APPLICATION OF CIVITAS IN LATE FALL IMPROVES TURF QUALITY IN THE SPRING

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OBJECTIVES

Winters in Nebraska often cause lethal stress on maintained turf as season-long snow cover is infrequent due to limited precipitation, high winds and open terrain. Turf managers often utilized protective tarps and winter-watering to ensure survival during the winter. These are expensive and time-consuming practices. The objective of this research was to investigate how different Civitas + Harmonizer rates and ratios affect plant health during desiccating winter conditions.

METHODS

Site Characteristics

This study was conducted at the John Seaton Anderson Turfgrass (JSA) Research Facility in Mead, NE during the winter of 2014-2015. The plot space consisted of a mature stand of 'L-93' creeping bentgrass (*Agrostis stolonifera*) managed as a putting green, and the root zone was constructed following the recommendations of the USGA (2004). Plots were irrigated to replace 80% of potential evapotranspiration (pET) prior to the initiation of the study the following fall. To promote desiccation and evaluate Civitas + Harmonizer throughout the course of the winter, snow/precipitation was withheld throughout the course of the study by covering the plots with a 6 mm clear impermeable tarp prior to precipitation events. Tarps were then removed immediately after the snow/precipitation event.

Similar studies were conducted in Kearney, NE and Mullen, NE. The Kearney study was conducted on a on a mature 'T-1' creeping bentgrass fairway grown on a native sandy soil at Awarii Dunes Golf Course. The Mullen study was also conducted on a mature fairway grown on a native sandy soil at the Sand Hills Golf Club. The fairway was a mixture of fine fescues (*Festuca* ssp.), annual bluegrass (*Poa annua*) and perennial ryegrass *(Lolium perenne*). Both sites were irrigated to prevent water stress prior to the winter. Natural precipitation was not withheld from these sites during the winter.

Experimental Treatments

Various mixtures of Civitas and Harmonizer were evaluated relative to a non-treated control and are summarized in Table 1. At the Mead site, the first application was applied on 24 October while the turf was actively growing, and again on 17 November when the turf was dormant. At the Kearney and Mullen sites, a single application of each treatment was applied on 20 November. The turf had stopped growing but still had some green color left in the leaf tissue when treatments were applied at those sites. All treatments were applied using a CO_2-powered backpack sprayer with three TeeJet AL 8005 nozzles at 34 PSI, calibrated to deliver a spray volume of 2 gallons 1000 ft^{-2}.

Data Collection

Turf Quality

Visual turf quality (TQ) of each plot was monitored weekly at the Mead site from 26 March to 15 April in the spring to evaluate turf recovery after potential winter injury. At the Kearney site, ratings were taken

on 11 March, 25 March, and 1 April. At the Mullen site, ratings were taken on 11 March. Visual TQ ratings were taken on a 1-9 scale where 1 represented dead or brown turf, 9 represented dark green, uniform turf, and 6 was minimally acceptable for late spring turf quality.

Crown Moisture Content

At the Mead site, crown moisture content (CMC) was evaluated on 17 February and 17 March. These dates were chosen because early spring is a critical period for recovering turfgrasses as they are rapidly losing cold-hardiness and turf crown moisture content is starting to increase (Beard, 1973). Crown moisture content was monitored monthly by taking one 2" x 2" plug from each plot, and randomly selecting 27 crowns per plug. Crowns were defined as the portion of the plant where the stem and roots joined, and this region was approximately 1/8" in thickness. Once plant material was collected, the crowns were immediately isolated and weigh to determine fresh weight. Crowns were then oven-dried at 70°C for 24 h. Crown moisture content was calculated as the difference between the fresh and dry weight divided by the fresh weight of the plant material for each plot.

