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Seasonal Changes in Carbohydrate and Protein Content of Seeded Bermudagrasses and Their Effect on Spring Green-Up

M. Schiavon[1], S. Macolino[1], B. Leinauer[2] & U. Ziliotto[1]

1 Department of Agronomy, Food, Natural Resources, Animals, and Environment, University of Padova, Legnaro (PD), Italy

2 Department of Extension Plant Sciences, New Mexico State University, LasCruces, NM, USA

Keywords

crude protein; days to reach 80 % green cover; transition zone; warm-season grasses; water-soluble carbohydrates

Correspondence

S. Macolino

Department of Agronomy, Food, Natural Resources, Animals, and Environment

University of Padova

35020 Legnaro (PD) Italy

Tel.: +390498272834

Fax: +390498272839

Email: stefano.macolino@unipd.it

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Abstract

The widespread use of warm-season turfgrasses in transition zones of Europe, such as northern Italy, has been hampered by the long dormancy periods. To encourage the conversion from cool- to warm-season grasses, research is needed to identify cultivars that exhibit early spring green-up. A 2-year study was conducted at the agricultural experimental farm of Padova University from November 2006 to October 2008 to compare water-soluble carbohydrate and protein content in stolons of four bermudagrass [*Cynodon dactylon* (L.) Pers.] cultivars and determine their effect on spring green-up. Samples of 'La Paloma', 'NuMex Sahara', 'Princess 77' and 'Yukon' were collected monthly, and water-soluble carbohydrates (WSC) and crude protein (CP) content of stolons were measured. Dry weight values of WSC and CP for each cultivar were regressed against days needed to reach 80 % green cover in spring (D80). 'Yukon' exhibited the highest rhizome dry weight and WSC content during the winter months and was the fastest to reach 80 % green cover. Conversely, 'Princess 77' was the slowest cultivar to green-up in both years. Regression analysis revealed a stronger relationship between D80 and WSC than between CP content and D80.

Introduction

The use of potable water to irrigate lawns and other landscape areas is being questioned as turfgrasses are considered a non-essential crop or ground cover. Additionally, a shortage of water in many urban areas resulted in polices that mandate a shift towards the use of drought-resistant, water-use-efficient plants. Consequently, warm-season turfgrass species are increasingly used for lawns and other recreational landscapes in transition zones such as the Mediterranean Europe area because they require less or no irrigation compared to cool-season grasses. Moreover, the climate of the Po River Valley in northern Italy is considered humid and subtropical during the summer months and does not favour the growth of cool-season turf species (Köppen 1936). Warm-season grasses generally use water more efficiently than cool-season grasses, and their growing period in transition zones is shorter, which results in less annual irrigation needed to sustain turf areas. Nonetheless, the loss of colour during the winter months has been a major impediment for more widespread use of these species in these zones, particularly in the European Mediterranean area (Macolino et al. 2010, Rimi et al. 2012). Moreover, quality of warm-season grasses can be affected by a lack of cold tolerance; they can suffer from freeze damage, thereby resulting in a delay in spring green-up when grown in transitional zones. Proper maintenance practices and the choice of cultivars adapted to the specific environment can ease these problems (Anderson et al. 2002, Patton et al. 2008). The use of cultivars that can tolerate low temperatures coupled with the ability to green-up early in the spring would be an important factor to shorten warm-season turfgrasses' dormancy period which, in return, would lead to a greater acceptance of warm-season species.

Among the warm-season species, bermudagrass [*Cynodon dactylon* (L.) Pers.] is one of the most widely used for landscape areas in Europe (Croce et al. 2004). This species enters dormancy when soil temperatures drop below 10 °C and begins spring recovery or green-up when temperatures rise above the same threshold (Youngner 1959). Turf-type bermudagrass cultivars, such as 'NuMex Sahara', 'Princess 77' and 'Yukon' recently, have been most used in Italy on new athletic fields, parks and lawns (Macolino et al. 2010). These cultivars can be overseeded in the fall with perennial ryegrass (*Lolium perenne* L.) or annual ryegrass (*Lolium multiflorum* Lam.) when utilized for athletic fields (Gaetani et al. 2004, 2007) to provide green colour over the winter months. Conversely, when turfgrasses are used primarily during the summer months, overseeding can be omitted. Cold tolerance and winter hardiness, coupled with the ability to green-up quickly in the spring, are important criteria for selecting cultivars in transitional zones with rigid winters.

Total non-structural carbohydrates (TNC) can be used to measure the physiological status of the plants (Sheffer et al. 1979). Non-structural carbohydrate content tends to be lower when favourable conditions for rapid growth and metabolic activity occur. Conversely, more TNC are accumulated when environmental conditions permit high photosynthetic rates, but plant growth is reduced (Hull 1992). White (1973) reported that non-structural carbohydrates represent the major reserves for plants and these include reducing (glucose and fructose), non-reducing sugars (sucrose), as well as fructosans and starch, and can also be divided into water-soluble (WSC) and water non-soluble carbohydrates, such as saccharides (e.g. starch).

