

# Humic Acid Application Does Not Improve Salt Tolerance of Hydroponically Grown Creeping Bentgrass

Chunhua Liu<sup>1</sup> and R.J. Cooper<sup>2</sup>

Department of Crop Science, North Carolina State University, Raleigh, NC 27695-7620

ADDITIONAL INDEX WORDS. *Agrostis stolonifera*, humate, turfgrass

**ABSTRACT.** Growth and mineral nutrient content of creeping bentgrass [*Agrostis stolonifera* (L.) var. *palustris* (Huds.) Farw.] in response to salinity and humic acid (HA) application were investigated, and the effects of HA application on salinity tolerance was evaluated. Bentgrass plugs were grown hydroponically in one-quarter-strength Hoagland's nutrient solution containing HA at 0 or 400 mg·L<sup>-1</sup> with salinity levels of 0, 8.0, or 16.0 dS·m<sup>-1</sup>. Clipping dry weight (DW), tissue water content, and net photosynthesis (P<sub>N</sub>) were measured weekly for 1 month. Maximum root length, and root DW from 0 to 10 cm and >10 cm root zones were determined 31 days after treatment (DAT). The turfgrass plugs were mowed three times weekly, with clippings collected and dried for mineral nutrient analysis. Salinity was inversely related to clipping DW, tissue water content, P<sub>N</sub>, and maximum root length. Salinity had less effect on root growth than top growth. HA treatment did not affect tissue water content, P<sub>N</sub>, or root growth of salt-stressed turf. Salinity decreased uptake of N, P, K, Ca, and S; increased uptake of Mg, Mn, Mo, B, Cl, and Na; and had no influence on uptake of Fe, Cu, and Zn. Application of HA at 400 mg·L<sup>-1</sup> during salinity stress neither increased uptake of the mineral nutrients inhibited by salinity, nor decreased uptake of nutrients which were excessive and toxic in the salinity solution. In general, application of HA did not improve salinity tolerance of creeping bentgrass.

Creeping bentgrass (*Agrostis stolonifera* var. *palustris*) has been characterized as having moderate salinity tolerance among the cool-season turfgrasses (Turgeon, 2002). Even so, increased salt tolerance in bentgrass is desired 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). Physiological responses of turfgrasses to salinity include shoot dehydration, lowered osmotic potential, and/or a loss of turgor potential, resulting in reduced growth rate (Levitt, 1980; Neumann et al., 1988; Peacock and Dudeck, 1985). Dudeck et al. (1993) reported that leaf water potential, leaf osmotic potential, and leaf turgor potential of St. Augustinegrass [*Stenotaphrum secundatum* (Walter) Kuntze] decreased linearly with increased salinity. Relative top growth, tissue water content, and accumulation of cytoplasmic solutes have been used to evaluate salt tolerance of turfgrasses (Marcum and Murdoch, 1990, 1994). When grown at high salinity, creeping bentgrass has been known to exhibit inhibition of root water absorption and reduced shoot water content (Marcum and Murdoch, 1990, 1994). Relative root growth is a reasonable indicator of the complex of factors determining salt resistance (Kit, 1989), and reduced turf quality has also been reported to accompany declining root production due to salt stress (Cordukes and Maclean, 1973).

During salinity stress, the concentration of mineral nutrients in turfgrass shoots and roots, as well as their balance, can fluctuate

greatly. With increasing soil salt concentration, leaf tissue chloride concentration has been reported to increase in Kentucky bluegrass (*Poa pratensis* L.), creeping red fescue (*Festuca rubra* L. ssp. *rubra*), and perennial ryegrass (*Lolium perenne* L.) (Cordukes and Maclean, 1973). Ahmad et al. (1981) reported that in the presence of salt, accumulation of Na and Cl in both shoots and roots of creeping bentgrass was accompanied by a decline in tissue K content. Peacock et al. (1993) reported that as salinity increased, tissue Cl increased linearly, P levels decreased linearly, while Ca and Mg decreased nonlinearly. Dudeck and Peacock (1985) have reported that the tissue content of Ca, K, and Mg decreased in seashore paspalum (*Paspalum vaginatum* Swartz.) with increased salinity. Avoidance of Na and Cl toxicity may involve ion exclusion at the root cortex (Jeschke, 1984) or redistribution of excess ions to senescing leaves or other plant parts (Yeo and Flowers, 1984). Marcum and Murdoch (1994) theorized that salinity tolerance is associated with exclusion of Na and Cl from shoots.

