Carbohydrate Metabolism in Creeping Bentgrass as Influenced by Two Summer Irrigation Practices

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ABSTRACT. This field study was conducted to investigate carbon metabolic responses to deep and infrequent (DI) versus light and frequent (LF) irrigation in ‘Providence’ creeping bentgrass (*Agrostis stolonifera* L.). LF irrigation was performed daily to wet soil to a depth of 4 to 6 cm, whereas DI irrigation was performed at leaf wilt to wet soil to a depth of ≥ 24 cm. The creeping bentgrass was seeded into a sand-based root zone in 2005 and was maintained as a putting green during the 2006 and 2007 study years. Canopy net photosynthesis (P_n) and whole plant respiration (R_w) were monitored, and water-soluble carbohydrates [WSC (i.e., glucose, fructose, and sucrose)], storage carbohydrates [SC (i.e., fructan and starch)], and total nonstructural carbohydrates [TNC (i.e., the sum of water soluble and storage sugars)] in leaf and root tissue were quantified. Creeping bentgrass subjected to DI irrigation had a lower P_n and a generally similar R_w compared with LF-irrigated bentgrass. DI irrigated bentgrass generally had greater levels of WSC and TNC in leaf tissue in 2006 and similar levels in 2007 when compared with LF-irrigated bentgrass. Leaf SC levels were higher in DI- than LF-irrigated bentgrass in both years. Creeping bentgrass roots subjected to DI irrigation generally had greater SC and TNC levels in both years than were found in LF-irrigated plants. Root WSC levels were higher (2006) or similar (2007) in DI- versus LF-irrigated bentgrass. Irrigating creeping bentgrass at wilt rather than daily to maintain moist soil generally resulted in higher carbohydrate levels in leaves and roots, which may enable creeping bentgrass to better tolerate and recover from drought and other stresses.

Careful water management is critical to growing quality creeping bentgrass during summer stress periods, especially in sand-based root zones. Many golf course superintendents in the mid-Atlantic region and elsewhere irrigate creeping bentgrass greens lightly and frequently (LF) or deeply and infrequently (DI). LF irrigation involves applying water before wilt is evident and maintaining soil moisture at or near field capacity (Fry and Huang, 2004). DI irrigation is defined as irrigating at the first sign of leaf wilt to replenish the root zone with water (Fry and Huang, 2004). DI irrigation generally is recommended for maintaining cool-season grasses in summer (Beard, 1973; Fry and Huang, 2004).

Carbohydrate metabolism in leaves and sheaths, including photosynthesis, respiration, and carbon translocation, are major physiological processes that form the basis of healthy plant function. Creeping bentgrass summer performance may be improved by maximizing carbohydrate production through photosynthesis, while minimizing carbohydrate consumption from respiration (Xu and Huang, 2000). TNC availability has been widely used as a physiological measure of stress tolerance because carbohydrates provide energy and solutes for osmotic adjustment. The major TNC found in grasses include water-soluble (i.e., glucose, fructose, sucrose) and storage (i.e., starch and fructan) sugars (Smith, 1972).

Understanding the many physiological factors affecting rooting is critical because roots can be a nutrient sink and

obviously can contribute to overall plant health maintenance. Efficient carbon allocation to roots might increase the probability of plant survival during periods of drought stress (DaCosta and Huang, 2006a; Sisson, 1989). Several investigations have found that turfgrass plants subjected to drought stress accumulate more carbohydrates in leaves, stems, and roots when compared with well-watered plants (DaCosta and Huang, 2006a; Huang and Fu, 2000 and 2001; Huang and Gao, 2000). Soil drying reduces the proportion of newly photosynthesized carbon allocated to leaves, while increasing the proportion of carbon allocated to tall fescue (*Festuca arundinaceae* Schreb.) roots (Huang and Fu, 2000; Huang and Gao, 2000). This allocation of carbon to roots occurred to a greater extent in the more drought-tolerant tall fescue cultivars evaluated (Huang and Gao, 2000). Similarly, DaCosta and Huang (2006a) reported that newly photosynthesized carbon increased during the early phase of drought stress in creeping bentgrass roots, but not in leaves and stems.

