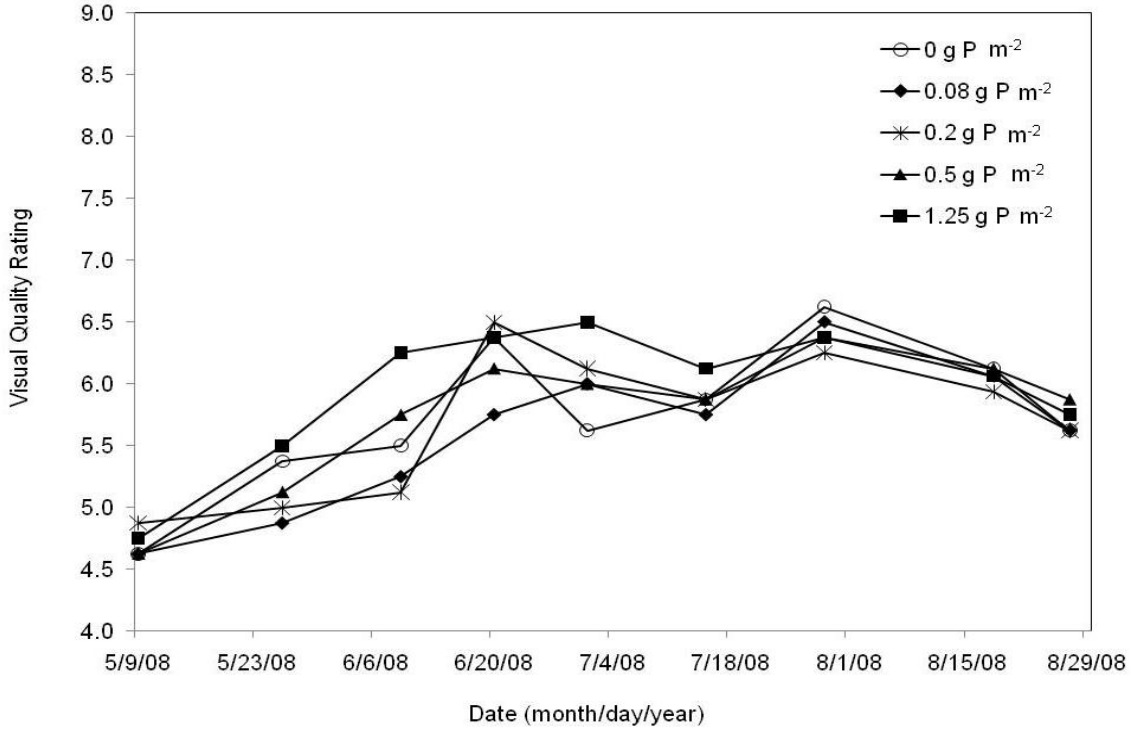
depths greater than 30 cm, the root length density in St. Augustinegrass was significantly greater than in zoysiagrass cultivars (Bowman et al., 2002).

Overall, P partitioning was not affected by P supply rate in either species (Table 5-9, Table 5-10). Only in the case of St. Augustinegrass during the first growing season, increasing P supply increased P partitioning to leaf tissue (Table 5-9). Phosphorus content in the thatch layer per m^2 was from 19 to 23-fold greater in St. Augustinegrass and from 53 to 56-fold greater in zoysiagrass than the cumulative P content accumulated in leaf tissue harvested during the growth season (Table 5-9, Table 5-10). The thatch layer represents a large P reservoir that can release P over time as it is mineralized by soil microorganisms. Berndt (2008) noted different microbial decomposition rates of thatch from two hybrids ('Tifdwarf' and 'Tifeagle') of bermudagrass (*Cynodon dactylon* L. Pers x *Cynodon transvaalensis* Burtt-Davy) associated to differences in lignin content and C:N ratio of these turfgrasses. On average across evaluation years the P reservoir in the thatch layer of the control treatment was as large as 26.2 kg P ha^{-1} in St. Augustinegrass and 29 kg P ha^{-1} in zoysiagrass (Table 5-9, Table 5-10). Maximum P application rate per year recommended in the state of Florida to urban turfgrass is 10.7 kg P ha^{-1} (State of Florida, 2007). In a soil with low P concentration, P supply from mineralization of the thatch layer could maintain adequate levels of P in leaf tissue for a long period of time. Phosphorus concentration in leaf tissue from turfgrass that had not been fertilized with P for over three years was 3.97 g P kg^{-1} in St. Augustinegrass and 2.90 g P kg^{-1} in Zoysiagrass, which in both cases are excessively high (Table 5-2).

