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Leaf physiological traits and their importance for species success in a Mediterranean grassland

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Abstract

We related leaf physiological traits of four grassland species (*Poa pratensis*, *Lolium perenne*, *Festuca valida*, and *Taraxacum officinale*), dominant in a Mediterranean grassland, to their origin and success at community level. From early May to mid-June 1999, four leaf samplings were done. Species originating from poor environments (*P. pratensis*, *F. valida*) had low carbon isotope discrimination (Δ), specific leaf area (SLA), leaf water and mineral contents, and net photosynthetic rate on mass basis (P_{mass}) but high chlorophyll content. The reverse traits were evident for the fast-growing species (*L. perenne*, *T. officinale*). Under the resource-limiting conditions (soil nitrogen and water) of the Mediterranean grassland, the physiological traits of *P. pratensis* and *F. valida* showed to be more adapted to these conditions leading to high species abundance and dominance.

Additional key words: carbon isotope discrimination; chlorophyll; leaf water content; nitrogen; photosynthesis; specific leaf area.

Introduction

Plants are grouped, according to their Relative Growth Rate (RGR), to fast- and slow-growing species with fast-growing ones being more adapted to rich environments while slow-growing ones succeed better in poor habitats (Poorter and Bergkotte 1992, van Arendonk and Poorter 1994, Reader 1998). Leaf anatomy and chemical composition are related to species RGR and considered to be a determinant factor for species adaptation to different environments (Lambers and Poorter 1992).

In general, fast-growing species are characterized by high specific leaf area (SLA, the ratio of the leaf area per g leaf dry mass) or low areal leaf mass (ALM, the ratio of leaf dry mass per leaf area unit, *i.e.* the SLA reciprocal), high water content, high nitrogen content, and low investment in the photosynthetic machinery. Their leaves have a short life span and show lower construction cost [kg(glucose) kg^{-1}(dry matter)], compared to the slow-growing species (Poorter and Remkes 1990, Poorter *et al.* 1990, Poorter and Bergkotte 1992, Shipley 1995, Poorter and Evans 1998, Baruch and Goldstein 1999). Furthermore, significant differences exist between fast- and slow-growing species in leaf anatomy and chemical composition, as previously mentioned. Specifically, fast-growing species are characterized by thick leaves with wide mesophyll and high contents of minerals and organic N-compounds. Slow-growing species have more rigid cell walls and invest more in secondary compounds such as lignin and (hemi-)cellulose (van Arendonk and Poorter 1994, van Arendonk *et al.* 1997). Species from resource-rich environments are directed to resource capture and thus show higher net photosynthetic rates (P_N) and photosynthetic nitrogen use efficiency (PNUE, the amount of assimilated CO_2 per unit leaf nitrogen), compared to species from poor environments (Lambers and Poorter 1992, Poorter and Evans 1998).

In water limited environments (poor environments), high water use efficiency (WUE) has been considered as a trait contributing to species success at the community level (Ehleringer 1993, Tsialtas *et al.* 2001). Since carbon isotope discrimination (Δ), which depends on the ratio of intercellular and ambient partial pressures of CO_2 (p_i/p_a), is negatively related to transpiration efficiency for C_3 species (Farquhar *et al.* 1989, Turner 1997), it is expected that species would be differentiated in their Δ values

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depending on their habitat of origin.

Much work has been conducted on identifying and correlating physiological traits with plant growth rates under controlled conditions or by selecting leaf samples from different environments. However, data are lacking on the relation of physiological traits of coexisting species with their success at community level.

The aim of this work was to relate species success at community level with specific leaf physiological traits of four species, two from poor (*Poa pratensis*, *Festuca valida*) and two from rich environments (*Lolium perenne*, *Taraxacum officinale*). The study was conducted in Mediterranean grassland whose species composition was studied for three years (Tsialtas *et al.* 2001).

