Biomass partition, leaf gas exchange and water relations of alfalfa and milkvetch seedlings in response to soil drying

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Abstract

This study compared physiological and growth responses to water stress of two legume species during the seedling stage. Potted alfalfa (*Medicago sativa* L. cv. Algonquin) and milkvetch (*Astragalus adsurgens* Pall. cv. Pengyang early-maturing vetch) seedlings were grown under well-watered [soil water content (SWC) maintained at 14.92% daily] or water-stressed conditions (drying) for 15 days. Net photosynthetic rate (P_N), transpiration rate (E) and stomatal conductance (g_s) of both species decreased parabolically. When SWC decreased to 7.2% and 10.3%, g_s values for alfalfa and milkvetch were significantly different from those of the respective well-watered plants ($p<0.05$). When SWC decreased to 6.6% for alfalfa and 6.8% for milkvetch, leaf water potentials (ψ_L) were significantly different from those of the well-watered plants ($p<0.05$). Thus the difference between the SWC thresholds for a nonhydraulic root signal (nHRS) and a hydraulic root signal (HRS) were 0.6% and 3.5% for alfalfa and milkvetch, respectively. Milkvetch had a lower g_s than alfalfa for a given SWC ($p<0.05$). Although alfalfa seedlings had a higher dry mass (DM) and root:shoot ratio (R/S) than milkvetch in both treatments ($p<0.05$), we concluded that milkvetch seedlings had greater drought tolerance than alfalfa.

Additional key words: hydraulic root signal; root:shoot ratio; nonhydraulic root signal; water stress.

Introduction

Plant response to drought depends on various factors such as the duration and magnitude of stresses, previous exposure to drought, the developmental phase of the plant, and the plant species (Shan *et al.* 2000, Zewdie *et al.* 2007). When the root system of a plant is subjected to water stress, the control of stomatal conductance (g_s) is the primary means by which plants regulate water flow (Saliendra *et al.* 1995). Decreasing g_s may or may not be associated with decreased leaf water potential (ψ_L), depending on the presence of either a nonhydraulic (chemical) root signal (nHRS) or of a hydraulic root signal (HRS) (Li *et al.* 2000, Yao *et al.* 2001, Comstock 2002). The presence of nHRS and HRS is analyzed by the g_s and ψ_L responses of well-watered and water-stressed plants (Croker *et al.* 1998, Li *et al.* 2000, Mencuccini *et al.* 2000). Li *et al.* (2000), Xiong *et al.* (2006) and Xu *et al.* (2006a) suggest the use of nHRS and HRS responses to decreasing soil water content (SWC) to evaluate plant-species drought tolerance.

Alfalfa (*Medicago sativa* L.) and milkvetch (*Astragalus adsurgens* Pall.) have been widely cultivated as the main palatable, leguminous, and forage crops grown in northern China. Considerable research has studied *M. sativa* and *A. adsurgens* shoot and root biomass production under water stress conditions (Xu *et al.* 2006b). However, few studies have been carried out on seedlings.

Our objectives were (*1*) to characterize the differences

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Abbreviations: BT – before water treatment; DM – dry mass; E – transpiration rate; FC – field capacity; g_s – stomatal conductance; HRS – hydraulic root signal; nHRS – nonhydraulic root signal; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; RWC – relative water content; R/S – root:shoot ratio; SWC – soil water content; TWUE – transpiration water-use efficiency; ψ_L – leaf water potential.
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in shoot:root biomass ratios and physiological responses of the two species during the seedling stage under water-stress conditions; (*2*) to determine the SWC at which nHRS and HRS occurred in the two species during soil drying. These characterizations would improve our understanding for predicting their field performance and increase seedling plantation possibilities in the region.

Materials and methods

Plant materials: The species used in this study included alfalfa (*Medicago sativa* L. cv. Algonquin) and milkvetch (*Astragalus adsurgens* Pall. cv. Pengyang early-maturing shadawang). Algonquin alfalfa, introduced from Canada, has been widely planted in semiarid areas of the Loess Plateau of China (Xu *et al*. 2007). Pengyang early-maturing shadawang was bred by the Institute of Soil and Water Conservation, Chinese Academy of Sciences (CAS) in the 1970s and 1980s by selection and treatment of the mature seeds with ^{60}Co-γ (Ma and Sun 1990). Seeds of the two species were collected from experimental fields at the Ansai Research Station of CAS (36°51´30"N, 109°19´23"E; altitude 1,068–1,309 m a.s.l.).

