

## The effect of cutting on water balance of alfalfa plants

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Plants of alfalfa were grown in the field under different water and harvesting regimes (well watered and rainfed, harvested and non-harvested) in order to evaluate cutting effects on stems' internal water status. Stem water potential, transpiration rate and stomatal conductance were measured and specific hydraulic conductance was calculated. Plants that had been harvested maintained higher stem water potential than non-harvested plants. This occurred in spite of the higher transpirational water loss, which was the consequence of high stomatal conductance and higher specific conductance. Plants with high water loss compensated by higher water uptake as plant resistance to water uptake was reduced. Cutting resulted in altered plant internal water status.

**Keywords:** alfalfa, cutting, water stress, water uptake, specific conductance.

### INTRODUCTION

Alfalfa (*Medicago sativa* L.) is one of the most widely used perennial legumes, predominantly for hay and silage (Frame *et al.*, 1998). Water availability is usually the main factor decreasing yield of alfalfa (Peterson *et al.*, 1992). The ability of alfalfa to tolerate cutting is related to several mechanisms proposed to explain the compensatory growth that occurs as a result of defoliation (Chatterton *et al.*, 1977; Brummer & Bouton, 1991, 1992; Kim *et al.*, 1993). The climatic changes, with major feature the increase of temperature and the prevalence of drought, suggest that the effects of cutting on the internal water status of the leaf tissues would be of importance (Pereira & Chaves, 1995). This status depends on the difference between absorbed and transpired water and regulates stomatal opening, CO<sub>2</sub> uptake and photosynthetic rate (Jones, 1998; Patakas *et al.*, 2003). Moreover it could be an indicator of the water deficit established in the leaf tissues due to drought (Hailey *et al.*, 1973; Berkowitz, 1998). Previous experiments on other species have shown that cutting mitigates to some degree water stress (Tsiou-

varas *et al.*, 1986; Georgiadis *et al.*, 1989; Paez & Gonzalez, 1995), although the specific mechanisms contributing to this mitigation have not been completely investigated.

On the other hand, in order to avoid water deficit in leaves, the plant can either improve water uptake by reducing the hydraulic resistance from root to leaves or avoid water loss by closing the stomata aperture (Kramer, 1983; Steudle, 2000). The ability of the plant to control water uptake and/or water loss may be an adaptive mechanism to dry conditions (Tomar & O'Toole, 1982). However, little is known about the relation between cutting and water deficit as well as whether cutting alters plants water balance.

The objective of this study was to determine the physiological mechanisms established in the leaf tissues under long-term water deficit when alfalfa is subjected to frequent harvesting and furthermore to examine whether harvesting alters the internal water status of the leaf tissues.

### MATERIALS AND METHODS

The experiment was performed in the Tobacco Institute of Drama (41°09' N, 24°09' E, 130 m above sea level), Macedonia, Greece. The climate is semi-

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arid with a mean annual temperature of 15.2°C, a mean annual precipitation of 589 mm and a dry period from the middle of June to late September. The mean monthly temperature and precipitation over the two years of experimentation (1996, 1997) are shown in Fig. 1a. At microclimate level, relative humidity % (RH), leaf and air temperature were measured a few centimeters above the green cover, in order to calculate the Vapour Pressure Deficit (VPD) (Landesberg, 1986), as an integral indicator of the microclimate parameters (Aphalo & Jarvis, 1991).

*Medicago sativa* L. cv. Yliki, bred at the Forage Crops and Pastures Institute of Larisa, Greece, was established by a sowing rate of 4 g m<sup>-2</sup>. The textural class of the soil was silt loam, pH was 7.6 and the organic matter was 1.94% (w/w) for the upper layer (0-20 cm) and 1.8% for the lower one (20-40 cm). A fertilizer, containing 22% N and 11% P<sub>2</sub>O<sub>5</sub>, was applied once just before sowing. All the area of sowing was irrigated until the plants became well established.

The experiment consisted of two blocks (each one 6 × 5 m, representing one watering treatment). The first one was frequently irrigated (IP), by sprinkler system, up to the point of field capacity, in order to maintain high water availability in the soil. The system was controlled by soil moisture sensors, connected with electronic valves. The valves transmitted the signal of irrigation to the sprinkler system each time the soil sensor indicated soil moisture lower of field capacity. The second block was rainfed (RP). In each block there were eight plots of 1 × 1 m.

