Sequence of drought response of maize seedlings in drying soil

Urs Schmidhalter, Michel Evéquoz, Karl-Heinz Camp and Christoph Studer

Leaf elongation in monocotyledonous plants is sensitive to drought. To better understand the sequence of events in plants subjected to soil drying, leaf elongation and transpiration of maize seedlings (Zea mays L.) of 4 cultivars were monitored continuously and the diurnal courses of the root and leaf water relations were determined. Results from this study indicate the following sequence of drought response: Leaf elongation decreased before changes in the leaf water relations of non-growing zones of leaf blades were detected and before transpiration decreased. Reductions in leaf elongation preceded changes in the root water potential (Ψ_w). Root Ψ_w was not a very sensitive indicator of soil dryness, whereas the root osmotic potential (Ψ_s) and root turgor (Ψ_p) were more sensitive indicators. The earliest events observed in drying soil were a significant increase in the largest root diameter class (1 720 to 1 960 µm) and a decrease in leaf elongation ($P = 0.08$) 2 days after withholding water. Significant increases in root length were observed 2 days later. Soil drying increased the number of fine roots with diameters of <240 µm. Slight increases in soil strength did not affect leaf elongation in the drying soil.

Key words – Leaf elongation, leaf water relations, root diameter, root length, root osmotic potential, root water potential, root water relations, Zea mays.

U. Schmidhalter (corresponding author, e-mail schmidhalter@weihenstephan.de) and K.-H. Camp, Lehrstuhl für Pflanzenernährung, Technische Univ. München, D-85350 Freising-Weihenstephan, Germany; M. Evéquoz, Inst. of Land Improvement and Water Management, ETH Zürich, PCG-Project, B. P. 309, Tahoua, Niger; C. Studer, Icrisat Sahelian Centre, Niamey, Niger.

Introduction

The traditional view of how plants respond to a reduction in soil water availability was that they reduce their water uptake, resulting in a reduction in the water content, water potential (Ψ_w), or turgor (Ψ_p) in the leaves (e.g. Kramer 1988). There is no doubt that, under many circumstances, this does occur, but this is probably not always the case (Davies et al. 1990). Recent studies show that plants may react to drying soil well before their leaves wilt and even before there is a detectable change in leaf Ψ_w (Bates and Hall 1981, Davies and Zhang 1991). The growth rate of the leaves may fall (Passioura 1988, Saab and Sharp 1989), and their stomata may close (Blackman and Davies 1985, Gollan et al. 1986), apparently in

response to signals received from the roots in drying soil (Davies and Zhang 1991, Passioura 1994). Decreases in Ψ_w of drying roots may trigger an early signal (Zhang and Davies 1989, Davies and Zhang, 1991).

There are few reports on the inhibition of leaf expansion, which occurs independently of changes in leaf water relations (Munns 1987, Passioura 1988, Saab and Sharp 1989, Augé et al. 1994). There are also only very few studies of changes in leaf elongation and transpiration of monocotyledonous plants in drying soils as compared with changes in root and leaf water relations (e.g. Sharp and Davies 1979). There are no studies comparing early events in drying soil among different cultivars.

To better understand the sequence of events in plants subjected to soil drying an experiment was set up in which leaf elongation and transpiration of 4 maize cultivars were monitored continuously. The diurnal water relations of both roots and leaves were measured several times during the experiment. Root growth of the whole root system was measured and expressed as root length and specified for different root diameter classes. This study emphasizes the specific reaction of individual root diameter classes.

Leaf elongation is generally accepted to be sensitive in monocotyledonous plants subjected to soil drying. Direct sensing of the soil water status would have to occur in the roots. Abscisic acid (ABA) produced in dehydrating roots may enable the plant to 'measure' the water status of the soil (Zhang and Davies 1989, 1991). These authors speculated that dehydration of a few fine roots may promote the production of a chemical signal which will restrict not only leaf conductance, but also leaf expansion (Masle and Passioura 1987, Rosa da Costa et al. 1987), thereby increasing carbohydrate availability to the root system. At low soil Ψ_w, roots can shrink considerably (Huck et al. 1970). At early stages of soil drying or with mild drought, however, roots may not become substantially dehydrated. In contrast, decreases in leaf elongation can be observed at very early stages of soil drying. Decreases in root Ψ_w as sensitive means of detecting the degree of early soil drying may therefore not constitute a signalling system. This hypothesis was tested in the present work with 4 different maize cultivars. As an alternative we investigated whether early soil drying might be more sensitively indicated in changes in Ψ_s and Ψ_p of whole roots.

It is difficult to measure water relations in growing tissues, but essential for a causal analysis of growth regulation. This was not attempted in this work. Our measurements of root and leaf water relations were restricted to whole organs. We postulate, however, that changes in the water relations of whole organs might also be reflected in changes in the water relations of actively expanding cells. Decreases in Ψ_w and Ψ_s of mature regions of roots and leaves, representing the major part of these organs, have been shown to be associated with decreased values in the respective actively expanding tissues (Westgate and Boyer 1985). Our investigation at the whole organ level should therefore allow to more specifically focus on relevant processes occurring at early stages of soil drying in actively expanding tissues.

Abbreviations – DAW, days after beginning of different watering treatments; LVDT, linear variable differential transducer; Ψ_m, soil matric potential; Ψ_p, turgor; Ψ_s, osmotic potential; Ψ_w, water potential.

Materials and methods

Description of experiments

Two experiments were conducted under the same experimental conditions. Details will be given in Cultivation of plants (below). In the first experiment, the influence of soil drying on leaf elongation, root and shoot biomass, and leaf and root water relations of 4 cultivars of *Zea mays* L. (Issa, Irat 42, Irat 32, Costeño de Culiacan) were determined. The cultivar Issa was used for the second experiment in which the influence of soil drying on soil strength and root growth was measured. Changes in soil strength may represent an early trigger of decreased shoot growth and were therefore determined in this work. Growth of root and shoot biomass in well-watered and drought treatments were comparable in both experiments. All treatments were replicated at least three times and all experiments were repeated with similar results.

