

# Growth of the Maize Primary Root at Low Water Potentials<sup>1</sup>

## II. Role of Growth and Deposition of Hexose and Potassium in Osmotic Adjustment

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### ABSTRACT

Primary roots of maize (*Zea mays* L. cv WF9 × Mo17) seedlings growing in vermiculite at various water potentials exhibited substantial osmotic adjustment in the growing region. We have assessed quantitatively whether the osmotic adjustment was attributable to increased net solute deposition rates or to slower rates of water deposition associated with reduced volume expansion. Spatial distributions of total osmotica, soluble carbohydrates, potassium, and water were combined with published growth velocity distributions to calculate deposition rate profiles using the continuity equation. Low water potentials had no effect on the rate of total osmoticum deposition per unit length close to the apex, and caused decreased deposition rates in basal regions. However, rates of water deposition decreased more than osmoticum deposition. Consequently, osmoticum deposition rates per unit water volume were increased near the apex and osmotic potentials were lower throughout the growing region. Because the stressed roots were thinner, osmotic adjustment occurred without osmoticum accumulation per unit length. The effects of low water potential on hexose deposition were similar to those for total osmotica, and hexose made a major contribution to the osmotic adjustment in middle and basal regions. In contrast, potassium deposition decreased at low water potentials in close parallel with water deposition, and increases in potassium concentration were small. The results show that growth of the maize primary root at low water potentials involves a complex pattern of morphogenic and metabolic events. Although osmotic adjustment is largely the result of a greater inhibition of volume expansion and water deposition than solute deposition, the contrasting behavior of hexose and potassium deposition indicates that the adjustment is a highly regulated process.

Growing cells must produce or import solutes to maintain osmotic potential ( $\psi_s$ ) as the existing solutes are diluted by water uptake or removed from the osmotic pool. The  $\psi_s$  is essential for maintenance of turgor pressure and thus for continuing expansion. Plants experiencing water stress often have higher tissue solute concentrations, and lower  $\psi_s$ , than

plants supplied with abundant water. When the elevated solute concentrations are not merely the result of tissue dehydration, this phenomenon is known as 'osmotic adjustment.' Osmotic adjustment is thought to benefit the plant by increasing the driving force for water uptake, permitting the cells to retain more water at reduced water potentials ( $\psi_w$ ) (2, 8, 13). Osmotic adjustment in growing regions could occur by two basic mechanisms: an increase in the net rate of osmoticum deposition in the growing zone and/or a reduction in rate of tissue volume expansion.

Osmotic adjustment at low  $\psi_w$  has been observed in growing regions of leaves (10, 16, 27, 29), stems (11, 12, 29), shoot apices (15), and roots (7, 19, 29). It is often observed, however, that root growth is less inhibited than shoot growth at low  $\psi_w$  (4, 19, 29). Consistent with these observations, we have shown that maize primary roots continue to grow, albeit at reduced rates, at low  $\psi_w$  which are completely inhibitory to shoot growth. Analyses of the spatial and temporal distribution of root growth revealed a complex pattern of developmental changes involving inhibition of both longitudinal and radial expansion and a shortening of the growth zone (20).

As a step toward understanding the physiology of osmotic adjustment in growing regions, it is important to analyze the relative contributions of solute deposition and growth to the observed changes in  $\psi_s$ . Investigators have recognized that local import and metabolic production act to increase soluble density, while growth-associated water uptake and solute utilization decrease the density (12, 27). A quantitative approach to this problem is to apply principles of growth kinematics (21, 22) to calculate and compare the spatial distributions of net solute and water deposition rates in the growing zone at various  $\psi_w$ . In this paper we show that in the growing zone of the primary root of maize, osmotic adjustment involves decreased rates of solute deposition per unit length but increased solute deposition rates on a water volume basis. The results are discussed in terms of the role of osmotic adjustment in the maintenance of root elongation at low  $\psi_w$ .

### MATERIALS AND METHODS

#### Plant Culture

Seedlings of *Zea mays* L. (cv WF9 × Mo17) were germinated in moist vermiculite, transplanted into Plexiglas boxes containing vermiculite ranging in  $\psi_w$  from  $-0.03$  to  $-1.7$  MPa, and grown in the dark at 29°C and near-saturation

<sup>1</sup> Supported by National Science Foundation Grant DCB 8417504 to W. K. S. and T. C. H. and U.S. Department of Agriculture Grant 87-CRCR-1-2492 to R. E. S. Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 10982.

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**Table I.** Vermiculite Water Content and Water Potential Corresponding to Treatments Indicated in Figures 2–9

The different treatments represent vermiculite water content as percentages of the water content at the highest water potential. The same lot of vermiculite, well mixed, was used for all the experiments to obtain approximately the same water potential at the same water content. Water potentials are means  $\pm$  1 SD of four to eight experiments.

Treatment	Vermiculite Water Content	Vermiculite Water Potential
%	$g\ g^{-1}$	MPa
100	3.72	$-0.03 \pm 0.00$
16	0.60	$-0.20 \pm 0.01$
4	0.15	$-0.81 \pm 0.06$
2	0.08	$-1.60 \pm 0.15$

humidity, as previously described (20). The different  $\psi_w$  were obtained by mixing the vermiculite with various amounts of  $10^{-4}$  M  $CaCl_2$ , and were measured by isopiestic thermocouple psychrometry (3). For most experiments, the four treatments used (Table I) were the same as for the measurements of expansive growth distribution described in the preceding paper (20).

#### Osmotic Potential and Solute Contents

When the primary roots had attained a length of approximately 5 cm, seedlings were selected for uniformity of root elongation rate (within  $\pm 15\%$  of the mean), as measured by periodically marking under dim light the position of the root apices on the angled Plexiglas face against which they were growing. Preliminary experiments established that both root elongation rate (see Fig. 1 of Sharp *et al.* [20]) and root tip  $\psi_s$  (Fig. 1, inset) were constant with time in all treatments in roots of this length (20–45 h after transplanting, depending on treatment). Adhering vermiculite was removed, and the primary roots were placed on moist (but not wet) graph paper. The apical 0.5 mm was excised to remove a major part of the root cap, and batches of 5 roots were sectioned into 10 1-mm serial segments as measured by the scale on the paper. The segments were collected by position along the root and held on ice in capped vials until the desired number of roots had been sampled. For subsequent computations, the  $\psi_s$  or solute content of the segment extending from 0.5 to 1.5 mm from the apex was defined to be at 1 mm. Root manipulation and harvesting were carried out inside a chamber of near-saturation humidity.