Humic acids (HAs) have been reported 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). Humic substances may also reduce plant uptake of certain toxic metal ions, Cd for example, adsorbing them from the soil solution (Strickland et al., 1979). In recent years, application of humic substances to golf course turf has become increasingly common. In previous research, humic substances have been shown to enhance photosynthesis, root growth, and uptake of Mg, S, and P in creeping bentgrass (Cooper et al., 1998; Liu et al., 1998). Since humic substances may enhance uptake of some mineral nutrients and reduce the uptake of certain toxic elements, one might reason that application of HA could improve plant response to salinity. However, there is a lack of research regarding HA application and its effects on plant salinity tolerance. Therefore, the objective of this research was to evaluate the effects of HA on creeping bentgrass salt tolerance by studying shoot growth, water uptake, photosynthesis, root growth and mineral nutrient uptake during salinity stress. This research

Received for publication 14 Aug. 2000. Accepted for publication 31 Oct. 2001. This work was supported in part by state and Hatch Act funds allocated to the North Carolina Agricultural Experiment Station. Additional support was provided by the Turfgrass Council of North Carolina.

<sup>1</sup>Former graduate student.

<sup>2</sup>Professor and corresponding author; e-mail: Rich\_Cooper@ncsu.edu.

was designed to evaluate several basic responses of creeping bentgrass to HA application using a controlled, solution-culture environment. The research was not intended to mimic field conditions and it would be inappropriate to assume that field-grown creeping bentgrass would necessarily respond similarly.

### Materials and Methods

**GENERAL PROCEDURES.** This research was conducted from 3 Apr. to 20 June 1997 in a greenhouse located at North Carolina State Univ., Raleigh, N.C., at lat. 35° N. No supplemental lighting was used during the study. Average maximum and minimum temperatures in the greenhouse were 38 and 21 °C, respectively. ‘Crenshaw’ creeping bentgrass sod was cut into 10.8 cm diameter plugs and transferred on 3 Apr. 1997 to six recirculating solution culture systems containing one-quarter-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<sup>-3</sup> H<sub>2</sub>SO<sub>4</sub>. Solutions were replaced every 2 weeks to ensure nonlimiting nutrition and were aerated vigorously throughout the study.

This experiment was a randomized complete block design with a factorial arrangement of six treatments and four replications. The six treatments were combinations of three salinity levels (0, 8.0, and 16.0 dS·m<sup>-1</sup>) and two rates of HA (0 and 400 mg·L<sup>-1</sup>). A salt mixture was formulated to approximate the average individual salt composition of seawater (Svedrup et al., 1959). The salt mixture contained (g·kg<sup>-1</sup> dry salt) 788 NaCl, 94 MgSO<sub>4</sub>, 73 MgCl<sub>2</sub>, 32 CaCl<sub>2</sub>, 7 KHCO<sub>3</sub>, and 6 KCL (Peacock et al., 1993). To avoid potential salinity shock, beginning on 13 May salinity levels were increased gradually by adding salt at a rate of 2.5 g·L<sup>-1</sup> at 2-d intervals over 8 d, or until final treatment levels were reached. On 20 May 1997, when final salinity levels (0, 8.0, and 16.0 dS·m<sup>-1</sup>) were attained, a commercial formulation of HA (Sustane HA, 100% HA a.i., Sustane Corp., Cannon Falls, Minn.) was added to solution culture systems to obtain final concentrations of 0 or 400 mg·L<sup>-1</sup>. The HA product had an elemental content

