CHAPTER 3

INFLUENCE OF HUMIC ACID ON MOISTURE RETENTION IN SIMULATED USGA PUTTING GREENS

ABSTRACT

Humic substances are routinely applied to creeping bentgrass putting greens during times of stress to improve turf health. Confounding effects of added nutrients and other ingredients make it difficult to separate the effects caused by the organic material from additional effects of the supplemental ingredients. Claims made in advertising and marketing of the effectiveness of humic substance products are often unsubstantiated, and the claim of increasing soil moisture retention has not been studied. Humic substance effects are also difficult to evaluate in the field because of confounding factors and variability, so pure humic acid without added nutrients or commercial additives was evaluated in a research greenhouse using simulated USGA putting greens. Organic acids, including a humic acid, tannic acid and citric acid, were applied at normalized carbon rates of 250 mg C L^{-1} as watering solutions through an automated irrigation system to creeping bentgrass. None of the organic acids improved moisture retention in sand. Humic acid actually decreased soil water holding capacity of the soil, drying down more quickly and requiring more frequent irrigations than the control. No increase in tissue phosphorus concentration was observed after treatment with humic acid, and no differences in top-growth, biomass production or dry shoot mass, were observed. Root growth and distribution was affected by the humic acid treatment, as turf irrigated with the humic acid produced significantly deeper roots than the control. All organic acids

induced hydrophobic tendencies in the sand, and root depth increases may have been influenced by this hydrophobicity. Surface soil hydrophobicity appeared to facilitate the movement of water into the subsurface, and consequently root growth may have followed water distribution. Golf course superintendents looking to reduce water and phosphorus fertilizer applications may not see a benefit using humic substance products.

INTRODUCTION

Creeping bentgrass (*Agrostis palustris* L.) is the predominant cool season grass grown and managed on putting greens in the Intermountain West region of the United States. While adapted to golf course conditions, both the climate and calcareous soil of the Intermountain West can impose difficult growing conditions for this turfgrass species. The transpiration gradient created by climatic factors during the summer can influence the amount of water needed for bentgrass growth. Plus, sand root zones have low water holding capacity that contributes to the increased frequency of irrigation needed on putting greens. Calcareous sand common in the Intermountain West may also buffer soil in an alkaline pH range (~ 7.5-8.5), making phosphorus and some micronutrients less available to the turf. Many golf course superintendents are often expected to reduce water use, especially during droughts, and minimize fertilizer applications while still maintaining extremely high quality turf. Thus, superintendents are always seeking for ways to be more efficient with their management practices while improving turf health. One practice that has gained popularity for its anecdotal ability to reduce irrigation and fertilizer applications is the use of natural organic products including those containing humic substances. However, there are still many questions regarding the effectiveness of these products, and what exactly these products can do for the putting green turf.

Humic substances are popular natural organic products used on golf courses. Humic substances are a component of soil humus, which can be divided into fractions of fulvic acid, humic acid and humin depending on their solubility as a function of pH (Stevenson, 1982). These fractions represent an operationally defined heterogeneous mixture of organic materials (MacCarthy et al., 1990) that are characterized as being yellow or black in color, of high molecular weight, and refractory (Aiken et al., 1985). Humic substances have been studied and used on a variety of agricultural crops for years, but only in the last twenty years have they been studied on turfgrass systems. Of the humic substances that have been studied humic acid is the most common, but responses by creeping bentgrass have been highly variable (Cooper et al., 1998).

It has been reported that humic substances have hormone-like effects on plant growth and metabolism (Chen and Aviad, 1990). This includes auxin effects (O'Donnell, 1973) and increased cytokinin levels in creeping bentgrass when treated with humic acid (Zhang and Ervin, 2004). However, Pertuit et al. (2001) suggested that growth responses from humic substances are due to increased micronutrient availability. Work done by Grossl and Inskeep (1991, 1992) showed that humic substances prolonged bioavailability of phosphorus in solution, and others have reported increased tissue levels of iron (Ayuso et al., 1996; Chen et al., 2004; Mackowiak et al., 2001), zinc (Ayuso et al., 1996; Carey and Gunn, 2000; Chen et al., 2004) and manganese (Ayuso et al., 1996; Liu et al., 1998). However, less mineral effects from humic substances have been reported on creeping bentgrass adequately supplied with nutrients (Cooper et al., 1998; Schmidt et al., 2003).

