

# Diurnal Growth Trends, Water Potential, and Osmotic Adjustment of Maize and Sorghum Leaves in the Field

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## ABSTRACT

The daily cycle of leaf elongation rate, water potential, and solute potential of maize and sorghum, as well as temperature, were monitored in the field. Major climatic features were high radiation and a minimum air temperature of about 12 C. Leaf elongation of both crops was slowest at night, presumably because of low temperature. Peak elongation rates were in daytime when leaf water potential ( $\Psi$ ) was low. Solute potential also decreased during daylight, thus permitting the maintenance of appreciable turgor pressure, a critical parameter for cell expansion.

Leaf  $\Psi$  versus relative water content (RWC) curves were developed by sampling detached leaves at intervals as they dried quickly in the laboratory. At a given RWC,  $\Psi$  was lower in leaves at midday than early in the morning, which is evidence that the decrease in  $\psi_s$  at midday was caused by an increase in the amount of solute in the tissue. Estimates of  $\psi_s$  at 100% RWC were 4 bars lower at midday than early in the morning in both crops. Soluble sugars, mainly nonreducing, accounted for most of the observed  $\psi_s$  decrease in sorghum leaves. Shading the leaves from light eliminated most of the solute buildup.

Diurnal variations in leaf water potential ( $\Psi$ )<sup>3</sup> in response to diurnal changes in evaporative demand have been documented for diverse plant species in the field (17). An important question is whether the lower plant  $\Psi$  in daytime affects plant processes. Midday depression or stoppage in expansive growth (7) as well as stomatal closure (15) are frequently observed in the field and normally attributed to water stress. Growth especially deserves close attention because it is one of the most sensitive to stress among all plant processes examined (11). Small and sudden reduction in tissue  $\Psi$  quickly reduces growth, at least for a short time (2). Boyer (4) deduced from his curves of leaf  $\Psi$  and leaf enlargement of sunflower that most growth should occur only at night and confirmed this deduction with data on day and night growth in the greenhouse. On the other hand, midday depression in growth is not observed or is minimal in some field studies (13, 20) which did not include  $\Psi$  data, and Watts (23) and Bunce (6) have suggested that growth in the field might be less sensitive than in the greenhouse or growth chamber to reduction in tissue  $\Psi$ .

A still broader but related question is whether  $\Psi$  is a good indicator of plant water status in terms of plant functions. This question is timely since during the last decade  $\Psi$  has become the most widely used indicator. In some processes, such as expansive

growth, however, a key parameter is known to be only one of the components of  $\Psi$ , namely, pressure potential or turgor pressure ( $\psi_p$ ) (14).

Variations in  $\psi_p$  would reflect variations in  $\Psi$  only if the other major component of  $\Psi$ , solute or osmotic potential ( $\psi_s$ ), remains relatively constant. Information or diurnal trends in components of water potential is scant.

In this study, we monitored through the daily cycle leaf growth,  $\Psi$ , and  $\psi_s$  and temperature of maize and sorghum leaves. The work was conducted in the field in a high solar radiation environment, where large diurnal variation in leaf  $\Psi$  may be expected.

## MATERIALS AND METHODS

The crops were grown on Yolo clay loam, fertilized prior to sowing, at Davis, California in rows 76 cm apart. Most of the data were obtained in 1974. In that year maize (*Zea mays* L., cv. Dekalb hybrid XL22) and sorghum (*Sorghum bicolor* [L.] Moench, cv. Pioneer 846) were planted on May 17 and 19 at densities of 69,800 and 132,000 plants per hectare, respectively. The plots (7.6 × 9.1 m) were either irrigated with about 5 cm of water weekly or not irrigated at all through the season, and were parts of two broader experiments (1, 8). The only rain during the season fell on July 6 and 7 and totaled 16.5 mm.

Measurements were taken on 10 to 20 plants selected for uniformity from center rows of a plot. Leaf length was measured frequently from the apex to a reference line to within 1 mm with a ruler. The reference line was marked on the stem of the same plant close to the ligule of a recently fully expanded leaf for maize, and on a stake driven into the soil for sorghum. Thus, some stem internodal elongation is included in the measured increases in leaf length, although this error was small in the case of the dwarf sorghum cultivar we used and in maize prior to the period of rapid stem elongation (starting about 42 days after planting).

