

Seasonal Changes in Leaf Tissue Rehydration of One Annual and Two Perennial Grass Forage Species Induced by Bioclimate

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Abstract

Bioclimate signifies the continuous interplay between plants and climate factors (primarily drought) and has a direct impact on the water relations and the duration of the rehydration process in water stressed plants. To explore the association between bioclimate and water physiology of forage species in semi-arid Mediterranean grasslands, we determined the seasonal variation in leaf water potential, turgid weight and relative water content in wild growing *Dactylis glomerata* L., *Bromus inermis* Leyss (perennial) and *Bromus sterilis* L. (annual) during the growing season. The study was conducted at the farm of the Aristotle University of Thessaloniki. The results of the current study reveal that *B. sterilis* maintained high levels of water potential most probably by accelerating its biological cycle and decreasing water content because it fails to sustain turgidity. *Dactylis glomerata* and *B. inermis* presented even higher water contents than *B. sterilis* for the same water potential. *Dactylis glomerata* exhibited substantially higher water potential and content than *B. inermis* by keeping the rehydration duration stable. The extensive creeping rhizome seems to allow *B. inermis* to sustain high values of water potential and content possibly ensuring turgidity. Regardless of the grass species the duration of rehydration ranged from 2.5 to 3.5 hours throughout the growing season. Our findings demonstrate that (a) *D. glomerata* and *B. inermis* are better adapted to Mediterranean semiarid conditions than *B. sterilis* and (b) turgid weight in Mediterranean forage species can safely be determined after a rehydration period of 3,5 hours.

Keywords: relative water content, turgid weight, semiarid grassland, water deficit, water potential

Introduction

Water in a natural vegetation is directly associated with environmental factors, especially climate (Trejo *et al.*, 2011). The constant interaction between plant communities and climate factors is known as bioclimate (Vlachos, 2006; Savo *et al.*, 2012). One of the most important climatic factors affecting plant viability is the annual amount and especially the annual distribution of precipitation. This is due to the physical and chemical properties of water, which render it essential for all physiological processes taking place in plants (Lambers *et al.*, 2008). In fact, water availability is the main factor limiting plant growth and production during the growing season, and regulating plant diversity in an ecosystem (Henkin *et al.*, 2010; Mitra *et al.*, 2013).

Over the last few decades, major human interventions in the natural environment have brought significant changes to global climate (Trejo *et al.*, 2011). In the aftermath of greenhouse effect intensification, the increase of average air temperature, along with the reduction and uneven distribution of precipitation have caused drought frequency and severity to become more intense (IPCC, 2007; Xu *et al.*, 2010; Savo *et al.*, 2012). Drought causes water deficit in plant tissues, which in turn leads to the disruption (stress) of their standard physiological and metabolic functions

(Kusaka *et al.*, 2005). Mild water deficit induces a decline in plant growth rate, as a consequence of a decrease in cell turgidity and the restriction of photosynthesis. Severe water deficit results in the accumulation of osmolytes in plant tissues, the formation of air cavities in the conduit tissue, even the deconstruction of protoplasm, as plants reach the permanent wilting point (Aranjuelo *et al.*, 2011). Water deficit mostly affects plants in arid and semiarid regions of the world (Wood, 2005), such as those lying along the coast of the Mediterranean Sea, where the drought (or xerothermic) period coincides with the growing season of many important forage species (Galmés *et al.*, 2007; Savo *et al.*, 2012).

Plants address severe xerothermic periods by employing certain adaptive mechanisms, which ensure survival by avoiding and/or tolerating drought (Karatassiou *et al.*, 2009; Kostopoulou *et al.*, 2010). These mechanisms include the development of both short-term and long-term adjustments in plant organisms, such as the emergence of specific phenotypic characteristics, and changes in anatomy and morphology, as well as physiology (Lambers *et al.*, 2008; Hayano-Kanashiro *et al.*, 2009; Blum, 2011). However, drought adaptation mechanisms may differ among plant species and genotypes (Mohsenzadeh *et al.*, 2006; Norton *et al.*, 2007; Aslam *et al.*, 2013). Annual species (ephemerals) mainly use drought avoidance mechanisms that

may be facilitated by phenotypic plasticity, in order to quickly complete their biological cycle so that occurrence and growth coincide with periods of abundant rainfall (Chaves *et al.*, 2002; Moreno *et al.*, 2008). Perennial species, on the other hand, may utilize both mechanisms of drought avoidance and/or tolerance (Volaire, 2008).

Drought adaptation mechanisms contribute greatly to the improvement of water relations in plants (Grzesiak *et al.*, 2006) and to the preservation of relatively constant water content in plant tissues (Iannucci *et al.*, 2002; Keyvan, 2010). Through the process of transpiration, soil water flows through plants and into the atmosphere, along a gradient of decreasing water potential that forms between the three components of Soil-Plant-Atmosphere Continuum (Blum, 2011). Therefore, maintaining high water content and also high water potential values in plant tissues ensures plant growth and reproduction during a xerothermic period (Galmés *et al.*, 2007; Rahimi *et al.*, 2010).

