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Changes of the photosynthetic behaviour in annual C_3 species at late successional stage under environmental drought conditions

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Abstract

Differences in structural, physiological, and biochemical features between C_3 and C_4 species resulted in different water-use efficiencies and different adaptations to climate. This paper aimed at investigating, at a late successional stage, the water-use efficiency of two forage species, *Dichanthium ischaemum* and *Dasypyrum villosum*, which exhibit different growth forms (perenial, annual) and photosynthetic mechanisms (C_4 and C_3, respectively). The annual C_3 species *Avena fatua,* at an early successional stage, was included in our experiments to contrast its behaviour against *D. villosum.* The experiment was conducted during the growing season in low-elevation grasslands of North Greece. Midday leaf water potential, net photosynthetic rate, transpiration rate and stomatal conductance were measured. Instantaneous water-use efficiency (WUE) and intrinsic water-use efficiency (WUE_i) were calculated in *D. ischaemum, D. villosum*, and *A. fatua.* The results suggest that, under natural rainfall conditions, the annual C_3 grass species *D. villosum* exhibits a similar WUE with higher values of WUE_i than the perennial C_4 species *D. ischaemum* at late stage of succession on the low elevation Mediterranean grasslands. Moreover, *A. fatua* at an early successional stage, exhibited different photosynthetic behaviour than *D. villosum* at a late successional stage. These findings indicate that the annual C_3 species *D. villosum* under drought and at a late successional stage seems to modify the WUE obtaining values similar to those of C_4 species. The extent to which the ecophysiological characteristics of *D. villosum* are environmentally or intrinsically determined remains to be answered.

Additional key words: instantaneous water-use efficiency; intrinsic water-use efficiency; net photosynthetic rate; stomatal conductance; water stress.

Introduction

Water shortage and high air temperature influence plant gas exchanges for shorter or longer periods during the year (Filella *et al.* 1998, Gratani *et al.* 2008) particularly in arid and semiarid areas. Native plants have developed an array of adaptations to drought, resulting in a high diversity of growth forms, photosynthetic mechanisms and life spans (Llorens *et al.* 2004).

In Mediterranean grasslands, plants can be categorized according to their photosynthetic mechanism into C_3 and C_4 types that bear different structural, physiological, and biochemical features with different water-use efficiencies and adaptations to climate (Ehleringer *et al.* 1997, Wang 2004). Normally, C_4 plants have higher photosynthetic rates than C_3 ones under high-irradiance and high-temperature conditions (Ehleringer and Monson 1993). Nevertheless, some C_3 species can tolerate extreme temperatures, light, and drought and exhibit photosynthetic rates similar to C_4 species (Niu *et al.* 2005).

Stomatal conductance (g_s) and net photosynthetic rate (P_N) depend upon conditions such as solar radiation, leaf temperature, vapour pressure deficit (VPD), soil and plant water/mineral nutrient availability (Massonnet *et al.* 2007). Also, in short term and at leaf scale, stomatal contractions regulate trade offs between P_N and transpiration rate (E) and hence WUE (Tambussi *et al.* 2007). WUE is defined as the ratio between carbon gain and water loss at the leaf level (Bacon 2004), and represents a reliable index of plant survival under water-deficit conditions (DaMatta *et al.* 2003). This index varies among plants with different photosynthetic mechanisms, morphological and ecological characteristics. Environmental factors such as humidity,

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Abbreviations: *E* – transpiration rate; g_s – stomatal conductance; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; T_a – air temperature; VPD – vapour pressure deficit; WUE – instantaneous water-use efficiency; WUE_i – intrinsic water-use efficiency; Ψ – leaf water potential.

temperature, exposure to sunlight, and severity of water stress can also affect WUE (Hou *et al.* 2007).