Materials and methods

Study site and plant sampling: The study site was an old, natural grassland (40º26'N, 22º00'E, 1 115 m a.s.l.) dominated by C_3 perennial grasses [*Poa pratensis* L., *Lolium perenne* L., *Festuca valida* (Uechtr.) Penzes] and forbs (*Trifolium repens* L., *Taraxacum officinale* Weber *ex* Wigg, *Plantago lanceolata* L., *Achillea millefolium* L.). The area is grazed by cattle and sheep from early May to autumn. For a more detailed description of the site see Tsialtas *et al.* (2001).

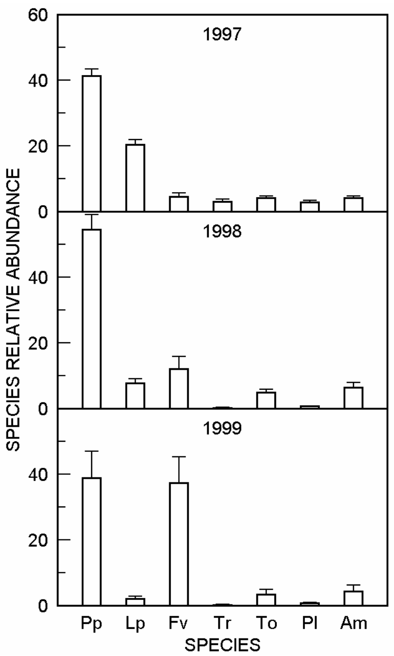

Fig. 1. Relative abundance of the dominant species of the grassland. Pp = *Poa pratensis*, Lp = *Lolium perenne*, Fv = *Festuca valida*, Tr = *Trifolium repens*, To = *Taraxacum officinale,* Pl = *Plantago lanceolata*, and Am = *Achillea millefolium* (from Tsialtas *et al.* 2001).

An area 15×25 m, representative of the grassland, was selected in early May 1996. Four species (*P. pratensis*, *L. perenne*, *F. valida*, and *T. officinale*) constituted 70 % (in 1997) to 80 % (in 1999) of the above-ground biomass of the community. *L. perenne* and *T. officinale* are species from resource-rich habitats (habitats with high availability of soil nutrients and water) while *P. pratensis* and *Festuca* spp. are usually found in poor sites (Poorter and Remkes 1990, Vázquez de Aldana and Berendse 1997). *P. pratensis* was the dominant species, *L. perenne* showed an abrupt decrease of its participation in the community's biomass between 1997 and 1999, and the reverse was evident for *F. valida*, while dandelion's (*T. officinale*) relative abundance was low and constant between years (Fig. 1).

In the 1999 growing season, leaf samples of the four species mentioned above were collected on four successive sampling dates (9 and 23 May, 6 and 20 June). From each species, three randomly selected samples of 30 intact, fully expanded, sun-lit leaves were collected each time. They were put in firmly closed plastic bags, laying on an iced chest, and transferred to the laboratory in a portable refrigerator. They were divided into two equal sub-samples. One was used for physiological trait determinations and the other was oven-dried (75 °C for 48 h).

Leaf parameter determinations: Chlorophyll (Chl) *a* and *b* contents were determined in four samples of fresh leaves. Extraction was made according to Hill *et al.* (1996). Extracts were assayed in a UV-Vis spectrophotometer *(Perkin-Elmer 403*, Wellesley, MA, USA) at 664 and 647 nm and Chl contents were calculated using the equations of Leegood (1993).

Specific leaf area (SLA, the ratio of leaf area to the dry mass of the leaf) and area leaf mass (ALM, the ratio of leaf dry mass to leaf area) were determined in three samples of three leaves each (a total of 9 leaves). Leaf area was measured on the fresh samples using an image analysis system (*DT-Scan*, *Delta-T Devices*, UK). Then, fresh leaf samples were weighed and oven-dried at 75 °C for 48 h to constant mass. The leaf water content (LWC) was calculated according to Garnier (1992).

