

DO HUMIC SUBSTANCES INFLUENCE MOISTURE RETENTION
AND PHOSPHORUS UPTAKE IN PUTTING GREENS?

by

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ABSTRACT

Do Humic Substances Influence Moisture Retention and
Phosphorus Uptake in Putting Greens?

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Humic substances are popular natural organic products used by golf course superintendents on putting greens because of benefits that may improve turf health. Claims have been made in advertising and marketing about their effectiveness in turf management, but are often unsubstantiated and have no objective research to support their effects. One claim that has not been studied is that humic substances increase the moisture retention of soil. This study evaluated pure humic acid and four commercial humic substance products applied to creeping bentgrass in a greenhouse and to golf course putting greens. In the greenhouse, organic acids, including a humic acid without commercial additives, were applied at 250 mg C L^{-1} as irrigation treatments through an automated irrigation system to simulated USGA putting greens. In the field, commercial humic substances were applied at label rates, along with pure humic acid at 9 kg ha^{-1} , to golf course putting greens and a research green during the summer growing season. Differences in soil volumetric water content, leaf tissue concentration of phosphorus,

chlorophyll content, and plant growth were evaluated. Humic substances did not increase moisture retention of soil. The application of humic acid to putting greens significantly decreased soil volumetric water content, and dried down the root zone, requiring more frequent watering than the control. Phosphorus uptake of creeping bentgrass did not improve with the addition of humic substances, as tissue concentration was significantly decreased when humic acid was applied. No differences in chlorophyll content, or top-growth of creeping bentgrass were observed. Root distribution was affected as root depth significantly increased in turf irrigated with humic acid. Golf course superintendents looking to reduce water and phosphorus fertilizer applications on putting greens may not see a benefit by using humic substance products.

(117 pages)

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Adam Van Dyke

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CHAPTER 1

INTRODUCTION

Creeping bentgrass (*Agrostis palustris* L.) is a cool season grass used on golf courses around the world, and is the predominant turf grown and managed on putting greens in the Intermountain West region of the United States. The climate of the Intermountain West can impose difficult growing conditions for turfgrass species, especially creeping bentgrass on sand putting greens. Summer months of June through August have high temperatures, low humidity and little rainfall, intensifying drought conditions. The transpiration gradient created by these climatic factors can cause wilting (Liu et al., 2002) and loss of roots (Huang and Liu, 2003) during periods of drought stress. Drought conditions are common around the globe, and can influence water needs for bentgrass survival during the summer. Although water is needed by bentgrass to meet evaporative demands and growth, turfgrass managers are frequently required to conserve water, especially during drought and are still expected to maintain extremely high quality turf.

In addition to plant water requirements, soil can influence the quality of bentgrass putting greens. Sand is used for putting green root zones to reduce compaction, and calcareous sand in the Intermountain West has high levels of calcium carbonate that buffer soil in the alkaline pH range (~ 7.5-8.5), consequently reducing availability of phosphorus and other micronutrients. These climatic and soil factors contribute to supplemental irrigation and fertilizer requirements needed for high quality creeping bentgrass putting greens. These factors are not limited to the Intermountain West, as

similar climate and soil conditions exist in other regions of the world where creeping bentgrass putting greens are managed.

The public demand for efficient water and fertilizer use by golf courses is what drives many superintendents' resource conservation goals. Other reasons for conservation are economic and regulatory, including the imposition of water restrictions. However, golf course superintendents continually try to be more efficient with their management practices while improving turf health. A management practice that has gained popularity for potentially reducing irrigation and fertilizer applications on putting greens is the use of natural organic products, including those containing humic substances. Yet, there is limited scientific basis supporting these claims, and many questions exist regarding the effectiveness, and what exactly they can do for putting green turf due to confounding effects of fertilizers and other ingredients often contained in these products.

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CHAPTER 2

LITERATURE REVIEW: HUMIC SUBSTANCES USE, STUDY

AND EFFECTS ON PLANTS

ORGANIC MATERIAL AND TURF MANAGEMENT

Using organic materials in the management of turfgrass is nothing new. The practice of top-dressing and composting manure on grasslands dates back to Roman times, and was influential on all European agriculture (Carrier, 1923). Composts and organic sources of nutrients are again being promoted and used because of concerns related to nutrient leaching and increasing organic matter in the soil, but also for unproven benefits that may or may not improve turf health (Farrell-Poe et al., 1997; Nelson, 1998). Because of these possible benefits to turf, golf course superintendents are incorporating the use of organic materials into course management practices, including use on putting greens.