In warm-season species, carbohydrates are accumulated and stored inside the plant during the fall when growth and metabolism are depressed. In the spring, during green-up, carbohydrates are metabolized and used as energy for the growth of new shoots (Rogers et al. 1975, Di Paola and Beard 1992). Levels of WSC and TNC are generally at their lowest during late spring/early summer months as they are metabolized by the plants through respiration (Thom et al. 1989).

Plant stress is an additional factor that influences TNC. Hoffman et al. (2010) showed that perennial ryegrass accessions with a higher freezing tolerance accumulate more WSC during the cold acclimation period than accessions with a lower freezing tolerance. Water-soluble carbohydrates (WSC) not only protect the plant by lowering intracellular freezing point, but also prevent protein denaturation and maintain membrane integrity when cells dehydrate due to cold (Santarius 1982, Anchordoguy et al. 1987). Zhang et al. (2006) showed that the TNC content of cold-tolerant cultivars of bermudagrass such as 'Riviera' is almost double that of cold-susceptible cultivars during cold acclimation. However, the relationship between TNC and freezing tolerance remains unclear. The inconsistent relationship between carbohydrate content and freezing tolerance has been reported in St. Augustinegrass [*Stenotaphrum secundatum* (Walter) Kuntze] (Fry et al. 1991, Maier et al. 1994), forage-type cultivars of perennial ryegrass (Pollock et al. 1988, Thomas and James 1993) and bermudagrass (Dunn and Nelson 1974).

Although carbohydrates in well-established bermudagrass are usually stored in both rhizomes and stolons, it has been suggested that carbohydrate content in stolons is most important for winter survival (Dunn and Nelson 1974). The majority of the studies that investigated the relationship between carbohydrate content and spring recovery concluded that a positive correlation exists between the two, regardless of the location of the carbohydrates (Rogers et al. 1975, Dunn et al. 1980, Macolino et al. 2010). Therefore, Macolino et al. (2010) recommended the development of cultivars with increased WSC content to accelerate spring green-up. Conversely, Goatley et al. (1994) did not find any correlation between TNC content in rhizomes and speed of green-up in mature Tifgreen hybrid bermudagrass.

Aside from carbohydrates, proteins may also play an important role in freezing tolerance of bermudagrass. Increased protein content has been documented in 'Midiron' bermudagrass crowns, which has been correlated with greater freezing tolerance when compared to 'Tifway' bermudagrass (Gatschet et al. 1996). Higher protein content in cold-tolerant 'Riviera' leaves during cold acclimation has been reported by Zhang et al. (2006). Zhang et al. (2010) documented higher protein levels in three cold-tolerant bermudagrass cultivars ('Patriot', 'Riviera', and 'Tifway') compared to cold-sensitive 'Princess 77' and 'GA-851'. The authors concluded that higher levels of N metabolites resulted in faster green-up and recovery from cold periods. Giolo et al. (2013) conducted a study to correlate WSC and crude protein (CP) contents in March with green-up speed in six different cultivars of bermudagrass. The authors reported no correlation between WSC content and days to reach 80 % green cover during spring, and a correlation between CP and spring green-up was found only in the first of a 2-year investigation. Therefore, the authors concluded that CP content in March might only be an aleatory predictor for spring green cover. However, the study investigated only the correlation between the WSC and CP contents in March and bermudagrass green-up; a better correlation might be found if days to reach 80 % green cover are correlated with sugars and protein content in late winter or early spring.

More research is needed to determine whether both CP and WSC content during the winter months are good predictors of spring green-up. Furthermore, only few studies have been conducted in the transitional Mediterranean area to investigate the suitability of warm-season turfgrasses in this climatic zone. The objectives of our study were to identify green-up patterns of bermudagrass cultivars that are suitable for the northern Italian environment. Four cultivars were selected ('La Paloma', 'NuMex Sahara', 'Princess

77' and 'Yukon'), and their WSC and CP contents were quantified monthly over a 2-year period. Finally, we investigated whether or not there is a correlation between these compounds and spring green-up.