The oven-dried (75 °C, 48 h) sub-sample of 15 leaves was ground to fine powder using a *KIKA A10* mill (*Labortechnik*, *Janke and Kunkel*, Staufen, Germany). In three aliquots of ~1 mg, the leaf N content and the ^{13}C natural abundance ($\delta^{13}C$) were determined at the Scottish Crop Research Institute at Dundee, UK, on a continuous flow isotope ratio mass spectrometer (*CF-IRMS*, *Europa Tracer Mass*, Crewe, England) which was interfaced with an elemental analyzer (*Roboprep*) for on line sample preparation. $\delta^{13}C$ [‰] was calculated as:

$$[(R_{sample} - R_{standard})/R_{standard}] \times 10^3,$$

where R_{sample} and $R_{standard}$ are the ratios $^{13}C/^{12}C$ of leaf sample and standard, respectively. The universally accepted standard is Pee Dee Belemnite (PDB) limestone (a fossil formation of *Belemnitella* spp. found in South Carolina). Carbon isotope discrimination (Δ) was calculated as:

$$\Delta\ [‰] = \delta_{air} - \delta_{plant}/[(1 + \delta_{plant}) \times 10^{-3}$$

where the $\delta^{13}C$ of air CO_2 is ca. –8 ‰.

The mass-based photosynthetic capacity, P_{mass} [μmol kg^{-1} s^{-1}] was calculated using the formula given by Reich *et al.* (1998):

$$\log_{10}P_{mass} = -2.03 + 2.02\ \log_{10}N_{mass} + 1.43\ \log_{10}SLA - 0.551\ (\log_{10}N_{mass}\ \log_{10}SLA),$$

where N_{mass} is the mass-based leaf N [g kg^{-1}] and SLA is the specific leaf area [m^2 kg^{-1}].

Leaf ash content was determined by dry combustion of 0.3–0.5 g samples, for at least 4 h at 500 °C in a muffle furnace and by weighing the residue. There were three replications for each species and sampling occasion.

Soil water content: On each sampling date, four randomly selected soil samples were taken from the depths of 5 and 15 cm using an auger. Soil water content was estimated gravimetrically after drying at 105 °C for 24 h.

Calculations and statistical analysis: Leaf nitrogen content [g(N) m^{-2}] was estimated as: N_{mass}/SLA. We used the average values of three replications of N_{mass} for leaf nitrogen calculations.

All parameters measured or calculated were subjected to ANOVA as a Completely Randomized Design with species and leaf sampling dates as main factors. Means were compared by LSD test ($p = 0.05$). Statistical analyses were conducted using the *MSTAT-C* (version *1.41*, Crop and Soil Sciences Department, Michigan State University, USA) statistical package.

Results

Soil moisture content tended to decrease with the progress of growing season but a rainfall in mid-June increased the available soil water content. Soil water content was higher at the surface soil layer (Fig. 2).

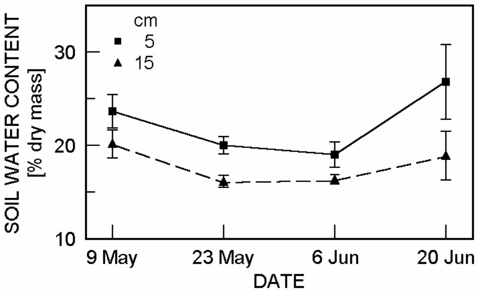

Fig. 2. Seasonal changes of soil water content at two soil depths (5 and 15 cm). Means ± SE, $n = 4$ per sampling occasion.

The species fell into two groups based on Δ (δC) [‰] time pattern. The two species from resource-rich environments, *L. perenne* and *T. officinale*, had always higher Δ (20.46–21.40 %) than the two species from poor habitats, *P. pratensis* and *F. valida* (17.20–19.28 %) (Fig. 3*A*). Similar were the trends of the ash content in the leaves with the means for *T. officinale* being the highest and statistically different from the means for all the other species throughout the sampling period (Fig. 3*B*).

