International Journal of Plant Production 10 (2), April 2016
ISSN: 1735-6814 (Print), 1735-8043 (Online)
www.ijpp.info

GUASNR

Water stress, nano silica, and digoxin effects on minerals, chlorophyll index, and growth of ryegrass

S. Mahdavi[a], M. Kafi[b,*], E. Fallahi[c], M. Shokrpour[d], L. Tabrizi[e]

[a]Ph.D. Graduate student conducting dissertation research jointly between Department of Horticultural Science and Engineering, University of Tehran, Iran, and University of Idaho, USA.
[a, b, d, e]Department of Horticultural Science and Engineering, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.
[a, c]University of Idaho, Parma Research and Extension Center, 29603 U of I Lane, Parma, ID, USA.
*Corresponding author. E-mail: mkafi@ut.ac.ir

Received 26 October 2015; Accepted after revision 17 November 2015; Published online 5 March 2016

Abstract

Water shortages worldwide mandate minimum use of irrigation water with maximum efficiency and productivity in all agricultural crops, landscape plants and turf. The objective of this experiment was to study the effect of two levels of evapotranspiration-based (ET_c) drought in combination with two levels of nano silicon dioxide (SiO_2; $NanSi_1$ = 1 mM and $NanSi_2$ = 2 mM), two levels of digoxin (Dig_1 = 0.25 mg.l^{-1} and Dig_2 = 0.5 mg.l^{-1}) and Dig_1 plus $NanSi_1$ on mineral nutrient concentration, chlorophyll index and visual performance of perennial ryegrass (*Lolium perenne*) under climatic conditions of southwest Idaho in the Intermountain West Region of the United States. Clippings with 50% ET_c had higher percentage dry weight but lower chlorophyll index and visual rating than those with 75%. Clippings of 75%ET_c treatment had significantly higher concentrations of nitrogen (N), potassium (K), zinc (Zn) and copper (Cu) but lower sodium (Na), than those receiving the 50%ET_c treatment. Considering all mineral nutrient values, chlorophyll indices and visual performance ratings, we conclude that application of 75%ET_c is sufficient for maintaining a healthy lawn with satisfactory appearance while we can saving 25% water as compared to application of water at 100%. Root growth was un affected by any of these treatments. Based on the results of this study, applications of $NanSi_1$ or Dig_1, either individually or simultaneously, can slow the process of quality decline in perennial ryegrass turf under extremely severe drought conditions.

Keywords: Drought; Lawn quality; *Lolium perenne*; Nano particle; Turf physiology; Water shortage.

Introduction

Water shortage is becoming one of the most critical issues worldwide and thus water rationing is mandated in some regions (Brown, 2015). Under this condition, priority for water use will be given to the food crops and as a result, landscape plants such as turf will be subjected to additional water rationing. This action will likely have sociological and economical ramifications as ornamental and landscape plants have major role in flood and run off control, heat reduction, noise and dust reduction, human emotional and physical health and they are integral parts of recreational areas and golf courses.

The use of various horticultural, technological and environmental means to increase turf tolerance to drought is timely and necessary. Among these means are water scheduling based on precisely measured evapotranspiration (ET) values, use of efficient micro jets sprinklers and drip systems, breeding for tolerant cultivars and the use of biostimulants such as glycine betaine, proline, trehalose, mannitol and other osmoprotectants, gibberellic acid, 1- aminocyclopropane1-carboxylic acid, abscisic acid (ABA), jasmonates and salicylic acid (Farooq et al., 2009).

Next to oxygen, silicon is the most abundant element on the surface of the earth. Although silicon is not considered as an essential element for higher plants, this element has major roles in leaf turgidity of monocots and cell wall structure (Gong et al., 2003; Epstein, 1999). Silicon can be effective against both biotic and abiotic stresses, including insects, herbivores, bacteria, fungi, wind, cold, heat, water logging, salinity, mineral and water shortage and mineral toxicities (Ma, 2004; Epstein, 1999). Gong et al. (2003) reported that application of silicon increased relative water content, leaf fresh weight and thickness. They implied that leaf thickness improvement could be due to the reduction of transpiration and maintaining relative water content, resulting in higher water potential. Gong et al. (2003) also suggested that application of silicon could improve growth of wheat in arid or semi-arid areas. Hattori et al. (2005) reported that nano silicon enhanced drought tolerance in two cultivars of *Sorgum bicolor.* They measured diurnal transpiration rate and reported that silicon-treated sorghum plants extracted a larger amount of water from drier soil and maintain a higher stomatal conductance.