Leaves similar to those measured for length were sampled for  $\Psi$  determination. Pieces were cut from the middle length of the blade of two (maize) or one (sorghum) plant and quickly placed in a thermocouple psychrometer cup (1.9-cm diameter, 2.5-cm height) to line the bottom and wall. The cup interior had been coated with a film of petroleum jelly. The cup was immediately closed and placed in an insulated container, taken to the laboratory within 20 to 30 min, and equilibrated in a precision constant temperature bath. The psychrometer was the wet-loop type (18).  $\Psi$  and  $\psi_s$  were measured isopiastically (5) at 30 C. For each sample, outputs were obtained with distilled H<sub>2</sub>O and two known concentrations of sucrose near the isopiestic point on the ring. After determining  $\Psi$ , the psychrometer cup and its contents were quickly frozen to eliminate cell  $\psi_p$ , thawed, and placed again in the bath for a second measurement. Since matric potential ( $\psi_m$ ) should be negligible compared to  $\psi_s$  at the RWC encountered in this study, the second measured value is taken as  $\psi_s$ .

Air or leaf temperature was recorded continuously on selected

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<sup>3</sup> Abbreviations:  $\Psi$ : leaf water potential;  $\psi_p$ : pressure potential;  $\psi_s$ : solute potential; RWC: relative water content.

days, using copper-constantan thermocouples (80- $\mu$ m wire diameter). Junctions of the thermocouple were shaded by leaves when measuring air temperature, or were taped to the abaxial side of expanding leaf blades with small pieces (3  $\times$  3 mm) of Magic Mending tape (3M Co., Minneapolis, Minn.) for measuring leaf temperature. Five thermocouples, taped one to a leaf, were connected in parallel to a mv recorder to obtain the mean temperature.

Leaf moisture release curves ( $\Psi$  versus RWC) were obtained by drying excised leaves. Thirty to 40 blades were collected, brought to the laboratory ( $25 \pm 1$  C), and spread to dry under light (around  $40 \mu\text{E m}^{-2} \text{s}^{-1}$  of PAR). Periodically, 10 or 12 discs, 9 mm in diameter, were cut from the blade on one side of the midrib for RWC determination (3); the other side was sampled for  $\Psi$ . Most of the data presented were obtained with a drying period of less than 3 h.

In the summer of 1978, leaves (fourth from the top) of sorghum of the same cultivar, growing on stored soil water, were measured for  $\Psi$ ,  $\psi_s$  and soluble sugars and organic acids, 42 and 50 days after planting. Artificial shades were placed over some leaves the previous night before sampling at midday. After storage at  $-15$  C, 4 g of the sampled tissue were homogenized in 15 ml 80% ethanol. The homogenate was increased to 25 ml with 80% ethanol, and centrifuged at 1,750 rpm for 10 min. The supernatant was analyzed colorimetrically for reducing sugars (16, 19) before and after hydrolysis with 1.33 N  $\text{H}_2\text{SO}_4$ . The organic acids in the supernatant were titrated with NaOH to the phenolphthalein end point (21) after the supernatant was first extracted three times with equal volumes of chloroform.

## RESULTS

**Diurnal Trends of Water Potential and Components, and of Leaf Growth and Temperature.** During the 1974 and 1975 growing seasons, most days were completely sunny. Daily insolation followed the classical bell-shaped curve.  $\Psi$  of the upper leaves exposed to the sun described a sinusoidal curve with time, with a minimum around solar noon (Fig. 1a). This appeared to be the general pattern for both maize and sorghum, as indicated by diurnal  $\Psi$  curves of expanding and fully expanded leaves on a number of other days in the season (not shown). Data points in Figure 1a represent individual samples and are indicative of the scatter commonly encountered in this study.

In spite of the low daytime leaf  $\Psi$ , length of growing leaves increased more during the day than at night in all measurements, as shown by the leaf length data (Fig. 1b) of the same population of leaves sampled for  $\Psi$  on the same data.

