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Winter Colour Retention and Spring Green-Up of Zoysiagrass Genotypes in Southern Europe

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Summary

Zoysiagrass (*Zoysia* spp. Willd.) is a sustainable choice for the Mediterranean area, being identified as grass species adapted to low-input environments (limited water requirements, no fertilizer or pesticides after establishment). A distinct drawback in the transition zone is winter dormancy, a temporary suspension of visible growth, followed by discoloration and loss of functionality. In order to investigate the genotype/species effect in relation with agro-meteorological conditions, 10 cultivars/experimental lines were screened for their ability to extend the winter colour retention. A two years field study was conducted on mature swards and evaluated during the onset of dormancy and spring green-up using digital image analysis. For both parameters, in both years, the Authors observed a wide range of cultivar responses. *Zoysia japonica* genotypes in our study provided significantly shorter dormancy period than fine-leaved genotypes. In some instances, there were as much as 35 and 20 d difference between entries in respect to the winter colour retention and spring onset, respectively. The identification of genotypes with shorter dormancy than 'Meyer' (industry standard) may reduce use of cultural practices, as late-season nitrogen or iron application, normally used to extend the winter colour retention of zoysiagrass in late fall or early spring, showing different levels of success.

Key words. cultivar evaluation – digital image analysis – growing degree days – Japanese lawn grass – manilagrass – mascarene grass

Introduction

Important differences between plant species characterized with different photosynthetic carbon cycle, with C_4 species being active at higher temperatures than C_3 species, may reduce overlap in optimal growing periods within a growing season. This is especially relevant in the transition zone, between the temperate and subtropical climates, leading to asynchrony in growth among species, lowered interspecific competition, a complementarity effect (Loreau and Hector 2001), and higher species diversity.

Zoysiagrass (*Zoysia* spp. Willd.), a warm-season grass characterized with the Hatch-Slack pathway (C_4), has an optimum growth temperature between 27 and 35 °C – which is approximately 10 °C higher than C_3 plants (Leegood 1993) – and it has typically been more widely utilized in the colder transition zone as the most low temperature hardy among the warm-season grasses. Since first reports, Japanese lawn grass (*Z. japonica* Steud.) has been identified as the most low temperature hardy among the warm-season grasses (except buffalograss – *Buchloe dactyloides* (Nutt.) Engelm.), and has shown better spring green-up and less winter injury in comparison with Manilagrass (Z. *matrella* (L.) Merrill) (Forbes and Ferguson 1947; Daniel 1955). In the last two decades, the universities of Southern Europe have developed research programs directed to the study of zoysiagrass, in particular their adaptability to the Mediterranean environment (Volterrani et al. 2008; Lulli et al. 2012; Macolino et al. 2012; Rimi et al. 2012; Ntoulas et al. 2013). Thanks to these traits, zoysiagrass has a unique niche in northern transition zones, where bermudagrass (*Cynodon* spp.) fails because of its inability to cope with severe winters. The species is valued to be a low-input sustainable turf (Fry et al. 2008), and the excellent heat and drought tolerance made it an excellent choice for Mediterranean areas, although an additional barrier to widespread use is the winter dormancy, due to a loss of aesthetic and functional growth (De Luca et al. 2008).

Zoysiagrass discoloration, enhanced by stronger light intensity (Youngner 1961), starts when the average minimum air temperature for 15 consecutive days was below 15 °C followed by termination of shoot growth when below approximately 10 °C (Wei et al. 2008), and soil temperature less than 16 °C (Baltensperger 1962).

Dormant zoysiagrass turf is characterized by unique brownish-white colour and provides an excellent playing surface while maintaining numerous functional and recreational purposes including golf course fairways, recreational areas as well as ornamental lawns and parks. In South Korea, *Z. japonica* – commonly called "Golden Grass" for these particular and noteworthy characteristics – has been used as ground cover for family and royal tombs, to impart a 'golden colour' when dormant in winter. However, in functional areas under the pressures of increased use, dormant zoysiagrass will not withstand heavy traffic. Cool-season grasses are often fall overseeded into zoysiagrass to provide a temporary green, lush and growing playable surface (Hurley et al. 1989). Bermudagrasses have much better tolerance to overseeding and provide a better spring transition from cool-season overseeded grasses than zoysiagrasses. In particular, spring transition can have devastating consequences to zoysiagrass, as the zoysiagrasses have difficulty recovering in the spring when overseeded, as well as fall renovation increases the winterkill (Hurley et al. 1989).