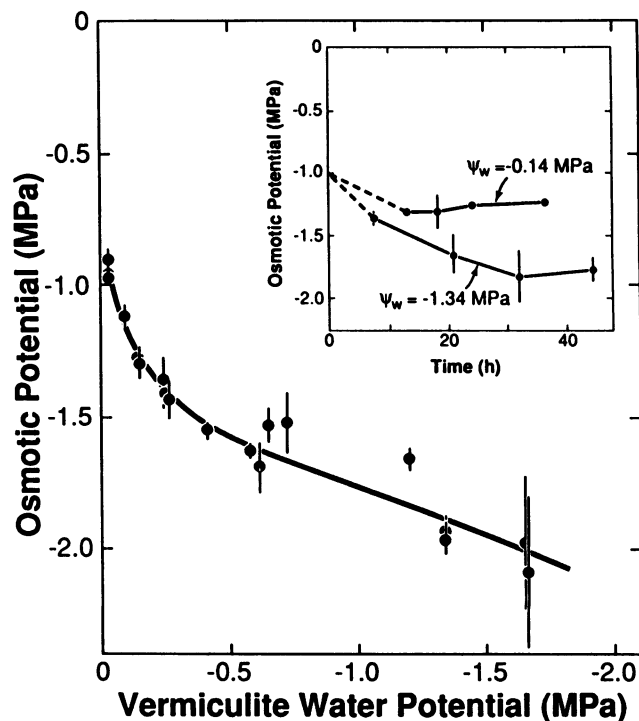
Measurements of  $\psi_s$  at each position were made on three to seven sets per treatment of 30 to 50 segments per position. Immediately after sampling, the segments were frozen over dry ice, thawed, and macerated with a small glass rod. Melting points of approximately 25  $\mu$ L of the tissue macerate were determined three times with a miniaturized freezing point depression apparatus (22). Standard KCl solutions of known  $\psi_s$  were used for calibration. 'Root tip'  $\psi_s$  (Fig. 1) were measured after combining the 10 serial segments (0.5–10.5 mm) from three to four roots.

The contents and concentrations of soluble carbohydrates

and potassium at each position along the root were measured on four sets per treatment of 40 segments per position. The samples were wrapped in preweighed aluminum foil packets, weighed, freeze dried, and reweighed to obtain the weight of water by difference. The samples were then extracted three times for 15 min at 80°C in small volumes of 80% ethanol. The extracts were combined, evaporated to dryness at 80°C under a stream of nitrogen, taken up in 10 mL of distilled water, and frozen until analysis. Preliminary tests showed that extracting a fourth time, and including tissue homogenization, did not yield detectable quantities of either soluble carbohydrates or potassium. An additional two sets of 40 roots per treatment were sampled for water content only.

Reducing sugars and sucrose were analyzed colorimetrically by the Somogyi-Nelson method (17, 25) before and after hydrolysis with 150 mM HCl (100°C, 1 h), respectively, using D-glucose standards. Reducing sugars were assumed to be hexoses, and sucrose was assumed to be the sole source of reducing sugars released by hydrolysis. Potassium was analyzed by flame photometry with a Beckman DU spectrophotometer using KCl standards.

Osmoticum contents and the  $\psi_s$  contributions of the different solute species were estimated using the mean measured values of  $\psi_s$ , water content, and solute content at each location



**Figure 1.** Osmotic potential of the apical 10 mm of maize primary roots growing at various vermiculite water potentials. Measurements were made at least 15 h after transplanting to the different water potentials, when the roots were 5 cm long and both elongation rates and osmotic potentials were approximately steady. The inset shows the time course of change in osmotic potential of the apical 10 mm after transplanting to different water potentials. Data are means  $\pm$  1 SD ( $n = 3-6$ ).

with the conventional formula:

$$\psi_s = - \frac{RTn}{V} \quad (1)$$

where  $R$  is the gas constant,  $T$  the absolute temperature,  $V$  the volume of water, and  $n$  the number of moles of solutes. Because the measured  $\psi_s$  values were not corrected for the dilution by apoplastic water after freezing and thawing, the  $\psi_s$  contributions of the different solutes were calculated on the basis of total tissue water volume.

### Numerical Methods

The methods were adapted from Silk *et al.* (24). Net local deposition rates,  $d$  (amount per mm of root length per h), were calculated from the continuity equation:

$$d = \frac{\partial S}{\partial t} + \frac{\partial(Sv_z)}{\partial z} \quad (2)$$

local rate      growth associated  
of change      deposition rate

where  $S$  is the local density (content per mm length of osmoticum, water, hexose, or potassium resolved at 0.5 mm intervals by linear interpolation),  $t$  is time (h),  $z$  is distance from the root apex (mm), and  $v_z$  is local (at distance  $z$ ) rate of longitudinal displacement from the root apex due to growth ( $\text{mm h}^{-1}$ ). Equation 2 confirms that deposition may occur even when the local density is unchanging in time, *i.e.* when the first term on the right-hand side is negligible, and shows the importance of knowing the local growth velocity. Spatial distributions of growth velocity resolved at 0.5 mm intervals were obtained from the preceding paper (20). Expansion of the growth-associated deposition rate gives:

$$\frac{\partial(Sv_z)}{\partial z} = \underbrace{S(\partial v_z / \partial z)}_{\text{growth dilution term}} + \underbrace{v_z(\partial S / \partial z)}_{\text{convective term}} \quad (3)$$

The growth dilution term represents the deposition rate necessary to maintain the local density in the face of tissue expansion. The convective rate of change represents change due to movement of cells away from the apex, and may be considered the deposition rate necessary to maintain any spatial gradient in density.

Numerical differentiation was performed with the five-point differentiating formula of Erickson (6). Because the midpoint formula was used, derivatives could be evaluated only between the third and third-to-last values of the abscissa. Equation 2 gave deposition rates per millimeter of root length, evaluated at 0.5 mm intervals. Division by the local water volume ( $\text{mm}^3$  per mm length) gave deposition rates per  $\text{mm}^3$  of tissue water.

### Definitions of Terms Used in Deposition Rate Analysis

As described above, 'deposition rate' refers to the local, net rate of addition of matter. In the case of solutes this rate includes production (by breakdown of complex polymers and/or biosynthesis of osmotica), import of osmotically active species, and removal from the osmotic pool (by respiration or biosynthetic processes). Rates may be expressed per unit length or volume. In this paper, expression per unit length

was necessary to determine whether the total rate of solute entry into the osmotic pool was altered in the root growing zone at low  $\psi_w$ , as tissue and water volumes per unit length varied among the treatments. Similarly, 'osmoticum accumulation' refers to an increase in total solutes per unit root length, in contrast to the common usage in nongrowing tissues to indicate increases in solute content per cell. This distinction is necessary in growing regions to determine whether changes in  $\psi_s$  are due to changes in the absolute amount of solutes in the tissue, as cell volumes may differ at different  $\psi_w$ . 'Osmotic adjustment' denotes a decrease in  $\psi_s$  (*i.e.* an increase in volumetric solute concentration) that results from either osmoticum accumulation and/or reduced tissue volume expansion. Making careful distinctions among these different terms allows us to explore and communicate the physiological and ecological significance of the experimental results.