Humic substances increased plant antioxidant levels in Kentucky bluegrass under moisture stress conditions (Zhang and Schmidt, 1997), and increased photosynthesis in creeping bentgrass (Liu et al., 1998; Zhang and Ervin, 2004; Zhang et al., 2003b). Additionally, root mass (Liu et al., 1998) and length (Cooper et al., 1998) increased in creeping bentgrass treated with humic acid. However, similar growth responses have not been observed in the field (Carey and Gunn, 2000; Ervin et al., 2004). One possible reason for this lack of response is that the effects of humic substances are difficult to isolate due to confounding effects of nutrients and other ingredients often included in the products (Karnok, 2000).

Increasingly, products containing humic substances are appearing in the turf industry market. Claims have been made in advertising and marketing that humic substances increase soil moisture and nutrient availability. While positive growth effects of humic substances on creeping bentgrass have been well documented, the claim of improved moisture retention effects on putting greens has not been studied. This study tested natural organic acids, without nutrient additives, including a pure humic acid in a research greenhouse using simulated putting greens to test the effects humic acid might have on 1) increased water retention in sand putting greens, and 2) improved uptake of phosphorus by creeping bentgrass turf in calcareous sand.

MATERIALS AND METHODS

Creeping bentgrass (*Agrostis palustris L.*) sod was cut from a Utah State University research putting green at the Greenville Farm in North Logan, Utah, and grown in 24cm x 36cm x 30cm deep (9.5in x 14in x 12in deep) plastic tubs with calcareous sand (Staker-Parson Companies, Brigham City, UT) in a research greenhouse. The sand was mixed with sphagnum peat moss at 90% sand and 10% peat on a volume basis to match the soil of the sod, and roots were trimmed to 12cm (4.7 inches) before planting. This procedure followed that of Cooper et al. (1998), with some notable exceptions, including the different type of sand used. Once the sod was planted, tubs were placed in pairs of two into a larger plastic tub 48cm x 58cm x 24cm deep (18.9in x 22.8in x 9.5in deep) on top of 4cm (1.6 inches) of gravel, and holes were drilled in both tubs to provide drainage. The setup simulated a USGA putting green (Moore, 2004) and was a modification of methods previously described by Slavens (2006). Sheets of cardboard covered with aluminum foil were wrapped around the tubs to reflect solar radiation, and prevent excess heating of the root zones. The experiment was repeated three separate times with each run lasting three months, after an establishment period of approximately three months. The first run began 21 June, 2006 and went until 25 August, 2006, and was repeated 16 January, 2007 to 4 April, 2007 (run 2) and 30 July, 2007 to 30 October, 2007 (run 3). The experiment was a split-plot design with run as the whole-plot factor, and organic acid treatment the sub-plot factor. The tubs in individual runs were completely randomized with three replications (Figure 3-1).

Treatments

Three organic acids were applied to the turf as watering solutions during irrigation, and evaluated against a control treatment of de-ionized water. The organic acids used were reagent grade materials consisting of leonardite humic acid (Sigma-Aldrich Inc., St. Louis, MO), gallo-tannic acid (J.T. Baker Chemical Co., Phillipsburg, NJ) and citric acid, monohydrate (Mallinckrodt Chemicals, Phillipsburg, NJ). The materials were applied at normalized carbon rates of 250 mg C L^{-1} and over a range of oxygen functional groups. Humic acid contains 9% functional groups, with tannic acid containing 13% and citric acid 50% per molecule (Grossl and Inskeep, 1991, 1992). Humic acid is a humic substance, while citric acid and tannic acid are common ingredients in household products, including use as preservatives. Tannic acid was used instead of fulvic acid, a humic substance, due to the high cost associated with obtaining a sample of fulvic acid from the International Humic Substances Society. However, these two organic acids have similar functional group content (Grossl and Inskeep, 1991, 1992).