Leaf elongation rates were derived by fitting a cubic spline function to the leaf length data and taking the first derivative. Elongation rates of both unirrigated and well-watered maize leaves exhibited two daily maxima, one in the forenoon and the other late in the afternoon (Fig. 2, a and e, respectively). Leaf  $\psi_s$  (Fig. 2d) also followed a sinusoidal curve as leaf  $\Psi$  but appeared to lag by about 1 to 2 h. The nearly in-phase oscillation in  $\Psi$  and  $\psi_s$  permitted the maintenance of positive turgor even at noon (Fig. 2, c and g), when leaf  $\Psi$  reached its lowest values. Presumably this accounted for the growth of leaves during the day in spite of the daytime depression of leaf  $\Psi$ .

Summer nights are usually cool at Davis. On the days of maize growth measurements, temperature dropped to as low as 12 C at night and rose rapidly after sunrise (Fig. 2, b and f). Temperature appeared to influence growth as much as plant water status. Figure 2 shows that at night, elongation was minimal although leaf  $\psi_p$  was near the highest point. Further, as leaf  $\psi_p$  continued to drop in the morning after sunrise, elongation rate actually increased, apparently because of rising temperature.

Growth of sorghum leaves also took place mostly during the daytime (Fig. 3a). As in maize,  $\psi_s$  of sorghum leaves oscillated diurnally almost in phase with  $\Psi$  (Fig. 3c). Consequently  $\psi_p$  in

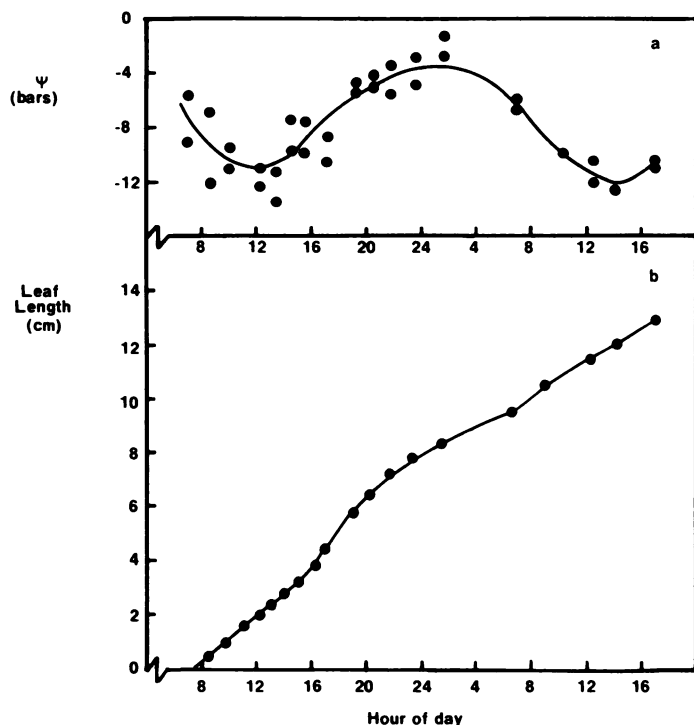


FIG. 1. a: Diurnal pattern of leaf water potential ( $\Psi$ ) in expanding leaves of unirrigated maize 41 to 42 days after planting. Data points represent individual measurements. b: Diurnal course of increase in length of leaf no. 13 in unirrigated maize. Measurements were taken concurrently with the  $\Psi$  measurements depicted in (a).

sorghum leaves remained substantial during the day (Fig. 3b), making daytime growth possible. Similar patterns of growth and leaf  $\Psi$  and components were observed repeatedly in 1974 and 1975.