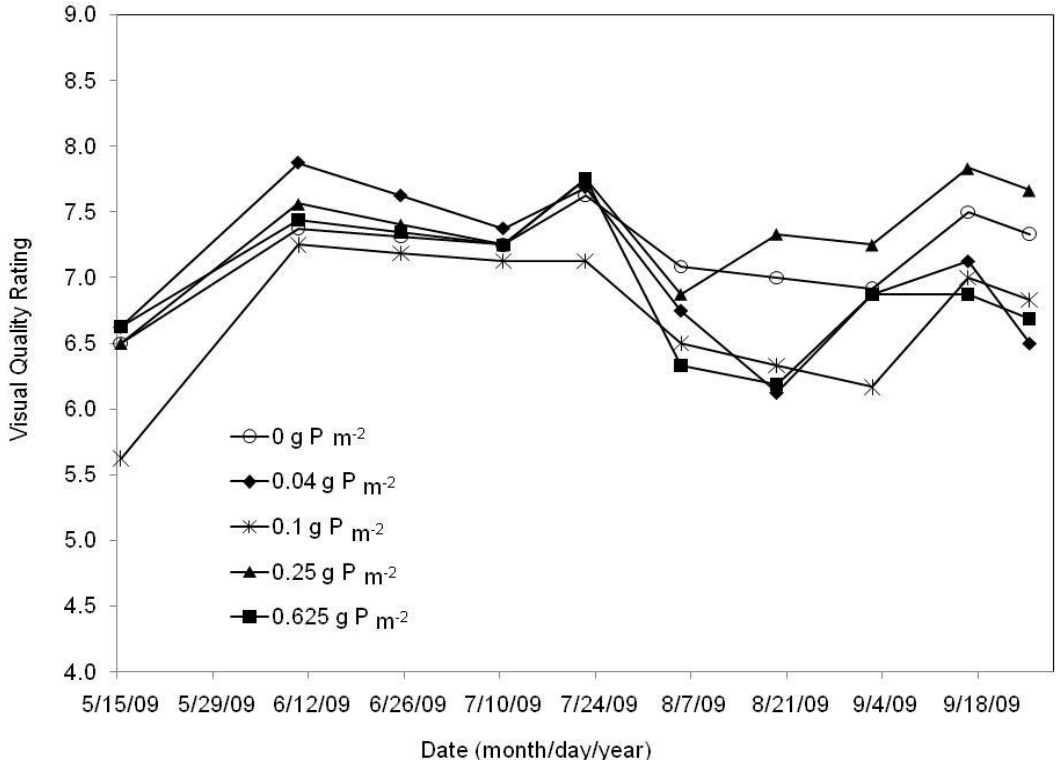
Phosphorus Use Efficiency

Phosphorus use efficiency was calculated as the ratio of leaf growth rate and P accumulation rate in leaf tissue over time (kg leaf DM per gram of P allocated to leaf tissue). Phosphorus use efficiency was inversely related to P application rate (Table 5-11, Table 5-12). On average across treatments and evaluation years, PUE of Zoysiagrass was 1.6-fold greater than in St. Augustinegrass. Averaged across rates and years St. Augustinegrass P uptake rate was 123% greater than in Zoysiagrass (Table 5-11, Table 5-12). Floratam St. Augustinegrass showed a tendency for luxury P consumption because it continued to accumulate P at a high rate even when the P concentration in leaf tissue was greater than the critical for maximum leaf growth (Figure 3-6). Since St. Augustinegrass leaf tissue P at the beginning of the experiment was above the critical, additional P uptake at a high rate would necessarily result in lower PUE. Under these conditions (excessive leaf tissue P concentration) any additional increase in leaf tissue P concentration would result in negative returns in terms of biomass accumulation. Hylton et al. (1965) noted a decrease in PUE of Italian ryegrass (*Lolium multiflorum* Lam.) with increasing P supply. In addition, they observed that the PUE of this turfgrass species reached a minimum at a leaf tissue P concentration near the critical for maximum growth. Furthermore, leaf tissue P concentration in St. Augustinegrass was significantly greater than in zoysiagrass (Table 5-1, Table 5-2) and PUE was inversely related to leaf tissue P concentration (Figure 3-7) and P supply (Table 5-11, Table 5-12). Consequently, under the conditions of this experiment comparing PUE in St. Augustinegrass and zoysiagrass without accounting for differences in leaf tissue P concentration is inadequate and misleading.

Differences in leaf tissue P concentration between species can be accounted for by expressing the PUE in relative terms, that is, in a scale between 0 and 1. The relative PUE of St. Augustinegrass was significantly greater than in zoysiagrass during both growing seasons (Table 5-11, Table 5-12). These results are in agreement with the relationships between RPUE and leaf tissue P concentrations obtained for these species under glasshouse conditions in hydroponic culture (Figure 3-8).

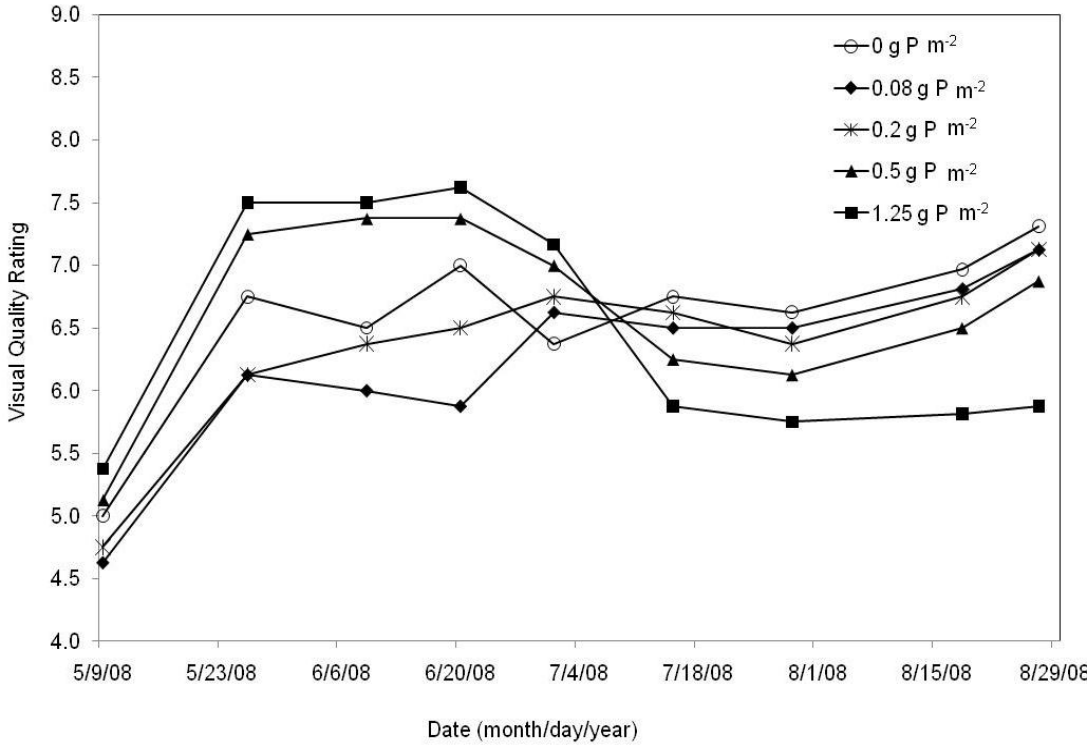


A

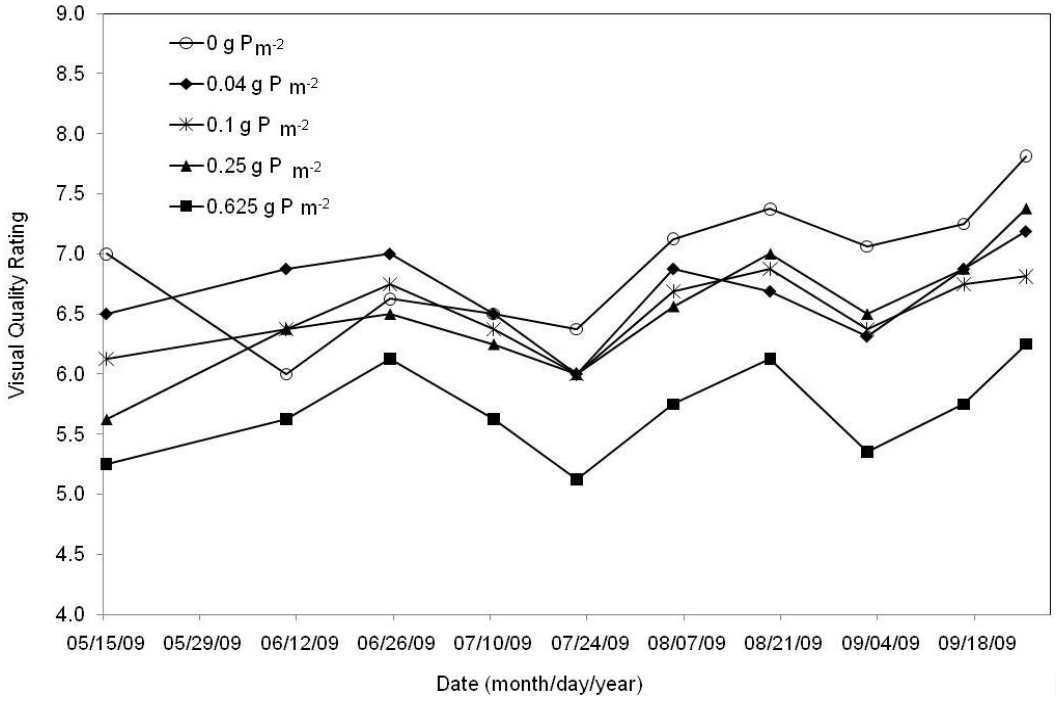


B

Figure 5-1. Empire zoysiagrass visual quality over time in response to phosphorus application rate. A) first growing season (2008) and B) second growing season (2009).



A



B

Figure 5-2. Floratam St. Augustinegrass visual quality over time in response to phosphorus application rate. A) first growing season (2008) and B) second growing season (2009).

Table 5-1. Mehlich 1 extractable soil phosphorus, leaf growth rate, phosphorus concentration in leaf tissue and visual quality of Empire zoysiagrass and Floratam St. Augustinegrass as influenced by phosphorus application rate during the first growth season (2008).