Solution samples of the treatments were analyzed for total organic carbon using a carbon analyzer that oxidized solution carbon to CO_2, which was subsequently detected by an infrared gas analyzer (Phoenix 8000 Tekmar-Dohrmann, Cincinnati, OH). The normalized carbon application rate was achieved by weighing 11.04g (0.39 ounces) of the humic acid, 9.44g (0.33 ounces) of the tannic acid and 10.05g (0.35 ounces) of the citric acid into bottles, and were filled up to 1L (33.8 fl. oz) with de-ionized water to make 2000 mg C L^{-1} stock solutions. From the stock solution, 312ml (10.5 fl. oz) of humic acid, 275ml (9.3 fl. oz) of tannic acid and 312ml (10.5 fl. oz) of citric acid were measured into bottles and filled up to 4L (135 fl. oz) with de-ionized water to make the 250 mg C L^{-1} watering solutions that were applied to the turf. This application rate and weight amounts of each organic acid were determined in a pilot study (Appendix A), and the application rate of the humic acid is similar to other greenhouse studies with creeping bentgrass (Cooper et al., 1998; Liu et al., 1998). Because the treatments were applied with every irrigation, the application rate was approximately 10 times the recommended field rate of a commercial humic acid product (H-85, Redox Chemicals Inc., Burley, ID). This application method simulated the repeated use of humic acid over consecutive years to test the effect the pure material had on moisture retention, nutrient availability and other growth benefits.

Sensor calibration

The treatments were delivered to the turf through an automated irrigation system that was triggered by the volumetric water content (VWC) of the soil as measured by a soil moisture probe. A capacitance soil moisture probe (ECH_20 probe model EC-20, Decagon Devices Inc., Pullman, WA) 20cm (8 inches) in length, was buried 13cm (5 inches) deep in the profile of each tub and connected to an AM16/32 multiplexer and CR10X datalogger (Campbell Scientific, Logan, UT). These probes have been reported to be an effective tool for measuring water content of soils (Blonquist et al., 2005; Jones, et al., 2005). ECH_2O probes were calibrated for the soil according to manufacturers' specifications (Decagon Devices Inc., 2007) to derive soil specific volumetric water content. This calibration was similar to Slavens (2006), but is described as follows. The calibration was performed in a separate set of tubs 31.5cm x 18.5cm and 10.0cm deep (12in x 7in and 4in deep). Each probe was individually calibrated to ensure accuracy and reduce variation. A probe was oriented horizontally within the tub containing the sand/peat media previously described. The media was packed with a closed fist around the probe to ensure good contact, and the probe was buried 6 cm (2.4 inches) below the soil surface to reduce air temperature fluctuations. The voltage output of the sensor was recorded, and the mass water content was obtained by averaging three soil samples taken from the tub. Calibration was done over a range of soil water contents by adding 250ml (8.5 fl. oz) of water to the soil after voltage outputs and mass water contents were recorded. Calibration began with dried soil (~ -1500 kPa) and water was added until the soil was completely saturated (~ 33 kPa).

Volumetric water contents were obtained by multiplying the mass water content by the bulk density of the soil (1.65 g cm^{-3}) divided by the density of water (1 g cm^{-3}). These values were plotted against the millivolt (mV) output of the ECH_2O probes (Figure B-1). A calibration equation was obtained from the slope of the line (y= 0.0007x - 0.2328) which was used in the datalogger program. The calibration was verified by gravimetric measurements of soil cores taken near the sensors at the end of each run to ensure the probes were accurately measuring water content (Table B-1).

Irrigation system

Based on work done by Slavens (2006), and evaluations of turf health during establishment, 10 % volumetric water content was determined to be the lowest soil moisture level that did not cause stress to the turf in this study. When the volumetric water content of the soil decreased below 10% in any individual tub, irrigation was triggered to that tub by a SDM16AC relay controller (Campbell Scientific, Logan, UT) which turned on a pump and valve to supply the treatments to the turf.

