Humic Acid Application Affects Photosynthesis, Root Development, and Nutrient Content of Creeping Bentgrass

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Abstract. Humic acids (HA) reportedly enhance the growth of numerous crops; however, little information is available as to their effects on turfgrasses. Experiments were conducted to evaluate the effect of a commercial preparation of HA on the photosynthesis, chlorophyll concentration, rooting, and nutrient content of 'Crenshaw' creeping bentgrass (*Agrostis stolonifera* L.). Bentgrass plugs were grown hydroponically in one-quarter-strength Hoagland's nutrient solution containing HA at 0, 100, 200, or 400 mg·L⁻¹ with measurements made weekly for 1 month. The photosynthetic rates of plants growing in 100 or 200 mg·L⁻¹ rarely differed from that of the control, but 400 mg·L⁻¹ significantly enhanced net photosynthesis on all four observation dates. Chlorophyll content was unaffected by HA rate on all observation dates. Root dehydrogenase (DH) activity and root mass regrowth were significantly increased by HA at 400 mg·L⁻¹ on all dates. The 100 and 200 mg·L⁻¹ rates increased root DH activity on two of four observation dates, but root regrowth was unaffected. At one or more of the rates used, HA increased tissue concentrations of Mg, Mn, and S and decreased those of Ca, Cu, and N, but had no influence on the concentrations of P, K, Fe, Mo, and Zn.

Humic substances have been defined by Aiken et al. (1985) as "a category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterized as being yellow to black in color, of high molecular weight, and refractory." They can be divided into humic acids (HA), fulvic acids (FA), and humins, based upon their solubility in acid and alkali. Humic acid is produced by extracting an organic matter source with an acidic solution (pH = 2.0); any fraction that is insoluble below pH 2.0, but soluble above, is considered a humic acid. In order to produce a soluble commercial product, HA can be treated with a basic compound to produce a soluble salt with a near neutral pH. This soluble salt of HA is referred to as humate.

The positive effects of humic substances on the growth of plants in the Gramineae family has been well documented (Chen and Aviad, 1990). Kononova and Pankova (1950) reported that the root length and root number of corn (*Zea mays* L.) growing in solution culture doubled in response to addition of humate at 4 to 5 mg·L⁻¹. Lee and Bartlett (1976), also working with corn grown in solution culture, found that rooting was enhanced significantly at a humate concentration of 8 mg·L⁻¹. Vaughan and Malcolm (1985) compared root and shoot growth of wheat grown in

water alone, or a complete (Hoagland's) nutrient solution, both with and without 50 mg·L⁻¹ HA. Addition of humic acid increased root growth 58% in water alone, and 25% in nutrient solution.

Chen and Aviad (1990) cite several studies demonstrating the influence of humic materials on nutrient uptake by plants. Dormaar (1975) reported increased N uptake by rough fescue (*Festuca scabrella* Torr.) in response to application of humic substances extracted from three soils, while P, K, Ca, Mg, and Na uptake was unaffected. Varshovi (1991) found no increase in N uptake of bermudagrass (*Cynodon dactylon* L.) following application of a commercial humate material at 0, 268, and 803 mg·L⁻¹. Dorer and Peacock (1997) reported no increase in leaf tissue concentration of N, P, or K following application of liquid and granular humate to a creeping bentgrass putting green.

Recently, many commercial products containing HA have been promoted for use on turfgrasses. While the effects of humic substances on cereal grasses and numerous other plants have been well documented, the growth response of turfgrasses has not been studied extensively. The purpose of this research was to investigate the effects of HA on the net photosynthesis, chlorophyll concentration, root DH activity, and nutrition of creeping bentgrass.

Materials and Methods

This research was conducted in a greenhouse located at North Carolina State Univ. in Raleigh. Average maximum and minimum temperatures were 31 and 21 °C, respectively. 'Crenshaw' creeping bentgrass sod was trimmed of roots below the thatch layer and cut into 10.8-cm-diameter plugs. Plugs were

transferred on 20 Nov. 1996 to four recirculating solution culture systems, each containing 58 dm³ of 0.25 strength Hoagland's solution (Hoagland and Arnon, 1950). The pH of the culture solution was maintained at 6.0 ± 0.1 by automated additions of 100 mol·m⁻³ H₂SO₄. Solutions were replaced every 2 weeks in order to assure nonlimiting nutrition and were aerated vigorously at all times.