Application Rate	Floratam St. Augustinegrass				Empire Zoysiagrass			
	Mehlich 1 extractable	Leaf P concentration	Leaf growth rate	Visual quality rating	Mehlich 1 extractable	Leaf P concentration	Leaf growth rate	Visual quality rating
	mg P kg^{-1}	g P kg^{-1}	g m^{-2} day^{-1}		mg P kg^{-1}	g P kg^{-1}	g m^{-2} day^{-1}	
0 g P m^{-2}	2.27	4.21^{c}	3.32^{b}	6.7	2.52	2.50^{cd}	2.49	5.8
0.08 g P m^{-2}	1.61	3.81^{d}	2.38^{d}	6.3	2.92	2.38^{d}	2.14	5.6
0.02 g P m^{-2}	1.90	4.31^{c}	2.79^{cd}	6.4	3.15	2.67^{bc}	2.35	5.7
0.5 g P m^{-2}	2.70	4.83^{b}	3.17^{bc}	6.7	3.55	2.86^{ab}	1.96	5.8
1.25 g P m^{-2}	2.91	5.35^{a}	3.90^{a}	6.5	3.40	3.08^{a}	2.02	6.0
p-value	0.1966	<0.0001	<0.0001	0.1469	0.3968	<0.0001	0.6207	0.5448

Data points labeled with the same letter within a given column are not significantly different at p = 0.05 by single degree of freedom contrasts analysis.

Table 5-2. Mehlich 1 extractable soil phosphorus, leaf growth rate, phosphorus concentration in leaf tissue and visual quality of Empire zoysiagrass and Floratam St. Augustinegrass as influenced by phosphorus application rate during the second growth season (2009).

Application Rate	Floratam St. Augustinegrass				Empire Zoysiagrass			
	Mehlich 1 extractable	Leaf P concentration	Leaf growth rate	Visual quality rating	Mehlich 1 extractable	Leaf P concentration	Leaf growth rate	Visual quality rating
	mg P kg^{-1}	g P kg^{-1}	g m^{-2} day^{-1}		mg P kg^{-1}	g P kg^{-1}	g m^{-2} day^{-1}	
0 g P m^{-2}	4.56^{b}	3.97^{d}	3.14^{a}	6.8^{a}	3.79^{c}	2.90^{cd}	1.83	7.1^{ab}
0.04 g P m^{-2}	5.00^{b}	4.28^{c}	2.84^{b}	6.5^{a}	5.76^{bc}	2.83^{d}	1.81	6.9^{b}
0.01 g P m^{-2}	5.48^{b}	4.32^{bc}	2.70^{b}	6.4^{a}	6.54^{b}	3.11^{c}	1.89	6.7^{c}
0.25 g P m^{-2}	5.84^{b}	4.58^{ab}	2.64^{b}	6.5^{a}	9.66^{a}	3.38^{b}	2.05	7.3^{a}
0.625 g P m^{-2}	9.75^{a}	4.65^{a}	2.45^{b}	5.6^{b}	9.53^{a}	3.76^{a}	1.61	6.8^{b}
p-value	<0.0001	<0.0001	0.0258	<0.0001	<0.0001	<0.0001	0.4755	0.0069

Data points labeled with the same letter within a given column are not significantly different at p = 0.05 by single degree of freedom contrasts analysis.

Table 5-3. Chlorophyll index, percent green turf cover and dark green color index (DGCI) in Floratam St. Augustinegrass during the second growing season (2009) as influenced by phosphorus application rate.

Application Rate	Chlorophyll Index		Percent Green Turf Cover		Dark Green Color Index	
	2009-1	2009-2	2009-1	2009-2	2009-1	2009-2
0 g P m^{-2}	312[b]	331	90[b]	88	0.34	0.34
0.04 g P m^{-2}	354[a]	336	92[b]	89	0.34	0.34
0.1 g P m^{-2}	341[a]	345	93[a]	89	0.35	0.34
0.25 g P m^{-2}	311[b]	315	88[b]	88	0.34	0.33
0.625 g P m^{-2}	289[b]	326	88[b]	89	0.34	0.34
p-value	<0.0001	0.0858	0.0104	0.1979	0.286	0.1648

Data points labeled with the same letter within a given column are not significantly different at p = 0.05 by single degree of freedom contrasts analysis.

Table 5-4. Chlorophyll index, percent green turf cover and dark green color index (DGCI) in Empire zoysiagrass during the second growing season (2009) as influenced by phosphorus application rate.

Application Rate	Chlorophyll Index		Percent Green Turf Cover		Dark Green Color Index	
	2009-1	2009-2	2009-1	2009-2	2009-1	2009-2
0 g P m^{-2}	262[c]	278	87[a]	89	0.34[a]	0.35[a]
0.04 g P m^{-2}	284[ab]	273	88[a]	88	0.34[ab]	0.33[b]
0.1 g P m^{-2}	300[a]	287	87[a]	87	0.34[a]	0.33[b]
0.25 g P m^{-2}	282[abc]	291	88[a]	88	0.34[a]	0.34[b]
0.625 g P m^{-2}	270[bc]	269	82[b]	88	0.33[b]	0.33[b]
p-value	0.0073	0.4970	0.0406	0.7784	0.0313	0.0188

Data points labeled with the same letter within a given column are not significantly different at p = 0.05 by single degree of freedom contrasts analysis.

Table 5-5. Floratam St. Augustinegrass leaf growth rate (g DM m^{-2} day^{-1}) per fertilizer application period during each evaluation year as influenced by phosphorus application rate.

Application Period	g P m^{-2} 0(0)	0.08(0.04)	0.2(0.1)	0.5(0.25)	1.25(0.625)*	p-value**
2008-1	1.10c	0.44c	0.68c	0.91c	0.88c	0.0961
2008-2	3.71ab (a)	2.25b (b)	2.77b (ab)	3.65ab (a)	4.44ab (a)	0.0023
2008-3	4.77a (b)	3.80a (b)	4.32a (b)	4.49a (b)	5.70a (a)	0.0175
2008-4	2.60c	2.24b	2.45b	2.77b	3.45b	0.2281
Overall-2008	3.32	2.38	2.79	3.17	3.90	
2009-1	2.73b (a)	2.08b (b)	2.01b (b)	2.22b (ab)	1.60b (b)	0.0126
2009-2	3.55a	3.59a	3.39a	3.06a	3.30a	0.7279
Overall-2009	3.14	2.84	2.70	2.64	2.45	

* P application rates placed outside parentheses correspond to P application rates during 2008 (g P m^{-2} every 4 weeks). Values within parenthesis correspond to the P application rates during 2009 (g P m^{-2} every 8 weeks).

Data points labeled with the same letter within a given column and year are not significantly different at p = 0.05 by single degree of freedom contrasts analysis.

Data points labeled with the same letter between parentheses within a given row (fertilizer application period) are not significantly different at p = 0.05 by contrasts analysis.

** p-value for phosphorus application rate effect comparisons within a given fertilizer application period.

Table 5-6. Empire Zoysiagrass leaf growth rate (g DM m^{-2} day^{-1}) per fertilizer application period during each evaluation year as influenced by phosphorus application rate.

Application Period	g P m^{-2} 0(0)	0.08(0.04)	0.2(0.1)	0.5(0.25)	1.25(0.625)*	p-value**
2008-1	0.44b	0.41b	0.43c	0.33c	0.50b	0.8061
2008-2	3.49a	2.93a	3.23ab	2.22ab	2.92a	0.7397
2008-3	3.45a	2.93a	3.20a	3.04a	2.74a	0.8732
2008-4	1.71ab	1.65ab	1.84ab	1.43bc	1.11b	0.3303
Overall-2008	2.49	2.14	2.35	1.96	2.02	
2009-1	2.44a	2.38a	2.53a	2.47a	2.00a	0.8861
2009-2	1.22b	1.25b	1.25b	1.63b	1.23b	0.5612
Overall-2009	1.83	1.81	1.89	2.05	1.61	

* Values outside parentheses correspond to P application rates during 2008 (g P m^{-2} every 4 weeks). Values within parenthesis correspond to the P application rates during 2009 (g P m^{-2} every 8 weeks).

