Sensitivity of root and leaf water status in maize (*Zea mays*) subjected to mild soil dryness

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U. Schmidhalter[A], Z. Burucs[B] and K.H. Camp

Technische Universität München, Chair of Plant Nutrition, 85350 Freising-Weihenstephan, Germany.
[A]*Author for correspondence; e-mail: Schmidha@edv.agrar.tu-muenchen.de*
[B]*Present address: Water Management Department, Pannon University of Agriculture, Georgicon Faculty, 8361 Keszthely, Hungary.*

Abstract. The aim of this work was to test the sensitivity of the water potential (Ψ_w), osmotic potential (Ψ_s) and turgor (Ψ_p) of roots and leaves of maize seedlings (*Zea mays* L. cv. Carla) subjected to a mild stress in drying soil in a growth chamber. To the best of our knowledge there are no experimental data which describes diurnal courses of Ψ_w in soil, roots, and leaves and the parallel changes in the osmotic potential (Ψ_s) and turgor (Ψ_p) of roots and leaves from plants grown in moist and drying soil. Root and leaf Ψ_w varied diurnally, the amplitude being much more marked in leaves than in roots. Root and leaf Ψ_w did not achieve equilibrium at predawn with the bulk soil matric potential (Ψ_m) but became higher. Our results are at variance with data indicating root Ψ_w is a sensitive indicator of soil dryness. Root Ψ_w in the well-watered and drought-stressed treatments did not differ, whereas daytime leaf Ψ_w in the droughted treatment was lower 6 days after water was withheld. Diurnal changes in Ψ_s and Ψ_p were more marked in leaves than in roots. Withholding water lowered leaf Ψ_p, whereas root Ψ_p substantially increased after only 3 days of withholding water. Early mild stress can be more easily and more quickly identified by changes in root Ψ_s, increases in root Ψ_p, or the divergence in root and leaf Ψ_p than by a lower Ψ_w of root or leaf. Relative water contents of roots and leaves measured in the light period indicated also sensitively falling Ψ_m.

Introduction

There have been very few attempts to measure experimentally the diurnal water potential (Ψ_w) gradients between soil, root, and leaf. Therefore, practically all descriptions of water flow in the soil-plant-atmosphere continuum are based on one or more assumptions. To the best of our knowledge there are no experimental data which describe diurnal courses of Ψ_w in soil, roots, and leaves and the parallel changes in the osmotic potential (Ψ_s) and turgor (Ψ_p) of roots and leaves from plants grown in moist and drying soil. This knowledge is required to determine the parameters in the root and leaf water status which react sensitively to water stress. Whereas diurnal changes in leaf Ψ_w have been demonstrated, comparative diurnal courses in root Ψ_w have rarely been described. There are only a few studies that give some evidence of the diurnal behaviour of root Ψ_w (De Roo 1969; Hellkvist *et al.* 1974; Nnyamah and Black 1977). Changes in leaf Ψ_w and root surface Ψ_w and soil matric potential (Ψ_m*) as transpiration proceeds in a plant rooted in initially wet soil were represented schematically by Slatyer and Denmead (1964) (after Taylor 1962) and Slatyer (1967). This representation has been reproduced many times (Nobel 1991; Mohr and Schopfer 1992) and has considerably influenced ideas about diurnal Ψ_w gradients in the soil-root-leaf continuum.

Progress in the field of root water relations has been slow, mainly because simple methods of measurement were not available. Psychrometric measurements permit only a few measurements per day; calibration and measurements are time-consuming, and many errors can arise from inappropriate handling of root samples. Experienced researchers, aware of the many pitfalls of this technique, are needed to obtain good results. The pressure chamber technique was first applied by De Roo (1969) in studying root water relations, and his ideas were taken up by Gee *et al.* (1973, 1974). Controversial ideas about the correctness and validity of the pressure chamber technique for measurements of root water relations remain. Since then this technique seems largely to have been forgotten because roots are more difficult to handle than leaves. Seven years ago, the pressure chamber technique was successfully applied to the measurement of root Ψ_w for the relatively thin roots of various desert succulents (Nobel and Lee 1991) and for single roots (Petrie and Hall 1992; Schmidhalter *et al.* 1992*a*) and whole root systems of various agricultural plants (Schmidhalter *et al.* 1992*b*). By combining this technique

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*Abbreviations used: DAW, days after beginning different watering treatments; Ψ_m, soil matric potential; RWC, relative water content.

with osmometric measurements, Ψ_s and Ψ_p in roots can be described.

Many recent studies describing sensitive reactions of plants in drying soils are characterized by the induction of rather drastic decreases in soil Ψ_w within a few days. This is not typical of most agricultural soils in drought prone areas except those that are marginally productive, for example sandy soils, and is also not realistic for most soils in more temperate regions characterized by infrequent droughts.

Roots may become substantially dehydrated in drying soil (Wang *et al.* 1991). However, there are no data demonstrating unambiguously a higher sensitivity of the root water status or the leaf water status to mild soil dryness. We consider evidence obtained so far indicating that Ψ_w or Ψ_p in root tips may drop, when there are still no changes observed in leaf water status, as rather speculative. An experiment was therefore conducted with soil-grown maize seedlings (*Zea mays* L.) to determine diurnal variation in Ψ_w, and additionally Ψ_s, and Ψ_p of maize roots and leaves in moist and drying soil.

Materials and methods

Experimental conditions

The experiment was conducted in a growth chamber under constant conditions and lasted 15 days (light period/dark period 12/12 h; temperature 23/23°C for 3 days after beginning different watering treatments (DAW 1–3) on day 9 and thereafter 20/18°C for another 3 days (DAW 4–6); relative humidity averaged 60% and varied from 50 to 70% during the light period and was about 90% during the dark period; daytime VPD-deficit ranged from 0.84 to 1.4 kPa for DAW 1–3, and ranged from 0.69 to 1.16 kPa for DAW 4–6; photosynthetic photon flux density at plant height was 450 μmol m^{-2} s^{-1}). Temperature was lowered on DAW 4 to induce a slow drying of the soil.

Cultivation of plants

Maize seeds (*Zea mays* L., cv. Carla), pre-germinated for 1 day, were sown in pots (200 mm high, 100 mm in diameter) in moist soil at a depth of 20 mm. Four seeds were sown in each pot. The pots contained 1.32 kg (dry weight) illitic-chloritic silt loam (fine mixed mesic Aquic Ustifluvent) which was 9.1% clay, 59.5% silt, 31.4% sand, and 0.85% organic matter (Schmidhalter *et al.* 1994). Dry soil was mixed with 330 mL 1/4-strength Hoagland's nutrient solution to produce a Ψ_m of –0.035 MPa, using a previously established soil water retention curve (see below) and covered with a 8 mm layer of quartz sand (grain size 1–2 mm). Pots were covered with transparent polyethylene sheets to prevent further evaporation from the soil surface. Seedlings grew and were watered through small holes in the polyethylene sheets. Plants were thinned to two seedlings per pot after emergence.

