

Effective Acidification of Calcareous Sand Rootzones Underlying Penn A-Series Creeping Bentgrass Putting Greens



Date: 30 June, 2014
Submitted to: The Ontario Turfgrass Research Foundation (OTRF)
Submitted by: Derek T. Pruyne and Maxim J. Schlossberg
The Pennsylvania State University Center for Turfgrass Science

Rootzone pH highly influences nutrient availability during establishment and maintenance of Penn A-series creeping bentgrass (*Agrostis stolonifera* L.) putting greens. Reference soil pH levels (1:1 mass soil:H₂O) supporting optimal health of creeping bentgrass reside within a range of 5.5–6.5 (Beard, 1973).

Calcareous soils are alkaline soils containing free lime (CaCO₃), and are typically characterized by pH levels between 7.2 and 9. They contain enough calcitic parent material to visibly effervesce when wet by ≥0.5% hydrochloric (HCl) acid solution, and possess calcium carbonate equivalencies (CCE) as high as 30% by dry mass (Carrow et al., 2001).

Justification

Calcite (CaCO₃) is a common impurity of quartz sand deposits in the Midwest and Western regions of North America, yet has become increasingly prevalent in commercially-available sands throughout the Mid-Atlantic and Northeastern US. When used to formulate putting green rootzones, quartz sands containing as little as 1% calcareous parent material result in persistent alkaline reactions (soil pH >7). Due to the inherently low nutrient holding capacity of sand rootzones, such supraoptimal pH levels contribute to nutrient deficiencies that become exacerbated in creeping bentgrass putting greens over time (Johnson et al., 2003).

A common cultural practice employed by managers of alkaline creeping bentgrass putting greens is the use of ammonium-N fertilizers. Greenhouse and field research has proven this to be a worthwhile effort towards lowering soil pH levels (Glinski et al., 1990), as well as increasing both tolerance to patch diseases (Hill et al., 2003; Thompson et al., 1993; Thompson et al., 1995) and availability of macro-/micro-nutrients (Schlossberg and Schmidt, 2007). It is important to note, however, that the degree of benefits observed in these studies was correlated to rate of ammonium-N fertilizer application (Schlossberg, 2006). Thus, exclusive use of ammonium-N in 'lean and mean' fertilizer programs is less likely to reduce soil pH than in programs satisfying the 15 to 45 kg N ha⁻¹ requirement of creeping bentgrass putting greens each month (Carrow et al., 2001).

Furthermore, soil/rootzone acidification through use of primarily-ammonium N fertilizer(s) can be negated by other frequent cultural practice(s). Topdressing with kiln-dried and/or calcareous sand introduces surprising quantities of Ca/Mg/K oxides and/or calcium carbonate to putting green rootzones. Likewise, irrigation water is a common and often significant input of alkalinity to turfgrass rootzones, especially in arid or drought-affected regions (Carrow, 2012).

Adverse effects of supraoptimal soil/rootzone pH levels on creeping bentgrass health may extend beyond nutrition and disease susceptibility. Pruyne and Schlossberg (2012) observed sand pH levels ≥7.0 to

significantly restrict creeping bentgrass root length density in the 8 to 24-cm depth of rootzone, despite frequent and liberal application of a complete fertilizer solution (including chelated micronutrient forms). Thus, while countless golf course superintendents currently do a superb job managing Penn A-series creeping bentgrass on alkaline ($\text{pH} > 7$) putting green rootzones, the task relies heavily on frequent and costly cultural practice(s). Which serves as a worthwhile segue to this classic quote by the late Dr. C.E. Kellogg: 'If we wanted to, we could grow crops on billiard balls. It's just a matter of the economics.'

Expense associated with current alkaline/calcareous creeping bentgrass putting green management practice, i.e., applying high rates of systemic fungicides to limit summer/take-all patch susceptibility, as well as frequently applying foliar fertilizers to optimize nutrition; compels investigation and discovery of a more effective approach. Specifically, how can soil amendments be used to safely lower supraoptimal rootzone/soil pH levels in maintained creeping bentgrass putting greens?

Elemental sulfur oxidation

The most common method for reducing an undesirably high soil pH is application/incorporation of elemental sulfur (S_0). Soil bacteria of the *Thiobacillaceae* family biochemically oxidize S_0 by the following reaction: $2\text{S}_0 + 3\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{H}_2\text{SO}_4$. The resulting production of sulfuric acid acts to dissolve free calcium carbonate within the soil.

The *Thiobacillaceae* family of chemoautorrophic bacteria gains useful energy from sulfur oxidation and carbon dioxide reduction via the Calvin cycle (Paul and Clark, 1996). Members of the *Thiobacillus* genera are motile, nonspore forming, rod-shaped bacteria, typically $0.3 \times 2\text{-}\mu\text{m}$ in size. While some *Thiobacilli* species thrive in acidic soil/wetland environments, more are indigenous to arable soils having near-neutral pH levels. The optimal soil temperatures for these bacteria are observed in the 25 to 35 C range. Thus in temperate climates, the majority of such biochemical S_0 oxidation occurs in summer.

Thiobacillus thioparus thrives at a near 6.9 soil pH level and is classified as a facultative anaerobe, meaning it is adaptable to short-term anoxic conditions. However, in the case of porous soils/rootzones constructed to support turfgrass systems, *Thiobacilli* species of interest are those adapted to use S_0 as the primary electron donor under aerobic and near-neutral soil conditions. In this instance, *T. thioparus* most effectively oxidizes S_0 , thiosulfate ($\text{S}_2\text{O}_3^{2-}$), and thiocyanate (SCN^-) using oxygen as the electron acceptor, but has the ability to reduce nitrite equally as well (Sylvia et al., 2001).

In the typical oxidation of S_0 , oxygen functions as the terminal electron acceptor. *Thiobacillus denitrificans* possesses the unique ability of substituting nitrate for oxygen as the electron acceptor by the following reaction: $2\text{NO}_3^- + 2\text{S}_0 + 2\text{H}_2\text{O} \rightarrow 2\text{H}_2\text{SO}_4 + \text{N}_2$ (Paul and Clark, 1996). The resulting denitrification, or gaseous loss of soil nitrate to the atmosphere, constitutes an undesirable transformation. Yet this N fate is unlikely, as the mobility and measureable CCE of nitrate fertilizers make them poor candidates for application to well-drained turfgrass systems having supraoptimal pH levels.

Elemental sulfur as an acidification treatment

Publications describing elemental sulfur (S_0) treatment of turfgrass systems are surprisingly limited. A recent two-year study evaluating S_0 treatment of a creeping bentgrass putting green has been reported by Bell et al. (2001). Three years prior to treatment initiation, the 'SR 1020' bentgrass was established to a slightly-alkaline, 17:3 sand:peat (v:v) root zone ($\text{pH}_w=7.1$), and continuously irrigated with alkaline water

(pH=8.4). Monthly broadcast applications of granular 'Sulfur 95', 950 g kg⁻¹ S_o (Sulfur Works Inc., Alberta, Canada) were made to deliver S_o at a 5.6 or 24.6 kg ha⁻¹ rate of each study year, Apr. to Nov. Thus, annual S_o rate totaled 44.8 or 196.8 kg ha⁻¹. Despite continued irrigation with alkaline water, second year measures of the upper 10 cm soil pH revealed marginal, yet statistically significant, reductions (0.2 units) by S_o treatment (Bell et al., 2001). Extractable sulfate (SO₄⁻²) in the upper 10 cm of the root zone was well correlated with S_o rate, yet no significant differences in bentgrass root mass or leaf nutrient concentrations were observed. Monthly S_o application rates as high as 74 kg ha⁻¹ did not cause bentgrass injury, even on days when air temperature exceeded 38 C (Bell et al., 2001).