(g·kg<sup>-1</sup>) of 625 C, 32 H, 317 O, 20 P, 6 S, and 2 N. On the same day, the roots of all turf plugs were trimmed to a length of 10.0 cm before initiating HA treatments to reduce variability among root systems in case any particular plug had developed better rooting during the grow-in period. For the remainder of the study, the Hoagland’s solution and treatment solutions were replaced each week to maintain salinity, HA, and nutrient levels. The turf plugs were mowed at 6 mm three times per week throughout the study. No pesticides were applied.

**MEASUREMENT OF CLIPPING DRY WEIGHT AND WATER CONTENT.** Clippings were collected on 25 May, and 1, 8, and 15 June 1997 [5, 12, 19, and 26 days after treatment (DAT)], respectively. Fresh weight (FW) was determined immediately after clippings were collected, and dry weight (DW) was determined after oven drying at 75 °C for 48 h. Clipping water content was calculated using the equation: WC = (C<sub>FW</sub> - C<sub>DW</sub>) / C<sub>DW</sub>, where WC = water content (g/g DW), C<sub>FW</sub> = clipping FW (g); and C<sub>DW</sub> = clipping DW (g). Water content was expressed as grams of water per 1 g of DW.

**MEASUREMENT OF NET PHOTOSYNTHESIS.** Net photosynthesis (P<sub>N</sub>) was determined on 26 May, and 2, 9, and 16 June 1997 (6, 13, 20, and 27 DAT, respectively). Photosynthesis was measured between 0830 and 1030 HR on each measurement date, using a portable gas exchange system (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<sup>-2</sup>·s<sup>-1</sup> during measurements using supplemental lighting. Turf plugs were acclimated under the lights for 5 min before photosynthesis determination to assure light saturation.

**MEASUREMENT OF MAXIMUM ROOT LENGTH AND DW.** On 20 June 1997 (31 DAT), each plug and its associated root system were removed from the solution tank. Maximum root length was determined, and roots were divided into 0 to 10 cm and >10 cm sections for root mass determination. Roots were oven-dried at 75 °C for 48 h, and then weighed.

**MINERAL NUTRIENT ANALYSIS.** To examine the effect of HA on plant nutrition during salt stress, leaf tissue was harvested from

Table 1. Clipping dry weight (DW) and tissue water content response of creeping bentgrass grown under salinity stress in a solution containing humic acid (HA) at 400 mg·L<sup>-1</sup>.

Solution characteristics	df	Clipping DW (g)				Tissue water content (g·g <sup>-1</sup> DW)			
		May 25	June 1	June 8	June 15	May 25	June 1	June 8	June 15
Salinity (dS·m <sup>-1</sup> ) <sup>z</sup>									
0		0.68 a <sup>y</sup>	1.22 a	0.97 a	1.67 a	3.48 a	3.71 a	2.74 a	3.77 a
8.0		0.58 a	0.87 b	0.66 b	1.06 b	3.42 a	3.40 b	2.20 b	3.22 b
16.0		0.35 b	0.70 c	0.55 b	0.75 c	2.97 b	2.87 c	1.81 c	2.54 c
HA (mg·L <sup>-1</sup> ) <sup>x</sup>									
0		0.55	0.82 b	0.73	1.23 a	3.29	3.38	2.27	3.28
400		0.51	1.04 a	0.71	1.09 b	3.28	3.27	2.23	3.07
ANOVA (source)									
Salinity	2	***	***	***	***	***	***	***	***
Linear effect	1	***	***	***	***	***	***	***	***
Quadratic effect	1	NS	NS	NS	**	**	NS	NS	NS
HA	1	NS	***	NS	*	NS	NS	NS	*
Salinity × HA	2	NS	NS	NS	NS	NS	NS	NS	***

<sup>z</sup>Salt stress was initiated 13 May 1997. Salinity data represent an average across all HA treatments.

