Humic Substances
Their Influence on Creeping Bentgrass Growth and Stress Tolerance

Chunhua Liu, Ph.D., Clemson University and Richard J. Cooper, Ph.D., North Carolina State University

In the June issue, we summarized the nature and properties of humic substances and the possible ways that they influence plant growth. In this article, we present our research results dealing specifically with the effects of humic substances on creeping bentgrass (*Agrostis stolonifera* L.) growth and stress tolerance.

Although there has been substantial research concerning the effects of humic substances on field crops, information regarding application of humic substances to turfgrass has been limited. In 1975, Dormaar reported an increase in N uptake by rough fescue (*Festuca scabrella* Torr.) in response to application of some humic substances extracted from three soils while P, K, Ca, Mg, and Na uptake were unaffected.

Manufacturers claim massive root systems, better establishment, improved vigor, better tolerance to salt, heat and stress, increased nutrient uptake, improved soil structure, and greater fertilizer and pesticide effectiveness.

Varshovi (1991) studied the influence of a humate on growth and N uptake of bermudagrass (*Cynodon dactylon* Pers.), and concluded that application of humate alone did not increase N uptake and growth. He speculated that applying humates to established turfgrass with already sufficient organic matter in the rootzone could yield no extended response.

Dorer and Peacock (1997) evaluated liquid and granular humate applications to a creeping bentgrass putting green and reported no increase in leaf tissue concentration of N, P or K. Meanwhile, many commercial humates and humic acid (HA) products are being promoted for use on turfgrasses, especially on creeping bentgrass.

Creeping bentgrass is the grass of choice for putting greens in the northern United States and in the transition zone due to its superior quality. However, bentgrass is often difficult to manage during summer, when heat stress often results in a shallow root system and less healthy bentgrass is more prone to disease and insect damage.

Manufacturers of commercial humic products often claim benefits to turfgrasses, including: a more massive and deeper root system; increased grass establishment; improved plant vigor and survivability; improved salt, heat and other stress tolerances; increased nutrient uptake; improved soil structure; and increased effectiveness of fertilizers and pesticides.

In an effort to better understand how humic substances might affect creeping bentgrass, studies were conducted over a three-year period at North Carolina State University. The purpose of our research was to investigate the effects of application of humic substances, including both humates and humic acids, on the growth and stress tolerance (heat and salinity) of creeping bentgrass.

Photosynthesis, chlorophyll concentration, rooting and nutrition

Greenhouse experiments were conducted using a solution-culture (hydroponic) system to evaluate the effect of a commercial HA on the photosynthesis, chlorophyll concentration, rooting and nutrient content of "Crenshaw" creeping bentgrass. Bentgrass plugs were grown hydroponically in one-quarter-strength Hoagland's nutrient solution, which contained HA at 0, 100, 200, or 400 ppm (parts per million). Hoagland's solution contained all of the mineral nutrients needed for plant growth. Growing plants in Hoagland's solution ensured that they have adequate nutrient during the study.

Measurements of photosynthesis, chlorophyll concentration and root dehydrogenase (DH) activity were made weekly for one month. Root DH activity reflects the vigor and health of the roots. The more active the dehydrogenase is in the root tissue, the more healthy are the roots. All clippings harvested after HA application were combined for nutrient analysis. At the end of the study, root length and dry mass were determined.

The results showed that the photosynthetic rate of plants growing in 100 or 200 ppm solutions of HA rarely differed from that of the control. However, the 400 ppm treatment significantly increased net photosynthesis by as much as 20% (Fig. 1). Chlorophyll content did not vary in response to HA application on any sampling dates. Thus, it appears that the increase in net photosynthesis following HA application was due to some process other than increased chlorophyll production.