The common cultural practice of applying late-season nitrogen or iron has been used to extend the winter colour retention of zoysiagrass in late fall or early spring, showing different levels of success (Dunn et al. 1993; Gibeault et al. 1997; Volterrani et al. 2010). Late-season fertilization and its effects on warm-season grasses have been under scientific scrutiny for more than 45 years. To date, opinions fall into divergent camps, the success of a late-fall fertilization program varying between regions and climates primary because it appeared detrimental to freeze tolerance. In the transition zone, the early close mowing of *Zoysia* conducted before green-up promotes faster green-up and improves the functional quality of the surface (Hawes 1979). Moreover, it has been observed that zoysiagrass cultivars grown under a shade environment exhibited better winter colour than their counterparts grown under sunlight (Kurtz 1985). Besides, newly established turf usually exhibit better winter colour retention than mature swards (Sifers et al. 1992).

Recently, selective breeding programs have led to the release of genotypes exhibiting varying degrees of adaptation to temperate climate. Of the 38 zoysiagrass cultivars currently or previously used in the U.S., 24 were released after 1990 (Patton 2009). A specific breeding objective was to improve quality and develop genotypes with an extended green colour retention into winter, a characteristic of particular interest in the transition zones. Improving colour retention and spring green-up through breeding appears a desirable option, compared to the adoption of cultural practices like late-season nitrogen or iron applications, due to cost and, limited for nitrogen effects on freeze tolerance (Pompeiano et al. 2011, 2013).

The work reported herein is aimed at characterizing the dynamics of winter colour retention (discoloration) and green-up response of 10 zoysiagrasses under transition zone conditions in a field environment.

Materials and Methods

Experimental conditions

The study 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 the 2009 and 2010 growing seasons on mature zoysiagrass swards. On 10 July 2007, experimental entries and cultivars of zoysiagrass (Table 1) were manually transplanted at 10 plants m^{-2} on a silt loam soil, using single potted plants

Table 1. Zoysiagrass (*Zoysia* spp.) common names, species, cultivar/genotype and typical type of establishment method.

Common names	Species	Cultivar/genotype[a]	Type[b]
Japanese lawn grass	*Z. japonica*	'DeAnza'[c]	vegetative
Japanese lawn grass	*Z. japonica*	'El Toro'	vegetative
Japanese lawn grass	*Z. japonica*	'Meyer'	vegetative
Japanese lawn grass	*Z. japonica*	'Victoria'	vegetative
Japanese lawn grass	*Z. japonica*	'Zenith'	seeded
Hybrid zoysiagrass	*Z. japonica* × *Z. pacifica*[d]	'Emerald'	vegetative
Hybrid zoysiagrass	*Z. japonica* × *Z. pacifica*	'HT-210'	vegetative
Manilagrass	*Z. matrella*	'DALZ0101'	vegetative
Manilagrass	*Z. matrella*	'Zeon'	vegetative
Mascarene grass	*Z. pacifica*	–	vegetative

[a] Entries fine-leaved [width ≤ 2 mm] are 'Emerald', 'HT-210', 'DALZ0101', 'Zeon' and mascarene grass.
[b] All entries were established by vegetative plugs into plots for this study.
[c] Entries are sorted according to alphabetical order inside each species.
[d] Formerly *Zoysia japonica* × *Zoysia tenuifolia* (Forbes 1962) now *Z. japonica* × *Z. pacifica* (Anderson 2000).

(obtained from sprigs or seed and raised in peat-filled honeycomb alveoli of 5 cm^3). 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 a 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 aerification or pest control was carried out. A weather station onsite monitored daily air temperature (Fig. 1).