Water relations of soil, roots, and leaves

Gravimetric water contents were converted to soil matric potentials (Ψ_m) using a previously determined relationship (Fig. 1). This had been determined using the method of Klute (1986) with soil of the same bulk density as that used in the experiments and the soil water retention model of Van Genuchten (1980). Van Genuchten's retention function is given by

$$\theta = \theta_r + \frac{\theta - \theta_r}{\left[1 + (\alpha h)^n\right]^m},$$
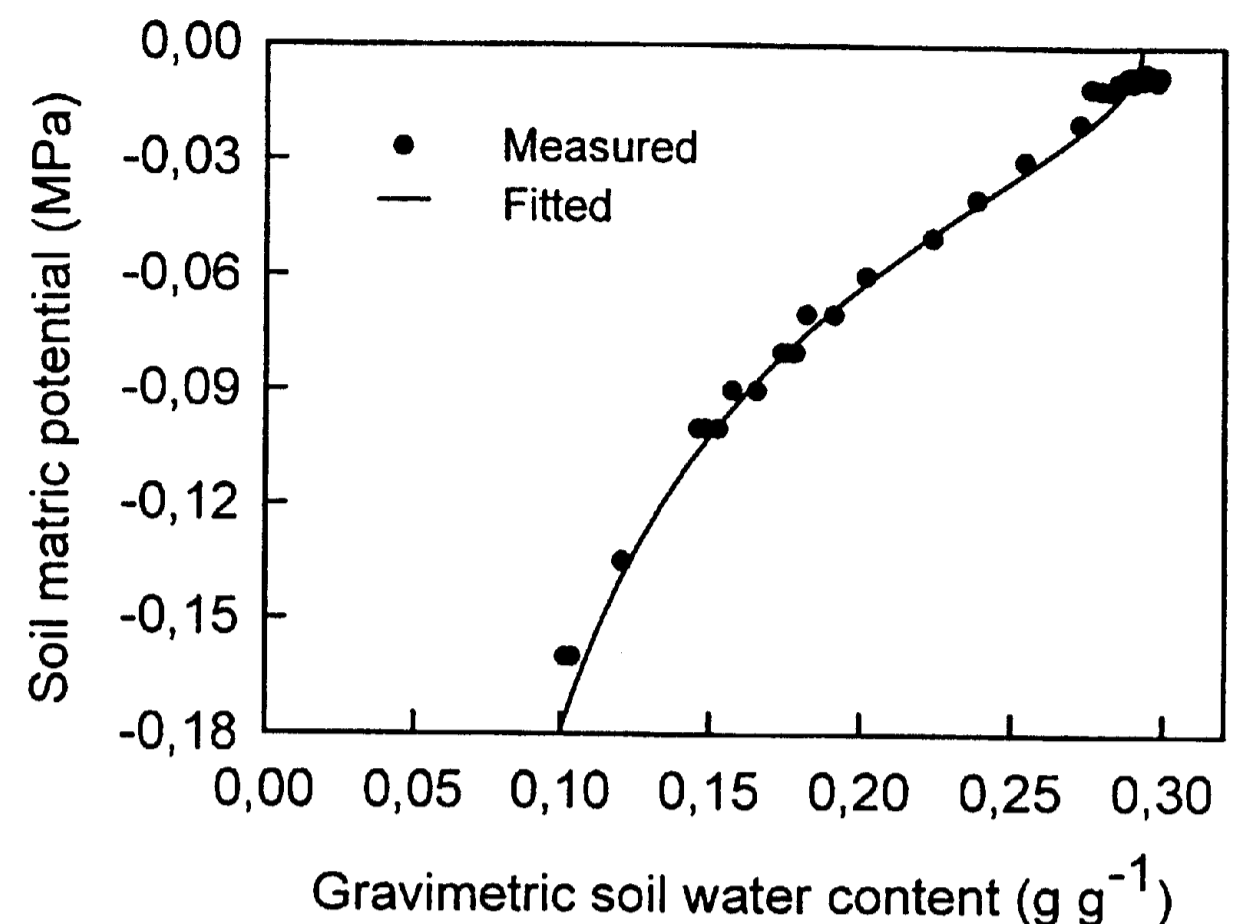

Fig. 1. Soil water retention curve of Charrat silt loam. The Van Genuchten function was fit to the water retention data. See Materials and methods for details.

where θ is the water content (g g^{-1}) at a given pressure head h (cm), θ_r is residual water content, θ_s is saturated water content, θ is an empirical parameter, m and n are dimensionless empirical shape parameters. The following values were determined for θ_s, θ_r, θ, n, and m: 0.293, 0.015, 0.0018, 2, and 0.5. The osmotic component of soil Ψ_w was higher than –0.02 MPa in the investigated range and was not included in further evaluation of the soil Ψ_w.

A pressure chamber was used to determine balancing pressures of root and leaf tissues. Previous investigations showed that the osmotic pressure of xylem sap in both tissues was in general smaller than 0.02 MPa (maximum values recorded were < 0.04 MPa at predawn). Because the xylem sap Ψ_s was small and sufficiently close to the soil Ψ_s this step was omitted in subsequent determinations. Values of soil and plant Ψ_w presented are, therefore, based only on pressure plate and pressure chamber determinations, respectively.

Water potential and Ψ_s of leaves were determined with a pressure chamber (PMS Instrument Co., Model 1002, Corvallis Co., Oregon, UT, USA) and a vapour pressure osmometer (Wescor 5500, Wescor Inc., Logan, USA), respectively. Turgor was calculated as the difference between Ψ_w and Ψ_s.

The same measurements of water status were made for whole roots using a pressure chamber/osmometer technique (Schmidhalter *et al.* 1992b). Given the heterogeneity of the water relations between sheathed and unsheathed maize roots (Wang *et al.* 1991), it might seem problematic to measure average values of Ψ_w and Ψ_s. However, no marked differences among roots of the same maize seedling were to be expected. Root systems of the investigated seedlings consisted of the primary root and a tier of four shoot borne roots and were less than 15 days old. Such young roots still have closed immature late metaxylem (Wenzel *et al.* 1989) and consequently a similar water status to each other. All roots were still sheathed with closely adhering soil which prevented water losses when used for the pressure chamber measurements (see below). Previous work indicated no significant Ψ_w difference among different root types in maize seedlings of this age (Schmidhalter *et al.* 1992a).

Leaf and root Ψ_w were determined sequentially, and alternatively for well-watered and droughted plants. A set of leaf and root Ψ_w determinations was made in less than 3 min. We previously checked with longer equilibration times that this time allowed for a true balancing pressure. The youngest fully developed leaf blade, cut one third from the leaf base, and the root system dissected at the mesocotyl were used for measurements.

Roots were cut from shoots only after the whole root system had been removed from the soil. For Ψ_w determinations, the bulk soil was gently shaken from the root system. Roots were first inserted through a metal ring (inner radius 14.8 mm, outer radius 16.1 mm, height 2.5 mm) and then through the hole of a rubber stopper (height 6.5 mm). The metal ring was manually pressed into a slightly narrower groove of the stopper facing the underside of the chamber top. The rubber stopper together with the root was inserted into the cone below the chamber top. The force applied to the tissue could be controlled and overpressurization of root cortical tissue was minimized. Using a stream of compressed air roots were cleaned more thoroughly within less than 30 s after the pressure chamber reading was taken. After the measurements leaf and root tissues were immediately sealed in plastic bags and kept in ice before transfer to the deep-freeze (–80°C). For Ψ_s determinations the tissues were plunged into liquid nitrogen and then equilibrated at room temperature. Sap was obtained from leaves and roots with a specially designed press and Ψ_s from sap determined with an osmometer. Osmotic potentials were not corrected for apoplastic dilution.

Transpiration and relative water contents of leaves and roots

Transpiration was determined by daily weighing and these values were checked by using water balance calculations based on water addition, gravimetric soil water contents at the beginning of the experiment and at each sampling for the plant water status. Evaporation losses were very small and could be neglected.

Relative water content (RWC) was calculated with the following equation: RWC% = 100 × [(fresh weight–dry weight)]/[turgid weight–dry weight]. Fresh and dry weights of leaves were determined gravimetrically, the latter after drying at 65°C for 48 hours. Values for roots were determined after the bulk of the soil had been gently shaken from the root system. The fresh weight of the root system with adhering soil (rhizoplane soil) was immediately thereafter determined gravimetrically. The root system was then dried at 60°C for 2 days and its dry weight determined. These roots were then briefly (to avoid significant losses of solutes) cleaned in water and redried, and the dry weight of the clean roots was again determined. By using a representative sample of rhizoplane soil, the water content of the soil was determined to enable calculation of the root fresh weight. Turgid weights can be obtained by resaturating the cut shoot (not the leaf), with only the cut shoot immersed in water. Our experience has shown that RWC of well-watered plants at predawn are very well approximated by this resaturating technique. The highest observed water contents of fully irrigated non-stressed leaves and roots were assumed to represent water content at 'full' hydration. This is a reasonable assumption because the water content in soil-grown roots and shoots will seldom exceed this value. Increased water contents due to artificial rehydrating represent artifacts. This risk is especially increased by immersing roots or leaves which leads to excessive rehydration of the intercellular space. Resultant negative consequences have been discussed by various authors (Meinzer et al. 1986; Parker and Pallardy 1988; Kubiske and Abrams 1991).