Growth conditions: The experiment was conducted at the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau. Seeds were soaked in deionized water for 24 h and 20 seeds for each species were sown in plastic pots (16 ×17 ×10.5 cm; height × upper inner diameter × lower inner diameter) containing 2.5 kg of oven-dried soil. A plastic pipe adjacent to the inner wall allowed water to be added to the bottom of the pots.

Soil was collected from the upper 20 cm of a cultivated field at Ansai Research Station. The soil was a sandy loam and was packed in the pots to give 55% porosity and a bulk density of about 1.2g cm^{-3}. SWC at field capacity (FC) and wilting point was 18.4 and 3.8%, respectively. At the beginning of the experiment, the soil in each pot was mixed with 0.1 g of pure nitrogen as urea, and 0.1 g K_2O, and 0.1 g P_2O_5 as KH_2PO_4. The top of the soil in each pot was covered with 15 g of perlite in order to reduce evaporation from the soil surface. The pots were put into a growth chamber (*PGV36, CMP3244, Conviron8601*, *Conviron*, Canada) for germination. During the seed germination and seedling emergence period the photosynthetic photon flux density (PPFD) was about 250 μmol m^{-2} s^{-1}, 1 m above the pots, the CO_2 concentration was about 390 ppm and the temperature was 22°C (7:30–19:30 h) or 14°C (19:30–7:30 h) inside the chamber. From the start of the water-stress treatment, the PPFD was adjusted to 375 μmol m^{-2} s^{-1} and the temperature to 26°C/18°C (day/night). The relative humidity inside the chamber was 75 ± 5% during the experiment.

Water consumption: The evapotranspiration was assessed daily from mass loss by weighing the pots at 17:00 h, and then SWC was maintained at 80% FC (SWC = 14.92%) at 18:00 h by adding the required amount of water through the plastic pipes. After seedling emergence, plants were thinned twice to obtain 10 similar, healthy seedlings in each pot before water treatments were imposed. Forty-five days after emergence, the seedlings were 16 cm in height and had about 6 leaves. Pots with each species were divided into two groups for the water treatments (5 pots for each combination of species and water treatment). Plants in group I were watered every afternoon to keep the soil water content (SWC) at 14.92% (well-watered), while plants in group II were allowed to dry (water-stressed). Water treatments lasted 15 days. Six pots of soil with an initial SWC of 14.92%, without plants, were used to estimate soil surface evaporation according to Xu *et al*. (2006a); 3 pots were kept well-watered and 3 were allowed to dry.

Leaf gas exchange parameters such as net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) were measured everyday after beginning the water treatments, between 10:00 and 11:00 h, using a portable photosynthesis measurement system (*CI-301PS, CID*, USA). During gas-exchange measurements, the PPFD was 375 μmol m^{-2} s^{-1}, 1 m above the canopy, the CO_2 concentration was about 390 ppm and the leaf temperature was 22°C. One leaf at maximum expansion in each pot was randomly selected for the gas-exchange measurements.

Leaf water potential (ψ_L) and relative water content (RWC) were measured on the same leaves used for gas-exchange measurements; ψ_L was measured using a pressure chamber (*Model 3005, Soil Water Equipment Corp.,* Santa Barbara, USA). After measuring the fresh mass (FM), leaves were left in deionized water for 24 h at 4°C in darkness to obtain the mass at full turgor (TM). DM was obtained after drying the samples in an oven at 70°C for 48 h. RWC was calculated from: RWC [%] = (FM – DM) × 100/(TM – DM).

Biomass allocation: Plant biomass was measured using 10 seedlings per pot. After separating plants into shoots and roots, DM was determined after drying for 48 h at 70°C, and the root:shoot ratio (R/S) was calculated as the ratio of root to shoot DM. Transpiration water-use efficiency (TWUE) was determined as the ratio of total or shoot DM to water consumption by transpiration [g(DM) kg(H_2O)$^{-1}$].

Statistical analysis: Data were expressed as mean ± standard error (SE). Differences in biomass, gas-exchange parameters, Ψ_L, RWC, and TWUE were tested using one-way *ANOVA* (p=0.05). Correlation analysis was used to analyze relationships between relative P_N, E, g_s, and SWC for the two legumes.