Agarie et al. (1999) reported that silicon decreased electrolyte leakage and increased cell walls polysaccharides in rice. They suggested that silicon could be involved in thermal stability of lipids in cell membranes and prevent structural and functional deterioration of cell membranes when rice plants are exposed to abiotic stresses. Haghighi et al. (2012) reported that application of nitrogen with silicon reduced the impact of salinity and improved seed germination of tomato. Azimi et al. (2014) reported that SiO_2 nanoparticles increased seed germination and dry weights in the shoot and root tissues.

Digoxin (Dig) has been exclusively used in human tissues. According to Lelievre and Lechat (2007), digoxin is a cardiac glycoside that binds to and inhibits sarcolemma-bound (Na^+/K^+-) Mg^{2+}-ATPase in humans. This ATPase catalyses both an active influx of 2 K^+ ions and an efflux of 3 Na^+ ions against their respective concentration gradients with the energy being provided by the hydrolysis of ATP. The inhibition induced by Dig leads to an efflux of K from the cell and, in proportion to the extent of inhibition of the ATPase, an increase in internal sodium ion concentration ([Na^+]) at the inner face of the cardiac membranes. This local accumulation of sodium causes an increase in free calcium concentrations via the Na^+–Ca^{2+} exchanger. This free cellular Ca is responsible for the inotropic action of Dig, secondary to the release of Ca^{2+} from the sarcoplasmic reticulum, including the clinical and molecular basis (Lelievre and Lechat, 2007). Edner et al. (1993) studied the influence of Dig on muscular and symphatoadrenergic activity and the serum potassium concentration. They conducted this study to determine whether this change in serum potassium concentration was dependent on inhibition of the Na-K-ATPase activity by Dig treatment. During Dig treatment, the serum K concentration significantly increased and they proposed that Dig-induced depression of Na-K-ATPase activity seems to be a prerequisite for the described change in serum K concentration.

Perennial ryegrass (*Lolium perenne*) is a popular cool season grass, which is commonly used alone and in mixtures with other species in managed landscapes. However, information on the use of various level of water stresses, nano particles and phytochemicals on this grass is lacking. Thus, our objective in this experiment was to study the effect of two levels of ET-based drought in combination with two levels of silicon nano particles (NanSi), two levels of Dig and one level of NanSi plus Dig on mineral nutrient concentration, chlorophyll index, visual performance of perennial ryegrass under climatic conditions of the southwest Idaho in the Intermountain West Region of United States. This region is similar to many climatic and soil conditions of the Alborz Province and Karaj in Iran.

Materials and Methods

Experimental site and turf establishment

This study was conducted in an area with a dimension of 30 x 80 m at the University of Idaho Parma Research and Extension Center, Parma, Idaho USA. The site was located at 43.7853° N, 116.9422° W, with a semi-arid climate and an annual precipitation of approx. 297 mm. The soil was sandy clay loam of approx. pH 7.3 and the soil chemical analysis revealed the following information: soil organic matter = 2.8%; soluble salts = 0.13%, nitrate = 2 ppm, ammonium = 1 ppm, phosphorus (P) = 11 ppm, potassium (K) = 497 ppm, sulphur (S) = 22 ppm, calcium (Ca) = 5614 ppm, magnesium (Mg) = 386 ppm, sodium (Na) = 99 ppm, zinc (Zn) = 2.2 ppm, copper (Cu) = 1.3 ppm, manganese (Mn) = 6 ppm, iron (Fe) = 6 ppm, boron (B) = 0.20 ppm, with a cation exchange capacity of 13 and percent base saturation of 229.

The ground was prepared and perennial ryegrass (*Lolium perenne)* was planted in May of 2013. This lawn was young and thriving without any diseases or insect damages and thus perfectly suitable for our research purpose. Nitrogen as urea (46%) was applied at the rate of 5 g actual N/m^2 at each application and watered immediately. Applications were made once in the middle of each month from April through July. Potassium and phosphorous were applied twice a year, in middle of May and June at the rate 5 g actual K/m^2 as KCl and 2.5 g actual P/ m^2 as P_2O_5 at each application. A complex compound of all micronutrients was applied to the lawn in middle of May and June every year to fulfill the micronutrient requirements. This study was started on July 20, 2015 and continued through October 20, 2015. With an exception to the irrigation regimes, NanSi and Dig treatments, all cultural practices were similar to those recommended for commercial lawns in the Intermountain West region of the United States.