Cultivation of plants

The experiments were conducted in a growth chamber under constant conditions (12/12 h day/night; 20/18°C day/night temperature; 420 µmol m^{-2} s^{-1} [PPFD]; 70% RH). Plants were grown in pots (20 cm in height, 10 cm in diameter) containing 1.32 kg of soil (dry weight basis). The soil used in this investigation was illitic-chloritic Charrat silt loam (fine mixed mesic Aquic Ustifluvent). The soil was 9.1% clay, 59.5% silt, 31.4% sand, and 0.85% organic matter (Schmidhalter et al. 1994). The initial soil matric potential (Ψ_m) was based on a soil water retention curve, as described in the next section. Four seeds of *Zea mays* L., pre-germinated for 2 days, were sown in the moist soil of each pot at a depth of 2 cm. The top of the soil was covered with a layer of sand, 1 cm thick (grain size 1–2 mm) to reduce the compacting action caused by watering the plants and to reduce evaporation from the soil surface. Evaporation was further decreased in the second experiment by covering the pots with transparent polyethylene sheets with small holes through which seedlings grew and were watered. Half the pots were watered daily to give a Ψ_m of −0.03 MPa, the rest were allowed to dry out from day 9 to day 17 (Exp. 1) and from day 9 to day 18 (Exp. 2).

Soil matric potential and soil strength

Gravimetric water contents were converted to Ψ_m based on a previously determined water retention curve (Schmidhalter 1997). A needle penetrometer with a stainless steel needle of 2 mm diameter and 30° internal angle was used to measure soil strength. The needle was driven into the soil at 2 mm s^{-1}. Readings were recorded from a balance and con-

160

verted to penetrometer resistance values by dividing the force by the cone's projected cross-sectional area. Penetration measurements were taken sequentially in the two treatments (control and drought), with 3 replicates per treatment. Each replicate represented one pot with 3 measurements averaged per pot. The sand layer was carefully removed prior to the measurements. Measurements in the soil were made at depths of 1, 4, 7, 10 and 13 cm. Measurements were made 2, 4, 6 and 7 DAW (days after beginning the different watering treatments).

Dry weight of roots and shoots

Dry weights of roots and shoots were determined in all experiments after drying the fresh material in an oven at 105°C for 48 h. Values represent pot averaged values of 4 pots from each treatment. Growth of root and shoot biomass in the control and drought treatments were comparable in all experiments and are reported for the second experiment (penetrometer resistance and root growth experiment). In this experiment, root and shoot biomass for both treatments were determined 2, 4, 6 and 7 DAW.

Development, length and diameter of roots

Development of roots was recorded at harvest time. The roots were washed manually for root length measurements. The roots were stained with Pararosaniline (Sigma Chemical Company, St Louis, MO, USA) and stored until measurement at 4°C in water. The roots were cut into small pieces and evenly distributed in a shallow, transparent tray for image recording with the image processing system IMCO/S (Kontron Elektronik, Eching, Germany) and a CCD camera ProgRes 3000 (Kontron Elektronik). The maximum resolution was 80 μm. Roots were divided into 8 classes with diameters being less than 240, 480, 720, 960, 1 200, 1 440, 1 680 and 1 920 μm, respectively. Images were analyzed for length and diameter of roots using the computer program ROOT DETECTOR (Walter and Bürgi 1996).

Water relations of leaves and roots

Leaf and root water relations were determined on 4 plants from different pots for each treatment on the same days at 0, 2, 5 and 8 h during the light period, except on DAW 8 where measurements were performed once, immediately before the light period commenced. The youngest not yet fully developed leaf blade, cut one third from the leaf base, and the root system cut at the mesocotyl were used for the measurements. Water potential was determined with a pressure chamber (PMS Instrument Co., Model 1002, Corvallis, OR, USA) and osmotic potentials (Ψ_s) with a vapor pressure osmometer (Wescor 5500, Wescor Inc., Logan, UT, USA).

Turgor was calculated as the difference between Ψ_w and Ψ_s. The same measurements of the water relations were made for whole roots using a recently adapted pressure chamber/osmometer technique (Schmidhalter et al. 1992). Measurements were first made on the detached leaf, leaving the remainder of the plant in the pot. Thereafter, only the bulk of the soil was gently shaken from the root/shoot system, and the intact root system, cut above the root/shoot junction, was used for the pressure chamber measurements. Further details have been reported elsewhere (Schmidhalter et al. 1998).

Pressure chamber measurements were not corrected for the Ψ_s of the xylem sap; previous investigations showed that it was higher than −0.05 MPa in leaves, whereas lower values may have occurred in roots. Xylem sap which was obtained by applying higher than balancing pressures to leaves and roots was contaminated with symplastic solutes. Uncorrected Ψ_s of the xylem sap were higher than −0.09 and −0.25 MPa for leaves and roots, respectively. Corrections applied for the measured contributions of glucose, fructose and sucrose increased these values to −0.05 and −0.16 MPa, respectively. We conclude that xylem sap Ψ_s of roots must have been markedly higher than −0.16 MPa. No significant difference was found in xylem sap Ψ_s between differently droughted and control roots. Leaf Ψ_w in these experiments were closely approximated by the measured balancing pressures, whereas root Ψ_w were significantly underestimated by the pressure chamber technique. Because comparable values of xylem sap Ψ_s were found in droughted and control roots changes in balancing and osmotic pressures can be used to characterize the sensitivity of the water relations to early soil drying.

After the balancing pressures were recorded 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 whole root systems with a specially designed press and Ψ_s from sap determined with an osmometer. Dilution of the sap due to apoplastic mixing was not taken into account. The xylar contribution to the dilution of root sap Ψ_s is comparatively small for normally fertilized plants in this experiment, whereas apoplastic liquids contained in the intercellular and cell wall space will increase root Ψ_s determined by osmometry. At the present there are no methods available to exactly quantify their contribution to apoplastic mixing. We assume that Ψ_s in control and droughted roots is similarly affected by the dilution due to apoplastic mixing.