Humic acid had no promotive effect on root length after the original roots were excised. However, 400 ppm significantly increased root dry mass on all sampling dates. Root DH activity of plants receiving HA at 400 ppm was significantly higher than that of nontreated plants, with the increases ranging from 35% to 108% (Fig. 2). Root DH activity was determined using the TTC (2, 3, 5 - triphenyl tetrazolium chloride) reduction method. The more active the dehydrogenase is in the root tissue, the more TTC is reduced. The large increases in TTC reduction due to HA treatment suggest that root respiration was increased substantially by humic substances.

Sladky (1959a, 1959b) also reported increased plant respiration in response to HA. There is a close connection in plants between the energy-releasing process of respiration and the energy-consuming process of growth. Thus, increases in root growth might be due to the stimulation of enzyme systems by increased respiration.

Although treatment with HA caused significant increase or decrease in concentrations of several nutrients, the changes were relatively small, and probably not of biological significance.

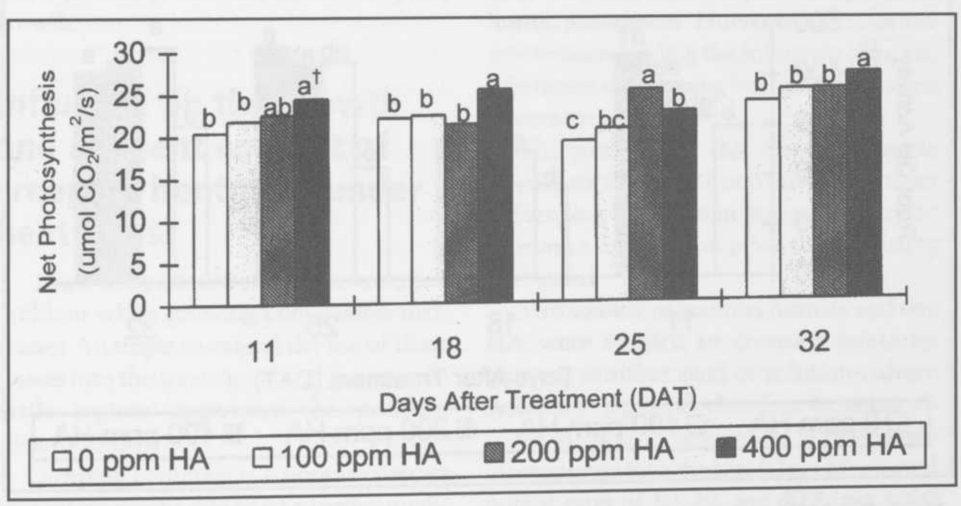

Fig. 1. Net Photosynthetic Rates of Creeping Bentgrass in Response to HA Application in One Greenhouse Solution-culture Experiment. + Means within the same DAT followed by the same letter are not significant at P=0.05 level.

Effects on root development, shoot growth, visual quality and nutrient concentration

The purpose of this experiment was to determine the potential of humic substances, including both humate and humic acid, to influence foliar growth, root growth and nutrient uptake of creeping bentgrass in sand-culture, solution-culture and under field conditions. In addition, the method of application (soil incorporated versus foliar application) was evaluated to determine if either method was preferable.

Creeping bentgrass rooting was increased by mixing granular humates into the top 10 cm of the rootzone or by surface application of humic acid to the rootzone prior to sod placement.

Greenhouse sand-culture, solution-culture experiments and one field experiment were conducted. Two commercially mined granular humates, a commercial HA and three IHSS (International Humic Substance Society) reference HAs extracted from leonardite, peat and soil were applied to creeping bentgrass growing in either sand, solution-culture or in the field. HA solution at different concentrations was either foliarly applied, or applied to the surface of the rootzone.

Creeping bentgrass rooting was increased by mixing granular humates into the top 10 cm of the rootzone (Table 1), or by surface application of humic acid to the rootzone prior to sod placement. This could have been due to more direct contact of humic substances with developing roots.

No single foliar-applied humic acid treatment consistently improved rooting compared to the control in either sand-culture or solution-culture experiments. Dorer and Peacock (1997) also reported no improvement in rooting for a "Cato"/ "Crenshaw" creeping bentgrass blend receiving foliar application of humates.