Materials and Methods

A field study was conducted at the Agricultural Experimental Farm of Padova University in Legnaro, north-east Italy (45°20′ N, 11°57′ E, and elevation 8 m) from November 2006 to October 2008, to study the temporal changes of carbohydrate and protein content in stolons, dry weight changes of stolons and rhizomes, and the influence of these parameters on spring green-up of four-seeded bermudagrass cultivars. Monthly average air and soil temperatures and precipitation during the research period are listed in Table 1. The climate at the location is similar to the climate described by the U.S. Department of Agriculture as plant hardiness zone 8. Bermudagrass cultivars selected were 'La Paloma', 'NuMex Sahara', 'Princess 77' and 'Yukon'. Except for 'NuMex Sahara', the cultivars used in the study represent grasses that are predominately used on both athletic fields and recreational areas. Plots were seeded on 4 July 2005 at a rate of 4 g m^{-2}. The soil at the site consisted of a coarse-silty, mixed, mesic, Oxyaquic Eutrudept (Morari 2006), containing 65 % silt, 16 % clay and 18 % sand. Soil chemical analysis, prior to the study, revealed a pH of 8.1 (1 : 5 soil–water solution), a total nitrogen content of 0.13 g 100 g^{-1} soil (Dumas combustion method), a phosphorous content of 38.3 mg kg^{-1} soil (Olsen method) and exchangeable potassium content of 178.1 mg kg^{-1} soil (buffered $BaCl_2$ method).

A slow release fertilizer (20N-2.2P-6.6K) was applied at a rate of 40 kg ha^{-1} $year^{-1}$ every other month from April to September to provide the grasses with macronutrients. During the growing period, the grasses were mowed weekly with a rotary mower at a height of 27 mm, and clippings were removed. The experimental design was a completely randomized block, and the plots were replicated three times. Individual plot size was 1.6 m by 4.2 m.

From November 2006 to October 2008, turfgrass samples measuring 20 cm in length, 20 cm in width and 4 cm in depth were collected monthly randomly from each plot. The cores were washed to remove all soil particles, and the stolons and rhizomes were separated from leaves and roots with scissors and subsequently frozen at −22 °C. Stolons were separated from rhizomes based on morphology and colour. Both storage organs were then freeze-dried and grounded, and their dry weight was recorded using an analytical balance.

Water-soluble carbohydrates were extracted from plant tissue using an 80 % ethanol solution followed by a hot water extraction (Suzuki 1968). The WSC content was determined by a colourimeter (Carlo Erba Scientific Instruments Division, Milan, Italy) after reaction with anthrone reagent (9,10-dihydro-9-oxoanthracene) using glucose as a standard (Van Handel 1968). The total N was extracted from stolon tissue using the Kjeldahl method (AOAC 1995), and CP was determined as $N \times 6.25$ (Jones 1991).

Spring green-up was assessed weekly visually on a scale from 0 % to 100 % green cover starting on 5 March 2007 [DOY (Day of Year) 64] and 8 March 2008 (DOY 68), until the end of May, at which all the plots reached complete green cover. A scatter plot of per cent green coverage vs. DOY suggested a non-linear relationship between the variables, and a sigmoidal model was identified to best describe spring green-up (GraphPad Prism 5.0 for Windows; GraphPad Software, La Jolla, CA, USA). Sigmoidal models were subsequently used to calculate DOY to reach 80 %

Table 1 Monthly average air temperatures (air temp; °C) and precipitation (mm) during the research period and long-term averages (1963–2007) for the agricultural experimental farm of Padova University, Legnaro, Italy

	January	February	March	April	May	June	July	August	September	October	November	December
	°C											
Air temperature												
2006	2.1	4.2	7.5	13.0	17.2	21.7	25.2	19.9	20.2	15.9	9.0	5.6
2007	5.6	6.9	10.4	16.1	19.1	22.1	23.7	22.1	17.4	13.3	7.5	3.2
2008	4.9	4.9	8.2	12.2	18.0	21.1	23.3	23.4	18.0	14.6	8.9	4.7
45 years avg.	2	4.1	7.8	11.6	16.6	20.2	22.3	21.9	18.2	13.1	7.2	2.9
	mm											
Precipitation												
2006	31	33	44	42	92	15	48	122	178	22	29	49
2007	17	60	79	1	146	61	31	48	105	36	23	31.6
2008	32	32	52	108	93	80	58	78	60	46	51	133
45 years avg.	53	49	56	69	74	82	74	76	72	80	76	60

green colour (D80) for each replicate separately. Stolons and rhizome dry weight, WSC content, protein content and D80 were subjected to ANOVA using SAS PROC MIXED (version 9.3; SAS institute; Cary, NC, USA), followed by means of differentiation using Fisher's protected least significant difference test at the 0.05 probability level. The stolon content of WSC and CPs from November to March was regressed against D80 to determine whether a fast green-up can be associated with higher WSC or protein content in plants. Fall colour retention was assessed in November, but no differences were found among cultivars (data not presented).

