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Carbohydrate Metabolism During Wintering Period in Four Zoysiagrass Genotypes

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Abstract: A key barrier to widespread use of warm-season grasses in the transition zone between the temperate and subtropical climates is represented by the winter dormancy, a temporary suspension of visible growth of any plant structure including the meristem. In this environment, species with different photosynthetic carbon cycle coexist, leading to asynchrony in growth among warm- and cool-season grasses. The objectives of the present study were (i) to assess the carbohydrate content in four representative zoysiagrasses *Zoysia japonica* ‘El Toro’, ‘Meyer’, *Z. pacifica*, and *Z. matrella* ‘Zeon’ during three successive phases of vegetation phenology, senescence, dormancy, and green-up, and (ii) to characterize the metabolic responses to phenological variations under natural acclimation. Japanese lawn grass genotypes ‘El Toro’ and ‘Meyer’ showed longer dormancy periods than fine-leaved *Z. pacifica* and ‘Zeon’. In Japanese lawn grass genotypes, the progressive decline of photosynthetic pigments was similar to that observed in the color retention. Over the experimental period, a significantly greater amount of starch reserves was observed in sprigs (horizontal stems) in the Japanese lawn grasses than in the fine-leaved zoysiagrasses. In general, total soluble sugars (TSS) in leaves and sprigs did not show evidence of sugar starvation during the senescence phase. TSS increased significantly during cold acclimation, resulting in a higher metabolic activity at the onset of green-up to support spring regreening in all zoysiagrasses except ‘Meyer’ leaves.

Key words: Digital image analysis, Japanese lawn grass, Manilagrass, Mascarene grass, Photosynthetic pigments, Soluble sugar, Starch.

The distribution and use of warm-season grasses (Chloroideae and Panicoideae subfamilies) are encouraged in the transition-zone, between the temperate and subtropical climates, due to enhanced water use efficiency, and capacity to acquire better thermotolerance by exposure to acute heat stress when compared to C₃ cool-season grasses (DiPaola and Beard, 1992). The downside is represented by the winter dormancy, a temporary suspension of visible growth of any plant structure containing a meristem (Lang et al., 1987). Therefore, species characterized by different photosynthetic carbon cycle coexist in this environment, leading to asynchrony in growth among species and a complementarity effect.

In the last decade, the Universities of Southern Europe developed research programs directed to the study of zoysiagrass (Macolino et al., 2012; Agati et al., 2013; Ntoulas et al., 2013), in particular focused on its adaptability to the Mediterranean environment (Volterrani et al., 2010; Pompeiano et al., 2011; Rimi et al., 2012).

Zoysiagrass (*Zoysia* spp. Willd.) has typically been more widely utilized in the upper transition zone, as the most low temperature hardy among the warm-season grasses. Dormancy, chill injury, and winter kill are different aspects of the response of plants to low temperatures. The severity of the plant’s response is associated with the rate of exposure to stress (change in temperature per unit time), duration and fluctuations (diurnal and seasonal). Significant differences in biometric characteristics, establishment growth rate, and abiotic stress tolerance among species and genotypes are known. For instance, Japanese lawn grass genotypes (*Z. japonica* Steud.) suffer less winter injury and exhibit higher freeze tolerance (LT₅₀) than Manila grass (*Z. matrella* (L.) Merr.) (Patton and Reicher, 2007).

Zoysiagrass discoloration, enhanced by higher intensity light (Youngner, 1961), starts when the average of minimum air temperature for 15 consecutive days was below 15°C followed by termination of shoot growth at

approximately 10°C or below (Wei et al., 2008), and soil temperature less than 16°C (Baltensperger, 1962). During cold acclimation in fall, the growth of the plant slows down while simultaneously adjusting biochemically, physiologically and metabolically. In particular, under low temperature treatments, most of the C₄ enzymes and ribulose-1,5-bisphosphate carboxylase (RuBPCase) decline as well as phosphoenolpyruvate carboxylase (PEPCase) and phosphoenolpyruvate carboxykinase (PCK) activities (Matsuba et al., 1997). Decarboxylation through PCK in *Z. japonica* was suggested to be one of the major limiting steps in photosynthesis under chilling conditions. Species sensitive to cool temperatures have a tendency to exhibit photochemical damage (photoinhibition of photosynthesis) when they are exposed to high radiance under low temperatures (Lyons, 1973). Photoinhibition occurs when leaves are exposed to more light than they can utilize, becoming in a state characterized by depressed rates of both light-saturated and light-limited photosynthesis. Symptoms of low-temperature damage in the photosynthetic apparatus are particularly sharp when substantial light intensity follows exposure to low temperature. Long-term treatment results in photooxidation of the pigments (Hendry et al., 1987) causing a straw-brown appearance in warm-season grasses.

In warm-season grasses, total nonstructural carbohydrates (TNC) show marked seasonal variation (White, 1973), whereas they are weakly affected by N source (Goatley et al., 1998; Rimi et al., 2013). As it is cool in autumn, photosynthesis is relatively high compared to respiration rates, which allows the plant to build carbohydrate reserves. Nonstructural carbohydrates, like starch, glucose, sucrose, and fructose are primarily involved in dormancy recovery, regrowth and recuperative capacity from cold stress (White and Schmidt, 1990; Munshaw et al., 2006). In particular, soluble sugars and starch reserves can play an important role as cryoprotectants to protect the cell during a freeze-thaw cycle, and stabilizing proteins structure and membranes during dehydration induced by low temperature, with both osmotic (Levitt, 1980; Santarius, 1982), and non-colligative functions in freezing resistance (Anchordoguy et al., 1987). Carbohydrate accumulation in rhizomes and stolons from nonacclimated and cold-acclimated zoysiagrass plants revealed that concentrations of soluble sugars increased considerably during acclimation, while starch concentrations decreased (Patton et al., 2007). In this latter study conducted in controlled-environment conditions, starch, sugar/starch ratio, glucose, and total reducing sugars in cold-acclimated plants were correlated with freeze tolerance. A correlation between freeze tolerance (LT₅₀) and total soluble sugars (TSS) in non-acclimated plant tissues was observed as well as between LT₅₀ or TSS and sucrose. These relationships indicate that genotypes with poor freezing tolerance

produce more soluble sugars during acclimation than genotypes with better freezing tolerance. Tolerant genotypes had a larger amount of reducing sugars in cold-acclimated tissues and total reducing sugars were related to LT₅₀. Starch concentration decreased during cold acclimation, and a correlation of this trait with freezing tolerance was observed. Total nonstructural carbohydrates were also found to promote early spring green-up in warm-season grasses (Macolino et al., 2010).