Associated disciplines, like horticulture and agronomy, offer additional results of acidifying S_o treatment. Neilsen et al., (1993) evaluated both finely- (0.062 mm) and coarsely-granulated (0.5 to 1.0 mm) S_o products as acidulents of an alkaline loam (pHw=8.1) topsoil within a peach orchard in southern British Columbia (Canada). Granular S_o products were surface-applied at a 4500 kg ha⁻¹ rate and either twice rotavated to a 15-cm depth (incorporated) or left undisturbed (surface). No injury to the perennial grass species residing in plots receiving surface treatments was observed and resident grasses within incorporated plots quickly re-established (Neilsen et al., 1993). The plots were regularly mowed, irrigated, and N-fertilized at rates totaling 100 kg ha⁻¹ (unknown source) each year of the 2-yr study.

In plots where S_o products were incorporated, significantly lower soil pHw values (0.6 to 0.8 units) and higher soil electrolytic conductivities (EC; 1.0 to 2.4 dS m⁻¹), reliable indicators of acid formation, resulted from application of the finer S_o product. While significantly-greater than untreated plots, an intermediate degree of acid formation resulted from incorporation of the coarse S_o product (Neilsen et al., 1993). Plots receiving surficial S_o treatments showed less acid-formation relative to the incorporated treatments, and only the finely-granulated S_o treatment resulted in significantly lower soil pHw values (0.3 to 0.6 units) and higher soil EC levels (0.5 to 0.8 dS m⁻¹) compared to the control treatment (Neilsen et al., 1993).

Janzen and Bettany (1987) collected 39 topsoils from Saskatchewan for incubation evaluations of sulfur oxidative capacity. Sub-samples of homogenized soils were evaluated for: extractable sulfate, nitrate, phosphate, and potassium; pHw; moisture content (at 0.03 MPa tension); particle size distribution (PSD); and organic carbon content (by mass). Soils were amended with 0.12-mm S_o at a mass ratio of 1000:32, or 35 869 kg (ha 15-cm)⁻¹, and incubated at 23 C for a 160-d period. Soils were vented every 2-d to permit adequate oxygen supply.

Sulfur oxidation rates ranged from 2 to 8 µg S cm⁻² d⁻¹, were normally distributed around a mean of 4.8 µg S cm⁻² d⁻¹, and decreased over time (Janzen and Bettany, 1987). Showing only significant effects in order of correlation strength, soil properties positively influencing rate of S_o oxidation were: soil pHw, air-filled porosity, sand content, organic carbon, and extractable phosphate. Sulfur oxidation rate was significantly, yet inversely, correlated with clay content. The positive correlation of sulfur oxidation rate and soil pHw ($r = 0.57$, $P = 0.003$) is consistent with the current literature (Nor and Tabatabai, 1977), and indicates acidophilic oxidizers such as *T. thiooxidans*, *T. ferrooxidans*, and *T. acidophilus* are of little significance in soil of neutral to alkaline reaction (Janzen and Bettany, 1987).

Experimental Objectives

The objectives of this research were to accurately measure Penn A-series creeping bentgrass root/shoot growth and soil pH response (by soil depth), to applications of flowable or granular elemental sulfur (S_0) amendments either incorporated-into or applied to the surface of calcareous sand-rootzone putting greens.

Materials and Methods

Study 1: Granular Elemental Sulfur (S_0) Treatments at Aggressive Application Rates

Study 1 was comprised of two identical experiments, the first initiated in late-May 2011 on a putting green (PG) comprised of a 1:1 blend of 'Penn A-1' and 'Penn A-4' creeping bentgrass, and the second initiated in mid-December 2011 on a Penn A-4 PG. The internally-drained PGs were constructed using USGA-specified 19:1 sand:sphagnum peat moss (by volume) rootzones, 30 cm in depth. At experiment initiation, the PGs had been managed within the Pennsylvania State University Joseph Valentine Turfgrass Research Center (University Park, PA) for approximately 7 years.

Each experiment featured four (4) blocks arranged to minimize spatial variability inherent to each PG. Soil cores were sampled from inter-plot alleyways, segmented, composited into 0-5-, 5-15-, and 15-27-cm depths, and stored to represent baseline conditions in subsequent soil analyses. Experimental plots (91 x 183 cm, 15-cm borders) were then treated with equivalent rates of 0, 293, 587, or 880 kg S_0 ha⁻¹ by a single application of Sulfur amendment (Martin Disper-Sul 90% S fairway-grade granules).

Non-zero rates of S_0 amendment were either surface-applied or incorporated. Plots receiving 'incorporated treatments' were initially cored (1.3-cm tines, 5.1-cm spacing, 4- to 5-cm penetration depth) using a walk-behind aerifier (ProCore 648, Toro). The following numbered steps were then conducted on a per-plot basis: (1) evacuated cores were harvested, (2) granular S_0 treatments were applied, (3) granules were gently push-broomed into the aerification holes, and (4) sand topdressing was applied (40 m³ ha⁻¹) and broomed into the remaining voids. Surface-applied plots simply received hand-applied treatments of granular S_0 at the specified rates. Following application of all surface and incorporated S_0 treatments, sand-topdressing (10 m³ ha⁻¹) was broadcast over the entire experimental area. Plots of Experiment 1 (May 2011 initiation) were then irrigated (0.5 cm) and flagged to discontinue daily mowing for a one week period. Plots of Experiment 2 (December 2011 initiation) were neither mowed nor irrigated until April 2012.

Management procedures conducted onsite were similar to standard golf course practice, except no growth regulators, herbicides or surfactants were applied. April to October of each year, the PGs were mowed 6±1-d week⁻¹ (3.1-mm cutting height), and clippings were not returned. Potable irrigation water was applied to prevent wilt, and plant protectants were used to control pests when necessary. Every 14±5-d, May through September, soluble fertilizers were foliarly applied to deliver both N and K₂O at rates of 5 to 7 kg ha⁻¹. The PGs were topdressed with 30 m³ ha⁻¹ straight rootzone sand in June and October.

Study 2: Moderate rates of Granular or Sprayable Elemental Sulfur (S_0) Treatments

Study 2 was comprised of two identical experiments (Exp. 3 and 4), each initiated in late-2012 on unique portions of the managed PGs described in Study 1. Both experiments were comprised of five (5) blocks of ten (10) 91 x 183 cm plots. Soil cores were sampled from inter-plot alleyways, segmented, composited into 0-5-, 5-15-, and 15-27-cm depths, and split for routine soil analysis by the Pennsylvania State University

Agricultural Analytical Services Laboratory (PSU-AASL) or calcium carbonate equivalency (%CCE) determination (Kane, 1996).

