Transpiration- and Growth-Induced Water Potentials in Maize[1]

MARK E. WESTGATE[*2] AND JOHN S. BOYER
Departments of Agronomy (M. E. W., J. S. B.) and Plant Biology (J. S. B.), University of Illinois; and
United States Department of Agriculture/Agricultural Research Service (J. S. B.), Urbana, Illinois 61801

ABSTRACT

Recent evidence from leaves and stems indicates that gradients in water potential (ψ_w) necessary for water movement through growing tissues are larger than previously assumed. Because growth is sensitive to tissue ψ_w and the behavior of these gradients has not been investigated in transpiring plants, we examined the water status of all the growing and mature vegetative tissues of maize (*Zea mays* L.) during high and low rates of transpiration. The ψ_w measured in the mature regions of the plant responded primarily to transpiration, while the ψ_w in the growing regions was affected both by transpiration and growth. The transpiration-induced potentials of the mature tissue formed a gradient of decreasing ψ_w along the transpiration stream while the growth-induced potentials formed a gradient of decreasing ψ_w from the transpiration stream to the expanding cells in the growing tissue. The growth-induced gradient in ψ_w within the leaf remained fairly constant as the xylem ψ_w decreased during the day and was associated with a decreased osmotic potential (ψ_s) of the growing region (osmotic adjustment). The growth-induced gradient in ψ_w was not caused by excision of the tissue because intact maize stems exhibited a similar ψ_w. These observations support the concept that large gradients in ψ_w are required to maintain water flow to expanding cells within all the vegetative tissues and suggest that the maintenance of a favorable gradient in ψ_w for cell enlargement may be an important role for osmotic adjustment.

Water entering the plant from the soil moves along gradients in ψ_w[3] or its components and, in general, realizes one of two fates. The bulk of the water moves through the xylem to the evaporating surfaces of the leaves where it eventually is transpired. A small fraction, however, moves to the growing regions where it causes expansion growth. These two types of water movement (*i.e.* for transpiration and growth) are so fundamentally different that often they are separated in time and space within the plant. As a result, the gradients in ψ_w associated with these processes are likely to differ in time and space as well. Yet these gradients may be interactive because conditions known to change transpiration can also change rates of growth (2, 7–9).

For both kinds of water movement, the relationship between water flux and driving force in the steady state can be approximated by the flow equation: $J_w = L_p (\Delta\psi_w)$, where J_w is the flux of water (cm^3 cm^{-2} s^{-1}), L_p is the hydraulic conductivity of the path (cm^3 cm^{-2} s^{-1} MPa^{-1}), and $\Delta\psi_w$ is the difference in water

potential at opposite ends of the path (MPa). This relationship assumes that water moves across membranes situated along the flow path and that the reflection coefficient (σ) for the solutes in the plant approaches 1.0. For those metabolites where measurements of the reflection coefficient are available for higher plants (33), this approximation holds. The flow equation has been used by several investigators to describe transpiration-induced water potentials in mature leaves (9, 16, 26). In each case, transpirational water movement through the plant was linearly related to the difference in ψ_w between the leaves and the water source in the soil. In growing leaves, however, a different relationship occurred when water movement for growth was considered with transpiration (8, 9, 19) because larger differences in ψ_w were required to move a unit of water for growth than for transpiration due to the lower hydraulic conductivity of the growth path than the transpiration path (8, 9).

Ray and Ruesink (28) were the first to suggest that large differences in ψ_w might be required to move water through growing tissues, and using an evaporation-immersion technique, calculated differences as large as 0.25 MPa in rapidly elongating oat coleoptiles. Subsequently, significant and persistent differences in ψ_w between growing tissue and the water supply were measured in stems (11, 12, 22), shoot apices (25), expanding leaves (7, 23), root apices (30), and developing grain (3).

These differences in ψ_w may be induced by the growth process itself if continued cell wall relaxation and irreversible wall extension prevent turgor (ψ_p) from increasing to its maximum despite water influx (7, 29). Since the ψ_w can be considered to consist primarily of the sum of ψ_s and ψ_p in the enlarging cells, ψ_w is kept low by the inability of ψ_p to rise. Consequently, ψ_w of the cells is maintained below that of the vascular supply and continued water movement into the expanding cells is favored. As the cells mature and wall extensibility decreases, continued influx of water would result in an increase in ψ_p until ψ_w of the cells equals the ψ_w of the vascular supply. At this point, the gradient in ψ_w should disappear.

Molz and Boyer (24) presented the physical basis for these growth-induced water potentials in elongating hypocotyl tissue of soybean seedlings. Their analysis indicated that significant growth-induced water potentials can arise in plant tissues even though individual cells are in near equilibrium with their local osmotic environment. The growth-induced potentials arise because those cells closest to the vascular supply transmit much larger fluxes than are required for their own enlargement and significant gradients in ψ_w result.

Since water movement through the plant will occur along the paths providing the greatest driving force and the highest conductivity, the rate at which water is extracted from the transpiration stream to support expansive growth should be a dynamic process affected by the transpiration-induced water potentials of the xylem, the growth-induced water potentials of the expanding cells, and the conductivity of the two paths. However, the interaction between the transpiration-induced and growth-induced gradients in ψ_w has not been examined in growing tissues. In this

[2] Current address: Arbeitsgruppe Membranforschung am Institut für Medizin, Kernforschungsanlage Jülich GmbH, Gebäude ICH/3, Postfach 1913, D-5170 Jülich, West Germany.

[3] Abbreviation: ψ_w, water potential.

study, we investigated the water status of all the growing and mature vegetative tissues of the maize plant to determine whether growth-induced water potentials are characteristic of all growing tissues and to what extent they interact with the transpiration-induced water potentials of the xylem.