Results

Spring green-up

Figures 1 and 2 represent average spring green-up of 'La Paloma', 'NuMex Sahara', 'Princess 77' and 'Yukon' in 2007 and 2008. The sigmoidal regression estimated spring green-up well, with coefficients of determination ranging from 0.98 to 0.99 (Figs 1 & 2). Days from January 1st needed to reach 80 % green cover (D80) were interpolated separately for each replicate plot. The analysis of variance of D80 values revealed a significant cultivar × year interaction (Table 2). In 2007, with the exception of 'Princess 77', all cultivars greened up in 105 days. Although statistically different from the other cultivars, 'Princess 77' needed only four more days (109) to reach 80 % cover (Table 3). 'Princess 77' was the slowest to green-up in 2008, with 135 days needed to reach 80 % ground cover (Table 3). 'La Paloma' and

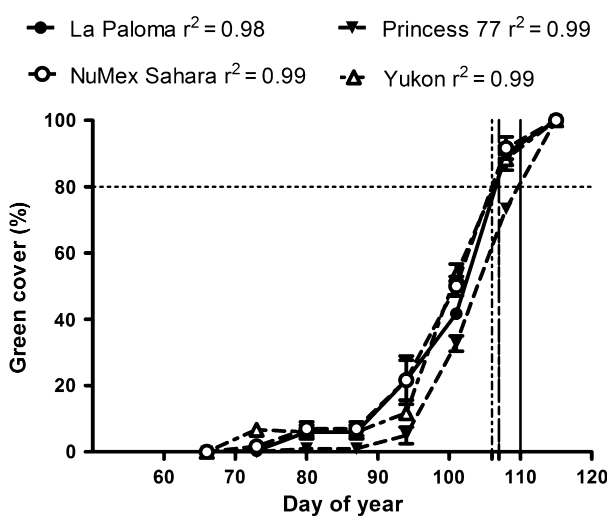

Fig. 1 Mean percentage of green cover during 2007 at days of the year of four bermudagrass cultivars ('La Paloma', 'NuMex Sahara', 'Princess 77' and 'Yukon'). Data points represent an average of three replications, and error bars indicate standard errors.

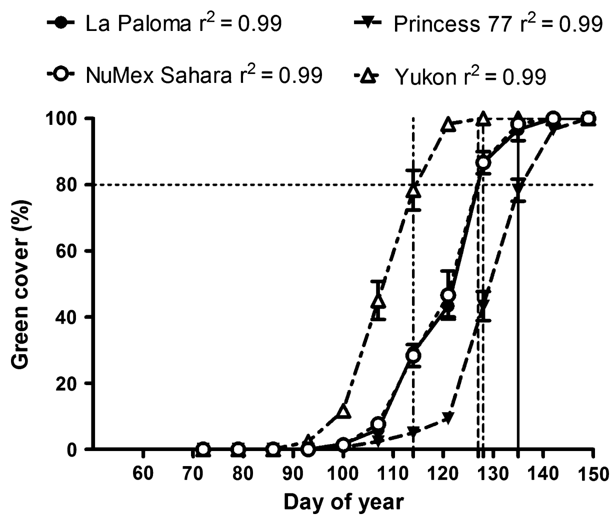

Fig. 2 Mean percentage of green cover during 2008 at days of the year of four bermudagrass cultivars ('La Paloma', 'NuMex Sahara', 'Princess 77' and 'Yukon'). Data points represent an average of three replications, and error bars indicate standard errors.

Table 2 Results of analysis of variance testing the effects of cultivar, month, year and their interactions on days to reach 80 % green cover (D80), rhizome dry weight, and stolon dry weight, water-soluble carbohydrate content and crude protein (CP) content in stolons

		Rhizome		Stolons	
Effect	D80	Dry weight	Mass	Water-soluble carbohydrates	CP
Cultivar (Cv)	***	**	**	***	**
Month (M)		***	***	***	***
Cv*M		*	ns	***	ns
Year (Y)	***	***	ns	***	***
Cv*Y	***	ns†	**	*	ns
Y*M		***	ns	***	***
Cv*Y*M		***	ns	**	*

*P < 0.05; **P < 0.01; ***P < 0.01; †ns, not significant at the 0.05 probability level.

Table 3 Days to reach 80 % ground cover in 2007 and 2008. Values represent an average of three replicates

Cultivar	2007	2008
La Paloma	107e	128b
NuMex Sahara	106e	127b
Princess 77	109d	135a
Yukon	105e	113c

Values followed by the same letter are not significantly different from one another.

'NuMex Sahara' required the same number of days (128 and 127, respectively), whereas 'Yukon' was the fastest to green-up, requiring only 113 days.

Rhizome dry weight

The analysis of variance of rhizome dry weight revealed a significant three-way interaction between cultivar, month and year (Table 2). During both years of investigation, rhizome dry weight exhibited a cyclic pattern, with a high dry weight during late summer and early fall (between July and September) followed by a rapid decrease in late fall and early winter (October to December). The highest dry weight during the 2-year study period was recorded during fall of 2007. 'Yukon' had the highest dry weight in 10 of 24 sampling months (Fig. 3). In August of 2008, rhizome dry weight of 'Yukon' (37 g m^{-2}) was lower than that of 'La Paloma' (132 g m^{-2}). With the exception of May 2007 and August 2008, 'Princess 77' rhizome dry weight was equal to that of 'NuMex Sahara' and 'La Paloma' throughout the research period (Fig. 3).