Experimental design and statistical analysis

A completely randomized design was used with three to six replications. All pots were first watered regularly for 9 days after emergence to maintain Ψ_w between –0.03 and –0.05 MPa. Thereafter, two treatments were applied: (i) a well-watered treatment (Ψ_{soil} –0.03 to –0.05 MPa) and (ii) a drought stress treatment in which water was withheld. Water relations of well-watered plants were measured 1, 4, and 6 days after beginning the different watering treatments and after 3, 4, 5 and 6 days in droughted plants. Leaf and root water relations were determined on at least three plants from different pots for each treatment at –1, 0, 2, 5, 8, 11, 12, 13 h in the 12-h light period. Measurements of soil water content and leaf and root dry matter were conducted at the same time and with the same number of

replications. Mean values are based on three observations from different pots except for day–night transitions in water status of plant and soil which are based on three to six observations. The whole experiment was repeated with similar results.

Characterization of drought response of specific traits can be derived from comparisons with well-watered control plants or by examining the trait as the soil dries. Statistical analysis was conducted on the combination of DAW (days after beginning the different watering treatments) and watering treatments to allow both comparisons. The combination of DAW × watering treatments was considered to be a fixed effect. The analysis was carried out separately for the two periods representing differences in VPD. The higher VPD treatment (DAW 1–3) included two levels (DAW 1 ww (well-watered) and DAW 3 ds (drought stress)) and the lower VPD treatment (DAW 4–6) included five levels (DAW 4 ds, DAW 5 ds, DAW 6 ds, DAW 4 ww, and DAW 6 ww). Analyses were performed separately with values from predawn measurements (–1 h in the light period) and with daytime values (measurements from 2, 5, 8 and 11 h into the light period). Data from the night/day (0 h into the light period) and day/night transitions (12 h into the light period) could not be analyzed, because they varied considerably from day to day. Prior to the analysis of variance, tests indicated homogeneity of variance among the treatment levels. Differences in root and leaf Ψ_w and components, and RWC of roots and leaves were analyzed using the SAS-GLM procedure (SAS/STAT 1990).

Results

Soil matric potential

In the drought stress treatment, Ψ_m decreased continuously from about –0.04 MPa to about –0.14 MPa after 6 days (Fig. 2). This represents a mild stress with slowly decreasing Ψ_m, ideal for examining the sensitivity of root and leaf water status as early indicators of drought stress.

Diurnal variation in water potential of roots and leaves

Variation in leaf and root Ψ_w for well-watered and droughted maize plants is shown in Fig. 2. Root and leaf Ψ_w changed diurnally. Leaf Ψ_w varied more than root Ψ_w. The diurnal decrease in Ψ_w of leaves is typical for growth chamber conditions where, after illumination there is a rapid transpiration-induced decrease in Ψ_w, followed by a slower gradual decrease during the day. The latter was less evident for root Ψ_w.

Leaf and root Ψ_w changed more at higher VPD on days 1 and 3 than on days 4, 5, and 6. The two periods (DAW 1–3 and DAW 4–6) were analysed separately for both predawn and daytime values in the water status. Withholding water for 3 days did not significantly affect predawn and daytime values of leaf and root Ψ_w. On days 4, 5, and 6, no difference in the root Ψ_w was found between well-watered and drought stressed treatments. Results of the statistical comparison of predawn and daytime values of root and leaf Ψ_w on DAW 4–6 (Tables 1 and 2) indicate leaf Ψ_w was significantly lower in the drought stressed treatment on day 6. Changes in leaf and root Ψ_w were greatest at the beginning and end of the light period. These were comparatively fast (< 10 min) when the light period commenced and ended (Fig. 3).
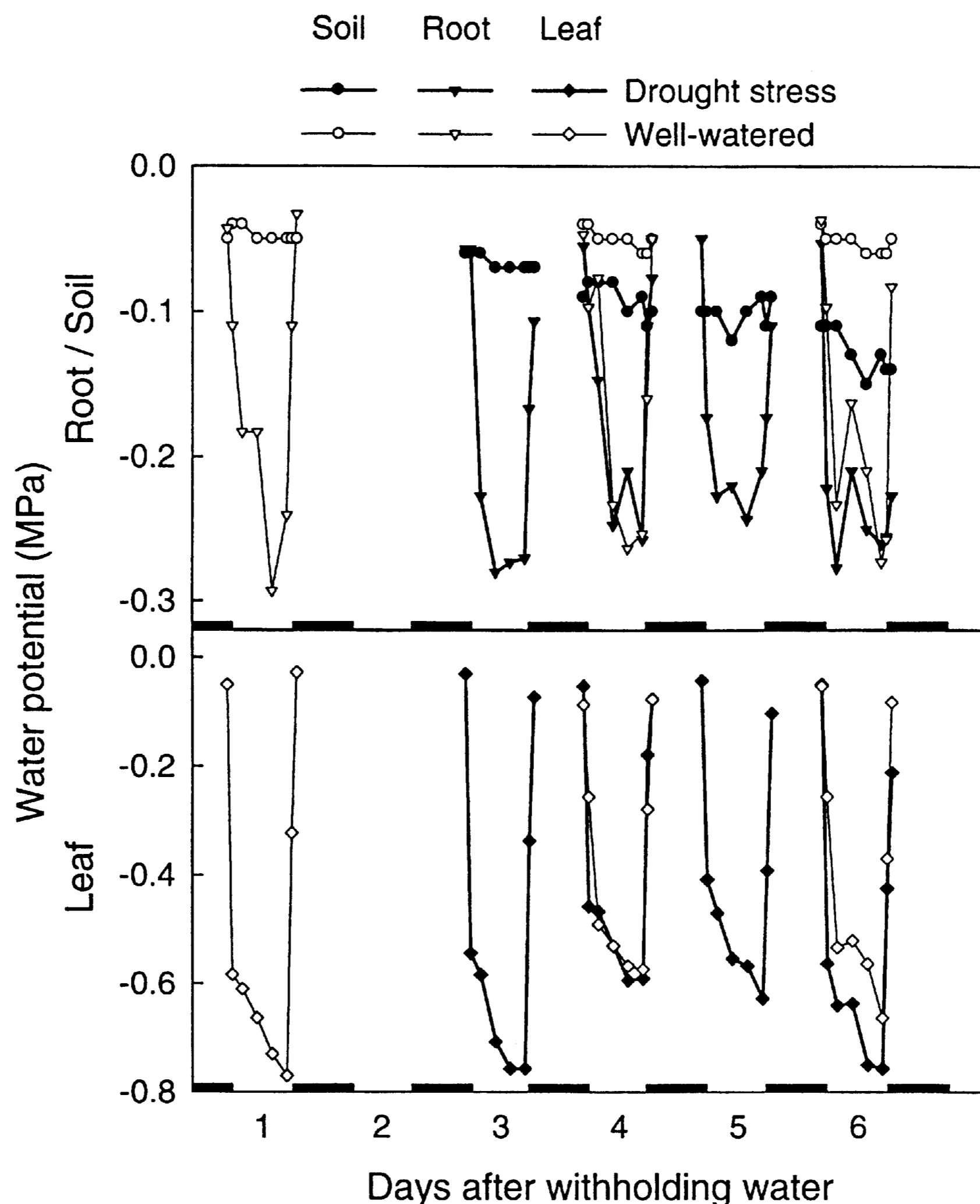
Fig. 2. Changes in Ψ_m, Ψ_w of leaves and roots of maize seedlings which were well-watered or subjected to drought stress by withholding water 9 days after emergence. The experiment was conducted in a growth chamber under constant conditions (12/12h dark/light period indicated by white/hatched bars on the *x*-axis; temperature 23/23°C until 3 days after withholding water, thereafter 20/18°C). Mean values of three to six observations are depicted. Error bars were omitted for reasons of clarity. Statistical information is given in Tables 1 and 2.

