



## Perennial-like adaptation mechanisms of annual legumes to limited irrigation

P. Kostopoulou\*<sup>1</sup>, M.S. Vrahnakis<sup>2</sup>, T. Merou<sup>3</sup> and M. Lazaridou<sup>3</sup>

<sup>1</sup>Laboratory of Range Science (236), Faculty of Forestry and Natural Environment,  
Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece

<sup>2</sup>School of Forestry and Management of Natural Environment, TEI of Larissa - 43100, Karditsq, Greece

<sup>3</sup>School of Forestry and Management of Natural Environment, TEI of Kavala - 66100, Droma, Greece

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**Abstract:** The hydrodynamic behavior of two annual legumes (*Trifolium angustifolium* L. and *Onobrychis caput-galli* (L.) Lam.) under water shortage was studied in a rain shelter experiment. Seeds were collected from natural grasslands of northern Greece and were sown in pots. Two months after seedlings' emergence, full irrigation (up to field capacity) and limited irrigation (40% of field capacity) were applied. During the vegetative period the leaf water potential and the relative water content were measured at seven day intervals in both treatments. *T. angustifolium* retained the lowest values of  $\psi$  both under full (-0.11 to -1.78 MPa) and limited irrigation (-0.16 to -2.90 MPa), while the highest values in both cases were those of *O. caput-galli* (-0.05 to -0.5 MPa). The results suggested that *T. angustifolium* was the species mostly affected by limited water supply. *T. angustifolium* seemed to display adaptation mechanisms to drought similar to those of perennial plants. *O. caput-galli* displayed a more isohydric behavior, by not altering its water potential under limited irrigation.

**Key words:** Drought escape, Drought tolerance, Water savers, Water spenders, Mediterranean

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### Introduction

The expected increasing drought phenomena for Mediterranean areas necessitate the study of ecophysiological mechanisms of plant adaptation to drought. According to Turner (1979) three types of drought resistance exist: a) drought escape, which is the ability of a plant to complete its lifecycle before serious water deficit develops, b) drought tolerance with high tissue water potential, which is the ability of a plant to endure dry periods while maintaining a high plant water status (also referred to as drought avoidance) and c) drought tolerance with low tissue water potential, which is the ability of a plant to endure low tissue water potential.

The identification of adaptation mechanisms to drought is of considerable importance especially for legumes, since they play significant ecological and economic roles. These species are considered valuable plant resources for revegetation of disturbed areas (Pugnaire *et al.*, 1996; Unkovich *et al.*, 1998; Zahran, 1999). Legumes exhibit high growth potential in substrates poor in nitrogen; they increase soil fertility and can resist large disturbances (Caravaca *et al.*, 2003; Rodriguez-Echeverria and Perez-Fernandez, 2005; Nair *et al.*, 2008). Apart from that, legumes are considered worldwide as valuable economic plant resources for livestock by providing high quality forage material (Frame, 2005). Even though legumes seem to have a significant and multi-functional role, their hydrodynamic responses to impending environmental drought remain little known (Iannucci *et al.*, 2002). Given that the ecophysiological mechanisms, triggered by water shortage, differ among plants, the attempt to indicate legumes sensitive to water deficit becomes significant. Leaf water deficit, developing as a consequence of soil water depletion, alters many physiological processes with eventual consequences for

biomass and seed yield (Hsiao, 1973). Many of these changes represent adaptive responses by which plants cope with water stress.

The aim of the present study was to assess the changes of leaf water potential and relative water content to water shortage in two annual legumes, occurring naturally in the grasslands of northern Greece, and further to infer about their ability of growing and producing during drought periods using physiological mechanisms of adaptation.

### Materials and Methods

**Plant material:** Seeds from two annual legumes (*Trifolium angustifolium* L. and *Onobrychis caput-galli* (L.) Lam.) were collected in July 2005 from grasslands of northern Greece. The seeds were mechanically scarified prior to germination and a rain shelter experiment was conducted in December 2005.