**Diurnal Osmotic Adjustment.** Leaf  $\psi_s$  can be reduced either by water loss and hence a concentration of existing solutes, or by a net increase in solutes in the tissue. The existence of a substantial  $\psi_p$  during the daytime period of low  $\psi_s$  suggested a substantial solute buildup, possibly coupled with some water loss. This was examined further in both maize and sorghum by comparing the moisture release curve ( $\Psi$  versus RWC) of leaf samples taken early in the morning with that of leaf samples taken at midday. The data (Fig. 4) show that for any given RWC of the leaf, the samples taken at midday had a more negative  $\Psi$  value than those taken early in the morning. Since comparisons are made at the same tissue RWC, the difference in  $\Psi$  cannot be attributed to water loss or dehydration but must be attributed to changes in the components of  $\Psi$ . The solute potential of the samples was also measured and plotted against RWC on a log-log scale (Fig. 5) to obtain  $\psi_s$  at 100% RWC (10). For both sorghum and maize,  $\psi_s$  at 100% RWC was about 4 bars more negative at midday than early in the morning, indicating a marked accumulation of solutes in the tissue. Since the RWC of the leaves in the field at midday would be less than 100%, the difference in  $\psi_s$  between the two times would be a bit larger. For example, a difference between  $-14$  and  $-10$  bars at saturation would be about 4.5 bars at a RWC of 90%. On typical days leaf  $\Psi$  dropped about 5 to 6 bars from 700 to 1200 h (Figs. 1a and 3c). The corresponding drop in  $\psi_s$ , however, compensated for most of the drop in  $\Psi$ . The other major component of water potential,  $\psi_p$ , remained substantially positive, making possible significant expansive growth at midday.

We suspected an association between photosynthesis and the observed diurnal osmotic adjustment; hence, a shading test was conducted in 1978. Sorghum leaves showed a decrease in  $\psi_s$  of 4