Results

SWC decreased linearly in response to the drying treatment for the two species (Fig. 1). The equations were y = −0.82 x + 14.67 (R^2 = 0.97) and y = −0.79 x + 14.52 (R^2 = 0.97) for alfalfa and milkvetch, respectively. On the 16th day, SWC values for alfalfa and milkvetch were not significantly different (Fig. 1).

RWC: The two species had a threshold SWC between 4.9% and 5.1% at which the leaf RWC decreased sharply during soil drying (Fig. 2). The mean leaf RWC of alfalfa was lower than that of milkvetch for SWCs above the threshold value, but was not significantly different (p>0.05). Below the threshold SWC at the end of the drying treatment, alfalfa leaf RWC was significantly higher than that of milkvetch (p<0.05) (Fig. 1).

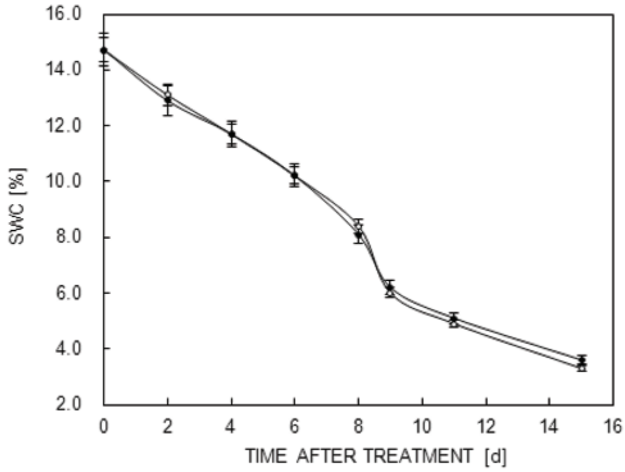

Fig. 1. Soil water content (SWC) change after withholding irrigation from alfalfa (○) and milkvetch (•) seedlings. Each value represents the mean ± standard error (n = 5).

Leaf gas-exchange *vs.* SWC: P_N, E, and g_s of each species decreased with decreasing SWC (Fig. 3). The relationships between the three-indices and SWC for both alfalfa and milkvetch were parabolic. When SWC had decreased to about 5.08%, the P_N value was 0 for alfalfa and became negative with further drying. Alfalfa had a significantly higher mean P_N than milkvetch when SWC was greater than about 6.0% (p<0.05); P_N values for milkvetch remained positive with further drying. When SWC had decreased to about 7.2%, the g_s of the water-stressed and well-watered plants were significantly different in alfalfa (p<0.05); this occurred for milkvetch when SWC was about 10.3% (well-watered plant data, not shown). In contrast, the ψ_L values for each species were not significantly different between water-stressed and well-watered plants at those SWCs (p>0.05).

ψ_L *vs.* SWC: In the well-watered treatment, the ψ_L values of milkvetch (between −0.17 MPa and −0.19 MPa) and alfalfa (between −0.11MPa to −0.15MPa) were significantly different (p<0.05) (data not shown). During soil drying, the relationship between ψ_L and SWC could be described by a power function curve; alfalfa leaf ψ_L values were lower than those of milkvetch (Fig. 4). The SWC, at which the ψ_L values of water-stressed plants were significantly lower (p<0.05) than those of well-watered plants, was about 6.6% and 6.8 % for alfalfa and milkvetch, respectively (Fig. 4).

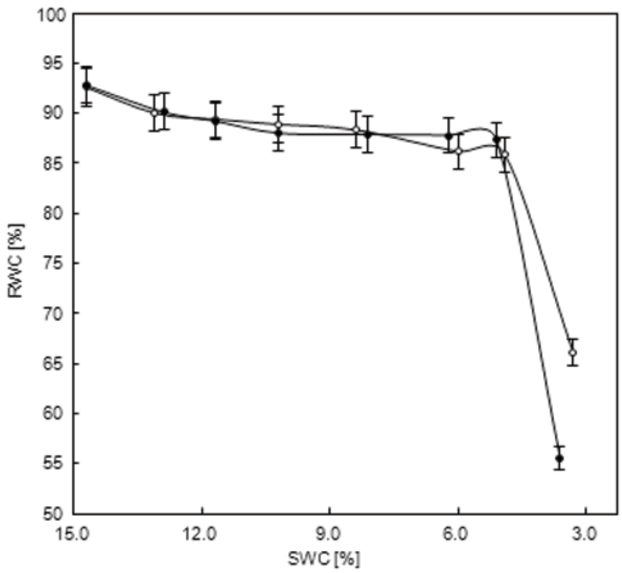

Fig. 2. Leaf relative water content (RWC) change with soil water content (SWC) for alfalfa (○) and milkvetch (•) seedlings. Each value represents the mean ± standard error (n = 5).