**Growing conditions and irrigation treatments:** Seeds from each species were separately sown in pots of 0.6 m height and 0.5 m diameter, filled with a mixture of 50% soil, 10% perlite and 40% sand (medium mechanical composition, pH=7.6). When seedlings were of approximately 10 cm height above soil surface they were thinned to nine plants per pot. Two months after seedlings' emergence (April, 2006) two irrigation treatments were applied: a) full irrigation (up to field capacity) and b) limited irrigation (40% of field capacity).

**Ecophysiological measurements:** Air temperature and relative humidity during the vegetative period were recorded with the use of a microclimatic polysensor (Novasima ms1, Novasima AG, Zurich, Switzerland). Vapor pressure deficit (VPD, KPa), used as a parameter that offers insight about the microclimatic conditions, was calculated according to the formula proposed by Brenner (1996).

\* Corresponding author: [giotakos@for.auth.gr](mailto:giotakos@for.auth.gr)

Leaf water status measurements were conducted every week during the growing season (April to June) of 2006. At each measurement three fully expanded, mature leaves of each species were randomly selected at predawn (06:00) in order to measure the leaf water potential ( $\psi$ , MPa), using a pressure chamber (ARIMAD-2, A.R.I. Kfar Charuv, Israel) and the relative water content (RWC, %). For the calculation of RWC leaf samples were weighed ( $W_{act}$ ) and were floated in water for 8 hr at 4-6°C in darkness. Leaves were then blotted dry and turgid weight was determined ( $W_{sat}$ ). The dry weight was measured after oven drying for 24 hr in 80°C ( $W_{dr}$ ). RWC was calculated by the formula (Koide et al., 1991):

$$RWC(\%) = \frac{W_{act} - W_{dr}}{W_{sat} - W_{dr}} \times 100$$

**Statistical analysis:** One-way analysis of variance for  $\psi$  and RWC was conducted for each species in order to indicate any significant effect of irrigation treatment. The t-test at the 0.05 level of significance was used to detect differences of the means (for the whole period of experimentation) between species due to irrigation treatment. Non-linear regressions of varying  $\psi$  in respect to RWC were fitted per species and irrigation treatment using a quadratic function at  $p < 0.05$  in order to infer about the dehydration behavior of leaf tissues. All statistical analyses were performed with the use of the SPSS program (SPSS 15.0 for Windows).

**Results and Discussion**

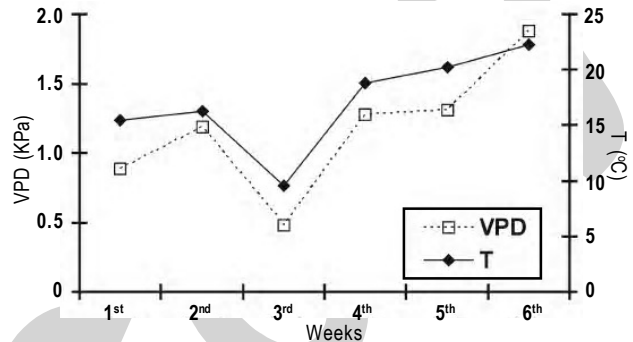
During the study period the temperature under the rain shelter ranged between 9.6 and 22.3°C with a mean value of 17.1°C, while the VPD ranged between 0.48 and 1.88 KPa with an average of 1.17 KPa (Fig. 1). These seasonal increases in air temperature and VPD were expected to influence the transpirational demands of the plants and, therefore, their water potential.

Water potential is considered an important and sensitive index of plant water status, ranging from 0 MPa in well-watered plants to negative values in water stressed plants (Kramer, 1983). Irrigation treatment significantly affected the mean period  $\psi$  values of *T. angustifolium* ( $p=0.0023$ ), while the values of *O. caput-galli* remained unaffected ( $p=0.454$ ) (Table 1). The pattern of temporal changes of both parameters ( $\psi$ , RWC) was not differentiated due to irrigation treatment. *T. angustifolium* retained the lowest values of  $\psi$  both under full (-0.11 to -1.78 MPa) and limited irrigation (-0.16 to -2.90 MPa), while the highest values in both cases were those of *O. caput-galli* (-0.05 to -0.5 MPa) (Fig. 2). In this sense, it seems that *T. angustifolium* exhibited a water spender behavior, while the other species could be rather considered as water saver (Larcher, 2003; Jones, 2004). The most important finding was that *T. angustifolium*, an annual species too, maintained its biological activity for a longer period (Fig. 2) compared to the other annual legume. This species seemed to achieve more efficient water use as  $\psi$  decreased to relatively low values.