There are two hydrodynamic parameters that demonstrate the magnitude of the resistance plants exhibit towards water deficit: leaf water potential (Ψ) and Relative Water Content (RWC) (González and González-Vilar, 2003; Blum, 2005; Dong *et al.*, 2011). Water potential reveals the level of water availability in a plant, thus its state of health (Wood, 2005). A force that has a large impact on water potential and leads plant tissues to lose water is Vapor Pressure Deficit (VPD), which signifies the transpirational demand of the atmosphere (Lambers *et al.*, 2008). Relative water content represents the relationship between water deficit in plant tissues and the absolute amount of water needed for them to reach full turgidity (González and González-Vilar, 2003; Ganji Arjenaki *et al.*, 2012). Hence, RWC values depict the relative volume of plant cells and reveal the extent of water deficit effects on a plant (Mohsenzadeh *et al.*, 2006).

Moreover, drought adaptation mechanisms also enhance the ability of plants to achieve rehydration when the opportunity occurs (Siopongco *et al.*, 2006). Rehydration process consists of two phases of water intake. In Phase I, a rapid passive absorption of water takes place, in order for the plant to replenish the water deficit in its tissues and reach turgidity. In Phase II, water intake continues at a very low rate that can hardly be defined. The Phase I can be completed in a relatively short period of time, which may vary depending on plant species and genotype, lasting from 2 to 4 hours (Chan and Fowler, 1992); however, turgid weight of stressed plant tissue can safely be determined only after a 24-hour rehydration period (Barrs and Weatherley, 1962). Yet, the minimum duration of rehydration needed by Mediterranean grasses in order to achieve turgidity as well as its seasonal variation remains unknown.

Rehydration leads to the recovery of standard plant function, as it contributes to the restoration of water relations, the maintenance of photosynthetic efficiency and the reactivation of biological activity in plant tissues (Mitra *et al.*, 2013). The recovery rate and its extent depend primarily on the intensity and duration of water deficit in plant tissues, on climatic conditions (precipitation, solar radiation) during present and especially past xerothermic periods (as they could have resulted in the restriction of meristem), on plant species, genotype, age, phenological stage of development and leaf structure, and also on the capacity for water intake that plants exhibit at the end of the drought period (Volaire *et al.*, 1998; Chaves and Oliveira, 2004; Siopongco *et al.*,

2006; Blum, 2011). Bajji *et al.* (2000), Pinheiro *et al.* (2004), Miyashita *et al.* (2005) and Rahimi *et al.* (2010) report that the great intensity and duration of water deficit and the small amount of time given for rehydration restrict the recovery of water in annual species. The above studies demonstrate a wide range of Ψ rescue (from 24% to 100%). Yet there are annual species in which the rate of water recovery varied considerably depending on the species and genotype (Grzesiak *et al.*, 2006; DaCosta and Huang, 2007; Izanloo *et al.*, 2008; Hayano-Kanashiro *et al.*, 2009). Similar effects of the intensity and duration of water deficit, and duration of rehydration on the recovery of water relations have been reported in perennial species (Awal and Ikeda, 2002; Mohsenzadeh *et al.*, 2006; DaCosta and Huang, 2007; Xu *et al.*, 2010). In these studies, RWC recovery ranged from 70% to 100%.

This paper aimed at examining how bioclimate regulates the seasonal changes in rehydration duration of annual and perennial grasses. To address the above objective we studied (a) the effect of bioclimate on water relations of three grass forage species, (b) the variance of rehydration duration in annual and perennial grass forage species throughout their life cycle, and (c) the ecophysiological mechanisms that grass forage species employ to address bioclimate changes.

Materials and Methods

Study area and climate

The experiments were conducted on the natural vegetation at the farm of the Aristotle University of Thessaloniki (Northern Greece) (longitude: 40°31'91", latitude: 23°59'58", altitude: 6 m a.s.l.), during the growing season of 2011. The climate of the study area is characterized as Mediterranean semiarid with cold winters following the bioclimatogram of Emberger (Dafis, 1986), which is typical of the Mediterranean semiarid type (Emberger *et al.*, 1963), with a mild meso-Mediterranean sub climate of cold winters, hot summers and a long dry period. The mean annual precipitation is 443 mm, and the mean annual temperature is 15.5 °C.

The Vapor Pressure Deficit (VPD), air temperature (T) and relative humidity (RH) were measured using a microclimatic sensor (Novasima MS1, Novatron Scientific Ltd, Horsham, UK) during the period of measurements. The values of VPD, RH and T given are averages of six measurements that have been recorded on the date and time that plant physiological parameters were measured (Figs. 1 and 2). Details regarding the experimental plan and measurements taken are given below.

Physiological measurements

Leaf water potential (Ψ), Relative Water Content (RWC) and Turgidity Weight (TW) were measured in two perennial grass species *Dactylis glomerata* L. and *Bromus inermis* Leyss and one annual grass species *Bromus sterilis* L. Both perennial species were chosen because they are important forage species in the low elevation Mediterranean grasslands, since they produce high quality forage during spring and early summer (Papanastasis and Karagiannakidou-Papadimitriou, 1983). The annual grass species was included in the study as a reference species in order to contrast its ecophysiological response (drought avoidance) against the two perennial species (drought avoidance and/or tolerance).