<sup>y</sup>Mean separation within columns for salinity and HA by Waller-Duncan K ratio (k = 100) t test (P < 0.05).

<sup>x</sup>HA treatments were initiated 20 May 1997. HA data represent an average across all salinity treatments.

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

each treatment three times per week. Clippings were dried at 75 °C for 48 h, ground and stored at room temperature until analysis. 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 C–H–N elemental analyzer (PE2400; Perkin-Elmer Corp, Norwalk, Conn.). A Plasma 2000 (Perkin-Elmer) was used to determine P, K, Ca, Mg, Cu, Fe, Mo, Mn, and Zn using 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). Chlorine (Cl) and Na were extracted according to the revised procedures of Gilliam (1971) (adding activated charcoal), and determined using a chloridometer (Haake Buchler, Saddle Brook, N.J.).

**DATA ANALYSIS.** All data were subjected to analysis of variance (ANOVA) procedures using Statistical Analysis System software (SAS Inst., Inc., 1985). Separation of significantly different treatment means was accomplished using preplanned orthogonal contrasts (Steel and Torrie, 1980). The Waller-Duncan K ratio *t* test was used for mean separation if the ANOVA F test indicated that source effects were significant.

## Results and Discussion

**CLIPPING DW AND WATER CONTENT.** Increasing salinity stress significantly decreased both clipping DW and tissue water content on all observation dates (Table 1). Plants subjected to a salinity level of 8.0 dS·m<sup>-1</sup> showed a significant DW reduction compared to control plants on the three observation dates during June (averaging 32% less DW over June observation dates). Tissue water content of plants subjected to a salinity level of 8.0 dS·m<sup>-1</sup> also showed a significant decrease compared to the control for all June observation dates, averaging a 14% reduction. At the 16.0 dS·m<sup>-1</sup> salinity level, the clipping DW and water content of creeping bentgrass decreased significantly compared to the control on all measurement dates, by an average of 47% for clipping DW and 26% for tissue water content. These results demonstrate

that reduced shoot growth and shoot dehydration are a common response of creeping bentgrass to salinity. This finding corroborates the report of Neumann et al. (1988).

Averaged over all salinity levels, HA treatment increased clipping DW 27% on 1 June, decreased clipping DW 11% on 15 June, and had no effect on growth on the other two observation dates (Table 1). The salinity × HA interaction was never significant. Averaged over all salinity levels, HA application generally did not influence tissue water content (Table 1). On 15 June, HA had no effect on water content at the salinity control level (0 dS·m<sup>-1</sup>), decreased water content at 8.0 dS·m<sup>-1</sup> salinity, and increased water content at 16.0 dS·m<sup>-1</sup> salinity level (data not presented). In general, HA application did not improve shoot growth or water retention of creeping bentgrass during salt stress.

**NET PHOTOSYNTHESIS.** Increasing salinity significantly decreased net photosynthetic rates on each observation date (Table 2). P<sub>N</sub> of turf subjected to the 8.0 dS·m<sup>-1</sup> salinity level was significantly reduced on two of four observation dates compared to the 0 dS·m<sup>-1</sup> treatment, with reductions of 9% and 16% measured on 26 May and 9 June, respectively. P<sub>N</sub> of turf subjected to the 16.0 dS·m<sup>-1</sup> salinity level was significantly reduced compared to the 0 dS·m<sup>-1</sup> treatment on all observation dates. P<sub>N</sub> was reduced by 13%, 20%, 27%, and 20% on 26 May, and 2, 9, and 16 June, respectively. HA application generally did not affect photosynthesis, and the salinity × HA interaction was not significant (Table 2).