Zoysiagrass winter colour retention and spring green-up were evaluated on a ten days basis, using digital image analysis (DIA) techniques (Richardson et al. 2001) to quantify the percent green turf colour for each plot (with 100 % being full retention – 0 % completely brown). Pictures were analyzed individually by SigmaScan Pro (version 5.0; Systat Software, San Jose, CA). In order to selectively identify zoysiagrass green leaves, a hue range from 45 to 100 and a saturation range from 0 to 100 were adopted after preliminary work on zoysiagrass. In both years and for each parameter, entries were evaluated for 70 d for the green-up (from 8 Feb.) and for 90 d for the green-down (from 9 Nov.), until all plots had reached 100 and 0 % green turf colour respectively for the spring green-up and winter colour retention. Growing degree days (GDD) were calculated during the whole green-up and -down period according to the experimental time using the formula GDD = Max. Temperature (°C) + Min. Temperature (°C)/2 – 5 °C (Patton et al. 2004).

Statistical analysis

Scatter plots of the percent green turf colour data versus number of days withheld during green-down and green-up

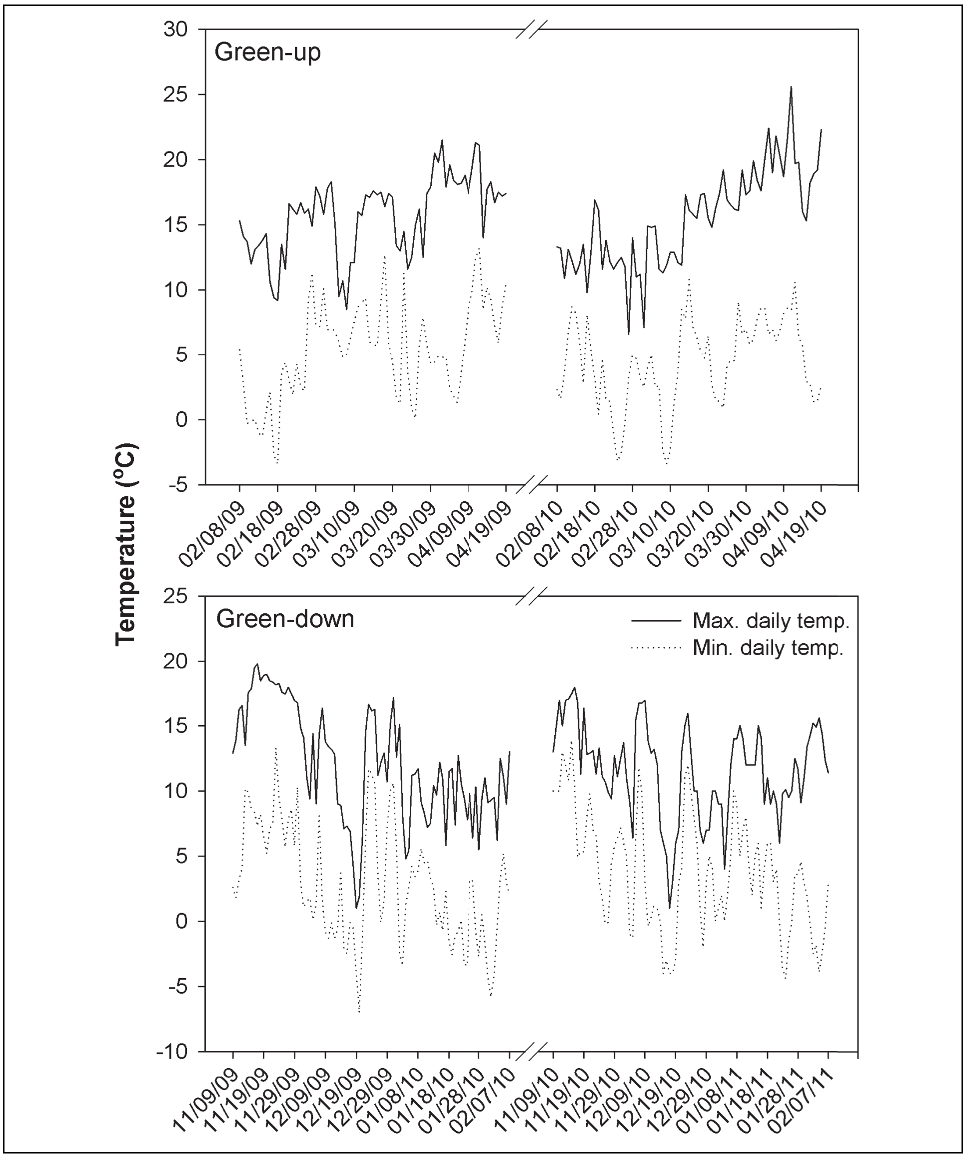

Fig. 1. Maximum and minimum daily temperature recorded at 2 m above soil surface during the whole green-up and -down period according to the experimental time.