Seasonal measurements were performed on clear sunny days

from 11:00am to 13:00pm at approximately 15-day intervals at the beginning of the growing season. As drought was becoming intense (middle-end of the growing season), measurements were conducted at short time intervals ranging from approximately 10 in the middle to 2 days at the end of the growing season. Two plants of each species were randomly selected along a 20-metre line. Three lines and a total of 6 plant species have been considered for each species (Cornelissen *et al.*, 2003). All measurements were obtained from mature, intact and fully expanded upper leaves that were randomly selected from each species. In each day for each parameter, the values presented are averages of six plants.

Leaf water potential (Ψ) was measured using the pressure chamber technique (Koide *et al.*, 1991; González, 2003). Relative water content (RWC) and turgid weight (TW) were determined on 5mm discs from leaves similar in age and orientation that came from the same plants that were used in measuring Ψ . The leaf discs were transferred to the laboratory where their fresh weights (FW) were determined in a saturated atmosphere. Subsequently, the discs were allowed to float in distilled water at 4-6 °C for 24 h, until they reached turgid weight (TW). The same discs were later dried at 70-80 °C for a period of 24-48 h, and were weighed again, in order to determine dry weight (DW). Relative Water Content was calculated using the following formula: $RWC = \frac{FW - DW}{TW - DW} \times 100$ (Iannucci *et al.*, 2002; González and González-Vilar, 2003).

Turgid weight (TW) of the leaf disks was also determined with the use of the improved relative turgidity technique by Barrs and Weatherley (1962). At first, the leaf disks were weighed directly in order to obtain the fresh weight. Afterwards, the disks were left to float in distilled water (fully saturated atmosphere) and remained in the dark, at a temperature of 4-6 °C. Every 30 min the disks were weighed until they were fully rehydrated and had reached a constant TW (Phase I of the rehydration process). The disks then remained floating in fully saturated atmosphere for 24 h and were ultimately reweighed to confirm their previously measured TW (Chan and Fowler, 1992).

Statistical analysis

In order to determine differences in the ecophysiological responses of the three species (first factor) during the growing season (second factor), we performed analysis of variance (ANOVA) on all parameters studied, using the univariate general linear model (dependent variables: Ψ , RWC and TW), the multivariate general linear model (dependent variable Ψ together with dependent variable RWC) and the bivariate (correlation) model (covariant Ψ together with covariant VPD, covariant RWC together with covariant VPD). The standard error of all means was also calculated while the same analyses and significance level for all tests were set at $\alpha = 0.05$ (Steel and Torrie, 1980). Following a significant one way ANOVA that ran at each measurement date, for each parameter, means of the three species were separated with the Fisher's PLSD test ($p \leq 0.05$). All statistical analyses were carried out using the SPSS v. 22.0 statistical package (IBM Corp., Armonk, NY, USA).

Results

Climatic conditions and water relations

The drought (xerothermic) period in the experimental area began in late May 2011 and lasted until approximately mid autumn. Mean annual temperature was 14.9 °C and annual precipitation reached 338 mm. During the experimental period, mean monthly temperature and mean monthly precipitation ranged from 12.8 °C (April 2011) to 26.9 °C (July 2011), and from 73.90 mm (May 2011) to 0.51 mm (July 2011) respectively (Fig. 1).

Statistical analyses revealed significant differences among the three forage species ($p \leq 0.0001$) for all tested parameters (Ψ , RWC and TW). Time of measurements also influenced significantly Ψ , RWC and TW ($p \leq 0.0001$). Moreover, the interaction between time of season (measurement) and species was significant ($p \leq 0.01$), suggesting that the species examined exhibited different physiological responses to water deficit conditions.

During the experimental period VPD values gradually increased ($p \leq 0.0001$) from 0.78 kPa in April 2011 to 2.06 kPa in July 2011. Ψ followed a declining trend in all species during the growing season, which for *D. glomerata* and *B. inermis* was facilitated by the negative correlation between VPD and Ψ ($r = -0.524$, $p \leq 0.05$ and $r = -0.823$, $p \leq 0.01$ respectively). Early in

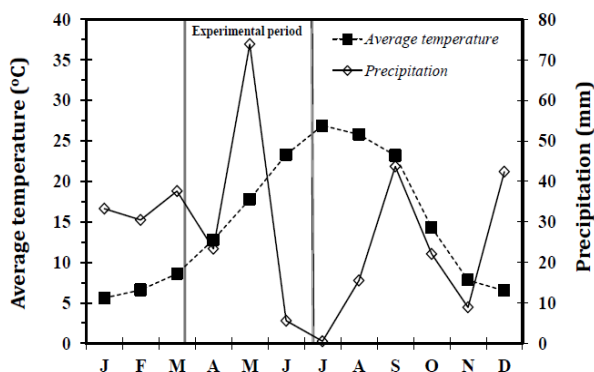


Fig. 1. Climatic diagram of the experimental area during 2011

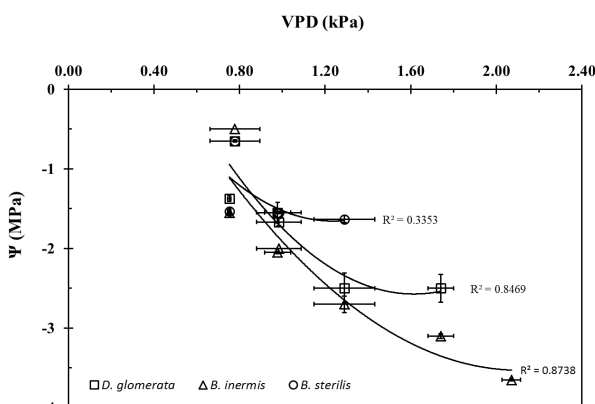


Fig. 2. The relationship between midday leaf water potential (Ψ) and vapor pressure deficit (VPD) for *Dactylis glomerata*, *Bromus inermis* and *Bromus sterilis*. Values present means \pm SE ($n = 6$)

spring, when VPD was low, the three species presented approximately similar values of Ψ (around -0.60 MPa), with *B. inermis* expressing the highest value. However, Ψ values differed among the species ($p \leq 0.01$) as the growing season progressed and VPD increased (Fig. 2).