We are not aware of any field studies that have investigated carbon metabolism in creeping bentgrass maintained as a putting green in response to summer irrigation practices. Therefore, the objectives of this field study were as follows: to quantify canopy net photosynthesis and whole respiration rates, and to quantify WSC, SC, and TNC levels in creeping bentgrass grown in a sand-based root zone in response to LF versus DI irrigation in the summer.

Materials and Methods

This study was conducted on a research green built to U.S. Golf Association (2004) recommendations at the University of Maryland Turfgrass Research Facility in College Park in 2006 and 2007. Soil was a modified sand mix (97% sand, 1% silt, and 2% clay) with a pH of 6.5 and 10 mg of organic matter per gram

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of soil. In Sept. 2005, the study site was treated with glyphosate and the sod was removed to expose bare ground. The area was seeded (50 kg·ha⁻¹ seed) with 'Providence' creeping bentgrass in Sept. 2005. The turf was fertilized (25 kg·ha⁻¹ N + 11 kg·ha⁻¹ P + 20 kg·ha⁻¹ K) eight times (20, 23, 28, and 30 Sept.; 18 and 20 Oct.; and 1 and 3 Nov.) in 2005 with a 20N-8.7P-16.6K fertilizer. A 19N-0.9P-15.8K fertilizer was applied on 11 Nov. to provide 50 kg·ha⁻¹ N, 2.3 kg·ha⁻¹ P, and 40 kg·ha⁻¹ K. A total of 250 kg·ha⁻¹ N was applied between 20 Sept. and 11 Nov. 2005. The bentgrass was fertilized biweekly with 4.9 kg·ha⁻¹ N from urea between 1 May and 7 June and then weekly through 24 Aug. for a total of 78.4 kg·ha⁻¹ N during the experimental period in 2006. In the autumn, bentgrass was fertilized (11.8 kg·ha⁻¹ N) six times between 20 Sept. and 17 Nov. 2006 to provide a total of 71 kg·ha⁻¹ N. In 2007, the bentgrass was fertilized weekly with 4.9 kg·ha⁻¹ N from urea between 30 Apr. and 27 Aug. to provide a total of 88.2 kg·ha⁻¹ N during the experimental period.

Iprodione (14.7 kg·ha⁻¹ a.i.) was applied biweekly in 2006 and 2007 to control dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) and brown patch (*Rhizoctonia solani* Kuhn). Iprodione was chosen because it has no known plant growth regulator effects. Deltamethrin (2.2 kg·ha⁻¹ a.i.) was applied on 26 July and 24 Aug. 2006 and on 18 July 2007 to control sod webworm (*Pediasia trisecta* Walker). Turf was mowed three times weekly to a height of 6 mm in Spring 2006. The mowing height was reduced to 4 mm on 3 July 2006 and the green was mowed at that height approximately five times weekly and clippings were removed throughout the remainder of the study. All eight plots were cored on 29 Oct. 2006 using a Toro Greens Aerator (Toro, Minneapolis, MN). The aerator was equipped with twelve 1.3-cm-diameter hollow tines that produced holes 12 cm deep on 5.7-cm spacing. Holes were filled to the surface with the previously described construction mix.