## RESULTS

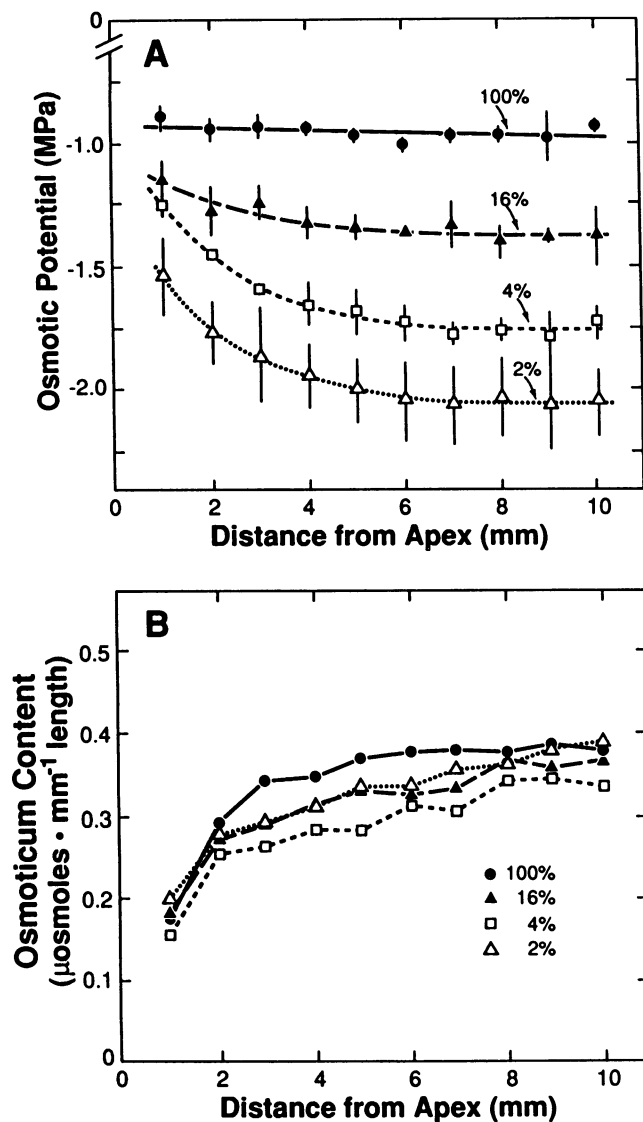
### Osmotic Adjustment in the Root Growing Zone

When seedlings were transplanted from moist vermiculite to vermiculite of various low  $\psi_w$ , the  $\psi_s$  in the apical 10 mm of the primary root decreased (Fig. 1). This region encompasses the elongation zone, which extends for approximately 10 mm from the apex at high  $\psi_w$  and is progressively shorter as the  $\psi_w$  decreases (20). The duration and magnitude of the change in  $\psi_s$  increased as the  $\psi_w$  decreased. The time courses of  $\psi_s$  change at two  $\psi_w$  are shown in the inset to Figure 1. Over the range of  $\psi_w$  examined,  $\psi_s$  were constant by the time the roots attained a length of 5 cm. The  $\psi_s$  shown in the main part of Figure 1 were determined in roots of this length. Therefore, the data can be compared with the steady (time invariant) response of root elongation rate over the same range of  $\psi_w$ , shown in Figure 1 of the preceding paper of this series (20). As the  $\psi_w$  decreased from  $-0.03$  to  $-1.7$  MPa, the  $\psi_s$  decreased from  $-0.9$  to  $-2.0$  MPa while the elongation rate declined to approximately one-third of the control rate of  $3.1 \text{ mm h}^{-1}$  (see also legends to Figs. 2 and 4). Both  $\psi_s$  and growth rate were more responsive above a  $\psi_w$  of  $-0.25$  MPa. These preliminary analyses showed that averaged over the growing region, elongation rate and  $\psi_s$  are both progressively decreased by increasing water stress.

Most of the observed changes in  $\psi_s$  can be attributed to osmotic adjustment. Relative water contents of the apical 10 mm were measured by weighing the tissue before and after floating for 3 h on an ice/distilled water mixture (to prevent growth but allow hydration), and after oven drying. Mean relative water contents of 96.4, 86.9, 81.7, and 79.6%, respectively, were obtained for roots growing at  $-0.03$ ,  $-0.20$ ,  $-0.78$ , and  $-1.60$  MPa vermiculite  $\psi_w$ . Also, fractional water volumes calculated from mean tissue volumes (20) and water content data were 82, 83, 82, and 86%, respectively. The contribution of dehydration to the changes in  $\psi_s$  was therefore minor.

### Spatial Distribution of Osmotic Potential and Osmoticum Content

The relationship between the adjustment of  $\psi_s$  in the growing zone and root growth inhibition was studied in greater



**Figure 2.** Spatial distribution of (A) osmotic potential, and (B) osmoticum content in the apical 10 mm of roots growing in vermiculite of various water contents. The treatments represent the vermiculite water content as percentages of the water content at high water potential (see Table I). Osmotic potentials are means  $\pm$  1 SD from three to seven experiments. Elongation rates of the roots were ( $\pm$  1 SD): 100% treatment,  $3.18 \pm 0.10 \text{ mm h}^{-1}$ ; 16% treatment,  $2.29 \pm 0.22 \text{ mm h}^{-1}$ ; 4% treatment,  $1.40 \pm 0.12 \text{ mm h}^{-1}$ ; 2% treatment,  $1.14 \pm 0.19 \text{ mm h}^{-1}$ . Osmoticum contents were calculated from the mean osmotic potential and mean water content at each position.