The objectives of the present study were (i) to assess carbohydrate content in four representative zoysiagrasses during three successive phases of vegetation phenology, senescence, dormancy, and green-up, and (ii) to characterize the changes in metabolism in response to phenological variations under natural acclimation.

Materials and Methods

1. Plant material and growth conditions

The research was conducted at the Department of Agriculture, Food and Environment Experimental Station in Pisa, Italy (43° 40' N, 10° 19' E; 6 m a.s.l.) during 2011 – 12 on mature zoysiagrass swards. On 10 July 2007, zoysiagrass plants were manually transplanted at 10 plants m⁻² on a silt loam soil, using single potted plants (obtained from sprigs or seed and raised in peat-filled honeycomb cell of 5 cm³). At the time of transplant, plants were 6 to 10 cm high and had never been trimmed down. Experimental plots were 1.5 by 2.3 m, arranged in a randomized complete block design with three replications. Following establishment, plots were maintained with a mowing height of 12 mm and monthly N applications (each year, from June through September) at the rate of 25 kg ha⁻¹ from urea (46N–0P–0K). Weeds were controlled manually and no aeration or pest control was carried out. Two cultivars of Japanese lawn grass *Zoysia japonica* Steud. cvs. 'El Toro' and 'Meyer' and two fine-leaved (< 2 mm) zoysiagrasses *Z. pacifica* (Goudswaard) Hotta & Kuroki (mascarene grass), and *Z. matrella* (L.) Merr. cv. 'Zeon' were selected in the study. The four zoysiagrasses were selected based on opposite winter color retention and spring green-up performances previously observed in the area of study (Pompeiano et al., 2014). A weather station onsite monitored daily air temperature (Fig. 1).

Winter color retention and spring green-up of zoysiagrass were evaluated every seven days, using digital image analysis (DIA) techniques (Richardson et al., 2001) to quantify the percent green turf color in each plot (with 100% being full retention – 0% completely brown). Pictures were analyzed individually with SigmaScan Pro (version 5.0; Systat Software, San Jose, CA). In order to selectively identify green leaves of zoysiagrass, a hue range from 45 to 100 and a saturation range from 0 to 100 were adopted after preliminary work on zoysiagrass. Each parameter was evaluated for 112 days for the green-down

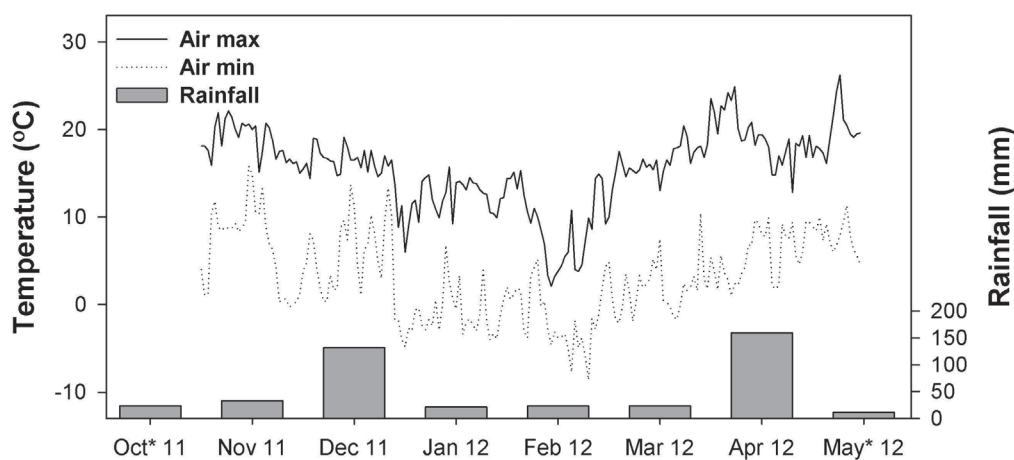


Fig. 1. Maximum and minimum daily air temperature recorded at 2 m above soil surface and monthly rainfall precipitation. October* and May* values are limited according to the experiment time.

Table 1. Hypothesis test summaries of difference in green turf color of the zoysiagrasses (*Zoysia* spp.) during green-down and on green-up (cultivar/genotype and species).

Sum of squares reduction test	Green-down	Green-up
Null hypothesis	Shared regression parameters (Slope and Days ₅₀) ^a for all entries	
Alternative hypothesis	Different regression parameters for each entry	
Numerator df	6	6
Denominator df	196	148
F-value	394.6	159.6
P-value	< 0.0001	< 0.0001

^aSlope and Days₅₀ values determine percent green turf color according to Eq. [1].

(from 21 October 2011) and for 84 days for the green-up (from 10 February 2012), until all plots had reached 0 and 100% green turf color respectively for the winter color retention and spring green-up. Plugs were harvested from each plot at biweekly intervals for biochemical analyses, and plants were separated into leaves and sprigs (stolons and rhizomes combined). Immediately after sampling and washing off soil with water and removing roots and shoots, all samples were frozen in liquid N₂ and stored at -80°C until analysis.