Within each water regime, two harvesting levels were applied, each one in four completely randomized replications: a) Plants harvested (HP) by cutting at a height of 7 cm above the soil surface. This happened four times over the growing season, during

spring and early summer, at intervals of about 20 days, beginning from April; b) Non-harvested plants (NHP). They were once cut at the stage of full maturity. Thus, there were four treatment plots: IHP, INHP, RHP and RNHP.

Measurements took place during two successive growing seasons (1996 and 1997), at noon, the first being in April 1996. Three mature leaves were measured at the center of each replicate plot, totally 12 leaves per treatment. Stomatal conductance (Gs) and transpiration rate (T) were measured by a porometer (Li-1600, Li-Cor Inc., Nebraska, U.S.A.), on the abaxial leaf surface. Stem water potential (Ψ) was also measured, using a pressure chamber (ARIMAD-2, Kfar Charuv, Israel).

Specific conductance (SC) was assessed by the equation  $SC = T/Gs$  and hydraulic resistance (R) by the equation  $R = \frac{\Delta\Psi}{\Delta T}$  under irrigation conditions, assuming that at these conditions soil water potential and soil resistance were close to zero (Hailey *et al.*, 1973; Tomar & O'Toole, 1982; Abdul-Jabbar *et al.*, 1984).

The experimental design was completely randomized. Statistical analysis was done using the SPSS statistical computer package (SPSS for Windows, 8.0). ANOVA and mean separation by LSD tests were used to compare the harvesting treatments ( $p \leq 0.05$ ).

## RESULTS

The seasonal changes of precipitation and VPD revealed that the period of drought, during the experimentation, began in May and went on over July (Figs 1a, b). The seasonal changes of midday stem

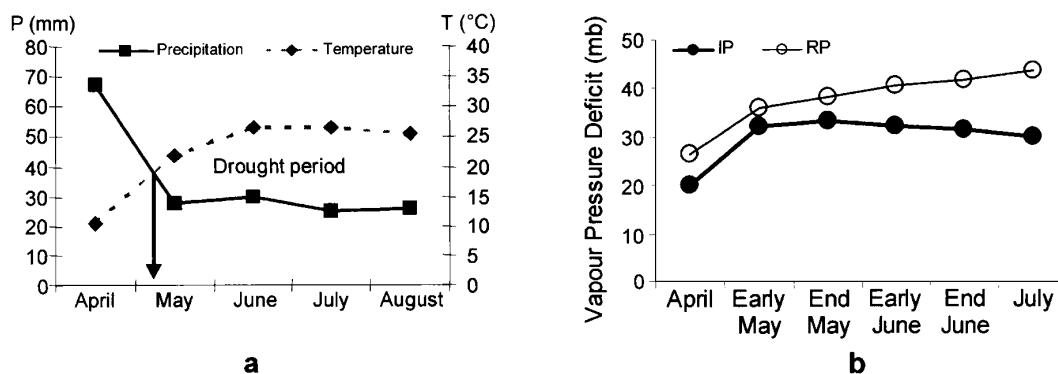


FIG. 1. Ombrothermic diagram showing the drought period of two years (1996, 1997) of experimentation. (a) Number of days of precipitation. (b) Seasonal changes of VPD in irrigated (IP) and rainfed (RP) plants. Each value is the mean of two years.

water potential ( $\Psi$ ) indicated that in both water regimes, the leaves of the harvested alfalfa presented significantly higher values of  $\Psi$  at the same time of the growing season (Figs 2a, b). Under irrigation, the differences became greater as season progressed. In the rainfed plants, the differences were significant in May and June. Moreover, no significant differ-

ences of  $\Psi$  between the RHP and INHP treatments were observed.

The transpiration rate ( $T$ ) – obtained at midday – was higher in HP compared with NHP in both water regimes at the same date after May (Figs 3a, b). A similar pattern was exhibited by the seasonal changes of stomatal conductance (Figs 4a, b).