Transpiration did not differ between the two treatments until DAW 4, but decreased on DAW 6 in the drought-stressed treatment. The Ψ_w gradient between root and leaf was increased by the higher VPD deficit during the first 3 days of the experiment and by withholding water on DAW 6 (Table 1). Predawn Ψ_w gradients between roots and leaves hardly differed (Table 2). We do not have information about the hydraulic resistances encountered in the water conducting pathway between root and leaf, because the respective surfaces where water uptake and water losses occurred were not known. For the maize seedlings transpiring at a steady rate (DAW 1–4), Ψ_w differences between soil and root were smaller than between root and

leaf over the range of Ψ_m from –0.03 to –0.11 MPa. A comparison of the Ψ_w difference between soil and root, and root and leaf in well-watered plants shows that the major resistance is found between root and leaf for a constant transpirational flux. In a drying soil, the resistance between root and leaf must have been increased as compared with the soil-root resistance. Increases in plant resistance with soil drying have been demonstrated by Blizzard and Boyer (1980).

Diurnal variation in root and leaf osmotic potential

Withholding water for 3 days significantly decreased predawn and daytime values of root Ψ_s ($P \leq 0.05$). Predawn

Table 1. Daytime water potential (Ψ_w), osmotic potential (Ψ_s), turgor (Ψ_p) and relative water content (RWC) of leaves and roots of well-watered or drought-stressed maize seedlings

Maize seedlings were well-watered or subjected to drought stress by withholding water 9 days after emergence. Measurements were conducted 2, 5, 8 and 11 hs after the light period commenced on days 4, 5, and 6 after beginning the different watering treatments (DAW). Means within each water potential component of leaves or roots not followed by the same letter are significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$)

DAW	Water supply treatment	Ψ_w (MPa)		Ψ_s (MPa)		Ψ_p (MPa)		RWC (%)	
					Leaf				
4	Drought stress	−0.55	a	−0.85	a	0.31	a	98.2	b
5	Drought stress	−0.57	a	−0.85	a	0.28	a	98.0	b
6	Drought stress	−0.67	b	−0.91	a	0.24	a	96.6	c
4	Well-watered	−0.53	a	−0.89	a	0.37	a	98.6	a
6	Well-watered	−0.59	a	−0.95	a	0.36	a	97.9	b
					Root				
4	Drought stress	−0.22	a	−0.78	b	0.57	b	96.8	b
5	Drought stress	−0.23	a	−0.81	b	0.58	b	96.3	b
6	Drought stress	−0.25	a	−0.93	c	0.68	a	95.4	c
4	Well-watered	−0.21	a	−0.65	a	0.44	c	98.5	a
6	Well-watered	−0.22	a	−0.68	a	0.46	c	98.0	a

Table 2. Predawn water potential (Ψ_w), osmotic potential (Ψ_s), turgor (Ψ_p) and relative water content (RWC) of leaves and roots of well-watered or drought-stressed maize seedlings

Maize seedlings were either well-watered or subjected to drought stress by withholding water 9 days after emergence. Measurements were conducted, immediately before the light period commenced, on days 4, 5, and 6 after beginning the different watering treatments (DAW). Means within each water potential component of leaves or roots not followed by the same letter are significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$)

DAW	Water supply treatment	Ψ_w (MPa)		Ψ_s (MPa)		Ψ_p (MPa)		RWC (%)	
					Leaf				
4	Drought stress	−0.05	a	−0.78	a	0.73	a	99.1	ab
5	Drought stress	−0.04	a	−0.70	a	0.66	a	99.1	ab
6	Drought stress	−0.05	a	−0.72	a	0.67	a	98.1	b
4	Well–watered	−0.09	a	−0.80	a	0.71	a	100	a
6	Well–watered	−0.05	a	−0.76	a	0.70	a	99.0	b
					Root				
4	Drought stress	−0.06	a	−0.87	c	0.81	a	96.7	b
5	Drought stress	−0.07	a	−0.77	b	0.70	b	96.3	b
6	Drought stress	−0.05	a	−0.91	c	0.86	a	97.3	b
4	Well–watered	−0.05	a	−0.61	a	0.57	c	100	a
6	Well–watered	−0.04	a	−0.61	a	0.57	c	99.0	ab

and daytime values of root Ψ_s were higher than leaf Ψ_s in well-watered plants (Fig. 4; Tables 1 and 2). With lower soil moisture, daytime values of Ψ_s for roots and leaves were comparable, and the predawn values of root Ψ_s were lower than leaf Ψ_s. Leaf Ψ_s of well-watered and drought-stressed maize seedlings were the same on DAW 4 to 6. In contrast, root Ψ_s was clearly lower in the stress treatment as compared with the well-watered treatment. Daytime root Ψ_s was lower in the drought stress treatment on DAW 6 as compared with DAW 4.

Diurnal variation in root and leaf turgor

Diurnal variation in Ψ_p was much more marked in leaves than in roots (Fig. 5). During the daytime, root Ψ_p values were slightly higher than leaf Ψ_p in well-watered plants and were much higher in drought-stressed treatments (Table 1). Withholding water lowered leaf Ψ_p only slightly compared to the well-watered treatment. A drastic effect, however, was observed in root Ψ_p. After only 3 days of withholding water, root Ψ_p values increased substantially during both the light
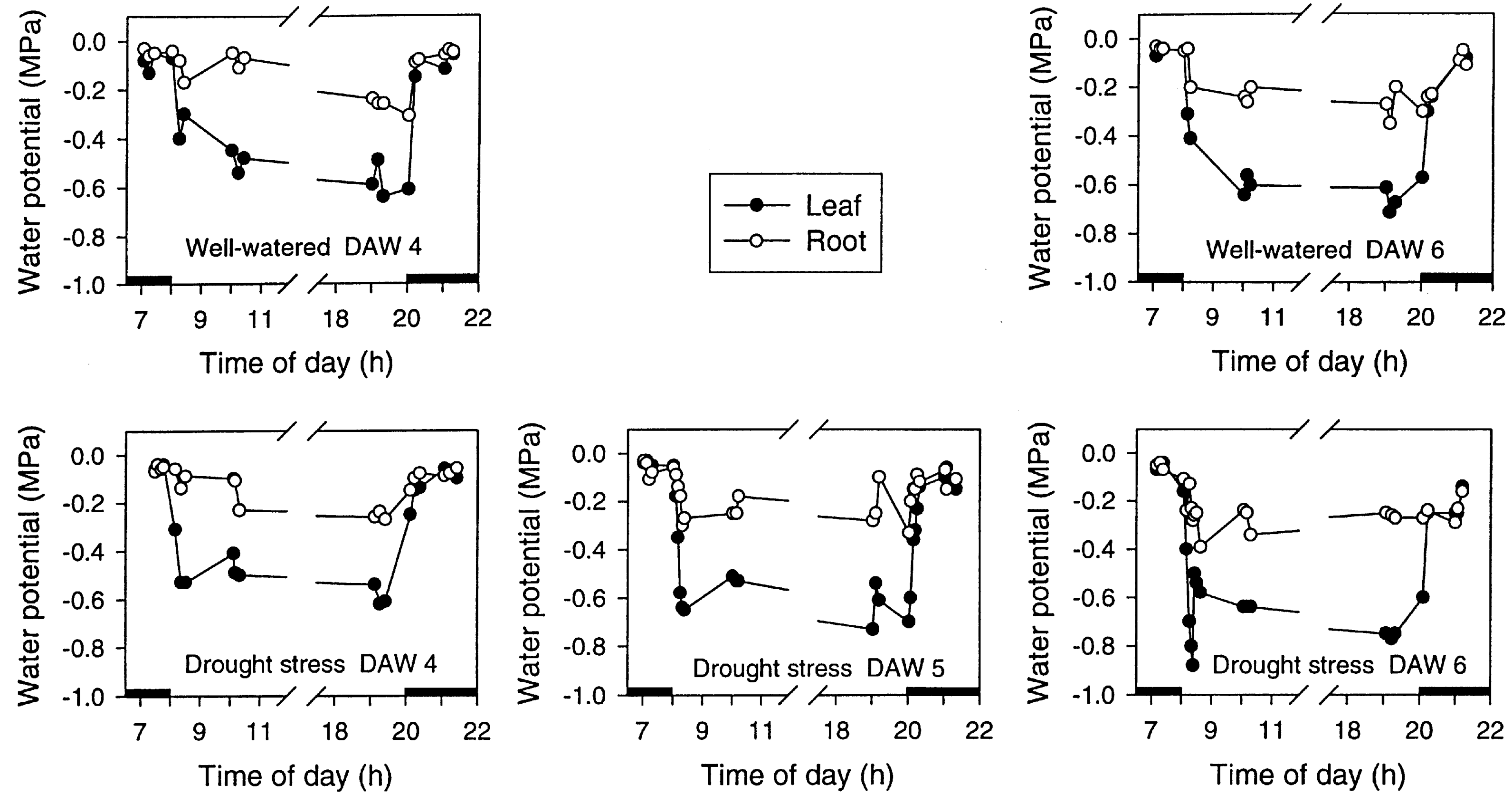
Fig. 3. Changes in Ψ_w of roots and leaves of well-watered and drought stressed maize seedlings with the onset and termination of the light period in a growth chamber under constant conditions (12/12h dark/light period indicated by white/hatched bars on the x-axis temperature 20/18°C). Drought stress was imposed by withholding water. Values shown are for days 4, 5, and 6 after beginning the different watering treatments.

