Research article

Changes in Turfgrass Leaf Chlorophyll Content and Some Soil Characteristics as Influenced by Irrigation Treatments

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Received 14 December 2011 Accepted 18 April 2012 (*: Corresponding Author)

Abstract In plants, less water causes photosynthesis reduction whereas excessive water reduces the soil nutrients. These effects induce stunted plant growth. The aim of this work was to study the changes in leaf chlorophyll content and some soil characteristics of the native common bermudagrass (*Cynodon dactylon* L.) as influenced by irrigation treatments. Two experiments, open bottom 4 x 1 m concrete blocks and closed bottom pots were set up during the dry season from January to May 2011 at the Horticultural Section, Faculty of Agriculture, Khon Kaen University. The experimental designs were randomized complete block design (RCBD) with 4 daily irrigation levels; 0.50 evaporation pan ($0.50E_{pan}$), $0.75E_{pan}$, $1.00E_{pan}$ (control) and $1.25E_{pan}$. The results showed that $1.25E_{pan}$ irrigation in open bottom concrete blocks significantly increased leaching of essential soil nutrients and decreased leaf chlorophyll content. The $0.50E_{pan}$ irrigation treatment in pot experiment which was meant to resemble drought conditions had the lowest values in leaf chlorophyll content for the first two months. However, there were no differences in leaf chlorophyll content in all the treatments for the last two months. Moreover, the soil nutrients in all the treatments were not leached out in the closed bottom pot experiment. Therefore, the optimum watering amount should be taken into consideration.

Keywords photosynthesis, soil nutrients, water logging, water stress

INTRODUCTION

Chlorophyll is a molecule that performs photosynthesis, and it is found in the chloroplasts of green plants. This molecule is also responsible for the visual green colour in plants (Cheng et al., 2007; Fu and Dernoeden, 2009). Changes in chlorophyll content could occur as a result of nutrients deficiencies or exposure to environmental stress such as water stress (Mohesenzadeh et al., 2006) and soluble salts (Hameed and Ashraf, 2008). Generally, high soil fertility has been associated with the increase in total chlorophyll concentration (Minotta and Pinzauti, 1996; Cheng et al., 2007). Moderate soil drying and infrequent irrigation was reported to have increased leaf chlorophyll content (Jiang and Huang, 2001; DaCosta and Huang, 2006; Fu and Dernoeden, 2009). However, Alshehhi et al. (2010) found out that chlorophyll *b* and total chlorophyll (*a*+*b*) contents did not present significant differences on the three warm season turfgasses studied under different irrigation regimes in arid region, which reflects that there was no water stress.

Irrigation is an important factor in achieving the desired functions and aesthetics of the turfgrasses. It is aimed at sustaining turf quality and performance by maintaining a favorable soil water balance which can be achieved with a well-scheduled irrigation together with other appropriate inputs such as fertilizers (Kneebone et al., 1992). Water is utilized by the turf for the transportation of organic molecules, inorganic molecules and in overall structural support (Decoteau, 2005). However, less or excessive water can have some detrimental effects on turfgrass growth due to water stress and the environment such as water logging and salinity problems (Qadir et al., 2003; Rose, 2004). Moreover, soil characteristics which are fundamental to turfgrass growth such as pH, EC, CEC, the rate of organic matter decomposition, nutrients and exchangeable cations are affected by either excessive or insufficient water (Decoteau, 2005; Barton et al., 2006; Harivandi et al., 2009).

Despite chlorophyll being an important parameter for plants growth and soil characteristics playing a major role on plants growth as well, an observation of inconsistent reporting by the previous researchers along these areas was noticed. Therefore, the present study was aimed at evaluating the changes in native common bermudagrass leaf chlorophyll content and some soil characteristics as influenced by the irrigation treatments. Moreover, soil nutrients leaching were confirmed by 2 experiments in open and closed soil systems.