Chlorophyll Content and Reactive Oxygen Species

Chlorophyll content was measured for the Mead study on 15 January, 17 February, and 17 March. Plant tissue was harvested from 2" x 2" plugs taken from each plot, and chlorophyll was released from plant tissue while suspended in dimethylformamide (DMF). The absorbance of each extract was measured at 470, 644, and 647 nm. Chlorophyll a, b, total chlorophyll content (CC), and total carotenoids (TC) were calculated using the equations of Inskeep and Bloom (1985). Reactive oxygen species (H_2O_2) were also measured monthly (January through March) at the Mead study. The assay was conducted using the Amplex Red Hydrogen Peroxide/Peroxidase method from Life Technologies (Grand Island, NY). This assay is extremely sensitive, with detection limits as low as 10 picomoles of H_2O_2 per mL of extract.

Statistical Analysis

Individual plots (5'x5') were arranged in a randomized complete block design with four replications. Data were subjected to Analysis of Variance (ANOVA) and treatment means were separated using Student's *t*-test, at the 0.05 probability level when appropriate. Turf quality was subjected to repeated measures analysis to reduce variability within treatments. Data were transformed when required to satisfy the equal variance assumption of ANOVA.

RESULTS

Turfgrass Quality

The plots at the Mead site (averaged across all treatments) had a mean TQ of 2.8 on 26 March, and TQ steadily improved to a mean of 4.2 by the 10 April rating date (Table 2). The application of Civitas + Harmonizer increased TQ in the spring (pooled across rating dates). The 32 oz Civitas + 2 oz Harmonizer treatment and the 16 oz Civitas + 4 oz Harmonizer treatment had the highest TQ of 4.3 and 4.1, respectively. The 8 oz Civitas + 0.5 oz Harmonizer treatment was similar to the untreated control, which had the lowest TQ. The 16 oz Civitas + 1 oz Harmonizer treatment was statistically similar to the 16 oz Civitas + 4 oz Harmonizer treatment, but had a lower TQ than the 32 oz Civitas + 2 oz Harmonizer treatment.

At the Kearney site (Table 2) on the 11 March rating date, the 16 oz Civitas + 4 oz Harmonizer had the highest TQ with a mean of 3.1. The 16 oz Civitas + 1 oz Harmonizer and the 32 oz Civitas + 2 oz Harmonizer treatments had TQs of 1.8 and 2.6, respectively, and these were higher than the untreated control, which had the lowest TQ with a value of 1.0. On all other rating dates there was no difference among treatments, likely due to removal of the pigments as the turf was mowed.

At the Mullen site (Table 2), we found that 16 oz Civitas + 1 oz Harmonizer, 32 oz Civitas + 2 oz Harmonizer, and 16 oz Civitas + 4 oz Harmonizer treatments were in the best performing category for TQ receiving TQ values of 2.3, 2.9, and 3.3 respectively. The control has the lowest mean observed indicating that all the rates and ratios had a positive effect on improving turf quality.

The data from these three different study sites provide evidence that the application of Civitas + Harmonizer in late fall can improve TQ in the spring following winter stress conditions.

Crown Moisture Content

While there was no treatment effect on CMC, there was a significant date effect as the CMC decreased from 40.7% in February to 29.0% in March ($P<0.036$). All plots were visually water-stressed, and through previous research, we have found that significant turf death occurs when crown moisture content drops below 35%. This suggests that crown moisture by the end of this study was low enough to result in some death of the turf plants.

Chlorophyll Content and Reactive Oxygen Species

Chlorophyll and carotenoid contents were relatively low, but there was a significant Treatment x Date interaction for chlorophyll a, b, total chl., and TC (Table 3). In January, the untreated control and the 8 oz Civitas + 0.5 Harmonizer rate contained more chlorophyll a, b, and total CC than the other treatments. The control had significantly more carotenoids than all other treatments.

Greater chlorophyll and carotenoid contents were observed in February. The untreated control contained more chlorophyll a, b, and total CC than the 8 oz Civitas + 0.5 oz Harmonizer rate, while all other treatments were statistically similar to the control. For TC, the 16 oz Civitas + 4 oz Harmonizer rate contained more TC than the 8 oz Civitas + 0.5 oz Harmonizer rate. All other treatments were statistically similar for TC during this time.