MATERIALS AND METHODS

Culture Conditions. Maize plants (*Zea mays* L., cv B73 × Mo17; Illinois Foundation Seeds, Inc.) were grown from seed in soil in controlled environment chambers (day/night temperature: 30°/20° ± 1°C; day/night RH: 50/95 ± 5% RH; photoperiod: 14 h). Fluorescent cool white lamps supplied 800 ± 50 μmol photons $m^{-2} s^{-1}$ (PAR) at the level of the upper leaves.

For most leaf and root data, plants were grown for 2 to 3 weeks in plastic pots having a 19.5-cm top diameter and containing 1.8 kg of soil (a 2:1:1 mix of soil:peat:perlite). One week prior to sampling, the entire pot was enclosed in two opaque plastic bags to maintain a uniform soil ψ_w. Previous work showed that soil ψ_w was within ±0.04 MPa using this technique (5). The plastic bags were sealed tightly around the stem and a tube (1 cm i.d.) was inserted along the stem to facilitate gas exchange. For stem data, plants were grown for 6 weeks in larger plastic pails containing 12.0 kg of the same mix of soil. In some instances, leaf, stem, and root samples were obtained simultaneously from these large plants. In all cases, seeds were sown in soil saturated with Hoagland solution 1 (20) without micronutrients. Thereafter, this nutrient solution was applied twice weekly beginning at 7 d after planting. After 3 weeks of growth, the nitrate concentration was doubled to meet the increased nutrient demand of the plants during internode elongation. Between nutrient additions, water was added to the soil if the soil surface appeared slightly dry. Sufficient water was added to flush excess nutrients from the soil.

Growth Measurements. *Leaves.* The zone of elongation of the leaves was delimited by placing pinholes at 1-cm intervals along the length of the leaf. After 12 h of growth, the plant was dissected and the position of the holes was determined. The elongation of a particular region of the leaf was measured as the increase in distance between consecutive holes during the growth period.

Stem Internodes. The zone of elongation of the stem internodes was delimited similarly except a longer growth period (16 h) was required due to the relatively slow rate of stem elongation. Elongation of the entire internode over a 16-h period was measured as the increase in distance between two needle marks placed at the apical and basal ends of the internode.

Nodal Roots. The zone of elongation of the nodal roots was measured on individual root axes which had reached the interface between the soil and the plastic pot. Roots were exposed by inverting the plant and gently removing the pot. The apical 30 mm of several roots were marked at 5-mm intervals with an oil base ink using a fine brush. The marking procedure usually required 20 to 30 s. After marking, the pot was replaced and the plant returned to the growth chamber. After 12 h of growth, the pot was again removed and the roots inspected. Elongation was measured as the increase in distance between consecutive marks during the growth period.

Water Potential Measurements. The plant organ to be sampled was excised from the plant and rapidly transferred to a humidity box for further dissection. All subsequent tissue manipulations were performed at saturating humidity within the box to minimize water loss from the tissue after excision. Tissue samples were taken from various positions along each plant organ in order to compare the water status of growing and mature tissues. Sampling time generally required less than 3 min for each organ.

Root axes (6–8 segments 10 mm long), leaf sections (2 cm long including midveins and midrib), and stem segments (removed from the stem by making two transverse slices 1.5–2.0

cm apart and one tangential slice 3–4 mm from the surface) were placed at the bottom of a psychrometer chamber which was coated with melted and resolidified petrolatum. Additional petrolatum was used at the base of the cup for stem tissue, which was placed cut surface down, to minimize the exposed cut surface area. Measurements of ψ_w were made using the isopiestic technique (10) and were corrected for heat of respiration (4). After the ψ_w measurement, the psychrometer chambers were removed from the psychrometer system, sealed, frozen for 10 min at −70°C to rupture cell membranes, thawed, and returned to the psychrometer system for ψ_s measurement. Osmotic potentials were measured by isopiestic technique in a fashion similar to ψ_w. Turgor was calculated as $\psi_w - \psi_s$.

Water Potentials of Intact Tissues. A comparison of the ψ_w of intact and excised tissue of corn seedlings was made using a specially designed psychrometer chamber similar to the instrument described by Boyer and Wu (11). The psychrometer was designed so that only the tissue of interest was exposed to the thermocouple. The rest of the plant was isolated from the psychrometer by the barrier formed by the psychrometer cup walls and petrolatum. The entire seedling could be placed in the instrument and sealed to maintain a humid and temperature-controlled environment throughout the measurement.

The ψ_w of the intact mesocotyl tissue was measured using the isopiestic technique and corrected for heat of respiration. The tissue was then excised using a circular blade which fit closely around the psychrometer cup. After excision, a second ψ_w measurement was made using isopiestic technique which was also corrected for heat of respiration. This measurement allowed a close comparison of the ψ_w of intact and excised tissue under conditions where water loss from the seedling and tissue under observation was prevented. At the same time, the seedling could grow in the apparatus until excision occurred. Thus, the ψ_w of the growing regions could be measured in the intact seedling while growth was occurring. For these measurements, seedlings were grown for 3 to 4 d in vermiculite saturated with 0.1 mM $CaCl_2$ in the dark at 28.5° ± 0.5°C and 100% RH.