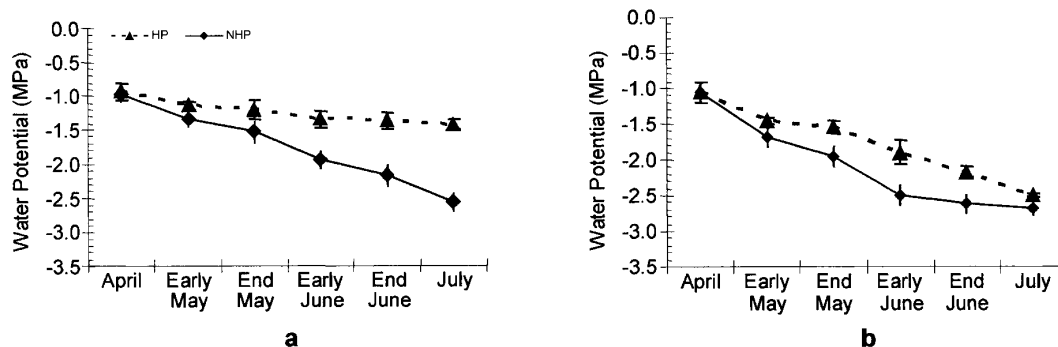


FIG. 2. Seasonal changes of stem water potential in harvested (HP) and non-harvested (NHP) plants. (a) Irrigated plants. (b) Rainfed plants. Bars indicate the standard error of mean of twelve replicates.

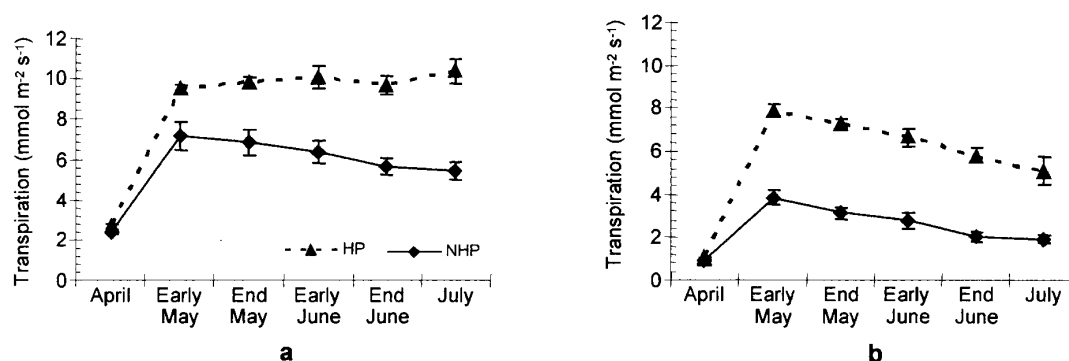


FIG. 3. Seasonal changes of transpiration rate in the leaves of harvested (HP) and non-harvested (NHP) plants. (a) Irrigated plants. (b) Rainfed plants. Bars indicate the standard error of mean of twelve replicates.

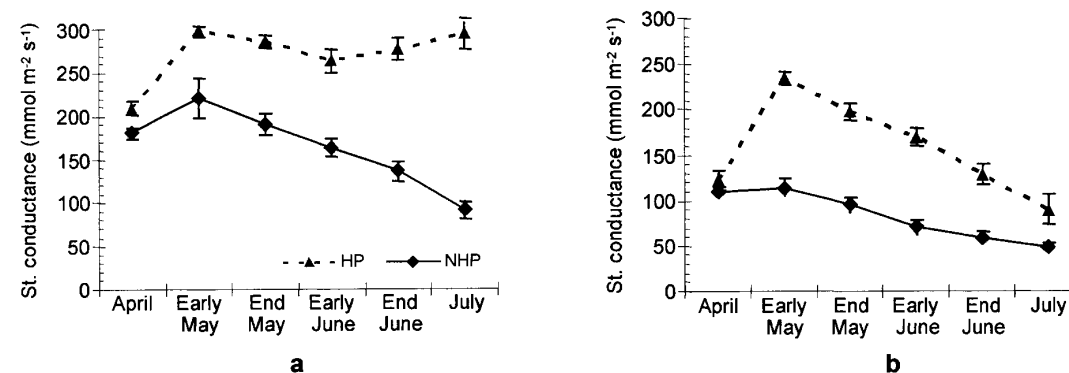


FIG. 4. Seasonal changes of stomatal conductance in the leaves of harvested (HP) and non-harvested (NHP) plants. (a) Irrigated plants. (b) Rainfed plants. Bars indicate the standard error of mean of twelve replicates.

