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Effect of limited water supply on *Melilotus officinalis* nutrient content

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Abstract. The aim of the present study was to investigate the influence of limited water supply on nutrient accumulation of *Melilotus officinalis* tissues. For this purpose a pot experiment was conducted, where half of the pots were fully irrigated (up to field capacity) and the rest were irrigated with half the quantity of water. Irrigation plan was applied for five weeks during the growing season. At the end of the experiment, randomly selected plant material, divided in roots, shoots and leaves, was dried and the content of K, Na, Mg, Ca, Cu, Fe, Zn and Mn was determined. The results showed that the limited water supply interacted with the mineral content of *M. officinalis* plants, increasing the concentration of K, Ca, Mg, Cu, Fe and Mn in the leaf tissues. Accumulation of these nutrients could serve as a means to alleviate the adverse impact of drought on important metabolic processes, enhancing *M. officinalis* tolerance to water stress.

Keywords. Drought – Macro-nutrients – Micro-nutrients.

***Effet de l'approvisionnement en eau limité sur le contenu en éléments nutritifs de* Melilotus officinalis**

Résumé. *L'influence de l'approvisionnement en eau limité sur l'accumulation des éléments nutritifs dans les tissus de* Melilotus officinalis *est étudiée. Un expérimente en pot a été menée, où la moitié des pots ont été entièrement irrigué (jusqu'à la capacité au champ) et le reste ont été irrigués avec la moitié de la quantité d'eau. Un plan d'irrigation a été appliqué pour les cinq semaines pendant la saison de croissance. À la fin de l'essai, la matière végétale choisie au hasard, divisée dans les racines, les tiges et des feuilles, est séché et le contenu de K, Na, Mg, Ca, Cu, Fe, Zn et Mn a été déterminée. Les résultats montrent que l'approvisionnement en eau limité a interagi avec la teneur en minéraux des plants de* M. officinalis*; les tissus foliaires indiquent une augmentation de la concentration de K, Ca, Mg, Cu, Fe et Mn. L'accumulation de ces nutriments pourrait servir comme un moyen d'atténuer l'impact négatif de la sécheresse sur d'importants processus métaboliques, cet-à-dire d'améliorer la tolérance de M.* officinalis *à un stress hydrique.*

Mots-clés. *Sècheresse – Macro-nutriments – Micro-nutriments.*

I – Introduction

Drought, a common phenomenon in Mediterranean areas especially during summer, has many adverse impacts on plants, inhibiting their growth (Asgharipour and Heidari, 2011) and affecting their mineral content (Hu and Schmidhalter, 2005). Drought is generally regarded to reduce nutrient uptake by roots and their translocation to the shoots due to its negative effect on transpiration rate, active transport and membrane permeability (Alam, 1999). Nevertheless, numerous studies have shown that many species accumulate inorganic ions as a means to adapt to water stress (Patakas *et al*. 2002; Zhu *et al*. 2005), increasing in this way their drought resistance.

Melilotus officinalis L. (yellow sweetclover) is a biennial legume species, considered as palatable forage of high quality both for livestock and wildlife (Mueggler and Stewart, 1980). In addition, this species produces strong and deep penetrating taproots that can loosen and aerate com-

pacted soil; these characteristics make *M. officinalis* a suitable candidate as a nurse crop in revegetation of derelict land (Stevens and Monsen, 2004). Aim of the present study was to determine the changes in nutrient content of *M. officinalis* under water stress.

II – Materials and methods

The study was conducted at the farm of Aristotle University, 14 km south of the city of Thessaloniki (40°32´N, 22°59´E), at an elevation of about 5 m a.s.l. in autumn 2002. The climate of the area could be characterized as Mediterranean semi-arid with cold winters. Seeds (commercial seedlot of Spanish origin) of yellow sweetclover (*Melilotus officinalis* L.) were sown in twenty 25-liter-pots. All pots were filled with a 5:4:1:1 (on a volume basis) peat:soil:manure:sand mixture. The mixture comprised of white peat medium bedding, enriched with nutrients and trace elements (Klassmann TS1, Klassmann-Deilmann GmbH, Geeste, Germany), grassland soil (0-20 cm), collected the same year from the farm of the Aristotle University of Thessaloniki, farmyard manure and sand.