On the other hand, intrinsic WUE_i, defined as the ratio of P_N to g_s (Comstock and Ehleringer 1992), is less dependent upon instantaneous environmental conditions (air temperature, atmospheric humidity), and is under tight genetic control in many species (Massonnet *et al.* 2007). Therefore, it reflects more closely plant physiological properties (Yu *et al.* 2005). Experimental efforts have been realised in order to investigate the differential effects of water-use on C_3 and C_4 grasses, without revealing a consistent pattern (Ibrahim *et al.* 2008). Moreover, the interpretation of these experiments in terms of photosynthetic mechanisms is difficult because the ancient origin of C_4 photosynthesis, into the grass family, has led to significant divergence between most C_3 and C_4 clades (Barker *et al.* 2001, Christin *et al.* 2008).

D. villosum (L.) P. Candargy, *A. fatua* L. and *D. ischaemum* (L.) Roberty are three monocot species which functionally differ in their carbon fixation and life span. *D. villosum* and *A. fatua* are annual C_3 grasses, very common in low-elevation semiarid Mediterranean grasslands growing together with the perennial C_4 grass species *D. ischaemum* and *Chrysopogon gryllus* (Karatassiou 1999). The fact that *D. villosum* (C_3 annual grass) dominates at a late succesional stage in higher percentage (23.25–12.40%) than the perennial C_4 species *D. ischaemum* (7.87–6.48%) (Karatassiou 1999, Karatassiou and Koukoura 2009) is itself an interesting phenomenon. This is paradoxical since *D. villosum* is an annual C_3 species, theoretically realizing an *r*-selection strategy (Gurevitch *et al.* 2002), and therefore, of lower competitive strength under semiarid conditions without grazing pressure at late successional stages compared to C_4 perennial species such as *D. ischaemum*. In fact, even the domination of *D. villosum* at a late successional stage raises questions.

The aims of this paper were: (*1*) to compare the water-use efficiency of two forage species, *D. ischaemum* and *D. villosum* that exhibit different growth forms (perennial, annual) and photosynthetic mechanisms (C_4, C_3); (*2*) to test whether the high participation of *D. villosum* in the vegetation at a late successional stages is due to a higher water-use efficiency as compared with *D. ischaemum*; and (*3*) to test whether the behaviour of C_3 plants differed in different successional stages. We also included in our experimentation *A. fatua,* a C_3 annual grass species predominating in grasslands at an early successional stage.

Materials and methods

Study area and plants: The experiment was carried out under natural conditions during 1994 on a low-elevation Mediterranean grassland. The climate of the experimental area is typical of the Mediterranean semiarid type with the dry period from late spring through summer (April to September). The mean monthly air temperature ranges from 4.4°C (January) to 24.7°C (August) and the mean annual precipitation from 1983 to 1993 was 409 mm. Photosynthetic photon flux density (PPFD), VPD and air temperature (T_a) were measured on dates and times when plant physiological parameters were measured as averages of ten measurements (Table 1).

The experimental area was located near Melissohori (40°58'N, 28°01'E), 25 km north of Thessaloniki, northern Greece, at an altitude 170 m a.s.l. All data were collected from two fenced (10 m × 20 m) neighboring areas with the same orientation (north-east) and slope (10–12%). Before fencing, the first area was an abandoned crop field (old field, grassland A) at an early successional stage dominated by annual grasses such as *A. fatua*, legumes, and forbs. The second area was a permanent grassland that had been grazed for at least twenty years prior to fencing (grassland B). This area was at a late successional stage, dominated by the perennial grasses (*D. ischaemum, C. gryllus*), the annual grass *D. villosum*, and legumes species. Also, there were a few patches of shrubs (for a detailed description *see* Papanastasis 2007, Karatassiou and Koukoura 2009). The two areas had been excluded from grazing since 1989 and prior to fencing both areas were freely grazed by sheep and goats.

Table 1. Midday photosynthetic photon flux density (PPFD), vapour pressure deficit (VPD), and air temperature (T_a) of the experimental area during the period of the measurements. Values present means ± SE (n = 10).