**MAXIMUM ROOT LENGTH AND ROOT DW.** Increasing salinity significantly decreased maximum root length, but significantly increased root DW in the 0 to 10 cm and >10 cm depth root zones (Table 3). Maximum root length of turf subjected to either 8.0 or 16.0 dS·m<sup>-1</sup> salt stress level was reduced by 8% and 16%, respectively, compared to the control. However, turf subjected to 16.0 dS·m<sup>-1</sup> salinity stress exhibited significantly increased root DW compared to the control. Root DW increased by 14% and 44% in the 0 to 10 cm and >10 cm root zones, respectively, relative to the control.

Cordukes and Maclean (1973) reported that reduced turf quality caused by salt stress was accompanied by declining root growth. Dudeck et al. (1993) observed that salinity had less effect

Table 2. Net photosynthetic rate of creeping bentgrass grown under salinity stress in solutions containing 0 or 400 mg·L<sup>-1</sup> HA.

Solution characteristics	df	Net photosynthetic rate (μmol·m <sup>-2</sup> ·s <sup>-1</sup> )			
		May 26	2	June 9	16
Salinity (dS·m <sup>-1</sup> ) <sup>z</sup>					
0		23.30 a <sup>y</sup>	28.33 a	31.22 a	27.45 a
8.0		21.24 b	26.69 a	26.22 b	27.41 a
16.0		20.20 b	22.53 b	22.71 c	22.00 b
HA (mg·L <sup>-1</sup> ) <sup>x</sup>					
0		21.28	25.41	27.27	24.75
400		21.88	26.29	26.17	26.49
ANOVA (source)					
Salinity	2	***	***	***	***
Linear effect	1	***	***	***	***
Quadratic effect	1	NS	NS	NS	*
HA	1	NS	NS	NS	NS
Salinity × HA	2	NS	NS	NS	NS

<sup>z</sup>Salt stress was initiated on 13 May 1997. Salinity data represent an average across all HA treatments.

<sup>y</sup>Mean separation within columns for salinity and HA by Waller-Duncan K ratio (*k* = 100) *t* test. (*P* < 0.05).

<sup>x</sup>HA treatments were initiated 20 May 1997. HA data represent an average across all salinity treatments.

NS,\*,\*\*\*Nonsignificant or significant at *P* = 0.05 or 0.001, respectively.

Table 3. Maximum root length and root DW of creeping bentgrass in response to humic acid (HA) application during salinity stress.

Solution characteristics	df	Max root length (cm)	Root DW (g)	
			0–10 cm	>10 cm
Salinity (dS·m <sup>-1</sup> ) <sup>z</sup>				
0		30.6 a <sup>y</sup>	0.90 b	0.09 b
8.0		28.1 b	0.95 b	0.10 ab
16.0		25.8 c	1.03 a	0.13 a
HA (mg·L <sup>-1</sup> ) <sup>x</sup>				
0		29.0 a	0.93	0.11
400		27.3 b	0.99	0.10
ANOVA (source)				
Salinity	2	***	**	*
Linear effect	1	***	**	*
Quadratic effect	1	NS	NS	NS
HA	1	**	NS	NS
Salinity × HA	2	***	*	*
HA in 0 dS·m <sup>-1</sup> salinity	1	***	NS	*
HA in 8.0 dS·m <sup>-1</sup> salinity	1	NS	**	NS
HA in 16.0 dS·m <sup>-1</sup> salinity	1	NS	NS	NS

<sup>z</sup>Salt stress was initiated 13 May 1997. Salinity data represent an average across all HA treatments.

<sup>y</sup>Mean separation within columns for salinity and HA by Waller-Duncan K ratio (k = 100) *t* test. (*P* < 0.05).

<sup>x</sup>HA treatments were initiated 20 May 1997. HA data represent an average across all salinity treatments.

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

in reducing root growth than top growth. In the present study, salinity decreased clipping DW, tissue water content, and maximum root length linearly. However, root DW in both the 0 to 10 cm and >10 cm root zones increased linearly with increasing salinity. Thus, these results tend to substantiate the findings of Dudeck et al. (1993).