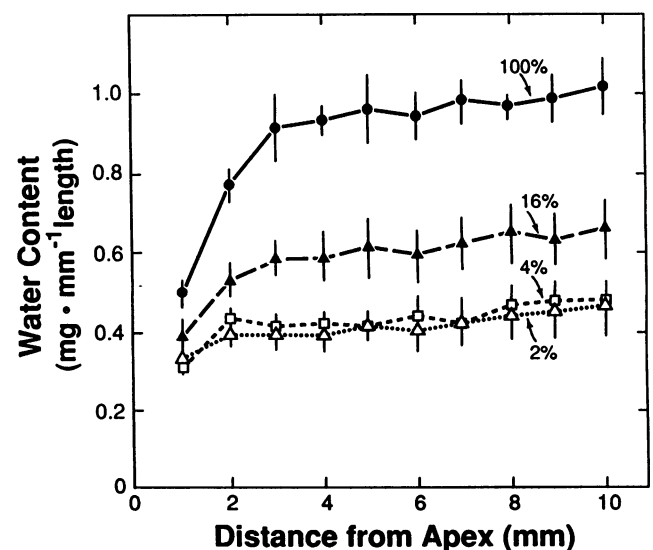
detail by determining the spatial distribution of  $\psi_s$  in roots growing at the same four levels of water availability used previously to evaluate the distribution of the growth response (20). The different treatments are indicated in Figures 2 to 9 by the vermiculite water contents expressed as percentages of the high  $\psi_w$  (100%) treatment. The mean vermiculite  $\psi_w$  measured for the treatments are given in Table 1. Figure 2A shows that  $\psi_s$  was virtually constant (ranged from  $-0.89$  to  $-1.00 \text{ MPa}$ ) along the length of the apical 10 mm of roots

growing at high  $\psi_w$ , in agreement with the result of Silk *et al.* (22). As the  $\psi_w$  decreased, however, a spatial gradient in  $\psi_s$  developed such that the  $\psi_s$  decreased with distance from the apex. At a  $\psi_w$  of  $-1.6 \text{ MPa}$  (2% treatment), for example, the  $\psi_s$  was only  $0.65 \text{ MPa}$  lower than at high  $\psi_w$  in the first millimeter, but decreased a further  $0.5 \text{ MPa}$  by the 7 mm location.

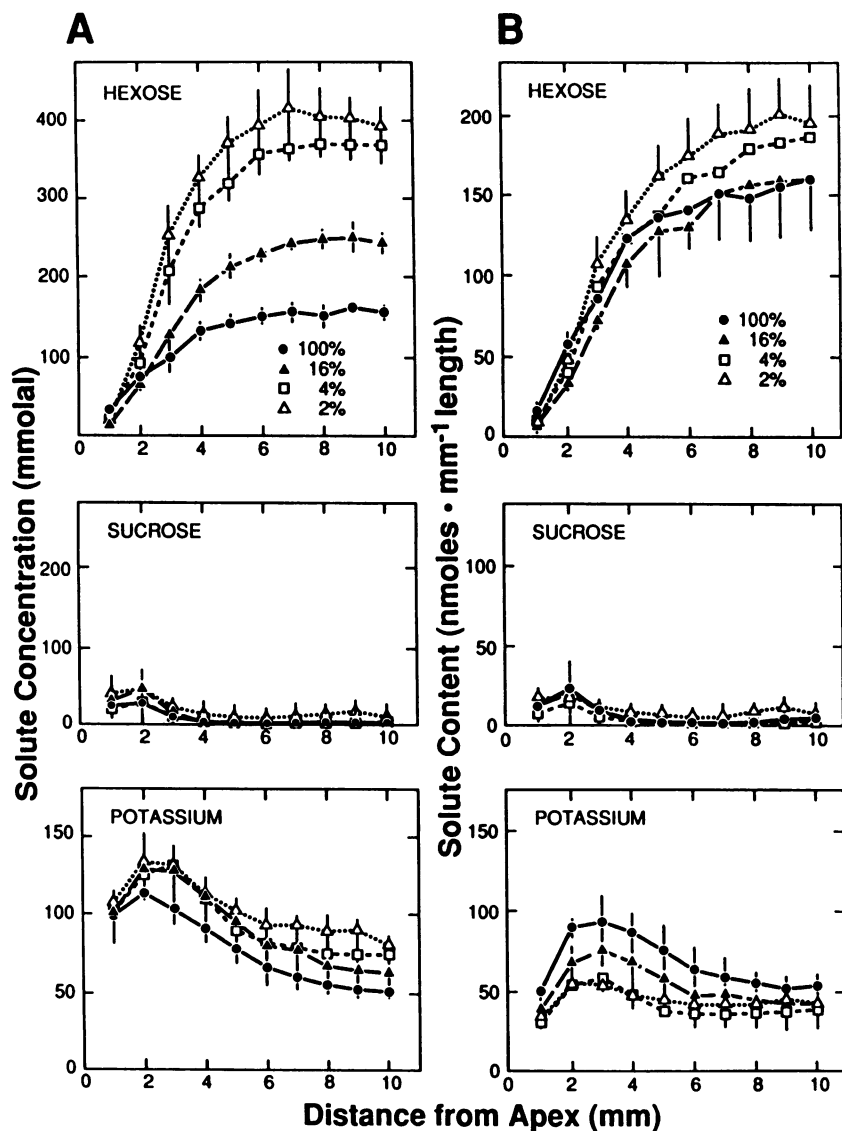
We showed previously that radial as well as longitudinal expansion was inhibited at low  $\psi_w$ , so that the stressed roots were thinner (20). Reflecting this, water content per millimeter length was progressively lower as the  $\psi_w$  decreased (Fig. 3). The data of Figures 2A and 3 were used with Equation 1 to calculate osmoticum content per unit root length. The results (Fig. 2B) show that osmotica did not accumulate at any location in any of the low  $\psi_w$  treatments. Indeed, except in the apical 2 mm, osmoticum content per mm of length appeared to be slightly higher in the roots growing at high  $\psi_w$ . Consequently, it was possible to have substantial osmotic adjustment (Fig. 2A) without osmoticum accumulation (Fig. 2B). Clearly, therefore, inhibition of radial growth was an important factor in the osmotic adjustment.

#### Spatial Distribution of Hexose, Sucrose, and Potassium

Total osmoticum contents were calculated from  $\psi_s$  measured by freezing point depression, a technique which measures colligative properties without resolving individual chemical species. However, osmotic adjustment could involve selective accumulation of particular solutes. Carbohydrates have been shown to make a major contribution to osmotic adjustment in growing regions of leaves (1, 15, 16), stems (12), and roots (18). In contrast, although potassium ions constitute a major fraction of the solute pool in growing regions at high  $\psi_w$  (including the maize primary root [22]), increases in potassium concentration in osmotically adjusted tissues are usually



**Figure 3.** Spatial distribution of water content in the apical 10 mm of roots growing at the various vermiculite relative water contents. Data are means  $\pm$  1 SD from six experiments.



**Figure 4.** Spatial distribution of (A) concentration and (B) content of (top) hexose, (middle) sucrose, and (bottom) potassium in the apical 10 mm of roots growing at the various vermiculite relative water contents. Data are means  $\pm$  1 SD from four experiments. Concentrations were calculated on the basis of total tissue water content. Elongation rates of the roots were ( $\pm$ 1 SD): 100% treatment,  $3.11 \pm 0.12$  mm h<sup>-1</sup>; 16% treatment,  $2.26 \pm 0.13$  mm h<sup>-1</sup>; 4% treatment,  $1.44 \pm 0.13$  mm h<sup>-1</sup>; 2% treatment,  $1.01 \pm 0.13$  mm h<sup>-1</sup>.

small (1, 5, 18). Here, we investigate the effects of low  $\psi_w$  on the spatial distributions of sugars and potassium in the root growing zone.