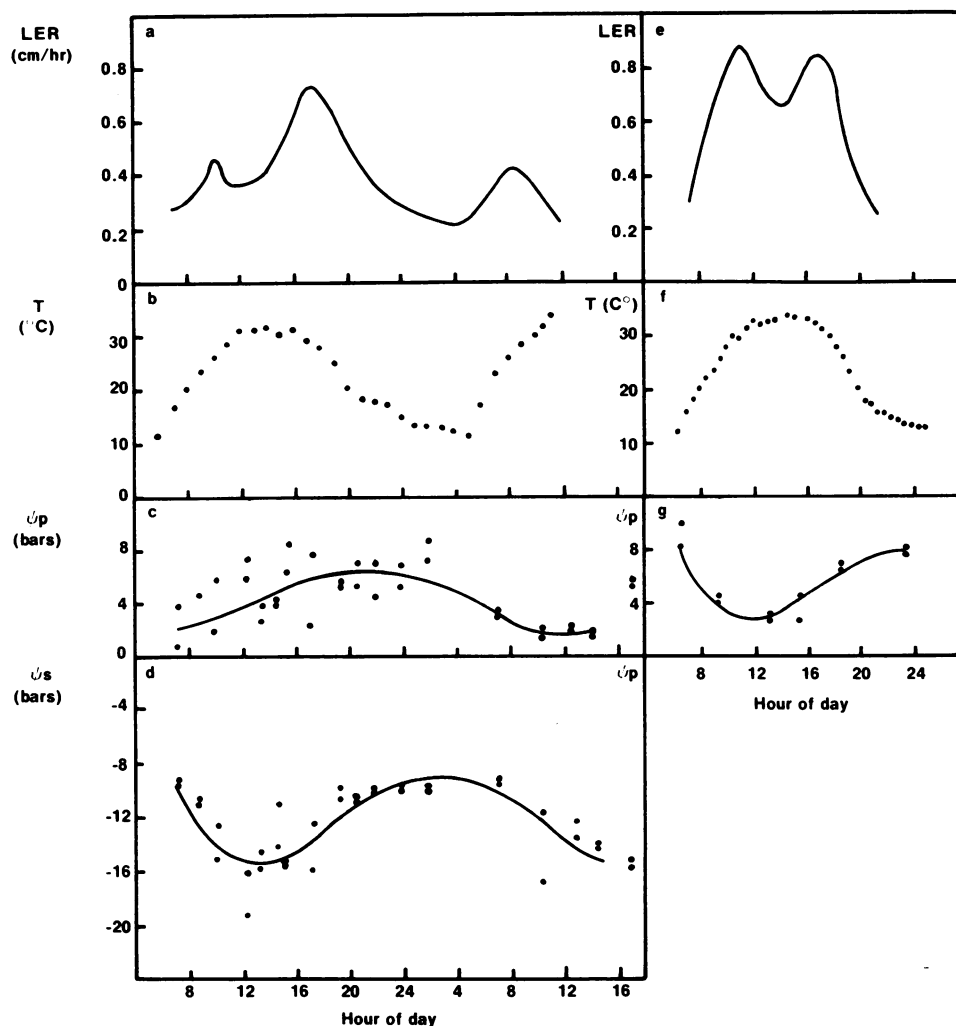


FIG. 2. Diurnal course of the following parameters: (a) leaf elongation rates (LER) calculated as the derivative of a cubic spline function fitted to the experimental data of Figure 1b; (b) leaf temperature; (c) turgor pressure,  $\psi_p$ , calculated as the difference between  $\Psi$  (Fig. 1a) and  $\psi_s$  (Fig. 2d); (d) solute potential,  $\psi_s$ , curve shown is fourth degree polynomial fitted to the experimental data; (e) leaf elongation rates (LER) calculated as indicated in (a); (f) air temperature, T; and (g) turgor pressure,  $\psi_p$ . (a) through (d) are for unirrigated maize, 41 and 42 days after planting. (e) through (g) are for irrigated maize, 37 days after planting.

bars from dawn to midday when exposed to sun but exhibited almost no diurnal change in  $\psi_s$  when artificially shaded (Table I). There was a large increase in the total sugar level of exposed leaves from dawn to midday and minor increases in reducing sugars and organic acids levels, as shown in Table I. Total sugars of shaded leaves at midday were below the observed values at dawn. This experiment was repeated 8 days later, and similar results were obtained.

## DISCUSSION

Osmotic adjustment as a response to water stress may be a common phenomenon in nature (12). We have shown (9) that seasonal osmotic adjustment occurs in field-grown sorghum and maize in response to moderate water stress. Wenkert *et al.* (25) reported seasonal osmotic adjustment in soybeans with a decrease of  $\psi_s$  at saturation of about 4 bars over a 2-month period. However, Turner *et al.* (22) did not find either diurnal or seasonal osmotic adjustment in two other soybean cultivars subjected to water stress in the field. This is consistent with their observations of no changes in soluble sugars on a diurnal basis.

In contrast, we have shown a marked diurnal trend in soluble sugars in sorghum (Table I) and similar trends in  $\psi_s$  of maize and sorghum. It may be possible that either differences among soybean

cultivars or in age of leaf sampled are responsible for the discrepancy between the two reports mentioned above.

The contribution of the measured solute changes to the  $\psi_s$  drop was evaluated using the formula

$$\psi_s = \frac{RT}{V_w} n_s$$

where  $n_s$  is the number of moles of solute (calculated assuming the sugars before and after hydrolysis to be glucose and sucrose, respectively), T is the absolute temperature,  $V_w$  the total volume of water, and R the gas constant. The volume of water acting as a solvent was assumed to be 74% of the tissue fresh weight (9). The calculations indicate that the increase in sugar concentration from dawn to midday was equivalent to  $-3.2$  bars or 82% of the observed decrease in  $\psi_s$  of  $-3.9$  bars (Table I) while the increase in organic acids represented less than  $-0.1$  bar.

In the field, leaf growth patterns cannot be interpreted solely on the basis of water status as other environmental parameters also fluctuate. Wenkert *et al.* (24) found that the sensitivity of soybean growth to water status was variable and that temperature and radiation partially dictated the pattern of leaf expansion in the field. Ambient temperatures may have an overriding effect at certain times on leaf expansion. In this study (Fig. 2), leaf elongation rate in the morning was not related to turgor pressure but