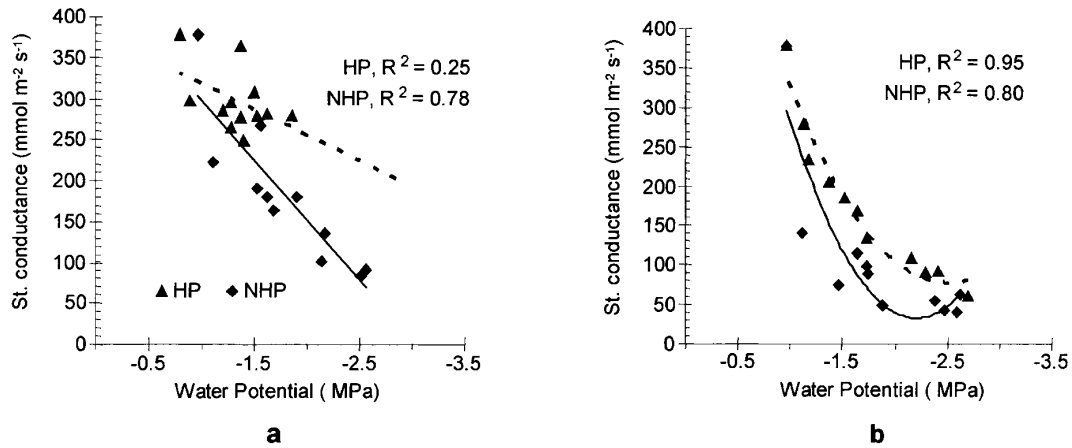


FIG. 5. Relationship between stomatal conductance and water potential in harvested (HP) and non-harvested (NHP) plants. (a) Irrigated plants. (b) Rainfed plants. Statistical analysis showed significant differences.

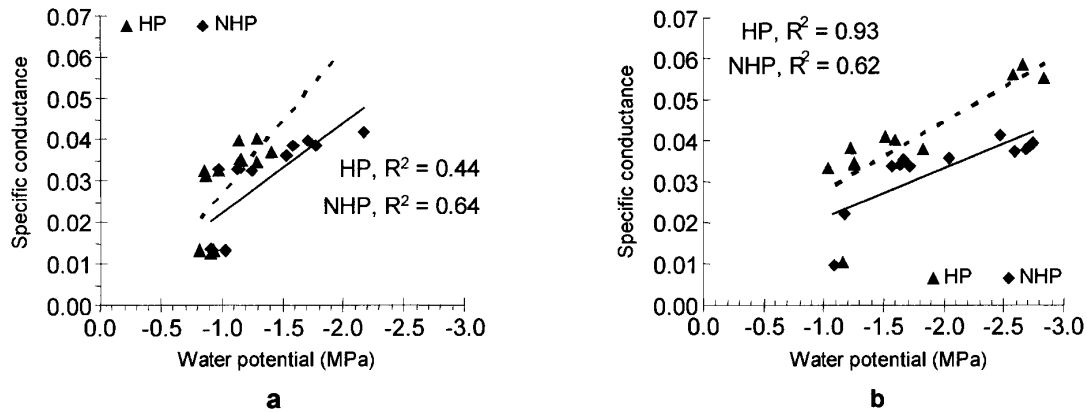


FIG. 6. Relationship between specific conductance and water potential in harvested (HP) and non-harvested (NHP) plants. (a) Irrigated plants. (b) Rainfed plants. Statistical analysis showed significant differences.

The changes of stomatal conductance in relation to  $\Psi$  revealed that in the irrigated harvested plants, stomatal conductance was significantly higher from that of the non-harvested plants at the same value of  $\Psi$  and it decreased in both harvested and non-harvested plants as  $\Psi$  decreased (Fig. 5a). The decrease of stomatal conductance in relation to  $\Psi$  became non-linear in the HR and NHR plants (Fig. 5b). In this case, stomatal conductance declined steadily until  $\Psi$  reached a value of  $-2.0$  MPa. Beyond this value, the changes of stomatal conductance were linear and almost asymptotic to the x axis.