After seedling emergence, the pots were thinned to a total of 10 seedlings per pot. Then the pots were transferred to a permanent rain shelter, a wooden construction of 2.5 m height, with a transparent plastic cover on top. The rain shelter was designed in such a way as to avoid rainfall reaching the pots, while keeping the atmospheric conditions unchanged. Ten pots, randomly selected, were irrigated frequently (Full Irrigation) so that the soil in the pots was always near field capacity, as determined by tensiometers. The other ten pots received 50% less water than the fully irrigated plants (Limited Irrigation). After six weeks, plant material from both treatments was collected. Roots, shoots and leaves were first separated and rinsed with deionized water, then dried for 48 h at 60^{o}C and ground through to ≤1 mm screen. A sample of 1 g from each tissue material was dry-ashed in a HCl 2N solution at 500^{o}C for 16 h. Potassium and Na concentration was determined using a flame photometer (PFP7, Jenway, Essex, England), while Mg, Fe, Cu, Ca, Mn and Zn concentration was determined with an atomic absorption spectrophotometer (AA-6300, Shimadzu Corporation, Tokyo, Japan).

A completely randomized experimental design was followed with ten replications for each treatment. Statistical analysis of the data was performed using ANOVA with the help of the SPSS® statistical software v. 17.0 (SPSS Inc., Chicago, IL, USA).

III – Results and discussion

The water supply significantly interacted with the majority of macro-nutrients in *M. officinalis* roots, stems and leaves (Table 1). More specifically, under limited irrigation, K and Ca content decreased in roots and stems and increased in leaves (Fig. 1). Potassium plays an important role in survival of plants under environmental stress (Waraich *et al*. 2011), contributing to osmotic adjustment (Utrillas *et al*. 1995; Patakas *et al*. 2002). Leaf K increase could have contributed to the osmotic adjustment of *M. officinalis* plants, acting as a mechanism to maintain turgor pressure and stomatal conductance (Patakas *et al*. 2002) and, hence, photosynthesis under drought conditions (Waraich *et al*. 2011). In compliance with this result, Kostopoulou *et al*. (2010) in their study with the same species under similar experimental conditions found that stomatal conductance was not affected by limited irrigation. Calcium, on the other hand, has a prominent role in maintaining cell structure (McLaughlin and Wimmer, 1999) and recovery from drought (Palta 1990). Increase of Ca under drought conditions has been reported by others (Utrillas *et al*. 1995; Patakas *et al*. 2002).

Little information is available on the effect of drought on Na and Mg. Under limited water supply root Na content of *M. officinalis* plants decreased (Fig. 1), while stem and leaf content was not affected (Table 1). A decrease in sodium content of *Cynodon dactylon* was also observed under water deficit (Utrillas *et al*. 1995). On the other hand, plants of *Sorghum bicolor* grown on different

irrigation regimes did not differ in the concentration of Na (Asgharipour and Heidari, 2011). Magnesium concentration decreased in stems and increased in leaves of *M. officinalis* plants (Fig. 1). Magnesium plays a significant role in reducing the generation of reactive oxygen species, protecting the chloroplasts from photo-oxidative damage under water stress (Waraich *et al*. 2011).

Table 1. Effect of irrigation treatment on nutrient content (in mg kg^{-1} dry matter) of *Melilotus officinalis* tissues

Plant part	K	Na	Ca	Mg	Cu	Fe	Zn	Mn
Roots	***	***	***	NS	NS	NS	*	NS
Stems	***	NS	***	***	*	NS	**	NS
Leaves	***	NS	*	**	**	**	NS	*

*P<0.05, **P<0.01, ***P<0.001, NS: non significant.