Data points labeled with the same letter within a given column and year are not significantly different at p = 0.05 by single degree of freedom contrasts analysis.

**p-value for phosphorus application rate effect comparisons within a given fertilizer application period.

Table 5-7. Leaf, thatch and root dry matter partitioning in Empire zoysiagrass and Floratam St. Augustinegrass as influenced by phosphorus application rate during the first growth season (2008).

Application Rate	Floratam St. Augustinegrass				Empire Zoysiagrass			
	Leaf	Thatch	Root 0–15 cm	Root 15-30 cm	Leaf	Thatch	Root 0-15 cm	Root 15-30 cm
	g DM m^{-2}	kg DM m^{-2}	kg DM m^{-2}	kg DM m^{-2}	g DM m^{-2}	kg DM m^{-2}	kg DM m^{-2}	kg DM m^{-2}
0 g P m^{-2}	29.75[ab]	3.41	0.46	nd	21.27	4.60	0.57	nd
0.08 g P m^{-2}	19.98[c]	2.54	0.61	nd	18.32	3.52	0.53	nd
0.02 g P m^{-2}	24.02b[c]	3.49	0.49	nd	20.10	2.92	0.64	nd
0.5 g P m^{-2}	28.30[ab]	2.87	0.55	nd	16.10	3.50	0.52	nd
1.25 g P m^{-2}	33.97[ab]	3.48	0.53	nd	17.77	3.37	0.42	nd
mean	27.21	3.16	.0.53	nd	18.71	3.58	0.53	nd
p-value	0.0044	0.4285	0.2381	nd	0.744	0.626	0.3257	nd

Data points labeled with the same letter within a given column and year are not significantly different at p = 0.05 by single degree of freedom contrasts analysis.
nd = data not available.

Table 5-8. Leaf, thatch and root dry matter partitioning in Empire zoysiagrass and Floratam St. Augustinegrass as influenced by phosphorus application rate during the second growth season (2009).

Application Rate	Floratam St. Augustinegrass				Empire Zoysiagrass			
	Leaf	Thatch	Root 0–15 cm	Root 15-30 cm	Leaf	Thatch	Root 0-15 cm	Root 15-30 cm
	g DM m^{-2}	kg DM m^{-2}	kg DM m^{-2}	kg DM m^{-2}	g DM m^{-2}	kg DM m^{-2}	kg DM m^{-2}	kg DM m^{-2}
0 g P m^{-2}	31.02	3.90	0.33	0.18	19.06	4.24	0.24	0.07
0.08 g P m^{-2}	27.38	4.33	0.34	0.19	18.77	5.07	0.26	0.06
0.02 g P m^{-2}	26.04	4.12	0.36	0.19	19.37	3.93	0.29	0.07
0.5 g P m^{-2}	26.10	3.69	0.35	0.14	20.76	4.09	0.29	0.06
1.25 g P m^{-2}	23.61	3.78	0.30	0.19	16.57	4.70	0.27	0.05
mean	26.83	3.96	0.33	0.18	18.91	4.41	0.27	0.06
p-value	0.2645	0.6266	0.3690	0.0684	0.8718	0.670	0.5864	0.4805

Data points labeled with the same letter within a given column are not significantly different at p = 0.05 by single degree of freedom contrasts analysis.

Table 5-9. Empire zoysiagrass and Floratam St. Augustinegrass leaf, thatch and root phosphorus content as influenced by phosphorus application rate during the first growth season (2008).

Application Rate	Floratam St. Augustinegrass				Empire Zoysiagrass			
	Leaf	Thatch	Root 0–15 cm	Root 15-30 cm	Leaf	Thatch	Root 0-15 cm	Root 15-30 cm
	g P m^{-2}	g P m^{-2}	g P m^{-2}	g P m^{-2}	g P m^{-2}	g P m^{-2}	g P m^{-2}	g P m^{-2}
0 g P m^{-2}	0.13[b]	2.51	0.35	nd	0.05	3.06	0.28	nd
0.08 g P m^{-2}	0.08[c]	1.59	0.46	nd	0.05	2.26	0.27	nd
0.02 g P m^{-2}	0.11[bc]	2.54	0.22	nd	0.06	2.08	0.38	nd
0.5 g P m^{-2}	0.14[b]	2.73	0.36	nd	0.05	2.85	0.36	nd
1.25 g P m^{-2}	0.19[a]	3.15	0.33	nd	0.06	2.98	0.30	nd
mean	0.13	2.50	0.34	nd	0.05	2.65	0.32	nd
p-value	<0.0001	0.1245	0.1389	nd	0.8978	0.6087	0.2764	nd

Data points labeled with the same letter within a given column are not significantly different at p = 0.05 by single degree of freedom contrasts analysis.
nd = data not available.

Table 5-10. Empire zoysiagrass and Floratam St. Augustinegrass leaf, thatch and root phosphorus content per evaluation year as influenced by phosphorus application rate during the second growth season (2009).

Application Rate	Floratam St. Augustinegrass				Empire Zoysiagrass			
	Leaf	Thatch	Root 0–15 cm	Root 15-30 cm	Leaf	Thatch	Root 0-15 cm	Root 15-30 cm
	g P m^{-2}	g P m^{-2}	g P m^{-2}	g P m^{-2}	g P m^{-2}	g P m^{-2}	g P m^{-2}	g P m^{-2}
0 g P m^{-2}	0.12	2.58	0.38	0.17	0.05	3.07	0.23	0.05
0.08 g P m^{-2}	0.12	2.75	0.33	0.14	0.05	3.14	0.26	0.04
0.02 g P m^{-2}	0.12	2.80	0.38	0.17	0.06	3.13	0.31	0.06
0.5 g P m^{-2}	0.13	2.64	0.39	0.11	0.07	3.54	0.30	0.05
1.25 g P m^{-2}	0.11	2.92	0.34	0.19	0.06	3.77	0.34	0.04
mean	0.12	2.74	0.36	0.16	0.06	3.33	0.29	0.05
p-value	0.9362	0.9171	0.5389	0.0696	0.6766	0.4404	0.0927	0.2634

Data points labeled with the same letter within a given column are not significantly different at p = 0.05 by single degree of freedom contrasts analysis.

Table 5-11. Empire zoysiagrass and Floratam St. Augustinegrass phosphorus uptake rate and use efficiency as influenced by phosphorus application rate during the first growth season (2008).

Application Rate	Floratam St. Augustinegrass			Empire Zoysiagrass		
	P Use Efficiency kg DM g^{-1} P	Relative P Use Efficiency	P Uptake Rate mg P m^{-2} day^{-1}	P Use Efficiency kg DM g^{-1} P	Relative P Use Efficiency	P Uptake Rate mg P m^{-2} day^{-1}
0 g P m^{-2}	0.26[b]	0.79[b]	15.0[bc]	0.43[b]	0.70[ab]	6.60
0.08 g P m^{-2}	0.29[a]	0.87[a]	10.6[d]	0.48[a]	0.75[a]	5.75
0.02 g P m^{-2}	0.25[b]	0.77[b]	13.5[cd]	0.42[bc]	0.67[bc]	7.01
0.5 g P m^{-2}	0.22[c]	0.68[c]	16.5[b]	0.38[cd]	0.61[bd]	6.27
1.25 g P m^{-2}	0.19[d]	0.61[d]	22.6[a]	0.35[d]	0.57[d]	6.88
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.7951

Data points labeled with the same letter within a given column are not significantly different at p = 0.05 by single degree of freedom contrasts analysis.