Calculations for ET_c and water applications

Water requirement was calculated based on the lawn evapotranspiration data. These data were gathered from the Agri-Met weather station that was located at about 300 m from the experimental site and recorded ET and other meteorological data for agricultural crops including lawns in the region every 15 minutes, 24 hours a day.

In this study, two irrigation systems at 50% ET_c or 75% ET_c of a full ET_c were established. Plots with 50% ET_c or 75% ET_c water stress initially received irrigation at 50% ET_c through a system in which polyethylene lines were buried in a 14-cm deep trench and connected to the main source of a well water. In this system, macro jet Maxi-Paw 08, Rain Bird sprinklers (Azusa, CA, USA) were installed at about 6 × 6 m spacing and operated at 48 PSI to deliver 8.22 $l/m^2/hr$) of water. However, in the 75% ET_c treatment, an additional irrigation system was built to deliver the extra 25% water to make it a total of 75% ET_c. Using an Excel spread sheet and the daily ET_c, water schedules for the 50% ET_c and 75% ET_c treatments were made.

This study was started on July 20, 2015 and continued through October 20, 2015. Water requirement was calculated based on ET_c where $ET_c = ET_r \times K_c$. In this equation, ET_r (Penman-Monteith reference evapotranspiration) (Allen et al., 1998) was taken from the University of Idaho Agri-Met Parma Weather Station and K_c was the crop coefficient for turf grass. During the first week of the experiment, plots in both irrigation regimes received water at a full ET_c level. The differentiation between irrigation regimes was started on July 27, 2015 (a week after the initial water). First, full ET_c was calculated and from that value and the irrigation water delivery, the required time for each of the 75% ET_c and 50% ET_c irrigation was calculated. Rainfall during the summer months was rare in the region. However, when rain occurred, the rain volume was deduced from the total ET_c requirement.

Nano silica and digoxin treatments

Nano silica and digoxin treatments were prepared and applied as follows:
1. Digoxin (Dig; Powder; DSM Pharmaceuticals Inc. Greenville, NC, USA) was dissolved in ethyl alcohol with 95% purity (VWR International, Westchester, PA, USA) and prepared final solutions with concentrations of 0.25 $mg.l^{-1}$ (Dig_1) and 0.5 $mg.l^{-1}$ (Dig_2).
2. Nano silica (NanSi; ≈ 99% purity, 20-30 nm, amorphous, US Research Nanomaterials Inc., Houston, TX, USA) was dissolved in distilled water and prepared final solutions with concentrations of 1 mM ($NanSi_1$) and 2 mM ($NanSi_2$).
3. Dig at 0.25 $mg.l^{-1}$ and NanSi solution at 1 mM prepared and applied separately (not in the same solution).
4. Non-treated control (water application only).

Each of the NanoSi and Dig solutions and non-treated control (water) was applied twice to the lawn, using a backpack sprayer with a fine cone pattern (15.1 L Solo, Model 425, New Port News, VA, USA) to a run-off point. No surfactant was used in any of the sprays in this study. The first application was made between 8 a.m. and 1 p.m. on July 28, 2015. On this day, temperatures during application ranged from 21 ^oC to 28 ^oC with a clear sky and wind was calm (less than 2 $km.hr^{-1}$). The second applications were made on August 11, 2015 (two weeks after the first application). On this day, temperatures during application ranged from 29 ^oC to 37 ^oC with a clear sky and the wind was calm (about 1 km/hr). Each solution was uniformly sprayed at the rate of 0.78 $l.m^2$. The non-treated control plots were also sprayed with water at the 0.78 $l.m^2$.

Soil moisture and lawn temperature measurements

A fully computerized soil moisture meter equipped with two 7.5-cm rods and designed for lawn moisture (FieldScout Digital Soil Moisture Meter, Model TDR 300, Spectrum Technologies, Aurora, IL, USA) was used to measure soil moisture (volumetric water content; VWC) before and after each irrigation and sometimes in between irrigations. At each time, VWC from three different locations within the same plot was measured and averaged. Although ET_c was the basis for irrigation scheduling, the soil VWC was measured to monitor and compare the ET_c-based water application with that shown with a soil moisture meter. Soil moisture measurements were made between July 27 and September 7.