Golf courses can consist of many acres of turfgrass that are visible to the public. Because of this exposure, superintendents want to continue being environmentally conscious and conservative with their management practices. Increased regulations by agencies like the Environmental Protection Agency (EPA) continue to restrict chemical use on golf courses, and decreased water resources are making water budgets and the conservation of water a necessity. Using natural organic products is one way superintendents attempt to improve turf health with minimized environmental impacts.

Golf course superintendents use natural organic products because they feel they are doing something good for the environment – a “warm fuzzy” feeling one might say.

In some cases using “environmentally sound” natural organic products may be a marketing tool to show their environmental sensitivity, similar to the Audubon Cooperative Sanctuary Program for golf courses (ACSP) (Yarrington, 2006). The marketing of natural organic products, although with little scientific basis, can be a deciding factor for superintendents using these products over established synthetics. It is critical that questions surrounding their use are studied, before these materials can be incorporated into sound management practices. With development of new products each year, there is little time for researchers to evaluate their true effectiveness, creating more uncertainty to superintendents interested in using these materials (Karnok, 2000).

A problem that continues to raise questions and contribute to the confounding factors is the term biostimulant. Many organic products have been called biostimulants, which is a generic label used to categorize numerous products that stimulate plant growth into a group. However, this group can include both organic and inorganic materials. Humic substances are one example of natural organic materials that are considered to be biostimulants. Humic substances are labeled for use on plants, but commercial organic products may also contain other biostimulants and fertilizers in addition to the humic material that may be incorrectly reported on the label (Rossi, 2004). This makes it difficult to separate the effect of the humic substance from the confounding effects from other additives when evaluating products. Regardless of the inconsistencies and uncertainties with ingredients, humic substances continue to be marketed and highly used on turfgrass (Nelson, 1998). The responses observed from these products, especially in times of stress (Schmidt et al., 2003; Zhang et al., 2002) have made humic substances

very popular in turf management, and are a common choice by golf course superintendents because of their potential benefits that may improve turf health.

HUMIC SUBSTANCES

Humic substances are an operationally defined, heterogeneous mixture of organic materials (MacCarthy et al., 1990). They are the soluble components of soil humus extracted in an alkaline solution. These materials are found in all terrestrial and aquatic environments (Aiken et al., 1985), and are formed as plant and animal residues decompose. Different organic materials and compounds combine during the formation of these molecules, making their formation variable and misunderstood (Stevenson, 1982). Because of humic substances abundance and variability of formation in the environment, their chemical nature can be dependent on a number of factors, often making them long-chained molecules with their shape and solubility dependant on pH of the ecosystem (Piccolo et al., 1996; Stevenson, 1982).

Humic substances can be differentiated into three fractions depending on solubility in the alkaline solution extracted from soil humus as a function of pH. These include fulvic acid, humic acid and humin. The humin fraction is completely insoluble, and the fulvic acid is completely soluble in the alkaline solution at all pH values. The humic acid fraction precipitates when the pH is lowered below 2, but is soluble at pH values greater than 2. Fulvic acid is the least weathered fraction followed by humic acid, then humin. Together these three components of humic substances make up a natural heterogeneous substance that is characterized as being yellow or black in color, of high molecular weight and refractory (Aiken et al., 1985).

Humic acid and fulvic acid fractions are the most common materials used in the production of natural organic products that are applied to turf. The disassociated form of humic acid is known as humate. The soluble humic substance fractions (humic acid and fulvic acid) are used in combination or alone, with humic acid the most common fraction that has been studied. These two fractions have similarities, but in general have distinguishing differences of molecular weight, carbon content, oxygen content and total acidity.

Humic acid is a large molecule (10,000+ Da) compared to the smaller fulvic acid (640 Da). Humic acid has more carbon (50 to 60%) than fulvic acid (40 to 50%), and less oxygen (30 to 35%) than fulvic acid (44 to 50%) (Stevenson, 1982). Total acidity is a function of the number of oxygen functional groups associated with the molecule and differs between the fractions. Major functional groups of humic substances are carboxyl, alcohol, phenolic, hydroxyl, and carbonyl (MacCarthy et al., 1990), and are negatively charged acidic reactive sites on the molecule (Hayes and Malcolm, 2001) where cation exchange, water retention and binding of soil particles may occur. Humic acid has 9% functional groups per molecule with a total acidity of $6 \text{ mmol}_c \text{ g}^{-1}$ and fulvic acid has 13% functional groups per molecule with a total acidity of $10 \text{ mmol}_c \text{ g}^{-1}$ (Grossl and Inskeep, 1991, 1992).