The irrigation system of each treatment included a 32L (8.5 gallon) plastic collection tub, one electric diaphragm pump and three solenoid valves, one for each tub in the replicate. Irrigation lines connected to the valves were polyurethane pipe, and four spray stake emitters (Landscape Products Inc., Tolleson, AZ) connected to the irrigation lines by drip tubing applied the watering solutions to the turf during irrigation. The emitters applied approximately 1L (33.8 fl. oz) of water per minute at 206.8 kPa (30 psi), and pressure was maintained with a bypass line back into the collection tub set with a pressure gauge that ensured an accurate application amount. Routine checks of the irrigation system were made every two to three weeks to ensure all emitters were working correctly, and the system was operating at the proper pressure. Based on a calculation of the soil root zone volume, a total of 3L (101 fl. oz) of water was needed to saturate the soil profile during irrigation. This was achieved by applying the treatments with a "cycle/soak" irrigation program consisting of three, one minute cycles with five minutes of soaking in between. New watering solutions were made based on the irrigation frequency of the treatments, to keep the collection tubs from becoming empty.

Turf management

Management of the turf simulated golf course putting green management. Turf was mowed at approximately 6mm (0.24 inches) with electric grass shears at least twice each week. Fertilization included 48 kg N ha^{-1} (1 lb N per 1000ft^2) with a 24-6-12 + minors granular fertilizer (Andersons, Golf Products, Maumee, OH) at planting to establish the turf. During the study weekly applications of KNO_3 (Fisher Scientific, Pittsburgh, PA) at 5 kg N ha^{-1} (0.1 lb N per 1000ft^2) were applied as a drench in 500mL (17 fl. oz) of water. No phosphorus was applied during the three month experiment, except for 10 kg P ha^{-1} (0.21 lb P per 1000ft^2) that was applied during establishment with the 24-6-12 + minors granular fertilizer. Top-dressing was used to control thatch. A wetting agent (Cascade, Precision Laboratories, Inc., Waukegan, IL) was applied according to label directions in two split applications of 3ml m^{-2} (8 oz per 1000ft^2) using a CO_2 backpack sprayer at 275.8 kPa (40 psi). The first application was applied at the beginning of each run in the experiment, and the second was applied one week later. An extra wetting agent application was applied to all the tubs during the third run of the experiment, because low infiltration rates of the watering solutions that occurred in some treatments approximately two months into the study. Syringing, the process of applying small amounts of water to cool the turf canopy, occurred as needed throughout the experiment to prevent dry spots and loss of turf. Syringing was done with a hose applying approximately 300mL (10 fl. oz) of water to each tub, even to those not showing dry spots, to keep the amount of water applied to each tub the same. Syringing was done more frequently at the beginning of each run in the experiment, and was eventually not required later in the study once the turf was well established. Syringing was required less often during run 2. This was likely due to lower light levels in the greenhouse during run 2.

Greenhouse temperature was maintained at 24°C (75.2°F) during the day and 16°C (60.8°F) at night throughout the study. High-pressure sodium lamps (1000w) were used to supplement solar radiation when light levels dropped below 300 watts m^{-2}, and day length was maintained at least 12 hours and controlled with a timer. No pesticides were used during the course of the study.

Evaluation of treatments

The volumetric water content data was stored in the datalogger and analyzed for possible differences in the number of days between irrigations and the total number of irrigations. Leaf tissue was collected during each mowing with a hand-held vacuum. The tissue was bagged, oven-dried at 80°C (176°F) for at least 24 hours, and combined over the length of each run. Tissue was also weighed for differences in total biomass production. Leaf tissue was collected at the end of each run and analyzed in a lab (USU Analytical Laboratories, Logan, UT) for elemental content, most notably for phosphorus. Tissue was also collected prior to each run of the experiment to provide a baseline of tissue elemental concentrations. Root length and mass measurements were made at the end of each run. Root length in each tub was measured by averaging the length of six cores pulled from the sod using a 30cm (12 inch) long soil probe with a 1cm (0.4 inch) inside diameter. Cores were taken from the center of the sod along the length of the soil moisture sensor, by pushing the probe to the bottom of each tub. Root length was determined from the first visible, attached root and measured from the crown. After measuring root length, the samples were washed, and leaf tissue was cut from the roots at the crown. The leaf (shoot) and root samples were bagged and oven-dried at 80°C (176°F) for two days. Tissue samples were weighed and the weights averaged for the respective shoot and root dry weights for that tub. Root and shoot tissue masses were evaluated for differences, as well as the root: shoot ratio, for a measure of overall plant health.