Seedling biomass and allocation: Soil drying significantly decreased alfalfa and milkvetch total DM by 34.2% and 37.8%, respectively (p<0.05) (Fig. 5). The R/S ratio of alfalfa had significantly increased by 17.9% following the entire drying period, and that of milkvetch increased by 1.5 times, when compared to the well-watered treatment (p<0.05) (Fig. 5).

TWUE: Alfalfa had a significantly higher total biomass TWUE than milkvetch in the well-watered treatment while, after soil drying, milkvetch TWUE was higher (p<0.05) (Fig. 6*A*). In both water treatments, milkvetch had a higher shoot biomass TWUE than alfalfa (p<0.05) (Fig. 6*B*). Soil drying reduced total biomass TWUE of alfalfa by 29.1% while there was a nonsignificant increase in that of milkvetch by about 3.0% when

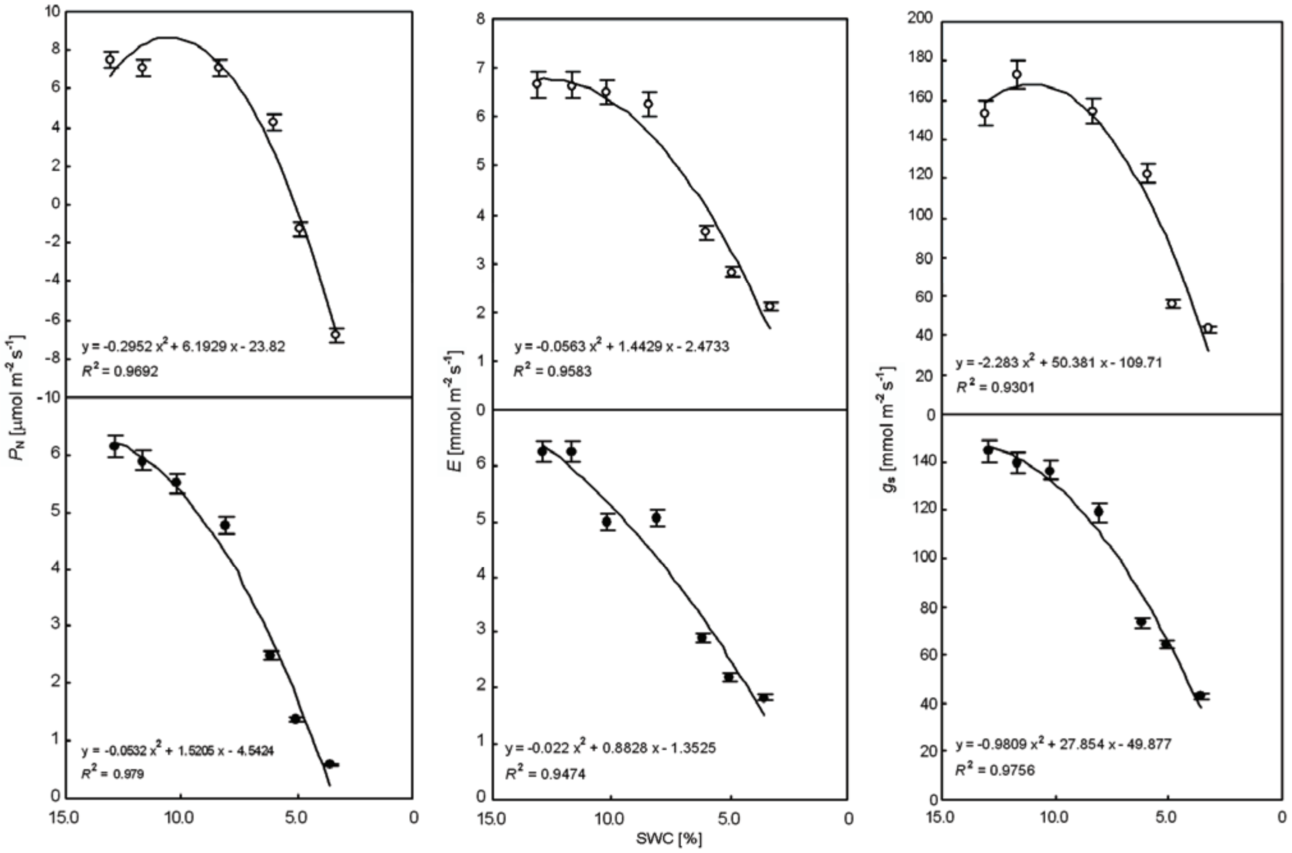

Fig. 3. Net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) changes with declining soil water content (SWC) for alfalfa (○) and milkvetch (●) seedlings. Each value represents the mean ± standard error (n = 5).

compared to the well-watered treatment (p>0.05) (Fig. 6*A*). Alfalfa TWUE based on shoot biomass was significantly lower than that of milkvetch, and water-stress significantly decreased the TWUE of both species by 34.1% and 24.8%, respectively (p<0.05) (Fig. 6*B*).

Discussion

Seedling growth and gas-exchange: A plant seedling can adapt to water stress by a number of mechanisms, for example, the ability to maintain viability at water potentials below the turgor loss point, rapid root growth to exploit the restricted soil water resources, and having a high R/S ratio (Ranney *et al*. 1990, Schütz *et al*. 2002). Our results were consistent with other studies on different plant species, in which water-stress decreased total biomass and altered biomass allocation to roots resulting in higher R/S ratios in stressed seedlings compared to well-watered plants (Figs. 5, 6) (Fotelli *et al*. 