Leaf elongation and plant transpiration

Instantaneous measurements of the growth of leaf 3 were made on 8 plants per treatment from different

pots by using linear variable differential transducers (LVDT) connected to the data logger. Measurements began when leaf 3 emerged above the enclosing sheath of the preceding leaf. The tip of the growing leaf was attached to the LVDT by a fishing line (0.22 mm diameter) which was attached to the leaf tip using a small clamp cushioned with mounting rubber to avoid damaging the leaf. The force on the fishing line was 10 g to minimize oscillations in the LVDT output; the force applied did not affect leaf elongation rate during measurement. LVDT readings corresponded to the elongation of leaf blades only. Leaf area expansion was derived from destructive leaf area measurements with a portable leaf area meter (LI-3000A, Li-Cor, Lincoln, NE, USA) on DAW 3, 5, 7 and 9. The diurnal course of transpiration was determined by weighing 8 pots per treatment every half hour and deducting evaporative losses from pots of soil without plants. Less frequent determinations of transpiration were made for the drought treatment before DAW 2. However, no difference in the average values of transpiration were found on DAW 1 between the two treatments. Values of transpiration have been expressed as amount of water lost per unit surface of the experimental pots (mm^3 mm^{-2} h^{-1}) by assuming the density of water to be 1 g cm^{-3}.

Statistical analysis

Values are presented as means with standard errors of 3 to 8 replications. Analysis of variance was carried out for root and shoot dry weight, total root length and root length by diameter class, leaf elongation, and transpiration rates for the first and second experiments. t-Tests were used for mean separations of the two treatments for the parameters leaf elongation, transpiration and diurnal water relations. Characterization of the drought response can be derived either from comparisons with the well-watered control or by examining the behaviour of the trait with increasing soil dryness. To allow both comparisons, the statistical analysis for leaf and root water relations was conducted on the basis of DAW and water supply treatments (with 5 levels: DAW 3 drought, DAW 5 drought, DAW 7 drought, DAW 4 well-watered and DAW 7 well-watered). Prior to the analysis of variance, tests indicated homogeneity of variances among these treatment levels. Differences in root and leaf Ψ_w components between these treatment combinations and genotypes were analyzed in a combined analysis of variance using the SAS-GLM procedure (SAS/STAT 1990).

Results

Soil water status

Ψ_m was maintained between -0.03 and -0.06 MPa in the well-watered treatment (control) (Fig. 1). A gradual
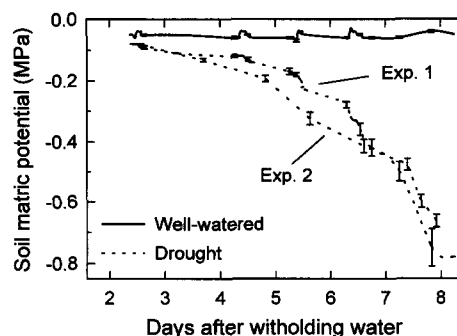

Fig. 1. Change in soil matric potential in a well-watered treatment and a soil drying treatment in which water was withheld 9 days after emergence of maize seedlings. Results are presented for experiments 1 and 2. Error bars indicate SE of the means of 4 observations.

decline in Ψ_m to -0.2 MPa was observed in the stress treatment until DAW 5, and thereafter a steeper decline, lasting 3 days, to about -0.7 MPa.

Soil penetrometer resistance

Penetrometer resistance was generally low (Fig. 2). Values recorded were about 300 kPa in the well-watered treatment and 400 kPa in the drought treatment except in the uppermost soil layer at DAW 7 (900 kPa).
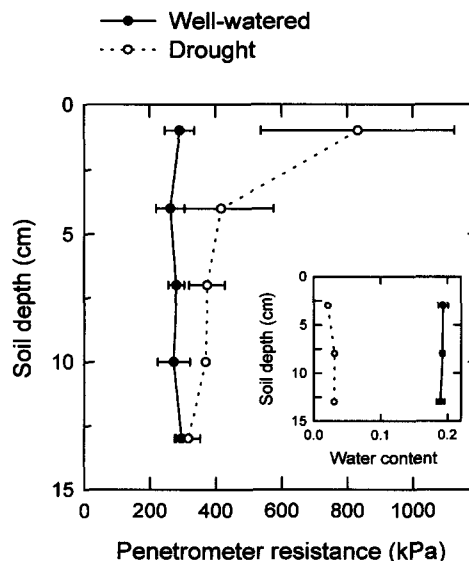

Fig. 2. Penetrometer resistance and soil water content at different soil depths in pots containing maize seedlings which were either well-watered (closed symbols) or watered for the last time 9 days after emergence (open symbols). Points are means $\pm$ SE of 4 observations and are indicated for 4 and 7 days after withholding water (DAW) and at the end of the experiment for the well-watered treatment.
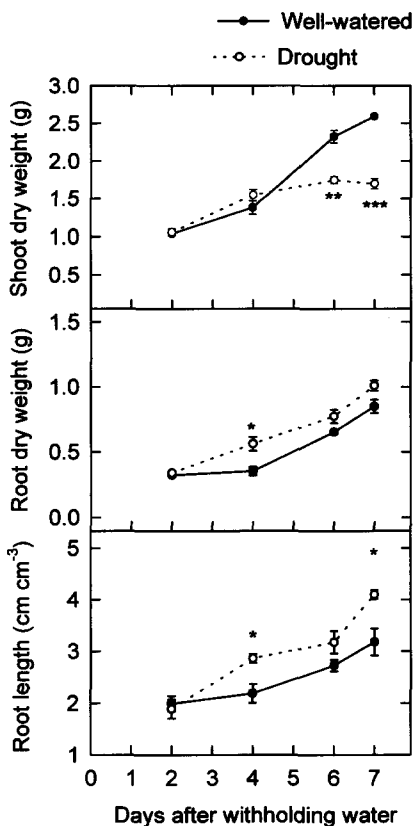
162

Fig. 3. Shoot and root dry weight production and root length of soil-grown maize seedlings, which were either well-watered or watered last (drought) on day 0 (9 days after emergence). Points are means ±SE of 4 observations. *, ** and *** indicate significant difference at $P \leq 0.05$, 0.01 and 0.005, respectively.