Table 5-12. Empire zoysiagrass and Floratam St. Augustinegrass phosphorus uptake rate and use efficiency as influenced by phosphorus application rate during the second growth season (2009).

Application Rate	Floratam St. Augustinegrass			Empire Zoysiagrass		
	P Use Efficiency kg DM g^{-1} P	Relative P Use Efficiency	P Uptake Rate mg P m^{-2} day^{-1}	P Use Efficiency kg DM g^{-1} P	Relative P Use Efficiency	P Uptake Rate mg P m^{-2} day^{-1}
0 g P m^{-2}	0.27[a]	0.87[a]	12.7	0.38[ab]	0.81[a]	5.46
0.08 g P m^{-2}	0.24[b]	0.79[b]	12.8	0.37[a]	0.82[a]	5.39
0.02 g P m^{-2}	0.24[b]	0.78[b]	12.2	0.34[bc]	0.75[b]	6.12
0.5 g P m^{-2}	0.23[b]	0.75[b]	12.5	0.32[c]	0.79[b]	7.10
1.25 g P m^{-2}	0.23[b]	0.75[b]	11.7	0.28[d]	0.63[c]	6.16
p-value	0.0005	0.0002	0.8378	<0.0001	<0.0001	0.3286

Data points labeled with the same letter within a given column are not significantly different at p = 0.05 by single degree of freedom contrasts analysis.

CHAPTER 6
SUMMARY AND CONCLUSIONS

Phosphorus is an essential plant nutrient. Adequate P nutrition is necessary to maintain sustainable levels of growth, density and quality of turfgrasses. Excessive P fertilization rates to turfgrasses growing in sandy soils with low P retention capacity may lead to P losses to the ground water. Enrichment of surface water bodies with P from anthropogenic sources has been link to eutrophication. Lawn turfgrasses represent an important land use in Florida; hence, losses of P associated to P fertilization of lawn turfgrasses could have an effect on P enrichment of ground water and surface water bodies.

St. Augustinegrass cultivar 'Floratam' is the most widely warm season lawn turfgrass species cultivated in Florida. In addition, 'Empire' Zoysiagrass is another widely adapted warm season turfgrass in Florida and the area planted with this turfgrass species has grown over the last years. Consequently, it is necessary to generate diagnostic tools that could allow assessing the need of P fertilization to these turfgrass species. A widely used concept to diagnose the nutritional status of plants is the critical nutrient concentration, either in soils or in plant tissues.

The critical leaf tissue P concentration in a turfgrass may be defined as the P concentration in leaf tissue that relates to maximum turfgrass growth, density and quality. Previous work on St. Augustinegrass conducted under glasshouse conditions revealed that the leaf tissue critical P concentration for this turfgrass species ranged between 1.6 g P kg^{-1} and 1.8 g P kg^{-1} (Liu et al., 2006; Liu et al., 2008). In these studies the critical leaf tissue P concentration determination was based solely on maximum turfgrass growth rate. The main objective of turfgrass culture is to obtain high quality

turfgrass from the aesthetics point of view; hence, maximum growth rate is not always a desirable attribute. Information regarding the critical leaf tissue P concentration in ‘Empire’ Zoysiagrass was not found. It is necessary to incorporate response variables associated with turfgrass aesthetic quality in the determination of critical P concentration in leaf tissue of these turfgrass species.

Phosphorus application rates in excess of plant requirements and the ability of the soil to retain P could lead to increased P leaching rates. Fluctuations in rainfall, especially when precipitation exceeds evapotranspiration could also affect the amount of P losses from fertilizer applied to turfgrasses. Previous research dealing with nitrate leaching from turfgrasses showed that differences in rooting depth among turfgrass species can have a significant impact on leaching losses (Bowman et al., 1998). There are many factors (and their interactions) that may influence P leaching from turfgrass systems. Limited information is available regarding P leaching from ‘Floratam’ St. Augustinegrass and ‘Empire’ zoysiagrass grown in Florida sands in response to inorganic P fertilizer application rates. Field studies to determine the maximum P application rate below which P leaching from these turfgrass species is minimized is warranted.

The overall objective of this research was to determine the critical leaf tissue P concentration for maximum sustainable levels of growth and quality in ‘Floratam’ St. Augustinegrass and ‘Empire’ Zoysiagrass and to identify the threshold P application rate that minimizes P leaching from these turfgrass species grown under field conditions. Specific objectives addressed in this study were the following:

(i) To determine critical P concentrations in leaf tissue of ‘Floratam’ St. Augustinegrass and ‘Empire’ Zoysiagrass based on leaf growth rate, visual quality and GC.

(ii) To evaluate the influence of leaf tissue P concentration on the rate of P depletion from solution (P influx) by these turfgrass species grown in hydroponic culture.

(iii) To study the effect of P supply and leaf tissue P concentration on dry matter and P partitioning as well as P-use efficiency in these turfgrass species under glasshouse and field conditions.

(iv) To evaluate the relationship between P supply and P_i leaching rate in ‘Empire’ Zoysiagrass and ‘Floratam’ St. Augustinegrass grown under field conditions.

(v) To assess the interaction between plant uptake, rainfall, irrigation, M1-P, soil PSR and SPSC with P_i leaching rate in these turfgrass systems.

(vi) To investigate the effect P supply rate, M1-P and leaf tissue P concentration on growth rate and visual quality in ‘Empire’ Zoysiagrass and ‘Floratam’ St. Augustinegrass grown under field conditions.

These objectives aimed to test the following hypotheses:

(I) Growth rate, visual quality and GC will increase with increasing leaf tissue P concentrations to a maximum leaf tissue P concentration beyond which no additional response to P supply will be observed.

(II) Rate of P depletion from solution by ‘Empire’ Zoysiagrass and ‘Floratam’ St. Augustinegrass grown in hydroponic culture will be inversely related to P leaf tissue P concentration.

(III) Greater P supply and leaf tissue P concentration will result in greater dry matter and P partitioning to leaf tissue.

(IV) Phosphorus use efficiency will be inversely related to P supply and leaf tissue P concentration.

(V) There is a maximum phosphorus application rate to ‘Floratam’ St. Augustinegrass and ‘Empire’ Zoysiagrass below which P_i leaching is minimized.

(VI) Rate of P_i leaching will be inversely related to plant growth, uptake rate and SPSC, and will increase with increasing rainfall and soil PSR.

(VII) Growth rate and turf visual quality will increase in response to increasing P application rate, Mehlich 1 extractable soil P and leaf tissue P.

In order to test these hypotheses, several experiments were conducted under glasshouse conditions to evaluate turfgrass response in terms of leaf growth rate, visual quality rating, CI, and percent GC to increasing P supply in hydroponic culture (0 to 456 mg P m^{-3} as KH_2PO_4). Percent green turf cover was measured using DIA and CI was determined with a CM 1000 chlorophyll meter. In addition, an experiment was carried out to evaluate the rate of P depletion from solution as related to P concentration in tissue. In this experiment, the change in solution P content was monitored over time and the total root length of absorbing roots was measured using root scanning techniques. Root depletion rate was expressed as µg P m^{-1} root hr^{-1}.

Field studies were conducted to evaluate the relationship between P application rate and P_i leaching using large HDPE lysimeters. Leachate samples were collected on a weekly basis and P_i concentration (leachate samples were filtered through 0.45 µm disposable filters) was measured within 48 hours of sample collection according to standard operating procedure approved by FDEP. Phosphorus application rates as concentrated superphosphate ranged between 0 and 5 g P m^{-2} $year^{-1}$ in 2008 and between 0 and 1.25 g P m^{-2} $year^{-1}$ during 2009. Moreover, the turfgrass responses to P application rate in terms of leaf growth rate, visual quality, CI, GC and DGCI were also evaluated.