Stolon dry weight

The analysis of variance of stolon dry weight revealed a significant interaction between cultivars and year, and a significant month effect (Table 2). Therefore, data were first averaged over month and presented separately for each year (Table 4). Subsequently, data were averaged over cultivar and year and presented separately for each month (Fig. 4).

Stolon dry weight was higher during the first year of the experiment for every cultivar except for 'Yukon' (Table 4). Stolon dry weight of 'Yukon' reached 798 g m^{-2} during 2007 and 750 g m^{-2} during 2008, which were the highest values among all the cultivars tested. Except for 'Yukon', dry weights of all cultivars declined significantly from 2007 to 2008, with the largest decrease observed in 'Princess' (105 g m^{-2}) (Table 4). Only 'Yukon' stolon dry weight differed significantly from other cultivars in 2008 (Table 4). When data were averaged over cultivar and year, the highest dry weight was recorded in December (901 g m^{-2}) (Fig. 4). However, values were also high throughout the fall. Stolon dry weight dropped rapidly shortly before green-up and remained low until late spring, with lowest values recorded in May. Beginning in late May, stolon dry weight gradually increased until winter (Fig. 4).

Water-soluble carbohydrates

The analysis of variance revealed a significant three-way interaction between cultivar, month and year for WSC in stolons (Table 2). Water-soluble carbohydrates content was highest in 'Yukon' from November 2006 to March 2007, in September of both years and from November 2007 to March 2008 (Fig. 5). Differences in WSC between 'Yukon' and the other cultivars were greatest in January 2007, when WSC content was 20 g kg^{-1} greater than in 'NuMex Sahara', 36 g kg^{-1} greater than 'La Paloma' and 63 g kg^{-1} more than 'Princess 77'. Conversely, 'Princess 77' had the lowest WSC content during fall 2006 and 2008 and winter 2007. No differences in WSC content among cultivars were observed in April of either year, in July 2007

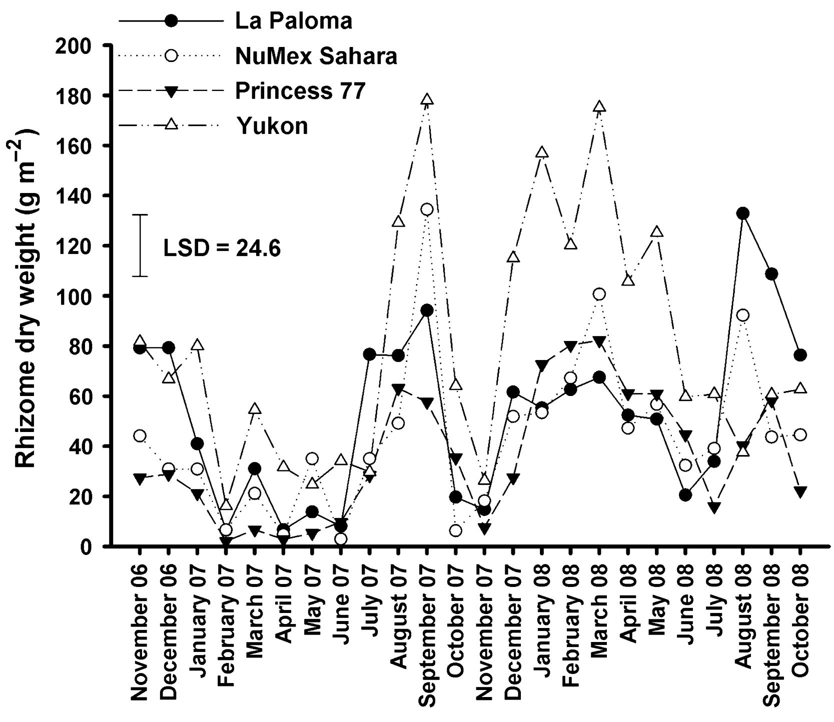

Fig. 3 Rhizome dry weight (g m^{-2}) of four bermudagrass cultivars ('La Paloma', 'NuMex Sahara', 'Princess 77' and 'Yukon') during 2 years of study (from November 2006 to October 2008). Data points represent an average of three replications.

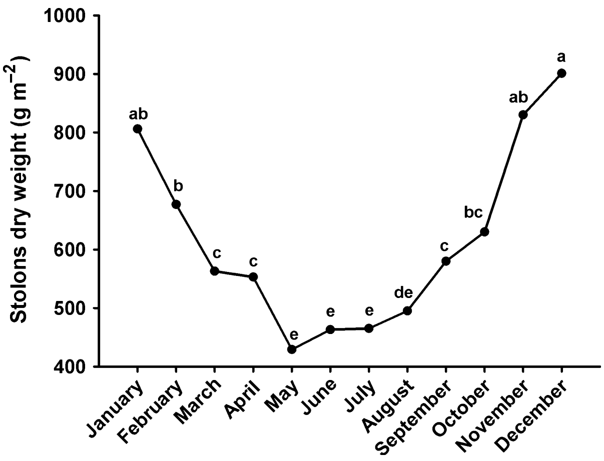

Fig. 4 Monthly variation in stolon dry weight (g m^{-2}). Data points represent an average of four cultivars ('La Paloma', 'NuMex Sahara', 'Princess 77' and 'Yukon'), 2 years, and three replications. Different letters indicate significant differences ($P < 0.05$) among sampling dates.