MATERIALS AND METHODS

Two experiments were set up during the dry season. In both experiments native common bermudagrass (*Cynodon dactylon* L.) was propagated using rhizomes. In experiment 1, 16 concrete blocks with open bottoms were used, each measuring 4 x 1 m while experiment 2 was comprised of 12 pots with closed bottoms buried to ground level, each measuring 0.55 m surface diameter and a depth of 0.35 m. The experiments were designed according to a randomized complete block design (RCBD) with four blocks and four replications for experiment 1, and three blocks and three replications for experiment 2. Four daily irrigation treatments were applied as follows; 50, 75, 100-control and 125% ($0.50E_{pan}$, $0.75E_{pan}$, $1.00E_{pan}$ and $1.25E_{pan}$) replacement of the evaporation pan (Class A pan; E_{pan}) which was installed at the study area for treatments 1-4 respectively. The watering was done manually.

Experimental units were filled up with a well prepared mixture of soil, chicken manure, rice husk and rice husk ash (1:1:1:1, v/v) to provide uniform soil conditions. The bulk density was 1.10 g cm^{-3} at 0-15 cm soil depth, and it contained 87.8% sand, 8.1% silt and 4.1% clay. The soil mixture was fermented for a month and some soil physical and chemical properties were determined as follows; pH (1:1 soil: distilled water extract) and electrical conductivity (EC; 1:5 soil: distilled water extract) were measured by conductivity meter (model: MPC227), cation exchange capacity (CEC) by ammonium saturation (Schollenberger and Simon, 1945), organic matter (OM) (Walkley, 1947), total nitrogen (N) content by Kjeldahl method and measured by flow injection analyzer (model: 5012 Analyser) (Bremner, 1965), available phosphorus (P) by Bray II method (Bray and Kurt, 1945), and both exchangeable potassium (K^+) and (Ca^{2+}) were measured by flame photometer (model: 410) after extraction with 1 N ammonium acetate pH 7.0. Also, soil characteristics were determined at the end of the study. The turf was maintained at 1 cm canopy height by cutting and removing the clippings from the experimental units.

Meteorological data was obtained from the agronomy section weather station, located 150 m away from the study area (Table 1).

Leaf chlorophyll content was measured on monthly basis following a method described by Arnon (1949). Chlorophyll was extracted by manual grinding 0.05 g of fresh leaf tissues with 80% acetone using a pestle and mortar in dark room at room temperature. After extracting the chlorophyll, the extract was topped up with 80% acetone to 20 mL, and filtered through a filter paper into a glass tube wrapped with aluminum foil. Residues were discarded, and the resulting solution was then transferred into a rectangular glass cuvette (path length 10 mm) and the absorbance was measured at 663 and 645 nm with a spectrophotometer (model: G10S UV-Vis). Final chlorophyll *a*, *b* and total chlorophyll (*a*+*b*) were calculated using the following equations.

$$Ca = [(12.7 \times D663) - (2.69 \times D645)] \times DF \quad (1)$$
$$Cb = [(22.9 \times D645) - (4.48 \times D663)] \times DF \quad (2)$$
$$Total\ chlorophyll\ (a+b) = [(8.02 \times D663) + (20.20 \times D645)] \times DF \quad (3)$$

Where *Ca* is leaf chlorophyll a (mg g^{-1} fresh weight), *Cb* is leaf chlorophyll *b* (mg g^{-1} fresh weight), *DF* is dilution factor, in this case was (20.05/50), *D663* and *D645* are spectrophotometer absorbance at *663* nm and *645* nm respectively.

The data collected was subjected to analysis of variance (ANOVA) using the STATISTIX-8 program, where a significant F-test was observed and means comparison test was carried out using Least Significant Difference (LSD) at $p \leq 0.05$ to separate treatment means.

RESULTS AND DISCUSSION

The temperature ranged from 14.5°C to 34.6°C and average relative humidity was 79.6%. The weekly E_{pan} reading was 33.4 mm or approximately 4.77 mm day^{-1} (Table 1). However, there was rainfall in mid March and April; so, compensation for rainfall by reducing the amount of watering was done when rainfall occurred.