HA treatment significantly reduced maximum root length, but did not affect root weight in either the 0 to 10 cm or >10 cm root zones (Table 3). The salinity × HA interaction was significant for both maximum root length, and root DW of the 0 to 10 cm and >10 cm root zones. For the salinity × HA interaction (data not presented), HA exposure decreased maximum root length of salt

Table 4. Mineral nutrient content of creeping bentgrass in response to HA application during salinity stress.

Solution characteristics	df	g·kg <sup>-1</sup>								mg·kg <sup>-1</sup>					
		N	P	K	Ca	Mg	S	Cl	Na	Fe	Mn	Cu	Zn	Mo	B
Salinity (dS·m <sup>-1</sup> ) <sup>z</sup>															
0		60.4 a <sup>y</sup>	6.2 a	37.9 a	6.3 a	3.0 c	5.4 a	7.3 c	0.7 c	215	240 b	31	85	10 b	64 c
8.0		58.2 b	5.7 b	26.5 b	3.4 b	3.4 b	5.0 b	16.9 b	8.4 b	228	274 a	28	84	12 a	102 b
16.0		56.7 c	5.0 c	24.2 c	3.4 b	3.6 a	5.0 b	20.9 a	9.2 a	234	275 a	27	85	13 a	143 a
HA (mg·L <sup>-1</sup> ) <sup>x</sup>															
0		58.3	5.6	29.5	4.5 a	3.3 b	5.2 a	15.0	5.9 b	243	227 b	31 a	88 a	11 b	101
400		58.6	5.7	29.6	4.3 b	3.4 a	5.0 b	15.1	6.4 b	209	299 a	27 b	82 b	12 a	105
Normal range <sup>w</sup>		45–60	3–6	22–26	5–7.5	2.5–3	3.4–4.3			100–300	50–100	8–30	25–75		8–20
ANOVA (source)															
Salinity	2	***	***	***	***	***	***	***	***	NS	***	NS	NS	***	***
Linear effect	1	***	***	***	***	***	***	***	***	NS	***	*	NS	***	***
Quadratic effect	1	NS	NS	***	***	*	**	***	***	NS	**	NS	NS	*	NS
HA	1	NS	NS	NS	*	*	***	NS	*	NS	***	**	***	**	NS
Salinity × HA	2	NS	NS	**	***	***	NS	***	NS	**	**	**	**	**	NS
HA in 0 dS·m <sup>-1</sup> salinity	1			**	***	***		NS		NS	***	***	***	NS	
HA in 8.0 dS·m <sup>-1</sup> salinity	1			NS	NS	**		**		NS	***	NS	NS	NS	
HA in 16.0 dS·m <sup>-1</sup> salinity	1			NS	NS	*		**		**	***	NS	NS	***	

<sup>z</sup>Salt stress was initiated 13 May 1997. Salinity data represent an average across all HA treatments.

<sup>y</sup>Mean separation within columns for salinity and HA by Waller-Duncan K ratio (k = 100) *t* test. (*P* < 0.05).

<sup>x</sup>HA treatments were initiated 20 May 1997. HA data represent an average across all salinity treatments.

<sup>w</sup>Normal nutrient range for creeping bentgrass suggested by Mills and Jones (1996).

