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Effect of Ozonated Water on Creeping Bentgrass Growth in a Sand Medium

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ADDITIONAL INDEX WORDS. greens, root zone, leachate, chlorophyll, mineralization, aeration, nitrate, phosphorus

SUMMARY. Irrigation of sand-based golf greens with ozonated water may affect grass growth and chemical processes in the root zone. The objective of this study was to evaluate the effects of ozonated and aerated water on bentgrass growth and root zone chemistry in sand-based greens over a 12-month period. Creeping bentgrass (*Agrostis stolonifera*) cores [10 cm diameter × 12 cm depth (3.9 × 4.7 inches)] were collected from a sand-based bentgrass nursery and placed in columns designed to collect leachate water. Cores were placed in a greenhouse and irrigated with 1) municipal tap water [6 to 8 mg·L⁻¹ (ppm) dissolved oxygen (DO)], 2) aerated tap water (12 mg·L⁻¹ DO), or 3) ozonated tap water (aerated plus 0.8 mg·L⁻¹ ozone). Leachate was periodically collected and analyzed for pH, electrolytic conductivity (EC), and nutrients. Grass clippings were weighed and analyzed for total nitrogen (N) and phosphorus (P). Roots were periodically collected from selected cores to determine root distribution. At 40 and 90 days after initiating water treatments, bentgrass irrigated with ozonated water had a higher chlorophyll index than bentgrass irrigated with tap water. After 128 and 157 days, bentgrass clipping weights were significantly greater for the cores irrigated with ozonated water and, to a lesser extent, aerated water. At 61 and 149 days, nitrate (NO₃-N) and EC levels were elevated in leachate from aerated and ozonated samples, sug-

gesting increased mineralization of organic matter in those bentgrass cores. Ozonated water increased bentgrass crown weights, but had no effect on root mass. Ozonated water did not affect bentgrass tissue N and P concentrations. Statistically significant effects from ozonated water occurred within the first few months, but sustained benefits were negligible.

Ozone (O₃) is a reactive molecule that is produced from atmospheric oxygen (O₂) by passing air through a high voltage corona discharge. Ozone has been used for disinfecting drinking water (Camel and Bermond, 1998), different types of wastewater (Beltran et al., 1999; Kearney et al., 1988), food processors (Restaino et al., 1995), and other applications where there is a need to reduce microbial populations. Ozone has been used to oxidize organic substances ranging from pesticides (Dong and Doo, 2000) to algae (Yun et al., 1997) and to degrade humic substances in water and reduce turbidity (Amir-sardari et al., 1998; Gracia et al., 1996). More recently, ozone has been added to irrigation waters with reported benefits that include increased water infiltration, increased disease resistance, and reduced need for fertilizers (Raub et al., 2001). Although there is limited scientific data to demonstrate these benefits, the results are consistent with the predicted effects one would see when adding a strong oxidizer, such as ozone, to soil. Ozone may oxidize soil organic matter and plant residues, thus releasing essential plant nutrients. Oxidation of organic matter coatings on clay particles could change their surface charges and cause them to flocculate (Raub et al., 2001). This effect could improve water infiltration by preventing clay dispersion, which can clog the soil pore system. The effects of using ozonated water in a sand-based growing medium, such as a putting green, are unknown. Although there may be beneficial short-term effects, it is possible that long-term effects would be detrimental due to the breakdown of organic matter. We need a greater understanding of the effects of ozonated water on chemical and physical properties in the rooting zone of a sand-based growing medium so that we can make knowledgeable decisions on how to use ozonated water in managing golf course greens. The

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primary objective of this study was to determine the effects of continuous irrigation with ozonated water on creeping bentgrass growth and on physical and chemical properties of a sand-based root zone mix.