In mid-September 2012, all experimental plots were amended by a single application of 0, 195, 390, or 586 kg S_o ha⁻¹. Surface and incorporated applications of granular S_o were made as described above in Study 1. Liquid/flowable S_o (Yellow Jacket Flowable Sulfur; 53% S) was applied using a single flat-fan nozzle (Teejet 110015E) CO₂ backpack sprayer at carrier volumes of 1500 to 2000 L ha⁻¹. Plots were immediately irrigated by approximately 1 cm of potable water and flagged to suspend daily mowing for a one week period.

Management and cultural practices conducted in 2013 were nearly identical those described above in Study 1. On 29 April, a greens-grade 17-0-19 fertilizer (8% N as methylene urea, K₂O as potassium sulfate) was applied to deliver 39 kg N ha⁻¹. Soluble urea and potassium nitrate fertilizers were foliarly applied at rates providing 4 to 7 kg N ha⁻¹ every 12±5 d from June through September. Both PGs were topdressed with the rootzone sand at a rate of 30 m³ ha⁻¹ in May and July. No herbicides, surfactants, or growth regulators were applied to either PG in 2013.

Studies 1 & 2: Data Collection and Analysis

Twice each week late-April through Sept., triplicate readings of 660- and 850-nm canopy reflectance were recorded from each plot using an ambient light-excluding FieldScout TCM-500 turfgrass chlorophyll meter (Spectrum Technologies Inc., Plainfield, IL). Likewise, green, red, and blue canopy reflectance was measured using a FieldScout TCM-500 RGB turfgrass color meter (Spectrum Technologies, Inc.). These measures of canopy reflectance were used to calculate normalized differential vegetative (NDVI) and dark green color indices (DGCI; Karcher and Richardson, 2003), and respectively quantify PG canopy density and color (Zhu et al., 2012).

Clippings were periodically collected over the 2011, 2012, and 2013 growing seasons. Clipping samples were oven-dried (55 C), ground to pass a 0.15-mm sieve, and submitted to PSU-AASL for acid-digest determination of leaf P, K, Ca, Mg, S, Fe, Mn, Cu, B, and Zn concentration (Miller, 1998) and total leaf N by medium temperature furnace combustion (Horneck and Miller, 1998).

Cores samples of the PG rootzones were periodically collected over the 2011, 2012, and 2013 growing seasons, and segmented by 0-5, 5-15, and 15-27-cm depths. After drying, the rootzone depth segments were analyzed for soil pH using a 1:1 DI H₂O to soil ratio (by mass). Thirteen to 15 months after initial treatment (MAIT), three (3) soil cores were sampled from the 15 to 27-cm (Study 1) or 8 to 16-cm (Study 2) soil depths (comprising 102- or 68-cm³ total rootzone volume, respectively) and analyzed for root length, density, and morphology.

Due to the different initiation dates of Experiments 1 and 2 in Study 1, separate analyses of all canopy quality reflectance, tissue nutrient concentration, soil chemical and root response data were analyzed using PROC MIXED in SAS/STAT (Ver. 8.2, SAS Institute, Cary, NC). Treatment levels comprised the sole model fixed effects. Significance of treatment effect was tested using block by treatment interaction term. Means within significant main treatment effects were separated by Fisher's least significant difference at a 0.05 α -level.

For Study 2, all canopy reflectance, tissue nutrient concentration, soil physicochemical, and root response data (from Experiments 3 and 4) were combined for global analysis in SAS/STAT (Ver. 8.2, SAS Institute, Cary, NC) using PROC MIXED (Zhu et al., 2012). Treatment levels and repeated time measures comprise the sole model fixed effects. The replicate experiments; conducted on either either the Penn A-4 or Penn A-1/A-4 blend putting green, comprised the lowest-order ANOVA source (EXP) and was analyzed as a random effect.

Significance of treatment effect, time effect, and associated contrasts and mean separations was tested using the expected mean squares of each respective experiment (EXP) interaction term, as determined by Model I of Hocking (1973) and later described by McIntosh (1983). The significance of treatment by time (repeated measures) was analyzed using its respective EXP interaction term and an appropriate time-series covariate structure. Means within significant main and/or interactive effects were separated by Fisher's least significant difference at an α -level appropriate of the source P -level indicated by ANOVA.

Results: Study 1

Table 1-1. Exp. 1 mean preliminary mean soil pHw (1:1 soil:DI H₂O) and post-treatment mean soil pHw; by S_o rate and application method (pooled sampling dates).

		pHw (1 : 1 soil : DI H ₂ O)		
		0-5 cm	5-15 cm	15-27 cm
Baseline levels (sampled May 2011)		7.41	7.55	7.58
Application method	S _o rate (kg ha ⁻¹)			
Control	0	7.13	7.60	7.77
Granular-incorp.	293	*6.95	7.60	7.79
	587	*6.76	7.57	7.71
	880	*6.69	*7.47	7.72
Granular-surface	293	*6.84	7.57	7.76
	587	*6.84	7.54	7.75
	880	*6.64	*7.53	7.74

* pHw value significantly less than same-depth control (Dunnett's one-tail separation, $\alpha=0.05$).

Table 1-2. Exp. 2 mean preliminary mean soil pHw (1:1 soil:DI H₂O) and post-treatment mean soil pHw; by S_o rate and application method (pooled sampling dates).