The water content of the droughted pots was reduced fairly homogeneously along the profile. Gravimetric water content was by less than 2% lower in the upper third of the pot than in the lower parts of the pot. The moderately higher penetrometer resistance recorded in 1 cm soil depth probably reflects a higher soil dryness and crusting effects caused by watering the plants before withholding water.

Shoot and root dry weight production

Withholding water caused a decrease in shoot dry weight and an increase in root dry weight (Fig. 3), resulting in an increased root to shoot dry weight ratio. Decreases in shoot dry weight were highly significant on DAW 6 and 7. Root dry weight was increased on DAW 4, 6 and 7 ($P < 0.05$, $P < 0.08$, $P < 0.07$). Whole plant dry weight was increased on DAW 4 and was significantly decreased on DAW 6 and 7 in the drought treatment as compared with the well-watered treatment.

Development, length and diameter of roots

Root systems of the investigated seedlings consisted of the primary root and a tier of 4 shoot-borne roots. No difference in the number of shoot-borne roots was observed between DAW 1 and 7 in the two treatments. In accordance with root dry weight the total root length (expressed as root length density) was significantly increased under drought beginning on DAW 4 (Fig. 3). Root length and root dry weight were significantly correlated (r = 0.77***). The distribution of root length by diameter class shows that increases in total root length were due mainly to roots having diameters less than 480 µm (Fig. 4). Increased growth of roots thicker than 1 920 µm was most likely caused by a proliferation of shoot-borne roots on DAW 2 and 4.

Leaf and root water relations

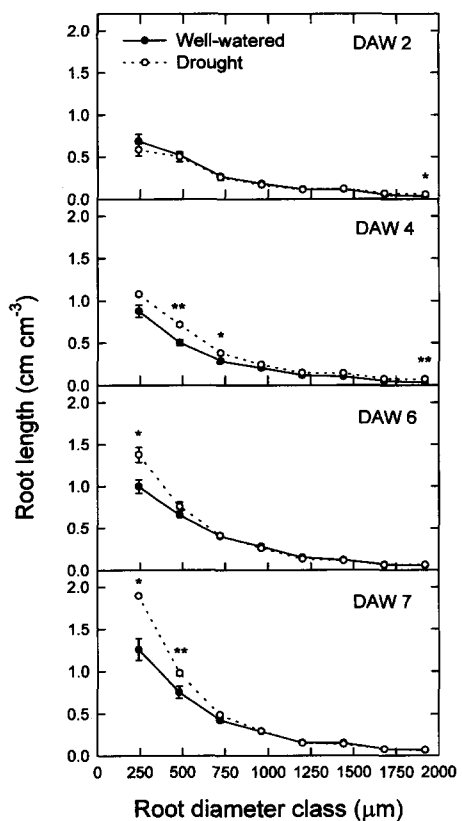The aim of this paper is to report the development of leaf and root Ψ_w in drying soil based on 4 maize

Fig. 4. Root length by diameter class of maize seedlings grown in well-watered soil and in soil that was watered last on day 0. Points are means ±SE of 4 observations. Results are shown for days 2 to 7 after withholding water (DAW). * and ** indicate significant difference at $P < 0.05$ and 0.01, respectively.

Tab. 1. Leaf water potential (Ψ_w), leaf osmotic potential (Ψ_s) and leaf turgor (Ψ_p) of maize seedlings which were well-watered or subjected to drought by withholding water 9 days after emergence. Measurements were conducted, 0 (predawn), 2, 5 and 8 h after the light period commmenced, on days 3, 4, 5 and 7 after beginning the different watering treatments (DAW). Four maize cultivars were included in the experiments. Means within each water potential component not followed by the same letter are significantly different at $P \leq 0.05$ according to the Student-Newman-Keuls test.

Leaf parameter	DAW	Water supply treatment	Light period (h)			
			0	2	5	8
Ψ_w (MPa)	3	Drought	−0.14b	−0.26a	−0.31a	−0.38a
	5	Drought	−0.15b	−0.36b	−0.37a	−0.44b
	7	Drought	−0.27c	−0.81c	−0.92b	−0.97c
	4	Well-watered	−0.09a	−0.23a	−0.33a	−0.35a
	7	Well-watered	−0.10a	−0.26a	−0.33a	−0.33a
Ψ_s (MPa)	3	Drought	−0.72ab	−0.75a	−0.84ab	−0.87a
	5	Drought	−0.73ab	−0.81b	−0.88b	−0.92b
	7	Drought	−0.86c	−0.97c	−1.14c	−1.18c
	4	Well-watered	−0.69a	−0.76a	−0.80a	−0.83a
	7	Well-watered	−0.75b	−0.79b	−0.84ab	−0.86a
Ψ_p (MPa)	3	Drought	.0.56b	0.47a	0.50a	0.46a
	5	Drought	0.55b	0.43a	0.48a	0.44a
	7	Drought	0.57ab	0.13b	0.19b	0.16b
	4	Well-watered	0.58ab	0.51a	0.44a	0.46a
	7	Well-watered	0.62a	0.46a	0.48a	0.49a

cultivars representing a broad genetic background. Therefore, results of the combined analysis of leaf and root water relations are presented in Tabs 1 and 2 with emphasis on the general response to soil drying, although some genotypic differences were found among the 4 maize cultivars (M. Evéquoz. 1993. Thesis, Swiss Federal Institute of Technology, Zurich, Switzerland).