An infrared thermometer (IR Temperature Reader, Spectrum Technologies, Aurora, IL, USA) was used to measure soil temperatures at about 1.0 m distance from the lawn on September 4, 2015.

Mineral nutrient analysis of clippings

A composite clipping sample from three locations within each plot was taken at a height of approximately 3 to 4 cm from the soil level on August 24, 2015. Clippings were washed in a mild solution containing 1% Liqui-Nox anionic detergent (AlcoNox Inc., White Plains, NY, USA), rinsed in three different 25-L containers of distilled water and dried in a forced-air oven at 65 $^{\circ}$C for 72 hours. Clippings' fresh weight (FW) and dry weight (DW) were measured and dry weight percentage (DW%) was calculated. The dried leaves were ground to pass a 40-mesh screen using a Cyclotec Sample Mill (Model 1093; Tecator, Hoganas, Sweden).

For mineral analysis, specific guidelines and methods described by Gavlak et al. (2005) were used. For this purpose, N and S concentrations were determined by combusting the dry leaf tissues using a LECO Carbon/Nitrogen/Protein/Sulfur analyzer (TruMac, LECO Corp., St. Joseph, MI, USA). Leaf tissues were analysed for P, K, Ca, Mg, Fe, Zn, Mn, Na, chlorine (Cl), boron (B) and Cu by dry ashing at 500 ºC, nitric/perchloric digestion and the use of Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) (Perkin-Elmer; Norwalk, CT, USA).

Root sampling

At the end of this study, a stainless steel cylinder with 17.9 cm diameter was manufactured and the sharp circular edge of the base was pushed against the turf, until it left a distinct mark. The, grass with its roots within that marked area down to 15 cm in the soil (beyond the extend of the root zone) was removed and roots were separated from the top with a knife. Roots were washed and fresh and dry weights of the roots and tops were measure before and after drying in an air-forced oven at 65 ^{o}C for 72 hours.

Chlorophyll index measurements

Chlorophyll indices between 10 a.m. to 2 p.m. were determined on 12 dates between July 29 and September 3, 2015, using a new equipment and technology (FieldScout Chlorophyll Meter, Model CM 1000, Spectrum Technologies, Aurora, IL, USA). With this equipment, the ambient and reflected 700 nm and 840 nm light were used to calculate the relative chlorophyll index. It measures conical viewing areas between, 30 and 180 cm from lens. The equipment measured index of relative chlorophyll content in a range from 0 to 999. At each time, chlorophyll indices of three different locations within the same plot were measured and averaged. The average values of chlorophyll indices over 12 dates were analyzed.

Visual performance ratings

Performance of the lawn was visually rated based on a combination of several factors, including greenness, growth, density and appearance from a scale of 1 to 9, according to the guideline recommended by Morris (2002). In this guideline, 1 is poor performance, 7 is acceptable and 9 is outstanding. The average values of visual performance ratings over 8.28 different dates were averaged and reported.

Layout, experimental design and statistical analyses

The experimental design was a randomized-complete-block split-plot design with two levels of irrigation as the main plot (main-effect) and nontreated control, NanSi, Dig and a combination of NanSi and Dig treatments as sub-plots, each with four blocks. The size of each sub-plot was 1.2 m × 7.2 m, in an east-west orientation. Sufficient buffer zones were kept to prevent cross contamination of the two levels of irrigation regimes (50% ET_c and 75% ET_c) within each block and between different blocks. Computing univariate analyses for all lawn responses in this study checked the assumption of a normal distribution of data. Analyses of variance and all possible correlation coefficients among different minerals, clippings and chlorophyll index were conducted using SAS Version 9.4 Programme (SAS Institute, Cary, NC, USA), with PROC GLM. Means were compared by Duncan's multiple range test at $P \leq 0.05$.

Results and Discussion

Interaction

Significant interaction between the main effects and sub-plots were absent for any of the parameters measured in this study. Thus, we used the average values for each of the main and sub-plot effects in the tables.

Soil volumetric water content (soil moisture)

Irrigation was scheduled based on daily ET_c data. Our goal was to keep the irrigation treatments at 50%ET_c and 75%ET_c. Plots with 50%ET_c received 141.1 mm water (equivalent to 143 $l.m^{-2}$) while those with 75%ET_c received 211.9 mm water (equivalent to 215 $l.m^{-2}$) during the term of this study. Our goal in this study was to keep 25% difference between 50%ET_c and 75%ET_c treatments and the water usage in these treatments confirms that we achieved this goal. Because: 0.5/0.75 = 0.66 which is exactly same as 141.1/211.9. Soil volumetric water content closely paralleled ET_c measurements in 50%ET_c and 75%ET_c blocks (data not shown).