In all treatments, the concentration of hexose increased with distance from the apex (Fig. 4A, top). Less than 50 mmolal hexose was present in the 1st mm, while at the 6-mm location hexose concentration was 150 mmolal at high  $\psi_w$  and 400 mmolal in the 2% treatment. In the middle and basal regions, the hexose concentration increased progressively as the  $\psi_w$  decreased. When hexose content is expressed per millimeter of root length, a tendency to accumulate at low  $\psi_w$  is evident (Fig. 4B, top). Approximately 200 nmol of hexose were assayed in the 9th mm in the 2% treatment, while 150 nmol were found in the same location at high  $\psi_w$ .

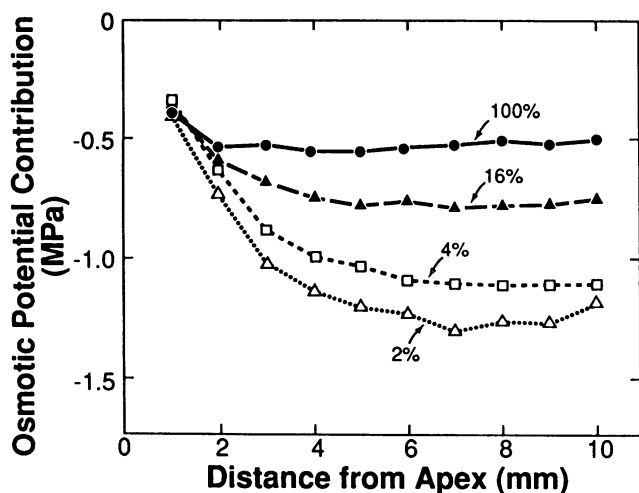
Sucrose concentrations and contents were much lower than those of hexose or potassium in all treatments (Fig. 4, A and B, middle). Unlike hexose, sucrose concentration was greatest at the 2-mm location.

The concentration of potassium was greatest at the 2- to 3-

mm location in all treatments (Fig. 4A, bottom). Potassium tended to be more concentrated at low  $\psi_w$ . Potassium accumulation, however, did not occur at any location in the low  $\psi_w$  treatments. Potassium content was reduced from a maximum of 90 nmol mm<sup>-1</sup> at high  $\psi_w$  to 50 nmol mm<sup>-1</sup> in the 2% treatment (Fig. 4B, bottom).

The contrast between concentration (Fig. 4A) and content (Fig. 4B) of the different solute species resulted from the differences in water content per unit length among treatments (Fig. 3).

To assess the importance of sugars and potassium to the osmotic adjustment of the root growing zone, we summed the solute concentrations and, using Equation 1, expressed the results as 'osmotic potential contribution' (Fig. 5). The solutes accounted for 60 to 70% of the osmotic adjustment that occurred at low  $\psi_w$  in the middle and basal regions, but for little in the apical few mm. In the basal 7 mm, the summed solute concentrations paralleled the overall  $\psi_s$  profiles for the different  $\psi_w$  treatments.



**Figure 5.** Spatial distribution of the summed osmotic potential contributions of hexose, sucrose, and potassium in the apical 10 mm of roots growing at the various vermiculite relative water contents.

### Deposition Rates

The profiles shown in Figures 2 through 5 were the result of transport and metabolism which occurred at the same time as tissue expansion and cell displacement away from the root apex. The uniformity of  $\psi_s$  along the length of the growing zone at high  $\psi_w$  indicates that osmoticum deposition rates were locally synchronized with growth, as shown earlier (22). Thus, osmoticum dilution by water uptake during tissue volume expansion was fully compensated in well-watered roots. In the low  $\psi_w$  treatments, the lower values and the spatial gradient in  $\psi_s$  imply that water stress caused the ratio of osmoticum to water deposition to increase, particularly at basal locations. This could have resulted from increased rates of total osmoticum deposition and/or from decreased water deposition due to inhibition of tissue volume expansion. To evaluate the behavior in quantitative terms, the information in Figures 2 through 4 was combined in Equation 2 with the previously published profiles of root growth velocity (20) to compute the spatial distribution of deposition rates of solutes and water.

The analysis shows that osmoticum deposition rates per unit length were not increased at any location in any of the low  $\psi_w$  treatments (Fig. 6A). Instead, the rate of osmoticum deposition per millimeter was independent of  $\psi_w$  in the apical 2 mm and, relative to the high  $\psi_w$  treatment, decreased progressively with distance from the apex as the  $\psi_w$  decreased. Osmoticum deposition was substantial for more than 9 mm at high  $\psi_w$ , but in the 2% treatment fell to low values beyond 6 mm, where elongation ceased (20). Thus, the total rate of osmoticum deposition in the apical 9 mm, calculated by integrating the rates over distance, was greatly decreased at low  $\psi_w$  (Fig. 6A, inset). In the 2% treatment, only one-third as much osmoticum per hour was deposited in the growing zone relative to the well-watered roots.

In contrast, when the osmoticum deposition rates are calculated per unit water volume, it is clear that low  $\psi_w$  caused a substantial increase in deposition rate in the apical few

millimeters (Fig. 6B). Even on the volumetric basis, however, low  $\psi_w$  caused a pronounced decrease in osmoticum deposition rate in basal regions.