The annual species *B. sterilis* presented a considerable decrease in Ψ during the early vegetative stages, as opposed to flowering, when it exhibited little variation in Ψ values. *Bromus sterilis* completed its biological cycle at the beginning of the xerothermic period in early June, at higher Ψ (-1.63 MPa) values ($p \leq 0.01$) and earlier than the other two species (Fig. 3). As far as the two perennial species are concerned, *D. glomerata* expressed higher Ψ values ($p \leq 0.01$) than *B. inermis* during the growing season. Specifically, *D. glomerata* presented an initial gradual decline in Ψ , followed by a temporal stabilization, which lasted from mid-May until the beginning of the xerothermic period. At the same time, *B. inermis* followed a similar course in Ψ , although having a steeper initial decrease than *D. glomerata*. As the xerothermic period progressed, Ψ decreased dramatically in *D. glomerata* until its water status was finally stabilized (-2.50 MPa) and preserved up to the end of the biological cycle in late June. Likewise, as the xerothermic period progressed, Ψ greatly decreased in *B. inermis* thus expressing the lowest value of the three species studied (-3.65 MPa) (Fig. 3).

Relative water content followed a declining pattern in all species during the growing season. In *B. inermis*, this declining trend was facilitated by the existing negative correlation between RWC and VPD ($r = -0.466$, $p \leq 0.01$). In early vegetative stages (April), substantial differences in RWC were detected among the three species ($p \leq 0.001$), with *B. inermis* presenting the highest (93.7%) and *B. sterilis* the lowest (91.01%) value. *Bromus sterilis* completed its biological cycle at a water deficit of 20.41%. From mid-May to late June, *D. glomerata* exhibited significantly higher values of RWC ($p \leq 0.001$) than *B. inermis*. Later in the growing season, *D. glomerata* managed to complete the fruiting stage, during the xerothermic period, by presenting the lowest RWC of the growing season (73.6%). *Bromus inermis* was able to continue its growth until water deficit reached a value of approximately 20% (Fig. 4) in the mid July.

The relationship between Ψ and RWC reveals that at a high value of Ψ (approximately -1.0 MPa), *B. inermis* presented the

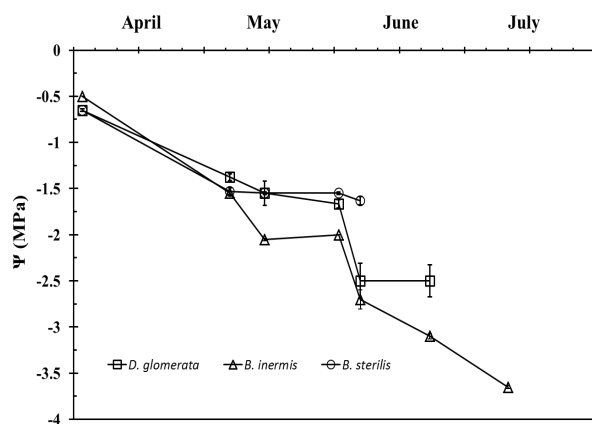


Fig. 3. Seasonal patterns of leaf water potential (Ψ) in *Dactylis glomerata*, *Bromus inermis* and *Bromus sterilis*. Values present means \pm SE ($n = 6$)

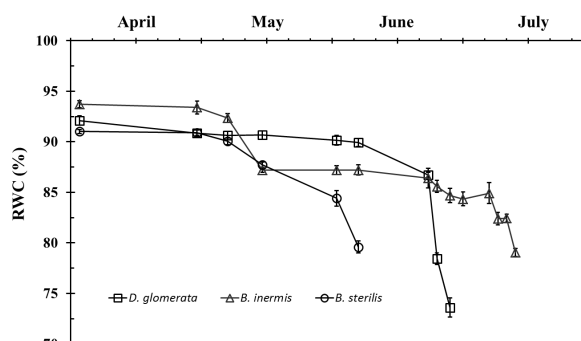


Fig. 4. Seasonal patterns of relative water content (RWC) in *Dactylis glomerata*, *Bromus inermis* and *Bromus sterilis*. Values present means \pm SE ($n = 6$)

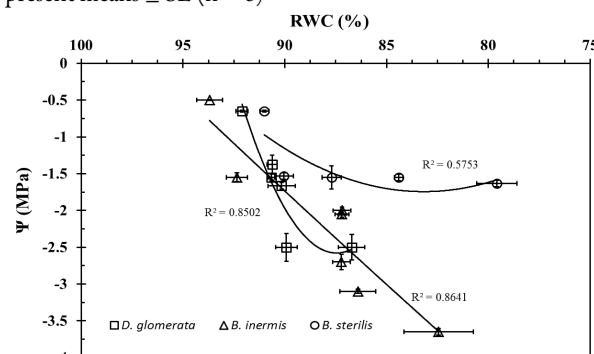


Fig. 5. Relationships between leaf water potential (Ψ) and relative water content (RWC) for *Dactylis glomerata*, *Bromus inermis* and *Bromus sterilis*. Values present means \pm SE ($n = 6$)

highest RWC, followed by *D. glomerata*, while *B. sterilis* presented the lowest RWC. At lower values of Ψ , *D. glomerata* presented the highest RWC, while *B. inermis* showed a substantially lower RWC (Fig. 5).