The chlorophyll and carotenoid values were lower in March than in February. The 16 oz Civitas + 4 oz Harmonizer had the most chlorophyll a, b, and total Chl, suggesting that this treatment had a positive effect on plant health. The control had the least chlorophyll a, although this wasn't statistically different from the 16 oz Civitas + 1 oz Harmonizer rate. A similar trend was observed for chlorophyll b, total Chl., and TC, where 16 oz Civitas + 4 oz Harmonizer resulted in higher values compared to all other treatments.

Reactive oxygen species were quantified in nM of H_2O_2 per g of fresh weight tissue. In general, ROS are produced in living cells as a stress response to high-light conditions, so greater ROS levels typically indicate a greater stress response by the plant. Overall, ROS were very low in samples from January, February, and March (Table 4). In January, there were no differences in ROS among the treatments. In February, the 16 oz Civitas + 4 oz Harmonizer treatment resulted in greater H_2O_2 concentrations than the untreated control. This could be attributed to the Harmonizer, considering that the 16 oz Civitas + 1

oz Harmonizer treatment has ROS concentrations similar to the control. In March, the 16 oz Civitas + 1 oz Harmonizer treatment resulted in greater ROS than all other treatments. Additionally, the same rate of Civitas applied with 4 oz of Harmonizer had similar ROS concentrations to the control. Despite the relatively low concentrations of ROS for all treatments, the presence of ROS could have implications on cellular viability in that the cells were functional and able to produce ROS.

CONCLUSION

In this study, we demonstrated the benefits of a late fall application of Civitas + Harmonizer for improving turfgrass quality in the spring following winter stress conditions in Nebraska. Applications of Civitas + Harmonizer improved quality in the spring following winter stress conditions in three different field studies in the winter of 2014-15. Particularly, the 32 oz Civitas + 2 oz Harmonizer, and 16 oz Civitas + 4 oz Harmonizer treatments were the most effective in improving turf quality in early spring at all sites. Excluding the 25 March and 1 April rating date at the Kearney location, all rates and ratios of Civitas resulted in a higher TQ's across all locations and rating dates. The inconsistent results observed at the Kearney site are likely due to more stressful winter conditions, resulting in more turf death.

Effects of Civitas + Harmonizer on chlorophyll content and ROS presence characteristics were less pronounced. In January, the 16 oz Civitas + 4 oz Harmonizer treatment actually decreased chlorophyll and carotenoids. However, the same treatment increased chlorophyll and carotenoids in April when the turf resumed growing, suggesting that this treatment had an overall positive result on plant health. While there were a few instances where Civitas + Harmonizer increased ROS, the actual concentrations were so low that these differences are likely negligible.

LITERATURE CITED

Beard, J.B. 1973. Turfgrass: Science and culture. Prentice-Hall, Inc. Englewood Cliffs, N.J.

Inskeep, W.P., and P.R. Bloom. 1985. Extinction coefficients of chlorphyll *a* and *b* in N,N-dimethylformamide and 80% acetone. Plant Physiol. 77:483-485.

Table 1. List of study sites, treatments, and application timings.

Location	Civitas + Harmonizer Rate	Application Date		
		24-Oct	17-Nov	20-Nov
	-----oz 1000 ft^{-2}-----			
Mead, NE	Control	X	X	
	8 + 0.5 (16:1)	X	X	
	16 + 1 (16:1)	X	X	
	32 + 2 (16:1)	X	X	
	16 + 4 (4:1)	X	X	
Kearney, NE	Control			X
	8 + 0.5 (16:1)			X
	16 + 1 (16:1)			X
	32 + 2 (16:1)			X
	16 + 4 (4:1)			X
Mullen, NE	Control			X
	8 + 0.5 (16:1)			X
	16 + 1 (16:1)			X
	32 + 2 (16:1)			X
	16 + 4 (4:1)			X

Table 2. Turf quality for Mead, Kearney, and Mullen sites.

