11

Growth

INTRODUCTION

Growth is the most obvious feature of plant activity because it causes plants to become larger or cells to become more numerous in a population. All the work of metabolism is directed toward it, and the ability to reproduce depends on it. Water plays a central part as can be seen in a maize plant that by the time it flowers has gained 800 g of weight of which 85% is water. Only 100 g make up the organic part of the plant together with some mineral salts. By this time, the plant has grown to about 300 cm and Table 11.1 shows that the various organs of maize grow rapidly when conditions are favorable; the leaves become visibly longer almost minute-by-minute. To grow this rapidly, the plant must gain an average of 14 ml of water content every day until flowering, and the water must be incorporated into each cell according to its rate of enlargement. Similarly in a population of single-celled plants, growth of the population occurs by cell division, and the daughter cells enlarge before they can reproduce again by dividing but much of the enlargement comes from an increase in water content of the cells.

This indicates that most of what we call plant growth is actually increased cellular water content. However, as discussed in Chapter 3, cells can change in size not only by growing but also by swelling and shrinking reversibly as they hydrate and dehydrate. The reversible swelling and shrinking are caused by the elastic nature of the cell walls as they are stretched, causing the cells to become turgid when water enters but flaccid as water departs. Gains in water content for growth need to be carefully distinguished from these reversible elastic effects

Table 11.1 Growth Rates in Various Parts of a Maize Plant[a]

Part	Growth rate ($mm \cdot hr^{-1}$)
Leaf	4.0
Style (silk)	2.6
Stem	1.3
Root (nodal)	1.1

Note. The rates were measured for 24 hr in plants supplied with adequate soil water and growing in day temperatures of 30°C and night temperatures of 20°C. Root and leaf rates are from the same plants and style and stem rates are from older plants.

[a]From Westgate and Boyer (1985b).

and we will define plant growth to be an irreversible increase in size to separate it from the reversible changes. To determine whether an increased water content is growth or simply an elastic change, the tissue can be dehydrated to its original water content and the size remeasured. An increased size at the original water content indicates that enlargement was irreversible and growth occurred. Also, continuously measuring the size of plant parts allows one to distinguish between the reasonably steady long-term increases characteristic of growth and the short term but reversible changes caused by elastic enlargement. In mature tissues, elastic changes are the only ones that occur and they are easily detected.

Growth is a prerequisite of reproduction in unicellular plants because the cell usually cannot divide unless it enlarges to about the size of the cell from which it came. Similarly, in multicellular plants the dividing cells of the meristematic tissues must double in size before they can divide again. Cell division generally is restricted to specific regions in multicellular plants, but cells outside of those regions can enlarge often by much more than doubling. It is common for cells in tissues to enlarge 10- to 15-fold after division and they divide no further but instead contribute to the increased size and water content of the tissue. In the process, the cells expand in directions that develop the form of the plant part. They also differentiate to form specialized tissues. Thus, division, enlargement, and differentiation together give rise to plant organs such as leaves, roots, and flowers.

Because water plays such a large role in these processes, its availability often becomes limiting. In the ocean, marine plants are surrounded by water but high concentrations of salt create an environment that can be osmotically unfavorable for some of them. On land, the roots are in contact with soil water and supply the rest of the plant but there is often an inadequate supply. Understand-

ing how growth is limited under these conditions has many applications in agriculture and in the management of natural plant communities.

GROWTH OF SINGLE CELLS

Much of our understanding of cell enlargement comes from studies of single cells of plants such as the large-celled algae *Nitella flexilis* and *Chara corallina* (Fig. 11.1). Among the algae, these species are probably some of the closest