Rehydration -Floating time

Dactylis glomerata generally kept higher relative water content ($p \leq 0.001$) (Fig. 4) and presented a much more stable rehydration (turgidity) during the growing season compared with the other two species (Figs. 6-9). The seasonal variation of rehydration duration in *B. sterilis* and *B. inermis* (Figs. 6-10) was generally consistent with the seasonal changes in their water content and water status (Figs. 3 and 4).

In April, when environmental conditions were favorable (average temperature 12.2 °C, VPD 0.78 kPa) (Fig. 1, 2) and the RWC of all species high (Fig. 4), *B. inermis* achieved turgidity sooner (2.5 h) than *B. sterilis* and *D. glomerata* (Fig. 6). The changes in leaf disk fresh weight over successive measurements were not affected by the grass species ($p > 0.05$). Floating time was a significant predictor of the changes in leaf disk fresh weight ($p \leq 0.001$). The interaction between plant species and floating time was not significant as well ($p > 0.05$) indicating no differences in the rehydration duration among the three species.

During May, the high rate of precipitation (73.9 mm) did not interrupt the upward trend of VPD (0.98 kPa) and the rise of temperature (18.7 °C), which in turn affected the relationship of Ψ and RWC in all three species. Plant species had no significant effect on the changes in leaf fresh weight ($p > 0.05$),

contrary to floating time ($p \leq 0.001$). The interaction between plant species and floating time was also significant ($p \leq 0.05$) highlighting variations in the rehydration duration among the three species. The perennial species *D. glomerata* and *B. inermis* were fully rehydrated after 3.5 hours in mid-May, while the annual species *B. sterilis* maintained rehydration duration stable during the growing season (Fig. 7).

At the beginning of the xerothermic period (early June), the grass species was not a significant predictor of the changes in leaf disk fresh weight over successive measurements ($p > 0.05$) but floating time was ($p \leq 0.001$). The interaction between plant species and floating time was also significant ($p \leq 0.001$) suggesting variations in the rehydration duration among the three species. Both *B. sterilis* and *B. inermis* expressed reduced rehydration duration (Fig. 8), although water deficit was increased; average temperature in June ranged from approximately 20 °C to 25 °C, while precipitation was 5.59 mm and VPD reached 1.71 kPa.

However, as water deficit intensified from late June to mid-July due to extreme temperatures (26.9 °C), low precipitation (0.55 mm) and the amplification of VPD (2.01 kPa), the changes in leaf disk rehydration weight over successive measurements were not influenced by the grass species ($p > 0.05$). Floating time had a significant effect on the changes in leaf disk rehydration weight ($p \leq 0.001$). The interaction between plant species and floating time was significant as well ($p \leq 0.001$)

suggesting variations in the rehydration duration between the two perennial species. *Bromus inermis* required more time to restore its water losses during the fruiting stage, completing its biological cycle in the middle of the xerothermic period with maximum rehydration duration of 3.5 h (Figs. 9 and 10).

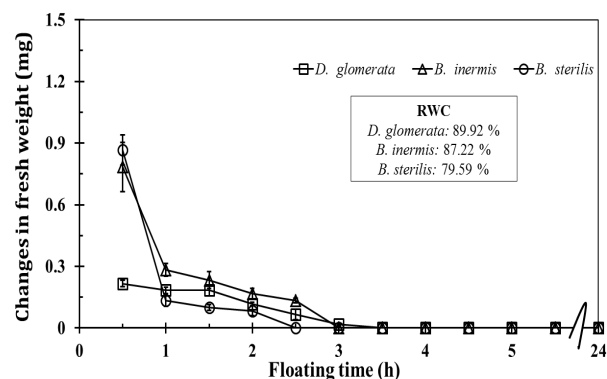


Fig. 8. Fresh weight changes (grX1000) of floating leaf disks obtained from *Dactylis glomerata*, *Bromus inermis* and *Bromus sterilis* as a function of time at the beginning of the xerothermic period (early June). Values present means \pm SE (n = 6)

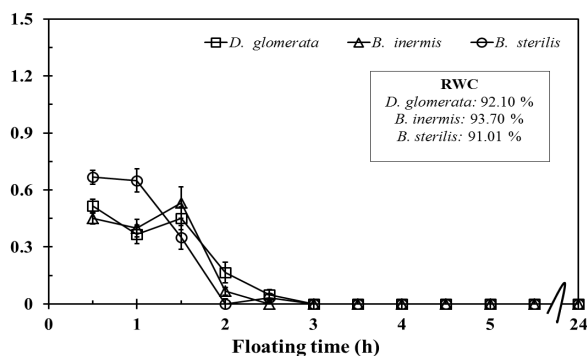


Fig. 6. Fresh weight changes (grX1000) of floating leaf disks obtained from *Dactylis glomerata*, *Bromus inermis* and *Bromus sterilis* as a function of time in April. Values present means \pm SE (n = 6)