Each plot measured 1.8 × 2.4 m and was bordered by fiber glass polymer edging (Easy Gardener Products, Waco, TX) set 10 cm deep in soil to minimize lateral movement of water. There also was a 60-cm creeping bentgrass perimeter border separating each individual plot. Each plot was individually irrigated between 0700 and 0800 HR using a hand-held hose equipped with a showerhead nozzle (400 PL Water Breaker; Damm Corp., Manitowoc, WI) and calibrated as described below. The quantity of water applied was monitored with a digital flow meter attachment having a 5% inaccuracy (model 01M31LM; Great Plains Industries, Wichita, KS). The flow rate to dispense a given amount of water was controlled with a ball valve to ensure an even distribution of water within each plot. The two irrigation regimes assessed were LF and DI. In the LF irrigation regime, water was applied daily to replace moisture lost due to evapotranspiration (ET). This ensured that soil was maintained in a moistened state to a depth of 4 to 6 cm each morning. An atmometer was used to estimate ET as described by Rosenberg (1974). Evapotranspiration was estimated every 24 h using an atmometer (ET Gauge; Spectrum Technologies, Plainfield, IL) located within 10 m of the center of the study site. In the DI irrigation regime, water was provided at the first visual sign of leaf wilt as determined by foot printing and/or the appearance of a bluish-gray leaf color. Footprinting refers to foot-shaped impressions left on turf due to wilt and flaccid leaves (Turgeon, 2008). The frequency of irrigation was variable and depended on weather conditions. Therefore, DI irrigation frequency was sometimes as often as every 3 days

or as infrequently as 7 days. Because soil was dried to about the same level at wilt, a standard amount of 50 L (11.6 mm) of water was applied to each DI plot when irrigated. Using a soil probe and ruler, it was determined that this amount of water wetted the soil to a depth of 6 to 8 cm within 5 min and water penetrated to a depth ≥24 cm within 20 min after irrigation ceased. On sunny days, plots were hand syringed with a showerhead nozzle (Cool Shot; Precision, Pompano Beach, FL) about three times daily, depending on weather conditions, to cool leaves during the experimental period. When syringed, the canopy was moistened, but little or no water wet the thatch-mat layer. To minimize the impact of rain, two tarps (each 3.3 × 11 m) were used to cover all eight plots before the onset of rain between 22 May and 31 Aug. 2006 and 2007. The tarps were constructed from 12 mil black/white reinforced polyethylene sheeting (model 12 BW; Integra Plastics, Madison, SD). The white side of the tarps faced up and the tarps usually were removed within 15 min after the weather had cleared. Six rain events in 2006 (total 16.3 mm) and five events in 2007 (21.6 mm) occurred before the plots could be covered. On those days, LF-irrigated plots were not irrigated. An additional 59.7 mm of rain fell on uncovered plots on 20 and 21 Aug. 2007. Plots were uncovered because bentgrass in the DI-irrigated plots was wilting and water was needed at this time. Irrigation treatments were initiated on 22 May and ended on 6 or 7 Sept. in both years.

Canopy net photosynthesis (P_n) and whole respiration (R_w), including plant and soil microbe respiration, were measured on 2-to 3-week intervals between 7 June and 7 Sept. 2006 and on 3-to 4-week intervals between 31 May and 6 Sept. 2007 using a portable gas exchange system (LI-6400; LICOR, Lincoln, NE). Photosynthesis and respiration were determined by enclosing the turf canopy (126.6 cm²) in a transparent Plexiglas chamber (15 × 10 × 10 cm) attached to the LI-6400 CO₂ analyzer as described by Fu et al. (2007). When whole respiration was measured, the transparent Plexiglas chamber was wrapped with a double layer of black plastic to prevent sunlight from entering the chamber. Measurements were taken between 3 and 5 d following DI irrigation on clear sunny days between 1100 and 1400 HR. Measurements of P_n and R_w were obtained between 12 and 24 h after mowing in one location of each plot on each date, and data were expressed as CO₂ uptake and evolution per unit area.