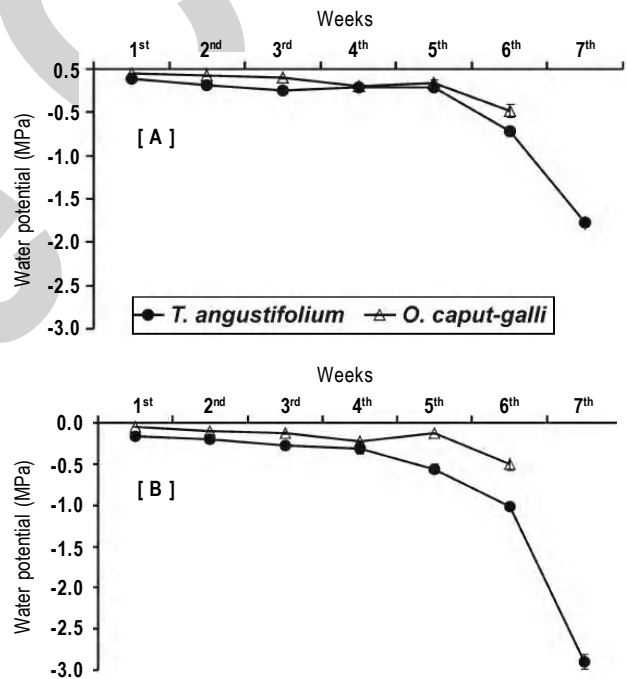
The preservation of  $\psi$  in less negative values is generally regarded as a way to avoid water deficit establishment in plant tissues (Kusaka et al., 2005). The low  $\psi$  values could be attributed

**Table - 1:** Leaf water potential  $\psi$  (MPa) of *Trifolium angustifolium* and *Onobrychis caput-galli* under full and limited irrigation. The numbers represent means  $\pm$  SE. Different letters in the same row indicate statistical differences at 0.05 level

Species	Irrigation	
	Full	Limited
<i>T. angustifolium</i>	- 0.50 $\pm$ 0.13 a	- 0.78 $\pm$ 0.20 b
<i>O. caput-galli</i>	- 0.18 $\pm$ 0.04 a	- 0.19 $\pm$ 0.04 a

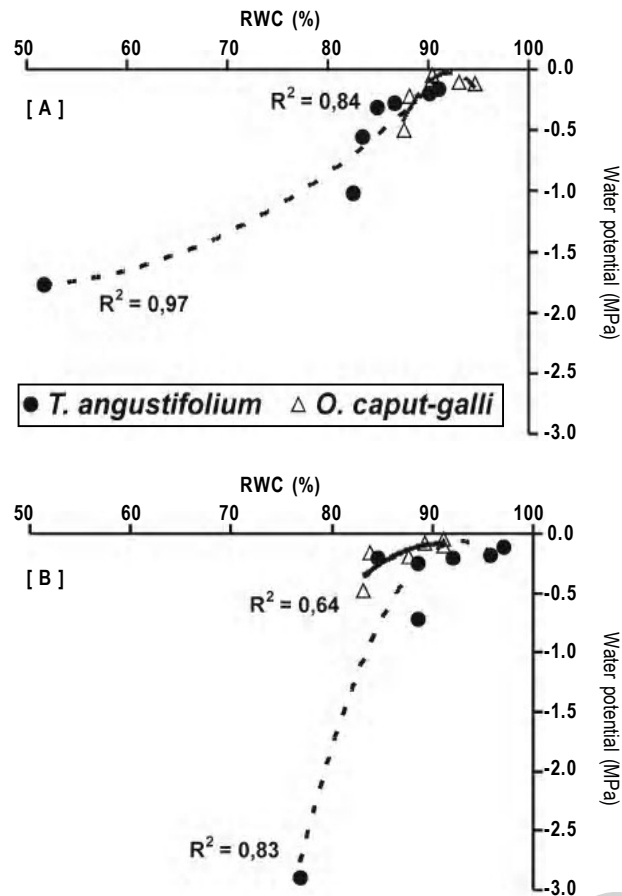


**Fig. 1:** Seasonal change of vapor pressure deficit (VPD) and air temperature (T) at predawn under the rain shelter during the experiment