In general, application of humic substances did not affect clipping dry weight, and did not result in improved visual quality compared to untreated turf. These results corroborated those of Varshovi (1991) and Dorer and Peacock (1997). Nitrogen and calcium concentration of leaf tissue were relatively unaffected by the application of humic substances, regardless of application rate.

Phosphorous uptake in sand-culture was increased by incorporated granular

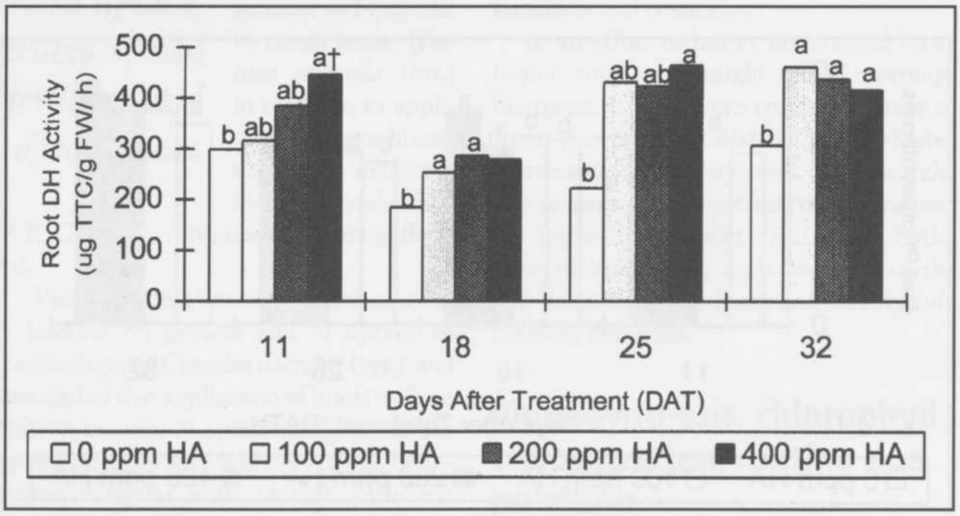

Fig. 2. Root DH activity of creeping bentgrass in response to HA application in greenhouse solution-culture. + Means within the same DAT followed by the same letter are not significant at P=0.05 level.

EFFECTS ON CREEPING BENTGRASS ROOT DEVELOPMENT

Table 1. Effects of humic substances on creeping bentgrass root development in sand-culture.

Humic Substance	*Formulation*	— *Rootzone section (cm)* — *0-10*	*10-20*	*>20*	*Maximum Root Length*
		Root Dry Mass (g)			*— cm —*
Menefee Humate	G+	0.96 a +	0.36 a	0.17 a	36.9 a
Soil HA	S	0.81 b	0.32 ab	0.15 a	33.0 ab
Peat HA	S	0.79 bc	0.28 b	0.11 ab	33.8 ab
Leonardite HA	S	0.79 bc	0.31 ab	0.15 a	36.1 ab
Sustane HA	S	0.80 bc	0.28 b	0.09 ab	34.1 ab
Control	—	0.66 c	0.26 b	0.04 b	32.2 b

+ Mean separation within columns by Waller-Duncan K ratio (K=100) t-test. Means within columns followed by the same letter are not significantly different at P=0.05 level. + (G) Granular formulation incorporated into the top 10 cm of sand. (S) soluble formulation applied as a foliar spray.

humates, as well as by several of the foliarly-applied humic substances. Iron uptake was increased in the field, but not in sand or solution-culture experiments.

The influence of HA application on the nutrition of solution-grown plants was minimal. The lack of improved rooting was related in part to a lack of P uptake response in solution-culture and field-culture experiments. The leaf tissue concentration of several other nutrients were significantly affected by treatment application; however, the differences were so small that they were probably not important to plant growth.