2. Analysis of pigments

Pigments were extracted and analyzed as reported in Pompeiano et al. (2013b).

3. Soluble carbohydrates

Samples (0.5 g fresh weight, FW) were ground to a powder and extracted as described by Tobias et al. (1992). Samples were assayed with coupled enzymatic assay methods (Pompeiano, et al. 2013a) that measure the increase in A₃₄₀. The accuracy of the method was tested using standards with known amounts of carbohydrates.

Recovery experiments evaluated the loss during

extraction. Two tests were done for each metabolite by adding a known amount of authentic standards to the samples prior to the extraction. The concentrations of the standards added were similar to those estimated to be present in the tissues in preliminary experiments. The percentage of recovery ranged between 93 and 105% depending on the sugar. The quantity of soluble carbohydrates was corrected on the basis of the recovery percentages for each sample, and expressed as μ moles hexose equivalents g⁻¹ FW.

4. Starch analysis

Sprigs (rhizomes and stolons) (100 mg FW) were ground in a mortar and resuspended in 100 mL of 10 mM KOH, and boiled for 1 min. One milliliter of 1 N HCl was then added to each sample. The starch standard solution was prepared using 100 mg of potato soluble starch dissolved in 100 mL dH₂O and boiled 1 min. To both samples (50 (L) and standards (from 0 to 100 (L), adjusted to 150 μ L with dH₂O), was added 1 ml of fresh iodine solution (0.13% K₂ and 0.3% KI, dissolved in dH₂O) and the absorbance was read at 595 nm immediately. Starch concentration was expressed as mg starch g⁻¹ FW.

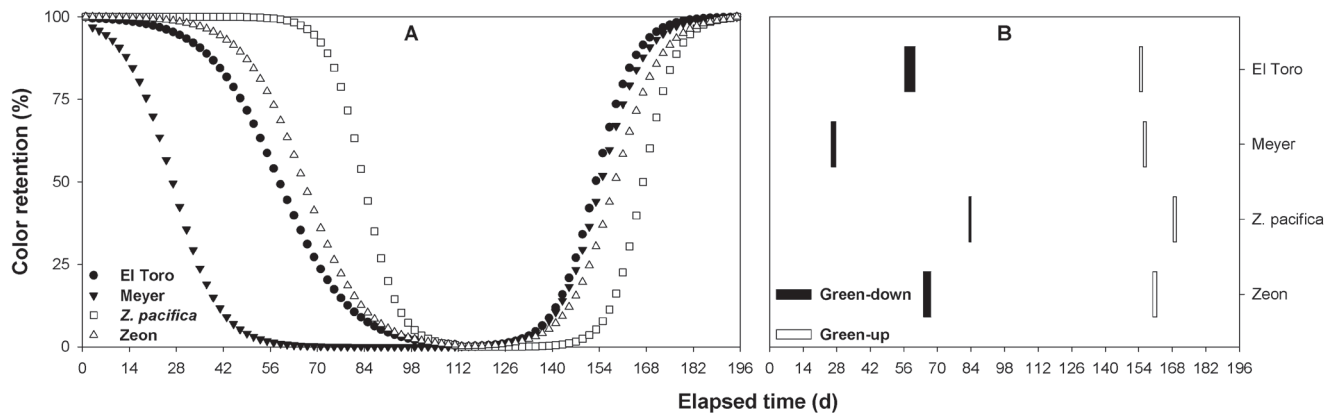


Fig. 2. (A) Predicted green color retention curves during the fall green-down and spring green-up vegetation stages for zoysiagrass (*Zoysia* spp.) cultivar/genotype. (B) Confidence intervals (95%) for the number of days until each cultivar/genotype retain 50% green color during the green-down and green-up phases. Entries with overlapping bars were not significantly different ($P=0.05$).

Table 2. Statistical parameters for predicting green-down and green-up characteristics of zoysiagrass (*Zoysia* spp.) entries and species. Smaller slope values indicate broadening changes over time. Days₅₀ is the predicted number of days until 50% green turf color is attained.

Cultivar/genotype	Green-down					Green-up				
	Slope	SE ^a	Days ₅₀	SE ^b	R ²	Slope	SE	Days ₅₀	SE	R ²
<i>Z. japonica</i> El Toro	-0.042	0.004	58.7	1.13	0.95	0.073	0.004	42.9	0.32	0.99
<i>Z. japonica</i> Meyer	-0.062	0.004	26.9	0.53	0.98	0.069	0.003	44.5	0.31	0.99
<i>Z. pacifica</i>	-0.084	0.004	83.8	0.27	0.99	0.089	0.006	57.0	0.35	0.99
<i>Z. matrella</i> Zeon	-0.049	0.004	65.9	0.83	0.97	0.063	0.003	48.7	0.42	0.99
Mean	-0.059		58.8		0.97	0.074		48.3		0.99
Species										
Japanese lawn grass ^c	-0.030	0.003	41.6	1.91	0.79	0.070	0.002	43.7	0.24	0.99
Fine-leaved ^d	-0.055	0.006	75.5	1.04	0.89	0.068	0.006	53.2	0.59	0.96

^aStandard error of Slope.

^bStandard error of Days₅₀.

^c*Zoysia japonica* genotypes El Toro and Meyer

^dFine-leaved genotypes [width ≤ 2 mm] *Z. pacifica* and *Z. matrella* 'Zeon'.

5. Statistical analysis

The statistical analyses of biometric and physiologic traits were performed using one-way analysis of variance (ANOVA) to determine whether significant differences among cultivars did exist. When significant differences were found, the means were compared using the least significant difference (LSD) test. Significant differences for all statistical tests were evaluated at the level of $P=0.05$. All computations were performed with R 3.0.2 (R Core Team, 2013), and the R package *agricolae* (de Mendiburu, 2014) was used.