Clippings were the source of mostly leaf plus some sheath tissue (hereafter leaf or leaves) used to measure WSC and SC levels. Clippings were collected between 1100 and 1500 HR using a walk-behind reel mower equipped with a basket. Clipping were collected 2 to 9 d following DI irrigation on 15 June, 13 July, 8 Aug., and 7 Sept. 2006, and on 1 and 28 June, 17 July, 15 Aug., and 6 Sept. 2007. Roots also were sampled by removing four soil cores (2.5 cm diameter × 20 cm deep) from each plot on the aforementioned dates. Green leaf tissue and the thatch-mat layer of each soil core were removed with scissors. The four soil cores from each plot were mixed and roots were washed free of soil. Leaves and roots were placed in separate plastic bags and placed immediately in liquid nitrogen and stored in a freezer at -80 °C until they were analyzed. Frozen tissue samples were killed at 105 °C for 30 min, oven dried at 70 °C until there was no further weight loss, ground in liquid nitrogen, and passed through a 0.4-mm sieve. For analysis of WSC (mainly glucose, fructose, and sucrose) and SC (mainly fructan and starch), 50 mg of dry tissue was placed in a 2.0-mL microtube, extracted three times in 1.0 mL of 92% ethanol,

vigorously shaken for 10 min, and then centrifuged at 20,000 g_n for 10 min as described by Wang and Jiang (2007). The supernatant containing reducing sugars (i.e., glucose and fructose) and sucrose was collected in a 10-mL test tube. The microtubes were left open and placed in an oven at 70 °C to evaporate the ethanol. The residue was used for SC analysis.

A 3-mL sample of combined supernatant containing glucose, fructose, and sucrose was diluted with distilled water to 10 mL. A 2-mL sample of the diluted supernatant was transferred to a test tube with 2 mL of 4% H₂SO₄ (w/v), mixed, and boiled for 15 min to hydrolyze sucrose to reducing sugar (i.e., glucose and fructose). The solution then was neutralized with 1.0 mL of 1.0 N NaOH. Ferricyanide reagent and arsenomolybdate solution were used to quantify reducing sugars as described by Ting (1956). A 0.2-mL amount of the above solution was transferred into a 20-mL volumetric tube, and 0.8 mL of distilled water and 1.25 mL of ferricyanide reagent were added (Ting, 1956). The solution was boiled in a water bath for 10 min and then cooled in ice water. A 2.5-mL aliquot of 2.0 M sulfuric acid was added to neutralize partially the solution. After neutralization, the tube containing the solution was shaken until gas evolution ceased, and 1.0 mL of arsenomolybdate solution was added (Ting, 1956). The solution was again shaken and diluted with distilled water to volume (20 mL). The absorbance of the solution was measured at 515 nm (OD₅₁₅) using a spectrophotometer (Beckman Coulter, Fullerton, CA). The amount of sugar in the solution was calculated using a glucose standard curve as described by Ting (1956). Therefore, WSC, SC, and TNC levels were expressed as milligrams of glucose per gram of dry weight.

Fructan and starch, which were obtained from the previously described residue, were hydrolyzed into reducing sugars using the method described by Smith (1981). For quantifying fructan and starch, 0.5 mL of distilled water was added to each microtube containing the residue recovered after the extraction of WSC from ethanol. The microtubes were sealed and placed on a heating block at 100 °C for 10 min, removed from the heating block, and cooled to room temperature. A 0.4-mL portion of 200 mM acetate buffer (pH 5.1) and 0.1 mL of enzyme solution were added to each microtube as described by Smith (1981). Final enzyme concentrations were 0.2 units of amyloglucosidase (product A 1602; Sigma, St. Louis) and 40 units of α -amylase (product A 2643; Sigma) per microtube. The microtubes were sealed, vortexed, and incubated at 55 °C for 16 to 24 h. When the incubation was completed, the tubes were centrifuged at 20,000 g_n for 10 min. The extract containing glucose (decomposed from starch) and fructan was diluted with distilled water 10 times (1:10, by volume). A 0.9-mL sample of the extract was placed in a 2.0-mL microtube and 0.1 mL of 1.0 N H₂SO₄ was added to hydrolyze fructan to fructose. The mixed solution was boiled on a heat block at 100 °C for 15 min, removed, and cooled to room temperature. The solution was neutralized with 0.1 mL of 1.0 N NaOH. The reducing sugar derived from starch and fructan was measured using the previously described method. The TNC content represents the sum of all water soluble sugars (i.e., glucose, fructose, and sucrose) and storage sugars (i.e., starch and fructan).