indicated a strong nonlinear relationship. A nonlinear regression analysis was performed using this sigmoid variable slope model:

green turf colour (%) =
$100/\{1 + 10^{[(Days_{50} - X) Slope]}$ [1]

where X is number of days after a discretionary time zero (DGU and DGD, respectively for green-up and green-down), and $Days_{50}$ and Slope are estimated model parameters. $Days_{50}$ is the number of days passed until each entry reached 50 % green turf colour, and Slope defines the steepness of the curve. A sum of squares reduction F-test was used to determine if zoysiagrass entries significantly affected green turf colour during green-down and green-up. The F-test compared the sum of squares from a global model (all varieties share $Days_{50}$ and Slope values) against the cumulative sum of squares from models where $Days_{50}$ and Slope values were determined separately for each variety. If the sum of squares were reduced significantly ($P < 0.05$) using separate parameter values, variety effects were determined to be significant. Parameters estimates were used to calculate confidence intervals (95 %) for number of days withheld until each entry reached 50 % green turf colour (Motulsky and Christopoulos 2003). At the selected green colour retention percentage, entries were considered significantly different if their confidence intervals did not overlap. When differences were examined between species, 'Emerald' and 'HT-210' (*Z. japonica* × *Z. pacifica*), as well as *Z. pacifica*, were grouped within fine-leaved zoysiagrass because of their similarities in colour, texture, and density with respective species. The data were fitted to a logistic regression model using the generalized linear model (glm) function. All computations were performed with R 3.0.2 (R Core Team 2013) and R package *agricolae* (De Mendiburu 2012) was used.

Results

Spring green-up

Environmental conditions during 2009 growing season were overall more favourable for the spring green-up (Fig. 1), and partially supported from the cumulative GDD indicating a higher accumulation since 19 DGU when compared to the second year of study pattern (data not shown). A steeper average slope of the sigmoid curve was also found significantly different in the 2009 compared to 2010 (0.068 *vs.* 0.059, $P < 0.001$). At this initial stage during the first year of study, the daily minimum air temperatures were 6 times below 0 °C, and a difference of 27.3 GDD was observed 10 DGU, whereas 7 d the temperatures dropped down during the 2010, of which 3 between 28 and 30 DUP. At the end of the green-up, the first year reached 391.2 GDD, 43.2 higher then the 2010.

Zoysiagrass entries ($P < 0.0001$) and species ($P < 0.0001$) significantly affected spring green-up in both years under review (Table 2). The sigmoid models provided a representative fit of the data to describe the dynamics of the predicted spring green-up curves (Fig. 2), resulting in average R^2 values of 0.97 and 0.98, respectively in 2009 and 2010 (Table 3). The average number of days for the entries included in the study to reach 50 % green colour was 28.9 in the first year, 46.0 in the second year. In this latter period of the study, 'Zenith' and 'Meyer' exhibited early green-up among the *Z. japonica* genotypes, reaching 50 % green colour at 40.3 and 42.9 DGP. Among the fine- leaved zoysiagrass, 'Emerald' and 'Zeon' exhibited significantly fastest green-up in 2009. In both years, Japanese lawn grass had a minimum, but significantly earlier spring green-up than fine-leaved entries, 3.4 and 3.5 d respectively in 2009 and 2010.

Table 2. Hypothesis test summaries for zoysiagrass (*Zoysia* spp.) entries effects on green turf colour during green-down and on green-up (cultivar/genotype and species).

Sum of squares reduction test	Green-down 2009	Green-down 2010	Green-up 2009	Green-up 2010
Null hypothesis	Shared regression parameters (Slope and $Days_{50}$)[a] for all entries			
Alternative hypothesis	Different regression parameters for each entry			
Numerator df	18	18	18	18
Denominator df	280	280	310	310
F-value	133.0	263.7	17.74	18.80
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a] Slope and $Days_{50}$ values determine percent green turf colour according to Eq. [1].