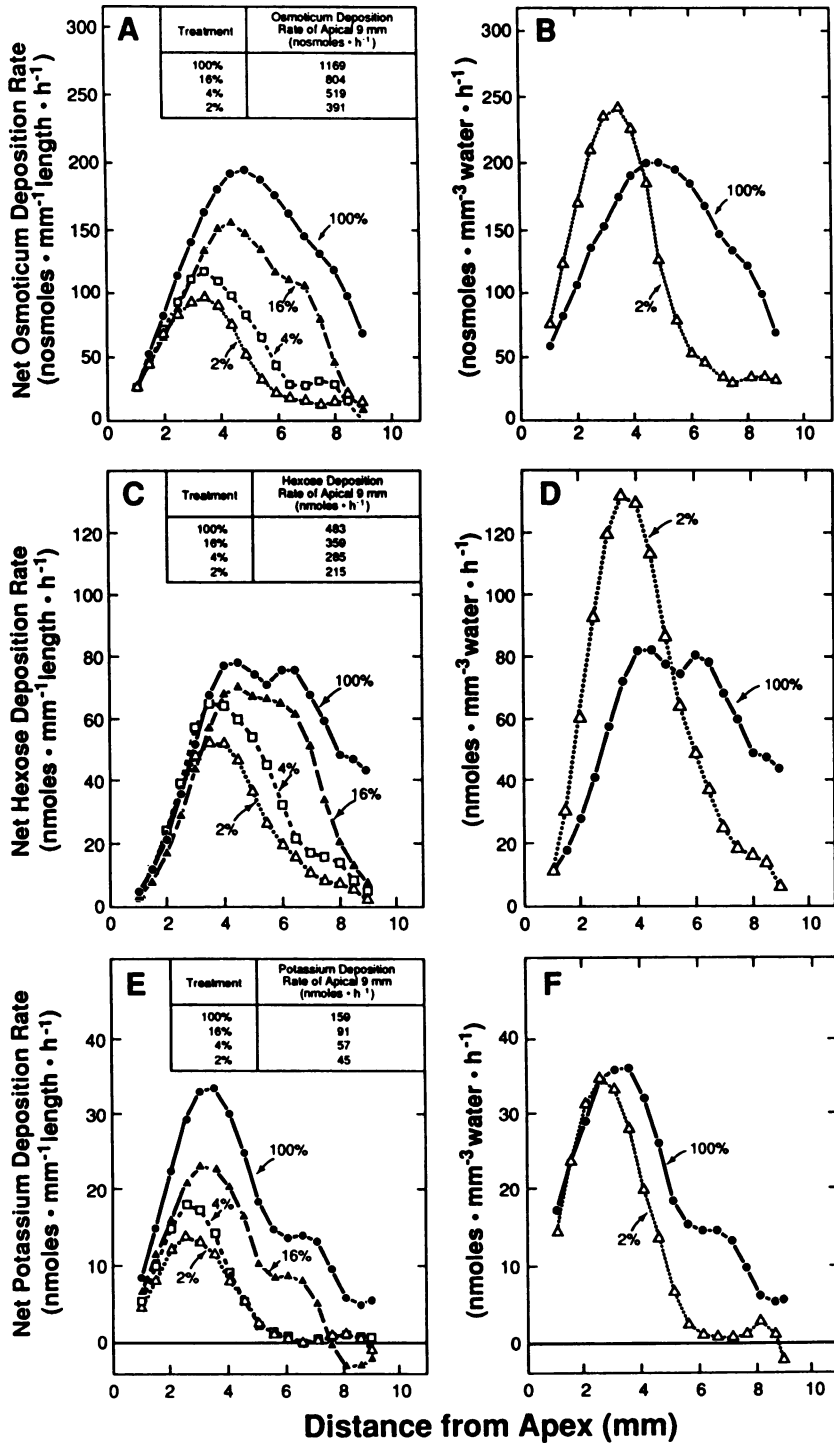
Deposition rate profiles for hexose were rather similar to those for total osmoticum (Fig. 6, C and D). Per millimeter of root length, there was no detectable effect of low  $\psi_w$  on the rate of hexose deposition in the apical 3 mm (Fig. 6C). Again, low  $\psi_w$  caused a shortening of the length of tissue for which deposition rate was high. Numerical integration revealed that water stress caused more than a twofold decrease in the rate of hexose deposition in the apical 9 mm (Fig. 6C, inset). Per unit of water volume, however, the hexose deposition rate of the roots in the 2% treatment exceeded that of the well-watered roots in the apical 5 mm (Fig. 6D).

Per unit length, low  $\psi_w$  caused a decrease in potassium deposition rate at all locations (Fig. 6E). Integrated over the apical 9 mm, the potassium deposition rate declined almost fourfold in the 2% treatment. Comparison between Figure 6 C and E demonstrates that low  $\psi_w$  caused a greater diminution in the deposition of potassium than of hexose. Per unit water volume, potassium deposition rates in all treatments were similar in the apical 3 mm (Fig. 6F), in contrast to the increased volumetric rates of total osmoticum and hexose deposition that occurred in this region at low  $\psi_w$  (Fig. 6, B and D).

The differences between the left and right sets of curves in Figure 6 were due to the inhibition of root radial growth at low  $\psi_w$ , again indicating the importance of this response to osmotic adjustment in the growing zone. This can also be seen by comparing the profiles of osmoticum deposition (Fig. 6A) with profiles of water deposition (Fig. 7) on the same basis of per unit root length. In the low  $\psi_w$  treatments the rate of water deposition decreased more than osmoticum deposition throughout the growing region, such that in the 2% treatment the total rate of water deposition in the apical 9 mm was reduced to one sixth of the rate at high  $\psi_w$ . As expected, the profiles of water deposition resembled more closely the previously published profiles of rate of root volume increase than those of longitudinal growth (20). The role of reduced radial growth was particularly clear in the apical 3 mm, where the rate of water deposition decreased progressively with decreases in  $\psi_w$  even though longitudinal expansion in this region was not affected (20).

The profiles of the ratio of deposition rates for solutes and for water differed among the solute species. For hexose:water, the ratio increased slightly from 1 to 4 mm and remained constant from 5 to 10 mm from the apex of roots growing at high  $\psi_w$  (Fig. 8A). In the 2% treatment, the ratio increased greatly with distance from the apex, reaching a maximum at the 6-mm location that was sixfold higher than the corresponding value at high  $\psi_w$ . In more basal regions the ratio fell steeply at low  $\psi_w$ , indicating dilution of the hexose pool. However, the hexose concentration was very high (Fig. 4B) and the water deposition rate was very low (Fig. 7) in this region. Therefore, the absolute amount of hexose dilution was small (Fig. 4B).

The pattern for the ratio of potassium to water deposition was very different (Fig. 8B). The ratio was highest in the apical region and fell quite steeply with increasing distance from the