	PPFD [μmol m^{-2} s^{-1}]	VPD [kPa]	T_a [°C]
20 April	643.8 ± 11.2	1.37 ± 0.05	23.8 ± 0.3
12 May	1584.1 ± 17.9	1.52 ± 0.02	25.0 ± 0.2
21 May	1660.5 ± 33.5	3.15 ± 0.09	32.0 ± 0.4
4 June	1767.7 ± 19.3	3.61 ± 0.06	34.1 ± 0.7
20 June	1946.4 ± 13.6	4.17 ± 0.01	36.3 ± 0.6

Physiological measurements: Leaf water potential (Ψ), P_N, E, and g_s were measured for the dominant species: the annual C_3 grass *A. fatua* from grassland A and the annual C_3 grass *D. villosum* and the perennial C_4 grass *D. ischaemum* from grassland B. All measurements were conducted around solar noon (12:00–14:00 h) on clear days in approximately 15-day intervals, on mature, intact, and fully expanded upper leaves. For each parameter, the presented values are averages of five plants.

Leaf water potential for all species was measured using the pressure-bomb technique (Scholander *et al.* 1965, Turner 1988). At the same time, leaves similar to those used for the leaf-water-potential measurements were sampled to measure P_N, E, and g_s at ambient CO_2 concentration [approximately 370 μmol(CO_2) mol^{-1}] with a portable photosynthesis system (*LI-6200, LiCor Inc*., Lincoln, NE, USA). The WUE and the WUE_i were calculated as the ratio P_N/E and P_N/g_s, respectively (Bacon 2004, Yu *et al.* 2005, Guo *et al.* 2006).

Statistical analysis: To determine differences in the ecophysiological response of the three species during the growing season we performed a two-way analysis of variance (*ANOVA*) on all parameters studied (Steel and Torrie 1980). Means were compared using the LSD test. Regression analysis was used to determine the relationship between P_N, E, g_s, WUE, WUE_i, and Ψ for all species. All statistical analyses were performed using the *SPSS* statistical package *v. 16.0* (*SPSS Inc*., Chicago, IL, USA).

Results

The two-way analysis of variance reveals significant differences ($P<0.05$) among the species for all tested parameters (*i.e.* P_N, E, g_s, Ψ, WUE, and WUE_i). Also, time (season) significantly affected ($P<0.0001$) all parameters. The interaction between time (during the growing season) and species was significant, indicating differential physiological response of the three species throughout the season ($P<0.0001$).

For all species Ψ showed a declining trend during the growing season (Fig. 1). However, *D. ischaemum* presented significantly higher values of Ψ compared with *D. villosum* and *A. fatua*. Only at the early vegetative growth stage (mid-April), *D. villosum* exhibited significantly higher value of Ψ compared to other species. *A. fatua* completed its biological cycle in the beginning of June while *D. villosum* at the end of June.

Patterns of E and g_s in relation to Ψ (Fig. 2*A,B*) were similar for *D. ischaemum* and *D. villosum*. As the growing season proceeded E and g_s decreased and presented the lower values at the end of June when the lowest value of Ψ was recorded. The two species stabilized their E at about 7 mmol m^{-2} s^{-1} after Ψ reached values <–3.0 MPa. *D. ischaemum* had significantly higher g_s for most of the growing season. Only at the lowest value of Ψ, that is, –3.5 MPa and –3.8 MPa for *D. villosum* and *D. ischaemum*, respectively, the two species presented the same g_s (approximately 0.18 mol m^{-2} s^{-1}). On the other hand, *A. fatua* exhibited completely different behaviour. With the progression of the growing season and the decrease in Ψ, E remained relatively stable, while g_s showed a significant decrease. *A. fatua* exhibited significantly higher E and g_s than *D. ischaemum* or *D. villosum* during most part of the growing season.

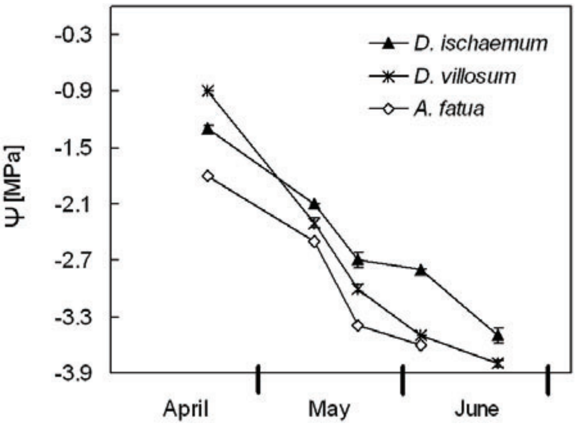

Fig. 1. Seasonal patterns of leaf water potential (Ψ) in *D. ischaemum*, *D. villosum*, and *A. fatua.* Values present means ± SE (n = 5).