Table 4 Stolon dry weight (g m^{-2}) of bermudagrass 'La Paloma', 'NuMex Sahara', 'Princess 77' and 'Yukon' for year 1 (November 2006–October 2007) and year 2 (November 2007–October 2008) of the study. Data are averaged over 12 months and three replicates

Cultivar	Year 1	Year 2
La Paloma	753bc	665de
NuMex Sahara	690cd	649e
Princess 77	814a	709cde
Yukon	798ab	750bc

Values followed by the same letter are not significantly different from one another.

or during the 2008 summer. Water-soluble carbohydrates content was generally highest in all cultivars during the winter. The peak of WSC content was reached in January 2007 for year 1 and in February 2008 for year 2. This was followed by a rapid decrease in WSC in association with spring green-up. The drop in WSC observed between January 2007 and March 2007 ranged from 62 g kg^{-1} for 'Yukon' to 16 g kg^{-1} for 'Princess 77' (Fig. 5). The drop in WSC in 2007 was more drastic than in 2008, because the peak reached in 2008 was lower than that of 2007. Carbohydrate content did not change between May and August for either year, but declined in October of both 2007 and 2008.

Crude protein content

Analysis of variance revealed that CP content in stolons was influenced by a significant three-way interaction among cultivar, month and year. From November 2006 until July 2007, 'Princess 77' had the highest CP content of all the tested cultivars (Fig. 6). Both 'Princess 77' and 'Yukon' had higher CP contents than the other two cultivars in August and September of 2007. From October 2007 until May 2008, 'Yukon' had the highest' CP content, whereas from June 2008 until the end of the study, no differences were detected among cultivars. Values were highest in February 2007 for 'Princess 77' and 'Yukon' (92 and 84 g kg^{-1}, respectively), and in January 2007 for 'La Paloma' and 'NuMex Sahara' (82 and 80 g kg^{-1}, respectively). From February to October 2007, the CP content decreased by 31 % in 'Yukon', 38 % in 'NuMex Sahara', 41 % in 'La Paloma' and 53 % in 'Princes 77'. From November 2007 onwards, CP content increased in all the cultivars, followed by another decrease during spring of 2008.

Predicting spring green-up from WSC and CP content

The regression analysis revealed a negative relationship between winter WSC content and D80, except during March 2008 (Table 5). Regression coefficients ranged from 0.23 (December 2007) to 0.7 (January 2008). Conversely, there was a significant positive relationship between CP content and D80 on 7 of 10 sampling months (Table 5), with regression coefficients ranging from 0.15 to 0.54.

Discussion

The replacement of cool-season turfgrass species with warm-season counterparts has been actively promoted in transition zones (Patton et al. 2008). The use of turf species that can survive drought conditions or sustain long periods without precipitation while still providing acceptable quality has been proposed as a strategy to reduce the amount of potable water used to irrigate turf and landscape areas (Leinauer et al. 2012, Schiavon et al. 2012). Therefore, it is important to identify warm-season species and cultivars well-adapted to cooler, transition zone climates. To date, there have been few studies which have investigated the potential of using bermudagrass in the northern region of Italy. The wide scale use of this species in the Venetian plain has been hindered by its 5-month-long dormancy period (Rimi et al. 2012).

Results of our study support those of previous studies (Macolino et al. 2010, Rimi et al. 2011, 2013a) which reported an early spring green-up of 'Yukon' compared to other cultivars. Climatic conditions were drastically different during the two winters of our study. Average monthly winter temperatures were higher in 2007 than in 2008 and higher than the 45-year average (Table 1). These higher temperatures during the first winter led to a more uniform spring green-up among cultivars, with 'La Paloma', 'NuMex Sahara' and 'Yukon'all reaching 80 % green coverage in 105 days, which was only 4 days faster than 'Princess