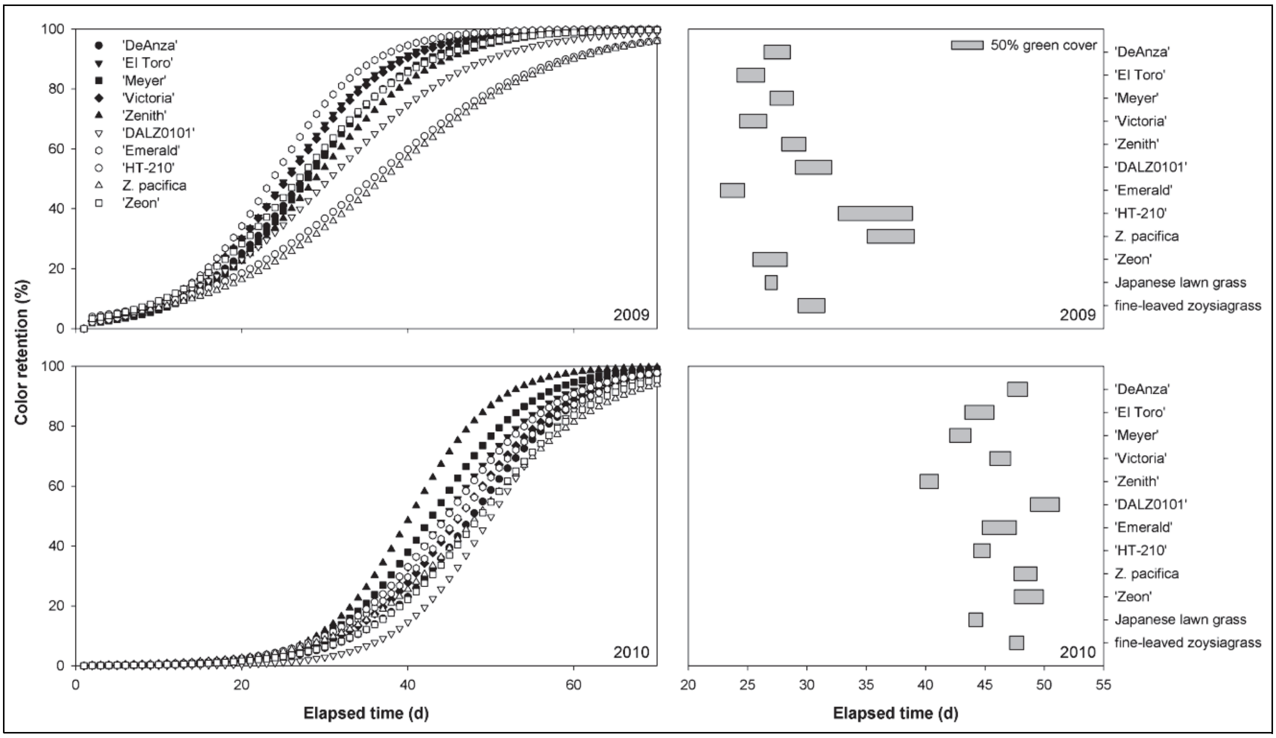

Fig. 2. (A) Predicted spring green-up curves for zoysiagrass (*Zoysia* spp.) cultivar/genotype. (B) Confidence intervals (95 %) for the number of days after 8 Feb. of each year until each cultivar/genotype, and species, retain 50 % green colour during the green-up. Entries with overlapping bars were not significantly different. Fine-leaved entries (leaf width ≤ 2 mm) were 'Emerald', 'HT-210', 'DALZ0101', 'Zeon', and mascarene grass.

Green-down

Slight differences were observed for max and min daily temperature patterns during the two years of study, as average min. daily temperatures were 2.8 and 3.8 °C, max. 12.1 and 11.6 °C during the 2009 and 2010 seasons, respectively (Fig. 1). Nevertheless, an early occurrence of freezing air temperature (below 0°C) was observed in 2010–11 when compared to the first season, 17 *vs.* 30 DGD, respectively.