Results

1. Color retention - dormancy

Green color retention during the fall green-down and spring green-up vegetation stages differed significantly ($P < 0.0001$) with the zoysiagrass genotype (Table 1). The sigmoid models provided a representative fit of the data to

describe the dynamics of the predicted curves (Fig. 2), resulting in average R^2 values of 0.97 and 0.99, respectively for fall green-down and spring green-up (Table 2). Japanese lawn grass 'El Toro' retained green color better than 'Meyer' (58.7 vs. 26.9 Days₅₀), although overall, they exhibited poor color retention (41.6 vs. 75.5 Days₅₀) and faster green-up (43.7 vs. 53.2 Days₅₀) compared to fine-leaved zoysiagrass genotypes. 'Zeon' reached the threshold of 50% green-down color 17.9 d earlier than mascarene grass, which provided better winter color retention. However, 'Zeon' had significantly faster spring green-up, on average 8.3 d earlier compared to the latter genotype. As a consequence, the period of time when color retention was lower than 50% was significantly longer in 'Meyer' (129.7 d), than in 'El Toro' (96.2 d), 'Zeon' (94.8 d), and mascarene grass (85.2 d).

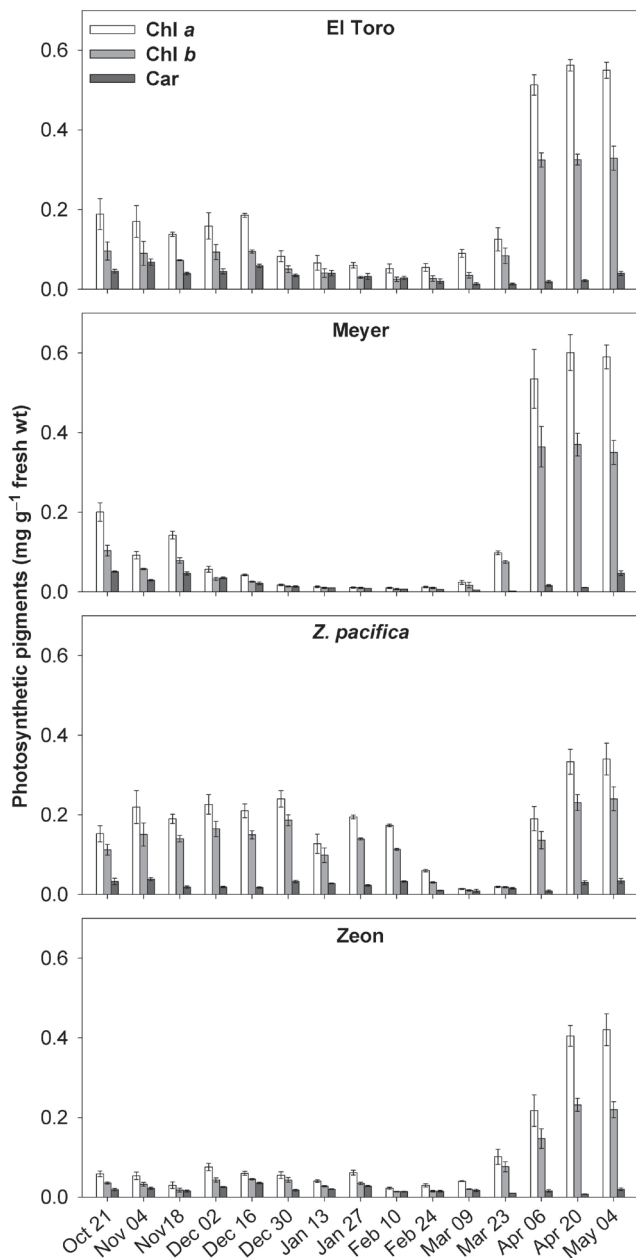


Fig. 3. Leaf photosynthetic pigments in zoysiagrass (*Zoysia* spp.) cultivar/genotype observed during three successive phases of vegetation phenology, senescence, dormancy, and green-up. Error bars represent standard error of the mean ($n = 3$).

2. Leaf photosynthetic pigments

Under field conditions, changes in chlorophyll *a-b* pigments and carotenoid contents were usually observed, although entries had a different behavior (Fig. 3). After the onset of green-down, Japanese lawn grass genotypes showed a progressive decline in photosynthetic pigments. In 'Meyer', pigments were almost completely degraded on 30 December, whereas on the same date, 'El Toro' still retained > 40% of the total chlorophylls detected at the onset of the senescence. In this genotype, the complete pigments degradation during the dormancy did not occur