Table 1 Weekly averages of meteorological data measured during the period of the study

Month	Week	Min_Temp (°C)	Max_Temp (°C)	RH (%)	Epan (mm)	Rainfall (mm)
Jan	3	14.5	27.9	85.6	34.0	-
	4	16.2	29.4	81.9	48.0	-
Feb	1	15.9	28.5	83.6	30.0	-
	2	19.1	33.8	80.0	30.0	-
	3	19.3	33.7	82.1	33.0	-
	4	22.7	33.5	85.1	25.0	-
Mar	1	22.4	34.6	75.9	37.0	-
	2	20.6	33.5	76.9	36.0	-
	3	18.6	29.4	76.9	33.0	-
	4	18.9	28.4	76.9	32.0	12.0
Apr	1	19.8	34.5	77.0	37.0	-
	2	19.7	33.3	76.1	24.0	18.0
	3	18.7	32.9	77.3	38.0	-
	4	17.6	33.1	79.4	30.0	6
Total					467.0	36.0

Note: Dates for total evaporation in January week 3 and week 4 ranges from 12th - 21st and 22nd - 31st respectively. Total rainfall in March week 4 was from 22nd - 31st

Soil characteristics

pH, electrical conductivity (EC), and cation exchange capacity CEC: The pH tended to increase in high irrigation treatments in both experiments. The values in this work were in the range of the general suggestion, which recommends soil pH between 5.5 and 7.5 for nutrients uptake by turfgrasses (Bell, 2011). The comparison between open and closed systems clearly differed in EC parameter. The open system had lower EC while the closed system had higher EC as compared to the composite soil sample value. This evidence indicated that the open system had soil nutrients leaching in the root zone to the deeper soil profile. The consideration in the 4 treatments for both experiments showed that high amount of watering ($1.25E_{pan}$) decreased the EC (Table 2). Nevertheless, the observed increase in EC from the closed system experiment could be attributed to the fertilizer applied two times in March and April.

The CEC in open system decreased with the increase in irrigation amount while the opposite was revealed in the closed system. Also, the reduction in CEC with high irrigation could be related with nutrients leaching out from the root zone (Qadir et al., 2003; Rose, 2004; Bell, 2011).

Organic matter (OM), Nitrogen (N) and Phosphorus (P): In open system experiment, OM significantly decreased in $1.25E_{pan}$ irrigation and all treatments were under the composite soil sample (Table 2). This evidence may be the same with EC and CEC; where high leaching of the soil nutrients was observed in open system experiment. Normally, OM is related with N in the soil. Our results revealed that the open system had the lowest OM and N as compared to the closed system. In addition, high irrigation in open system had the lowest OM and N. Comparing OM and N with the composite soil sample, and with the standard soil value (Bell, 2011), the values in this experiment were higher in OM and N levels.

Similarly, the closed system experiment had higher P when compared to open system. Also, for the high irrigation treatment in open system, P was leached out (Table 2).

Exchangeable cations; Potassium (K^+) and Calcium (Ca^{2+}): When comparing exchangeable K^+ of the two experiments, the results revealed high decrease in open system which was lower than the composite soil sample. This relates to the observed decrease in EC (Table 2). The watering induced leaching of exchangeable K^+ from the top soil. Bell (2011) reported that it is more difficult to maintain K^+ levels on soils with very high sand contents because K^+ is not tightly bound and leaches out easily. However, the closed system had accumulated exchangeable K^+. In detail, the treatments in closed system experiment, $0.75E_{pan}$ and $1.00E_{pan}$ irrigation had lower exchangeable K^+ as compared to $1.25E_{pan}$ irrigation. Mathowa et al. (2011) reported increase in shoot biomass in $0.75E_{pan}$ and $1.00E_{pan}$ irrigation treatments as opposed to the $1.25E_{pan}$ irrigation. Therefore, the observed decrease could also be attributed to exchangeable K^+ being utilized for shoot biomass production.

On the contrary, the irrigation treatments seem to have had no effect on exchangeable Ca^{2+} leaching (Table 2), because of the characteristic of Ca^{2+} as an immobile nutrient.