A distinct trend was observed in this stage on winter colour retention of genotypes, as measured by days to 50 % colour retention, among zoysiagrass species (Table 3, Fig. 3). Differences between Japanese lawn grass cultivars were observed, with 'DeAnza', 'El Toro', and above all in the 2009–10, 'Victoria' (53.9 and 44.1; 54.3 and 41.9; 61.5 and 44.4 $Days_{50}$, respectively in 2009 and 2010) that retained green colour better than 'Meyer' (45.6 and 28.4 $Days_{50}$). Additionally, 'Zenith', the seeded *Z. japonica* genotype exhibits the worst colour retention compared to the range of zoysiagrasses selected for this trial.

Among the fine-leaved entries tested in the present study, a lower variability was found although 'HT-210' in both years showed a significantly faster decline (52.9 and 41.4 $Days_{50}$). Overall, Japanese lawn grass exhibited poor colour retention (50.3 and 36.8 $Days_{50}$) compared with fine-leaved zoysiagrass genotypes (59.3 and 48.2 $Days_{50}$).

Discussion

The results of the present study clearly demonstrate that zoysiagrass entries and species have a significant impact on turf performances during spring green-up and winter colour retention, supporting first reports that zoysiagrass was able to survive in severe conditions, with Japanese lawn grass having better low temperature hardiness and spring green-up than Manilagrass (Forbes and Ferguson 1947). More recently, similar results were obtained by the National Turfgrass Evaluation Program (2001), comparing 8 of 10 entries evaluated in the present study ('DALZ0101' and mascarene grass were not included). In particular, 'DeAnza' and 'Victoria' showed a significantly greater delay in the onset of green-up compared to the other Japanese lawn grasses, as observed in the 2010, a season where late spring frost might be responsible for delayed onset of the spring green-up. Overall, the results of the present study concur with the objectives driving the release of 'El Toro', a japonica type developed by University of California in 1986, that provided better cool-weather colour, earlier spring green-up, and as desirable consequence, shorter dormant period (Gibeault and Cockerham 1988). 'Zenith', an improved seeded zoysiagrass cultivar currently available in today's market selected from the Beltsville, MD USDA Research Station, showed poor fall retention colour but earlier spring green-up similar to 'Meyer'.

Table 3. Statistical parameters for predicting green-down and green-up characteristics of zoysiagrass (*Zoysia* spp.) entries and species. Smaller slope values indicate a broadening changes over time. $Days_{50}$ is the predicted number of days until each entry reached 50 % green turf colour.

	2009					2010				
Cultivar/genotype	Slope	SE[a]	$Days_{50}$	SE[b]	R^2	Slope	SE	$Days_{50}$	SE	R^2
	Green-up									
'DeAnza'	0.063	0.004	27.5	0.54	0.98	0.068	0.004	47.8	0.42	0.99
'El Toro'	0.070	0.006	25.3	0.57	0.98	0.069	0.006	44.5	0.61	0.98
'Meyer'	0.066	0.004	27.8	0.48	0.99	0.073	0.005	42.9	0.44	0.99
'Victoria'	0.067	0.005	25.5	0.57	0.98	0.066	0.004	46.3	0.43	0.99
'Zenith'	0.060	0.004	28.9	0.50	0.99	0.085	0.006	40.3	0.38	0.99
'DALZ0101'	0.050	0.004	30.6	0.75	0.97	0.076	0.007	50.1	0.60	0.97
'Emerald'	0.076	0.006	23.7	0.50	0.98	0.061	0.005	46.2	0.71	0.97
'HT-210'	0.041	0.005	35.8	1.54	0.90	0.065	0.003	44.8	0.34	0.99
Z. pacifica	0.042	0.003	37.1	0.97	0.96	0.055	0.003	48.4	0.47	0.99
'Zeon'	0.059	0.005	26.9	0.71	0.97	0.063	0.005	48.7	0.60	0.97
Mean	0.059		28.9		0.97	0.068		46.0		0.98
Species										
Japanese lawn grass	0.064	0.002	27.0	0.26	0.98	0.069	0.003	44.2	0.30	0.97
Fine-leaved z.	0.049	0.003	30.4	0.58	0.91	0.062	0.002	47.7	0.30	0.97
	Green-down									
'DeAnza'[c]	−0.107	0.005	53.9	0.24	1.00	−0.099	0.003	44.1	0.19	1.00
'El Toro'	−0.074	0.005	54.3	0.41	0.99	−0.101	0.006	41.9	0.27	1.00
'Meyer'	−0.045	0.004	45.6	0.88	0.98	−0.058	0.004	28.4	0.65	0.98
'Victoria'	−0.055	0.004	61.5	0.69	0.98	−0.080	0.004	44.4	0.33	1.00
'Zenith'	−0.055	0.006	33.0	0.96	0.97	−0.092	0.008	22.3	0.43	0.99
'DALZ0101'	−0.074	0.005	63.6	0.41	0.99	−0.074	0.002	50.5	0.21	1.00
'Emerald'	−0.058	0.004	60.7	0.52	0.99	−0.079	0.003	50.0	0.23	1.00
'HT-210'	−0.138	0.008	52.9	0.22	1.00	−0.082	0.007	41.4	0.54	0.99
Z. pacifica	−0.078	0.004	62.5	0.35	0.99	−0.072	0.004	48.6	0.42	0.99
'Zeon'	−0.065	0.003	58.0	0.29	1.00	−0.076	0.003	49.4	0.24	1.00
Mean	−0.075		54.6		0.99	−0.081		42.1		0.99
Species										
Japanese lawn grass	−0.046	0.004	50.3	0.90	0.88	−0.054	0.005	36.8	0.86	0.88
Fine-leaved z[d]	−0.066	0.004	59.3	0.41	0.96	−0.069	0.003	48.2	0.32	0.98