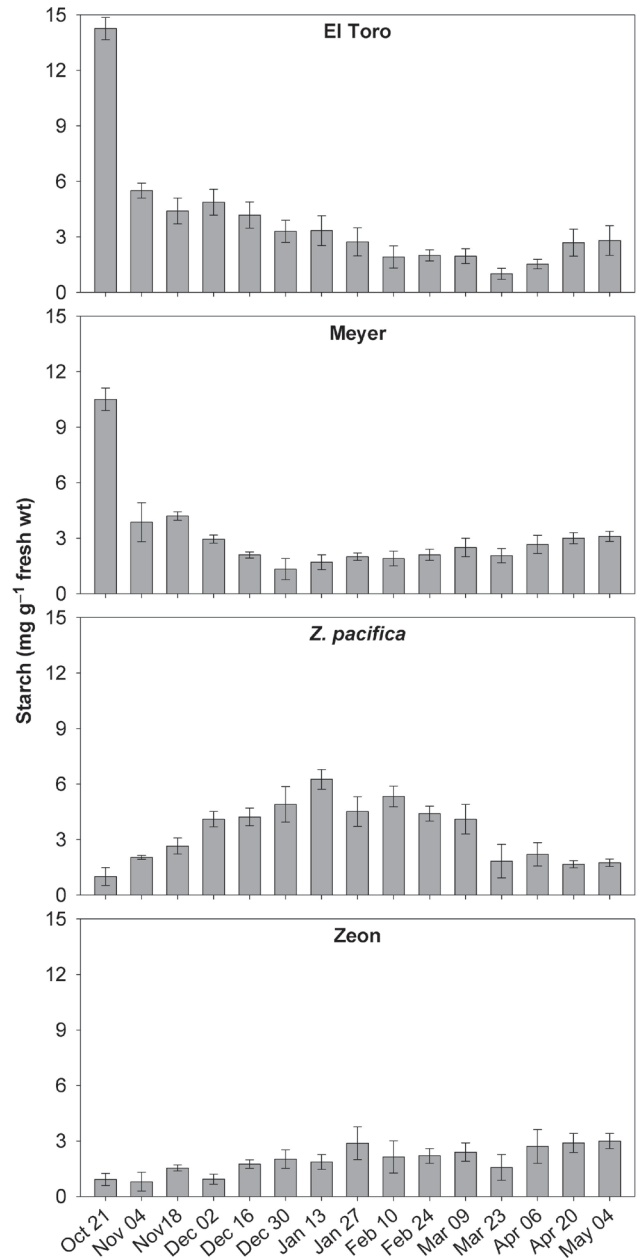


Fig. 4. Sprigs starch contents in zoysiagrass (*Zoysia* spp.) cultivar/genotype observed during three successive phases of vegetation phenology, senescence, dormancy, and green-up. Error bars represent standard error of the mean ($n = 3$).

under the present field conditions. In fine-leaved zoysiagrass, the degradation of pigments was less severe. A significant, but less pronounced decrease in chlorophylls and carotenoids levels was detected in 'Zeon' only after 10 February, at the end of the green-down stage. The overall pigments content was not significantly affected in mascarene grass during the senescence stage, although lower levels were observed at the onset of green-up.

The onset of spring green-up determined a sharp increase of chlorophyll and carotenoid contents in all the entries. A significantly higher chlorophyll content was

observed at the end of spring green-up stage compared to the beginning of the senescence, the concentrations averaging 3- and 4.5-fold higher respectively in *Z. japonica* and fine-leaved genotypes, whereas carotenoids level showed a lower variability. Overall, Japanese lawn grass genotypes attained a higher pigment level in May compared to the fine-leaved zoysiagrasses.

3. Starch

Significantly ($P < 0.01$) larger amounts of starch reserves were observed in sprig tissues of Japanese lawn grass genotypes than in fine-leaved zoysiagrasses over the experimental time course (Fig. 4). In this latter species, starch accumulation was sharply depressed by an average of 62% at the onset of the senescence (4 November compared to 21 October). As senescence progressed, a progressive decline in starch concentrations was evident in both Japanese lawn grass genotypes, although a sharper decline was detected in 'Meyer', reaching the minimum content at the end of December. In contrast, the lower content of starch reserve in 'El Toro' was reached on 23 March, during the onset of spring green-up. Relative few changes in starch levels were observed in 'Zeon' throughout the successive phenology stages, whereas in *Z. pacifica* an accumulation of this reserve carbohydrate was clearly evident during the senescence, reaching 6.3 mg g^{-1} FW on 13 January, and it gradually declined thereafter.

4. Soluble carbohydrates

In all the tissues examined, Japanese lawn grasses contained a significantly ($P < 0.01$) larger amount of soluble sugars than the fine-leaved zoysiagrasses throughout the experimental time (Figs. 5 and 6). Overall, the average amount of total soluble sugars (TSS) was 115.0 and $102.7 \text{ } \mu\text{mol g}^{-1}$ FW, respectively, in leaf and sprig tissues in Japanese lawn grass plants, but 104.3 and $88.9 \text{ } \mu\text{mol g}^{-1}$ FW in fine-leaved zoysiagrass plants. Among the single soluble carbohydrates that contributed to the differences in total content between the entries, sucrose was the prevalent one.

As previously observed with other parameters, TSS content of leaves showed different behavior, with the exception of 'Meyer' where no significant difference was observed over time. In all other zoysiagrasses, TSS in leaves gradually increased as senescence progressed, reaching a peak value corresponding to the end of the green-down stage ($254.3 \text{ } \mu\text{mol g}^{-1}$ FW in *Z. pacifica* on 10 February). In both fine-leaved zoysiagrasses, TSS content was lower in this tissue in correspondence to the onset of spring green-up.

Consistently, as observed in leaves, an analogous trend was observed in sprig tissues, where all the genotypes tested exhibited a higher TSS accumulation during the senescence phase compared to the onset of spring green-up. The fluctuation was less marked in 'Meyer', although a