Statistical analysis

Days between irrigations, number of irrigations, tissue phosphorus level, biomass production, root length, root mass, shoot mass and root-shoot ratio were all analyzed for differences using the PROC MIXED analysis (SAS Institute, 2003). Analysis was done as a split-plot design, with run as the whole-plot factor, and organic treatment as the sub-plot factor. Replication, or block, and all interactions with replication were considered random. Residual diagnostics were done to confirm the approximate normal distribution of all data. Random factors and error terms were assumed to be identically distributed with equal variances.

RESULTS AND DISCUSSION

Irrigation frequency

Days between irrigations were significantly influenced by the treatments (P=0.02) (Table 3-1; Table D-5). Citric acid (14 days) and the control (13.9 days) went the longest between irrigations, both having a significantly higher number of days between irrigation than the humic acid treatment (11.3 days) which dried down the quickest (Table 3-1). Even though the humic acid dried down quicker than the control, the number of irrigations were not significantly influenced by the treatments (P=0.07), suggesting the tubs received approximately the same amount of water. However, the humic acid treatment did have the highest number of irrigations on average (Table 3-1). Zhang and Ervin (2004) were not evaluating differences in volumetric water content, but they reported uniform moisture loss in creeping bentgrass plugs in pots treated with humic acid during a 28 day dry down study. However, Zhang and Ervin (2004) used a different media, (2:1; silt loam to sand by volume), that had a higher water holding capacity than the sand used in this study.

All organic acid treatments, as well as the control demonstrated hydrophobic properties that repelled water throughout the study, getting worse as the study went on. In fact, the humic acid treatment contributed to lower moisture retention than the control. This seemed to decrease the amount of water in the soil due to hydrophobic properties (Karnok and Tucker, 2001), thus reducing the amount of water available to the roots in the profile. The infiltration of water was so poor during the third run of the experiment that an additional wetting agent application was needed. Similar results were found in the field with these organic acids and commercial humic substance products, as the volumetric water content of sand was not increased in putting greens. The volumetric water content of soil treated with humic acid was found to be approximately 1% lower than the control (Chapter 4). This reduction in moisture retention of soil may increase the frequency of water needed to be applied to the turf, and may not reduce or conserve water. Prolonged use of humic substances may contribute to greater hydrophobicity (Murphy et al., 1990), and increased localized dry spots (Karnok and Tucker, 1999; Miller and Wilkinson, 1979) requiring the use of wetting agents (Karnok and Tucker, 2001) or high pH treatments (Karnok et al., 1993) together with the humic substances as a potential way to deal with this problem.

Phosphorus uptake

Phosphorus uptake as measured by tissue concentration not significantly influenced by the treatments (P=0.22) (Table 3-1; Table D-5). Experimental run had a significant effect (*P*=0.003), and was most likely due to the different light intensity levels of run 2 during the winter months, that reduced plant vigor, and had lower tissue levels of phosphorus (Table B-2). The tissue concentration of phosphorus was highest in the control (0.38%) compared to the organic acids, where turf irrigated with tannic acid had the lowest concentration on average (0.34%) (Table 3-1). The uptake of phosphorus was not improved in the field with humic acid, as the control actually had significantly higher levels than turf treated with humic acid (Chapter 4).

Variable results of improved phosphorus uptake by humic acid have been reported in other studies with creeping bentgrass, as there was no increase in tissue concentration reported in sand (Liu et al., 1998) or solution (Cooper et al., 1998) with foliar application, but was increased when humic acid was incorporated into sand (Cooper et al., 1998). No phosphorus increases were found in rough fescue (Dormaar, 1975) or perennial ryegrass (Guar, 1964). Humic substance products may not improve uptake of phosphorus in turfgrass systems because grass plants are efficient at obtaining phosphorus (Christians, 2004). In fact, creeping bentgrass has been reported to obtain adequate amounts of phosphorous even at low levels (Johnson et al., 2003). Additionally, high doses of humic acid may cause excess chelation of nutrients, reducing availability to the roots (Ayuso et al., 1996).