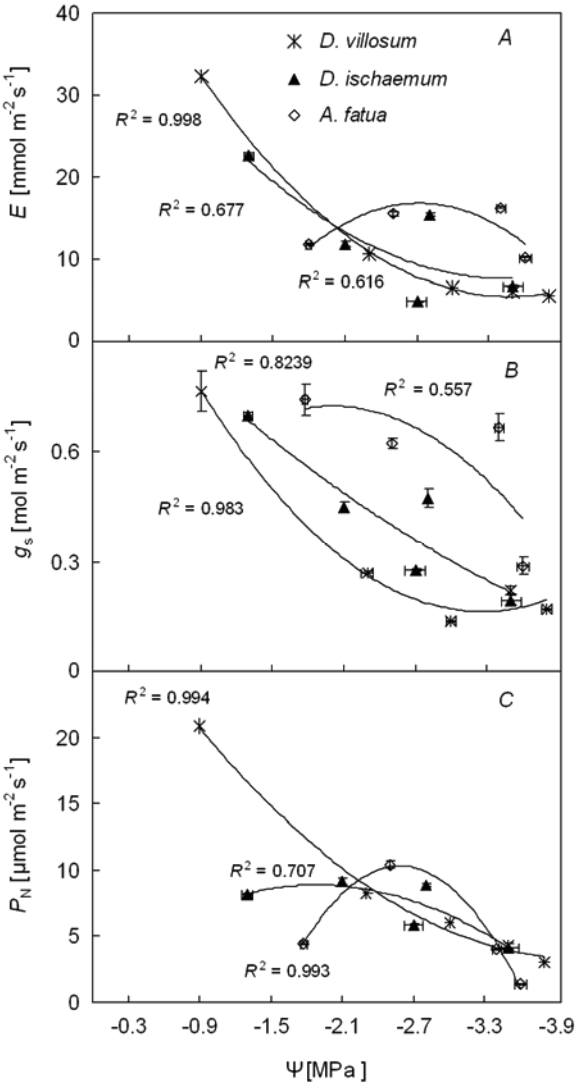

Fig. 2. The relationship between leaf water potential (Ψ) and *A*: transpiration rate (E), *B*: stomatal conductance (g_s), and *C*: net photosynthetic rate (P_N) for *D. ischaemum*, *D. villosum*, and *A. fatua.* Values present means ± SE (n = 5).

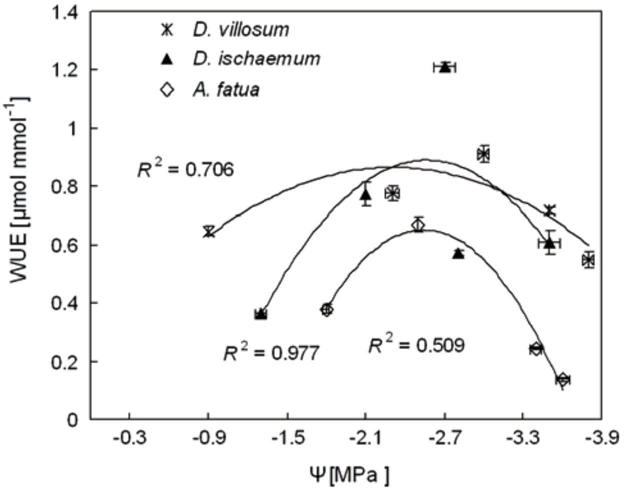

Fig. 3. The relationship between leaf water potential (Ψ) and instantaneous water-use efficiency (WUE) for *D. ischaemum*, *D. villosum*, and *A. fatua.* Values present means ± SE ($n = 5$).