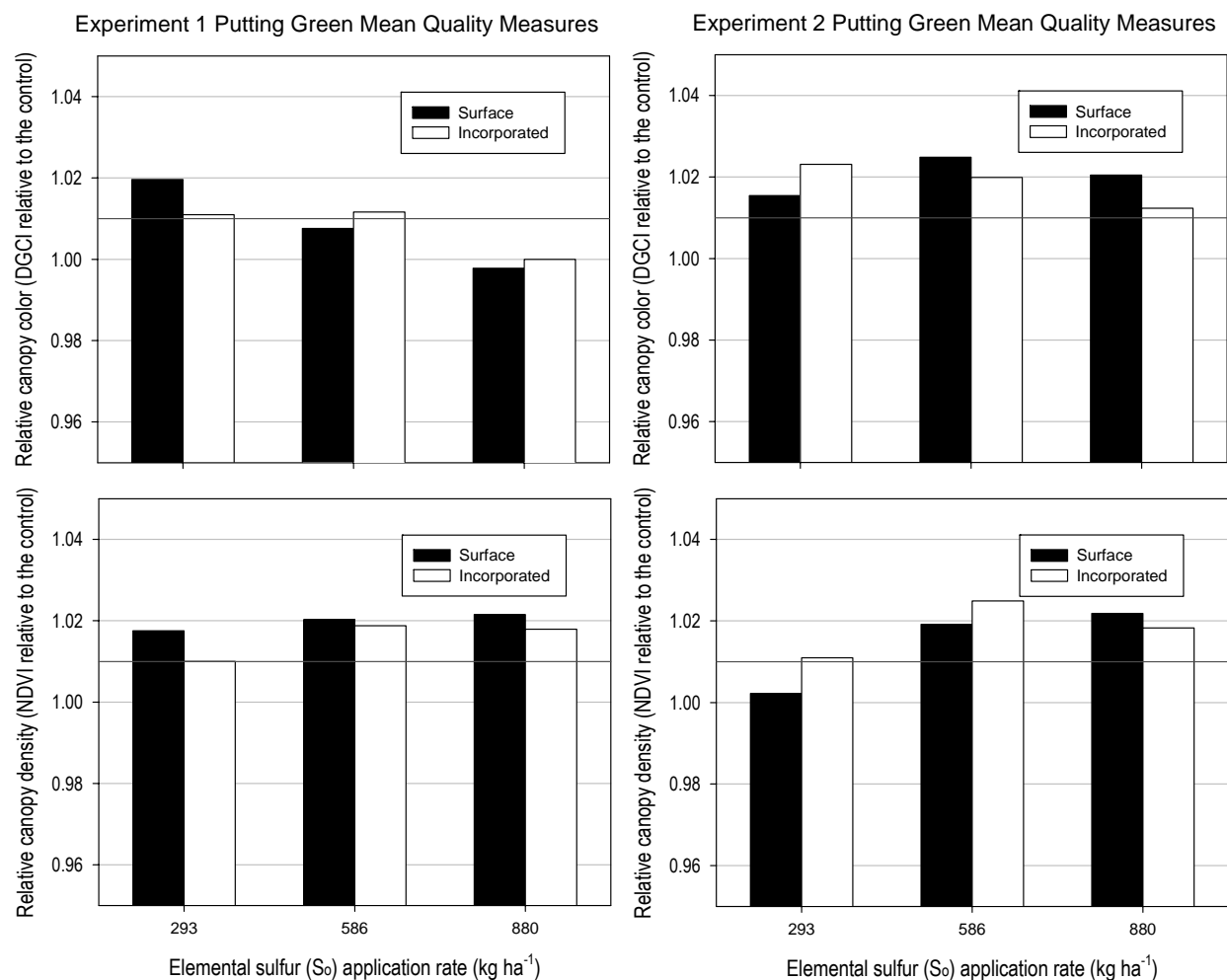
		pHw (1 : 1 soil : DI H ₂ O)		
		0-5 cm	5-15 cm	15-27 cm
Baseline levels (sampled Dec. 2011)		7.31	7.29	7.46
Application method	S _o rate (kg ha ⁻¹)			
Control	0	7.06	7.19	7.38
Granular-incorp.	293	*6.96	7.18	7.35
	587	*6.86	7.20	7.34
	880	*6.74	7.19	7.31
Granular-surface	293	*6.93	7.14	7.34
	587	6.97	7.13	7.35
	880	*6.80	7.14	7.33

* pHw value significantly less than same-depth control (Dunnett's one-tail separation, $\alpha=0.05$).

Table 1-3. Exp. 2 mean root length density (RLD) and specific root length (SRL) by S_o rate and application method sampled from 15-27 cm depth.

Application method	S _o rate (kg ha ⁻¹)	RLD (cm/cm ³)	SRL (m/g)
Control	0	19.88	257
Granular-incorp.	293	26.50	283
	587	12.79	233
	880	14.58	198
Granular-surface	293	23.84	299
	587	19.43	313
	880	17.17	223

Figure 1-1. Study 1 (Exp. 1, left; Exp. 2, right) mean relative canopy color (top, dark green color index units, relative to the control) and mean relative canopy density (bottom, normalized differential vegetative index units, relative to the control) by S_o application method and rate (pooled over sampling dates). All treatment means exceeding the red reference line are significantly different from the mean control value ($\alpha=0.05$).





Figures 1-2. Experiment 1 visible canopy response in early-Sept. 2011 (3 months after treatment). Red numbers indicate elemental sulfur (S₀) application rates in lbs 1000 ft⁻²; 6=293 kg S₀, 12=586 kg S₀, and 18=880 kg S₀ ha⁻¹.

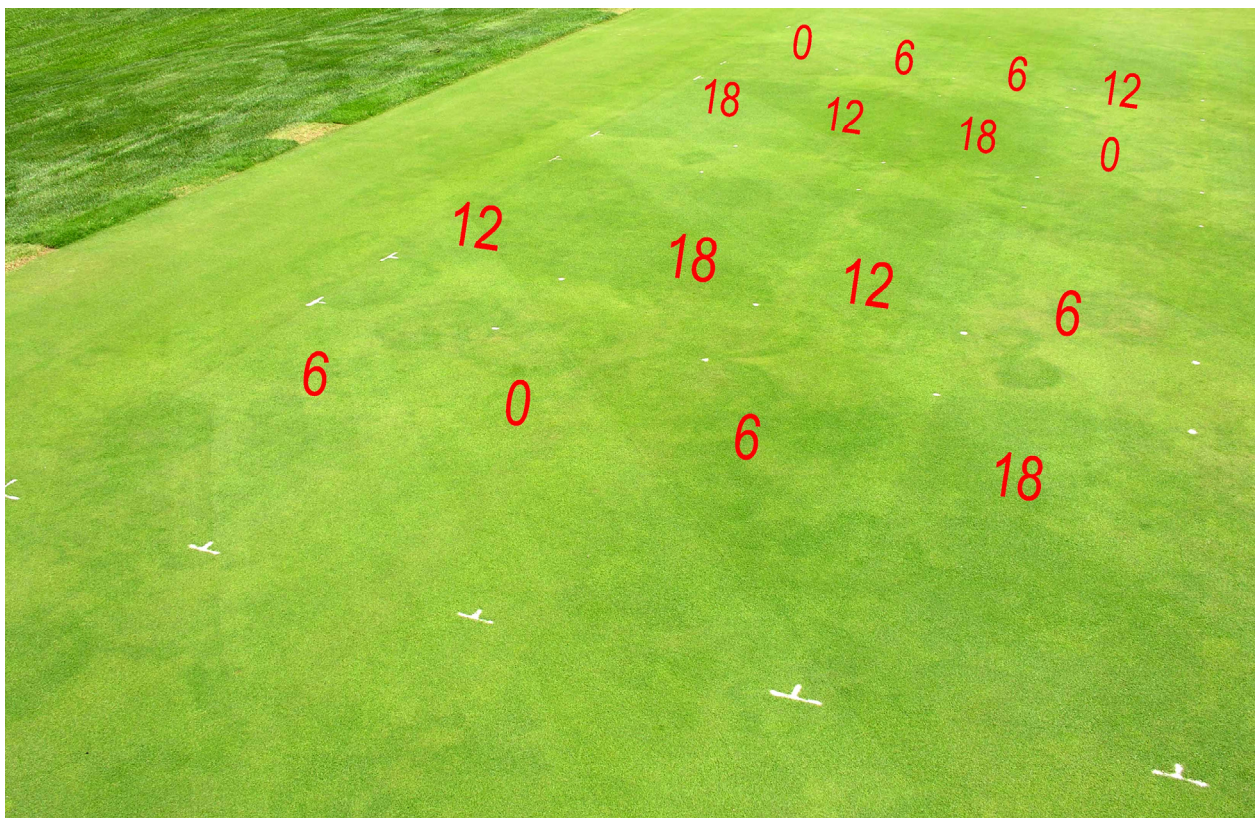


Figure 1-3. Study 1 (Exp. 1, left; Exp. 2, right) mean relative creeping bentgrass leaf sulfur (S, top) and manganese (Mn, bottom) concentrations, relative to the control, by S₀ application method and rate (pooled over sampling dates). All treatment means exceeding the red reference line are significantly different from the mean control value (alpha=0.05).