Humic acid and fulvic acid are operationally defined, hence there are differences in chemical characterization based on isolation of source materials. This will also affect the fraction of humic acid versus fulvic acid in the source material. Pure fractions of both can be obtained through the International Humic Substances Society (IHSS), but can be

costly due to labor involved with isolation and characterization of materials dependent on source material.

Humic substances have been studied and used on various agricultural and horticultural crops for years. Franz Karl Achard performed the first recorded studies involving humic substances in the early 1800's (MacCarthy et al., 1990), but the study and use of humic substances on turfgrass did not begin until thirty years ago. Research focused on golf course putting green turf did not begin until twenty years ago. Previous research applying humic substances to a variety of agricultural crops indicates documented growth responses that may be beneficial for turfgrass systems. Growth responses observed on crops and turfgrasses has been reported to be caused by either a hormone response, or increased bioavailability of nutrients.

HORMONE RESPONSE OR NUTRIENT BIOAVAILABILITY

O'Donnell (1973) reported that humic substances have an auxin-like hormone affect on plants. This has also been documented by Chen and Aviad (1990), who suggest the fulvic acid fraction is responsible for the hormone-like activity in plants. The lower molecular size and higher acidity of fulvic acid may influence the biological activity of plants (Piccolo et al., 1992), because it can be taken up by the plant while humic acid can only interact with cell walls (Nardi et al., 2002; Zalba and Quiroga, 1999). Recently that theory has been challenged because humic acid has also been associated with increasing cytokinin levels in creeping bentgrass (Zhang and Ervin, 2004).

Evidence supports the claim that humic substances have hormone-like effects on plants, but it's not clear if growth responses are due to the chemical structure of humic

substances, or the hormones released from microbes associated with the humic material (Nardi et al., 2002). Alternatively, some researchers have hypothesized that growth responses observed by humic substances are due to increased micronutrient availability (Chen et al., 2004; Pertuit et al., 2001). Improved fertility would also provide a growth stimulus, which may be difficult to decipher from a hormone response.

Hormone responses can be a beneficial tool for turf management, but hormones are present in plants in small amounts (Davies, 2004), and if humic substances do contain hormones, their use may raise hormones beyond normal plant levels producing an inhibitory or undesirable effect (Nelson, 1998). Whether humic substances have a hormone-like effect, improve nutrient uptake, or do nothing for plants, their use on plants including golf course turf has increased, which has led to more research evaluating their effects on crop and turf systems.

AGRICULTURAL CROP RESEARCH

Humic acid has been associated with improved plant growth. Ayuso et al. (1996) reported that humic acid encouraged top growth over root growth in barley. However, increased root growth has also been observed in marigolds, peppers and strawberries (Arancon et al., 2004), zinnia and marigold (Dudley et al., 2004). Top growth and root growth increased in corn, millet, beans, geraniums, begonia (Chen and Aviad, 1990) and maize (Sharif et al., 2002). Even small amounts of humic acid have encouraged plant growth by increasing micronutrient metal uptake of plants (Ayuso et al., 1996; Mackowiak et al., 2001; Schmidt, 2004), while high levels have resulted in no effect (Sharif et al., 2002) and even necrosis (Ayuso et al., 1996; Pertuit et al., 2001; Türkmen

et al., 2004). No growth response was observed in non-fertilized media (Pertuit et al., 2001), but in fertilized media nitrogen, phosphorus and potassium uptake was increased (Ayuso et al., 1996).

On recreational turfgrass, humic substances improved turf quality (Mueller and Kussow, 2005; Zhang and Ervin, 2004; Zhang et al., 2003b), shoot growth (Zhang and Ervin, 2004; Zhang and Schmidt, 2000) and root growth (Cooper et al., 1998; Zhang et al., 2003a, 2003c). Humic substances increased tissue levels of iron (Chen et al., 2004; Schmidt et al., 2003), zinc (Carey and Gunn, 2000; Chen et al., 2004) and manganese (Liu et al., 1998), but have had limited effects when adequately supplied with nutrients (Cooper et al., 1998).

From these studies, it is apparent that humic substances induce growth responses in a variety of plants, but the responses are variable and not conclusive. Many biostimulants, including humic substances, have been marketed for use during times of stress because they reportedly maintain plant health (Zhang et al., 2002). Using humic substances may also enhance drought resistance of turfgrass (Zhang and Ervin, 2004; Zhang and Schmidt, 2000) by increasing plant antioxidant levels (Zhang et al., 2003b).