Water stress effects

Clippings with 50% ET_c had significantly higher percentage dry weight but lower chlorophyll index and visual rating (performance) than those with 75% (Table 1) and the correlation coefficient between chlorophyll index and visual rating was extremely strong (r = 0.96). The lower volume of water in the 50% ET_c treatment resulted in lower water content and thus higher percentage dry matter in these treatments. Root fresh weight, root dry weight and root percentage dry weight were unaffected by water stress treatments (Table 1).

Clippings from 75%ET_c treatment had significantly higher concentrations of N, K, Zn and Cu but lower Na, than those of 50%ET_c treatment (Tables 2 and 3). Concentrations of nitrate, P, Ca, Mg, Mn and B also tended to be higher in the clippings from the 75% than those from 50%ET_c treatment, although differences were not statistically significant (Tables 2 and 3). The higher N and chlorophyll index in the 75% ET_c treatment indicate that photosynthesis in this treatment was higher than those in 50%ET_c, leading to a more efficient uptake of minerals. A significant correlation coefficient existed between N in the clippings and chlorophyll index (r = 0.96). The lower concentrations of K in the 50%ET_c treatment is in agreement with a similar study in apple (*Malus domestica*) by Fallahi et al. (2015) and in cocksfoot (*Dactylis glomerata*) by Volaire and Thomas (1995).

Comparing the clipping nutrient concentrations in our study with the standard values for *Lolium perenne* clippings reported by Mills and Jones (1991) revealed that our minerals were about15to 20% lower than the standard thresholds. This trend was expected because clippings for developing standard values are collected from growing points of lawns from well-irrigated plots (Mills and Jones, 1991) whereas results from our study were gathered from clippings that have received stress at either 50%ET_c or 75%ET_c levels.

Considering all mineral nutrient values, chlorophyll indices and visual performance ratings, we concluded that application of 75%ET_c was sufficient for maintaining a healthy lawn with satisfactory appearance while we can saved 25% water as compared to application of water at 100%. Although we did not have a 100% ET_c treatment in this study, high visual ratings (average ratings as high as 8.28 out of 9) for the 75%ET_c is a clear indication that this level of irrigation was sufficient. However, 50%ET_c treatment was too low and may lead to a poor lawn quality over a long time of application.

Table 1. Effect of two levels of water stress, calculated based on crop evapotranspiration, on clipping weight and chlorophyll index and root weight of *Lolium perenne*.

Water stress level†	Water used during the period of study		Clipping fresh wt ($g.cm^2$)	Clipping dry wt ($g.cm^2$)	Clipping dry wt (%)	Root fresh wt (g)	Root dry wt (g)	Root percentage dry wt	Average chlorophyll Index†	Visual rating†
	mm	$L.m^{-2}$								
50% ET_c	141.1	143	$22.5^{bΨ}$	8.9^{b}	40.5^{a}	37.3^{a}	11.5^{a}	30.5^{a}	346.25^{b}	5.88^{b}
75% ET_c	211.9	215	44.4^{a}	13.4^{a}	30.4^{b}	64.7^{a}	17.3^{a}	27.4^{a}	502.28^{a}	8.28^{a}

† Abbreviations: ET_c = Lawn evapotranspiration; wt = weight; Average chlorophyll index =average of chlorophyll measurements over 12 dates between July 29 and September 3; each index value at each time was an average of three measurements. Each value of visual rating is an average of 7 measurements between July 29 and September 3.
Ψ Mean values in each column followed by a different letter (s) were significantly different at $P \leq 0.05$ as determined by Duncan's multiple ranges test.

Table 2. Effect of two levels of water stress that was calculate based on crop evapotranspiration, on clipping macro nutrient concentration (% dry weight) of *Lolium perenne.*

Water stress level†	N	P	S	Ca	K	Mg	Na
50% ET_c	$2.61^{bΨ}$	0.29^{a}	0.20^{a}	0.38^{a}	1.07^{b}	0.07^{a}	0.02^{a}
75% ET_c	2.81^{a}	0.33^{a}	0.20^{a}	0.44^{a}	1.38^{a}	0.08^{a}	0.03^{b}

† Abbreviations: ET_c = evapotranspiration of lawn.
Ψ Mean values in each column followed by a different (s) were significantly different at $P \leq 0.05$ as determined by Duncan's multiple ranges test.