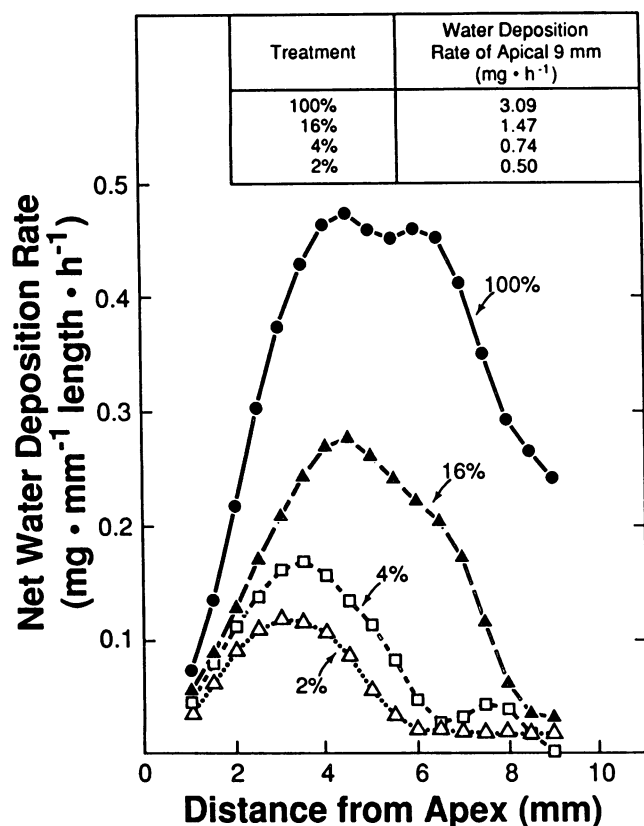


**Figure 6.** Spatial distribution of net deposition rates of osmoticum (A, B), hexose (C, D), and potassium (E, F) in the apical 10 mm of roots growing at the various vermiculite relative water contents. Deposition rates per unit root length (A, C, E) were calculated from information in Figures 2B and 4B, and growth velocity distributions from Sharp *et al.* (20) using Equation 2. Division by water volume per unit length gave deposition rates per unit volume of tissue water (B, D, F). The insets show deposition rates of the apical 9 mm obtained by integrating rates per mm length over distance.

apex, and was influenced only minimally by low  $\psi_w$ . We emphasize that the deposition rates for hexose and potassium integrated over the apical 9 mm were both diminished at low  $\psi_w$ , but the respective local rates were changed in different ways to give a varied, species-dependent effect on the osmotic pool size.

The deposition rate profiles shown in Figures 6 and 7 were calculated assuming that the water and solutes associated with

a spatial location were constant, *i.e.* local rates of change (the first component of Eq. 2) were negligible. The assumption of steady solute concentration is supported by comparison of  $\psi_s$  profiles for roots 41 and 50 h after transplanting to the 2% treatment (Fig. 9). However, we observed that in all treatments the roots grew slowly thinner as they grew longer (data not shown). Thus, the water and solute deposition rates were slightly overestimated because local rates of change were, in



**Figure 7.** Spatial distribution of net water deposition rate per unit root length in the apical 10 mm of roots growing at the various vermiculite relative water contents. Data were calculated from mean root water contents at each location (Fig. 3) and growth velocity distributions from Sharp *et al.* (20) using Equation 2. The inset shows water deposition rates of the apical 9 mm obtained by integrating over distance.

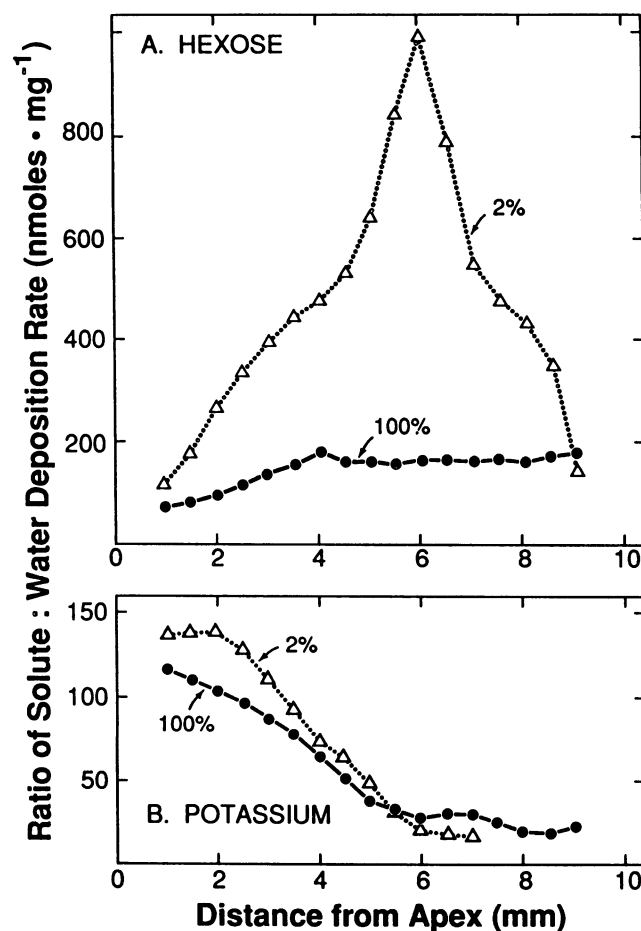
reality, slightly negative. Estimates based on the rate of change in root diameter suggest that these errors were small (less than  $8 \text{ nosmol mm}^{-1} \text{ h}^{-1}$  at all locations in all treatments in the case of osmoticum deposition), and would not have altered the interpretation of the data.

### DISCUSSION

The results presented in this and the preceding paper of this series (20) show that growth responses of the maize primary root to low  $\psi_w$  involve a complex pattern of morphogenic and metabolic events. Close to the apex, longitudinal expansion rate of roots growing at low  $\psi_w$  equals that of well-watered roots. However, the rate of radial growth is inhibited in this region and, consequently, rates of water deposition are decreased. Deposition rate of total osmotica per unit length is unaffected in the young tissue element, so that  $\psi_s$  is lower than at the same location of roots growing at high  $\psi_w$ . Later in development, when the stressed tissue is located 3 to 10 mm from the apex, rates of osmoticum deposition decrease relative to well-watered roots. The rate of water deposition decreases even more, however, due both to the smaller root diameter and to a stress-induced shortening of the growth

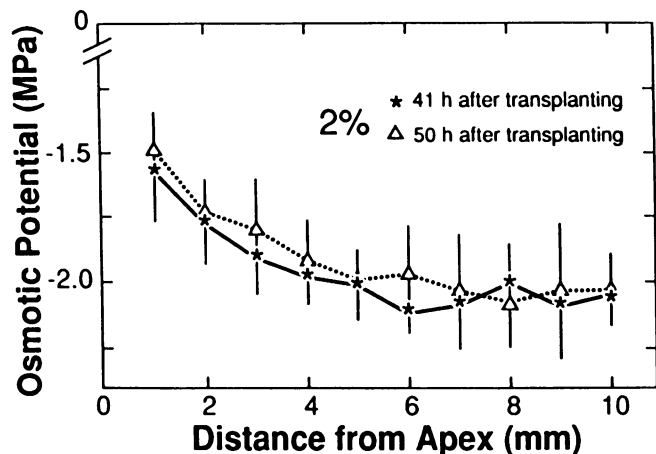
zone. Thus, osmoticum dilution is prevented and  $\psi_s$  declines further as the tissue element is displaced toward the base of the growth zone.