RESULTS

Figure 1 shows that during high rates of transpiration late in the day (Late Day), a gradient in ψ_w could be measured in the mature tissue progressing from −0.04 MPa in the soil to −0.58 MPa in the leaf. This gradient was transpiration-induced because it became negligible at night (Predawn) when transpiration was low (Fig. 1). Under these conditions, the mature cells rehydrated so that the ψ_w of the mature tissue throughout the plant was nearly equilibrated with that of the soil. Water potentials measured in the growing regions of these organs also decreased along the transpiration stream during the day, but were more negative than in the adjacent mature tissue in each case. Although these water potentials returned to somewhat higher values at night, large differences in ψ_w (0.31–0.35 MPa) persisted between the mature tissue and the growing regions. Thus, in contrast to the conditions in the mature tissue, the ψ_w in the growing regions of the plant did not equilibrate with that of the water supply even though adequate water was available. Therefore, several experiments were conducted to determine the nature of this disequilibrium and its relationship to the growth process.

The anatomical structure of maize generally necessitated the dissection and excision of the growing regions for measurement of ψ_w. This raised the possible complication that the ψ_w measured in the growing regions could have resulted from cell wall relaxation and turgor loss after excision. In order to test whether the ψ_w of excised tissues was similar to that of intact tissues, the ψ_w of maize mesocotyls was monitored before and after excision in the psychrometer for intact plants (11). Table I shows that the ψ_w of the mature tissue after excision in the psychrometer was
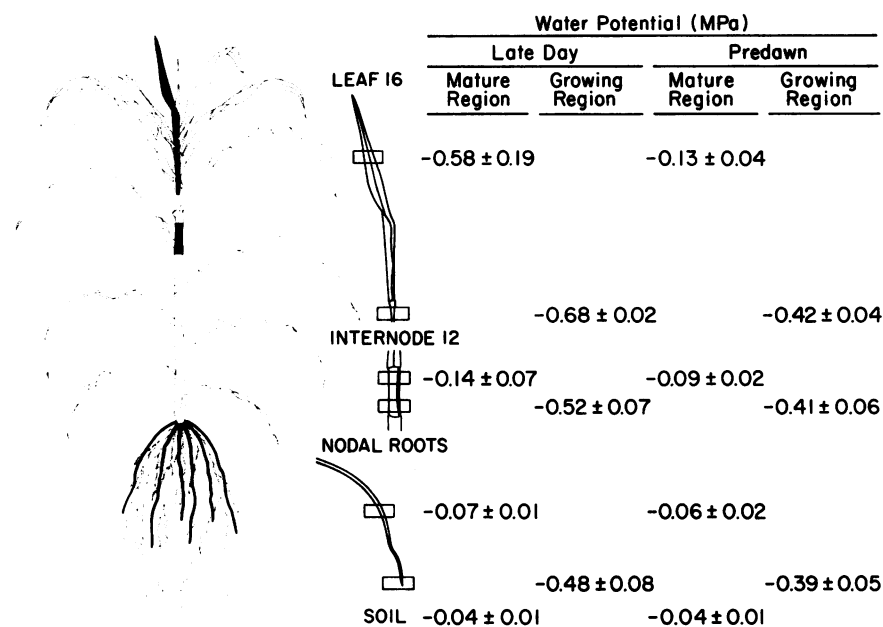
Water Potential (MPa)

	Late Day		Predawn	
LEAF 16	Mature Region	Growing Region	Mature Region	Growing Region
	-0.58 ± 0.19		-0.13 ± 0.04	
INTERNODE 12	-0.68 ± 0.02		-0.42 ± 0.04	
	-0.14 ± 0.07		-0.09 ± 0.02	
NODAL ROOTS	-0.52 ± 0.07		-0.41 ± 0.06	
	-0.07 ± 0.01		-0.06 ± 0.02	
	-0.48 ± 0.08		-0.39 ± 0.05	
SOIL	-0.04 ± 0.01		-0.04 ± 0.01	

FIG. 1. Water potentials of the mature and growing regions of the vegetative organs of a maize plant during periods of maximum (Late Day) and minimum (Predawn) transpiration 42 d after planting. The rate of transpiration was approximately 0.5 mg m^{-2} s^{-1} at night (Predawn) and 75.7 mg m^{-2} s^{-1} during the day (Late Day). Plant parts that were sampled are indicated in black. Elongating regions (basal region of leaf and internode, and apical region of nodal root axis) and mature regions of the three plant organs were sampled simultaneously and from the same plant except that the mature region of the leaf was sampled in a plant 51 d old because of the extra time necessary for full maturation of the leaf blade. The location of each sample is indicated in the expanded views to the right of the plant. The data are the mean ± 1 SD of three measurements.

Table I. *Water Potentials of Intact and Excised Mesocotyl Tissue of Maize Seedlings*

The water potential of the water supply (vermiculite) was −0.01 MPa.

	Water Potential	
	Intact	Excised
	MPa	
Elongating Zone	−0.42	−0.42
	−0.39	−0.40
	−0.44	−0.41
	−0.48	−0.48
	−0.43 ± 0.04[a]	−0.43 ± 0.04[a]
Mature Zone	−0.07	−0.10
	−0.10	−0.11
	−0.09	−0.10
	−0.07	−0.07
	−0.08 ± 0.02[a]	−0.10 ± 0.02[a]

[a] Mean ± SD.

within 0.02 MPa of that determined for the same tissue when intact. Also, these tissues were in near equilibrium with the ψ_w of the water supply (Table I). Water potentials measured in the elongating region of the mesocotyl also showed close agreement between values measured before and after excision in the same tissues. In contrast to the mature tissue, the ψ_w in the elongating region of the mesocotyl was approximately 0.43 MPa more negative than the ψ_w of the water supply. Similar results have been obtained with intact stems of soybean seedlings (11, 12) and attached sunflower leaves (7). Since the ψ_w of excised and intact tissue was similar, we made all subsequent measurements on excised tissues.