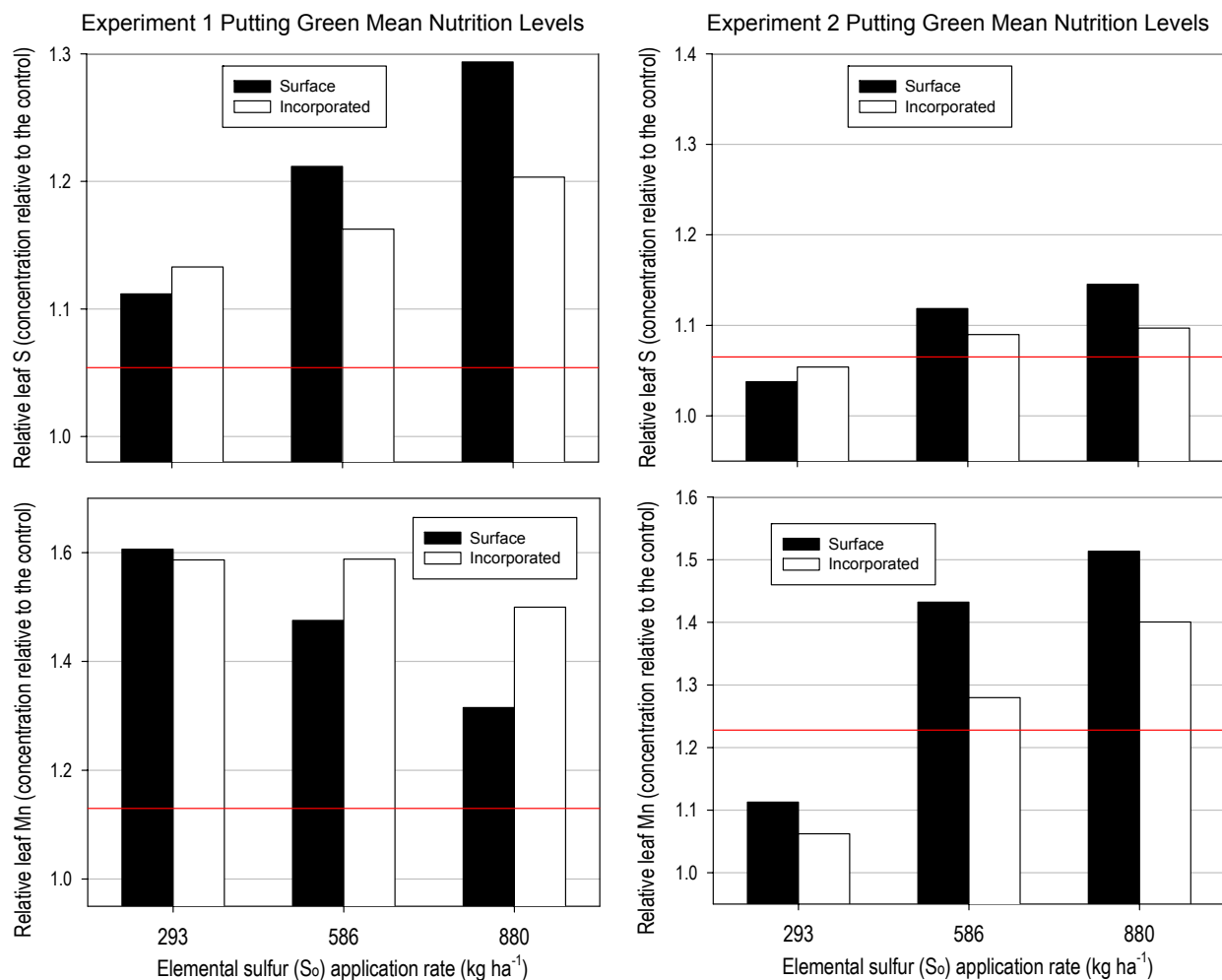
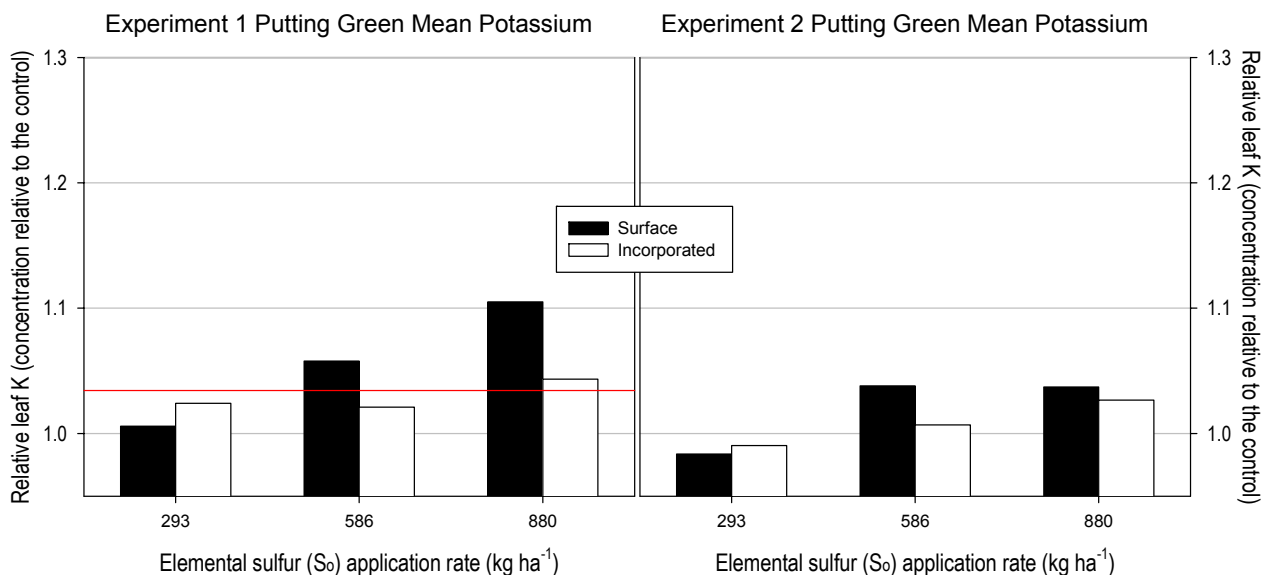


Figure 1-4. Study 1 (Exp. 1, left; Exp. 2, right) mean relative creeping bentgrass leaf potassium (K) concentrations, relative to the control, by S_o application method and rate (pooled over sampling dates). All treatment means exceeding the red reference line (where shown) are significantly different from the mean control value ($\alpha=0.05$).



Results: Study 2

Figure 2-1. The Penn A-1/A-4 blend creeping bentgrass putting green immediately following granular-incorporated, -surface, and flowable elemental sulfur (S_o) treatments in September 2012 (Study 2).



Table 2-1. Mean preliminary (Sept. 2012) soil chemistry data from Study 2 (2012-2013).