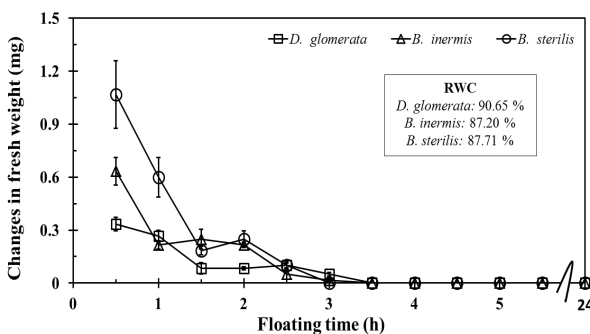


Fig. 7. Fresh weight changes (grX1000) of floating leaf disks obtained from *Dactylis glomerata*, *Bromus inermis* and *Bromus sterilis* as a function of time in mid-May. Values present means \pm SE (n = 6)

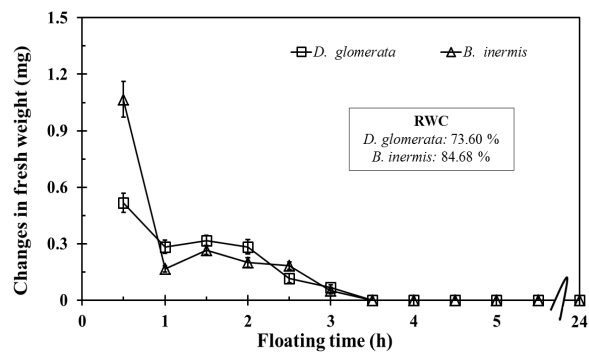


Fig. 9. Fresh weight changes (grX1000) of floating leaf disks obtained from *Dactylis glomerata*, *Bromus inermis* and *Bromus sterilis* as a function of time at the middle of the xerothermic period (late June). Values present means \pm SE (n = 6)

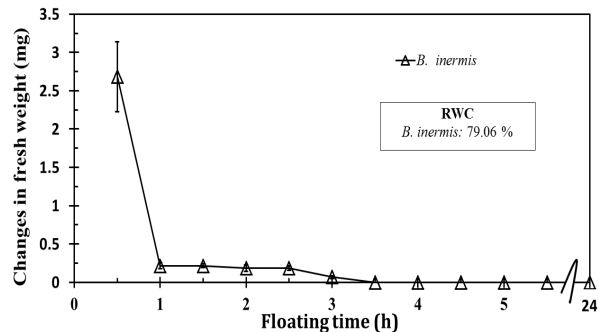


Fig. 10. Fresh weight changes (grX1000) of floating leaf disks obtained from *Dactylis glomerata*, *Bromus inermis* and *Bromus sterilis* as a function of time at the middle of the xerothermic period (mid-July). Values present means \pm SE (n = 6)

Discussion

In the study area, plant vegetative and flowering stages coincide with the xerothermic period. During the experimental period, the rise in average temperature and the simultaneous reduction in the amount of precipitation led to a gradual increase in vapor pressure deficit (VPD) (Figs. 1 and 2), especially in late spring and early summer. Our results are similar to Galmés *et al.* (2007) and Lambers *et al.* (2008). Elevated VPD equals increased demands in transpiration, which induce an extensive water loss by plants, a considerable reduction in available soil water and the establishment of intense water deficit in plant tissues (González and Reigosa, 2003; Chaves and Oliveira, 2004). The effect of elevated VPD on the water status of the three species studied was confirmed by the seasonal changes in both leaf water potential (Ψ) (Fig. 3) and relative water content (RWC) (Fig. 4).

In general, the three species considered in our study presented different physiological responses to water deficit conditions. During the growing season, the seasonal variance in Ψ (Fig. 2, 3) revealed a continuous variance in the water status of the three species, which must have strongly affected their growth. The variance in Ψ among the three species could be due to differential adaptation and survival capacity under water deficit conditions (Chaves *et al.*, 2002; Ganji Arjenaki *et al.*, 2012). At the end of its biological cycle, the annual forage species *B. sterilis* presented a higher Ψ than the perennial species (Fig. 3). Similar results were obtained by Volaire (2003), who report that the annual forage species *Hordeum vulgare* completed its life cycle at a higher Ψ than the perennial species *D. glomerata*. Also Galmés *et al.* (2007) indicate that, compared to other perennial species, the annual forage species *Diploaxis ibicensis* managed to maintain a higher Ψ during the final stages of its biological cycle. The maintenance of a higher Ψ value suggests that *B. sterilis* must have employed specific mechanisms in order to cope with drought conditions (Kusaka *et al.*, 2005; Aslam *et al.*, 2013).