The first objective of this research was to determine the critical leaf tissue P concentration in St. Augustinegrass and zoysiagrass. Evaluations were conducted during the period of the year of greater solar radiation and temperature, which coincide with greater plant growth and uptake rates.

Leaf growth rate increased quadratically with increasing leaf tissue P concentration. The critical leaf tissue P concentration for maximum zoysiagrass leaf growth rate was 1.67 g P kg^{-1} and for maximum turf visual quality was 1.7 g P kg^{-1}. The critical leaf tissue P concentration for maximum St. Augustinegrass leaf growth rate was 1.73 g P kg^{-1}.St. Augustinegrass visual quality increased linearly with leaf tissue P concentration; thus, no critical leaf tissue P concentration was identified for this explanatory variable. Acceptable St. Augustinegrass turf visual quality was attained at 1.15 g P kg^{-1}.

Digital image analysis could precisely measure differences in GC associated with changes in leaf tissue P concentration. Percent green turf cover increased quadratically with increasing leaf tissue P. The critical leaf tissue P concentrations for zoysiagrass and St. Augustinegrass maximum GC were 1.35 g P kg^{-1} and 1.48 g P kg^{-1}, respectively. The critical leaf P concentration for maximum growth rate was also sufficient to promote maximum turf density. Consequently, a P concentration in leaf tissue of 1.35 g P kg^{-1} and 1.67 g P kg^{-1} in the case of zoysiagrass and 1.48 g P kg^{-1} and 1.73 g P kg^{-1} for St. Augustinegrass could be used as the threshold concentrations for maintenance of maximum green turf density and maximum growth and recovery rates, respectively.

A CI level between 641 and 654 promoted maximum growth rate and turf visual quality of zoysiagrass. In addition, maximum GC in zoysiagrass was attained at a CI level of 479. Both leaf growth rate and turf visual quality of St. Augustinegrass increased linearly with increasing CI. A CI level of 319 or greater resulted in acceptable St.

Augustinegrass visual quality and maximum GC in St. Augustinegrass was attained at a CI level of 363.

Under the conditions of this experiment, an initial solution P concentration of 370 mg P m^{-3} and 382 mg P m^{-3} maximized zoysiagrass visual quality and leaf tissue growth rate, respectively. Maximum zoysiagrass density (evaluated with GC) was obtained at an initial P concentration in solution of 303 mg P m^{-3}. An initial solution P concentration of 335 mg P m^{-3} maximized GC of St. Augustinegrass, but no threshold initial solution P concentration was identified for St. Augustinegrass visual quality. Maximum St. Augustinegrass leaf growth rate was associated with an initial solution P concentration of 374 mg P m^{-3}. An initial solution P concentration of 203 mg P m^{-3} was necessary to maintain acceptable St. Augustinegrass visual quality.

The second and third objectives aimed to evaluate the effect on P supply and leaf tissue P concentration on the rate of P depletion from solution, dry matter and P partitioning and PUE in St. Augustinegrass and zoysiagrass grown in solution culture. The fraction of total DM per unit area produced by zoysiagrass allocated to leaf tissue increased with increasing P supply. Greater zoysiagrass leaf:root ratio was associated with increasing P supply. The percent of total DM accumulation in St. Augustinegrass allocated to leaves was positively related to P supply rate whereas the fraction of DM accumulation allocated to thatch decreased with increasing P supply. No treatment effect on DM partitioning to roots was observed in St. Augustinegrass. Zoysiagrass P concentration in leaf and thatch tissue was positively related to P supply rate.

Over 65% of the total P content per unit area of Zoysiagrass was accumulated in the thatch layer. Moreover, 58%, 26% and 16% of the total P content per unit area of St.

Augustinegrass turf was accumulated in thatch, leaf and root tissue, respectively. The fraction of the total P content allocated to zoysiagrass and St. Augustinegrass leaves increased with increasing P supply. Both P content in leaves as well as leaf tissue P concentration were positively related to the P storage in the thatch layer in both turfgrass species. The rate of P accumulation by zoysiagrass and St. Augustinegrass into leaf tissue over time increased linearly with increasing P supply.

Phosphorus depletion from the nutrient solution by zoysiagrass was inversely related to the P concentration in leave tissue. Phosphorus depletion rate from the nutrient solution by St. Augustinegrass did not change in response to P concentration in tissue. Phosphorus use efficiency of zoysiagrass and St. Augustinegrass were inversely related to P supply and P concentration in leaf tissue. Consequently, the factor that appears to limit leaf growth rate of St. Augustinegrass as the leaf tissue P concentration increases is the associated decrease in the PUE. Zoysiagrass minimum P depletion rate from solution as well as minimum PUE were related to a P concentration in leaf tissue between 1.58 and 1.65 g P kg^{-1} DM. These values were very close to the critical tissue P concentration required for maximum leaf growth rate (1.67 g P kg^{-1} DM). Minimum St. Augustinegrass PUE was reached at an initial P concentration in solution of 377 mg P m^{-3} and P concentration in leaf tissue of 1.65 g P kg^{-1}. These values are in close agreement with the P concentration in solution (374 mg P m^{-3}) and the leaf tissue P concentration (1.73 g P kg-1) required for maximum leaf growth rate of St. Augustinegrass. Over a wide range of leaf tissue P concentration St. Augustinegrass P influx rate and RPUE were greater than those measured in zoysiagrass.

The fourth and fifth objectives of this research were to determine the threshold P application rate to the turfgrass species that would not increase P leaching with respect to unfertilized turf, and to study the interaction between plant uptake, rainfall, irrigation, M1-P and soil PSR with P_i leaching rate.

Orthophosphate leaching rate and P_i concentration in leachate from zoysiagrass were greater than from St. Augustinegrass. Phosphorus uptake rate over time in St. Augustinegrass was greater than in zoysiagrass. The root system of St. Augustinegrass measured in this study was more extensive and deeper than in zoysiagrass and it likely helps to explain the greater P leaching measured in zoysiagrass. In the case of zoysiagrass, a P application rate of 0.8 g P m^{-2} $year^{-1}$ (4 applications of 0.2 g P m^{-2} $application^{-1}$) during 2008 and 0.2 g P m^{-2} $year^{-1}$ (2 applications of 0.1 g P m^{-2} $application^{-1}$) did not increase P_i leaching rate with respect to the unfertilized control treatment. In St. Augustinegrass plots, a P application rate of 5 g P m^{-2} $year^{-1}$ (4 applications of 1.25 g P m^{-2} $application^{-1}$) during 2008 and 0.5 g P m^{-2} $year^{-1}$ (2 applications of 0.25 g P m^{-2} $application^{-1}$) did not increase P_i leaching rate with respect to the unfertilized control treatment.

Rate of P_i leaching was positively related to amount of rainfall plus irrigation received by the turf. Phosphorus fertilization over time resulted in an increase of M1-P, PSR and a reduction of the SPSC. Greater volume-weighted P_i concentrations in leachate were measured from treatments with greater M1-P and PSR values and lower SPSC values. A volume-weighted P_i concentration in leachates ≤ 0.01 mg P l^{-1} (in compliance with most strict USEPA proposed numeric nutrient water quality criteria for the state of Florida surface waters) was measured in St. Augustinegrass treatments with

a soil PSR as high as 0.6. Total estimated amount of P leached from fertilizer application was fairly low in both turfgrass species.