Soil parameter	Units	0-5 cm		5-15 cm		15-30 cm	
		Exp. 3	Exp. 4	Exp. 3	Exp. 4	Exp. 3	Exp. 4
pHw (1:1 soil : DI H ₂ O)	-	7.34	7.28	7.54	7.51	7.49	7.83
CaCO ₃ equiv.	% mass	1.8	1.5	1.4	1.5	1.3	1.3
Mehlich 3 extractable							
CEC	meq / 100 g	4.76	4.14	5.32	4.48	5.74	3.05
Ca		3.68	3.49	4.63	3.69	5.14	2.47
K		0.25	0.11	0.078	0.045	0.069	0.04
Mg		0.83	0.54	0.607	0.74	0.53	0.54
P	ppm	37	31	26	16	18	10
S		10	15	9	7	10	4
Cu		1.2	1.7	1.2	1.7	0.5	0.7
Zn		1.1	3.9	1.1	3.9	0.3	0.6

Table 2-2. Mean soil pHw (1:1 soil:DI H₂O) by S_o rate and application method (pooled across experiments and sampling dates).

Application method	S _o rate (kg ha ⁻¹)	pHw (1 : 1 soil : DI H ₂ O)		
		0-5 cm	5-15 cm	15-30 cm
Control	0	7.40	7.67	7.92
Granular-incorp.	195	7.32	7.66	7.96
	390	*7.18	7.64	7.93
	586	*6.97	7.62	7.95
Granular-surface	195	*7.21	7.64	7.95
	390	*7.02	*7.61	7.95
	586	*6.73	*7.57	7.90
Sprayable	195	7.25	7.65	7.95
	390	*6.85	*7.61	7.94
	586	*6.60	*7.60	7.91

* pHw value significantly less than same-depth control (Dunnett's one-tail separation, $\alpha=0.05$).

Figure 2-2. Study 2 mean relative canopy color (top, dark green color index units, relative to the control) and mean relative canopy density (bottom, normalized differential vegetative index units, relative to the control) by S_o application method and rate (pooled over Experiments and sampling dates). All treatment means exceeding the red reference line are significantly different from the mean control value ($\alpha=0.1$).

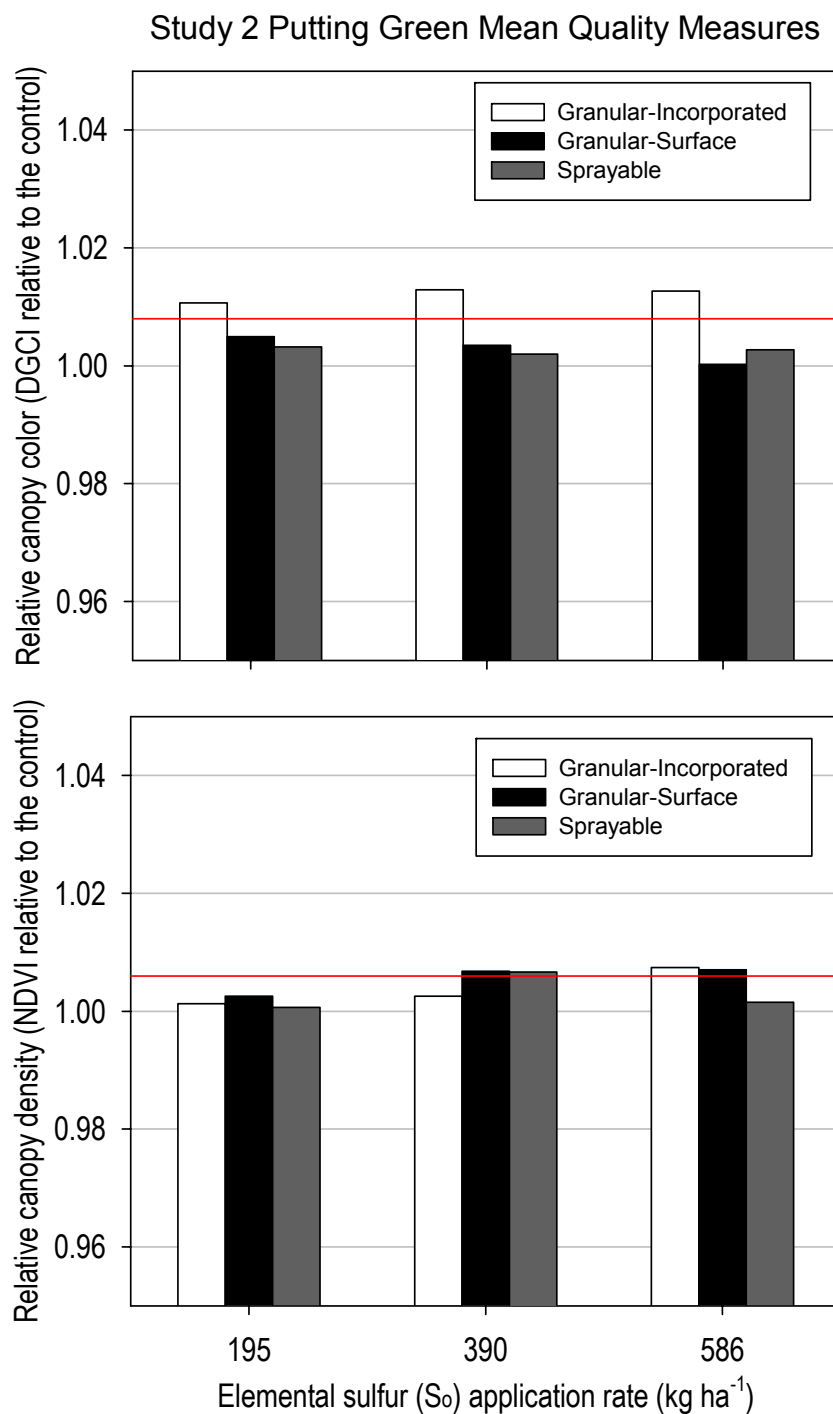


Table 2-3. Mean leaf macronutrient concentrations by S₀ rate and application method (pooled across experiments and sampling dates).

Application method	S ₀ rate (kg ha ⁻¹)	Leaf nutrient concentrations g kg ⁻¹			
		K	Ca	Mg	S
Control	0	25.2	5.4	2.9	5.4
Granular-incorp.	195	25.9	5.3	2.8	5.4
	390	*26.1	5.1	*2.6	5.5
	586	*26.5	*4.9	*2.5	*5.7
Granular-surface	195	25.9	5.2	2.7	5.4
	390	*26.7	*4.9	*2.6	*5.8
	586	*27.4	*4.6	*2.5	*5.9
Sprayable	195	25.3	5.5	2.8	5.3
	390	25.2	5.2	2.8	5.1
	586	25.4	5.1	2.7	5.0

* Concentration values significantly different than control (Dunnett's one-tail separation, $\alpha=0.05$).

Table 2-4. Mean leaf micronutrient concentrations by S₀ rate and application method (pooled across experiments and sampling dates).

Application method	S ₀ rate (kg ha ⁻¹)	Leaf nutrient concentrations - parts per million (ppm)			
		Mn	Fe	Cu	Zn
Control	0	98	348	17.1	47.6
Granular-incorp.	195	126	315	16.9	47.0
	390	*141	301	16.9	*45.8
	586	*135	369	16.7	*44.8
Granular-surface	195	*132	334	16.8	47.2
	390	*155	302	17.1	46.3
	586	*154	386	16.7	*45.6
Sprayable	195	125	326	16.4	47.4
	390	*131	351	*15.5	*45.4
	586	120	427	*14.9	*43.9

* Concentration values significantly different than control (Dunnett's one-tail separation, $\alpha=0.05$).