The experiment was arranged in a completely randomized block design with four replications. Treatment effects were determined by analysis of variance using the general linear model procedure of SAS (SAS Institute, Cary, NC). Significantly different means were separated by Fisher's protected least significant difference test ($P \leq 0.05$). The analysis of

variance revealed an interaction among treatments and years. Therefore, data from each year are shown.

Results

Canopy photosynthesis and whole plant respiration were determined six times between 7 June and 7 Sept. 2006 and five times between 31 May and 6 Sept. 2007 (Tables 1 and 2). Creeping bentgrass subjected to DI irrigation had a lower P_n on all six measuring dates in 2006 when compared with LF-irrigated bentgrass. Hereafter, LF-irrigated and DI-irrigated will be referred to as LF and DI, respectively. DI bentgrass had a lower R_w on 21 June, but a greater R_w on 4 July 2006 when compared with LF bentgrass. No differences in R_w were observed on the other four measuring dates between the two irrigation regimes in 2006. In 2007, P_n was lower on four measuring dates (i.e., 31 May, 2 and 24 July, and 14 Aug.) in DI versus LF bentgrass (Table 2). No difference in P_n was observed between regimes on 6 Sept. Except on 14 Aug. 2007 when R_w was lower in DI bentgrass, there were no differences in R_w between irrigation treatments in 2007.

WSC content in leaf tissue was similar on 15 June and 13 July and was greater on 8 Aug. and 7 Sept. 2006 in DI versus LF creeping bentgrass (Table 3). Creeping bentgrass subjected to

Table 1. Photosynthesis and respiration in response to light and frequent (LF) versus deep and infrequent (DI) irrigation in 'Providence' creeping bentgrass in 2006.

Treatments ^a	2006					
	7 June	21 June	4 July	26 July	16 Aug.	7 Sept.
Photosynthesis rate ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$)						
LF	6.7 a ^y	5.3 a	5.6 a	6.6 a	6.8 a	8.4 a
DI	5.1 b	3.5 b	3.5 b	4.0 b	5.5 b	7.6 b
Respiration rate ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$)						
LF	10.7 a	10.7 a	8.6 b	10.3 a	7.9 a	6.7 a
DI	10.5 a	8.8 b	9.9 a	9.5 a	6.6 a	7.3 a

^aLF irrigation was performed daily in the absence of rain to wet soil to a 4 to 6 cm depth. DI irrigation was performed at leaf wilt to wet soil to a depth of ≥ 24 cm.

^yMeans in a column within each parameter followed by the same letter are not significantly different based on Fisher's protected least significant difference test at $P \leq 0.05$.

Table 2. Photosynthesis and respiration in response to light and frequent (LF) versus deep and infrequent (DI) irrigation in 'Providence' creeping bentgrass in 2007.

Treatments ^a	2007				
	31 May	2 July	24 July	14 Aug.	6 Sept.
Photosynthesis rate ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$)					
LF	4.1 a ^y	6.2 a	6.5 a	6.8 a	4.1 a
DI	2.2 b	3.6 b	5.1 b	5.0 b	4.3 a
Respiration rate ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$)					
LF	11.5 a	8.8 a	8.7 a	8.9 a	9.8 a
DI	10.1 a	8.8 a	8.2 a	7.6 b	10.2 a

^aLF irrigation was performed daily in the absence of rain to wet soil to a 4 to 6 cm depth. DI irrigation was performed at leaf wilt to wet soil to a depth of ≥ 24 cm.

^yMeans in a column within each parameter followed by the same letter are not significantly different based on Fisher's protected least significant difference test at $P \leq 0.05$.

DI irrigation had similar SC levels in leaf tissue between 15 June and 8 Aug. in 2006 and a greater SC in leaf tissue on 7 Sept. compared with LF bentgrass. TNC content in leaf tissue was less on 15 June, similar on 13 July, and greater on 8 Aug. and 7 Sept. 2006 for DI versus LF bentgrass. On the final rating date in 2006, WSC, SC, and TNC were greater in bentgrass leaf tissue subjected to DI than LF.