and dark periods. Daytime root Ψ_p in plants subjected to drought increased on day 6. During the light period, differences between root and leaf Ψ_p increased with the duration of drought stress.

Diurnal variation in relative water content of leaves and roots

Predawn and daytime values in leaf RWC decreased on DAW 3 in the droughted treatment as compared to the well-watered treatment on DAW 1. Daytime values of RWC in leaves and roots decreased in the droughted treatment on DAW 4 and 6 (Table 1). RWC decreased during daytime and with increasing stress.
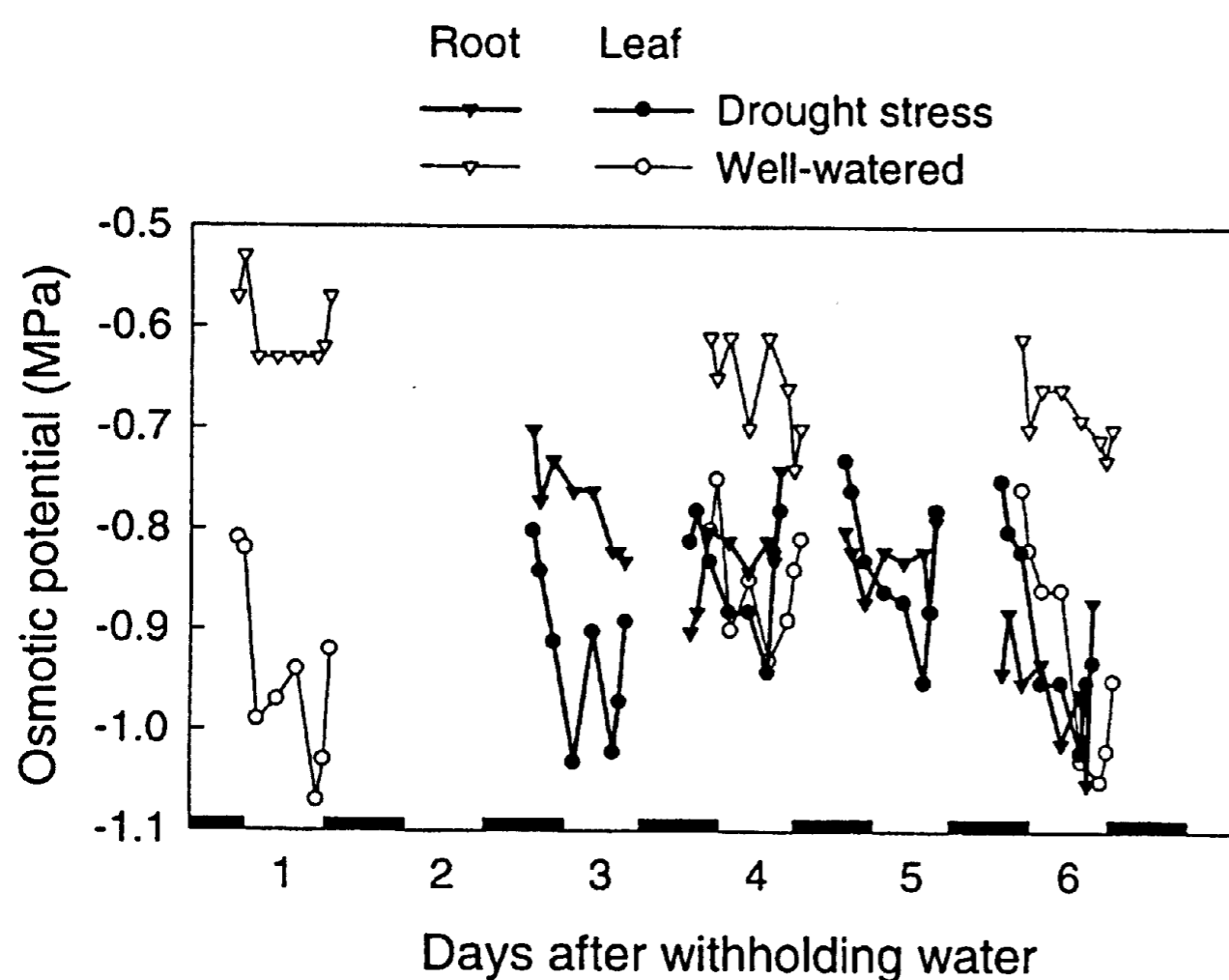