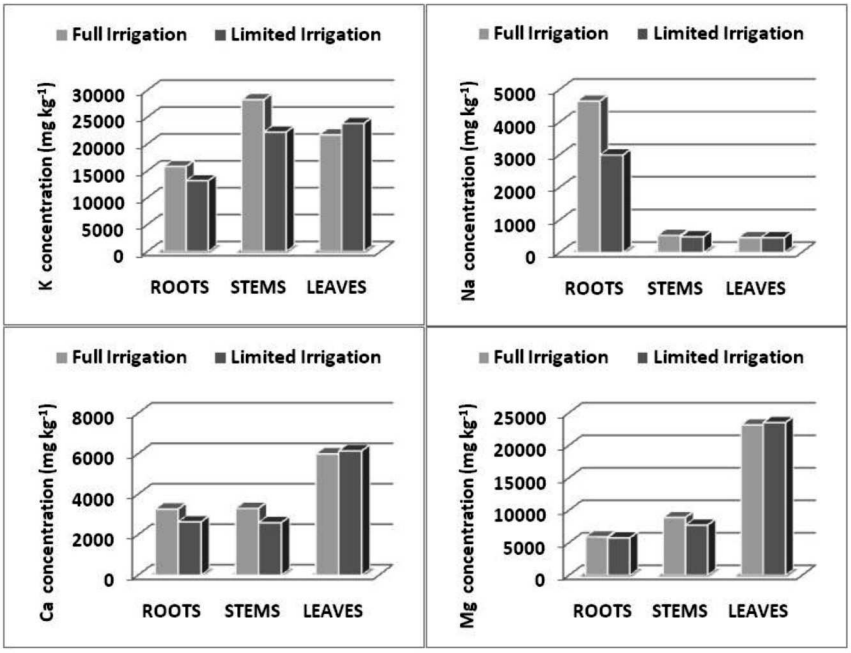

Fig. 1. Macro-nutrient content of *Melilotus officinalis* tissues under full and limited irrigation.

Information dealing with the effect of drought on the concentration of micro-minerals in plant tissues is rather scarce. In the present study, water supply significantly affected the micro-nutrient content of *M. officinalis* tissues (Table 1). Generally, drought reduces nutrient uptake by roots through the decrease in the diffusion rate of nutrients in the soil towards the absorbing root surface (Pinkerton and Simpson, 1986; Alam, 1999). According to Hu and Schmidhalter (2005) low soil moisture can induce deficiencies in Mn, Fe and Zn. In this study we found similar results for Zn, which decreased in roots and stems of *M. officinalis* (Table 2). On the contrary, Cu content increased in stems and leaves, while Fe and Mn increased in leaves (Table 2). Copper is required for lignin synthesis, needed for cell wall strength and prevention of wilting. Increase of Cu content could alleviate the adverse effects of drought by reducing dieback of stems, yellowing of

leaves and stunted growth (Waraich *et al*. 2011). Further research is needed in order to determine the role of Fe and Mn in *M. officinalis* under drought conditions. Nonetheless, accumulation of inorganic ions may contribute to the adaptation of this species to drought stress.

Table 2. Micro-nutrient content (in mg kg^{-1} dry matter) of *Melilotus officinalis* tissues under full and limited irrigation. Data represent means ± standard deviation

Plant part	Irrigation	Cu	Fe	Zn	Mn
Roots	Full	9 ± 0.4	918 ± 28	66 ± 6	41 ± 3
	Limited	9 ± 0.6	972 ± 47	53 ± 4	44 ± 2
Stems	Full	8 ± 0.2	104 ± 7	56 ± 2	19 ± 1
	Limited	11 ± 1.4	114 ± 18	45 ± 2	21 ± 2
Leaves	Full	10 ± 0.9	485 ± 17	55 ± 2	68 ± 4
	Limited	14 ± 0.6	900 ± 87	61 ± 4	79 ± 3

IV – Conclusions

The results of the present study showed that water supply interacted with the mineral content of *M. officinalis* plants. Increased accumulation of K, Ca, Mg, Cu, Fe and Mn in the leaf tissues of this species under drought conditions could serve as a means to alleviate the adverse impact of drought on important metabolic processes, enhancing *M. officinalis* tolerance to water stress.

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