Although not an original objective other nutrient levels in creeping bentgrass, as measured by tissue concentration, were significantly influenced by the treatments (Table B-3; Table D-6). Most notably was the high amount of sodium (Na) associated with the humic acid treatment (1185 mg kg^{-1}), but the tissue concentration was not significantly higher than the control. The high concentration of Na observed with the humic acid treatment is most likely due to high levels still present from the sodium hydroxide extraction process. High Na may not be present in all humic substances products applied to turf, but Rossi (2004) found increased levels in some commercial products. Excess Na may contribute to poor soil structure and poor water infiltration, but may not be a concern on sand. However, other problems such as inhibition of other cations being absorbed by the plant may be a problem (Carrow and Duncan, 1998).

Macronutrient tissue concentrations of potassium (K) and calcium (Ca) were significantly influenced by the treatments, but tissue concentrations of magnesium (Mg) and sulfur (S) were not affected (Table B-3; Table D-6). Tissue concentration of K was significantly higher in turf irrigated with citric acid (2.64%) and the control (2.59%), compared to turf irrigated with the tannic acid treatment (2.20%). The Ca tissue concentration was significantly higher in turf irrigated with tannic acid (0.56%), compared to turf irrigated with the humic acid treatment (0.47%). Differences in Ca uptake observed between these two treatments may have been influenced by the Na in the soil, based on Na tissue concentrations. Turf irrigated with the humic acid treatment had tissue levels of 1185 mg Na kg^{-1}, compared to turf irrigated with tannic acid which had 46 mg Na kg^{-1} on average.

Other work has reported no differences in K tissue levels with humic acid application in greenhouse sand culture (Cooper et al., 1998; Liu et al., 1998). However, tissue concentrations of K were decreased in solution culture (Cooper et al., 1998). Calcium tissue levels were decreased (Liu et al., 1998), with no difference in solution culture (Cooper et al., 1998). Magnesium levels were not different in solution culture (Cooper et al., 1998), but increased in sand culture (Liu et al., 1998) while tissue levels of

S were increased in sand culture (Liu et al., 1998), but decreased in solution (Cooper et al., 1998). Tissue concentration of S was also decreased in the field on fairway height creeping bentgrass, with no differences reported for K, Ca or Mg (Carey and Gunn, 2000).

Micronutrient metal tissue levels of copper (Cu), zinc (Zn), and manganese (Mn) were significantly influenced by the treatments, but tissue concentrations of iron (Fe) were not affected (Table B-3; Table D-6). Tissue concentration of Cu, Zn, and Mn were significantly higher in turf irrigated with tannic acid compared to the other treatments. This suggests that lower molecular weight humic substances (fulvic acid) are better at forming metal bridges thus improving availability of these nutrients supporting results of Grossl and Inskeep (1991, 1992), Zalba and Peinemann (2002) and Nardi et al. (2002).

In similar studies where humic acid was applied to creeping bentgrass, no tissue differences were observed for Fe (Cooper et al., 1998; Liu et al., 1998), Cu was decreased (Liu et al., 1998) and Zn was unaffected (Liu et al., 1998). In the field, only Zn and Cu tissue levels were increased on fairway height creeping bentgrass, with Mn tissue levels unaffected (Carey and Gunn, 2000). Although irrigation with organic acids caused significant increases or decreases in tissue concentration of nutrients in this study, the differences were relatively small, and do not appear to play a biological role.

Tissue growth

Above ground biomass was not significantly influenced by the treatments (P=0.47) (Table 3-1; Table D-5). Experimental run had a significant effect (P=0.02), and may be explained by turf mowing techniques becoming improved over time. This improvement in the methods collected more tissue, and resulted in average tissue biomass increases for each experimental run (Table B-2). Shoot dry mass was not significantly influenced by the treatments (*P*=0.13) (Table 3-1; Table D-5), but experimental run had a significant effect (*P*=0.02). This can be explained by improved methods over time for the washing and separation of the leaf tissue samples (Table B-2).

This result of no increase in shoot dry mass in this study is different than reports from Zhang and Schmidt (2000) and Zhang and Ervin (2004). Due to the methodology of measuring shoot mass in this study, few differences were expected due to the same size of the soil core, the dense growth habit of creeping bentgrass and the low mowing height imposed on the turf. Overall biomass production was a better determination of the possible enhanced top growth by the organic acids in this experiment and no significant differences were observed between the treatments. However, the humic acid treatment had the greatest biomass production on average (Table 3-1). Although the experimental runs had a statistically significant effect for biomass production and shoot mass dry weight, the same collection procedures were followed for each tub in each run. Given the lack of statistical significance between the treatments for biomass (*P*=0.47) and shoot mass (*P*=0.13) the differences do not appear to play a biological role.