Except at predawn (shortly before commencing the light period), leaf and root Ψ_w on DAW 3 in the drought treatment were comparable to the corresponding values in the well-watered treatment (DAW 4 and 7). Leaf Ψ_w in the drought treatment decreased from DAW 3 to 7. In contrast, root Ψ_w was only decreased on DAW 7. Root Ψ_w decreased slightly during the light period. A marked decrease was observed on DAW 7, 8 h after the light period commenced. Two hours after beginning the light period transpiration decreased to very low levels. Root Ψ_w were lower or very similar to the bulk Ψ_m on DAW 3 and 5 (Tab. 2, Fig. 1, Exp. 1). A remarkable exception to this was observed on DAW 7, where the decrease in the root Ψ_w lagged behind the decreasing bulk Ψ_m, except after 8 h where both values became again similar.

Leaf Ψ_s and Ψ_p on DAW 3 in the drought treatment were comparable to the corresponding values in the well-watered treatment throughout the experiment. In contrast, leaf Ψ_s and Ψ_p were markedly lower in droughted plants by DAW 7 once the plants were illuminated. Root Ψ_s between DAW 3 and 7 was very stable in the well-watered treatment but declined by about 0.4 MPa over the 4 days in droughted plants. Root Ψ_p showed an almost commensurate rise over the same period. The rise in root Ψ_p should be contrasted with sharp decreases in leaf Ψ_p.

Transpiration and leaf elongation

Leaf area expansion increased linearly between DAW 3 and 9. Plant leaf area between the two treatments was not significantly different before DAW 5. No difference was to be expected in describing the sensitivity of transpiration to soil drying during DAW 0 to 5 by expressing gravimetric transpirational losses either per unit plant leaf area or per unit experimental surface. The latter has been preferred because frequent gravimetric measurements of transpiration were performed whereas only few measurements of plant leaf area were available throughout the course of the experiment. Decreases in transpiration were observed after DAW 5, 14 days after emergence (Fig. 5). In contrast, slight reductions in leaf elongation were observed after DAW 1 and were clearly manifest after DAW 2 (Fig. 5). Withholding water slowed down elongation to a greater extent during the light period than during the dark period. Mean values of transpiration and leaf elongation measured from the third to the fifth hour of the light period are represented in Fig. 6 for the drought and control treatment. Statistically significant differences in leaf elongation were observed on DAW 3 ($P < 0.05$) in the stress treatment as compared with the control treatment. A less significant difference had already been observed on DAW 2 ($P < 0.1$). Only on DAW 5 was transpiration significantly decreased ($P < 0.01$) in the stress treatment. This figure clearly shows that leaf elongation started to decrease well before transpiration was reduced. Statistically significant differences in dark period elongation were observed after DAW 6 ($P < 0.05$). The decline in leaf elongation in the control treatment was due to the leaf's normal development, where after a linear growth phase (DAW −1 to 2) a declining rate of leaf elongation is observed. Treatment

effects in our study were not confounded by changes due to development.

Discussion

Does increased soil strength represent an early trigger of decreased leaf elongation?

A decrease in leaf expansion rate has been interpreted as a response to changes in mechanical impedance as a consequence of soil drying in two studies (Masle and Passioura 1987, Passioura 1988). Soil strength can be increased in rapidly drying surface soil as shown by Weaich et al. (1992) and is also indicated in our study. Mechanical impedance greater than 2 MPa is likely to reduce total root length and root elongation rate by at least 50% (Atwell 1993) and root elongation ceases at root penetration resistances of about 1 MPa (Bengough and Mullins 1990). The low values of soil strength in our study lead to the conclusion that changes in soil strength did not reduce leaf elongation. Results of this study are not unexpected, because the low soil bulk density of 0.9 kg m^{-3} made it unlikely that soil strength interfered with shoot elongation. This study shows that decreases in leaf elongation in drying soil do not necessarily involve soil strength as a primary factor, whereas this may be different in other soils with higher soil bulk densities.

Soil water status and leaf elongation

It is generally accepted that leaf elongation is one of the most sensitive processes in monocotyledonous plants subjected to soil drying. This is also demonstrated by the results of this study in which small decreases in leaf elongation were observed one day after withholding water. Acevedo et al. (1971) reported that the elongation of maize leaves was highly sensitive to slight reductions in soil Ψ_w and stopped completely at -0.25 MPa soil Ψ_w. This agrees with results from our study (Fig. 1, Exp. 1 and Fig. 6).

Water status of non-growing leaf blades and leaf elongation

Previous reports have shown that elongation reacted very sensitively to slight reductions in leaf Ψ_w (Boyer 1970, Acevedo et al. 1971). Leaf elongation decreased very drastically at leaf Ψ_w lower than -0.2 to -0.3 MPa in a dark and humid growth chamber (Boyer 1970, Acevedo et al. 1971). Both studies indicated that leaf elongation nearly ceased when the leaf Ψ_w reached -0.7 to -0.8 MPa. In our study, predawn leaf Ψ_w was -0.14 MPa when elongation decreased significantly and predawn and daytime leaf Ψ_w were -0.7 and -1.2 MPa, respectively, when leaf elongation stopped. Lower elongation rates during the night as compared to the day, in spite of significantly higher leaf Ψ_w, suggest other limiting factors. A higher leaf meristem temperature during daytime might partly account for the higher elongation rate as compared to nighttime, but this remains speculative because meristem temperatures were not determined. Decreases in daytime leaf elongation between and within treatments do not seem to be closely related to the leaf Ψ_p in non-growing leaf blades. There are several studies which suggest changes in growth rate without corresponding changes in Ψ_p (Matsuda and Riazi 1981, Michelena and Boyer 1982, Shackel et al. 1987).