In 2007, DI bentgrass had greater WSC in leaf tissue on two (17 July and 15 Aug.) of five measuring dates and similar WSC on three dates (1 and 28 June, and 6 Sept.) compared with LF bentgrass (Table 4). SC levels in leaf tissue were similar between 28 June and 15 Aug. 2007 and greater on 1 June and 6 Sept. in DI versus LF bentgrass. Creeping bentgrass subjected to DI had a greater amount of leaf TNC on 1 June, but similar TNC levels on all other measuring dates compared with LF

bentgrass. Unlike 2006, only SC levels were higher in DI bentgrass on the final measuring date.

Creeping bentgrass subjected to DI had greater WSC and TNC content in roots on all four measuring dates in 2006 compared with LF bentgrass (Table 5). SC levels in roots were similar on 13 July and 8 Aug. 2006, but were greater on 15 June and 7 Sept. 2006 compared with LF bentgrass. In 2007, root WSC was greater on 1 June in DI versus LF bentgrass (Table 6). However, no differences in root WSC were observed on the other four measuring dates between the two irrigation regimes in 2007. Creeping bentgrass subjected to DI had a greater SC and TNC levels in roots on four (1 June, 17 July, 15 Aug., and 6 Sept. 2007) of five 2007 measuring dates when compared with LF bentgrass. No difference in SC or TNC in roots was observed on 28 June 2007 between the two irrigation regimes.

Table 3. Water-soluble carbohydrate (WSC), storage carbohydrate (SC), and total nonstructural carbohydrate (TNC) in 'Providence' creeping bentgrass leaf tissue in response to light and frequent (LF) versus deep and infrequent (DI) irrigation in 2006.

Treatments ^z	2006			
	15 June	13 July	8 Aug.	7 Sept.
	WSC [glucose (mg·g ⁻¹ dry wt)]			
LF	39.9 a ^y	48.6 a	39.4 b	40.4 b
DI	34.5 a	50.4 a	52.6 a	44.1 a
	SC [glucose (mg·g ⁻¹ dry wt)]			
LF	70.6 a	73.9 a	33.1 a	47.4 b
DI	70.9 a	70.5 a	33.8 a	52.7 a
	TNC [glucose (mg·g ⁻¹ dry wt)]			
LF	110.5 a	122.5 a	72.6 b	88.0 b
DI	105.4 b	120.9 a	86.5 a	96.9 a

^zLF irrigation was performed daily in the absence of rain to wet soil to a 4 to 6 cm depth. DI irrigation was performed at leaf wilt to wet soil to a depth of ≥24 cm.

^yMeans in a column within each parameter followed by the same letter are not significantly different based on Fisher's protected least significant difference test at $P \leq 0.05$.

Table 4. Water-soluble carbohydrate (WSC), storage carbohydrate (SC), and total nonstructural carbohydrate (TNC) in 'Providence' creeping bentgrass leaf tissue in response to light and frequent (LF) versus deep and infrequent (DI) irrigation in 2007.

Treatments ^z	2007				
	1 June	28 June	17 July	15 Aug.	6 Sept.
	WSC [glucose (mg·g ⁻¹ dry wt)]				
LF	43.6 a ^y	43.1 a	36.4 b	33.4 b	44.0 a
DI	43.2 a	40.7 a	38.8 a	38.7 a	43.5 a
	SC [glucose (mg·g ⁻¹ dry wt)]				
LF	83.4 b	88.4 a	101.0 a	88.6 a	79.7 b
DI	90.7 a	87.9 a	100.4 a	87.8 a	83.6 a
	TNC [glucose (mg·g ⁻¹ dry wt)]				
LF	127.1 b	131.5 a	137.3 a	122.1 a	123.8 a
DI	133.8 a	128.2 a	139.1 a	126.4 a	127.1 a

^zLF irrigation was performed daily in the absence of rain to wet soil to a 4 to 6 cm depth. DI irrigation was performed at leaf wilt to wet soil to a depth of ≥24 cm.