PLANT ANTIOXIDANT PRODUCTION

Antioxidants are molecules naturally produced by plants that provide a number of functions related to maintaining plant health during stress (Mittler, 2002). Antioxidants react with harmful free radicals inside cells that cause damage to plant tissue (Liu and Huang, 2000; Zhang and Ervin, 2004). Free radicals are molecules of reactive oxygen species produced during times of plant stress (Schmidt et al., 2003) that damage cell

membranes through lipid peroxidation (Huang, 2004). In plant cells equilibrium exists between antioxidants and free radicals, and antioxidants scavenge and “breakdown” reactive oxygen species (Huang, 2004) preventing damage to cells (Zhang and Schmidt, 1997). During periods of plant stress, such as prolonged drought, the ability to maintain this equilibrium can become impaired (Mittler, 2002), and the severity and duration of the stress dictates when free radicals overwhelm the antioxidant defense system (Schmidt et al., 2003). For more in depth review on plant antioxidants in turfgrass, refer to (Ervin and Zhang, 2008).

During times of stress a plant must maintain production of antioxidants to continue efficient photosynthesis. Reduced photosynthesis can limit carbohydrate allocation in the plant, and it has been reported that applying humic acid to turf increased photochemical efficiency (Zhang et al., 2003a, 2003c), and increased photosynthesis of creeping bentgrass (Liu et al., 1998; Liu and Cooper, 2000; Zhang et al., 2003b). However, studies have also reported no increases in photosynthesis (Ervin et al., 2004). Additionally, foliar applied nutrients alone maintained photosynthetic activity of creeping bentgrass during heat stress (Fu and Huang, 2003), and it is expected that soil applied nutrients could also improve turf health and stress tolerance. Development of a deeper root system by plants could obtain more of the nutrients applied to the soil, and root growth is a documented response that humic substances have had on plants including creeping bentgrass turf.

ROOT GROWTH RESPONSE

Foliar applied humic acid increased rooting of Kentucky bluegrass sod (Zhang et al., 2003c), improved root strength of tall fescue sod (Zhang et al., 2003a) and increased root mass of Kentucky bluegrass seedlings (Zhang and Ervin, 1997). On creeping bentgrass plugs, an increase in root dehydrogenase activity was reported with humic acid applied at a rate of 400 mg L⁻¹ suggesting root respiration is influenced by humic substances (Liu et al., 1998). It was suggested from this research that increased root growth may be due to enhanced enzyme systems stimulated by the increased respiration. Addition of granular humic acid into sand at rates of 490 to 1960 kg ha⁻¹ increased root mass 45% and root depth 15% over the control, but foliar applied humic acid did not improve root growth in hydroponics or sand (Cooper et al., 1998).

It has been stated that based on the low application rates, humic substances may be a cost effective management tool to increase rooting (Liu and Cooper, 2000). This was tested in a study that applied granular humate with a fertilizer on a newly constructed putting green, but no increase in root mass density of the seedlings was observed (Kaminski et al., 2004). Lack of improved rooting in the hydroponic solution supplied with fertilizer (Cooper et al., 1998) supports the hypothesis that humic acid has limited affects on turf adequately supplied with nutrients.

In the Intermountain West, specifically Utah, many soils contain high levels of calcium carbonate which buffers soil pH above 7.0. Calcareous sand used for putting green root zones in the Intermountain West contains sufficient phosphorus, but it is present in forms not always available to the plant.

PHOSPHORUS BIOAVAILABILITY

Phosphorus fertilization of turfgrass is often not required unless the planting is new, but too often excess phosphorus is applied to putting greens. Management practices need to reduce leaching loss of this and other nutrients. As the limiting nutrient in eutrophication, phosphorus can be an environment contaminant. Organic acids, like humic substances, enhance the effectiveness of fertilizers by increasing nutrient availability (Frankenberger and Arshad, 1995). Increasing phosphorus availability could reduce the amount applied to putting greens, saving money and possibly reducing environmental contamination.

Phosphorus is a macro nutrient needed in large amounts by plants. In recreational turfgrass, optimum tissue levels are 0.20 to 0.55% (Carrow et al., 2001). This element is responsible for energy functions in the plant including the production of adenosine triphosphate (ATP). Phosphorus is taken up by the plant as the phosphate ion, and humic substances have been reported to improve plant uptake of phosphorus. Humic substances improve phosphorus levels in solution by coating soil particles, preventing sorption onto soil surfaces and prolonging the bioavailability (Grossl and Inskip, 1991, 1992; Zalba and Peinemann, 2002). Humic substances may also lower rhizosphere pH making some nutrients more available to plants (Chen et al., 2004). Work by Inskip and Silvertooth (1988) suggests that large organic acid molecules, such as humic acid and fulvic acid, increase phosphorus bioavailability more than smaller weight molecules like citric acid.