Even though visual ratings were not performed in this study, it appeared that turf irrigated with citric acid had small, narrow leaf blades that were light-green in color, whereas turf irrigated with humic acid had wider, darker green leaf blades, similar to what was reported on tomato (Pertuit et al., 2001). However, the dark color observed with the humic acid treatment could have been due to residual staining from the material, which may have given the leaf tissue the appearance of being darker. However, no overall differences in top-growth (biomass) were measured with humic acid application. It should be noted that the application rate of 250 mg C L^{-1} with the humic acid every time the turf was irrigated did not result in necrosis or any visual damage to the turf.

Root growth

Root depth was significantly influenced by the treatments (*P*=0.0006) (Table 3-1; Table D-5). At planting roots were trimmed to 12cm (4.7 inches), and by the end of the experiment roots in tubs irrigated with humic acid measured 21.5cm (8.5 inches) and were significantly longer than roots for the tannic acid, control and citric acid treatments (Table 3-1). Over the course of the study, roots in tubs irrigated with humic acid grew an average of 9.5cm (3.7 inches) compared to roots of the control which grew 5.7cm (2.2 inches). This is a 60% increase in root depth associated with irrigating turf with humic acid in this study. Experimental run had a significant effect (*P*=0.0006), and can be explained by improved methods over time (Table B-2), but the same measurement procedures were followed for each tub in each experimental run. Irrigating with humic substances is not practical in the field, but the results imply that repeated use of humic acid may increase root depth on putting greens.

Root dry mass was not significantly influenced by the treatments (*P*=0.39) (Table 3-1; Table D-5). However, turf irrigated with humic acid had the highest root mass on average (Table 3-1). The root: shoot ratio was not significantly influenced by the treatments (*P*=0.25) (Table 3-1; Table D-5), although turf irrigated with humic acid had the greatest ratio of root growth to shoot growth (Table 3-1). Experimental run had a significant effect (*P*=0.007). This can be explained by improved methods over time.

Improving the cleaning methods of the samples resulted in an average decreases for the root: shoot ratio with each experimental run (Table B-2). However, the same measurement procedures were followed for each tub in each run.

Increased rooting with application of humic acid was found on Kentucky bluegrass (Zhang and Ervin, 1997; Zhang et al., 2003c) tall fescue (Zhang et al., 2003a) and other crop plants (Arancon et al., 2004; Pertuit et al., 2001; Sharif et al., 2002), but results on creeping bentgrass have been variable. Humic acid had no effect on root re-growth, and actually reduced root length at low levels, although 400 mg humic acid L^{-1} visually produced more developed roots (Liu et al., 1998). Additionally, no overall differences of root length have been reported with five different humic acid materials, but the incorporation of a granular Menefee humic acid into the rootzone has produced significantly longer roots, and produced greater root mass deeper in the root zone (Cooper et al., 1998). Root mass increased on creeping bentgrass seedlings (Zhang and Schmidt, 2000), but root mass of bentgrass plugs treated with humic acid did not increase unless applied in combination with seaweed (Zhang and Ervin, 2004). Additionally, root growth of salt stressed creeping bentgrass was not increased by humic acid (Liu and Cooper, 2002). Finally, root growth responses of creeping bentgrass in the field have also been variable with increases reported (Hunter and Anders, 2004), no increases also observed (Dorer and Peacock, 1997; Carey and Gunn, 2000), and no increase in root strength reported (Ervin et al., 2004). Perhaps root growth responses observed in these studies, or lack thereof, are related to the application rate of the humic substances to the soil.