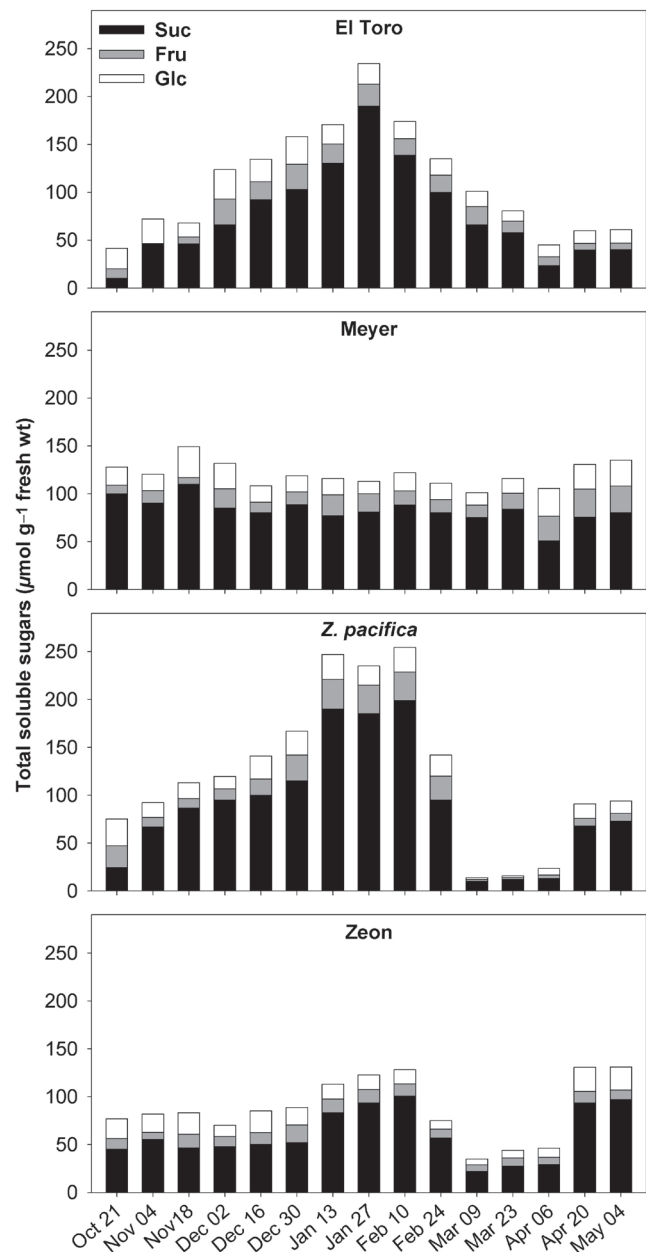


Fig. 5. Glucose, fructose, and sucrose (as hexose equivalents) contents of leaves in zoysiagrass (*Zoysia* spp.) cultivar/genotype observed during three successive phases of vegetation phenology, senescence, dormancy, and green-up.

lower TSS content was found on 23 March, as well as in mascarene grass where TSS content averaged $72.5 \text{ } \mu\text{mol g}^{-1}$ FW since 27 January ($101.2 \text{ } \mu\text{mol g}^{-1}$ FW the average before the latter date).

Discussion

Color retention during fall senescence and spring green-up may differ significantly with the genotype, and as a consequence, cause large differences in the dormancy period. In the present study, there was as much as a 44.4 d difference between genotypes in respect to the period of

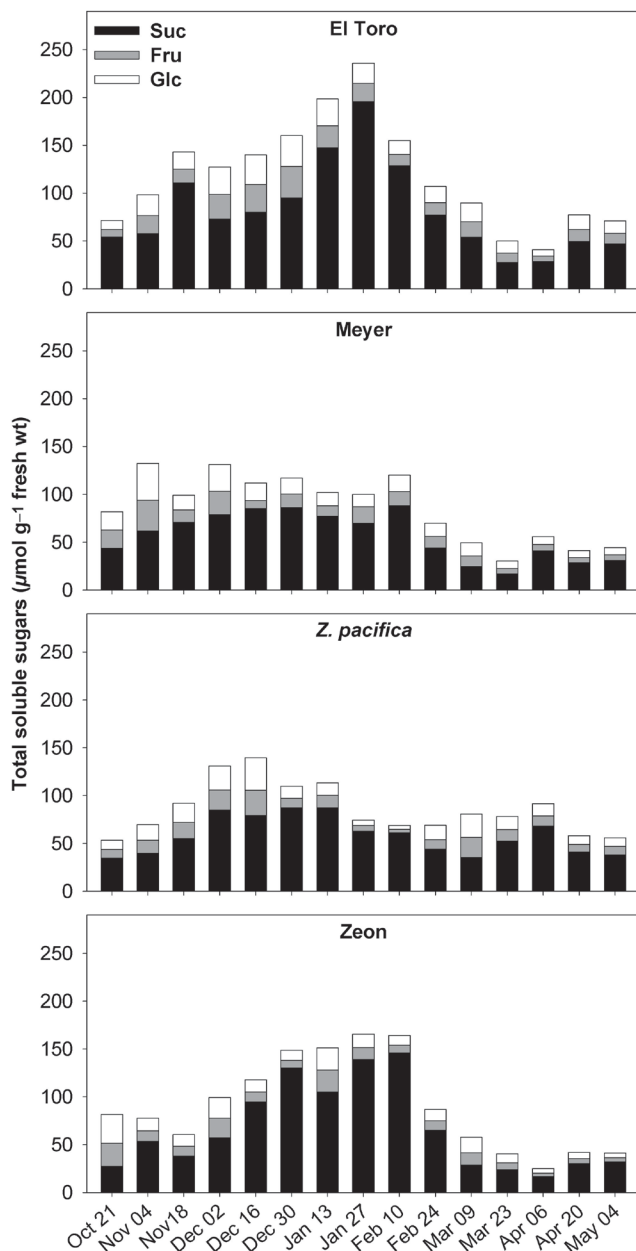


Fig. 6. Glucose, fructose, and sucrose (as hexose equivalents) contents of sprigs in zoysiagrass (*Zoysia* spp.) cultivar/genotype observed during three successive phases of vegetation phenology, senescence, dormancy, and green-up.

time when color retention attained lower than 50%. ‘El Toro’, a Japonica type released in 1986 from University of California, Riverside, was developed with the objectives of providing better cool weather color, earlier spring green-up, and shorter dormant period compared to ‘Meyer’, the industrial standard in the transition zone because of its excellent freezing tolerance (Gibeault and Cockerham, 1988). In the present study, these traits were confirmed, as well as the overall performance of ‘Zeon’ compared to the Japanese lawn grasses (National Turfgrass Evaluation Program, 2001).