In maize, expansion growth is confined to specific regions of the roots, stems, and leaves. Thus, growth-induced water potentials should be present only in those regions. To test this, we measured the ψ_w of each organ at various positions from the growing regions to the mature tissue. To insure that transpiration was minimal and that measured potentials were associated primarily with water uptake for growth, all tissues were sampled after at least 3 h in darkness at 95% RH to allow ample time for tissue hydration (8). Under these conditions, transpiration was approximately 0.5 mg m^{-2} s^{-1}. Transpirational water loss was

inhibited further because the roots were protected in wet soil and the growing regions of the leaves and stem were enclosed by outer tissues which formed a relatively dark and humid environment. Since the ψ_w measured in the mature tissue adjacent to each growing region should approximate the ψ_w of the xylem, and the ψ_w in the growing region is the average for all the expanding cells, measurements at various positions along each organ allowed an estimate of the difference in ψ_w between the xylem and the expanding cells.

Figure 2 shows that elongation in the leaf was confined to the basal 5 cm or so, although lateral expansion extended to about 15 cm from the leaf base (data not shown). At night, when transpiration was minimal, the ψ_w of the mature, nongrowing region of the leaf blade was −0.12 MPa and in near equilibrium with the ψ_w of the soil (−0.08 MPa; Fig. 2). However, in the elongating region at the base of the leaf, the ψ_w was −0.55 MPa. The lower potential was primarily a consequence of the low turgor generated by the expanding cells, 0.39 MPa, compared to the 1.0 to 1.1 MPa of turgor in the mature zone (Fig. 2). Michelena and Boyer (23) have recently presented a similar profile of water status for the expanding maize leaf.
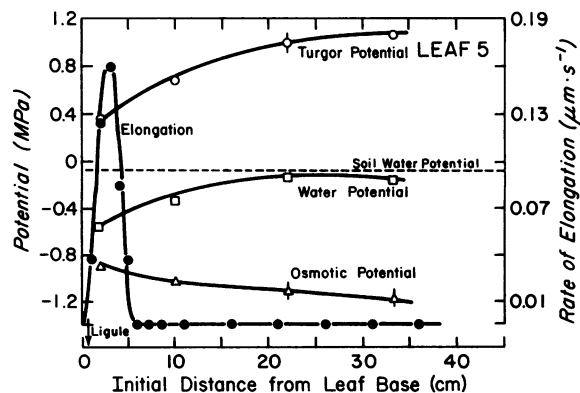
FIG. 2. Elongation and water status of a growing leaf of maize. The water potential profile was constructed by sampling 2-cm segments at several positions along leaf five during the dark period 16 d after planting. The zone of elongation (●——●) was determined on similarly treated plants using a pinning technique described in "Materials and Methods." Data are the mean ±1 SD of three samples. Soil ψ_w: −0.08 MPa.

Since the soil and mature leaf tissue had similar water potentials, the ψ_w of the xylem throughout the leaf must have been close to that of the mature blade (−0.12 MPa). Therefore, the low ψ_w measured in the zone of elongation (−0.55 MPa) must have reflected the low ψ_w of the expanding cells surrounding the xylem. Using the ψ_w of the mature tissue as an estimate of the ψ_w of the xylem, the difference in ψ_w driving water uptake from the xylem to the surrounding cells in the elongating region was approximately 0.43 MPa for the leaf.

For the roots, elongation along the main axis was confined to the apical 1 cm (Fig. 3). The ψ_w in the mature region was −0.08 MPa, which was close to the ψ_w of the soil (−0.05 MPa; Fig. 3). However, the ψ_w in the elongating region was more than 0.43 MPa below the ψ_w of the soil. As in the leaf, this low potential was confined to the zone of elongation. The difference in ψ_w between the mature and elongating regions was associated with both a lower turgor and a more negative ψ_s in the elongating regions than in the mature regions. A similar profile of the water status of the nodal roots of maize has been reported by Sharp and Davies (30). The difference in ψ_w between the xylem and the expanding cells in the root was approximately 0.40 MPa.

In the stems, the basal internodes elongate first followed by the younger, apical internodes and, in plants 42-d-old, internodes 11 through 15 were actively elongating (Fig. 4A). The zone of elongation of internode 12 was confined to the basal 3 to 4 cm of the internode (Fig. 4B). The ψ_w in the mature region was approximately −0.13 MPa, whereas the ψ_w of the expanding cells at the basal end of the internode was −0.45 MPa. This low ψ_w, confined to the expanding region, was due primarily to a lower turgor. The magnitude of the difference in ψ_w between the xylem and the expanding cells was approximately 0.32 MPa. This difference could not be observed in mature internodes having no elongating region (Fig. 4C).

The correspondence between the position of the elongating regions and the position of the disequilibrium in ψ_w in each organ supported the concept that these water potentials were growth-induced and suggested that the disequilibrium should disappear during maturation of the organ. Furthermore, the finding that the xylem equilibrated with the water supply when neither transpiration nor growth were occurring in the tissue (i.e. the mature tissue at night) suggested that the growth-induced water potentials were generated outside the xylem, as proposed by Molz and Boyer (24), and would be dependent on the ψ_w of the xylem. To investigate these possibilities, we followed the ψ_w of the mature and elongating regions of the leaf as it matured.

FIG. 4. Elongation and water status of the stem of maize. A, Profile of stem internode elongation 42 d after planting. B, Water status of rapidly elongating internode twelve from plants of similar age. C, Water status of internode nine, which was mature, on the same plant. (●), Elongation rate. Profiles of ψ_w were constructed by sampling stem segments at three positions along the internode during the dark period. Stem elongation and the zone of elongation of internode 12 were delimited by a pinning technique described in "Materials and Methods" on similarly treated plants. Data are the mean ±1 SD of three measurements.

By making the measurements diurnally, we could impose various transpiration-induced water potentials and observe the response in the growth-induced water potentials.