Osmotic adjustment occurs with substantial decreases in net solute deposition rates. Other workers have suggested that it is contradictory to consider osmotic adjustment as a beneficial process for maintaining growth at low  $\psi_w$  if the contributing solutes increase in concentration only because growth, and hence the rates of solute utilization and osmoticum dilution, decrease (14, 26, 30). However, spatial and dimensional aspects of growth responses to low  $\psi_w$  have not been considered previously. Our results (20) show that close to the root apex, longitudinal expansion is insensitive to  $\psi_w$  as low as  $-1.6 \text{ MPa}$ , and this must be attributed to the osmotic adjustment that occurred in this region. Certainly, positive turgor, and therefore cell elongation, would not have been maintained in this range of  $\psi_w$  had osmotic adjustment not occurred. Thus, our results demonstrate that osmotic adjustment serves an essential role in the maintenance of maize primary root elongation at low  $\psi_w$ , even though the increase



**Figure 8.** Spatial distribution of the ratio of net deposition rates of (A) hexose to water, and (B) potassium to water, in the apical 10 mm of roots growing at high (100% treatment) or low (2% treatment) vermiculite relative water contents. Data were calculated by dividing solute deposition rates per unit length (Fig. 6, C, E) by water deposition rate per unit length (Fig. 7).





**Figure 9.** Spatial distribution of osmotic potential in the apical 10 mm of roots growing at low (2% treatment) vermiculite relative water content, 41 h and 50 h after transplanting. Data are means  $\pm$  1 SD from three to four experiments.

in total osmoticum concentration occurs because the rate of root volume expansion decreases.

The conclusion that osmotic adjustment in the root growing zone occurs despite substantial decreases in the rate of osmoticum deposition is similar to those drawn from results with soybean hypocotyls (12) and maize leaves (27). In those studies, the osmotic adjustment was associated with considerable solute accumulation per unit length, as has generally been observed at low  $\psi_w$  in shoot growing regions (1, 15). It is worth emphasizing that because the maize roots growing at low  $\psi_w$  were thinner, substantial decrease in  $\psi_s$  was possible without osmoticum accumulation. We believe there is little question that the roots growing at low  $\psi_w$  in this study should be considered to have adjusted osmotically. Therefore, in growing regions the definition of osmotic adjustment should not be restricted to situations where solute accumulation occurs. Accordingly, caution should be applied when assessing osmotic adjustment from measurements of solute contents per growing region.

There seems to be considerable adaptive value to the mechanism for osmotic adjustment observed in our experiments. Nutritional supplies are conserved, utilization of resources is concentrated in a small but essential region of tissue, and the root retains the ability to explore new soil volume for water. Whether the mechanism is of general physiological importance remains to be seen. As discussed in a preliminary report of these results (23), there is evidence that modulation of development is important in regulation of long-term osmotic adjustment in natural environments. Whether roots grow thinner at low  $\psi_w$ , however, depends partly on soil properties. The vermiculite used in our system did not impose much resistance to root penetration. In contrast, many soils increase greatly in strength as their water content decreases, and a common response to mechanical impedance is root thickening.

Osmotic adjustment, though pronounced, was apparently not the only physiological change contributing to root growth at low  $\psi_w$ . Although we have emphasized the role that osmotic

adjustment must have played in the insensitivity of local elongation rate to low  $\psi_w$  close to the root apex, osmotic adjustment in this region was less than in more basal locations (Fig. 2A). The  $\psi_s$  at the 2-mm location declined from  $-0.9$  MPa at high  $\psi_w$  to  $-1.8$  MPa in the roots growing at a  $\psi_w$  of  $-1.6$  MPa (2% treatment). Thus, the change in  $\psi_s$  (0.9 MPa) did not fully compensate for the decrease in  $\psi_w$  (1.57 MPa). Turgor pressure of the segment, therefore, was probably much lower in the 2% treatment than at high  $\psi_w$ . To attain similar longitudinal growth rates at the reduced turgor, the cell walls must have become much more longitudinally extensible at low  $\psi_w$ . A study using a pressure microprobe to measure cell turgor (9) also concluded that the cell walls of growing maize primary roots became more extensible under osmotic stress.

It is of interest to explore the relationship between osmotic adjustment and the inhibition of growth at low  $\psi_w$  in more detail. If increases in osmoticum concentration were merely an inevitable result of the inhibition of tissue volume expansion when  $\psi_w$  (and hence turgor) decrease, then it might be expected that similar osmotic adjustment will occur when growth is inhibited under conditions other than decreased water availability (26). There is evidence that this does not occur. Meyer and Boyer (11) observed substantial osmotic adjustment in the growing region of soybean hypocotyls when growth was inhibited at low vermiculite  $\psi_w$ . Osmotic adjustment did not occur, however, when the hypocotyls were exposed to increased pressure, although the sensitivity of growth to low tissue  $\psi_w$  increased. It was concluded, logically, that the slow growth that continued at low vermiculite  $\psi_w$  was dependent on the osmotic adjustment that occurred (11, 12). A related example is the comparison by Greacen and Oh (7) of osmotic adjustment in the growing zone of pea roots in response to soil  $\psi_w$  or mechanical impedance. Mechanical impedance caused a greater inhibition of growth but less osmotic adjustment. These results suggest that osmotic adjustment in growing regions is not an inevitable result of growth inhibition, but depends on the uncoupling of volumetric growth from net solute deposition as the growth rate slows down. Our data showing the different effects of low  $\psi_w$  on the ratios of hexose:water and potassium:water deposition in the root growth zone support this suggestion (Fig. 8). At low  $\psi_w$ , potassium deposition decreased in close parallel with water deposition at all locations and, therefore, potassium contributed little to the osmotic adjustment. In contrast, the ratio of hexose:water deposition increased greatly at low  $\psi_w$ . Taken together, these results indicate that either the rate of hexose utilization decreased more than water deposition as growth slowed, or the ratio of hexose to potassium import increased. We conclude that osmotic adjustment at low  $\psi_w$  in the maize primary root is likely to constitute a highly regulated process, involving the selective increase in concentration of particular solutes as well as modulation of the pattern of expansive growth.

In the basal 7 mm of the root growing zone, the summed concentrations of soluble carbohydrates and potassium accounted for much of the osmotic adjustment at low  $\psi_w$  (Fig. 5). However, these solutes accounted for little of the change in  $\psi_s$  in the apical 2 to 3 mm, indicating that other solute species were preferentially deposited in this region. Recent investigations have revealed that proline accounts for as much

as 50% of the osmotic adjustment in the apical region (28). Characterization of the pattern of proline deposition will be the subject of the next paper in this series.

#### ACKNOWLEDGMENTS

We wish to thank Gary Voetberg for the soluble carbohydrate and relative water content analyses, and Dr. Imad Saab and Dr. William Spollen for critical reading of the manuscript.

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