As far as the perennial species are concerned, *D. glomerata* presented low Ψ values during the xerothermic period (Fig. 3), due to the negative correlation between its Ψ and VPD. Volaire and Lelièvre (2001) found that other genotypes of *D. glomerata*, which are more drought resistant (Currie, Medly and Lutetia), presented even lower Ψ values. During the same period, *B. inermis* expressed the lowest Ψ of all species studied (Fig. 3), results that concur with the negative correlation existing between VPD and Ψ for this species and also with those obtained by Karatassiou *et al.* (2011). Low Ψ can be consistent either with a high rate of transpiration and/or a restricted water intake and/or low hydraulic conductivity in plants (Iannucci *et al.*, 2002; Moreno *et al.*, 2008).

The seasonal pattern of RWC in the three species studied (Figs. 4 and 5) gives a more complete picture of their water status. During the growing season, the declining trend of RWC (Fig. 4) was proportional to the gradual increase of water deficit in the tissues of the three species (Keyvan, 2010). However, the three species exhibited varying RWC values (Fig. 4), as a result of their distinct ability to sustain stable water contents (Mamolos *et al.*, 2001). Towards the beginning and in the middle of the xerothermic period, when weather conditions became acute (Figs. 1 and 2), RWC was reduced in all species (Fig. 4) as a result of their lack of rehydration, which may be caused by the seasonal reduction in the available soil water (Machado and Paulsen,

2001; Mohsenzadeh *et al.*, 2006). With increasing water deficit, species maintaining higher water content are considered to be more drought-resistant (Keyvan, 2010; Ganji Arjenaki *et al.*, 2012). There are several *D. glomerata* genotypes that can withstand even higher water deficits (Voltaire, 2003). *Bromus inermis*, on the other hand, can maintain its water status unaffected for a long period of time (Karatassiou *et al.*, 2011).

The relationship between Ψ and RWC (Fig. 5) confirms that the three species have a diverse ability of water deficit resistance (Iannucci *et al.*, 2002). Higher Ψ and RWC values were determined in all species at the beginning of the experimental period (Fig. 3, 4, 5), when there was a considerable amount of soil water available for the plants (Fig. 1) (Lambers *et al.*, 2008; Dong *et al.*, 2011) and also due to low VPD (Fig. 2). On the contrary, lower Ψ and RWC values were recorded at the end of the growing season (Figs. 3, 4, 5), probably due to increased transpiration and the reduction of plant cell turgidity (Chimenti *et al.*, 2006).

Distinct responses to drought were observed between the annual *B. sterilis* and the perennial species, as expected, as well as between the two perennial species. As an annual C₃ species, *B. sterilis* grows and develops when weather conditions are favorable and ceases growing when water deficit becomes intense (Mohsenzadeh *et al.*, 2006; Karatassiou and Noitsakis, 2010). This is due to the fact that annual species exploit available soil water better in early spring, when there is less competition from other species (Moreno *et al.*, 2008; Galmés *et al.*, 2007). During the growing season, *B. sterilis* presented the smallest decrease in Ψ (Fig. 3, 5) and the largest decrease in RWC (Figs. 4 and 5) compared to the perennial species, highlighting its inability to achieve adequate rehydration and to preserve a good water status (Voltaire, 2003).

The perennial species were less affected by water deficit since they maintained growth and development until mid-summer, supposedly by making a better use of the limited available soil water during early flowering, at the beginning of the xerothermic period (Awal and Ikeda, 2002). Plants that preserve good and relatively constant water content at low Ψ values are deemed capable of handling water deficit better and maintaining usual growth rate (Blum, 2005; Voltaire, 2008; Rahimi *et al.*, 2010). The occurrence of low values of RWC for the same low values of Ψ (Fig. 5) means that *B. inermis* was less adequate to manage water deficit than *D. glomerata*.

Further analysis of the hydrodynamic parameters during the growing season of the three forage species suggested that different adaptation mechanisms (avoidance and tolerance) were deployed to deal with water deficit. These adaptation mechanisms protected plants from the adverse effects of water deficit and enhanced their survival chances; they contributed to the achievement and maintenance of high water content in plant tissues both during and after the xerothermic period, while they satisfactorily facilitated the continuation of plant growth (Chaves *et al.*, 2002; Blum, 2005; Hayano-Kanashiro *et al.*, 2009). The annual species *B. sterilis* completed growth and reached flowering and fruiting in a short time, supposedly by accelerating its biological cycle in order to avoid intense water deficit (Bajji *et al.*, 2000; Machado and Paulsen, 2001; Galmés *et al.*, 2007).

Dactylis glomerata responded well to water deficit by being capable of forming a sufficient water status in its tissues. This was

probably achieved through the deep root system that this species often develops in order to extract water from deeper soil layers (Chaves and Oliveira, 2004). Its adequate root volume possibly reinforced soil water intake (Kusaka et al., 2005; Xu et al., 2010; Aslam et al., 2013), enabling *D. glomerata* to maintain Ψ and RWC stable for longer periods of time. Volaire and Lelièvre (2001) also observed high water content values in three drought-resistant genotypes of *D. glomerata*, which had developed an extensive root system. However, Kostopoulou et al. (2010) point out that a rangeland ecotype of this species managed to avoid intense dehydration by reducing transpiration losses through the restriction of stomatal aperture. At the beginning of the xerothermic period, though presenting a higher RWC than the other species, *D. glomerata* exhibited signs of early drying. Norton et al. (2007) mark that in order to tolerate water deficit certain genotypes of *D. glomerata* coming from semiarid or arid regions often express a level of summer dormancy. Volaire (2003, 2008) confirmed that a drought-resistant *D. glomerata* genotype, presenting high Ψ and RWC values, exploited the same mechanism in order to tolerate water deficit. So it is possible that the species studied here applied the same tolerance mechanism as well.