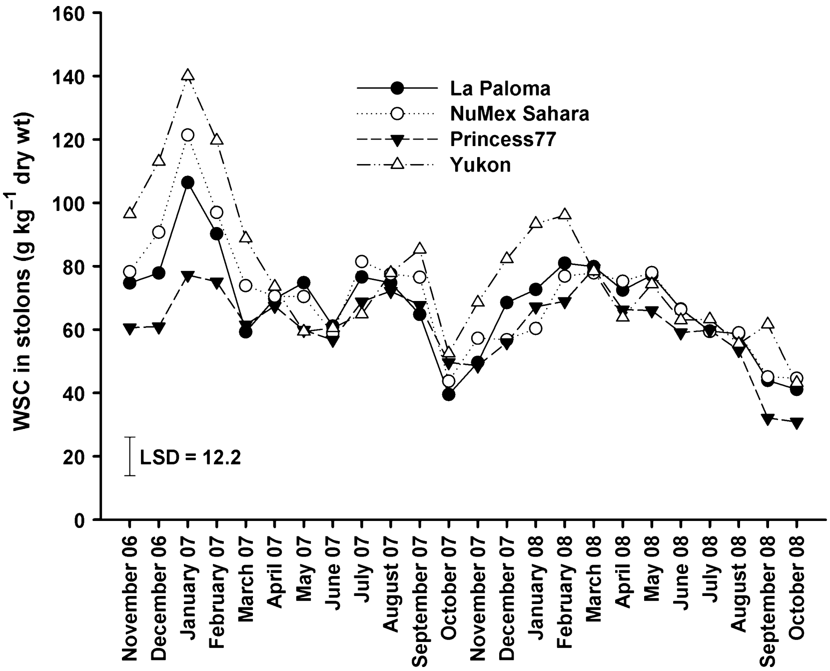

Fig. 5 Water-soluble carbohydrates content (g kg^{-1} dry weight) of four bermudagrass cultivars ('La Paloma', 'NuMex Sahara', 'Princess 77' and 'Yukon') during 2 years of study (from November 2006 to October 2008). Data points represent an average of three replications.

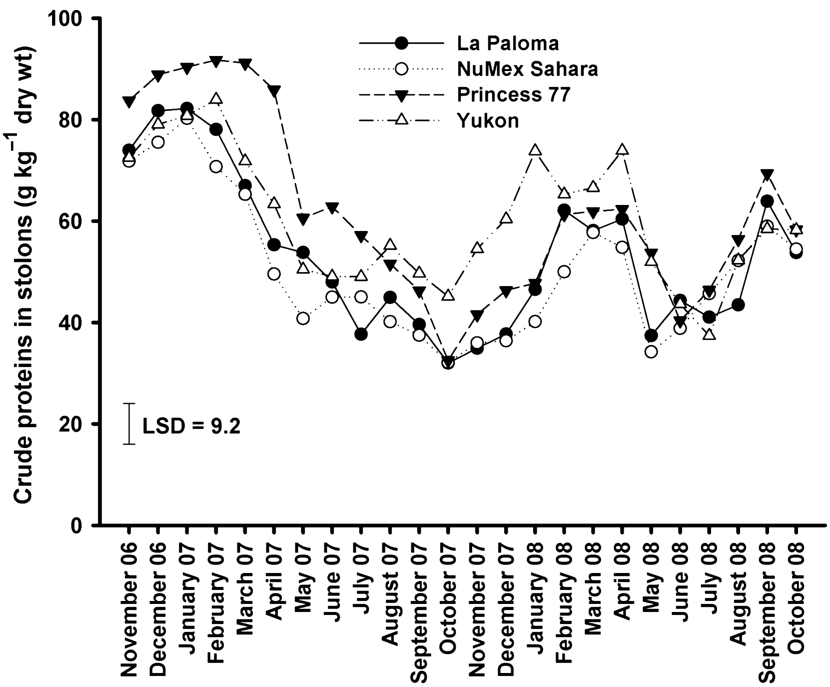

Fig. 6 Crude protein content (g kg^{-1} dry weight) of four bermudagrass cultivars ('La Paloma', 'NuMex Sahara', 'Princess 77' and 'Yukon') during 2 years of study (from November 2006 to October 2008). Data points represent an average of three replications.

77'. In contrast, 'Yukon' reached 80 % green cover 8 days later in 2008 than in 2007, but 2 weeks earlier than 'La Paloma' and 'NuMex Sahara', and 22 days sooner than 'Princess 77'. These results differed from those of Rimi et al. (2013a) who reported consistency in D80 between years. However, average monthly temperatures during summer and spring did not differ greatly between years of their study period (Rimi et al. 2013a). The results we obtained in the second year of our study were in agreement with those reported by the National Turfgrass Evaluation Program (NTEP 2011), which listed 'Yukon' as the fastest cultivar on average to green-up throughout the United States, and 'NuMex Sahara' as faster to green-up than 'Princess 77'.