Table 3. Effect of two levels of water stress that were calculate based on crop evapotranspiration, on clipping micro nutrient concentrations ($mg.kg^{-1}$dwt) of *Lolium perenne.*

Water stress level†	Zn	Fe	Cu	Mn	B	Cl
50% ET_c	$9.06^{bΨ}$	219.49^{a}	3.36^{b}	101.68^{a}	32.79^{a}	5.28^{a}
75% ET_c	14.40^{a}	165.11^{a}	8.58^{a}	106.37^{a}	34.62^{a}	5.15^{a}

† Abbreviations: ET_c = evapotranspiration of lawn.
Ψ Mean values in each column followed by a different letter (s) were significantly different at $P \leq 0.05$ as determined by Duncan's multiple range test.

Effects of nano silica (NanSi) and digoxin (Dig)

Application of NanSi and Dig at different levels, either individually or combinations, failed to affect fresh and dry weight of clippings and roots (Table 4). Applications of $NanSi_1$, $NanSi_2$, Dig_1, Dig_2 and Dig_1 plus $NanSi_1$ significantly increased average chlorophyll index and thus visual rating (Table 4). However, Dig_2 could not improve either chlorophyll index or visual rating as compared to control (Table 4). Applications of $NanSi_1$, Dig_1 or $NanSi_1$ plus Dig_1 significantly increased N and K in the clipping tissue but Dig_2 failed to affect these nutrients (Table 5). $NanSi_2$ was less effective and Dig_2 was ineffective in uptake N and K the clipping tissue, chlorophyll index and visual rating as compare to $NanSi_1$ or Dig_1. These results indicate that although applications of NanSi at 1mM ($NanSi_1$) and Dig at 0.25 $mg.l^{-1}$ (Dig_1) can reduce the levels of stress, perhaps due to maintaining or increasing uptake of nutrient particularly K, the use of these compounds at higher rates adds to the cost of materials and labour while lacking beneficial effects and thus not recommended.

Our results underscore the importance of NanSi application in reduction of drought effects and this area deserves further studies. Our study was conducted at a large scale and under field conditions which are more similar to a realistic commercial setting. However, our results in perennial ryegrass was consistent with an earlier study by Hattori et al. (2005) who reported a higher chlorophyll content in the NanSi-treated Sorghum (*Sorgum bicolor*) plants grown under abiotic stress conditions in a greenhouse. They did not include digoxin in their study.

Application of Dig for stress reduction of lawn has never been reported elsewhere. Although this chemical at 0.25 $mg.l^{-1}$ (Dig_1) showed a positive impact on N and K uptake and thus chlorophyll and visual improvement, the cost for such application should be taken into account. This chemical is used for heart patients in the medical communities and is too expensive for use in agricultural crops. However, further studies are required to determine if the positive impacts would justify mass production and perhaps lower cost of this compound for various agricultural crops and landscaping plants.

Concentrations of K in clippings had a highly positive correlation with visual rating ($r = 0.74$) and negative correlations with lawn temperatures ($r = -0.47$) and with clipping electrolytes leakage ($r = -0.67$), which confirms the positive relationship between K and drought resistance. Visual ratings also had a strong positive correlation with Cu in the clippings ($r = 0.67$) and this subject deserves further study.

A strong linear regression between the chlorophyll index and visual rating ($R^2 = 0.92$, data not shown) revealed that when using FieldScout C-1000 Chlorophyll meter, any index at or above 450-460 corresponded with a visual rating of 8 or greater which was considered an acceptable rating and good to great lawn performance.

Table 4. Effect of digoxin and nano silica treatments on clipping weight, chlorophyll index and root weight of *Lolium perenne*.