Tab. 2. Root water potential (Ψ_w), root osmotic potential (Ψ_s) and root turgor (Ψ_p) of maize seedlings which were well-watered or subjected to drought by withholding water 9 days after emergence. Measurements were conducted, 0 (predawn), 2, 5 and 8 h after the light period commmenced, on days 3, 4, 5 and 7 after beginning the different watering treatments (DAW). Four maize cultivars were included in the experiments. Means within each water potential component not followed by the same letter are significantly different at $P \leq 0.05$ according to the Student-Newman-Keuls test.

| Root parameter	DAW treatment	Water supply	Light period (h)			
			0	2	5	8
Ψ_w (MPa)	3	Drought	−0.08b	−0.15ab	−0.15a	−0.20a
	5	Drought	−0.09b	−0.14ab	−0.16a	−0.19a
	7	Drought	−0.12c	−0.16b	−0.17b	−0.39b
	4	Well-watered	−0.05a	−0.14ab	−0.16a	−0.16a
	7	Well-watered	−0.06a	−0.12a	−0.18a	−0.16a
Ψ_s (MPa)	3	Drought	−0.53a	−0.53a	−0.53a	−0.55a
	5	Drought	−0.61bc	−0.62b	−0.63c	−0.67b
	7	Drought	−0.90d	−0.91c	−0.93d	−0.96c
	4	Well-watered	−0.59b	−0.60b	−0.60b	−0.62b
	7	Well-watered	−0.62c	−0.63c	−0.64c	−0.66b
Ψ_p (MPa)	3	Drought	0.43d	0.37c	0.35c	0.34d
	5	Drought	0.51c	0.47b	0.45b	0.42c
	7	Drought	0.74a	0.72a	0.75a	0.55a
	4	Well-watered	0.53bc	0.45b	0.42b	0.44bc
	7	Well-watered	0.55b	0.45b	0.46b	0.48b
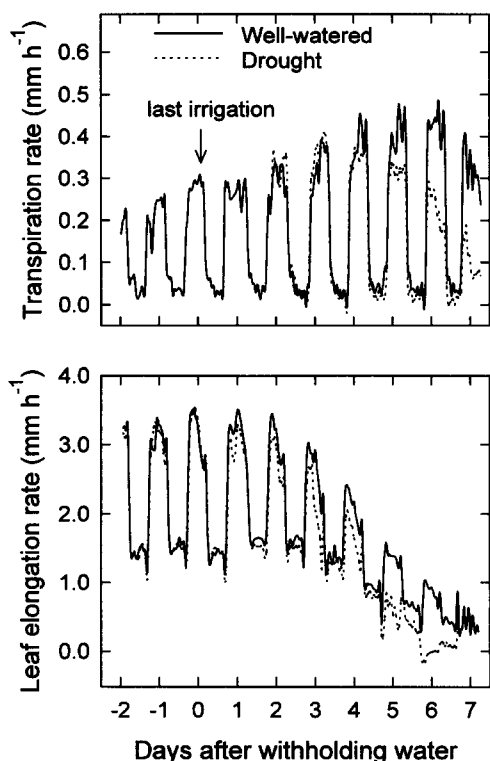
Fig. 5. Diurnal and nocturnal values of transpiration and leaf elongation of maize seedlings in a well-watered treatment and a drought treatment in which water was withheld 9 days after emergence. Light/dark periods were 12 h. Nocturnal values of transpiration and elongation are reflected in markedly lower values as compared with diurnal values. Lines represent mean values of 8 observations per treatment.

Root water status and leaf elongation

In our study, root Ψ_w was hardly affected by early soil drying in contrast to leaf elongation. Decreasing root Ψ_s, whereby root Ψ_p was maintained or increased, may be a more sensitive indicator of early soil dryness. Decreases in Ψ_s may result from decreased leaf growth and thereby decreased sink strength in leaves, thus leading to a shift of assimilates from leaves to roots and to increased root growth as observed in this study. Our measurements showed that Ψ_p was maintained in the whole root system. Continued root growth suggests maintenance of Ψ_p above the Ψ_p threshold value in growing root tips. This casts some doubt on the belief that decreases in root Ψ_w and/or decreased root tip Ψ_p are early events indicating soil dryness. We suggest instead that this role may be played by decreased root Ψ_s and increased root Ψ_p resulting from reduced leaf growth and continued assimilation. Maintenance of root Ψ_p in drying soil was shown by other researchers as well (Sharp and Davies 1979, Westgate and Boyer 1985), whereas decreases in Ψ_p were only found in severely stressed plants (Spollen and Sharp 1991); de-

creased root tip Ψ_p and Ψ_w may also have been caused by cell wall relaxation of still growing root tissue in this study. Furthermore low values can result if non-living root tips were sampled from very dry topsoil. Higher root Ψ_s and lower root Ψ_p, which were observed only at an early stage of stress (drought, DAW 3 vs well-watered, DAW 4), might be a result of the increased root growth, as demonstrated in the second experiment (Fig. 3). Increased growth will cause dilution of imported solutes. The main result, however, is a continuous and significant decrease in root Ψ_s and an increase in root Ψ_p of the droughted plants.