Table 2 Soil characteristics as influenced by irrigation treatments in both open bottom concrete blocks and closed bottom pots experiments

	pH 1:1 H_2O	EC 1:5 H_2O dS m^{-1}	CEC (cmol kg^{-1})	OM (%)	Total N (%)	Available P (mg kg^{-1})	Exchangeable K^+ (mg kg^{-1})	Exchangeable Ca^{2+} (mg kg^{-1})
Composite	7.2	0.7	9.9	3.9	0.12	2199.5	1461.0	1300.0
SD	0.3	0.1	1.6	0.4	0.02	430.3	114.4	127.3
Treatments	Open bottom concrete blocks							
$0.50E_{pan}$	7.0^b	0.5^a	11.0^a	3.4^b	0.12^b	1852.4^c	1051.9^b	1900.0^a
$0.75E_{pan}$	7.1^b	0.5^a	10.6^b	3.5^a	0.13^a	2275.0^a	1149.3^a	1450.0^c
$1.00E_{pan}$	7.0^b	0.4^c	10.3^d	3.3^c	0.11^b	2142.3^b	1042.2^b	1400.0^d
$1.25E_{pan}$	7.3^a	0.2^d	10.4^c	3.2^d	0.10^b	1749.2^d	866.9^c	1850.0^b
F-test	*	**	**	**	*	**	**	**
LSD 0.05	0.18	0.01	0.02	0.02	0.02	10.73	17.79	8.22
CV (%)	0.80	0.62	0.05	0.15	4.73	0.17	0.54	0.16
Treatments	Closed bottom pots							
$0.50E_{pan}$	7.0^b	1.8^a	10.0^d	3.9^c	0.15^c	2893.6^a	3141.1^d	1452.5^c
$0.75E_{pan}$	7.0^b	1.7^b	10.8^c	3.9^c	0.16^b	2771.2^c	3335.9^c	1550.0^b
$1.00E_{pan}$	7.1^b	1.7^b	11.7^a	4.6^a	0.18^a	2845.8^b	3555.1^b	1150.0^d
$1.25E_{pan}$	7.6^a	1.4^c	11.4^b	4.2^b	0.15^c	2412.5^d	4139.5^a	1700.0^a
F-test	**	**	**	**	**	**	**	**
LSD 0.05	0.13	0.05	0.02	0.01	0.01	15.16	17.08	8.88
CV (%)	0.57	0.87	0.05	0.10	2.17	0.17	0.15	0.19

** significant at $p\leq0.05$, ** highly significant at $p\leq0.01$, standard deviation (SD). Means separated by LSD Test at $p\leq0.05$, means within columns followed by the same letters are not significantly different.*

Leaf Chlorophyll content

Chlorophyll a, b and total chlorophyll (a+b): In open system, chlorophyll *a* seemed to be positively correlated with the treatments for the first two months with $0.50E_{pan}$ irrigation significantly reducing the content. Contrary to the first two months, $0.50E_{pan}$ and $0.75E_{pan}$ showed the highest content (Fig. 1A) The same trend was revealed for chlorophyll *b* and total chlorophyll *(a+b)* contents (Fig. 1B and 1C). Similar results; that is, the increase in chlorophyll content in $0.50E_{pan}$ and

0.75E_{pan} irrigation treatments were reported by Jiang and Huang (2001), and DaCosta and Huang (2006) found out that drought or lower amount of irrigation increase the chlorophyll content. It seems as if turfgrass chlorophyll contents increase serves as an activator for biochemical processes such as photosynthesis. Moreover, a sharp decrease in chlorophyll content for the control and 1.25E_{pan} irrigation at the end of the study could be attributed to the excessive irrigation which led to some essential soil nutrients such as N and exchangeable K^+ being leached out to the deeper soil profile as shown in Table 2.

Similar trend in closed system (Fig. 2) to the one shown in open system (Fig.1) was revealed for the first two months. However, there were no differences in the third and fourth months (Fig.2). Water moves downwards to the deeper soil profile after rain or irrigation and it moves upwards to evaporate or transpire from the soil surface or plant leaves (Plaster, 2003). Therefore, the closed bottom pot experiment was set up to counteract the upward movement of water. As a result no compensation for less water in closed system, hence water stress could possibly explain the high differences among the treatments as opposed to the open system for the first two months (Fig.1 and 2). Due to the fact that the closed pots were able to retain essential soil nutrients such as N, P and K^+ (Table 2), this could be attributed to the increase observed in chlorophyll contents at the end of the study. In support of our findings, Minotta and Pinzauti (1996) and Cheng et al. (2007) suggested that chlorophyll content progressively increase in high fertility soils.