T. officinale also showed a higher P_N, statistically similar to those of *L. perenne* from the 23 May to the 6 June. *L. perenne* showed a rise of P_N, on the 23 May followed by a gradual decrease. *F. valida* exhibited the same time pattern but at a lower value level. On the other hand, P_{mass} of *P. pratensis* leaves increased on the final sampling day (20 June), after insistent reduction, similarly to *T. officinale* (Fig. 4*A*). Chl content per unit area was significantly higher in *Festuca* (150.61–268.75 mg m^{-2}) followed by *Poa*. In the other two species the Chl contents fluctuated between 38.12–92.96 mg m^{-2} (Fig. 4*B*). The ratio of Chl *a/b* was 1.37–2.15. This ratio for the last sampling (20 June) of both *L. perenne* and *T. officinale* was similar and higher than those of *P. pratensis* and *F. valida* (Fig. 4*C*).

The species also fell into two groups, as previously described for Δ values, based on N and water contents of leaves. Specifically, *P. pratensis* and *F. valida* had, generally, higher ALM and N content per leaf area and lower water content (LWC) compared to *L. perenne* and *T. officinale* (Fig. 5). Both ALM and N contents were the highest and showed a pronounced decrease (by 46 and 37 %, respectively) between the two first sampling days (9 and 23 May) in *Festuca* leaves. On the contrary, the

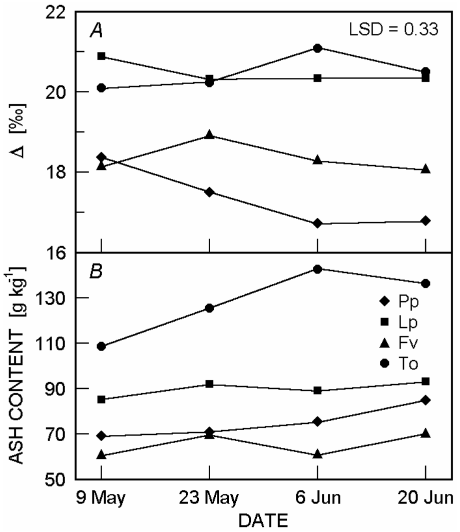

Fig. 3. Variation of Δ values and ash content in leaves of the four species for the four samplings conducted. LSD of means was calculated for $p = 0.05$. Pp = *Poa pratensis*, Lp = *Lolium perenne*, Fv = *Festuca valida*, and To = *Taraxacum officinale*.

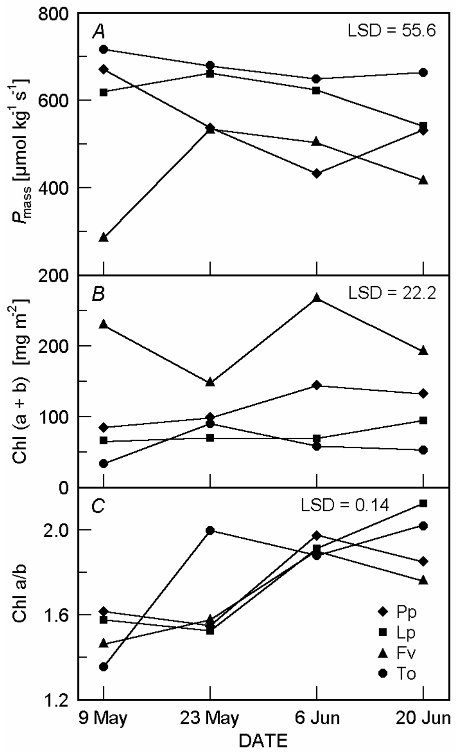

Fig. 4. Variation of mass based photosynthetic capacity (P_{mass}), chlorophyll (Chl) (a+b) content per unit leaf area, and Chl *a/b* ratio in the leaves of the four grassland species. LSD of means was calculated for p=0.05. Pp = *Poa pratensis*, Lp = *Lolium perenne*, Fv = *Festuca valida*, and To = *Taraxacum officinale*.