Figure 5 shows that the basic pattern of expansion growth for the fifth leaf of maize involved a rapid increase in blade length followed by an increase in sheath length, which was complete about 21 d after planting. Expansion growth continued as the sheath increased in thickness and width as measured near the base of the leaf. At 27 d after planting, all measurable expansion growth was complete.

The ψ_w at the tip of the leaf blade initially was about −0.50 MPa and exhibited little diurnal variation (Fig. 6A). At this point, the leaf was still enclosed within the whorl and the entire leaf was expanding. By 15 d after planting, however, the tip of the blade was beyond the zone of elongation and lateral expansion and had matured. The ψ_w at the tip then reached −0.10 MPa at night and maintained this value throughout subsequent development. Late in the day, the ψ_w was as much as 0.30 MPa lower than at night. This transpiration-induced potential was independent of leaf development beyond 15 d after planting. Osmotic potentials also were lower late in the day than at night which resulted in a higher turgor in the mature leaf blade (Fig. 6A).

In the growing region of the same leaf, the ψ_w at night initially was −0.40 to −0.55 MPa, which was 0.30 to 0.40 MPa lower than in the mature leaf tip. The ψ_w gradually increased until 27 d after planting (Fig. 6B). After this time, growth ceased and the ψ_w in the basal region of the leaf was about −0.10 MPa, which was similar to that at the mature leaf tip (Fig. 6A). The ψ_w measured late in the day was −0.55 to −0.72 MPa, which was generally 0.20 to 0.25 MPa lower than at night. Thus, while the leaf was growing, the ψ_w in the growing region underwent a diurnal change similar to that in the leaf tip but was always lower than the ψ_w in the mature tissue.

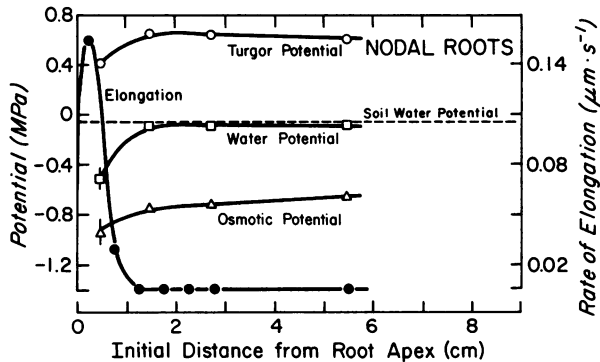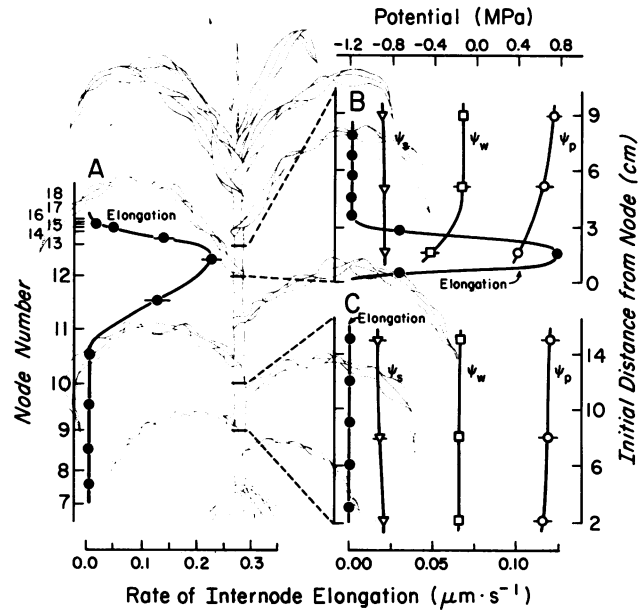This interaction between the growth-induced and transpira-

FIG. 3. Elongation and water status of a nodal root of maize. The water potential profile was constructed by sampling 1-cm segments along the length of the root beginning at the apex during the dark period 21 d after planting. The zone of elongation (●——●) was measured on roots not used for water potential measurement but on the same plant. Data are the mean ±1 SD of four plants. At least six roots were sampled from each plant. Soil ψ_w: −0.05 MPa.
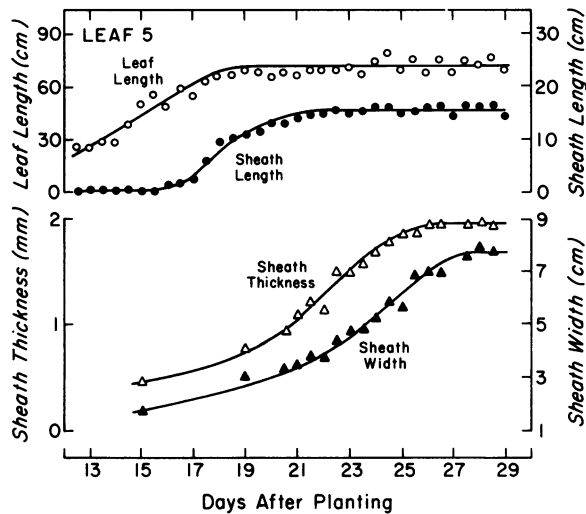
FIG. 5. Pattern of expansive growth of a leaf of maize. Total leaf length (O) was measured as the distance from the point of leaf insertion on the stem to the leaf apex. Sheath length (●) was measured as the distance from the point of leaf insertion to the ligule. Sheath thickness (△) was measured 1 cm above the point of leaf insertion in the area adjacent to the midrib using a micrometer, and sheath width (▲) was measured with a rule at the same position. Data were collected on leaf five both at the end of the dark period and at the end of the light period on consecutive days until all growth was complete. Each point is from an individual plant.

tion-induced water potentials within the developing leaf is apparent in Figure 7A which shows that, during rapid expansion growth, the ψ_w within the growing region of the leaf was consistently 0.30 to 0.40 MPa lower than the ψ_w of the xylem (mature leaf tissue) and oscillated in concert with the xylem as evaporative demand changed diurnally (Fig. 7A). These diurnal shifts in ψ_w resulted in the maintenance of a fairly constant difference in ψ_w between the xylem and the expanding cells over the course of the day until the leaf matured when they had similar ψ_w.