Plugs were grown to allow production of sufficient roots for analysis, and on 10 Feb. 1997 a commercial HA product was added to the solution culture systems to obtain final concentrations of 0, 100, 200, or 400 mg·L⁻¹. The product had a nutrient content (g·kg⁻¹) of 595 C, 42 H, 320 O, 0 P, 1 S, and 9 N. The turf plugs were mowed at 6 mm three times per week throughout the study. The experimental design was completely randomized with four replications. No pesticides were applied.

Measurement of net photosynthesis and chlorophyll content. Net photosynthesis and leaf chlorophyll content were determined on 21 and 28 Feb. and on 7 and 14 Mar. 1997. Photosynthesis was measured between 0830 and 1030 HR on each evaluation date, using a portable gas exchange system (Model LI-6200; LI-COR, Lincoln, Nebr.) and a custom-made chamber designed specifically to accommodate the bentgrass plugs. Photosynthetically active radiation was maintained at 1000 μmol·m⁻²·s⁻¹ during measurements using supplemental lighting. Turf plugs were acclimated under the lights for 5 min prior to measurement to assure light saturation. Clippings were harvested and extracted in 80% acetone at room temperature overnight, and chlorophyll content was determined by the method of Arnon (1949).

Root development. DH activity and regrowth. Root DH activity was assessed at 7-d intervals for 4 weeks beginning 24 Feb. Roots were uniformly excised at the base of each plug and root DH activity was determined using the TTC (2,3,5-triphenyl tetrazolium chloride) reduction method of Yoshida (1966). Reduction of TTC is a reaction that has been widely used as a simple and rapid assay for plant tissue respiration (Bennett and Loomis, 1949; Leigh and Mazur, 1975). The TTC is reduced by dehydrogenase in root tissue. Reduced TTC (which is red) is extracted using ethyl acetate and measured using a spectrophotometer at a wavelength of 485 nm. The more active the dehydrogenase is in the root tissue, the more TTC is reduced. Root DH activity was expressed as the amount of TTC reduced per gram of root fresh mass per h (μg·g⁻¹·h⁻¹). After roots were harvested on each date, the turf plugs were returned to the culture systems to allow root regrowth. Rooting was assessed on 23 Mar., after 13, 20, and 27 d of regrowth by excising the new roots from each plug and determining root length and mass (dried at 75 °C for 48 h).

Nutrient analysis. To examine the effect of humic substances on plant nutrient content, leaf tissue was harvested from each treatment during routine mowing three times each week. Clippings were dried at 75 °C for 48 h, ground and stored at room temperature until analysis.

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Because of the small area of each plug, the clippings from all harvest dates were pooled to produce a composite sample large enough for analysis. Nitrogen was determined using a Perkin-Elmer PE2400 CHN Elemental Analyzer (Perkin-Elmer Corp., Norwalk, Conn.). A Perkin-Elmer Plasma 2000 was used to determine P, K, Ca, Mg, Cu, Fe, Mo, Mn, and Zn by inductively coupled plasma emission spectrometry, using a slight modification of the technique described by Grewling (1976). Sulfur was analyzed according to the wet-ashing procedure of Novozamsky et al. (1986).

Data analysis. All data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, 1985). The Waller-Duncan K ratio *t* test was employed for mean separation if the F-value indicated that treatment effects were significant. Orthogonal contrasts for regression (linear, quadratic, and cubic effects) were tested using the F-test when treatment effects were significant (Steel and Torrie, 1980).

Results and Discussion

Net photosynthesis and chlorophyll concentration. Gas exchange was determined on a canopy area, rather than a leaf area basis, because of the very small size of bentgrass leaves. Consequently, the data reflect the integrated effects of a large and diverse population of leaves, including expanding, fully mature and active, and senescent leaves. The 400 mg·L⁻¹ HA treatment increased average net photosynthesis rate 17% over the four measurement dates, whereas the 200 mg·L⁻¹ treatment increased photosynthesis rates significantly only on 7 Mar. (Table 1). The 100 mg·L⁻¹ treatment never affected net photosynthesis. There were highly significant linear effects of HA rate on CO₂ exchange on all four dates ($P \leq 0.001$, Table 1). Quadratic and cubic effects were significant on two and one of the four dates, respectively.

Application of HA did not affect chlorophyll content on any sampling date (data not presented). Values for all HA rates and dates averaged 1206 µg·g⁻¹ FM vs. 1148 µg·g⁻¹ FM for nontreated plants. Our results contrast with those of Sladky (1959a, 1959b) who reported increases in chlorophyll content of 63% for tomato (*Lycopersicon esculentum* L.) and 7% for begonia (*Begonia semperflorans* L.) treated with HA at 50 and 100 mg·L⁻¹, respectively. While the highest level of HA clearly increased net photosynthesis, this was not the result of increased chlorophyll production.