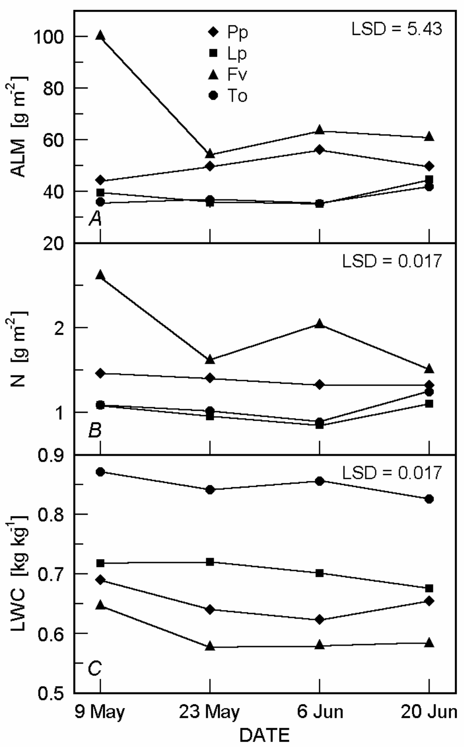

Fig. 5. Seasonal variation of ALM, N content, and leaf water content (LWC) in the leaves of the four species studied. LSD of means was calculated for p=0.05. Pp = *Poa pratensis*, Lp = *Lolium perenne*, Fv = *Festuca valida*, and To = *Taraxacum officinale*.

same species had the lowest water content, in contrast to *Taraxacum* whose water content was always highest and significantly different compared to the other three species.

Significant linear correlation was found between the parameters studied, except for the Chl *a/b* whose fluctuation was independent of the other traits. Δ and SLA were correlated only to each other (r = 0.73, p<0.01). On the other hand, ALM (1/SLA) was related to the ash content, P_{max}, Chl per leaf area, and Chl *b* content. All the other traits were mutually correlated positively or negatively, with few exceptions (Table 1).

Discussion

Species leaf physiological traits were analogous to their habitat of origin. Species derived from poor environments (*P. pratensis*, *F. valida*) were characterized by low Δ with a decreasing trend toward summer. Low Δ, *i.e.* high water use efficiency (WUE) is an adaptive trait to low water availability environments (Ehleringer 1993, Tsialtas *et al.* 2001). On the other hand, species from resource rich environments showed the highest Δ values with small seasonal changes. On dry sites, species with high WUE were more fitted if they had large leaves (Arntz *et al.* 2000). In the present work, this was evident for *P. pratensis* since it had the lowest Δ values but a higher SLA compared to *F. valida* which followed *P. pratensis* as regards Δ values. Virgona and Farquhar (1996) found negative relationship between RGR and Δ amongst sunflower (*Helianthus annuus*) genotypes but, contrary to our results, there was no significant correlation between Δ and SLA. However, a positive correlation between Δ and SLA was reported by Thumma *et al.* (1998) in *Stylosanthes scabra*. Based on that, it could be supposed that Δ is positively related to RGR since Wright and Westoby (2001) found a strong positive correlation between RGR and SLA. Accordingly, *T. officinale* and *L. perenne,* for which high Δ was recorded, probably show high RGR. These two species had also high leaf ash contents. Accumulation of minerals in plant leaves is a trait related to species growth rate. In accordance with our results, fast growing species (species from rich environments) have a high mineral content in their leaves (Poorter and Bergkotte 1992).

T. officinale and *L. perenne* had the highest P_{mass} and showed small plasticity for this trait during the sampling

Table 1. Correlation coefficients and significance for the leaf physiological traits determined. *, **, ***, ****: significant coefficients at $p<0.05$, <0.01, <0.001, or <0.0001, respectively; P_{mass}: photosynthetic capacity per area base [μmol kg^{-1} s^{-1}]; Chl$(a+b)_{area}$ [g m^{-2}]; SLA: specific leaf area [cm^2 kg^{-1}]; ALM: areal leaf mass [g m^{-2}]; N: leaf nitrogen [g m^{-2}]; Chl/N: Chl $(a+b)$ per N content [mg kg^{-1}]; LWC: leaf water content [kg kg^{-1}].