The last objective was to assess the influence of P supply rate, M1-P and leaf tissue P concentration on leaf growth rate, turf visual quality, CI, GC and PUE on St. Augustinegrass and zoysiagrass grown under field conditions. Mehlich 1 extractable soil P and P concentration in leaf tissue were positively related to P application rate. Turf visual quality was inversely related to P application rate and P concentration in leaf tissue. Leaf growth rate was detrimentally affected by increasing P concentration in leaf tissue. The decrease of visual quality and growth rate in response to increasing P rate and leaf tissue P concentration is likely due to the excessively high P concentration in leaf tissue measured in this experiment.

Chlorophyll index, GC, and DGCI were not affected by P supply rate. No significant treatment effect on dry matter partitioning was observed. Only in the case of St. Augustinegrass during the first growing season, increasing P supply increased P partitioning to leaf tissue. The greatest fraction of the total dry matter and P accumulation per unit area of turf was allocated to thatch tissue. It is possible that mineralization of organic P from the thatch layer could supply P to growing tissues (i.e., leaves and roots) and maintain adequate tissue P concentrations over long periods of time. The leaf tissue P concentration in the unfertilized control treatments remained very high over the evaluation period (more than three years).

Phosphorus use efficiency was inversely related to P rate and P concentration in tissue. Phosphorus uptake rate and RPUE were greater in St. Augustinegrass than in zoysiagrass. The P uptake feedback mechanism suggested for zoysiagrass may allow

this species to avoid excessively high P concentration in tissue and maintenance of adequate visual quality and growth in naturally high P concentration soils or in P impacted soils from anthropogenic activities.

Based on the results of the various studies conducted, we can conclude that P fertilization is required to maintain adequate growth and quality of St. Augustinegrass and zoysiagrass. Excessively high P concentrations in leaf tissue associated with unnecessary P fertilization (when leaf tissue P concentration is above the critical level for maximum growth rate) to these turfgrass species would result in decreased quality and growth. Excessive P application rates to these turfgrass species can result in an increased rate of P leaching. Hence, if P fertilization is required based on tissue analysis and the SPSC is positive, a maximum P supply of 0.2 g P m^{-2} $application^{-1}$ or 0.8 g P m^{-2} $year^{-1}$ to zoysiagrass would be an environmentally safe application rate. Under the same conditions, a maximum P supply of 0.54 g P m^{-2} $application^{-1}$ or 1.07 g P m^{-2} $year^{-1}$ to St. Augustinegrass would not increase P leaching losses. These results indicate that the maximum P application rate to urban turfgrasses currently permitted in the state of Florida is adequate for St. Augustinegrass; however, it should be modified to account for turfgrass species and soil P status influence on the risk of P leaching.

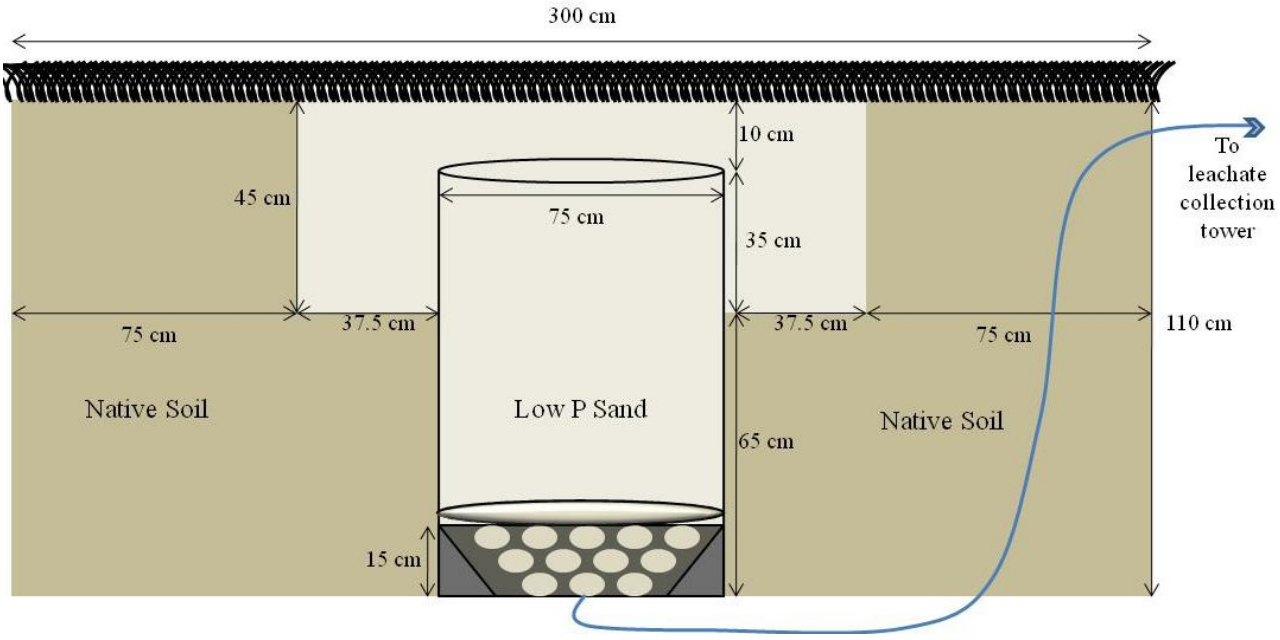


Figure A-1. Schematic description of lysimeter installation.

Table A-1. Empire Zoysiagrass and Floratam St. Augustinegrass root biomass, root length density, root surface area, root volume and average root diameter as influenced by phosphorus application rate.

Application Rate	Floratam St. Augustinegrass					Empire Zoysiagrass				
	Root Dry Matter (kg DM m^{-3})	Root Length Density (cm cm^{-3})	Root Surface Area (cm^2 cm^{-3})	Root Volume (cm^3 cm^{-3})	Root Diameter (mm)	Root Dry Matter (kg DM m^{-3})	Root Length Density (cm cm^{-3})	Root Surface Area (cm^2 cm^{-3})	Root Volume (cm^3 cm^{-3})	Root Diameter (mm)
2008					0-15 cm					
0 g P m^{-2}	2.64	2.42	0.37	4.46	0.49	3.23	3.02	0.36	3.53	0.38
0.08 g P m^{-2}	3.68	2.46	0.40	5.10	0.51	3.00	2.80	0.34	3.52	0.39
0.02 g P m^{-2}	2.82	2.51	0.36	4.02	0.48	3.66	2.99	0.40	4.38	0.43
0.5 g P m^{-2}	3.20	2.42	0.39	4.99	0.52	2.96	2.87	0.34	3.19	0.38
1.25 g P m^{-2}	2.75	2.65	0.39	4.60	0.47	2.40	2.53	0.28	2.39	0.35
Mean	3.02	2.49	0.38	4.63	0.49	3.05	2.84	0.34	3.40	0.38
p-value	0.2354	0.9410	0.9178	0.6838	0.7790	0.3301	0.1942	0.1335	0.1240	0.3182
2009					0-15 cm					
0 g P m^{-2}	2.18	1.18	0.21	3.06	0.57	1.63	1.22	0.17	2.04	0.44
0.08 g P m^{-2}	2.24	1.24	0.22	3.07	0.56	1.73	1.24	0.18	2.27	0.48
0.02 g P m^{-2}	2.37	1.23	0.21	3.16	0.57	1.90	1.27	0.19	2.49	0.48
0.5 g P m^{-2}	2.35	1.26	0.23	3.35	0.58	1.91	1.32	0.19	2.29	0.47
1.25 g P m^{-2}	1.97	1.27	0.21	2.88	0.53	1.77	1.26	0.18	2.20	0.46
Mean	2.22	1.24	0.21	3.11	0.56	1.79	1.26	0.19	2.26	0.47
p-value	0.0923	0.8814	0.7737	0.5027	0.5530	0.5669	0.2320	0.2595	0.4520	0.7980
2009					15-30 cm					
0 g P m^{-2}	1.18	0.75	0.13	1.85	0.54	0.44	0.58	0.07	0.59	0.34
0.08 g P m^{-2}	1.24	0.83	0.14	1.77	0.50	0.37	0.57	0.05	0.41	0.31
0.02 g P m^{-2}	1.26	0.86	0.14	1.93	0.52	0.48	0.58	0.06	0.58	0.35
0.5 g P m^{-2}	0.91	0.75	0.11	1.37	0.47	0.42	0.58	0.06	0.51	0.33
1.25 g P m^{-2}	1.26	0.88	0.15	1.98	0.50	0.36	0.52	0.05	0.44	0.32
Mean	1.17	0.81	0.14	1.78	0.51	0.41	0.56	0.06	0.51	0.33
p-value	0.0678	0.0525	0.0062	0.0334	0.1347	0.4200	0.9388	0.5545	0.3627	0.2460