The suitability of pressure chambers to estimate root water status should be questioned because of an anomaly in our study on DAW 7. Higher root Ψ_w as compared to the bulk Ψ_m in our study were most likely due to an uneven depletion of water in the bottom of the pots in the first experiment which is not represented by the bulk Ψ_m. This was different in the second experiment where we could achieve fairly homogeneous soil water distributions throughout the soil profile. Bulk root Ψ_w may be biased towards the water status of the wettest part of the soil. This is also indicated by signifi-
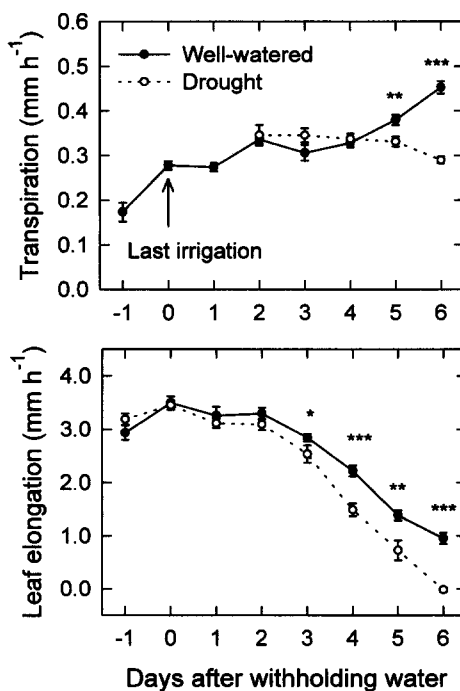

Fig. 6. Mean values of transpiration and leaf elongation of maize seedlings measured between 3 and 5 h after beginning the 12-h light period in a well-watered (closed symbols) and a drought treatment (open symbols) in which water was withheld 9 days after emergence. Points are means ±SE of 8 observations. *, ** and *** indicate significant difference at $P \leq 0.05$, 0.01 and 0.005, respectively. Significance symbols compare differences between treatments on the same day.

166

cantly higher root Ψ_w as compared with the surrounding soil found in dry pots in split-root experiments (Saab and Sharp 1989, Augé et al. 1994). On DAW 7, a sudden decrease in the leaf Ψ_w accompanied by a strongly decreased transpiration was observed 2 h after beginning the light period. Eight hours after commencing the light period a substantial drop in the root Ψ_w was observed. Continued water losses from the plant, albeit at a very much reduced rate, may have resulted in dehydration of roots. Values of root Ψ_w obtained by pressure chamber measurements were 'logical' throughout most of the time in this investigation. Together with the above plausible explanation we conclude that this technique can reliably be applied to measure balancing pressures of roots.

Root growth and leaf elongation

Root growth and leaf elongation seemed to react comparatively early to drying soil (Figs 3 and 6). Promotion of root growth and decreases in leaf growth under such conditions support previous reports. Low soil Ψ_w promoted lateral root initiation and elongation in *Lolium perenne* plants, the total length of lateral roots being between 3 and 5 times that of control plants (Jupp and Newman 1987). Increased proliferation of lateral roots responding to soil drying have been reported for woody plants, wheat and soybean plants (Read and Bartlett 1972, Sharma and Ghildyal 1977, Osonubi and Davies 1978, Huck et al. 1983). Early increases in the length of thick, most likely basal roots are puzzling and may raise the question as to whether these roots react especially sensitively. This study emphasizes the specific reaction of individual root diameter classes. Most studies investigating primary effects of water deficit have focused on the growth of fairly large primary roots in maize plants. Although this may be representative of an early stage of growth, this study shows that withholding water mainly increased the length of fine roots. Consequently, further studies will have to address these diameter classes specifically.

Sequence of drought response in maize seedlings

The earliest events observed in the water deficit treatment were decreases in leaf elongation and increases in the length of the largest root diameter class (1 720 to 1 960 µm) followed by increases in root length of fine roots and in root dry weight. Except at predawn, leaf and root Ψ_w reacted less sensitively and only after changes in root Ψ_s and Ψ_p had been observed. The least sensitive traits were transpiration and shoot dry weight. Results of this study agree with the observation of other authors that the inhibition of leaf elongation precedes decreases in transpiration (Acevedo et al. 1971, Davies et al. 1981, Passioura 1988, Saab and Sharp 1989) and in the water status of non-growing leaf blades (Saab and Sharp 1989). Decreases in root Ψ_w, however, as sensitive means of detecting the degree of soil drying, as suggested previously, are contrasted by our study and therefore do not seem to constitute a signalling system.

Conclusions

Reductions in leaf elongation can occur before significant changes in the water relations of non-growing leaf blades are observed. However, this is not necessarily the case for growing organs. Therefore, it is premature to conclude that growth reductions occur without parallel changes in the water relations of the growing cells. Reductions in leaf elongation precede changes in root Ψ_w. In contrast to root Ψ_w, changes in root Ψ_s and Ψ_p are more sensitive indicators of changes in drying soil and might constitute an early signalling system. Further work is required to determine whether reductions in leaf elongation precede decreases in root Ψ_s, which may be accompanied by increased root Ψ_p values, or whether early changes in the water relations of the leaf growth zone precede inhibition of leaf elongation. Early increases in the length of fine roots and the thickest roots represent a particularly interesting subject for further investigations. This may allow us to determine whether decreases in leaf elongation and increases in root length occur simultaneously or not. In this study soil strength can be excluded as an inhibiting factor.

References

Acevedo, E., Hsiao, T. C. & Henderson, D. W. 1971. Immediate and subsequent growth responses of maize leaves to changes in water status. – Plant Physiol. 48: 631–636.

Atwell, B. J. 1993. Responses of roots to mechanical impedance. – Environ. Exp. Bot. 33: 27–40.

Augé, R. M., Duan, X., Ebel, R. C. & Stodola, A. J. W. 1994. Nonhydraulic signalling of soil drying in mycorrhizal maize. – Planta 193: 74–82.

Bates, L. M. & Hall, A. E. 1981. Stomatal closure with soil water depletion not associated with changes in leaf bulk water status. – Oecologia 50: 62–65.

Bengough, A. G. & Mullins, C. E. 1990. Mechanical impedance to root growth: A review of experimental techniques and root growth responses. – J. Soil Sci. 41: 341–358.

Blackman, P. G. & Davies, W. J. 1985. Root to shoot communication in maize plants of the effects of soil drying. – J. Exp. Bot. 36: 39–48.

Boyer, J. S. 1970. Leaf enlargement and metabolic rates in corn, soybean, and sunflower at various leaf water potentials. – Plant Physiol. 46: 233–235.

Davies, W. J. & Zhang, J. 1991. Root signals and the regulation of growth and development of plants in drying soils. – Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 55–76.