Fig. 4. Variation in Ψ_s of leaves and roots of maize seedlings which were well-watered or subjected to drought stress by withholding water. Further details are given in the legend of Fig. 2.
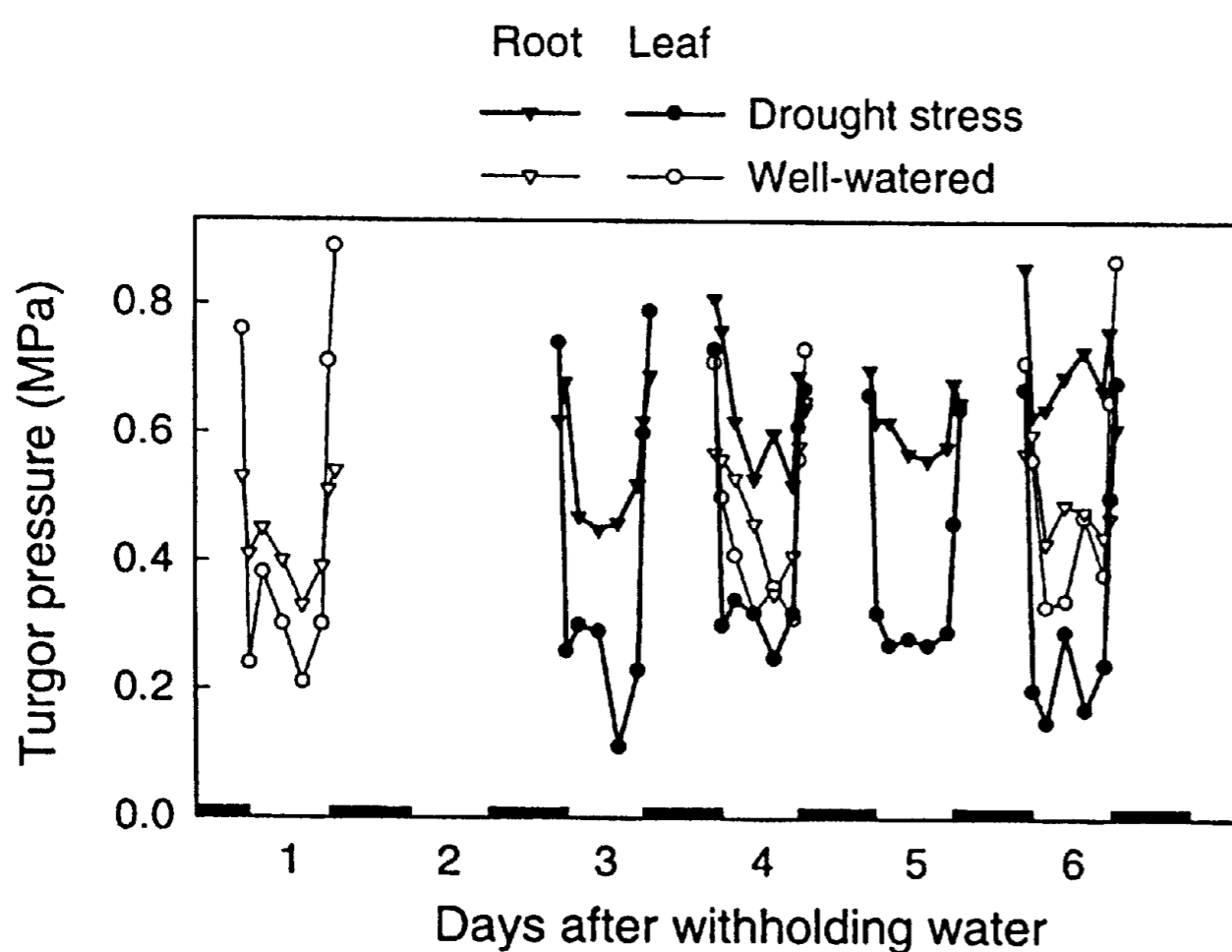

Fig. 5. Variation in Ψ_p of leaves and roots of maize seedlings which were well-watered or subjected to drought stress by withholding water. Further details are given in the legend of Fig. 2.

Discussion

The pressure chamber readings give a volume-averaged reading of Ψ_w or, more exactly, the pressure component of Ψ_w of the xylem sap that has come into equilibrium with the rest of the leaf (Passioura 1991). The osmotic potential of the xylem sap was sufficiently small to be neglected. Measurements taken during the day may not reflect equilibrium in Ψ_w between the xylem and surrounding tissues, which can be expected before dawn. Leaf and root Ψ_w were measured within 60 s after sealing the leaf or root. Longer equilibration times, however, were not necessary as indicated by previous investigations with comparably stressed plants.

Ψ_w gradients are the driving forces of water transport in plants. The diagrammatic representation of Ψ_w in the soil–root–leaf continuum by Slatyer (1967) and Fig. 2 show a distinct gradient between the root surface and the leaf, suggesting a major resistance. Our work suggests a somewhat stronger decrease in leaf Ψ_w than in root Ψ_w in transpiring plants, which is consistent with observations of Westgate and Boyer (1984). De Roo (1969) has observed that differences between the root and shoot must be caused by a rapid equilibration throughout the drier shoot and the wetter roots. He concluded that, since the root and shoot were joined at the point of decapitation and observation, the Ψ_w was originally the same at that point. Transpiration-induced gradients in Ψ_w disappeared within a few minutes after the light period ended (Fig. 3), and Ψ_w differences between root and leaf were eliminated. Observations of the initial decrease in Ψ_w with the onset of the light period revealed a quick decrease too. We do not know whether Ψ_w in the xylem is closer to the leaf or the root Ψ_w. Maize plants develop vessels that are continuous from the primary seminal root through the mesocotyl and the first node (Aloni and Griffith 1991). We expect, therefore, that Ψ_w within the xylem is similar for the root and leaf. Fig. 3 suggests that an internal resistance exists between the root and leaf. There must be a steep gradient in Ψ_w which, however, decreases rapidly with the onset of the dark period. If there is a major resistance at the endodermis, then Ψ_w in the xylem probably agrees more closely with the leaf Ψ_w. If it is at the endodermis, the cortex of the root will have a Ψ_w much closer to that of the soil than to the rest of the plant and hence it will be buffered against the diurnal changes experienced by the rest of the plant (Passioura 1988).

Fig. 2 shows that during daytime the root Ψ_w is closer to the soil Ψ_w (soil Ψ_m) than to the leaf Ψ_w. The subsequent parallel recovery of Ψ_w in roots and leaves to predawn values occurs more slowly compared to the rapid initial increase in root and leaf Ψ_w with the onset of the dark period. During this second stage, equilibrium is probably achieved with wetter sites in the soil. In contrast to the diagrammatic representation, we show that root and leaf Ψ_w

do not necessarily achieve equilibrium with the bulk Ψ_m but are higher. Other results support this by showing\ that plants do not respond to the average Ψ_m but rather to the highest Ψ_m, especially under predawn conditions (Schmidhalter *et al.* 1992*a*). This is to be expected, because root activity may have ceased in dry top soil and continued in wet subsoil. This mechanism enables plants to avoid severe dehydration. Consequently, water stress will develop more gradually in plants as long as the water demand can be met by uptake from wet sites in the soil due to new root growth. Therefore, the water status in leaves may not change although the bulk soil becomes drier. Root and leaf Ψ_w need not necessarily drop, and a higher Ψ_w may be found in the plant than in the bulk soil. The assumption in Slatyer's model that root Ψ_w falls with decreasing bulk soil water content because the soil hydraulic conductivity drops rapidly and inverted gradients between bulk soil and plant are not possible, is not generally correct. This is confirmed by a reversed water flow occurring from wetter roots in dry soil (e.g. Mooney *et al.* 1980). Roots are hydraulically interconnected and can thus transport water from wetter sites to drier sites (e.g. Baker and van Bavel 1986). Basic features, inherent in Slatyer's model representation of Ψ_w gradients in the soil-root-leaf-continuum, have been incorporated into many models describing Ψ_w gradients (Johnson *et al.* 1991; Lafolie *et al.* 1991) in the soil-plant continuum and need partial revision.

Root tip vs whole root studies

Previous studies demonstrating sensitive reactions in maize plants were based on water status measurements of root tips subjected to a rather drastic drying out of the soil. They differ from our work which includes measurements of whole root systems subjected to mild soil drying. However, results from our study can be extrapolated to more specific cases like studies of single roots or root tips. We also discuss some limitations of root tip studies to detect sensitive reactions in plants subjected to early and mild soil dryness.

It has been suggested for example, that root tips in drying soil have a lower turgor. Shallow fine roots in maize plants apparently dehydrated substantially even though larger roots (seminal and nodal) and deeper roots retained turgor (Zhang and Davies 1989). Decreases in root tip Ψ_w before such decreases in mature leaves occurred were observed in another study with maize plants (Sharp and Davies 1979) and in another species *Ceratonia siliqua* (Rhizopoulou and Davies 1989). A sensitive role in detecting early water stress was therefore ascribed to such decreases in root tip Ψ_w and Ψ_p.