Table 2-5. Mean root length density (RLD) and specific root length (SRL) by S_o rate and application method (pooled across experiments) sampled from 8-16 cm depth.

Application method	S_o rate (kg ha ⁻¹)	RLD (cm/cm ³)	SRL (m/g)
Control	0	3.27	258
Granular-incorp.	195	3.15	251
	390	3.08	255
	586	4.32	274
	195	3.04	250
Granular-surface	390	2.62	237
	586	3.75	257
	195	3.35	253
Sprayable	390	3.40	253
	586	2.81	228

Figure 2-3. Study 2 canopy injury resulting from 'edge-heavy' surface application of liquid/flowable elemental sulfur (S_o) at the 586 kg S_o ha⁻¹ rate. This photo of the Penn A-1/A-4 blend creeping bentgrass putting green was captured in early-May 2013 (8 months after treatment).



Figure 2-4. Study 2 canopy injury resulting from 'edge-heavy' surface application of liquid/flowable elemental sulfur (S_0) at the 586 kg S_0 ha⁻¹ rate. This photo of the Penn A-1/A-4 blend creeping bentgrass putting green was captured in September 2013 (12 months after treatment).



Figure 2-5. Study 2 canopy injury resulting from surface application of granular elemental sulfur (S_0) at the 586 kg S_0 ha⁻¹ rate. This photo of the Penn A-1/A-4 blend creeping bentgrass putting green was captured in June 2013 (9 months after treatment).



Discussion

When planning *in situ* amendment of rootzones underlying golf course putting greens (PGs), superintendents typically employ a method that minimizes disruption to the playing surface and a rate that precludes risk of plant injury. The experimental results describe soil chemical and plant physiological response of creeping bentgrass putting greens to a single elemental sulfur (S_0) application made in varying practical method, formulation, and rate combinations. All resulting observations are specific to internally-drained sand rootzones constructed to USGA specifications, containing $\approx 1.5\%$ $CaCO_3$ equivalency, demonstrating 1:1 pH levels of ≈ 7.4 , and maintained in a cool humid climatic region with mean annual temperature near 10.5 C.

Measureable acidification of rootzone pH was confined to the upper 15-cm of rootzone and influenced primarily by S_0 application rate. Over the one year experiment duration, and regardless of S_0 formulation or application method, amendment at the 390, 586, or 880 kg rate lowered mean soil pH 0.2- to 0.8-units in the 0-5 cm rootzone depth. Acidification in the 5-15 cm rootzone depth was of statistical, rather than practical, significance (≤ 0.1 -unit soil pH reduction); and was again a result of the 390, 586, and 880 kg S_0 application rates. In Exp. 1, either method used to apply the 880 kg ha⁻¹ granular S_0 rate resulted in the described 5-15-cm depth pH reduction. Surprisingly in Study 2, the only 390- or 586-kg S_0 rate applications that did not significantly lower 5-15 cm rootzone mean pH levels relative to the control were those incorporated into the upper 5-cm of the PG rootzones.

While the described pH reductions in the uppermost 15-cm of rootzone were statistically significant, no S_0 treatment lowered mean soil pH to levels optimal for creeping bentgrass (5.5-6.5). Yet certain turfgrass quality measures were observed to be favorably influenced by S_0 treatment.

Canopy color and density measurements effectively differentiated favorable relative responses to S_0 treatment in all Experiments and Studies. In Study 1, May application (Exp. 1), at the 586 or 880 kg S_0 rate, incited minor leaf desiccation that was detected by canopy color measures over the one year evaluation period. However, no acute/persistent canopy injury was observed to result from any rate in Exp. 1 or 2 of Study 1. One year following May application, the 293 kg rate resulted in significantly improved mean canopy color (relative to the control). Significant canopy density enhancement, relative to the control, was observed at all S_0 rates applied by either method in May; and was strongly correlated with the 0-5 cm depth mean pH level. In Study 1, the December application (Exp. 2) significantly improved mean canopy color, relative to the control, at all S_0 rates. Significant relative mean canopy density enhancement resulted only from surface applications of the 586 or 880 kg S_0 rate, but was observed at all incorporated rates of S_0 .

In Study 2, canopy color measures proved sensitive to localized canopy injury/desiccation resulting from surface applications of S_0 at the intermediate (390 kg) or high (586 kg) rates. Thus, the highest statistical grouping for mean color index was comprised solely of incorporated S_0 treatments. On the contrary, mean canopy density averaged over the 6-month measurement period, was highest in plots treated by the 586 kg (all formulations/application methods) or 390 kg (granular surface) S_0 rate; and strongly correlated with the 0-5 cm depth mean pH level.

During the 2011, 2012, and 2013 growing seasons, comprehensive cultural practice was uniformly-imposed over the experimental PGs for the purpose of preventing biotic and abiotic stress. Thus it is reasonable to conclude that the reported enhancements to the turfgrass quality parameters described, while significant at probability levels <0.05 (Study 1) to <0.12 (Study 2), resulted from S_o -induced change in the biochemistry of the PG rootzone and turfgrass rhizosphere. Established methods of plant tissue analysis undoubtedly comprise the most effective approach to assessing plant nutrient availability. Chemical analysis of clippings showed leaf/shoot concentration of several plant essential nutrients was significantly influenced by rate, formulation, and/or method of recent S_o application. While nearly all nutrient levels were observed to reside within their respective sufficiency ranges for creeping bentgrass, significant increases in mean leaf K, S, and Mn by S_o treatment showed moderate to strong correlations with mean soil pH and canopy density parameters. These results do prove interesting, particularly in the case of Mn; as no micronutrient fertilizers were applied to any of the PGs over the experimental periods.

The observed root length densities and specific root lengths of treated plots were not statistically different than control plots. Data generated was limited to only one collection date approximately one year after treatment. The absence of an observed treatment effect could be explained by the limited amount of time elapsed from amendment, and/or the fact that minimal change in rootzone pH level was observed within the rootzone depths sampled (15-27 cm or 8-16 cm).

Soils sampled from each PG for rootzone pH, nutrient, and root length analyses were additionally monitored for black layer development. There was no instance of black layer (metal sulfide) formation in any plot at any given point of the season. Because the experimental PGs were comprised of internally-drained, sand-based rootzones with relatively low CCE levels, adequate oxygen likely provided conditions suitable for S_o oxidation (versus reduction). We would like to reiterate that these described results may not readily transfer to putting greens established to native or minerals soils having less-dependable percentages of air-filled porosity.

As seasonal temperatures increased in 2013 (Study 2), mild (foliar) to significant (crown) plant injury was observed in plots treated by surface applications of 390 and 586 kg ha⁻¹ S_o (granular and flowable formulations). While canopy injury observed in the 390 kg ha⁻¹ S_o treated plots rebounded relatively quickly (within approximately one month), acute/crown injury resulting from the highest application rate (586 kg S_o ha⁻¹) left voids in the canopy that persisted over the entire growing season. It is important to note that this acute injury, associated with an 'edge-heavy' spray pattern (caused by partial nozzle-clogging), would be considered totally unacceptable under modern standards of PG management. There was no instance of foliar damage associated with any 195 kg ha⁻¹ S_o rate application method. Furthermore, plots that received granular-incorporated treatments did not show canopy injury at S_o application rates from 195 to 880 kg ha⁻¹.