^yMeans in a column within each parameter followed by the same letter are not significantly different based on Fisher's protected least significant difference test at $P \leq 0.05$.

Table 5. Water-soluble carbohydrate (WSC), storage carbohydrate (SC), and total nonstructural carbohydrate (TNC) in 'Providence' creeping bentgrass roots in response to light and frequent (LF) versus deep and infrequent (DI) irrigation in 2006.

Treatments ^z	2006			
	15 June	13 July	8 Aug.	7 Sept.
	WSC [glucose (mg·g ⁻¹ dry wt)]			
LF	36.0 b ^y	22.4 b	44.4 b	41.4 b
DI	54.2 a	57.6 a	50.6 a	47.6 a
	SC [glucose (mg·g ⁻¹ dry wt)]			
LF	15.8 b	10.0 a	9.2 a	10.8 b
DI	21.8 a	11.6 a	10.6 a	15.0 a
	TNC [glucose (mg·g ⁻¹ dry wt)]			
LF	51.6 b	32.6 b	53.6 b	52.2 b
DI	75.8 a	69.0 a	61.2 a	62.8 a

^zLF irrigation was performed daily in the absence of rain to wet soil to a 4 to 6 cm depth. DI irrigation was performed at leaf wilt to wet soil to a depth of ≥24 cm.

^yMeans in a column within each parameter followed by the same letter are not significantly different based on Fisher's protected least significant difference test at $P \leq 0.05$.

Table 6. Water-soluble carbohydrate (WSC), storage carbohydrate (SC), and total nonstructural carbohydrate (TNC) in 'Providence' creeping bentgrass roots in response to light and frequent (LF) versus deep and infrequent (DI) irrigation in 2007.

Treatments ^z	2007				
	1 June	28 June	17 July	15 Aug.	6 Sept.
	WSC [glucose (mg·g ⁻¹ dry wt)]				
LF	23.6 b ^y	24.0 a	17.5 a	17.7 a	20.3 a
DI	26.8 a	22.0 a	17.1 a	17.4 a	20.8 a
	SC [glucose (mg·g ⁻¹ dry wt)]				
LF	34.0 b	28.0 a	23.1 b	29.8 b	21.8 b
DI	44.5 a	28.2 a	28.5 a	33.3 a	25.1 a
	TNC [glucose (mg·g ⁻¹ dry wt)]				
LF	57.6 b	53.0 a	40.7 b	47.6 b	42.1 b
DI	71.6 a	49.2 a	45.6 a	50.7 a	46.0 a

^zLF irrigation was performed daily in the absence of rain to wet soil to a 4 to 6 cm depth. DI irrigation was performed at leaf wilt to wet soil to a depth of ≥24 cm.

^yMeans in a column within each parameter followed by the same letter are not significantly different based on Fisher's protected least significant difference test at $P \leq 0.05$.

Discussion

During the study period in both years, gravimetric soil moisture at the 0 to 10 cm depth averaged 9.3% and 15.7% in DI and LF plots, respectively (data not shown). Data showed that DI irrigation reduced P_n in both years, but generally had no effect on R_w . It is likely that LF irrigation encouraged more shoot growth, which would have resulted in a greater leaf surface area. A greater leaf area at the time of measurement may account for the higher P_n rate in LF versus DI bentgrass. Hence, photosynthesis was more sensitive to soil drying than respiration. It should be noted that P_n and R_w usually were measured 3 to 5 d following DI irrigation. Therefore, the creeping bentgrass was not under visible wilt stress at the time measurements were obtained. Huang and Fu (2000) previously reported that photosynthesis decreased in kentucky bluegrass (*Poa pratensis* L.) and tall fescue plants subjected to complete drying of the soil profile in a greenhouse study. When only the top 20 cm of the soil profile was allowed to dry, there was no reduction in photosynthesis when compared with well-watered plants (Huang and Fu, 2000). In a field study, however, it was shown that the P_n rate in creeping bentgrass was similar, regardless of being irrigated three times per week at 40%, 60%, or 100% of actual ET (DaCosta and Huang, 2006b). In the current study, only LF plots were irrigated to 100% ET on rain-free days. While R_w remained similar between irrigation regimes in our study, Huang and Fu (2000) reported that respiration decreased in kentucky bluegrass and tall fescue beginning 9 days after imposing drought stress in a greenhouse study.