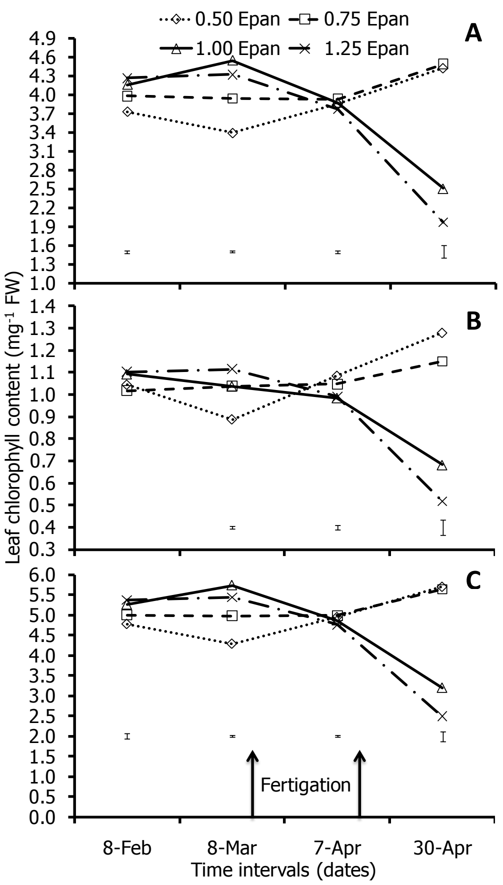

Fig. 1 Effect of irrigation regime on leaf chlorophyll content

chlorophyll a (A), chlorophyll b (B) and total chlorophyll (a+b) (C) of native common bermudagrass in open bottom concrete blocks. Vertical bars are LSD values (p≤0.05)

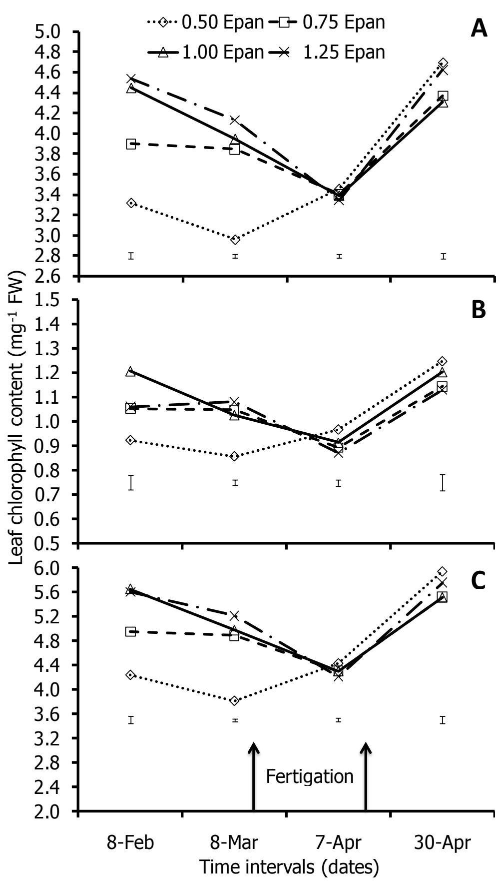

Fig. 2 Effect of irrigation regime on leaf chlorophyll content

chlorophyll a (A), chlorophyll b (B) and total chlorophyll (a+b) (C) of native common bermudagrass in closed bottom pots. Vertical bars are LSD values (p≤0.05)

CONCLUSIONS

Excessive irrigation in open system experiment led to leaching of essential plants nutrients and resulted in a significant decrease of leaf chlorophyll content. On the contrary, the closed system had no leaching of essential plants nutrients as proved by the increase in chlorophyll content in the third and fourth months. Therefore, optimum irrigation can retain nutrients and increase chlorophyll content; this should be taken into consideration for irrigation amount.

ACKNOWLEDGEMENTS

Thanks to the Graduate School, Khon Kaen University for financial support, TICA scholarship, and Agronomy Soil and Plant Analysis Laboratory technicians for technical assistance.

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