In Japanese lawn grasses, the progressive decline of photosynthetic pigments showed a similar trend as observed in the color retention curves. Under temperate climatic conditions such as coastal areas of Mediterranean basin, *Z. pacifica* showed high ability to retain fall color among zoysiagrasses (Pompeiano et al., 2012), and it was generally consistent with the pigment data, where a decline in chlorophyll *a-b* pigments and carotenoids content was not observed during the senescence phase. At the end of the green-up, carotenoid synthesis was slower compared to the chlorophyll levels.

As expected, the accumulation of starch reserves and soluble sugars in *Zoysia* spp. showed a dynamic system of energy balance during three successive phases of vegetation phenology under examination. As expected, marked differences were observed among genotypes. In both Japanese lawn grasses, a steep decrease in starch accumulation was evident at the beginning of the senescence, in agreement with Patton et al. (2007) where under induced cold acclimation, starch decreased in rhizomes and stolons of 13 zoysiagrass genotypes. Moreover, as senescence progressed, a continuous decline in starch content was observed in both Japanese lawn grasses. This is not in contrast with a previous study conducted on ‘Meyer’, where starch increased during cold acclimation and remained at relatively high level since December and sharply declined in March (Rogers et al., 1975). Differences in environmental conditions occurred during the two studies may explain the different pattern of starch accumulation. An analogous trend was observed in bermudagrass and seashore paspalum, where starch accumulation was observed before winter to support winter survival and spring regrowth (Rimi et al., 2013). In *Z. pacifica*, starch accumulation was detected during the senescence, although the levels reached were not pronounced, as corroborating evidence that the levels of reserves are primarily determined by growth rate. This genotype, one of the slowest growing among zoysiagrasses (Pompeiano et al., 2012), showed the latest greening in the present field study, indicating an active ability to accumulate reserves in the dedicated organs till the onset of the dormancy.

Usually, TSS in leaves and sprigs did not show evidence of sugar starvation as senescence progressed. With the exception of ‘Meyer’ leaves, TSS significantly increased during cold acclimation followed by a higher TSS consumption at the onset of green-up in both organs, in agreement with previous studies conducted on zoysiagrass (Rogers et al., 1975, Pompeiano et al., 2011), as well as on bermudagrass (Zhang et al., 2006, Macolino et al., 2010). The marked depletion of carbohydrates is associated with the utilization of carbohydrates as an energy source to support the regrowth. The larger amounts of TSS in ‘El Toro’ during senescence should be attributed to the

superior color retention ($\Delta = 30.6$ d in Days₅₀) compared to 'Meyer'.

Conclusions

Genotype interaction with winter color retention and spring green-up could be an important trait to consider in order to minimize the dormancy period in the Mediterranean transition zone and prolonging the aesthetic function, especially in low budget athletic fields, golf course fairways and recreational areas. Japanese lawn grasses showed longer dormancy periods than the fine-leaved zoysiagrasses. In the present study, different timing in phenological stages reflected significant changes in leaf photosynthetic pigments and carbohydrate metabolism among zoysiagrasses in the wintering period. Some genotypes are capable of postponing winter dormancy supported by accumulation and relocation of TSS more evident in the metabolic and storage sinks.