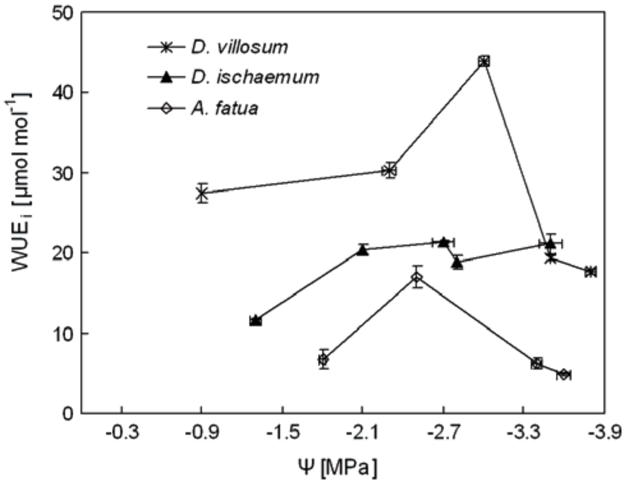

Fig. 4. The relationship between leaf water potential (Ψ) and intrinsic water-use efficiency (WUE$_i$) for *D. ischaemum*, *D. villosum*, and *A. fatua.* Values present means ± SE ($n = 5$).

P_N in relation to Ψ followed different patterns in all species (Fig. 2*C*). At high Ψ values *D. villosum* exhibited higher P_N than the other two species. However, as the growing season proceeded and Ψ decreased, the P_N of *D. villosum* decreased. In contrast, the P_N of *A. fatua* was increasing up to the beginning of May, and then sharply decreased (Fig. 2*C*). There were significant differences ($P<0.001$) between the above species in their P_N. In *D. ischaemum* P_N showed lower variability in relation to Ψ compared to other two species (Fig. 2*C*). Nevertheless, the relationship between P_N and Ψ was significant ($P<0.01$).

WUE in *D. villosum* showed a stabilizing trend during the growing season while that in *D. ischaemum* presented an increase from April to May (high Ψ) followed by a decrease and stabilization during June (low Ψ). At high Ψ, *D. villosum* presented higher WUE than *D. ischaemum* and *A. fatua* but as the growing season proceeded, the WUE of *D. ischaemum* increased, tending to present the same value with *D. villosum*, especially at Ψ lower than –2.1 MPa (Fig. 3). *D. villosum* and *D. ischaemum* presented approximately the same mean value of WUE during the study period (0.719 ± 0.029 μmol mmol^{-1} and 0.706 ± 0.066 μmol mmol^{-1}, respectively), while *A. fatua* exhibited a significantly lower value (0.358 ± 0.055 μmol mmol^{-1}). WUE$_i$ followed a completely different pattern (Fig. 4), being significantly higher ($P<0.0001$) in *D. villosum* and significantly lower in *A. fatua* for most part of the growing season. *D. villosum* and *D. ischaemum* presented similar WUE$_i$ only at very low Ψ. The mean values of WUE$_i$ for *D. villosum, D. ischaemum*, and *A. fatua* were 27.74 ± 2.18 μmol mol^{-1}, 18.69 ± 0.875 μmol mol^{-1}, and 8.67 ± 1.46 μmol mol^{-1}, respectively.

Discussion

The results suggest that under natural rainfall conditions at low-elevation Mediterranean grasslands and at a late stage of succession, annual C_3 grass species exhibit (*1*) a similar pattern of WUE and higher values of WUE$_i$ compared to C_4 perennial species, and (*2*) a different photosynthetic behaviour than C_3 species at early successional stages. It seems that the values of g_s of *D. villosum* are low and rather stable under natural drought conditions, at late stage of succession. This probably enables *D. villosum* to increase WUE at levels comparable to C_4 species. It is also worth noting that the two indexes of water-use efficiency provide different results.