[a] Standard error of Slope.

[b] Standard error of $Days_{50}$.

[c] Entries are sorted according to alphabetical order inside each species.

[d] Entries fine-leaved [width ≤ 2 mm] are 'Emerald', 'HT-210', 'DALZ0101', 'Zeon' and mascarene grass.

The green-down performance of these entries, obtained in a one-year trial conducted on modules injection-molded from high-density polyethylene plastic, were previously presented (Pompeiano et al. 2012). The two-years field performance data here presented are partially in agreement with the previous report, although differences in trial conditions (modular system *vs.* field) significantly affected the overall performances. In the present study, it has been shown that considerable variation exists in the colour retention pattern of the genotypes, where in both years, fine-leaved zoysiagrass demonstrated significantly better winter colour retention.

Analyzing the weather conditions, the premature senescence onset in 2010 appeared to be related to the earlier

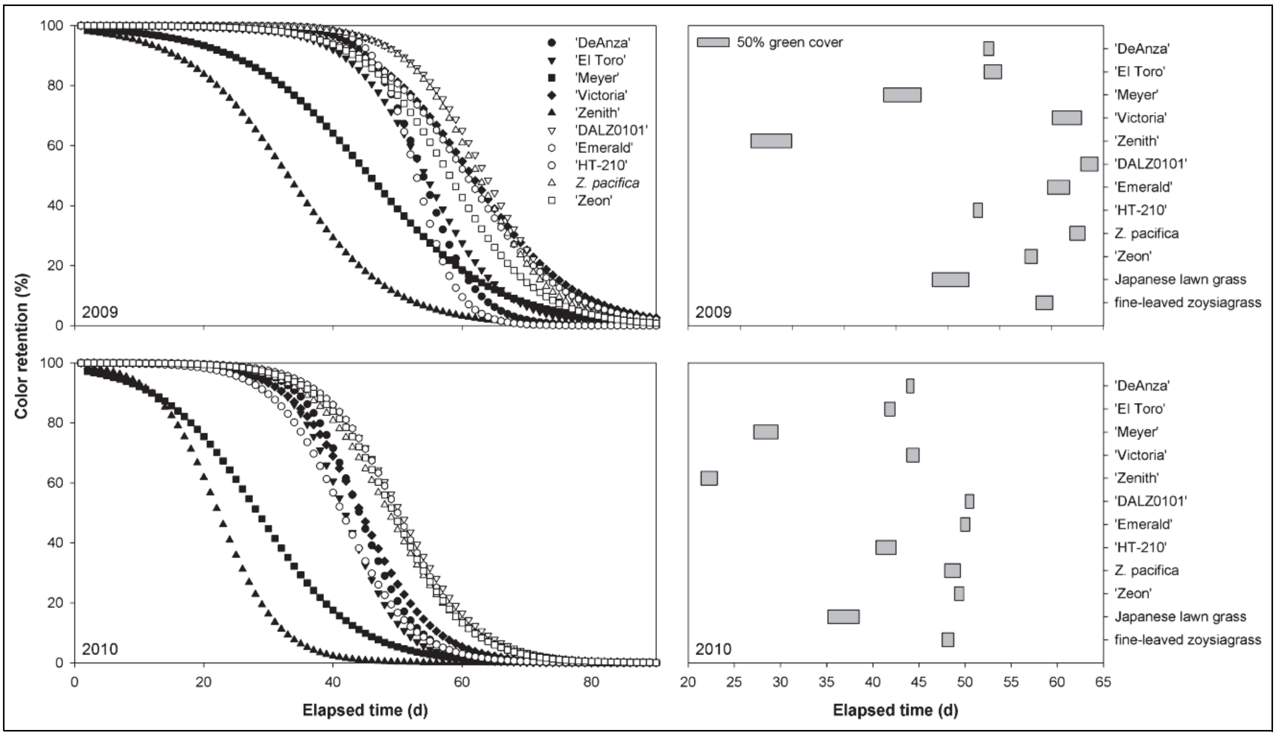

Fig. 3. (A) Predicted winter colour retention curves for zoysiagrass (*Zoysia* spp.) cultivar/genotype. (B) Confidence intervals (95 %) for the number of days after 9 Nov. of each year until each cultivar/genotype, and species, retain 50 % green colour during the green-down. Entries with overlapping bars were not significantly different. Fine-leaved entries (leaf width ≤ 2 mm) were 'Emerald', 'HT-210', 'DALZ0101', 'Zeon', and mascarene grass.

freezing events. Differences between *Z. japonica* genotypes were observed both years, with 'DeAnza', 'El Toro', and above all, 'Victoria' retained green colour better than 'Meyer'. These three cultivar were released by University of California, Riverside and the breeding program that developed 'DeAnza' and 'Victoria' specifically selected in part for grasses that had superior winter colour retention characteristics when compared to all commercially available (Gibeault et al. 1997). 'Emerald', a hybrid between Japanese lawn grass and manilagrass, showed better performance than 'El Toro' and 'Meyer', according to a previous report (Sifers et al. 1992). *Z. japonica* consistently exhibited a lower colour retention compared to fine-leaved genotypes, as a result of a faster entrance of dormancy.