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References

- Agati, G., Foschi, L., Grossi, N., Guglielminetti, L., Cerovic, Z.G. and Volterrani, M. 2013. Fluorescence-based versus reflectance proximal sensing of nitrogen content in *Paspalum vaginatum* and *Zoysia matrella* turfgrasses. *Eur. J. Agron.* 45: 39-51.
- Anchordoguy, T.J., Rudolph, A.S., Carpenter, J.F. and Crowe, J.H. 1987. Modes of interaction of cryoprotectants with membrane phospholipids during freezing. *Cryobiology* 24: 324-331.
- Baltensperger, A.A. 1962. Reduce dormancy of bermudagrass by soil heating. *Report on Turfgrass Research [Arizona]*. The University of Arizona, Tucson. 18-22.
- de Mendiburu, F. 2014. *Agricolae: statistical procedures for agricultural research*. R package version 1.1-7. 60.
- DiPaola, J.M. and Beard, J.B. 1992. Physiological effects of temperature stress. In Waddington, D.V., Carrow, R.N. and Shearman, R.C. eds., *Turfgrass. Agronomy Monographs* 32. ASA, CSSA, & SSSA, Madison. 231-267.
- Gibeault, V.A. and Cockerham, S.T. 1988. 'El Toro' zoysiagrass. *Calif. Turfgrass Cult.* 38: 1.
- Goatley, J.M., Jr., Maddox, V.L. and Hensler, K.L. 1998. Late-season applications of various nitrogen sources affect color and carbohydrate content of 'Tiflawn' and Arizona Common bermudagrass. *HortScience* 33: 692-695.
- Hendry, G.A.F., Houghton, J.D. and Brown, S.B. 1987. Tansley review No.11 - the degradation of chlorophyll - a biological enigma. *New Phytol.* 107: 255-302.
- Lang, G.A., Early, J.D., Martin, G.C. and Darnell, R.L. 1987. Endo-, para-, and eco-dormancy: Physiological terminology and classification for dormancy research. *HortScience* 22: 371-377.
- Levitt, J. 1980. Chilling, freezing, and high temperature stresses. In *Responses of plants to environmental stress*, 2nd ed., vol. 1, Academic Press, New York.
- Lyons, J.M. 1973. Chilling injury in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 24: 445-466.
- Macolino, S., Serena, M., Leinauer, B. and Ziliotto, U. 2010. Preliminary findings on the correlation between water-soluble carbohydrate content in stolons and first year green-up of seeded bermudagrass cultivars. *HortTechnology* 20: 758-763.
- Macolino, S., Ziliotto, U. and Leinauer, B. 2012. Comparison of Turf Performance and Root Systems of Bermudagrass Cultivars and 'Companion' Zoysiagrass. In Welbaum, G.E., Nektarios, P.A., Monteiro, J.A. and Fernández-Escobar, R. eds., *Xxviii International Horticultural Congress on Science and Horticulture for People*. Int Soc Horticultural Science, Leuven 1. 185-190.
- Matsuba, K., Imaizumi, N., Kaneko, S., Samejima, M. and Ohsugi, R. 1997. Photosynthetic responses to temperature of phosphoenolpyruvate carboxykinase type C₄ species differing in cold sensitivity. *Plant Cell Environ.* 20: 268-274.
- Munshaw, G.C., Ervin, E.H., Shang, C., Askew, S.D., Zhang, X. and Lemus, R.W. 2006. Influence of late-season iron, nitrogen, and seaweed extract on fall color retention and cold tolerance of four bermudagrass cultivars. *Crop Sci.* 46: 273-283.
- National Turfgrass Evaluation Program 2001. National zoysiagrass test - 1996: final report 1997-2000. *NTEP No. 01-15*. USDA-ARS, Beltsville, MD. 129.
- Ntoulas, N., Nektarios, P.A., Charalambous, E. and Psaroulis, A. 2013. *Zoysia matrella* cover rate and drought tolerance in adaptive extensive green roof systems. *Urban For. Urban Green.* 12: 522-531.
- Patton, A.J., Cunningham, S.M., Volenec, J.J. and Reicher, Z.J. 2007. Differences in freeze tolerance of zoysiagrasses: II. Carbohydrate and proline accumulation. *Crop Sci.* 47: 2170-2181.
- Patton, A.J. and Reicher, Z.J. 2007. Zoysiagrass species and genotypes differ in their winter injury and freeze tolerance. *Crop Sci.* 47: 1619-1627.
- Pompeiano, A., Guglielminetti, L. and Volterrani, M. 2011. Freeze tolerance of *Zoysia matrella* (L.) Merrill as affected by late-season nitrogen application, and changes in carbohydrates during cold acclimation. *Plant Biosyst.* 145: 885-892.
- Pompeiano, A., Grossi, N. and Volterrani, M. 2012. Vegetative establishment rate and stolon growth characteristics of 10 zoysiagrasses in Southern Europe. *HortTechnology* 22: 114-120.
- Pompeiano, A., Fanucchi, F. and Guglielminetti, L. 2013a. Amylolytic activity and carbohydrate levels in relation to coleoptile anoxic elongation in *Oryza sativa* genotypes. *J. Plant Res.* 126: 787-794.
- Pompeiano, A., Vita, F., Miele, S. and Guglielminetti, L. 2013b. Freeze tolerance and physiological changes during cold acclimation of giant reed [*Arundo donax* (L.)]. *Grass Forage Sci.*, DOI: 10.1111/gfs.12097.
- Pompeiano, A., Grossi, N., Guglielminetti, L. and Volterrani, M. 2014. Winter color retention and spring green-up of zoysiagrass genotypes in Southern Europe. *Europ. J. Hort. Sci.* 79: 158-166.
- R Core Team 2013. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna.
- Richardson, M.D., Karcher, D.E. and Purcell, L.C. 2001. Quantifying turfgrass cover using digital image analysis. *Crop Sci.* 41: 1884-1888.
- Rimi, F., Macolino, S. and Ziliotto, U. 2012. Rooting characteristics and turfgrass quality of three bermudagrass cultivars and a zoysiagrass. *Acta Agric. Scand. Sect. B-Soil Plant Sci.* 62: 24-31.

- Rimi, F., Macolino, S., Richardson, M.D., Karcher, D.E. and Leinauer, B. 2013. Influence of three nitrogen fertilization schedules on bermudagrass and seashore paspalum: II. Carbohydrates and crude protein in stolons. *Crop Sci.* 53: 1168-1178.
- Rogers, R.A., Dunn, J.H. and Nelson, C.J. 1975. Cold hardening and carbohydrate composition of 'Meyer' *Zoysia*. *Agron. J.* 67: 836-838.
- Santarius, K.A. 1982. The mechanism of cryoprotection of biomembrane systems by carbohydrates. In Li, P.H. and Sakai, A. eds., Plant cold hardiness and freezing stress: mechanisms and crop implications. Academic Press, New York. 475-486.
- Tobias, R.B., Boyer, C.D. and Shannon, J.C. 1992. Alterations in carbohydrate intermediates in the endosperm of starch-deficient maize (*Zea mays* L.) genotypes. *Plant Physiol.* 99: 146-152.
- Volterrani, M., Grossi, N., Gaetani, M. and Pompeiano, A. 2010. Zoysiagrass cultivar establishment rate and turf quality in central Italy. *Acta Hort.* 881: 313-316.
- Wei, S., Zhou, Y. and Li, D. 2008. Cold sensitivity and biochemical adjustments in zoysiagrass under low temperature stress. *Acta Hort.* 783: 195-206.
- White, L.M. 1973. Carbohydrate reserves of grasses: A review. *J. Range Manage.* 26: 13-18.
- White, R.H. and Schmidt, R.E. 1990. Fall performance and post-dormancy growth of 'Midiron' bermudagrass in response to nitrogen, iron, and benzyladenine. *J. Amer. Soc. Hort. Sci.* 115: 57-61.
- Youngner, V.B. 1961. Growth and flowering of *Zoysia* species in response to temperatures, photoperiods, and light intensities. *Crop Sci.* 1: 91-93.
- Zhang, X., Ervin, E.H. and LaBranche, A.J. 2006. Metabolic defense responses of seeded bermudagrass during acclimation to freezing stress. *Crop Sci.* 46: 2598-2605.
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