Root DH activity. Significant linear effects of HA on root DH activity were evident on all four sampling dates, and significant quadratic effects on two dates (Table 2). Root DH activity of plants receiving HA at 400 mg·L⁻¹ was significantly higher than that of nontreated plants on all observation dates, with the increases ranging from 35% to 108%. Humic acid supplied at 100 or 200 mg·L⁻¹ also increased root DH activity significantly on two of four sampling dates.

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 respiration in response to HAs. Syltie (1985) has suggested that HAs supply polyphe-nols that catalyze plant respiration. 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.

Root regrowth. Humic acid had no promotive effect on root length measured on 23 Mar., 13, 20, and 27 d after the original roots were excised, respectively. In fact, HA at 100 and 200 mg·L⁻¹ had reduced regrowth when measured after 27 d (Table 3). Similarly, root mass was not reduced by these lower rates until 27 d of regrowth. However, 400 mg·L⁻¹ significantly increased root dry mass on all sampling dates. Both Sladky (1959b) and Dorer and Peacock (1997) also observed this trend toward increased root mass without a corresponding increase in root length. The roots of plants growing in HA at 400 mg·L⁻¹ in our experiment were better developed and more highly branched than were the roots of control plants.

Nutrition. Tissue N, P, and K concentration averaged 60.6, 6.6, and 36.0 g·kg⁻¹, respectively, with no meaningful effect of HA (Table 4). Although tissue N concentration was significantly reduced at the 200 mg·L⁻¹

rate, no visible turf response was evident. Our findings support those of Dorer and Peacock (1997), who reported no increase in the concentration of N, P, or K in leaf tissue of creeping bentgrass following humate application.

Calcium content of bentgrass decreased linearly as HA rate increased; 200 and 400 mg·L⁻¹ significantly reduced Ca concentration relative to that of the control (Table 4). Even so, all plants had Ca levels within the sufficiency range (5–7.5 g·kg⁻¹) proposed by Mills and Jones (1996). Slight increases in Mg concentration were observed at 100 and 200, but not at 400 mg·L⁻¹. Tissue S increased significantly in response to HA treatment, with both linear and quadratic effects being significant.

Leaf Fe, Mo, and Zn concentration (data not presented) were not influenced by HA treatment and averaged 224, 10.4, and 78.5 mg·kg⁻¹, respectively. Tissue Cu concentration decreased in a linear fashion as rate of HA increased. Tissue Mn concentration increased significantly in response to 200 and 400 mg·L⁻¹, the response again being linear.

Although treatment with HA caused significant increases or decreases in concentrations of several nutrients, the changes were relatively small, especially for the macronutrients. Further, all nutrients were present in ranges considered adequate or even supraoptimal for bentgrass (Mills and Jones, 1996), probably because the nutrient supply in solu-

Table 1. Effects of humic acid (HA) addition¹ during hydroponic greenhouse culture on net photosynthetic rate of creeping bentgrass.

HA (mg·L ⁻¹)	Photosynthetic rate (µmol CO ₂ ·m ⁻² ·s ⁻¹)			
	21 Feb.	28 Feb.	7 Mar.	14 Mar.
0	20.4 b ²	22.1 b	19.2 c	23.8 b
100	21.8 b	22.4 b	20.7 bc	25.2 b
200	22.5 ab	21.3 b	25.3 a	25.3 b
400	24.4 a	25.4 a	22.7 b	27.2 a
ANOVA				
Source	df			
TRT	3	*	***	***
HA rate				
linear	1	***	***	***
quadratic	1	NS	**	NS
cubic	1	NS	NS	NS

¹Commercial humic acid added to solution on 10 Feb. 1997.

²Mean separation within columns by Waller-Duncan K ratio (K = 100) *t* test ($P \leq 0.05$).

NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2. Effects of humic acid (HA) addition¹ during hydroponic greenhouse culture on root dehydrogenase (DH) activity of creeping bentgrass.

HA (mg·L ⁻¹)	Root DH activity (µg TTC·g ⁻¹ FW·h ⁻¹)			
	24 Feb.	3 Mar.	10 Mar.	17 Mar.
0	299 b ²	185 b	223 b	307 b
100	316 ab	254 a	431 ab	461 a
200	384 ab	286 a	423 ab	437 a
400	446 a	279 a	464 a	415 a
ANOVA				
Source	df			
TRT	3	NS	**	NS
HA rate				
linear	1	*	**	*
quadratic	1	NS	*	NS
cubic	1	NS	NS	NS

¹Commercial humic acid added to solution on 10 Feb. 1997.