Recommended field application rates of commercial humic substance products may not influence bulk soil organic matter content. However, their use on putting greens may influence the natural effect of organic material breakdown, and coating sand particles causing hydrophobic conditions and localized dry spots (Karnok and Tucker, 1999). Even at label rates humic substance products decreased the volumetric water content of sand putting greens (Chapter 4). Applying humic substances at high rates, like in this study and other greenhouse experiments (Cooper et al., 1998; Liu et al., 1998), may contribute to greater soil hydrophobicity compounding the effects of natural localized dry spot formation (Karnok and Tucker, 1999; Murphy et al., 1990). This may explain the increased rooting found in sand culture, and why no rooting differences were seen in solution culture (Cooper et al., 1998). Incorporating humic acid into the root zone, or applying heavy, frequent amounts may increase already high levels of humic substances in reed sedge peat and other materials in root zone mixes that accumulate in putting greens over time (Kerek et al., 2002).

We hypothesize that root growth increases observed on creeping bentgrass in this study, are the result of the hydrophobic nature of humic substances decreasing water in the profile. We surmise that the humic acid bound onto the sand particles increasing their hydrophobicity, consequently facilitating the downward movement of the water through the sand profile. Thus, hydrophobicity enhanced downward channeling relative to systems comprised of sand only. Furthermore, this may have influenced root distribution in the profile, as the movement of water into the subsurface may have caused root growth to follow water distribution. This may have been a physiologic root growth response and not a stimulatory effect on root growth from the humic acid. Increased root respiration and carbohydrate allocation to plant roots reported in Liu et al. (1998), may be due to this possible physiological root growth response following water movement deeper in the profile. This possible influence on root distribution may have had an effect on the nutrient uptake of creeping bentgrass. Even though no phosphorus was applied during the study, except during establishment, fewer roots in the upper rootzone may not have accessed available phosphorus if fertilizers were surface applied, as would be done on a putting green. This may explain why no increases in tissue phosphorus levels were observed in other greenhouse studies with creeping bentgrass (Cooper et al., 1998; Liu et al., 1998) or in the field (Carey and Gunn, 2000; Hunter and Anders, 2004). In a greenhouse study where phosphorus uptake was increased, it was done so by incorporating humic acid into the top 10cm of soil (Cooper et al., 1998). This was a single application, and may have spread out the material in a greater volume of soil.

Future work should evaluate root distribution of creeping bentgrass treated with humic substances. This should be tested in simulated putting greens with other types of sand, and in field sites to characterize the hydrophobic tendencies of these materials and their influence on root growth and distribution in the profile.

Golf course superintendents looking to conserve water and reduce phosphorus fertilization may not see a benefit when using humic substance products. These products may offer other benefits to turf, but their use may require more frequent applications of water, and more maintenance on putting greens including wetting agent applications to reduce localized dry spots because of the hydrophobic tendencies of these materials.

LITERATURE CITED

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Table 3-1. Effect of organic acid application[†] on irrigation frequency, tissue phosphorus level, shoot growth and root growth of creeping bentgrass grown in simulated USGA putting greens.

Treatment		Irrigation frequency	Total irrigations	Phosphorus concentration	Tissue biomass	Shoot mass	Root mass	Root length	Root: shoot ratio
		days	#	%	g			cm	
Citric acid		14.02 a[‡]	6.56 a	0.36 a	15.49 a	0.33 a	0.89 a	17.56 b	3.25 a
Control		13.87 a	7.00 a	0.38 a	16.91 a	0.31 a	0.77 a	17.72 b	3.03 a
Tannic acid		13.47 ab	6.78 a	0.34 a	16.57 a	0.31 a	0.94 a	18.28 b	3.87 a
Humic acid		11.29 b	7.89 a	0.36 a	17.10 a	0.23 a	1.02 a	21.52 a	4.34 a
ANOVA									
Effect	**df**								
Run	2	ns	ns	**	*	*	ns	***	**
Treatment	3	*	ns	ns	ns	ns	ns	***	ns
Run × Treatment	6	ns	ns	ns	ns	ns	ns	ns	ns

*, **, ***, ns, significant at $P \leq 0.05$, 0.01, 0.001, or not significant respectively.

[†]Treatments were applied as irrigation treatments when soil moisture dropped below 10% volumetric water content as measured by soil moisture sensors triggering an automated irrigation system.

[‡]Means within same column with same letter are not different significantly P=0.05.

Figure 3-1. Creeping bentgrass (*Agrostis palustris* L.) sod planted in tubs with calcareous sand to simulate a USGA putting green.