However, *B. inermis* had a different response towards water deficit than *D. glomerata*. The species initially maintained favorable water content in its tissues and presented high values of Ψ and RWC (Figs. 3, 4, 5), probably by exploiting soil water reserves via its extended root system and extensive creeping rhizome (Karatasiou et al., 2011). However, when water deficit became intense, *B. inermis* possibly resisted drought through physiological adjustments, which include changes in cell wall elasticity and osmotic adjustment. These mechanisms help stressed plants maintain leaf turgidity, even when RWC is quite low (Mamolos et al., 2001; González and Reigosa Roger, 2003; Pinheiro et al., 2004; Dong et al., 2011).

Rehydration – Floating time

The time needed for the species to fully rehydrate fluctuated during growing season, as it was possibly affected by their genotype, stage of development, age, leaf structure, the observed climatic conditions (precipitation and solar radiation), the intensity and duration of water deficit and the water intake capability exhibited by the species (Siopongco et al., 2006; DaCosta and Huang, 2007). Rehydration duration did not vary greatly among the species during early vegetative stages (Fig. 6), when environmental conditions were favorable (Figs. 1 and 2). Nevertheless, *B. inermis* achieved turgidity sooner than the other two species (Fig. 6). Grzesiak et al. (2006) report similar results for *Triticale* sp. and *Zea mays*, which developed differential rehydration ability after being exposed to a short-term water deficit, by applying distinct adaptation mechanisms.

In mid-May, despite precipitation events, the perennial species needed more time to replenish water losses (Fig. 7); because of increased temperatures and VPD (Figs. 1 and 2), which led to the intensification of water deficit in plant tissues (Blum, 2005). However, rehydration duration was more extended in *B. inermis* (Fig. 7), suggesting that the increased VPD (Fig. 2) affected the ability of the species to maintain a favorable water content under intense water deficit (Izanloo et al., 2008), a fact that was confirmed by the significant negative correlation found between VPD and RWC of the species.

At the beginning of the xerothermic period, although VPD and water deficit increased significantly (Fig. 2), *B. sterilis* kept rehydration duration relatively short (Fig. 8) probably by using its root system to absorb recent water reserves on the soil surface (Chaves et al., 2002). *Bromus inermis* also managed to reduce rehydration duration (Fig. 8) by temporarily stabilizing its water content (Fig. 4) (Oliver et al., 1998; Karatasiou et al., 2009; Xu et al., 2010), through a decrease in Ψ (Fig. 3), possibly by the application of osmotic regulation (González and Reigosa Roger, 2003; Keyvan, 2010). At the same time, *D. glomerata* stabilized rehydration duration but failed to limit RWC decline (Fig. 4). This fact indicates that, at the final stages of its biological cycle, the species most likely did not succeed in fully saturating its tissues and recovering its normal function due to the onset of the decomposition of cell walls, the formation of air cavities in conduit tissue, and even the degradation of protoplasm (Bartoli et al., 1999). Mohsenzadeh et al. (2006) demonstrate that the perennial forage species *Aeluropus lagopoides* also failed to recover fully from the intense water deficit through rehydration. Similar results were obtained by Galmés et al. (2007), who state that the 24-h rehydration of the intensely water-stressed perennial species *Beta maritima* ssp. *maritima* and *Beta maritima* ssp. *macrostis* only led to a partial (70-80%) recovery of their water relations.

In the middle of the xerothermic period (Fig. 9), the extensive drought greatly hindered the recovery of water relations in the two perennial species (Figs. 4 and 5). As *D. glomerata* exhibited early drying signs, it is possible that it went into a state of dormancy during the last stage of its development (Chaves et al., 2002; Blum, 2005; Mitra et al., 2013). Unlike *D. glomerata*, *B. inermis* continued growing and producing under intense water deficit (Kapsali 2014). In order to prolong its development up to this point, *B. inermis* supposedly applied the appropriate adaptation mechanisms towards intense water deficit, such as osmotic regulation, which distinguishes the more drought-resistant species (Voltaire, 2003; Kusaka et al., 2005; Chimenti et al., 2006; Izanloo et al., 2008; Ganji Arjenaki et al., 2012).

Conclusions

In the Mediterranean region, bioclimate greatly affects water relations and the duration of rehydration process in forage species that are common in semiarid grasslands. Both leaf water potential (Ψ) and relative water content (RWC) varied greatly between the annual and perennial species studied, as well as between the two perennial species, revealing distinct physiological responses and the use of different adaptation mechanisms towards water deficit conditions. On the contrary, rehydration duration showed a limited variation among the three species, ranging from 2.5 hours during the first phenological stages to 3.5 hours during the xerothermic period. *Bromus sterilis* avoided intense water deficit conditions possibly by the acceleration of its biological cycle, which helped the species preserve its water status at a high level though it seems to have hindered its ability to maintain turgidity. *D. glomerata* and to a lesser extent *B. inermis* managed to maintain a favorable water balance under xerothermic Mediterranean conditions through physiological and/or morphological mechanisms and it is safe to determine their turgid weight after a rehydration period of four hours.

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