These results may beg the following question: Why did the surface applications of 390 and 586 kg granular S_o result in significant PG injury in Study 2, whereas surface applications of 586 and 880 kg granular S_o made in Study 1 did not? The answer may be related to both random and 'controlled' environmental conditions. As described, the rate of S_o oxidation in soil is a function of temperature and oxygen, as well as amount of substrate; i.e., S_o amendment. In 2011, the central Pennsylvania region was subject to above-average precipitation. Specifically, rainfall recorded in the months of April to October 2011 was 103.3 cm,

and well in excess of the 80 year average for annual rainfall (98.9 cm) in the region. Thus the rate of oxidation of S_0 applied in May 2011 (Exp. 1) may have been oxygen-limited compared to 2012 and 2013 seasons (having lesser rainfall accumulations). Regarding Exp. 2, the December 2011 application date likely resulted in a reduced rate of S_0 oxidation over the late-winter and spring months of 2012, reducing the residual substrate present at the onset of higher summer temperatures. The authors believe these important aspects of seasonal and environmental influence on S_0 oxidation rate, as well as amendment efficacy, deserve further attention.

Suggested Practice for Amendment of Putting Greens by Elemental Sulfur

Undesirably-alkaline soils/rootzones (as identified by recent soil testing) slated for renovation should NOT be amended by S_0 in the establishment or seedbed preparation process. Frequent/excessive irrigation typical of establishment practice will promote S_0 reduction rather than oxidation. For best renovation results, amend such alkaline soil with S_0 at the first opportunity and irrigate sparingly until seeded/sprigged. In the event the pH of the seedbed soil remains supraoptimal for the intended species prior to seeding, a preferable approach to certain soil pH reduction is the direct spray application of $\leq 1\%$ concentrated sulfuric or muriatic acid to the soil, not to exceed 10 L concentrated acid per hectare, followed by irrigation. The authors also recommend selecting a primarily ammonium- and/or urea-based starter fertilizer for application concurrent with seeding/sprigging, at a rate specified in soil test recommendations.

Caution should be exercised when applying S_0 to poorly-drained soils, low-lying areas or basins, and turfgrass subjected to high seasonal water tables. Where xeric (Mediterranean) soil moisture regimes or tropical climates prevail; S_0 should be applied just before the onset of the 'dry' season, and not immediately before or during the 'wet' season.

The authors reiterate these experimental results are specific to internally-drained sand rootzones constructed to USGA specifications, demonstrate $\approx 1.5\%$ $CaCO_3$ equivalency, 1:1 pH levels of ≈ 7.4 , and are maintained in cool humid climatic regions with mean annual temperatures near 10.5 C. It is imperative that superintendents planning *in situ* S_0 amendment of rootzones underlying golf course PGs select rates and timings that minimize risk of plant injury.

Due to their predominately air-filled porosity, internally-drained sand rootzones make good candidates for S_0 incorporation at rates that should never exceed 586 kg ha⁻¹ annually. While this incorporation method of S_0 amendment was not most effective at lowering soil pH in the 5-15 cm rootzone depth, use of granular S_0 and topdressing sand to backfill recently-evacuated core aerification holes proved safer to creeping bentgrass PG surfaces than equal broadcast applications. Typical early-Spring and/or early-Fall scheduling of such PG cultivation coincides with ideal climatic conditions for S_0 application, when the likelihood of temperature-induced 'runaway' generation of sulfuric acid is low.

Despite the fact these results showed surface application of granular S_0 proved more effective at reducing soil pH than incorporation of granular S_0 , the authors believe incorporation to be the best method for applying granular S_0 to maintained PGs. Superintendents who core PGs twice yearly, and are considering S_0 amendment at every opportunity, are encouraged to assess soil pH levels prior to the second cultivation event. Again, the authors do not recommend incorporating >586 kg S_0 ha⁻¹ per year. Superintendents

preferring to surface apply granular S_o forms are recommended to do so following discontinuation of green mowing activities in late-fall or early-winter, and at a rate not exceeding $292 \text{ kg } S_o \text{ ha}^{-1}$.

Of all application methods employed, spray application of the 53% S_o flowable 'Yellow Jacket' product in Study 2 undoubtedly proved least disruptive to the playing surface. Flowable S_o applications should be followed by a $>0.5\text{-cm}$ irrigation or precipitation event to prevent removal by mowing. Results of Study 2 showed the most considerable drawback to spray application of flowable S_o , particularly at rates of 390 to $586 \text{ kg ha}^{-1} S_o$, was an edge-heavy spray pattern. This non-uniform distribution of flowable S_o from the flat-fan nozzle in Study 2 caused an undesired concentration of S_o at the pattern edges and caused acute plant injury in May and June of 2013.

A small-scale follow-up experiment, initiated in July 2013 on a 'push-up' style 'Penn G-2' creeping bentgrass PG (1:1 pH=7.2), evaluated flowable S_o spray application of 97, 195, 292, or 390 kg ha^{-1} rates using both an air-induction even flat spray tip/nozzle (TeeJet AI9509EVS) and a flat fan nozzle (TeeJet TP9508E; similar to the nozzle used in Study 2). The initiation date of this experiment was chosen to favor rapid S_o oxidation in a purposeful worse-case scenario. Seven weeks following, significant injury was observed at the edges of the 292 and $390 \text{ kg } S_o \text{ ha}^{-1}$ spray application patterns originating from the flat fan nozzle. No damage resulted from spray applications originating from the air-induction even flat spray tip/nozzle (TeeJet AI9509EVS).

Despite the enhanced uniformity of application, and relative safety, associated with use of air-induction nozzles/tips, the authors suggest spray applications of flowable elemental sulfur be limited to rates of 24 to $195 \text{ kg } S_o \text{ ha}^{-1}$ in carrier volume exceeding 2000 L ha^{-1} , and only be made using air-induction flat fan or hollow cone nozzle types. When spraying flowable S_o at rates $>97 \text{ kg } S_o \text{ ha}^{-1}$, managers must recognize the inherent risk to PG surfaces and commit due diligence to uniform spray application; perhaps by instructing a second individual to follow the sprayer and promptly communicate the status of all spray nozzle patterns to the operator.

Superintendents managing 'push up'-style PGs, having 5+ cm-deep 'sand caps' overlying mineral/native soil, should exercise extreme caution in efforts toward lowering soil pH by S_o amendment. Relative to under-drained sand profiles, air-filled porosity within push-up rootzones is often limited and irregularly-distributed. While incorporation minimized crown/shoot exposure to S_o and prevented acute plant injury on the sand-based PGs, the potential for iron and/or manganese sulfide formation from S_o exists. Incorporating S_o into depths of an oxygen-limited and/or less than well-drained profile may exacerbate this already challenging condition by instigating black layer formation. Cultural practices that maximize air-filled porosity in the upper portion of the root zone (e.g. coring, verticutting) may help limit the potential for reduction of recently-applied S_o .

Acknowledgements

The authors sincerely thank the Ontario Turfgrass Research Foundation and The Pennsylvania Turfgrass Council for their financial support of this research. Likewise, we appreciate the technical assistance provided by Mr. Tom Bettie, Kyle Hivner, Chase Rogan, Victor Faconti, Brad Bartlett, and the Pennsylvania State University Agricultural Analytical Services Laboratory.

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