Materials and methods

SET UP. A greens cup cutter was used to collect 72 bentgrass cores measuring 10-cm diameter $\times$ 12-cm depth from a nursery at the Trophy Club Golf Course in Trophy Club, Texas. Thirty-six cores were collected in Aug. 2001 and another 36 cores were collected 3 weeks later. Each core was placed in a modified polyvinyl chloride (PVC) column and transferred to a greenhouse at the Texas A&M University Research and Extension Center in Dallas. The PVC columns were designed with an upper chamber for supporting the bentgrass core and a detachable lower chamber for collecting leachate. Bentgrass cores fit snugly into the PVC columns in such a way that air was able to enter the cores primarily through the surface. However, it is likely that some air entered the root zone through the bottom of the cores whenever the detachable lower chamber was removed to collect leachate. Cores were fertilized with ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4, 21\text{N}-0\text{P}-0\text{K}; \text{Fisher Scientific, Pittsburgh}]$ at a rate of $48.8 \text{ kg}\cdot\text{ha}^{-2}$ ($1 \text{ lb}/1000\text{ft}^2$) N and this fertilizer application was repeated approximately every 2 months. The bentgrass cores were watered daily with greenhouse tap water. Each time the columns were irrigated, sufficient water was added to saturate the greens cores. Excess water drained freely from the cores within 1 h after irrigation, but it was likely that a small saturated layer temporarily remained at the bottom of each column.

WATER TREATMENTS. On 17 Jan. 2002, bentgrass columns were divided into three groups of 24 columns. From this date forward for the next 12 months, each group was exclusively watered with one of three water treatments. One group was watered with normal city tap water, the second group with aerated tap water, and the third group with ozonated tap water. Ozonated water was generated using an OxionTM ozone generator (Thunderstorm Technology, Flowermound, Texas), which passes ambient air through high voltage corona discharge columns before introducing

the ozonated air into irrigation water with a venturi-type aspirator. Ozone concentrations were measured at various flow rates using the indigo colorimetric method (Greenberg et al., 1992). Aerated water was generated with the same unit by decreasing the voltage on the corona discharge tubes to zero. Dissolved oxygen concentrations in the tap water ranged from 6 to $8 \text{ mg}\cdot\text{L}^{-1}$, and were increased to 12 to $14 \text{ mg}\cdot\text{L}^{-1}$ in the aerated and ozonated water. Dissolved oxygen was measured with a dissolved oxygen meter (Orion Model 862A, Orion Research, Beverly, Mass.). Elevated ozone and dissolved oxygen levels in the irrigation water were temporary and generally decreased to initial levels within 1 to 2 min when the treated water was left in an open 1-L beaker.

GRASS MEASUREMENTS. Chlorophyll content is a good indicator of the overall nutrient status of a plant, especially nitrogen. At 40 and 90 d after initiating water treatments, we measured the relative chlorophyll content of the bentgrass cores using a chlorophyll meter (Fieldscout CM1000; Spectrum Technologies, Plainfield, Ill.). Chlorophyll content was measured at five spots on the surface of each bentgrass core and the five readings were averaged to obtain a single relative chlorophyll value.

Aboveground bentgrass was clipped to a height of 12.7 mm (0.5 inch) whenever growth reached 25.4 to 38.1 mm (1.0 to 1.5 inches). On average, grass was clipped every 4 to 5 weeks but actual time between clippings depended on a variety of conditions, including frequency of fertilization and watering as well as seasonal changes in greenhouse growing conditions. Grass clippings were dried at 60°C (140.0°F) and weighed to determine aboveground biomass production. At 103 and 131 d after initiating treatments, grass clippings were ground to pass a 1-mm (0.04 inch) sieve and analyzed for total nitrogen content.

LEACHATE CHEMISTRY. On three occasions after initiating water treatments, leachate water was collected and analyzed for nitrate ($\text{NO}_3\text{-N}$), orthophosphate ($\text{PO}_4\text{-P}$), electrolytic conductivity (EC), and pH. On the first two occasions, 300 mL (10.1 fl oz) of the appropriate water treatment was added to each column, but only 150 mL (5.1 fl oz) was used for the third leaching event. Excess water was

allowed to drain from the bentgrass cores for several hours before leachate was removed from the lower column reservoir. Nitrate, EC, and pH were measured on unfiltered leachate and $\text{PO}_4\text{-P}$ was measured on leachate filtered through a $0.45\text{-}\mu\text{m}$ cellulose nitrate membrane filter.

ROOT WEIGHTS. Four randomly selected soil cores were removed from each treatment at 70, 171, and 274 d after initiation of water treatments. Each core was divided into the crown and three depth increments of 0–3.81, 3.81–7.62, and 7.62–11.43 cm (0–1.5, 1.5–3.0, and 3.0–4.5 inches). Roots were separated from the sand growing medium by washing, dried at 60°C , and then weighed in order to determine root mass distribution with depth.