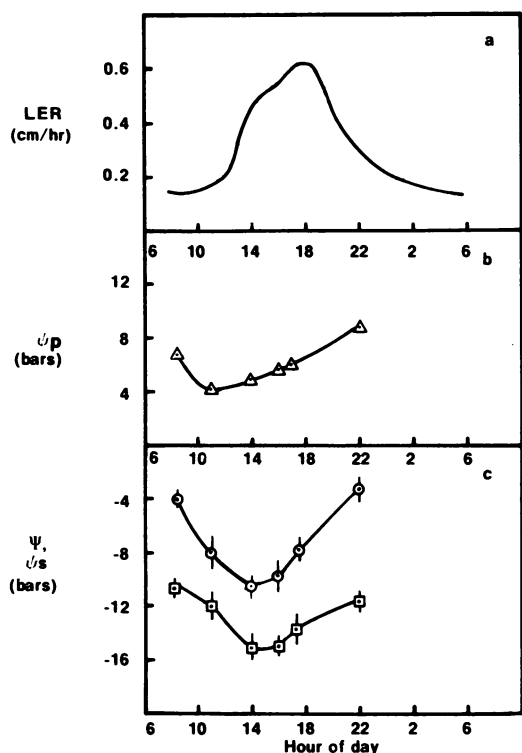


FIG. 3. Diurnal course of: (a) leaf elongation rate (LER) calculated as the derivative of a cubic spline function fitted to experimental data similar to that of Figure 1b; (b) turgor pressure; and (c) total and solute potential for unirrigated sorghum 39 days after planting. Bars in (c) indicate the standard deviation of the mean of three measurements of  $\Psi$  and  $\psi_s$ .

Table 1. Changes in Water Potential and Solute Potential, Soluble Sugars and Organic Acids in Exposed and Artificially Shaded Sorghum Leaves from Dawn to 1300 Hours

Values are averages of six to eight determinations  $\pm$  one standard error.

	0600 hours	1300 hours	
		Exposed	Shaded
Photosynthetically active radiation ( $\mu\text{E m}^{-2} \text{s}^{-1}$ )	—	>1500	30–50
$\Psi$ (bars)	$-1.1 \pm 0.5$	$-9.5 \pm 0.7$	$-7.5 \pm 0.5$
$\psi_s$ (bars)	$-9.3 \pm 0.3$	$-13.2 \pm 0.4$	$-10.1 \pm 0.6$
Reducing sugars before hydrolysis (mg/g fresh weight)	$12.3 \pm 2.3$	$15.2 \pm 2.1$	$10.9 \pm 2.2$
Reducing sugars after hydrolysis (mg/g fresh weight)	$54.6 \pm 1.9$	$88.2 \pm 2.1$	$33.4 \pm 2.4$
Titrateable organic acids ( $\text{mol} \times 10^{-2}$ )	$1.13 \pm 0.04$	$1.32 \pm 0.10$	$1.01 \pm 0.08$

correlated linearly with temperature, as also reported by Watts (23) for maize. On the other hand, elongation was linearly related to turgor pressure when temperature was not low and thus limiting (1, 8). It is clear that in an environment with high solar radiation and cool nights, the interwoven pattern of water and temperature determines the diurnal trends of expansive growth of cold-sensitive plants such as maize and sorghum. We have shown (2) that under a nearly constant but relatively low light environment a small decrease in  $\Psi$  markedly inhibits maize leaf expansion, apparently due to a decrease in turgor pressure (12). Diurnal osmotic adjustment, in the field, must then play a key role in permitting rapid leaf expansion in maize and sorghum during the day despite the observed low  $\Psi$  values (Figs. 2 and 3).