Table 5 Linear regression parameters ($y = a + bx$) and regression coefficients for predicting days to reach 80 % green cover from water-soluble carbohydrates (WSC) or crude protein (CP) content of four bermudagrass cultivars 'La Paloma' 'La Paloma', 'NuMex Sahara', 'Princess 77' and 'Yukon'. Regressions are based on four cultivars and three replicates

Month	Parameter	WSC	CP
November 2006	*a*	114.646	94.694
	b	−1.016	1.600
	r^2	0.55***	0.54***
December 2006	*a*	111.350	96.299
	b	−0.534	1.261
	r^2	0.36***	0.59***
January 2007	*a*	111.492	90.303
	b	−0.424	1.975
	r^2	0.32***	0.48***
February 2007	*a*	114.465	102.174
	b	−0.805	0.567
	r^2	0.32***	0.15***
March 2007	*a*	113.705	–
	b	−0.978	–
	r^2	0.41***	ns†
November 2007	*a*	139.415	146.483
	b	−2.280	4.942
	r^2	0.50***	0.31***
December 2007	*a*	155.194	144.854
	b	−4.635	4.201
	r^2	0.23***	0.28***
January 2008	*a*	150.730	144.856
	b	−5.445	−3.590
	r^2	0.70***	0.37***
February 2008	*a*	157.674	–
	b	−4.387	–
	r^2	0.62*	ns
March 2008	*a*	75.117	–
	b	6.646	–
	r^2	0.33**	ns

*P < 0.05; **P < 0.01; ***P < 0.01; †ns, not significant at the 0.05 probability level.

'Yukon' exhibited the highest rhizome dry weight from August 2007 until July 2008 and the highest stolon dry weight during the second year of the study. These results suggest an excellent adaptability of 'Yukon' to the northern Italian climate. In contrast, 'Princess 77' was the slowest cultivar to green-up in 2008, indicating a poorer adaptability to these climate conditions. The poor adaptability of 'Princess 77' to northern Italian climate was also noted by Rimi et al. (2013b), as further evidenced by its low stolon dry weight compared to 'Riviera'.

Temperature plays a key role in the accumulation and mobilization of WSC and protein. During the first winter of our study, WSC content was highest in January for all cultivars. Stolon WSC content rapidly decreased in the spring as plants began to green-up. In contrast, WSC content was highest between February (for 'Yukon', 'La Paloma and 'NuMex Sahara') and March ('Princess 77') during the second year. February was also the month when Rimi et al. (2013b) recorded the highest WSC content in bermudagrass and seashore paspalum. Increased accumulation of carbohydrates as a result of cold acclimation has also been reported by Zhang et al. (2006) for 'Princess 77' and 'Riviera'. These WSC are used as source of energy during the spring to support green-up (Rogers et al. 1975). Thus, the delayed green-up observed during the second year of the study may be due to a corresponding delay in WSC mobilization from stolons to new photosynthesizing organs. In 2008, the stolon WSC of 'Princess 77' increased until March 2008, but 80 % green cover was not reached before 15 May. A positive relationship between cold acclimation and protein content, which was used to explain the greater cold tolerance of 'Midiron' bermudagrass, was also reported by Gatschet et al. (1996). Crude protein content during the first year increased steadily until February for 'La Paloma' and 'NuMex Sahara' and until March for 'Yukon' and 'Princess 77', and did not begin to decline significantly until May 2008. This suggests that unusually high winter temperatures can lead to early metabolism of carbohydrates and protein and a subsequent faster green-up in the spring. Elevated CP content during cold acclimation has been previously reported by several authors, confirming the key role amino acids, and soluble proteins play in preventing cell freezing (Hsiao 1973, Gatschet et al. 1996, Munshaw et al. 2006, Rimi et al. 2013b).

Studies involving freeze tolerance of warm-season species have reported the positive association between carbohydrate content and speed of spring green-up (Rogers et al. 1975, Di Paola and Beard 1992, Macolino et al. 2010). Our results support these studies and also reveal that WSC content during the winter months is negatively correlated to D80. Protein content was also significantly correlated with D80, with greater protein content leading to shorter times to green-up. However, the relationship was not as strong, as evidenced by lower regression coefficients, and a lack of significant coefficients during some months of the year. Overall, the higher and more consistently significant regression coefficients between WSC and length of time to green-up compared to those between protein content and D80 suggest that the CP is not as important as carbohydrates for an early spring green-up.

Our results demonstrate that even newly available seeded turf-type bermudagrass can be grown successfully in northern Italy without facing significant cold damage during the winter months. Although no differences were detected in fall colour retention, choosing an appropriate cultivar can significantly increase the length of time during which turf is green. Our results indicate that choosing a cultivar with earlier green-up could extend the functionality period up to 3 weeks, hence resulting in a greater acceptance of warm-season turfgrasses by the end user. In our study ‘Yukon’ exhibited the highest dry weight of stolons and rhizomes and the highest concentration of both WSC and CP in the stolons. The study also revealed that early spring green-up of bermudagrass seems to be more strongly related to carbohydrate content than protein content. Breeding for traits such as high stolon dry weight and high WSC may help develop cultivars that have short winter dormancy and that are not affected by winter kill. Improved cultivars with those characteristics will represent an excellent choice for transition zone areas, such as northern Italy.

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