Treatment †	Clipping fresh wt. (g. cm^2)	Clipping dry wt. (g.cm^2)	Clipping percentage dry wt.	Root fresh wt. (g)	Root dry wt. (g)	Root Percentage dry wt.	Average chlorophyll Index†	Average visual rating†
Control	$35.28^{a\Psi}$	11.70^{a}	37.41^{a}	53.23^{a}	15.62^{a}	29.43^{a}	396.2^{c}	6.71^{b}
Dig_1	31.88^{a}	10.18^{a}	33.45^{a}	47.53^{a}	13.38^{a}	28.74^{a}	439.8^{a}	7.34^{a}
Dig_2	28.03^{a}	9.90^{a}	36.89^{a}	58.10^{a}	15.35^{a}	26.55^{a}	403.0^{bc}	6.69^{b}
$NanSi_1$	35.23^{a}	11.67^{a}	33.92^{a}	47.67^{a}	12.73^{a}	27.51^{a}	436.3^{a}	7.29^{a}
$NanSi_2$	38.95^{a}	13.20^{a}	36.05^{a}	47.15^{a}	13.83^{a}	31.08^{a}	432.6^{ab}	7.15^{a}
$NanSi_1Dig_1$	31.57^{a}	10.38^{a}	35.02^{a}	52.25^{a}	15.48^{a}	30.31^{a}	437.7^{a}	7.30^{a}

† Abbreviations: Dig_1= Digoxin at 0.25 $mg.l^{-1}$; Dig_2= Digoxin at 0.5 $mg.l^{-1}$; $NanSi_1$= Nano silica at 1mM; $NanSi_2$= Nano silica at 2mM; wt = weight; Average chlorophyll index =average of chlorophyll measurements over 12 dates between July 29 and September 3, each index value at each time was an average of three measurements; Each value of visual rating is an average of seven measurements between July 29 and September 3.

Ψ Mean values in each column followed by a different letter (s) were significantly different at $P \leq 0.05$ by Duncan's multiple range test.

Table 5. Effect of digoxin and nano silica treatment on clipping macro elements (% dry wt) of *Lolium perenne*.

Treatment[†]	N	P	S	Ca	K	Mg	Na
Control	$2.53^{b\Psi}$	0.31^{a}	0.19^{a}	0.43^{a}	1.10^{b}	0.07^{a}	0.03^{a}
Dig_1	2.82^{a}	0.30^{a}	0.20^{a}	0.41^{a}	1.32^{a}	0.08^{a}	0.025^{a}
Dig_2	2.69^{ab}	0.30^{a}	0.20^{a}	0.42^{a}	1.11^{b}	0.07^{a}	0.025^{a}
$NanSi_1$	2.83^{a}	0.30^{a}	0.20^{a}	0.39^{a}	1.26^{a}	0.07^{a}	0.028^{a}
$NanSi_2$	2.62^{ab}	0.33^{a}	0.19^{a}	0.41^{a}	1.27^{a}	0.07^{a}	0.026^{a}
$NanSi_1Dig_1$	2.74^{a}	0.33^{a}	0.20^{a}	0.40^{a}	1.33^{a}	0.08^{a}	0.03^{a}

[†] Abbreviations: Dig_1= Digoxin at 0.25 $mg.l^{-1}$; Dig_2= Digoxin at 0.5 $mg.l^{-1}$; $NanSi_1$ = Nano silica at 1mM;
$NanSi_2$ = Nano silica at 2mM; wt= weight;
[Ψ] Mean values in each column followed by a different letter (s) were significantly different at $P \leq 0.05$ by Duncan's multiple ranges test.

Table 6. Effect of digoxin and nano silica treatments on clipping micro nutrient concentration ($mg.kg^{-1}$) of *Lolium perenne*.

Treatment[†]	Zn	Fe	Cu	Mn	B	Cl
Control	10.04[aΨ]	265.15[a]	6.31[a]	94.25[bc]	33.12[a]	5.09[a]
Dig_1	11.63[ab]	152.99[a]	5.53[a]	112.50[a]	31.25[a]	5.21[a]
Dig_2	9.65[b]	195.01[b]	5.00[a]	91.71[c]	34.00[a]	5.09[a]
$NanSi_1$	10.61[ab]	162.62[b]	5.72[a]	108.87[ab]	35.8[a]	5.34[a]
$NanSi_2$	15.57[a]	176.77[b]	6.48[a]	105.50[abc]	34.00[a]	5.16[a]
$NanSi_1Dig_1$	13.10[ab]	213.76[ab]	6.50[a]	111.29[a]	34.00[a]	5.41[a]

[†] Abbreviations: NanSi= Nano silica; Dig= Digoxin; $NanSi_1$= 1mM; $NanSi_2$= 2mM; Dig_1= 0.25 $mg.l^{-1}$; Dig_2= 0.5 $mg.l^{-1}$

[Ψ] Mean values in each column followed by a different letter (s) were significantly different at $P \leq 0.05$ by Duncan's multiple range test.