Maintaining healthy turf under limited water conditions may depend on the availability of carbohydrates. Leaf tissue from DI bentgrass contained higher levels of WSC and TNC than LF bentgrass on the final measurement date in Sept. 2006, whereas SC levels in leaf tissue were higher on the final date in both years. On the final measurement dates in Sept. 2006 and 2007, SC and TNC levels in roots were higher in DI than in LF bentgrass. On average, root SC levels were 39% (2006) and 15% (2007) higher on the final measurement date in each year in DI versus LF bentgrass. On the final measurement date in each year, root TNC levels were 9% to 20% higher in DI bentgrass. DaCosta and Huang (2006a) subjected creeping bentgrass to drought stress for 6 to 18 d in a greenhouse and observed that the proportion of newly produced TNC carbon was highest in roots, intermediate in stems, and lowest in leaves within 12 d of inducing drought. In our study, higher TNC levels generally were found in leaves versus roots. We, however, did not measure newly produced carbon as reported by DaCosta and Huang (2006a). The present study also showed that creeping bentgrass subjected to DI irrigation had root TNC levels averaging 23% higher in both years compared with LF bentgrass. Therefore, although TNC levels were greater in leaves, roots accumulated more TNC when grown under DI irrigation. Hence, the results from our field studies are in agreement with greenhouse studies that have shown the TNC levels in leaves and roots increase in response to soil drying (DaCosta and Huang, 2006a; Huang and Fu, 2000; 2001). Soil drying also results in a significant reduction in leaf growth rate (Fry and Huang, 2004; Huang and Fu, 2001). While not quantified, it is very likely that creeping bentgrass leaf growth was restricted more in DI than LF plots. Because P_n was less in DI bentgrass, the increases in carbohydrate levels of DI

bentgrass leaves and roots likely were a result of a reduction in plant growth. That is, because growth was restricted by dry soil conditions, plants used less carbohydrate.

The maintenance of a favorable water status is essential for plants adapted to conditions with limited water availability. Osmotic adjustment is an important physiological mechanism of water retention and cell turgor maintenance. The accumulation of solutes such as WSC is associated with active osmotic adjustment when plants are subjected to soil water deficits (Morgan, 1984). DaCosta and Huang (2006a) reported that creeping bentgrass plants osmotically adjust to dehydration stress by accumulating WSC. Similarly, Jiang and Huang (2001) observed that drought-preconditioned kentucky bluegrass had 21% to 44% higher leaf WSC than nonpreconditioned plants. In this study, higher levels of WSC were observed in leaves in 2006 (8 Aug. and 7 Sept.) and 2007 (17 July and 15 Aug.). Higher WSC levels also were observed in roots of DI versus LF bentgrass on all dates in 2006 and on 1 June 2007. These WSC data suggest that creeping bentgrass plants subjected to DI irrigation were adapting to drought stress.

In summary, this field study showed that creeping bentgrass exhibited reduced P_n , but generally unchanged R_w rate in response to DI irrigation. DI irrigation resulted in higher levels of WSC, SC, and TNC in creeping bentgrass leaves in 2006 and higher SC and TNC levels in roots in both years. Carbohydrates accumulated more in DI bentgrass because plant growth was restricted by frequent periods of wilt stress. Higher WSC levels in tissues of DI creeping bentgrass might contribute to improved drought tolerance by providing for a more negative osmotic pressure in tissues in response to prolonged periods of water stress. Accumulated TNC also would be available to assist plants in their recovery from wilt and other stresses.

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