The relationship between specific conductance (SC) and  $\Psi$  indicated that for the same values of  $\Psi$  and stomatal conductance, the transpiration rate was higher in the harvested plants than in the non-harvested plants in both irrigated and rainfed treatments (Figs 6a, b).

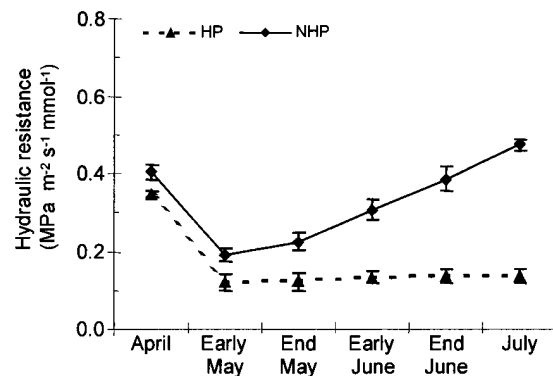


FIG. 7. Seasonal changes in plant resistance in irrigated, harvested (HP) and non-harvested (NHP) plants. Bars indicate the standard error of mean of twelve replicates.

The seasonal changes of hydraulic resistance ( $R$ ) over the growing period in the irrigated plants were lower in the H than in the NH plants (Fig. 7). The

values of R decreased in the H and NH plants until May. From then on, in the H plants they remained lower and relatively constant, while in the NH plants they increased.

## DISCUSSION

The higher values of midday stem water potential of the harvested plants can be probably explained by the fact that cutting kept the plants at the vegetative stage. At this stage, the leaves can maintain a more favorable internal water status (Wolf & Parrish 1982, Tsiouvaras *et al.*, 1986). On the contrary, the lower values of stem  $\Psi$  of the non-harvested plants in both water regimes might be due to the reduced ability of the stem to absorb or to conduct water from soil to leaves due to senescence (Noitsakis & Berger, 1984; Link *et al.*, 1990). The cutting effects on plant internal water status could be probably attributed either to the reduction of the overall transpiration rate and/or to the improving of the water absorption at a given soil water availability (Wolf & Parrish, 1982; Tsiouvaras *et al.*, 1986; Georgiadis *et al.*, 1989; Steudle, 2000).

The above first assumption was not verified by our results, since transpiration was higher in the harvested plants. It is known that transpiration rate is regulated by the atmospheric demand expressed in VPD (Kramer, 1983) as well as by the stomatal conductance (Kramer, 1983; Xue *et al.*, 2004). The higher specific conductance (T/Gs) observed in the harvested plants could be attributed to the fact that the canopy of these plants presumably had a lower density and thus air speed was higher. Air movement has been shown to induce variations in microclimate inside the stand and to enhance transpiration in the leaves and canopies (Roberts, 2000; Kitaya *et al.*, 2003). Since the seasonal pattern of stomatal conductance was similar to that of transpiration, it could be assumed that the transpirational water loss was regulated by the adjustment of stomatal conductance (Kramer, 1983; Bonham *et al.*, 1990; Collinson *et al.*, 1997). On the other hand, in spite of the higher values of transpiration rate, the higher values of  $\Psi$  in the harvested plants could be attributed to the reduced leaf area and consequently to the reduced total transpirational water loss from the leaves and the soil (Turner, 1986; Blum, 1997; Roberts, 2000; Anyia & Herzog, 2004). The decrease in stomatal conductance and transpiration in non-harvested plants, as phenological stage advances, is well known (Hailey *et al.*, 1973; Bonham *et al.*, 1990; Link *et al.*, 1990;