Conclusions

Considering all mineral nutrient values, chlorophyll indices and visual performance ratings, we conclude that application of 75%ET_c is sufficient for maintaining a healthy lawn with satisfactory appearance while we can save 25% water as compared to application of water at 100%. Although we did not have a 100% ET_c treatment in this study, high visual ratings (as high as a rating of 9 out of 9) in the 75%ET_c treatment is a clear indication that this level of irrigation was sufficient. However, 50%ET_c treatment is too low and may lead to a poor lawn quality over a long time of application. Based on the results of this study, application of $NanSi_1$, Dig_1 and $NanSi_1$ plus Dig_1 can slow the process of quality decline under extremely severe drought conditions (i.e. 50% ET_c). This area deserves further study to see if applications of stress levels such as 65%ET_c with $NanSi_1$ and Dig_1 can further reduce the stress and maintain the visual quality in the lawns. Also, the potential effects of various rates of digoxin on reduction of water stress in other agricultural crops and land scape plants deserve further study.

Acknowledgement

Authors wish to express their appreciation to Jon Taberna and Western Laboratories for their support and to several researchers at the University of Idaho Pomology and Viticulture Program, including Dr. Robert Tripepi, Mike Keister, Bahar Fallahi and Tom Elias for their assistance in different parts of this study.

References

Agarie, S., Uchida, H., Agata, W., Kaufman, P.B., 1999. Effects of silicon on stomatal blue-light response in rice (*Oryza sativa* L.). Plant Prod. Sci. 2, 232-234.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop evapotranspiration. Guidelines for computing crop water requirements. FAO Irrigation and Drainage Paper 56. FAO, Rome, Italy.

Azimi, R., Borzelabad, M.J., Feizi, H., Azimi, A., 2014. Interaction of SiO_2 nanoparticles with seed prechilling on germination and early seedling growth of tall wheatgrass (*Agropyron elongatum* L.). Polish J. Chem. Tech. 16 (3), 25-29.

Brown, E.G., 2015. Governor Brown Declares Drought State of Emergency https://www.gov.ca.gov/news.php?id=18368.

Edner, M., Ponikowski, P., Jogestrand, T., 1993. The effect of digoxin on the serum potassium concentration. Scandin. J. Clinic. Lab Invest. 53 (2), 187-189.

Epstein, E., 1999. Silicon. Annual Review of Plant Physiology and Plant Molecular Biology. 50, 661-664.

Fallahi, E., Rom, C.R., Fallahi, B., Mahdavi, S., 2015. Leaf and fruit mineral nutrient partitioning influenced by various irrigation systems in 'Fuji' apple over four years. J. Amer. Pomo. Soc. 69 (3), 137-147.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Barsa, S.M.A., 2009. Agronomy for sustainable development. Agr. Sustain. Develop. 29 (1), 185-212.

Gavlak, R., Hornek, D., Miller, R.O. 2003. Soil, plant and water reference methods for the western region. 3rd edition. 125p.

Gong, H.J., Chena, K.M., Chen, J.C., Wang, S.M., Zhang, C.L., 2003. Effects of silicon on growth of wheat under drought. J. Plant Nut. 26 (5), 1055-1063.

Haghighi, M., Afifipour, Z., Mozafarian, M., 2012. The effect of N-Si on tomato seed germination under salinity levels. J. Biol. Envi. Sci. 6 (16), 87-90.

Hattori, T., Inanaga, S., Araki, H., An, P., Morita, S., Luxova, M., Alexander, L., 2005. Application of silicon enhanced drought tolerance in sorghum bicolor. Physiol. Plantarum. 123 (4), 459-466.

Lelievre, L.G., Lechat, P., 2007. Mechanisms, manifestations and management of digoxin toxicity. Heart Metabo. 35, 9-11.

Mills, H.A., Jones, J.B., 1991. Plant analysis handbook 2. Micromacro publishing. 422p.

Ma, J.F., 2004. Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. Soil Sci. Plant Nut. 50, 11-18.

Morris, K.N., 2002. A Guide to NTEP Turfgrass Ratings. The national turfgrass evaluation program (NTEP). http://www.ntep.org/ reports/ratings.htm.

SAS Institute, 2007. SAS/STAT1 user's guide. Version 9.2, SAS Institute, Cary, NC.

Volaire, F., Thomas, H., 1995. Effects of drought on water relations, mineral uptake, water-soluble carbohydrate accumulation and survival of two contrasting populations of cocksfoot (*Dactylis glomerata* L.). Ann. Bot. 7p.