2001, Li *et al*. 2008). We observed that alfalfa grew rapidly, increasing its total biomass production, and had a relatively high TWUE when sufficient water was supplied, as reported by others (Grimes *et al*. 1992, Ray *et al*. 1999). We also observed a significant decrease in the TWUE of alfalfa under water-stressed conditions whereas there was a nonsignificant increase in that of milkvetch (p<0.05) (Fig. 6*A*). Mild water deficits will induce partial stomatal closure, and this can result in improvements in water-use efficiency because of the nonlinear relationship between g_s and P_N (Li *et al*. 2000, Xu *et al*. 2006a). The stability of the milkvetch TWUE based on total biomass was likely due to a more sensitive stomatal response (Table 1, Fig. 3) under water-stress than that of alfalfa, and a greater ability of the mesophyll cells to utilize intercellular CO_2 that was more directly related to photosynthesis than to stomatal aperture (Yao *et al*. 2001, Singh and Singh 2003).

Root signal: Water-stress results in reductions in many plant physiological processes, such as P_N, E, g_s, and metabolite accumulation. Furthermore, studies have shown that the sensitivity of leaf stomata to soil water change was not affected by leaf water conditions alone (Ranney *et al*. 1990, Yao *et al*. 2001, Zewdie *et al*. 2007). In our study, milkvetch responded to soil drying at a higher SWC threshold than alfalfa by reducing its

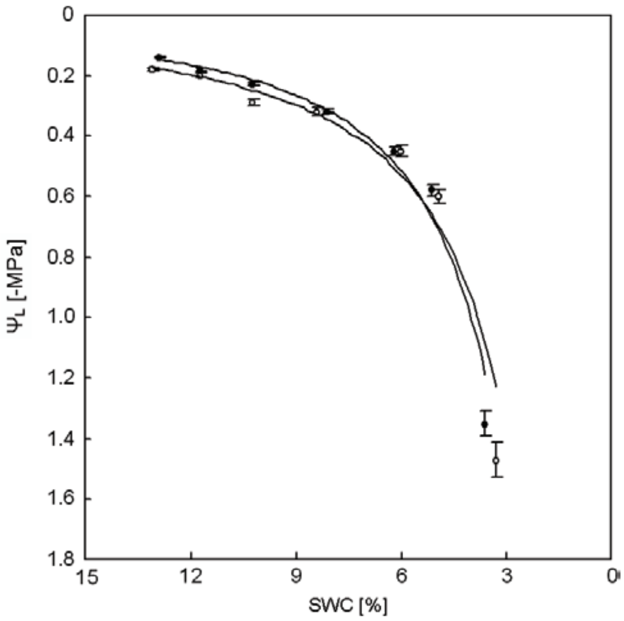

Fig. 4. Leaf water potential (ψ_L) change with declining soil water content (SWC) for alfalfa (○) and milkvetch (●) seedlings. Each value represents the mean ± standard error (n = 5).