Influence on the growth and nutrient content of creeping bentgrass under heat stress

High temperature stress is a common problem when growing cool-season turfgrasses. Attempts to extend the use of these grasses into the transitional and warmer climatic regions aggravates the problem (Beard, 1995).

Creeping bentgrass is a popular species for putting greens due to its superior quality; however, creeping bentgrass often declines during the summer months (Krans and Johnson, 1974, Carrow 1996), as it is extended beyond its normal regions of heat stress adaptation (Beard, 1995).

Improvement of heat tolerance in creeping bentgrass would enhance turfgrass quality and its use in warmer environments. Many efforts have been made to improve bentgrass heat tolerance (Beard 1995, 1997) including: modifying rootzone composition, using heat resistant cultivars, syringing and increasing air movement.

Manufacturers of humates and HA for commercial use often claim that plant heat tolerance might be improved by use of humic substances. However, no information exists regarding the influence of humic substances on creeping bentgrass heat stress tolerance.

The purpose of this research was to investigate the growth and nutrient content of creeping bentgrass in response to humic substance application prior to and during heat stress.

Two sources of granular humate and one HA were applied to creeping bentgrass growing in either sand or solution-culture systems in a growth chamber. In sand-culture, two sources of granular humate were incorporated into the top 10 cm of selected pots at rates of 10, 20, and 40 lb per 1000 sq. ft. The turf was grown for 31 days before heat stress was initiated.

In the solution-culture experiment, HA was added to nutrient solution at rates of 0, 100, 200, or 400 ppm immediately after heat stress was initiated.

In both sand and solution-culture experiments, creeping bentgrass was exposed to day/night (14h/10h) temperature regimes of 77/59, 95/77, and 104/86 F, for 38 days. Increasing day/night temperatures significantly reduced clipping dry weight, clipping water content, maximum root length and root dry weight in both experiments. Increasing day/night temperatures significantly decreased photosynthetic rates throughout the experiment. Humate application in the sand-culture experiment did not influence clipping dry weight, maximum root length, or root dry weight, and had minimal influence on water content. In solution-culture, HA application actually decreased clipping dry weight and water content in some measurements, but generally did not effect chlorophyll content or photosynthesis.

During salinity stress, HA application inconsistently influenced clipping dry weight and did not affect tissue water content, net photosynthesis or root growth.

Rooting was generally not improved by HA application during heat stress. Heat stress resulted in increased N content, decreased Ca, Mg, S, and B content, and had no influence on P and K content in sand-culture. Nitrogen, P, Mg, and S content increased, K and B content decreased, and Ca content was unaffected in the solution-culture experiment.

Increased level of nutrient uptake in solution-culture may have been due to less severe heat stress in the rootzone compared to sand grown plants. Application of humate did not influence the uptake of mineral nutrients in sand-culture. However, in solution-culture, HA application significantly reduced uptake of N, P and Mg, and increased uptake of K and B. Application of humic substances did not result in significantly improved heat tolerance.

Influence on the growth and nutrient content of creeping bentgrass under salt stress

Creeping bentgrass has been characterized as having very good salinity tolerance among the cool-season turfgrasses (Turgeon, 1996). Even so, increased salt tolerance in bentgrass is needed to minimize problems such as: increased salt accumulation in soil (Hoss, 1981), increased sea water encroachment into golf course irrigation sources and increased restrictions on use of potable water sources for irrigation (Marcum and Murdoch, 1990).

The adverse effects of salinity mainly involve two aspects: increased osmotic potential stress and possible toxic effects of excessive ions (Taiz and Zeiger, 1991). Humic acids have been reported in some studies to increase uptake of both macro and micronutrients (such as N, P, K, Fe and Zn), thereby improving the nutritional status of the plant (Gaur, 1964; Rauthan and Schnitzer, 1981).