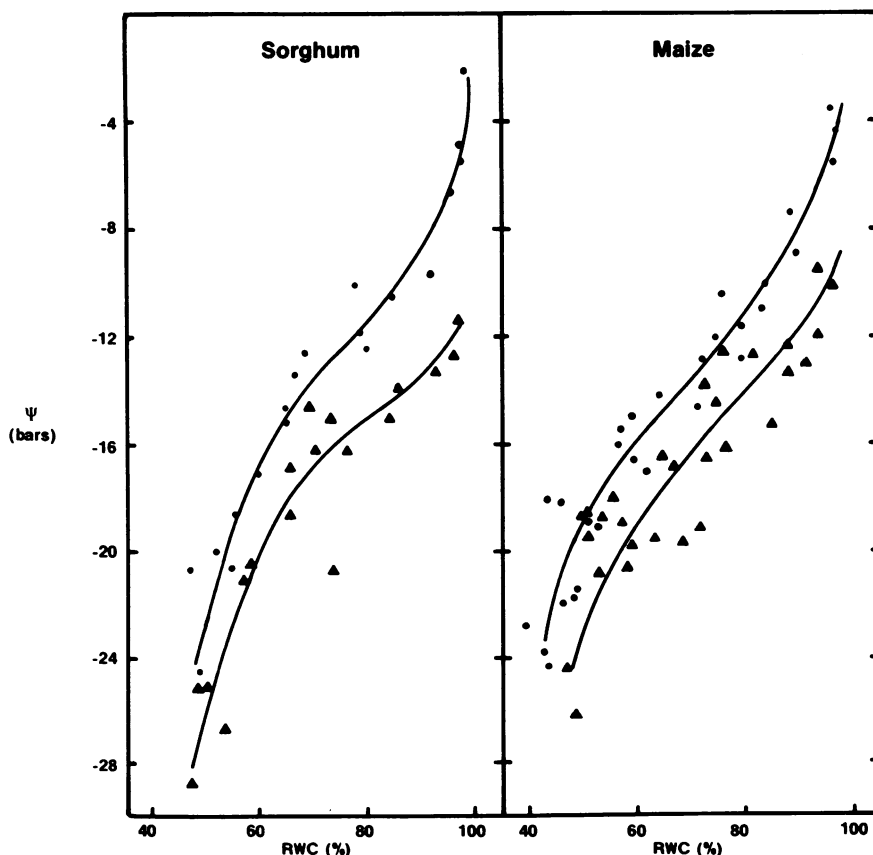


FIG. 4. Relationships between relative water content (RWC) and water potential ( $\Psi$ ) at two times of day for: (a) unirrigated sorghum, 50 days after planting at 0600 hours ( $\cdots$ ) and at 1300 hours ( $\blacktriangle$ ); and for (b) unirrigated maize taken at 0700 hours on day 45 ( $\cdots$ ) and at 1300 hours on day 47 ( $\blacktriangle$ ).

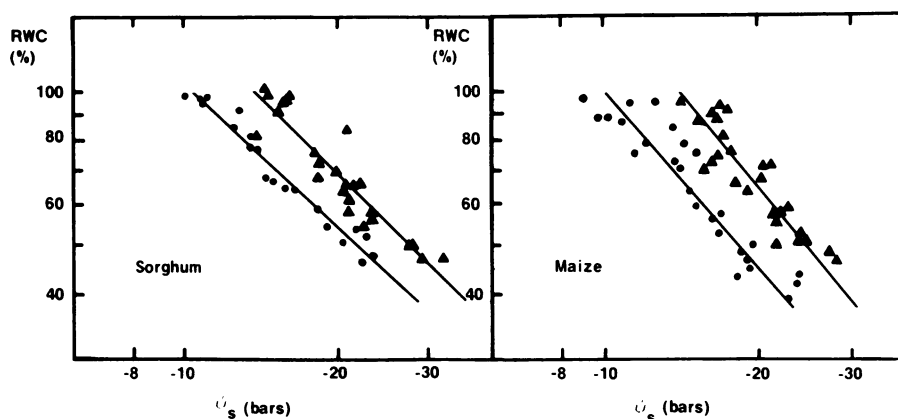


FIG. 5. Relationships between RWC and solute potential ( $\psi_s$ ) at two times of day for: (a) unirrigated sorghum at 0600 hours (· · ·) and at 1300 hours ( $\blacktriangle$ ); and (b) maize at 0700 hours (· · ·) and 1300 hours ( $\blacktriangle$ ).  $\psi_s$  measurements done on the same samples taken to measure  $\Psi$  in Figure 4.

Our data demonstrate that the relationship between  $\Psi$  and RWC is not unique and may even vary from hour to hour. Therefore, measurement of  $\Psi$  alone does not provide sufficient information on the impact of water stress on physiological processes mediated by tissue water content or cell turgor.

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