ROOT ZONE MIX. In order to evaluate the effect of water aeration and ozonation on organic matter content of the sand-based root zone mix, we determined organic matter content of the root zone mix by measuring loss of weight from a 1.0-g (0.035 oz) sample after heating at 400°C (752.0°F) for 16 h (Nelson and Sommers, 1996). All visible root residues were removed from the sample before the procedure was carried out. Soil organic matter was first measured at 0–3.81, 3.81–7.62, and 7.62–11.43 cm depth on samples collected 170 d after initiating water treatments. Soil organic matter was measured again in the upper 3.81 cm of the bentgrass cores 12 months after initiating water treatments.

Results and discussion

OZONE CONCENTRATION. Ozone concentration in the irrigation water varied with water flow rate through the ozone generator (Fig. 1). Ozone concentration in the irrigation water was $0.2 \text{ mg}\cdot\text{L}^{-1}$ when the greenhouse spigot was opened to allow a maximum flow of $24 \text{ L}\cdot\text{min}^{-1}$ (6.3 gal/min). Ozone concentration increased to approximately $0.9 \text{ mg}\cdot\text{L}^{-1}$ as flow rate was decreased to $3 \text{ L}\cdot\text{min}^{-1}$ (0.8 gal/min). We used a flow rate of approximately $5 \text{ L}\cdot\text{min}^{-1}$ (1.3 gal/min) when watering the bentgrass cores, resulting in an ozone concentration of 0.7 to $0.9 \text{ mg}\cdot\text{L}^{-1}$. Maier (1984) reported that when treating drinking water, ozone concentrations of 0.5 to $1.5 \text{ mg}\cdot\text{L}^{-1}$ resulted in maximum flocculation of colloidal material.

LEACHATE CHEMISTRY. Nitrate concentrations in leachate from the

bentgrass cores were affected by the water treatments more than other chemical constituents, but the effect was not apparent at every sampling date (Table 1). There was no difference in leachate $\text{NO}_3\text{-N}$ concentrations 18 d after initiating the water treatments, but after 61 and 149 d both the aerated and ozonated treatments had higher leachate $\text{NO}_3\text{-N}$ concentrations than the control. At 149 d, leachate $\text{NO}_3\text{-N}$ concentrations for the ozonated water treatment were significantly higher than both the aerated and control treatments. There was significantly less $\text{PO}_4\text{-P}$ in leachate from the aerated water treatment at 61 d after initiating water treatments, but no significant differences at the other sampling dates. Electrolytic conductivity was mostly unaffected by water treatments, and pH was significantly lower for the ozone water treatment at 149 d after initiation of treatments. Raub et al. (2001) reported lower pH and higher electrolyte concentrations in drainage water from agricultural soils treated with ozonated water compared to tap water. However, they used water with an ozone concentration of $10 \text{ mg}\cdot\text{L}^{-1}$, whereas the concentration in our study was much lower ($0.7\text{--}0.9 \text{ mg}\cdot\text{L}^{-1}$). Also, they used a native agricultural soil that had a higher organic matter content than the sand-based putting green used in our study. Overall, analysis of leachate from the sand-based bentgrass cores provided little evidence that ozonated or aerated water produced major changes in root zone chemistry.

BENTGRASS GROWTH. Chlorophyll content can be a good indicator of a plant's general health and nutritional status. Relative chlorophyll content of bentgrass irrigated with aerated and ozonated water was significantly higher than control bentgrass 40 d after initiating water treatments (Fig. 2). At 90 d, only bentgrass irrigated with ozonated water had higher relative chlorophyll content than the control bentgrass. It is possible that increased relative chlorophyll contents in bentgrass irrigated with ozonated and, to a lesser extent, aerated water were due to release of nutrients by increased mineralization of organic residues in the crown and surface layer of the bentgrass cores. Raub et al. (2001) found that ozonated water increased the concentration of electrolytes, including nitrate and ammonium, in soil leachate water. If

Fig. 1. Relationship between water flow rate through the ozone generator and ozone concentration in the irrigation water. Error bars represent ± 1 standard deviation of the treatment mean; $1 \text{ L}\cdot\text{min}^{-1} = 0.264 \text{ gal/min}$.