Since humic substances have been shown to enhance photosynthesis, rooting and increase the uptake of Mg, S and P of creeping bentgrass (Liu, et al. 1998; Cooper, et al. 1998), one might reason that application of humic acid could improve plant response to salinity. Manufacturers of humates and HA often claim that plant salinity tolerance might be improved by use of humic substances. However, there are no scientific reports about humic acid application and its effects on plant salinity tolerance.

The purpose of this study was to evaluate the effects of humic acid application on creeping bentgrass salt tolerance by studying shoot growth, water uptake, photosynthesis, plant rooting and nutrient uptake following application of humic acid during salinity stress. "Crenshaw" creeping bentgrass plugs were grown hydroponically in one-quarter-strength Hoagland's nutrient solution containing HA at 0 or 400 ppm with salinity levels of 0.48, 8.00 and 16.00 ds/m (EC = electric conductivity). A salt mixture was formulated to mimic the average salt composition of sea water (Svedrup et al. 1959). Clipping dry weight, tissue water content, and net photosynthesis were measured weekly for one month. Maximum root length, and root dry weights from 0 to 10 cm and >10 cm rootzones were determined 31 days after treatment (DAT).

Turf was mowed three times weekly and clippings were dried and analyzed. Increasing salinity decreased clipping dry weight, tissue water content, net photosynthesis, and root length, but increased root dry weights. Salinity had less effect in reducing root growth than top growth.

During salinity stress, HA application inconsistently influenced clipping dry weight and did not affect tissue water content, net photosynthesis or root growth. Salinity decreased the uptake of N, P, K, Ca and S; increased the uptake of Mg, Mn, Mo, B, Cl and Na; and had no influence on the uptake of Fe, Cu and Zn. Application of 400 ppm humic acid during salinity stress neither increased the uptake of the nutrients inhibited by salinity nor decreased the elements which were excessive and toxic in the salinity solution. In general, application of HA did not improve salinity tolerance of creeping bentgrass.

Summary

Application of HA materials at 400 ppm in solution-culture significantly increased root mass, compared to untreated turf on almost every sampling date in greenhouse studies. The response to lower rates were not as conclusive. Although the materials improved the amount of roots present, they did not affect root length. When granular humates were incorporated into the rootzone to a depth of four inches, the rooting effects were stronger than the effect of foliar sprays.

Keep in mind that these results were from plants growing in sand or hydroponic solutions containing little or no native organic matter or humic substances. Rooting responses might be less evident on a putting green containing significant organic matter or naturally occuring humic substances.

Photosynthesis is an important process in a plant because it provides the plant with carbohydrates for growth and recovery from stress injury. Applying HAs at 400 ppm in solution-culture increased photosynthesis, compared to untreated turf on most dates when photosynthesis was measured.

The root DH activity was enhanced due to HA application, suggesting plant root respiration can be increased substantially by humic substances. In all the experiments evaluating nutrient uptake, the differences normally were very small — so small, in fact, that it is doubtful that these differences would result in turfgrass quality in the field. Application of humic substances did not improve heat or salt tolerance.

Although rooting, photosynthesis, root dehydrogenase activity and nutrient content were often improved by the application of humic substances; turfgrass shoot growth and visual quality rarely differed from untreated turf. Even so, we remain open minded regarding the potential benefits of making supplemental applications of humic substances. Applying the materials to low fertility soils or newly seeded greens might be useful in some putting green situations. Also, given the very low application rates required, one might consider their use to be cost effective for potentially improving rooting during summer months.

Although rooting, photosynthesis, root dehydrogenase, and nutrient content were often improved by humic substances; turfgrass shoot growth and visual quality rarely differed from untreated turf.

Chunhua Liu, Ph.D., is a post-doctoral research scientist at Clemson University in Florence, SC. He can be reached at 843/662-3526 or e-mail at Cliu@Clemson.edu. Dr. Richard J. Cooper is a professor of Turfgrass Management at NCSU. He can be reached at 919/515-7600.

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