Location	Civitas + Harmonizer Rate	Turf Quality					
		11-Mar	25-Mar	1-Apr	10-Apr	15-Apr	Mean (all dates)
	-----oz 1000 ft^{-2}-----	----------1-9----------					
Mead, NE	Control	-	1.0d	1.9c	2.5b	3.0c	2.1c
	8 + 0.5 (16:1)	-	2.0c	2.0c	3.0b	3.5bc	2.6c
	16 + 1 (16:1)	-	3.0b	2.8b	4a	4.8ab	3.6b
	32 + 2 (16:1)	-	3.8a	3.5a	4.8a	5.3a	4.3a
	16 + 4 (4:1)	-	4.0a	3.6a	4.8a	4.3abc	4.2ab
p-value			<0.001	<0.001	0.004	0.049	<0.001
Kearney, NE	Control	1.0d	1.9a	2.3a	-	-	1.7c
	8 + 0.5 (16:1)	1.4cd	1.8a	2.5a	-	-	1.9bc
	16 + 1 (16:1)	1.8b	2.1a	2.6a	-	-	2.2abc
	32 + 2 (16:1)	2.6b	2.1a	2.6a	-	-	2.5ab
	16 + 4 (4:1)	3.1a	2.1a	3.1a	-	-	2.8a
p-value		<0.001	0.712	0.832			0.044
Mullen, NE	Control	1.3b	-	-	-	-	1.3b
	8 + 0.5 (16:1)	1.4b	-	-	-	-	1.4b
	16 + 1 (16:1)	2.3ab	-	-	-	-	2.3ab
	32 + 2 (16:1)	2.9a	-	-	-	-	2.9a
	16 + 4 (4:1)	3.3a	-	-	-	-	3.3a
p-value		0.001					0.001

*Different letters following quality scores denotes significant differences. Treatments are comparable by location within date.

Table 3. Chlorophyll a, b, total chlorophyll, and total carotenoid concentrations for January, February, and March at the Mead site.

Civitas + Harmonizer Rate	January			
	Chlorophyll			Total Carotenoids
	a	b	Total	
	--------µg g^{-1} fresh weight--------			--mg g^{-1} fresh weight--
Control	35.0a	71.7a	105.8a	10.85a
8 + 0.5 (16:1)	32.5ab	65.0ab	96.7ab	10.04b
16 + 1 (16:1)	21.6c	45.8c	67.5c	6.805c
32 + 2 (16:1)	20.8c	44.1c	66.7c	7.531c
16 + 4 (4:1)	32.5ab	65.0ab	96.7ab	10.04b
p-value	0.001	0.017	0.019	0.005
			February	
Control	68.3a	140.8a	207.5a	24.34ab
8 + 0.5 (16:1)	59.2b	125.8b	183.3b	22.20b
16 + 1 (16:1)	65.8ab	136.7ab	201.7ab	23.66ab
32 + 2 (16:1)	64.1ab	133.3ab	198.3ab	23.79ab
16 + 4 (4:1)	65.0ab	135.0ab	199.1ab	25.40a
p-value	0.238	0.356	0.256	0.168
			March	
Control	34.2c	71.7b	107.5b	13.59b
8 + 0.5 (16:1)	40.8b	83.3b	124.2b	15.00b
16 + 1 (16:1)	37.5bc	77.5b	115.8b	14.69b
32 + 2 (16:1)	40.8b	83.3b	123.3b	14.98b
16 + 4 (4:1)	49.2a	100.0a	149.2a	16.75a
p-value	<0.001	<0.001	<0.001	0.004

*Different letters following quality scores denote significant differences. Treatments are comparable within a column, by month.

Table 4. Reactive oxygen species concentration for the months of January through March at the Mead site.

Civitas + Harmonizer Rate	January	February	March
	Reactive Oxygen Species (ROS)		
-----oz 1000 ft^{-2}-----	----nM H_2O_2 g^{-1} fresh weight * 10^2----		
Control	2.24a	0.10b	0.09b
8 + 0.5 (16:1)	0.35a	0.00b	0.25b
16 + 1 (16:1)	0.14a	0.91ab	1.37a
32 + 2 (16:1)	3.71a	1.88ab	0.33b
16 + 4 (4:1)	2.98a	3.31a	0.06b
p-value	<0.001	0.014	0.005

*Different letters following quality scores denote significant difference within columns. Treatments are comparable within months.