– , Wilson, J. A., Sharp, R. E. & Osonubi, O. 1981. Control of stomatal behaviour in water-stressed plants. – *In* Stomatal Physiology (P. G. Jarvis and T. A. Mansfield, eds), pp.

163–187. Cambridge University Press, Melbourne. ISBN 0-521-23683-5.

–, Mansfield, T. A. & Hetherington, A. M. 1990. Sensing of soil water status and the regulation of plant growth and development. – Plant Cell Environ. 13: 709–719.

Gollan, T., Passioura, J. B. & Munns, R. 1986. Soil water status affects the stomatal conductance of fully turgid wheat and sunflower leaves. – Aust. J. Plant Physiol. 13: 459–464.

Huck, M. G., Klepper, B. & Taylor, H. M. 1970. Diurnal variation in root diameter. – Plant Physiol. 45: 529–530.

–, Ishimara, K., Peterson, C. M. & Ushijima, T. 1983. Soybean adaptation to water stress at selected stages of growth. – Plant Physiol. 73: 422–427.

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

Kramer, P. J. 1988. Changing concepts regarding plant water relations. – Plant Cell Environ. 11: 565–568.

Masle, J. & Passioura, J. B. 1987. The effect of soil strength on the growth of young wheat plants. – Aust. J. Plant Physiol. 14: 643–656.

Matsuda, K. & Riazi, A. 1981. Stress-induced osmotic adjustment in growing regions of barley plants. – Plant Physiol. 68: 571–576.

Michelena, V. A. & Boyer, J. B. 1982. Complete turgor maintenance at low water potentials in the elongating region of maize leaves. – Plant Physiol. 69: 1145–1149.

Munns, R. 1987. Does leaf turgor determine leaf expansion rates in dry or saline soil? – *In* Physiology of Cell Expansion during Plant Growth (D. J. Cosgrove and D. P. Knievel, eds), pp. 241–242. American Society of Plant Physiologists, Rockville, MD. ISBN 0-943088-11-9.

Osonubi, O. & Davies, W. J. 1978. Solute accumulation in leaves and roots of woody plants subject to water stress. – Oecologia 32: 323–332.

Passioura, J. B. 1988. Root signals control leaf expansion in wheat seedlings growing in drying soil. – Aust. J. Plant Physiol. 15: 687–693.

– 1994. The yield of crops in relation to drought. – *In* Physiology and Determination of Crop Yield (K. J. Boote, ed.), pp. 343–359. Am. Soc. Agron., Crop Sci. Soc. Am., Soil Sci. Soc. Am., Madison, WI. ISBN 0-89118-122-9.

Read, D. J. & Bartlett, E. M. 1972. The physiology of drought resistance in the soybean plant (*Glycine max*). I. The relationship between drought resistance and growth. – J. Appl. Ecol. 9: 487–499.

Rosa da Costa, A., Metcalfe, J., Lodge, T. A. & Davies, W. J. 1987. Soil drying and the resulting chemical and hydraulic effects on leaf growth. – *In* Plant Response to Stress (J. D. Tenhunen, F. M. Catarino, O. L. Lange and

W. C. Oechel, eds), pp. 267–275. Springer-Verlag, Berlin. ISBN 3-540-16082-5.

Saab, I. N. & Sharp, R. E. 1989. Non-hydraulic signals from maize roots in drying soil: Inhibition of leaf elongation but not stomatal conductance. – Planta 179: 466–474.

SAS/STAT. 1990. User's Guide, Vol. 2, GLM-VARCOMP. Version 6, 4th Ed. – SAS Institute Inc., Cary, NC. ISBN 1-55544-376-1.

Schmidhalter, U. 1997. Gradient between predawn rhizoplane and bulk soil matric potential: Relation to the predawn root and leaf water potential of four species. – Plant Cell Environ. 20: 953–960.

–, Evéquoz, M. & Oertli, J. J. 1992. Osmotic adjustment of roots and shoots. – *In* Root Ecology and its Practical Application – A Contribution to the Investigation of the Whole Plant (L. Kutschera, E. Hübl, E. Lichtenegger, H. Persson and M. Sobotik, eds), pp. 272–282. Verein für Wurzelforschung, Klagenfurt. ISBN 3-9500203-0-6.

–, Selim, H. M. & Oertli, J. J. 1994. Measuring and modeling root water uptake based on [36]chloride discrimination in a silt loam soil affected by groundwater. – Soil Sci. 158: 97–105.

–, Burucs, Z. & Camp, K. H. 1998. Sensitivity of root and leaf water status in maize (*Zea mays*) subjected to mild soil dryness. – Aust. J. Plant Physiol. 25: 307–316.

Shackel, K. A., Matthews, M. A & Morrison, J. C. 1987. Dynamic relation between expansion and cellular turgor in growing grape (*Vitis vinifera* L.) leaves. – Plant Physiol. 84: 1166–1171.

Sharma, R. B. & Ghildyal, B. P. 1977. Soil water-root relations in wheat: Water extraction rates of wheat roots that developed under dry and moist conditions. – Agron. J. 69: 231–233.

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

Spollen, W. G. & Sharp, R. E. 1991. Spatial distribution of turgor and root growth at low water potentials. – Plant Physiol. 96: 438–443.

Walter, S. & Bürgi, H. 1996. Report on the project ROOT DETECTOR. Computer aided evaluation of scanned images of roots. – Institute of Plant Sciences, ETH, Zurich.

Weaich, K., Bristow, K. L. & Cass, A. 1992. Preemergent shoot growth of maize under different drying conditions. – Soil Sci. Soc. Am. J. 56: 1272–1278.

Westgate, M. E. & 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. & Davies, W. J. 1989. Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. – Plant Cell Environ. 12: 73–81.

– & Davies, W. J. 1991. Antitranspirant activity in xylem sap of maize. – J. Exp. Bot. 42: 317–321.

Edited by L. Dolan