These findings are however at variance with results from this and several other studies. In contrast to the afore mentioned studies it has been observed quite often that maize root tip turgor decreased only slightly or was even maintained in dry soil (Sharp and Davies 1979; Westgate

and Boyer 1985). In both cases substantial decreases in the osmotic potential of maize roots were observed.

Maintenance of root Ψ_p in the whole root system makes it very likely that root Ψ_p is also maintained in the root tip. The same holds true for Ψ_w. If Ψ_w remains steady or decreases only a little in the whole root system under mild soil dryness then it is logical to assume that transpiration-induced changes in Ψ_w will be small in root tips.

Measurements of Ψ_w in growing root tips are much more difficult to interpret because they include growth-induced changes in Ψ_w. There seem to be hydraulic barriers in root tips which can be ascribed to incomplete xylar development, which creates additional resistances in the water conducting pathway. Results from several studies in fact would suggest that there is a reversal of the flow with water being directed from shortly behind the root tip with the higher Ψ_w to the root tip with the lower Ψ_w (Sharp and Davies 1979; Davies *et al.* 1986). This must be concluded from the observed gradients in Ψ_w. In the latter studies, decreases in root Ψ_w may have been caused by cell wall relaxation of still growing root tissues. It remains difficult to conclude from decreases in root tip Ψ_w to drought-induced sensitive reactions in plants. The analysis is complicated by growth-induced gradients and the risk of cell wall relaxation in psychrometer chambers. Studies which used a rather drastic drought stress or severe stress conditions encompass the risk that dead root tips were sampled for Ψ_w measurements. Substantial death of root tips was found at very low soil Ψ_m (Jupp and Newman 1987). Drought-induced death of root tips may result in erroneous estimates of the root water status. Measurements of a few single root tips cannot describe average reactions of plants when subjected to soil drying. However, it is clear that a comparison of whole roots and single root tips would be most appropriate and should be further investigated. Growing regions in roots should be compared most preferably with growth zones in leaves.

Root Ψ_s and root Ψ_p react more sensitively to early and mild soil drying than root Ψ_w

This study shows that precise measurements are required to demonstrate small drought-induced differences in Ψ_w. There is no indication that root Ψ_w reacted more sensitively to drought stress than leaf Ψ_w in this study. A significant decrease in leaf Ψ_w was detected before any changes in root Ψ_w became apparent which agrees with expected results from a resistance–capacitance network when plants are transpiring. Early mild stress can apparently be more easily and quickly identified in this species in changes in root Ψ_s (decreases in this work; Fig. 4) and increased root Ψ_p (Fig. 5) than in decreased root Ψ_w (Fig. 2). We consider these reactions, therefore, to be more sensitive for indicating soil dryness than are changes in root Ψ_w. Divergence in root and leaf Ψ_p (root Ψ_p rising) was also a highly sensitive indicator

of drought stress, appearing before changes in root or leaf Ψ_w were observed. Our results, therefore, question the suggested role of decreases in root Ψ_w and root Ψ_p as sensitive indicators of soil dryness. Particularly interesting are the observations that decreases in RWC of roots and leaves can be used as sensitive indicators of drought stress as well. Because roots seem to be highly elastic, relatively large changes in root volume can occur with small changes in Ψ_w. This suggests that RWC of the roots might be a better indicator of incipient drought stress than Ψ_w of the roots.

The drop in Ψ_w, resulting from a decreased RWC, was much more gradual in roots than in leaves of maize (Wang *et al.* 1991; Evéquoz 1993). This may suggest a higher sensitivity of the leaf Ψ_w than of root Ψ_w to mild and moderate dryness in the soil. Roots may be better protected from water losses by the presence of a further resistance at the endodermis.

Osmotic adjustment in roots and divergence in Ψ_p between root and leaf

Decreases in water availability are frequently detected first in decreased leaf elongation (Evéquoz 1993). If such decreases are accompanied by an increased transport of organic osmotica to roots, then changes in root Ψ_s might be observed at a very early stage of soil dryness. Decreases in root Ψ_s will result from a shift of assimilates from leaves to roots when leaf elongation decreases and photosynthesis is not yet much affected. In this work the decreases in root Ψ_s were mainly caused by active accumulation and not by passive lowering due to water losses. This can be concluded from the small decreases observed in predawn RWC (less than 3.7%). This indicates osmotic adjustment in a 'true' sense. Increases in total osmoticum concentration have been reported to occur because the root volume expansion decreases (Sharp *et al.* 1990). In contrast to shoots where osmotic adjustment does not occur without a decrease in the growth rate (Munns 1988), a positive association between increased osmotic adjustment and promotion of root growth (increase in total root weight and volume due to more and thinner roots) can temporarily exist. Osmotic adjustment of roots is also favoured by a high root elasticity as found in several other maize cultivars (Evéquoz 1993). Root Ψ_s decreased at an early stage of drought stress in this study. If root Ψ_w does not vary initially under decreasing water availability then root Ψ_p will increase with decreased root Ψ_s. Leaf Ψ_p decreased slightly in this work and root Ψ_p increased; this divergence can then be used to demonstrate sensitive changes in the plant water status to decreased water availability. Decreased root Ψ_s and increased root Ψ_p help to maintain or even favour root growth. A better exploitation of soil volume, where water is still available, will contribute to increased water uptake and thus support the ability of plants to withstand soil dryness. Increased root Ψ_p may be advantageous in overcoming a higher soil resistance in drying soils. Higher root Ψ_p may even be necessary to reestablish the effective driving force, because the turgor threshold may be increased, probably due to decreased cell wall extensibility. Small decreases in RWC of roots and leaves may be due to passive losses of water but can also result from changes in cell morphology, for example smaller cell size can be observed under drought stress (Farah 1979).

Summary and conclusions

In agreement with other studies (e.g. Davies and Zhang 1991) we found reactions in the root water status to a drying soil before there was a detectable change in the leaf Ψ_w. However, we did not find that Ψ_w in roots reacted more sensitively to early soil dryness than Ψ_w in leaves. This supports the notion of Boyer (1989) that shoots generally have lower Ψ_w than roots. However, a significantly higher hydraulic capacitance in roots than in leaves of maize plants (Schmidhalter and Evéquoz, unpublished; Wang *et al.* 1991) indicates that changes due to soil drying can be more sensitively indicated by a lowering of RWC in roots than in shoots. This was observed with mild stress in this study but is likely at moderate and more severe stress. This study shows that roots may also vary with soil dryness via other changes in the water status. Sensitive reactions in plants subjected to mild soil dryness were found in decreased root Ψ_s and increased root Ψ_p. Dehydration of roots may occur but seems to be too small in this study to account for the production of a chemical signal which eventually restricts leaf expansion. Leaf expansion is known to react very sensitively to early soil dryness. It has long been recognized that the accumulation of solutes in roots (decreased root Ψ_s as in this study) of stressed plants results from a decline in assimilate utilization by growing shoots (Kramer and Boyer 1995). A higher sensitivity of leaf growth as compared to root growth which is frequently found in drying soil may be due to increased hydraulic resistances leading to reductions in growth-sustaining Ψ_w gradients. Growth may be limited by a number of other factors like increased turgor threshold, decreased extensibility, lack of inorganic nutrients, hormonal limitations and other metabolic factors. Further studies investigating sensitive reactions in plants subjected to soil drying should concentrate on the simultaneous evaluation of changes occurring in leaf and root meristematic zones.

References

Aloni, R., and Griffith, M. (1991). Functional xylem anatomy in root-shoot junctions of six cereal species. *Planta* **184**,123–129.

Baker, J.M., and van Bavel, C.H.M. (1986). Resistance of plants roots to water loss. *Agronomy Journal* **78**, 641–644.

Blizzard, W.E., and Boyer, J.S. (1980). Comparative resistance of the soil and the plant to water transport. *Plant Physiology* **66**, 809–814.