It was expected that *A. fatua* and *D. villosum* would follow the same photosynthetic mechanism and present the same photosynthetic capacity; however, this was not the case. Also the patterns of photosynthetic characteristics varied considerably among species and seem to be independent of their photosynthetic mechanism. *A. fatua* as a representative annual C_3 grass species completed its biological cycle in the first days of June. On the contrary, the growth of *D. villosum* (C_3) and *D. ischaemum* (C_4) continued until the end of June when water was more limiting and temperature higher. Probably during succession in natural rainfall conditions, other physiological attributes, a life form, an allocation pattern, particular environmental conditions (drought) or the previous history (grazing) can often play a more important role than the photosynthetic mechanism alone (Pearcy *et al.* 1981, Pearcy and Ehleringer 1984). This is supported by the same P_N and WUE values in *D. villosum* and *D. ischaemum* during most part of the growing season, especially early in summer at very low Ψ. These two species seem to have the same ability to grow in semiarid Mediterranean grasslands at the late stage of succession.

It is well accepted that C_4 plants are adapted to warm/dry climatic conditions, and exhibit superior WUE than C_3 plants (McNaughton 1991, Nelson *et al.* 2004, Nayyar and Gupta 2006). However, the fact that observed WUE in *D. villosum* was approximately as high as that in *D. ischaemum*, could be attributed to low and constant g_s (Jones 1992) in the former, which is likely to account for the high WUE, especially during June. On the other hand, the paralleled decrease of E and g_s in relation to Ψ in *D. villosum* and *D. ischaemum* and the high positive correlation between E and g_s (r = 0.976, 0.954 respectively, P<0.01) could partly explain the decrease of E as a function of g_s. However, in *A. fatua* there was no correlation between E and g_s. Probably, the sensitivity of the stomatal apparatus increased at the late successional species in response to the decrease in Ψ and increase in VPD. The high WUE in *D. villosum* could be probably attributed to a lower E due to a better stomatal control, a mechanism that is usually occured in perennial species under water deficit conditions and not in annual ones (Gurevich *et al.* 2002, Karatassiou *et al.* 2009).

Contrary to instantaneous WUE, results regarding WUE_i clerly suggest a differentiation among the three species. Obviously the higher WUE_i in *D. villosum* and the lower one in *A. fatua* indicate that in late successional species the lower g_s could drastically reduce water loss, while it could decrease P_N proportionally less than in early successional species (Llambí *et al.* 2003). The high WUE_i of *D. villosum* could be attributed to g_s or to higher P_N or even in a combination of both (Tambussi *et al.* 2007). However, specific microclimatic characteristics may thus enable the C_3 species to persist, and even outcompete the C_4 species (Kubien and Sage 2003).

It seems that *D. ischaemum* is more adapted to drought than *D. villosum* or *A. fatua* because it showed relatively stable P_N during the growing season. It should be noted that the photosynthetic values observed in all species appeared reduced due to photoinhibition, which, however, most probably did not affect the photosynthetic machinery (Osmond 1994, Larcher 2003, Taiz and Zeiger 2006). Decline of P_N during the dry season seems to be a less serious factor for the drought-adapted species (Lambers *et al.* 2008). However, the same P_N in *D. villosum* and *D. ischaemum* for most part of the study period (especially at the end of spring and early summer) indicates that *D. villosum,* despite its annual C_3 chracteristics, is likely to be a drought-adapted species as well. The high photosynthetic capacity of *D. villosum* under natural drought conditions could probably explain its high percentage in the grassland at a late successional stage. Whether the ecophysiological characteristics that *D. villosum* exhibited were environmentally or intrinsically determined, remains to be answered.

According to Zámečník and Holubec (2005), *D. villosum* plants from semiarid Mediterranean areas presented relatively low carbon discrimination, an indication of a high WUE. It probably had stomata open and/or contained a high activity of Rubisco even under very dry conditions (Zámečník and Holubec 2005). Changing from one photosynthetic mechanism to the other may be induced by stress conditions (Niu *et al.* 2005) and may evolve through adaptations to environmental conditions (Ehleringer *et al.* 1997). The possibility that *D. villosum* is exhibiting C_4 photosynthetic mechanism and/or C_4 photosynthetic enzymes should be further determined. Further data to rigorously test the above hypothesis should include studies on leaf anatomy and carbon isotope ratios.

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