Jones, 1998). This can be explained by the relationship between  $\Psi$  and stomatal conductance, mainly in the rainfed plants (Carter & Sheaffer, 1983; Noitsakis, 1988; Dixon & Johnson, 1993). A crucial threshold for initiation of stomatal closure that occurs in many crop species (Carter & Sheaffer, 1983; Dixon & Johnson, 1993) was not observed here, despite the apparent sensitivity of the alfalfa stomatal conductance in relation to  $\Psi$  reduction. Generally, it is accepted that in the rainfed plants the decrease of stem  $\Psi$  is due to the soil water deficit, while in the irrigated plants is due to high VPD in the midday. The non-linear relationship between  $\Psi$  and stomatal conductance at very low  $\Psi$  values in stressed plants has been often reported in the literature (Carter & Sheaffer, 1983; Noitsakis & Tsiouvaras, 1990). In the irrigated harvested plants, the low regression coefficient suggests that stomata aperture is relatively insensitive to  $\Psi$ , and therefore, other environmental conditions like maximum air temperature and VPD may affect it (Carter & Sheaffer, 1983; Xue *et al.*, 2004). The high values of stomatal conductance might positively affect CO<sub>2</sub> uptake and therefore plant productivity (Noitsakis & Tsiouvaras, 1990; Paez & Gonzales, 1995; Patakas *et al.*, 2003). This fact in combination with the favorable internal water status suggests that harvesting improved the physiological efficiency of alfalfa.

The above second assumption concerning the improvement of water absorption was verified by the seasonal changes of the hydraulic resistance (R). The low hydraulic resistance (R) exhibited by the harvested plants after May indicates a better hydration of the leaf tissues (Tomar & O'Toole, 1982), since aging (a factor that increases R) was avoided by harvesting (Hailey *et al.*, 1973; Wolf & Parrish, 1982; Georgiadis *et al.*, 1989). Thus, the internal water status in the leaves of alfalfa apparently was affected by the improvement of the water absorption rather than by the regulation of the stomatal apparatus.

## CONCLUSIONS

The results suggest that harvesting of alfalfa seems to maintain a favorable internal water status in the leaf tissues, compensating the transpirational water loss probably by reducing the hydraulic resistance.

## REFERENCES

- Abdul-Jabbar AS, Lugg DG, Sammis TW, Gay LW, 1984. A field study of plant resistance to water flow in alfalfa. *Agronomy journal*, 76: 765-769.