²Mean separation within columns by Waller-Duncan K ratio (K = 100) *t* test ($P \leq 0.05$).

NS, *, **Nonsignificant or significant at $P \leq 0.05$, or 0.01, respectively.

Table 3. Effects of humic acid (HA) addition² during hydroponic greenhouse culture on root regrowth (root length and mass) of creeping bentgrass.

HA (mg·L ⁻¹)		Root length (cm)			Root mass (mg)		
		Days after treatment					
		13	20	27	13	20	27
0		19.8	24.0	28.8 a ^y	56 b	75 b	106 b
100		20.0	21.0	24.5 b	48 b	77 b	95 c
200		19.3	21.5	23.0 b	62 b	70 b	91 c
400		19.8	22.8	30.3 a	78 a	117 a	130 a
ANOVA							
Source	df						
TRT	3	NS	NS	**	**	***	***
HA rate							
linear	1	NS	NS	NS	**	***	***
quadratic	1	NS	NS	***	NS	**	***
cubic	1	NS	NS	NS	NS	NS	NS

²Commercial humic acid added to solution on 10 Feb. 1997.

³Mean separation within columns by Waller–Duncan K ratio (K = 100) *t* test ($P \leq 0.05$).

NS, **, ***Nonsignificant or significant at $P \leq 0.01$, or 0.001, respectively.

Table 4. Effects of humic acid (HA) addition² during hydroponic greenhouse culture on tissue nutrient concentration³ of creeping bentgrass.

HA (mg·L ⁻¹)	g·kg ⁻¹						mg·kg ⁻¹	
	N	P	K	Ca	Mg	S	Cu	Mn
0	61.0 a ³	6.60	35.5	6.90 a	3.13 c	5.20 c	59.8 a	177 b
100	60.6 a	6.63	36.0	6.63 ab	3.25 ab	5.45 b	48.5 b	197 ab
200	59.9 b	6.58	35.8	6.30 bc	3.33 a	5.78 a	42.6 bc	222 a
400	60.8 a	6.55	36.8	5.88 c	3.15 bc	5.48 b	36.8 c	214 a
Normal concentration ⁴	45–60	3–6	22–26	5–7.5	2.5–3.0	3.4–4.3	8–30	50–100
ANOVA								
Source	df							
TRT	3	*	NS	NS	**	**	***	*
HA rate								
linear	1	NS	NS	NS	***	NS	***	**
quadratic	1	**	NS	NS	NS	***	NS	NS
cubic	1	NS	NS	NS	NS	NS	NS	NS

²Commercial humic acid added to solution on 10 Feb. 1997.

³Nutrient concentration is an average of 16 leaf tissue samples obtained three times weekly from 17 Feb.–23 Mar.

⁴Mean separation within columns by Waller–Duncan K ratio (K = 100) *t* test ($P \leq 0.05$).

⁵Nutrient sufficiency range for *Agrostis stolonifera* suggested by Mills and Jones (1996).

NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

tion culture was nonlimiting. Dorer and Peacock (1997) also reported statistically significant, but small, differences in nutrient concentrations of bentgrass following humate application and questioned the biological significance of such differences.

In summary, HA at 400 mg·L⁻¹ consistently increased net photosynthesis in creeping bentgrass. Since the chlorophyll content of treated plants did not differ from that of the control, enhanced photosynthesis was due to some mechanism other than enhanced chlorophyll production. Humic acid increased root DH activity and root mass regrowth significantly at 400 mg·L⁻¹ on all dates; however, it had no promotive effect on root length and actually reduced length at rates of 100 and 200 mg·L⁻¹. In recent years, numerous anecdotal reports by turfgrass managers have indicated improved summer stress tolerance of creeping bentgrass following HA application. Our findings of improved photosynthesis, greater root DH activity, and improved root mass regrowth

of HA-treated turf in this study offer possible supportive evidence for such field observations. However, research should be conducted to determine if the effects noted in our work also occur under field conditions. While HA application resulted in statistically significant changes in leaf concentrations of certain elements, the differences were very small and probably not of biological significance. Thus, ascribing the positive growth effects of HA found in our study to a nutritional mechanism is questionable. However, nutrient limitation in soil systems is common, and beneficial responses to HA in the field could reflect improved nutrition.

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