**Fig. 2:** Seasonal change of leaf water potential ( $\psi$ ) of *Trifolium angustifolium* and *Onobrychis caput-galli* under full (A) and limited (B) irrigation

to low water uptake, low hydraulic flow rates within the plants or high water loss rates (Iannucci et al., 2002). Peculiar hydrodynamic behaviour was observed in *O. caput-galli*, the seasonal  $\psi$  pattern of which was not affected by limited irrigation, though its biological activity seemed to be stopped at the end of May. The fact that *O. caput-galli* did not present pronounced fluctuation of water content under well and limited irrigation during the growth period could be attributed to



**Fig. 3:** Relationship between leaf water potential ( $\psi$ ) and relative water content (RWC) of *Trifolium angustifolium* and *Onobrychis caput-galli* under full (A) and limited (B) irrigation

stomatal conductance regulation. This regulation implies energy consumption (Turner, 1979), reducing eventually the energy budget for other biological activities.

The relationship of  $\psi$  and RWC was expected to give a better insight into the strategy followed by plants under water-limited conditions. Indeed, this relationship has often been used in order to quantify the dehydration tolerance of tissues; tissues maintaining a high RWC with decreasing  $\psi$  are considered more tolerant to dehydration (Iannucci *et al.*, 2002). Under full irrigation, the RWC of *T. angustifolium* decreased almost linearly with the decrease in  $\psi$  (Fig. 3), indicating no limitation of transpirational demands. In this case, it is most likely that pathetic osmoregulation took place (increase of the osmolyte concentration due to water loss). When water was limited, though, the intense decrease in  $\psi$  to almost -3.0 MPa was accompanied by a modest decrease in RWC to 75%, indicating that possibly some physiological adaptation mechanism, such as osmoregulation, took place (Fig. 3). In this way, the plant achieved maintenance of RWC to relatively high levels. An important aspect of annual plants' adaptation to drought is the rapid phenological development and their developmental plasticity. The ability to produce flowers with a minimum of vegetative structure enables them to produce seeds on a limited supply of water. In addition, developmental plasticity offers the advantage of producing an

abundance of vegetative growth, flowers and seeds in seasons of abundant rain (Turner, 1979). These two mechanisms enable plants both to escape drought and to survive long periods without rain. But it seems that these are not the only mechanisms of adaptation to drought used by annual plants. Nowadays, it is recognized that some plants are adapted to photosynthesize despite the low water potential values, using perennial-like mechanisms that tend to maintain turgor, producing and conserving osmolytes in order to protect tissues from dehydration (Berkowitz, 1998; Jones, 2004). On the other hand, *O. caput-galli* showed an isohydric behavior, since it responded more conservatively to water shortage, attempting to maintain both RWC and  $\psi$  in high levels (Fig. 3), probably through stomatal closure induced by hydraulic or chemical signals (Jones, 1998). Isohydric plants tend to adjust their stomatal behavior in such a way as to maintain leaf water status relatively stable as environmental conditions change (Tardieu and Simonneau, 1998). The closure of the stomatal apparatus results in reduction of the plant's photosynthetic capacity and, hence, in limited biomass and seed production. These plants, like *O. caput-galli*, are usually characterized by shorter biological cycles as an adaptive mechanism to avoid the critical dry period (Larcher, 2003; Jones, 2004).

Nowadays that the effects of climate change are more pronounced it is important to explore the adaptation mechanisms to drought not only of crop plants (Evrendilek *et al.*, 2008) but also of strategic legume species of high ecological and economical importance. The knowledge of these mechanisms could play a key role in management practices that exploit the multi-functionality of legume species.

*T. angustifolium*, although an annual legume species, was found to use, apart from the typical drought escape mechanism of annual species (like short life duration), other mechanisms of adaptation to drought, usually found in perennial plants. In this way, it maintained its water content to relatively high levels under limited irrigation and continued to be productive even after the other legume had stopped its biological activity. On the other hand, *O. caput-galli* was found to maintain its water potential stable even under limited irrigation, displaying a clear isohydric behavior.

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