If transpiration effects are eliminated by comparing the ψ_w of the growing region and the mature tissue at night, the growth-induced water potentials, represented by the difference between the ψ_w of the growing tissue and the xylem (mature tissue), were present throughout expansion growth and disappeared completely about day 27 when expansion growth was complete (Fig. 7B). The disappearance of the growth-induced potentials was associated with a gradual increase in turgor in the cells at the base of the leaf to a value similar to that at the leaf tip.

DISCUSSION

In maize, as in other members of the Gramineae, expansion growth in the shoot occurs primarily in the 'intercalary meristem' at the base of each leaf and stem internode. As a consequence, water moving from one internode to another up the stem, or from the stem to the leaf blade, must pass through a region of comparatively immature and undifferentiated tissue. However, it has been shown that protoxylem elements are initiated early in the development of leaves and stem internodes and that functional protoxylem is present in these organs even before elongation begins (17). Although the protoxylem vessels are generally destroyed during growth, they differentiate in succession so that some intact and functional vessels are present throughout the period of elongation. Therefore, vascular strands pass through the growing regions of the shoot and are functional in water transport. They must serve as the supply of water for cell expansion as well as for transpiration in the exposed stem
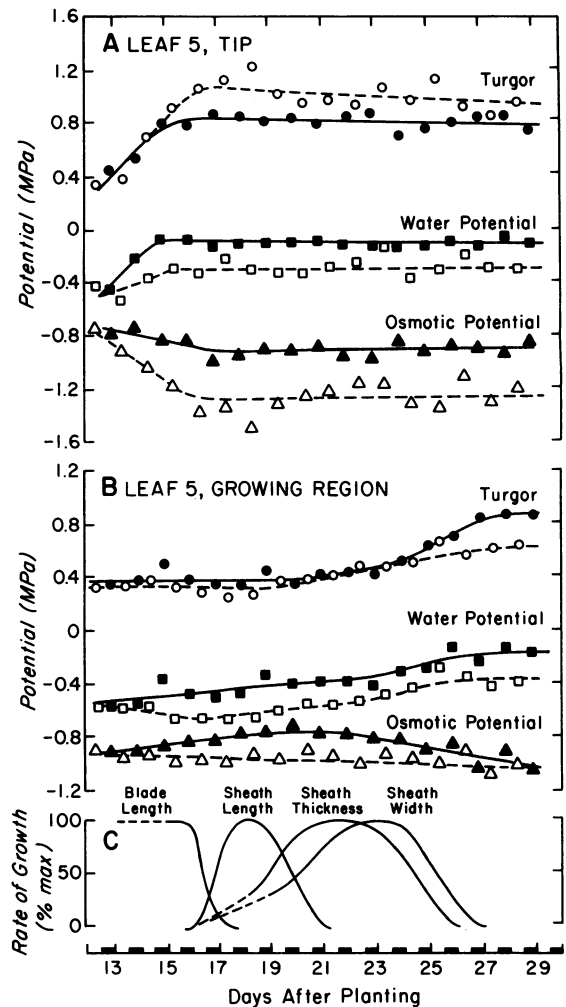

FIG. 6. Water status of the tip (A) and base (B) of the fifth leaf of maize during leaf development. The leaf tip was sampled at a point 20 cm from the leaf apex. The leaf base was sampled 2 cm above the leaf node in the zone of elongation. Samples were taken at the end of the day (O, □, △) and at the end of the night (●, ■, ▲) on consecutive days until all growth was complete. Each point is from one plant. The pattern of expansive growth is also shown (C) and is expressed as per cent of the maximum rate for each aspect of growth. Curves in C were generated from data in Figure 5.

and leaf blade. Similarly, protoxylem elements are differentiated approximately 1 mm from the root apex (18). Thus, functional xylem is also present in the elongating region of the roots.

We used the ψ_w of the mature, nongrowing tissue measured in the steady state to estimate the ψ_w of the vascular supply within the adjacent growing regions of the plant. These measurements indicated that gradients in ψ_w existed within the xylem when transpiration occurred, which is consistent with the movement of water from the soil to the evaporating sites in the leaves. The collapse of these transpiration-induced gradients at night further supports this concept, since water movement for transpiration at night was less than 1% of the transpiration rate during the day. Since the xylem supplying water for transpiration is continuous throughout the plant, the low ψ_w of the growing tissues at various points along the transpiration stream must have reflected the low ψ_w of the expanding cells outside the xylem.