Table 1. Effect of aerated and ozonated water on leachate chemistry at various times after initiation of the water treatments. For each date, treatment means followed by the same letter are not significantly different (Fisher's least significant difference, $P \leq 0.05$).

Treatment	Nitrate ($\text{NO}_3\text{-N}$) [$\text{mg}\cdot\text{L}^{-1}$ (ppm)]	Phosphate ($\text{PO}_4\text{-P}$) ($\text{mg}\cdot\text{L}^{-1}$)	Electrolytic conductivity ($\text{dS}\cdot\text{m}^{-1}$) ^z	pH
<i>4 Feb. 2002 (18 DAIT)^b (4 DALEA)^c</i>				
Control	10.0 a	0.13 a	1203 a	7.92 a
Aerated	9.5 a	0.16 a	1148 a	7.91 a
Ozonated	10.1 a	0.14 a	1174 a	7.91 a
<i>19 Mar. 2002 (61 DAIT) (47 DALEA)</i>				
Control	7.8 b	0.72 a	562 a	7.77 a
Aerated	8.7 a	0.38 b	588 a	7.78 a
Ozonated	9.6 a	0.53 ab	572 a	7.70 a
<i>18 June 2002 (149 DAIT) (49 DALEA)</i>				
Control	33.1 c	0.17 a	722 ab	8.44 a
Aerated	36.6 a	0.16 a	713 b	8.39 ab
Ozonated	34.8 b	0.25 a	753 a	8.33 b

^z $\text{dS}\cdot\text{cm}^{-1} = \text{mmhos}\cdot\text{cm}^{-1}$.

^bDays after initiation of water treatments.

^cDays after last fertilization application of ammonium sulfate nitrogen [$(\text{NH}_4)_2\text{SO}_4\text{-N}$] at $1 \text{ lb}/1000\text{ft}^2$.

Fig. 2. Effect of aerated and ozonated water on the relative chlorophyll index of bentgrass, measured with a chlorophyll meter (Fieldsout CM1000; Spectrum Technologies, Plainfield, Ill.), 40 and 90 d after initiating water treatments. For each sampling date, columns with the same letter are not significantly different (Fisher's least significant difference, $P \leq 0.05$).

Table 2. Effect of aerated and ozonated water on bentgrass clipping weights (g/pot) at various times after initiation of the water treatments. Treatment means for a given date followed by the same letter are not significantly different (Fisher's least significant difference, $P \leq 0.05$).

Date	DAIT ^z	DALFA ^y	Control	Aerated	Ozonated
-----Clipping wt (g/pot) ^x -----					
14 Feb. 2002	28	14	0.48 a	0.49 a	0.47 a
30 Apr. 2002	103	40	1.61 b	1.85 a	1.79 ab
28 May 2002	128	28	1.03 b	1.08 b	1.20 a
26 June 2002	157	57	0.72 b	0.93 a	0.95 a
30 July 2002	191	15	0.93 a	0.84 a	0.91 a
15 Sept. 2002	238	62	0.59 a	0.60 a	0.67 a
16 Oct. 2002	269	62	0.97 a	0.97 a	0.96 a
4 Nov. 2002	288	10	0.89 a	0.81 a	0.90 a
26 Nov. 2002	310	32	0.91 a	0.85 a	0.91 a
16 Dec. 2002	330	52	0.79 a	0.83 a	0.88 a

^zDays after initiation of treatments.

^yDays after last fertilization application of ammonium sulfate nitrogen $[(\text{NH}_4)_2\text{SO}_4\text{-N}]$ at 1 lb/1000 ft².

^x1.00 g = 0.353 oz.

ozonated water did increase soluble nutrients by oxidizing organic matter, then it is likely that the increased nutrients were taken up and utilized by bentgrass.

Bentgrass cores were clipped 10 times in the 330 d following initiation of the water treatments. The length of time between clippings ranged from 19 to 75 d and depended on a variety of conditions, including frequency of fertilization and watering as well as seasonal changes in greenhouse growing conditions. Aerated water significantly increased bentgrass clipping weights 103 d after initiating water treatments and ozonated water did the same at 128 d after initiating water treatments (Table 2). Both aerated and ozonated water increased clipping weights at 157 d after initiating the water treatments.