- Anyia O, Herzog H, 2004. Water-use efficiency, leaf area, and leaf gas exchange of cowpeas under mid-season drought. *European journal of agronomy*, 20: 327-339.
- Aphalo PJ, Jarvis PG, 1991. Do stomata respond to relative humidity? *Cell and environment*, 14: 127-132.
- Berkowitz GA, 1998. Water and salt stress. In: Raghavendra AS, ed. *Photosynthesis: A comprehensive treatise*. Cambridge University Press, London: 226-237.
- Blum A, 1997. Crop responses to drought and the interpretation of adaptation. In: Belhassen E, ed. *Drought tolerance in higher plants: genetical, physiological and molecular biological analysis*. Kluwer Academic Publishers, Dordrecht: 57-70.
- Bonham CD, Mack SE, Trlica MJ, 1990. Leaf conductance and transpiration of winterfat associated with 2 species of wheatgrass on disturbed sites. *Journal of range management*, 43: 501-505.
- Brummer EC, Bouton JH, 1991. Plant traits associated with grazing-tolerant alfalfa. *Agronomy journal*, 83: 996-1000.
- Brummer EC, Bouton JH, 1992. Physiological traits associated with grazing-tolerant alfalfa. *Agronomy journal*, 84: 138-143.
- Carter PR, Sheaffer CC, 1983. Alfalfa response to soil water deficits. II. Plant water potential, leaf conductance, and canopy temperature relationships. *Crop science*, 23: 676-680.
- Chatterton NJ, Akao S, Carlson GE, Hungerford WE, 1977. Physiological components of yield and tolerance to frequent harvests in alfalfa. *Crop science*, 17: 918-923.
- Collinson ST, Clawson EJ, Azam-Ali SN, Black CR, 1997. Effect of soil moisture deficits on the water relations of bambara groundnut (*Vigna subterranean* L. Verdec). *Journal of experimental botany*, 48: 877-884.
- Dixon MA, Johnson RW, 1993. Interpretation of the dynamics of plant water potential. In: Borghetti M, Grace J, Raschi A, eds. *Water transport in plants under climatic stress*. Cambridge University Press, London: 63-74.
- Frame J, Charlton JFL, Laidlow AS, 1998. *Temperate forage legumes*. Cab International, New York.
- Georgiadis NJ, Ruess RW, McNaughton SJ, Western D, 1989. Ecological conditions that determine when grazing stimulates grass production. *Oecologia*, 81: 316-322.
- Hailey JL, Hiler EA, Jordan WR, van Bavel CHM, 1973. Resistance to water flow in *Vigna sinensis* L. (Endl) at high rates of transpiration. *Crop science*, 13: 264-267.
- Jones HG, 1998. Stomatal control of photosynthesis and transpiration. *Journal of experimental botany*, 49: 387-398.
- Kim TH, Ourry A, Boucaud J, Lemaire G, 1993. Partitioning of nitrogen derived from N<sub>2</sub>-fixation and reserves in nodulated *Medicago sativa* L. during re-growth. *Journal of experimental botany*, 44: 555-562.
- Kitaya Y, Tsuruyama J, Shibuya T, Yoshida M, Kiyota M, 2003. Effects of air current speed on gas exchange in plant leaves and plant canopies. *Advances in space research*, 31: 177-182.
- Kramer PJ, 1983. *Water relations of plants*. Academic Press, London.
- Landesberg IJ, 1986. *Physiological ecology of forest production*. Academic Press, London.
- Link SO, Gee GW, Downs JL, 1990. The effect of water stress on phenological and ecophysiological characteristics of cheatgrass and Sandberg's bluegrass. *Journal of range management*, 43: 506-513.
- Noitsakis B, Berger A, 1984. Relations hydriques chez *Dactylis glomerata* et *Dichanthium ischaemum* cultivés sous deux régimes hydriques contrastés. *Acta oecologica*, 5: 75-88.
- Noitsakis B, 1988. Etude comparative de comportement hydrique journalier et saisonnier de *Medicago sativa* et *Festuca elatior* cultivés en conditions naturelles. In: Di Castri F, Floret C, Rambal S, Roy J, eds. *Time scales and water stress. Proceedings of the 5<sup>th</sup> international conference on Mediterranean-climate ecosystems*. IUBS, Paris: 215-226.
- Noitsakis B, Tsiouvaras C, 1990. Seasonal changes in components of leaf water potential and leaf area growth rate in kermes oak. *Acta oecologica*, 11: 419-427.
- Patakas A, Stavrakas D, Fisarakis I, 2003. Relationship between CO<sub>2</sub> assimilation and leaf anatomical characteristics of two grapevine cultivars. *Agronomie*, 23: 293-296.
- Pereira JS, Chaves MM, 1995. Plant responses to drought under climate change in Mediterranean-type ecosystems. In: Moreno JM, Oechel WC, eds. *Global change and Mediterranean-type ecosystems*. Ecological studies Vol. 117. Springer-Verlag, New York: 140-160.
- Peterson PR, Sheaffer CC, Hall MH, 1992. Drought effects on perennial forage legume yield and quality. *Agronomy journal*, 84: 774-779.
- Paez A, Gonzalez OME, 1995. Water stress and clipping management effects on guineagrass: II. Photosynthesis and water relations. *Agronomy journal*, 87: 706-711.
- Roberts J, 2000. The influence of physical and physiological characteristics of vegetation on their hydrological response. *Hydrological processes*, 14: 2885-2901.
- Steudle E, 2000. Water uptake by roots: effects of water deficit. *Journal of experimental botany*, 51: 1531-1542.
- Tomar VS, O'Toole JC, 1982. A field study on leaf water potential, transpiration and plant resistance to water flow in rice. *Crop science*, 22: 5-10.

- Tsiouvaras CN, Noitsakis B, Papanastasis VP, 1986. Clipping intensity improves growth rate of kermes oak twigs. *Forest ecology and management*, 15: 229-237.
- Turner NC, 1986. Adaptation to water deficits: a changing perspective. *Australian journal of plant physiology*, 13: 175-190.
- Wolf DD, Parrish DJ, 1982. Short-term growth responses of tall fescue to changes in soil water potential and to defoliation. *Crop science*, 22: 996-997.
- Xue Q, Weiss A, Arkebaue TJ, Baenziger PS, 2004. Influence of soil water status and atmospheric vapor pressure deficit on leaf gas exchange in field-grown winter wheat. *Environmental and experimental botany*, 51: 167-179.