This study shows that, although the ψ_w of the mature regions of each plant organ responded primarily to transpiration, the ψ_w of the growing regions responded both to growth and transpiration. A large difference in ψ_w between the vascular supply and
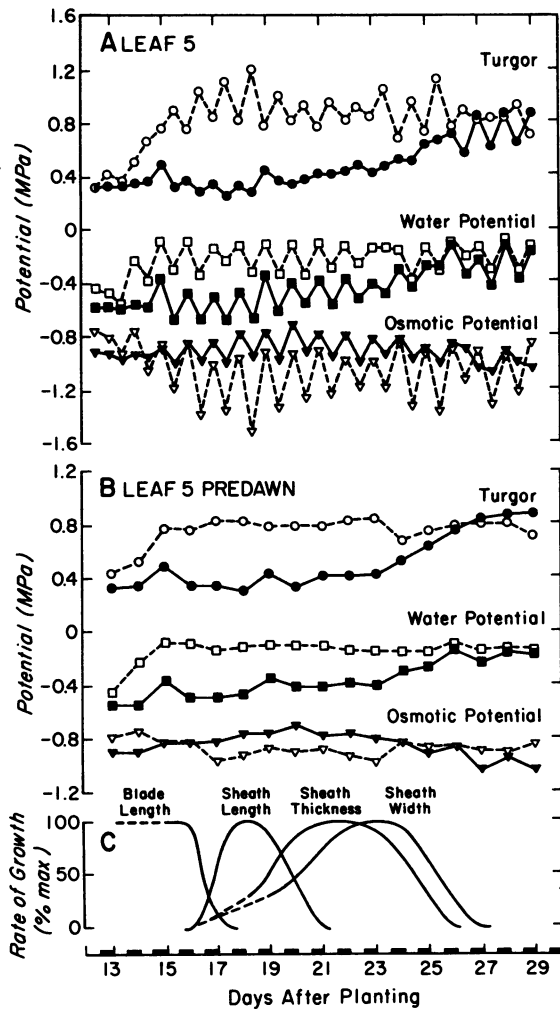
FIG. 7. Diurnal changes (A) in water status at the base ($\bullet$, $\blacksquare$, $\blacktriangledown$) and the tip (O, $\square$, $\triangledown$) of the fifth leaf of maize during leaf development, and comparative water status (B) of the base ($\bullet$, $\blacksquare$, $\blacktriangledown$) and the tip (O, $\square$, $\triangledown$) of the fifth leaf of maize measured at the end of the dark period (Predawn). The pattern of expansive growth is also shown (C) and is expressed as per cent of the maximum rate for each aspect of growth. Curves in C were generated from data in Figure 5.

the expanding cells was present in all the growing regions of the plant and was restricted to these regions. This difference in ψ_w was approximately 0.40 MPa during rapid growth and persisted until all aspects of expansive growth were complete. These observations support the concept that the ψ_w measured in growing tissues is growth-induced and that a gradient in ψ_w within growing tissues is required to maintain water flow to the expanding cells (24).

It has been proposed (13) that the low ψ_w of the expanding cells outside the xylem is controlled by a high solute content within the cell walls, and that the effective disequilibrium in ψ_w between the xylem and the expanding cells is small (about 0.05 MPa) (13). If true, the ψ_w of growing tissue measured with the pressure chamber, which does not measure free-space solutes (6), should require only small pressures (about 0.05 MPa). However, the ψ_w of growing tissue measured in the pressure chamber are similar to those measured with the thermocouple psychrometer (7, 8). Therefore, while some solutes undoubtedly are present in the cell wall, it is unlikely that the growth-induced water potentials measured in this study are caused solely by wall solutes.

The magnitude of the growth-induced ψ_w within the growing tissues was 0.32 to 0.43 MPa. These values are similar to those

reported for stems measured with the thermocouple psychrometer (11, 12, 24) but larger than those reported for growing pea epicotyls (0.02–0.10 MPa) calculated from tissue diffusivities obtained using the pressure probe technique (14). However, the calculated values were based on relatively slow growth rates (1–5% h^{-1}), only one cell type, and a hydraulic conductivity for the cortical cells which was larger, by an order of magnitude, than those reported for most higher plant cells. Assuming a growth rate of 10% h^{-1}, which is typical for the leaves, stem internodes, and roots of maize, and a hydraulic conductivity for the cells within the growing region which is typical for higher plant cells ($L_p = 1 \times 10^{-5}$ cm s^{-1} MPa^{-1}), much larger gradients in ψ_w could easily develop during rapid expansion growth in these tissues.

The ψ_w of the growing regions was associated with a lower turgor than in the adjacent mature tissue. These low turgors presumably are a consequence of cell wall relaxation during growth that prevents turgor from increasing to the maximum. If so, these water potentials are induced and maintained by the growth process. As growth ceases, cell turgor increases until the ψ_w of the cell equilibrates with that of the xylem. Then, turgor in this recently mature tissue is similar to that of the older mature tissue. The progressive nature of this process is evident in Figure 7B. It might be argued, however, that the low ψ_w measured in the growing regions are a result of continued cell wall relaxation after excision. Since water uptake is disrupted by excision, the turgor could decrease until the minimum turgor required for growth (Y) is reached, resulting in a lower ψ_w for the tissue. However, a comparison of the ψ_w of intact and excised mesocotyl tissue indicated that the effects of excision were small (Table I). Furthermore, the ψ_w of the intact tissue was lower in the growing regions than in the mature regions. Inasmuch as these potentials were measured while growth was occurring in intact tissue, they could not have been an artifact of excision. Thus, while a decrease in turgor may have occurred after excision, it was too small to affect the conclusions drawn here.

Measurements of ψ_w of excised tissue involved the prevention of water loss at the same time water uptake was disrupted by excision. Under these conditions, gradients in ψ_w within the excised tissue should have equilibrated and should have given a volume averaged ψ_w of all the cells within the tissue sample. As a result, the growth-induced water potentials measured in this study should be averages for the entire sample and do not reflect the complexity of gradients in ψ_w within a growing tissue. However, the gradients are likely to extend radially from the xylem to the bulk of the cells surrounding the vascular bundles, since water flux through the cells closest to the vascular supply must support the growth of cells distant from the xylem (24). Although the arrangement of such gradients within the expanding zone of a leaf or stem internode would necessarily be complex, the basic concept that gradients in ψ_w are generated within the tissue external to the vascular bundles and that these gradients persist throughout the expansion growth of each structure is supported by the simple analysis of mature and elongating tissues presented here.