Increased bentgrass yields could not reliably be attributed to increased N availability and uptake because the only significant difference in bentgrass N content was for the aerated treatment at 103 d (Fig. 3).

Bentgrass yield data suggests that if water ozonation and aeration were responsible for increased bentgrass clipping weights, the effects were temporary and occurred within the first 2 to 3 months after initiating the treatments. Ozonated and/or aerated water increased bentgrass clipping weights only for the second, third, and fourth cuttings after initiating the water treatments (Table 2). There were no significant differences among treatments for the last six clippings. If ozonated and aerated water accelerated the breakdown of soil organic matter,

as suggested by Raub et al. (2001), and this in turn stimulated increased bentgrass growth, then it is likely that the effect would be temporary and cease once the most easily oxidized organic matter was exhausted. Seasonal variations in greenhouse growing conditions probably contributed to the effect of ozonated and/or aerated water on bentgrass clipping weights as well. Moderate temperatures and increasing day length associated with late spring and early summer occurred near the start of this study when we observed increased clipping weights and, to a lesser extent, elevated $\text{NO}_3\text{-N}$ leachate concentrations with ozonated and/or aerated water. Later in the study, during summer months, bentgrass growth was probably limited by high greenhouse temperatures. This is evidenced by a general decrease in clipping weights during the last six harvests (Table 2). When cooler temperatures returned in the fall, the pool of organic matter that was easily oxidized by ozonated or aerated water had already been depleted due to continuous application of the water treatments.

CROWN/ROOT GROWTH AND SOIL ORGANIC MATTER. Bentgrass crown weights were significantly increased by ozonated water at 70 and 274 d after initiation of water treatments (Fig. 4). However, water treatments had no significant effect on root weights at any of the sampling depths or times. Twelve months after initiating the study, there was no difference among treatments in soil organic matter content in the upper 3.81 cm of the bentgrass cores (Fig. 5). Raub et al. (2001) calculated that the ozone in ozonated water penetrated to a depth of <2 mm (0.08 inch), indicating that the ozone-related reactions were limited to the soil surface. Therefore, it is likely that the ozone-treated water in our study had its greatest effect in the crown area of the bentgrass cores and the roots immediately below the crown area. If roots from the top 1.27 cm (0.5 inch) of the bentgrass core had been separated from the deeper roots, perhaps we would have measured a significant difference at that depth. Another possible reason that ozonated and aerated water did not affect root weights at deeper depths is that none of the roots in this study suffered from severe oxygen deprivation. Therefore, the effects of ozonated water on root growth in an anaerobic root zone were not tested.

Fig. 3. Effect of aerated and ozonated water on total nitrogen (N) content of bentgrass clippings 103 and 131 d after initiating water treatments, which also corresponded to 40 and 28 d after the most recent fertilizer application, respectively. For each sampling date, columns with the same letter are not significantly different (Fisher's least significant difference, $P \leq 0.05$).

Fig. 4. Effect of aerated and ozonated water on bentgrass crown and root weights at 70, 171, and 274 d after initiation of water treatments. Depth increments are equivalent to 0–1.5, 1.5–3.0, and 3.0–4.5 inches. Columns for each sampling data and depth labeled with the same letter are not significantly different (Fisher's least significant difference, $P \leq 0.05$); 1 g = 0.035 oz.

Fig. 5. Soil organic matter contents at 0–3.81 cm (0–1.5 inches) after 12 months irrigation with normal tap water, aerated water, or ozonated water. Columns with the same letter are not statistically different (Fisher's least significant difference, $P \leq 0.05$).

Conclusions

A 1-year greenhouse study provided only limited evidence that ozonated water had some beneficial effects on bentgrass growth in a sand-based medium, but the effects occurred primarily during the first few months after initiating the water treatments and were not consistent during the entire length of the study. The clearest evidence was in the form of short-term increases in

clipping weights and higher chlorophyll contents for bentgrass treated with ozonated water. These effects corresponded to increased $\text{NO}_3\text{-N}$ concentrations in leachate water, suggesting that improved growth was partially attributed to increased nutrients released by the breakdown of organic residues. Some of these same effects were also apparent for aerated water. It is likely that the effects observed in this study would be more prominent with higher levels of ozone in the irrigation water or with root zones suffering from anaerobic conditions. It also appears that the effects of ozonated water may become negligible after continuous application for a period of time.

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