	Ash	P_{mass}	Chl$(a+b)_{area}$	Chl *a*	Chl *b*	Chl$(a+b)$	Chl *a/b*	SLA	ALM	N	Chl/N	LWC
Δ [‰]	ns	ns	ns	ns	ns	ns	ns	0.73^{*}	ns	ns	ns	ns
Ash		0.64^{**}	-0.69^{**}	-0.53^{*}	-0.71^{**}	-0.60^{*}	ns	ns	-0.62^{**}	-0.65^{**}	-0.56^{*}	0.89^{***}
P_{mass}			-0.82^{****}	-0.53^{*}	-0.62^{*}	-0.57^{*}	ns	ns	-0.89^{***}	-0.75^{***}	-0.69^{**}	0.73^{**}
Chl$(a+b)_{area}$				0.83^{****}	0.90^{****}	0.87^{****}	ns	ns	0.84^{****}	0.84^{****}	0.82^{***}	-0.75^{***}
Chl *a*					0.93^{***}	0.99^{***}	ns	ns	ns	ns	0.94^{***}	-0.70^{**}
Chl *b*						0.97^{***}	ns	ns	0.56^{*}	0.61^{*}	0.90^{***}	-0.81^{***}
Chl$(a+b)$							ns	ns	0.47	0.51^{*}	0.94^{***}	-0.75^{***}
Chl *a/b*								ns	ns	ns	ns	ns
SLA									ns	ns	ns	ns
ALM										0.95^{****}	0.47	-0.57^{*}
N											ns	-0.55^{*}
Chl/N												-0.76^{***}

period, in accordance with previous reports (Poorter *et al.* 1990, Poorter and Evans 1998). High P_{mass} may be a trait of adaptation to rich environments, but it is disadvantageous when plants grow in stressful environments (Arntz and Delph 2001). Species from poor habitats invest more in Chl content as a mechanism to cope with the shortage of soil resources such as water and nitrogen (Westbeek *et al.* 1999). Indeed, *F. valida* and *P. pratensis* had significantly higher Chl content in their leaves and a negative relationship between P_{mass} and Chl content was evident. Also, P_{mass} was negatively correlated to ALM, N content, and Chl/N ratio showing that P_{mass} was restricted by increasing leaf rigidity, by diffusion of N in the leaf, and by investing more N in Chl synthesis (Poorter and Bergkotte 1992, van Arendonk and Poorter 1994, Poorter and Evans 1998). However, P_{mass} was promoted by the increased LWC which according to Garnier and Laurent (1994) means higher investment in assimilatory tissue, *i.e.* mesophyll.

Although Mendes *et al.* (2001) reported no plasticity to light or season for Chl *a/b* ratio under Mediterranean conditions, we found significant increases of Chl *a/b* toward the summer period. According to Kitajima and Hogan (2003), increase of Chl *a/b* is an indication of plant acclimation to N limitation and high irradiance, conditions occurring during spring to summer period under Mediterranean climates. Since no relationship between Chl *a/b* and leaf N was found, the increases of Chl *a/b* could be attributed to the increasing irradiance toward summer (Kull and Kruijt 1998).

In conclusion, the most abundant species (*P. pratensis* and *F. valida*) were characterized by traits related to species from poor environments such as high investment in Chl or low Δ values, P_{mass}, SLA, and LWC. These findings reflect low RGR and, consequently, high persistence in stressful environments. The other two species (*L. perenne* and *T. officinale*), restricted to low relative abundances in the community, had high Δ values, P_{mass}, SLA, and LWC but a low leaf Chl content. These traits characterize species from resource-rich environments and are related to high RGR. These species show a high competitive ability but under stressful environments like the Mediterranean ones, their success in the community is limited (Lambers and Poorter 1992, Poorter and Bergkotte 1992, van Arendonk and Poorter 1994).

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