Generally, rates of cell enlargement are considered to be determined by cell wall properties and turgor. However, in all the growing tissues examined, the calculated difference in ψ_w between the xylem and the expanding cells was large enough to influence growth rates. Following the analysis of Boyer and Wu (11), the relative size of the driving force for water movement and the turgor in the growing region can be compared according to:

$$m'/\alpha\,L_p' = (\psi_{xyl} - \psi_{ec})/(\psi_p - Y)$$

where m' is the tissue extensibility (s^{-1} MPa^{-1}), $\alpha = (1/V_{ec}^{tiss})$ $(dV_{ec}^{tiss}/dV_{ec}^w)$ is the change in tissue volume for a change in tissue water content of a unit of enlarging tissue (cm^{-3}), L_p' is the apparent hydraulic conductivity of the path for water flow within

the tissue (cm^3 s^{-1} MPa^{-1}), ψ_{xyl} is the water potential of the xylem within the growing tissue (MPa), ψ_{ec} is the average water potential of the expanding cells measured in the growing region (MPa), ψ_p is the average turgor of the expanding cells (MPa), and Y is the minimum turgor required for cell expansion (MPa). As a first approximation, we will assume that all the turgor generated by the expanding cells contributes to cell wall expansion, that is, that the minimum turgor required for cell expansion is zero. Thus, using the ψ_w of the mature tissue as an estimate of ψ_{xyl}, the values for $(\psi_{xyl} - \psi_{ec})/(\psi_p)$ for the root (Fig. 3), stem (Fig. 4B), and leaf (Fig. 2) are 1.0, 0.8, and 1.1, respectively. This analysis indicates that the difference in ψ_w required to maintain water flow to the growing regions is comparable in magnitude to the turgor and could contribute as much to cell expansion. If Y is not zero (22, 27), this conclusion is even stronger. Similar conclusions have been made for elongating soybean stems (11) and expanding sunflower leaves (27).

It is clear that water must cross at least one membrane (plasmalemma) for cell expansion to occur. However, it must be pointed out that the actual path for water flow from the xylem to the expanding cells within the growing tissues is not known. Theoretically, water could travel via a transcellular (through cells and their walls), symplastic (through the cytoplasm and plasmodesmata), and/or apoplastic (through the cell walls) pathway. Assuming a transcellular path, Molz and Boyer (24) calculated a gradient in ψ_w of approximately 0.2 MPa in growing soybean hypocotyls in close agreement with ψ_w measurements using the thermocouple psychrometer. More recently, results using the pressure probe technique, which measures directly the water transport characteristics of individual cells and whole tissues, have shown that the transcellular pathway could be dominating water transport through leaf (31), stem (14), and root (32) tissues. While such measurements have not been made in maize, these observations, in conjunction with the large gradients in ψ_w measured in this study, suggest that the transcellular pathway for water flow may also dominate in the growing regions of maize. This implies that water moves across a series of membranes as it passes from the xylem through the cortical parenchyma of each organ. If so, the relationship between the driving force ($\Delta P - \sigma\Delta\pi$) and water flow ($J_w$) will be linear only if the reflection coefficients (σ) for each membrane are uniform throughout the tissue (21). Thus, while a linear relationship between the driving force and transpirational water flow has been observed (9, 16, 26), the relationship between forces and flows in growing tissues may be more complex.

Diurnal changes in growth rate are often observed in growing plant tissues (1, 7, 15). Generally, these are attributed to changes in turgor. However, the rate of growth could vary with diurnal changes in xylem ψ_w even though little or no change in turgor occurs in the elongating cells. In such a case, a decreased turgor in the mature tissue would be transmitted via a decrease in the ψ_w of the xylem to the growing regions. Growth would then continue only if the gradient in ψ_w from the xylem to the expanding cells is maintained. Such behavior was evident in the expanding region of the maize leaf (Fig. 7A), and also has been proposed to explain the diurnal variations in the growth rate of rice leaves (15). In the maize leaf, however, the ψ_w of the growing region changed during the day and paralleled that of the xylem in the leaf. As a consequence, the gradient in ψ_w driving water to the expanding cells remained fairly constant throughout the day (Fig. 7A). This suggests that, during rapid expansion growth, the maintenance of a favorable gradient for growth occurs throughout the day in the growing regions of the plant.

Although the ψ_w measured in the growing regions responded to transpiration as the ψ_w of the local xylem shifted to lower values during the day, it is unlikely that the transpiration-induced component of the ψ_w in the growing region was due to

transpirational water loss directly from the growing tissues. The roots were protected in the wet soil and the growing regions of the stem and leaves were enclosed by outer tissues that form a relatively dark and humid environment. In addition, the lower ψ_w in the growing region of the leaf during the day was associated with a lower ψ_s (Fig. 7A). It is noteworthy that this decrease in ψ_s reflected the decrease in ψ_s in the leaf tip. Decreases in ψ_s in maize and sorghum leaves have been attributed to the accumulation of assimilates (osmotic adjustment) (1). This suggests that osmotic adjustment may be important for the maintenance of growth-induced water potentials. If so, the role of diurnal osmotic adjustment includes more than the maintenance of turgor and extends to the maintenance of the gradients in ψ_w that cause water entry for growth. Since the growing region of the leaf is comparatively nonphotosynthetic, distribution of photosynthate to the growing region may have contributed to the lower ψ_s. A decrease in assimilate supply has been shown to increase the sensitivity of growing soybean hypocotyls (22) and maize leaves (23) to low water potentials. This suggests that the control of solute partitioning to the growing regions of the plant under water limited conditions or high rates of transpiration may affect the sensitivity of these organs to growth inhibition at low water potentials.

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