stomatal conductance and, consequently, its transpiration rate (Fig. 3, Table 1). These kinds of reductions, when they occur before soil moisture contents in a drying soil reach a level that is critical to seedling survival, may ensure that milkvetch avoids irreversible damage due to the drought conditions by maintaining some measure of water availability. In order for this to happen, the milkvetch response depended on long-distance chemical signals (nHRS) (Li *et al.* 2000, Xu *et al.* 2006a). The milkvetch response to the drying soil occurred before the SWC was so low that water would no longer be transported quickly enough to respond to transpiration demand, which would have triggered plant water-stress due to a hydraulic signal (HRS) (Dingkuhn *et al.* 1989). The determinations of nonhydraulic and hydraulic signal appearances are commonly based on the difference in physiological indices (g_s and ψ_L) between plants that are well-watered and those growing under water-deficit conditions (Croker *et al.* 1998, Mencuccini *et al.* 2000, Li *et al.* 2000, Xiong *et al.* 2006, Xu *et al.* 2006a). The SWC threshold value at which the nHRS occurs and the difference in SWC threshold values between nHRS and HRS occurrences have been taken as gauges by which to

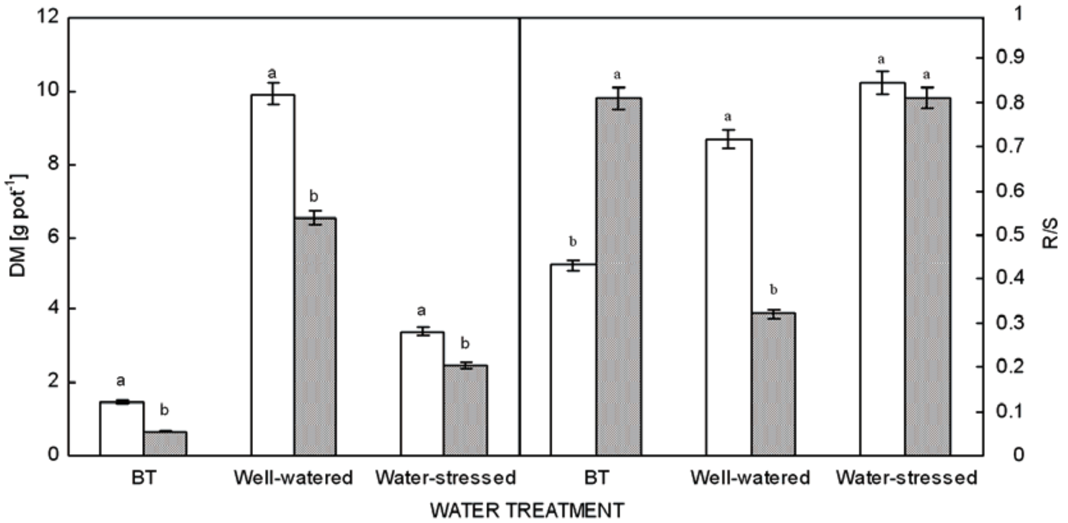

Fig. 5. Dry mass (DM) and root:shoot ratio (R/S) for each water treatment for alfalfa (*open bars*) and milkvetch (*grey bars*) seedlings. Each value represents the mean ± standard error (n = 5). BT – before water treatment; the same lowercase letters above bars indicate no significant difference between values for the two species within a given water treatment (p<0.05).

evaluate plant sensitivity and drought tolerance, and these values are also positively correlated with drought dehydration capability (Croker *et al.* 1998, Li *et al*. 2000, Xu *et al*. 2006a). Thus, milkvetch seedlings, by responding more quickly to soil drying, would be more tolerant of drought conditions than alfalfa seedlings (Table 1, Fig. 3). Furthermore, a smaller decline in SWC below the threshold value for alfalfa seedlings could expose alfalfa leaves to a lethal drought episode.