Boyer, J.S. (1989). Water potential and plant metabolism: Comments to Dr. P. Kramer's article 'Changing concepts regarding plant water relations', Vol. 11, Number 7, pp. 565–568, and Dr. J.B. Passioura's response, pp. 569–571. *Plant, Cell and Environment* **12**, 213–216.

Davies, W.J., Metcalfe, J., Lodge, T.A., and da Costa, A.R. (1986). Plant growth substances and the regulation of growth under drought. *Australian Journal of Plant Physiology* **13**, 105–125.

Davies, W.J., and Zhang, J. (1991). Root signals and the regulation of growth and development of plants in drying soils. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**, 55–76.

De Roo, H.C. (1969). Water stress gradients in plants and soil-root systems. *Agronomy Journal* **61**, 511–515.

Evéquoz, M. (1993). Adaptation osmotique et propriétés rhéologiques des parois cellulaires: Critères pour la sélection du maïs à la sécheresse? PhD Thesis No. 10234, ETH Zurich. 93 pp.

Farah, S.M. (1979). An examination of the effects of water stress on leaf growth of crops of field beans *Vicia faba* L. PhD Thesis, University of Reading.

Gee, G.W., Janes, B.E., and Tan, C.S. (1973). A chamber for applying pressure to roots of intact plants. *Plant Physiology* **52**, 472–474.

Gee, G.W., Liu, W., Olvang, H., and Janes, B.E. (1974). Use of pressure bomb measurements to estimate root water potentials. *Agronomy Journal* **66**, 75–78.

Johnson, I.R., Melkonian, J.J., Thornley, J.H.M., and Riha, S.J. (1991). A model of water flow through plants incorporating shoot/root >message= control of stomatal conductance. *Plant, Cell and Environment* **14**, 531–544.

Jupp, A.P., and Newman, E.I. (1987). Morphological and anatomical effects of severe drought on the roots of *Lolium perenne* L. *New Phytologist* **105**, 393–402.

Klute, A. (1986). Water retention: Laboratory methods. In 'Methods of Soil Analysis'. Part 1. 2nd edition (Ed. A. Klute). pp. 635–662. Agronomy Monograph 9. (ASA and SSSA: Madison, WI.)

Kramer, P.J., and Boyer, J.S. (1995). 'Water Relations of Plants and Soil.' (Academic Press: San Diego.)

Kubiske, M.E., and Abrams, M.D. (1991). Rehydration effects on pressure-volume relationships in four temperate woody species: variability with site, time of season and drought conditions. *Oecologia* **85**, 537–542.

Lafolie, F., Bruckler, L., and Tardieu, F. (1991). Modeling root water potential and soil-root water transport: I. Model presentation. *Soil Science Society of America Journal* **55**, 1203–1212.

Meinzer, F.C., Rundel, P.W., Sharifi, M.R., and Nilsen, E.T. (1986). Turgor and osmotic relations of the desert shrub *Larrea tridentata*. *Plant, Cell and Environment* **9**, 467–475.

Mohr, H., and Schopfer, P. (1992) (Eds) 'Pflanzenphysiologie.' (Springer-Verlag: Berlin.)

Mooney, H.A., Gulmon, S.L., Rundel, P.W., and Ehleringer, J. (1980). Further observations on the water relations of *Prosopis tamarugo* of the northern Atacama desert. *Oecologia* **44**, 177–180.

Munns, R. (1988). Why measure osmotic adjustment? *Australian Journal of Plant Physiology* **15**, 717–726.

Nnyamah, J.U., and Black, T.A. (1977). Field performance of the dew-point hygrometer in studies of soil-root water relations. *Canadian Journal of Soil Science* **57**, 437–444.

Nobel, P.S. (1991). (Ed.) 'Physicochemical and Environmental Plant Physiology.' (Academic Press: London.)

Nobel, P.S., and Lee, C.H. (1991). Variations in root water potentials: Influence of environmental factors for two succulent species. *Annals of Botany* **67**, 549–554.

Parker, W.C., and Pallardy, S.G. (1988). Leaf and root osmotic adjustment in drought-stressed *Quercus alba*, *Q. macropara*, and *Q. stellata* seedlings. *Canadian Journal of Forestry Research* **18**, 1–5.

Passioura, J.B. (1988). Water transport in and to roots. *Annual Review of Plant Physiology and Plant Molecular Biology* **39**, 245–265.

Passioura, J.B. (1991). An impasse in plant water relations? *Botanica Acta* **1040**, 405–411.

Petrie, C.L., and Hall, A.E. (1992). Water relations in cowpea and pearl millet under soil water deficits. III. Extent of predawn equilibrium in leaf water potential. *Australian Journal of Plant Physiology* **19**, 601–609.

Rhizopoulou, S., and Davies, W.J. (1991). Influence of soil drying on root development, water relations and leaf growth of *Ceratonia siliqua* L. *Oecologia* **88**, 41–47.

SAS/STAT (1990) 'User's Guide, Volume 2, GLM-VARCOMP.' Version 6, Fourth Edition, Volume 2 (SAS Institute Inc.: Cary, NC.)

Schmidhalter, U., Besson, A., and Oertli, J.J. (1992a). Water uptake by roots at spatially variable soil matric potentials. In 'Root Ecology and its Practical Application—a Contribution to the Investigation of the Whole Plant'. (Eds L. Kutschera, E. Hübl, E. Lichtenegger, H. Persson and M. Sobotik.) pp. 133–136. (Verein für Wurzelforschung: Klagenfurt.)

Schmidhalter, U., Evéquoz, M., and Oertli, J.J. (1992b). Osmotic adjustment of roots and shoots. In 'Root Ecology and its Practical Application—a Contribution to the Investigation of the Whole Plant'. (Eds L. Kutschera, E. Hübl, E. Lichtenegger, H. Persson and M. Sobotik.) pp. 272–282. (Verein für Wurzelforschung: Klagenfurt.)

Schmidhalter, U., Selim, H.M., and Oertli, J.J. (1994). Measuring and modeling root water uptake based on ^{36}chloride discrimination in a silt loam soil affected by groundwater. *Soil Science* **158**, 97–105.

Sharp, R.E., and Davies, W.J. (1979). Solute regulation and growth by roots and shoots of water-stressed maize plants. *Planta* **147**, 43–49.

Sharp, R.E., Hsiao, T.C., and Silk, W.K. (1990). Growth of the primar root at low water potentials. I. Spatial distribution of expansive growth. *Plant Physiology* **87**, 50–57.

Slatyer, R.O. (1967). 'Plant-Water Relationships', (Academic Press: London and New York.)

Slatyer, R.O., and Denmead, O.T. (1964). Water movement through the soil-plant-atmosphere system. In 'Water resources, use and management.' (Ed. E.S. Hills). pp. 276–289. (Melbourne University Press: Melbourne.)

Taylor, S.A. (1962). Water transport through plants from soil to atmosphere. (CSIRO Symposium on soil–plant–atmospheric water relationships: Canberra).

Van Genuchten, M.T. (1980). A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. *Soil Science Society of America Journal* **44**, 892–898.

Wang, X.L., Canny, M.J., and McCully, M.E. (1991). The water status of the roots of soil-grown maize in relation to the maturity of their xylem. *Physiologia Plantarum* **82**, 157–162.

Wenzel, C.L., McCully, M.E., and Canny, M.J. (1989). Development of water conducting capacity in the root systems of young plants of corn and some other C_4 grasses. *Plant Physiology* **89**, 1094–1101.

Westgate, M.E., and Boyer, J.S. (1984). Transpiration- and growth-induced water potentials in maize. *Plant Physiology* **74**, 882–889.

Westgate, M.E., and Boyer, J.S. (1985). Osmotic adjustment and the inhibition of leaf, root, stem and silk growth at low water potentials in maize. *Planta* **164**, 540–549.

Zhang, J., and Davies, W.J. (1989). Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. *Plant, Cell and Environment* **12**, 73–81.