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

## Literature Cited

- stress control plants ( $0 \text{ dS}\cdot\text{m}^{-1}$ ), but did not affect plants grown in either the  $8.0$  or  $16.0 \text{ dS}\cdot\text{m}^{-1}$  solution. HA treatment increased root DW ( $0$  to  $10 \text{ cm}$  root zone) in the  $8.0 \text{ dS}\cdot\text{m}^{-1}$  solution, but decreased root DW of the  $>10 \text{ cm}$  root zone of the control solution. Given these variable results, it appears that HA exposure had no meaningful effect on creeping bentgrass root development or growth during salt stress.
- MINERAL NUTRIENT CONTENT.** Increasing salinity significantly decreased leaf tissue content of N, P, K, Ca, and S; increased the content of Mg, Mn, Mo, B, Cl, and Na; and did not affect the content of Fe, Cu, and Zn (Table 4). The N, P, K, Ca, and S tissue content of turf subjected to either  $8.0$  or  $16.0 \text{ dS}\cdot\text{m}^{-1}$  decreased significantly compared to nonsalt-stressed turf. Averaged over both the  $8.0$  and  $16.0 \text{ dS}\cdot\text{m}^{-1}$  salt stress levels, Ca content decreased by 46% and K content decreased by 33% relative to the control. Magnesium, Mn, Mo, B, Cl, and Na content in tissue of turf subjected to either  $8.0$  or  $16.0 \text{ dS}\cdot\text{m}^{-1}$  increased significantly, with Na content increased  $\approx 12$ -fold, Cl by  $\approx 160\%$ , and B by  $\approx 90\%$ .
- Results herein corroborate findings of Ahmad et al., (1981), Cordukes and Maclean, (1973), Dudeck and Peacock, (1985), Marcum and Murdoch, (1990, 1994), and Peacock et al., (1993) regarding uptake of Cl, P, Na, Ca, and K during salinity stress. However, Dudeck and Peacock (1985) reported a decrease in Mg content with increased salinity in seashore paspalum. The present investigation showed Mg increasing linearly with increasing salinity in creeping bentgrass.
- HA treatment significantly increased Mg, Mn, Mo, and Na content, decreased Ca, S, Cu, and Zn content, and did not affect N, P, K, Fe, B, and Cl content (Table 4). Averaged over both salinity levels, the increase in Mg, Mn, Mo, and Na content was 2%, 32%, 9% and 5% respectively, and the decrease in Ca, S, Cu, and Zn content was 4%, 4%, 13%, and 7%, respectively.
- One negative effect of increasing salinity is inhibited uptake of essential mineral nutrients such as N, P, K, and Ca (Ahmad, et al., 1981; Peacock et al., 1993). Also, toxic accumulation of nutrients such as Cl, Na, and Mn has been reported (Taiz and Zeiger, 1991). Although inhibited by increased salinity, tissue concentrations of N, P, K, and S at either  $8.0$  or  $16.0 \text{ dS}\cdot\text{m}^{-1}$  salinity levels were still within the sufficiency range noted by Mills and Jones (1996) for creeping bentgrass, probably due to the nonlimiting nutrient supply in solution. However, tissue content of Ca at either  $8.0$  or  $16.0 \text{ dS}\cdot\text{m}^{-1}$  ( $3.4 \text{ g}\cdot\text{kg}^{-1}$ ) was below the normal range ( $5$  to  $7.5 \text{ g}\cdot\text{kg}^{-1}$ ). During salinity stress, tissue concentrations of Cl and Na were increased drastically. Chlorine concentration increased by 133% and 188%, and Na content increased by 1116% and 1238% for plants growing in the  $8.0$  and  $16.0 \text{ dS}\cdot\text{m}^{-1}$  salinity solutions, respectively. This was expected due to the high amounts of these elements present in the salt solutions.
- Exposure to HA during salinity stress did not increase uptake of N, P, K, and Ca, whose uptake were inhibited by salinity; nor did HA decrease uptake of the excessive, toxic elements of Cl and Na in salt solution (Table 4). Exposure to HA at  $400 \text{ mg}\cdot\text{L}^{-1}$  did not improve creeping bentgrass nutrient status during salt stress.
- In conclusion, increasing salinity stress significantly decreased clipping DW, tissue water content, and  $P_N$  of creeping bentgrass on all observation dates. Application of HA at  $400 \text{ mg}\cdot\text{L}^{-1}$  during salinity stress had no effect on creeping bentgrass growth, nor its nutrient uptake characteristics. Thus, under the conditions of this research, HA appears to have little promise as an agent to reduce salinity stress to creeping bentgrass.
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