Although the stomatal response of milkvetch and alfalfa was evident at SWC of 10.3% and 7.2%, respectively, evidence of water-stress in leaf potential values occurred at lower SWC (6.8% and 6.6%). Thus the difference between the SWC thresholds for nHRS and HRS were 0.6% and 3.5% for alfalfa and milkvetch, respectively. This phenomenon of plants adjusting to water-stress without detectable changes in leaf water status is due to osmotic adjustment (Davies and Zhang 1991), and the capacity for osmotic adjustment depends on the rate of soil drying (Auge *et al*. 2003). The soil-drying rate in our study was less than 1.0% per day, which would be classed as a slow soil-drying rate (Fig. 1) (Thomas 1991)

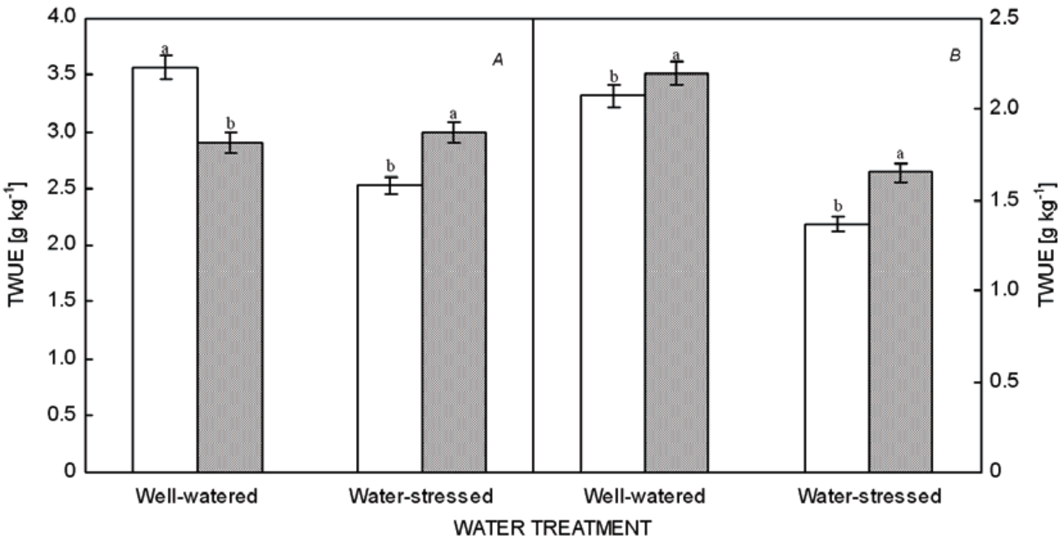

Fig. 6. Transpiration water-use efficiency (TWUE) of alfalfa (*open bars*) and milkvetch (*grey bars*) seedlings for two water treatments (*A*: based on total dry biomass; *B*: based on shoot dry biomass). The same lowercase letters above bars indicate no significant difference between values for the two species within a given water treatment ($p<0.05$). Each value represents the mean ± standard error ($n = 5$).

Table 1. Threshold soil water contents (SWC) at which nonhydraulic (nHRS)- and hydraulic root signal (HRS) occurred and the threshold range for two seedling species. Each value represents the mean ± standard error ($n=5$). Values within a column followed by different letters are significantly different ($p<0.05$).

Species	nHRS SWC [%]	HRS SWC [%]	SWC threshold range [%]
Alfalfa	7.2 ± 0.01^b	6.6 ± 0.01^a	0.6 ± 0.01^b
Milkvetch	10.3 ± 0.03^a	6.8 ± 0.02^a	3.5 ± 0.01^a

and plant osmoregulation was possible in both milkvetch and alfalfa. Under a more rapid onset of drought stress, the capacity of these species for plant osmoregulation would be impaired or lost (Arndt *et al*. 2000).

Conclusion: Soil drought conditions significantly affected the growth of both leguminous species studied. Although alfalfa and milkvetch exhibited similar responses in growth to cope with severe drought stress, there were significant differences in their gas-exchange and leaf water responses to soil drying. Our results showed that milkvetch seedlings adapted better to drought under the changing soil water conditions, whereas a sufficient water supply was beneficial for alfalfa seedling growth.

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