No increase in tissue phosphorus concentration was reported in rough fescue (Dormaar, 1975) or perennial ryegrass (Guar, 1964) in response to humic acid. However,

when granular humic acid was applied to creeping bentgrass grown in sand at levels ranging from 490 to 1960 kg ha⁻¹, the tissue phosphorus concentration was increased (Cooper et al., 1998). Application of humic acid did not provide an increase greater than the control in hydroponics, but in sand culture tissue phosphorus concentration of creeping bentgrass did increase with several foliar applied humic acid products (Cooper et al., 1998).

Using humic substances to improve phosphorus availability may not be justified because creeping bentgrass can obtain adequate concentrations of phosphorus even at low levels in the soil (Christians, 2004; Johnson et al., 2003). Additionally, phosphorus dissolution by organic acids did not account for all phosphorus mobilization (Hu et al., 2005). Alternatively, it has been mentioned that increased ligands with high doses of humic acid can cause excess chelation of nutrients, possibly reducing the amount available to the roots (Ayuso et al., 1996).

SUMMARY

Natural organic products are becoming a popular choice of golf course superintendents for the management of turf. Humic substances, specifically humic acid and fulvic acid, are an example of natural organic biostimulants being manufactured and sold to superintendents looking for advantages to reduce environmental impact and still maintain high quality putting greens. Humic substance products may induce growth responses in creeping bentgrass. As research has shown, humic substances increased tissue levels of cytokinins (Zhang and Ervin, 2004), iron (Schmidt et al., 2003), and phosphorus (Cooper et al., 1998), while improving photosynthesis (Liu et al., 1998) and

increasing root growth (Cooper et al., 1998). However, variable results have been reported by superintendents due to fertilizers and other ingredients that may be applied along with these humic substances. The responses from humic substances may only be observed over a period of continued use. This has made humic substances difficult to test and results difficult to interpret. Advertising and marketing claims for humic substance products often have little scientific evidence to support the effects on turfgrass, and little evidence exists in the literature to support the responses of the pure materials. More research is needed to understand the mechanisms of humic substances, including continued evaluation of new products arriving on the turf market.

Even though positive growth responses on creeping bentgrass have been reported, the association between humic substances and increasing soil moisture, a common claim in advertising and marketing, has not been studied. Zhang and Schmidt (2000), and Zhang and Ervin (2004) both studied humic acid applied to creeping bentgrass during drought conditions. These studies were evaluating plant growth responses, not their effects on water retention. However, Zhang and Ervin (2004) did report that visual quality and photosynthetic efficiency were postponed seven days on average with application of humic acid. This effect was reportedly due to greater leaf water status from increased cytokinin levels in plant tissue, not improved soil moisture retention by the humic substance.

The amount of water needed by creeping bentgrass grown on putting greens is determined by climactic and soil factors (Allen et al., 1998). Humic substances will not reduce the amount of water needed for plant transpiration and growth. However, as an organic material, humic substances may increase the water holding capacity in soil. This

may reduce the frequency of irrigation required on the putting green. Jordan et al. (2003) evaluated irrigation frequency on a creeping bentgrass USGA putting green, and found that irrigating every 4 days produced better turf quality, shoot density and root length density when compared to irrigating every one or two days. It is a common cultural practice for golf course superintendents to irrigate creeping bentgrass putting greens every day in the summer. With water restrictions increasing in the Intermountain West and other parts of the world, water budgets are becoming common for many golf courses. Humic substances may provide golf course superintendents with an effective management tool to reduce irrigation amounts on creeping bentgrass putting greens.

Potential benefits of this research include the evaluation of some commercially available humic substance products, on moisture retention and phosphorus availability effects in putting greens. This may help superintendents determine which humic substance products to use in management programs for reducing irrigation and phosphorus fertilizer applications on putting greens. The continued application of humic acid to the soil may also result in bridging and aggregation of soil particles, improving the structure of the soil. The results of this study may provide golf course superintendents with tools to reduce the input of resources and save money.

PROJECT OBJECTIVES

- 1) Determine if humic substances increase moisture retention in sand putting greens.
- 2) Determine if humic substances improve uptake of phosphorus by creeping bentgrass grown in calcareous sand.

LITERATURE CITED

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