The objective of a previous study conducted on these entries was to quantitatively determine basal growth temperatures, where stolon growth sensitivity to ground temperatures was found to be cultivar-specific (Pompeiano et al. 2008). The Japanese lawn grass genotypes that displayed higher growth arrest temperature ('Zenith' and 'Meyer' respectively with 11.3 ad 10.6 °C) in the present study had weak colour retention when compared with 'DeAnza', 'El Toro' and 'Victoria' (8.5, 10.1, and 9.6 °C). It is interesting to correlate this result to the fact that Japanese lawn grass generally exhibited less winter injury in the field and better freeze tolerance than Manilagrass (Patton and Reicher 2007), suggesting an improved cold acclimation mechanism during the onset of the dormancy.

Cultural practices have been used to extend the winter colour retention of zoysiagrass in late fall or early spring, showing different levels of success applying late-season nitrogen or iron (Dunn et al. 1993; Gibeault et al. 1997), and where application date also plays a critical role (Volterrani et al. 2010). The colour enhancement is largely due to new growth during the late fall, although the more susceptible plant is further exposed to frost and desiccation damage. In zoysiagrass, freeze tolerance response to late-season fertilization during cold acclimation and overwintering, and under natural conditions, control plants reached cold hardiness before, whereas the nitrogen treated ones were affected by a prolonged activity of the canopy (Pompeiano et al. 2011). Percent variations of standardized LT_{50} indicated that the 30-g m^{-2} N treated plants were most responsive throughout the experiment. They were less cold hardy in October, and, surprisingly, less sensitive in March. Under controlled conditions, zoysiagrass freeze tolerance was observed to be negatively affected by N fertilization since 2 weeks after treatment, and also N source provided consistent differences in freeze tolerance (Pompeiano et al. 2013).

Winter dormancy is the main impediment to a wide acceptance of warm-season turfgrasses in the transition zone due to a loss of colour during the winter months. In the present study, zoysiagrasses differ markedly in their ability to postpone winter dormancy in the Mediterranean transition zone.

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