Table A-2. U.S Environmental Protection Agency proposed numeric nutrient water quality criteria for Florida lakes.

Lake Type	Chlorophyll a (µg L^{-1})	Baseline Criteria	Modified Criteria [a]
		Total P (mg P L^{-1})	
Colored lakes[b]	20	0.050	0.050 – 0.157
Clear lakes, alkaline[c]	20	0.030	0.030 – 0.087
Clear lakes, acidic	6	0.010	0.010 – 0.030

[a]If Chlorophyll a in a given lake is less than the values indicated above for the corresponding lake type and there are representative data to calculate ambient-based, lake-specific, modified total P criteria, the Florida Department of Environmental Protection may calculate such criteria within these bounds from ambient measurements to determine lake-specific, modified criteria.
[b]Colored lakes have values of dissolved organic matter greater than 40 Platinum Cobalt Units (PCU). Clear lakes have values of dissolved organic matter ≤ 40 PCU.
[c]Acidic lakes have concentrations of $CaCO_3$ ≤ 50 mg L^{-1} $CaCO_3$.The concentration of $CaCO_3$ in alkaline lakes is > 50 mg L^{-1}.

Table A-3. U.S Environmental Protection Agency proposed numeric nutrient water quality criteria for free-flowing waters in Florida per watershed region.

Watershed region	In-stream protection value criteria
	Total P (mg L^{-1})
Panhandle	0.043
Bone Valley	0.739
Peninsula	0.107
North Central	0.359

Flowing waters include rivers, streams, creeks, canals (outside south Florida) and fresh water sloughs

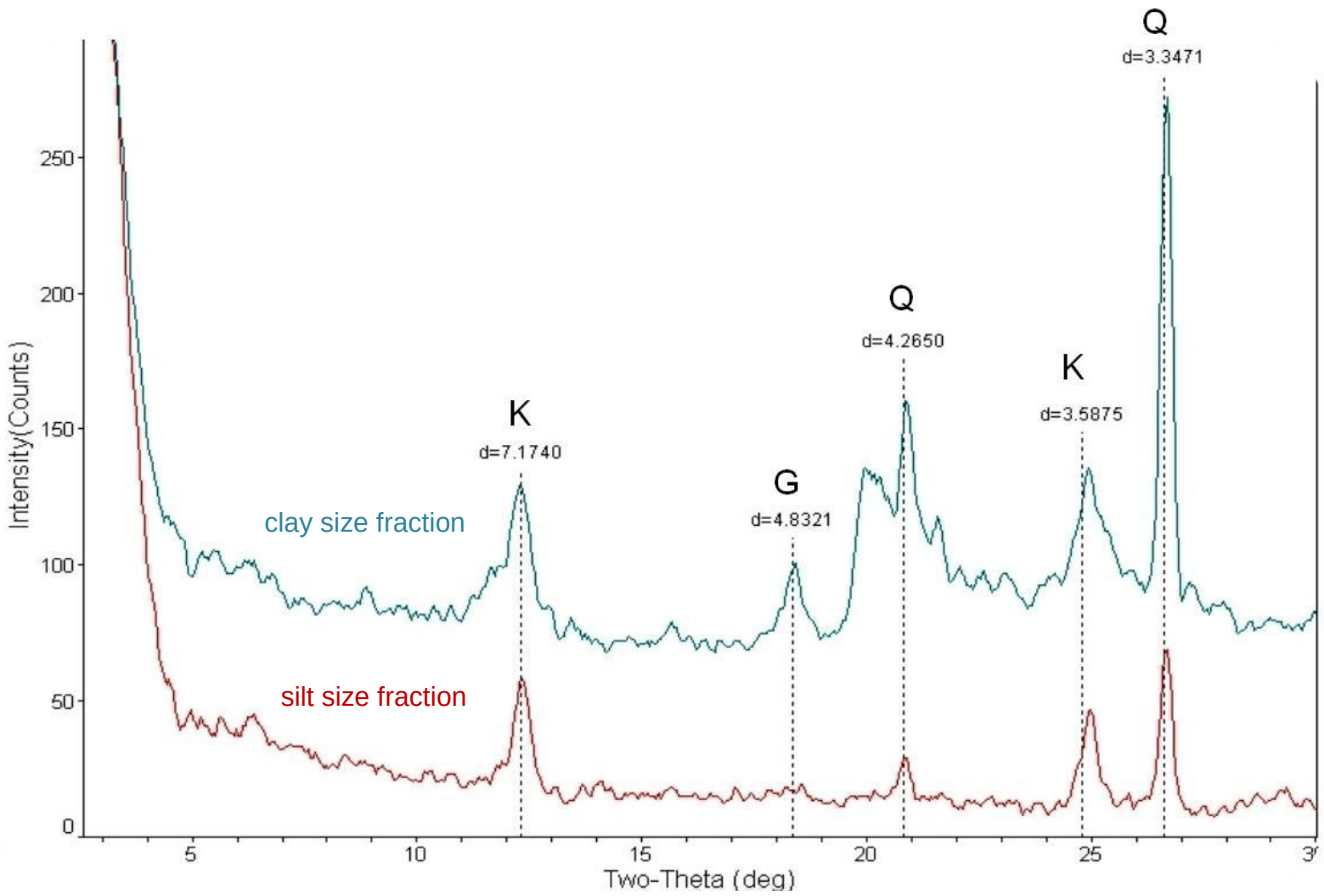


Figure A-2. X-ray diffraction patterns from the silt size fraction (red line) and clay size fractions (magenta line) of uncoated sand sampled prior to phosphorus application in 2008.The letters K, Q and G stand for kaolinite, quartz and gibbsite, respectively.

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BIOGRAPHICAL SKETCH

Ronald Gonzalez was born in the Costa Rican countryside. Ronald received a BS in Tropical Agriculture from EARTH University in Costa Rica. Later he joined the Soil Science Department of the University of Wisconsin-Madison where he obtained a master's degree. His research resulted in two publications: *Compost Effects on Soil Physical Properties* and *Field Nursery Production* which were published in *Compost Science and Utilization* and the *Journal of Environmental Horticulture*, respectively. Upon returning to Costa Rica, Ronald worked as an assistant scientist in soil fertility and pineapple nutrition. He applied to the Fulbright Program in 2006, and was selected as an International Fulbright Science and Technology Award grantee. In fall 2007, Ronald began his Ph.D. program in soil fertility and plant nutrition at the Soil and Water Science Department of the University of Florida in Gainesville.