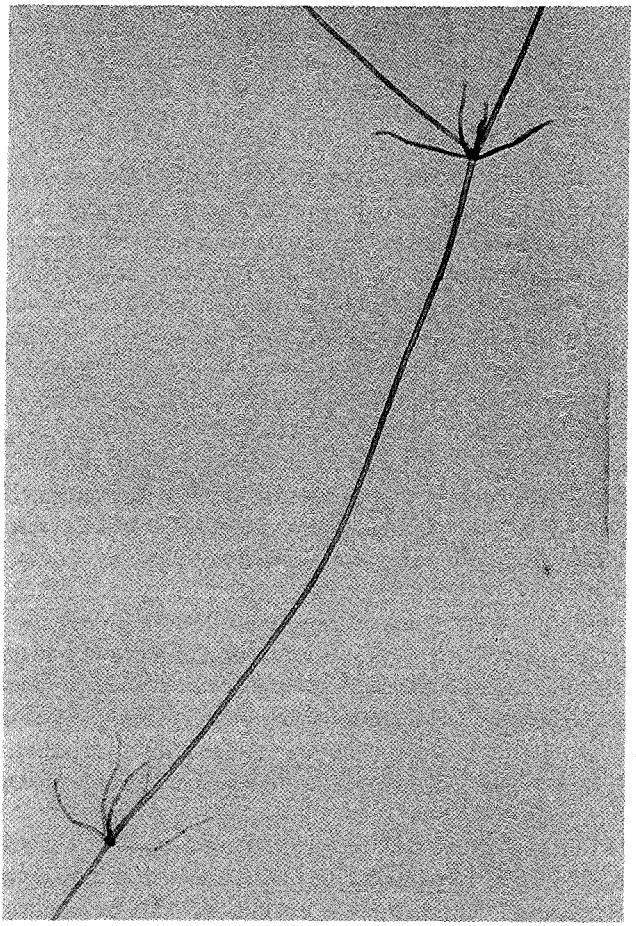

Figure 11.1 *Chara corallina* showing a giant internode cell and branches at the nodes. The internode cell is about 0.7 μm in diameter and frequently reaches a length of 10–15 cm.

relatives of land plants (Chapter 12). The internodal cells are large enough to observe easily, are in direct contact with the surrounding medium, and are photosynthetic and self-sufficient for substrate which simplifies growth experiments. Immature cells of these plants elongate steadily (Green *et al.*, 1971; Zhu and Boyer, 1992) and additionally undergo reversible changes in length as they hydrate and dehydrate (Kamiya *et al.*, 1963; Zhu and Boyer, 1992). They quickly develop turgor pressure when water enters (Green *et al.*, 1971; Zhu and Boyer, 1992), and in dilute solution the pressure rises to around 0.6 MPa where it balances the osmotic potential inside the cell (Zhu and Boyer, 1992). Bisson and Bartholomew (1984) found that there can be osmotic adjustment in some species as the external solution becomes more concentrated.

The structure which limits the expansion of the cell is the wall, which must be strong enough to prevent rupture under the force of internal pressure but also able to enlarge in a controlled pattern. Because the turgor pressure is multidirectional and would be expected to generate a spherical cell if the wall was isotropic, it follows that a cylindrical cell like an internode cell of *Nitella* or *Chara* (Fig. 11.1) must have an anisotropic wall that is reinforced to prevent lateral expansion. Probine and Preston (1962) found that isolated walls of *Nitella* stretched more longitudinally than laterally when subjected to a unidirectional pull, confirming the anisotropic nature of the wall. Cellulose microfibrils are embedded in the wall matrix and in young walls they are oriented in a transverse direction that strengthens the wall against lateral extension but allows longitudinal growth by spreading apart. The longitudinal growth is thus determined by the spreading of the matrix in which the strengthening microfibrils are embedded (Taiz *et al.*, 1981). The composition of the walls resembles that of dicotyledonous plants (Morrison *et al.*, 1993) and is about 32% cellulose, 26% hemicellulose, 34% pectin, and 5% protein with the hemicellulose/pectin/protein forming the matrix for the cellulose microfibrils (Taiz *et al.*, 1981).

As the cell grows, the wall does not get thinner but tends to thicken instead (Green, 1958; Métraux, 1982), indicating that wall polymers are continually synthesized. There are no instances of sustained wall growth without synthesis (Taiz *et al.*, 1981), which is strong evidence that synthesis is part of the growth process, but growth and synthesis often correlate only loosely. Growth often occurs only in localized bands of *Nitella* walls while deposition of new polymers occurs similarly in growing and nongrowing bands (Taiz *et al.*, 1981). Richmond *et al.* (1980) present evidence that the inner layers of the wall next to the plasmalemma control the directionality of growth. Green (1958) found that most synthesis occurred in the inner layers in *Nitella* but Ray (1967) also observed deposition throughout the wall in oat coleoptile cells. Synthesis continues after *Nitella* cells mature (Morrison *et al.*, 1993), and the new layers are richer in cellulose with a distinctly layered appearance. The new layers make up the secondary wall and it is possible that they play a part in preventing further

growth of the cells. It seems that a detailed knowledge of polymer deposition will aid in explaining the relationship between growth and wall synthesis.

Because turgor can increase cell size elastically, there has been much interest in the role of turgor in irreversible growth. Pfeffer (1900) considered turgor to be important and Probine and Preston (1962) showed that isolated walls of *Nitella* become irreversibly longer when they are stretched for long times. The lengthening occurs steadily and resembles the plastic deformation termed "creep" in polymers. Creep was faster in walls isolated from rapidly growing cells than in walls from slowly growing cells and was seen only when the force was above a threshold and in a range expected from the turgor of an intact cell. Green *et al.* (1971) measured turgor and growth simultaneously in intact *Nitella* cells and similarly observed a threshold turgor, although they thought the threshold was changeable.

Based mostly on the Probine and Preston (1962) finding that creep was faster when more force was applied above a threshold, Lockhart (1965a,b) formalized the concepts in an equation of the form

$$G = M(\Psi_p - Y), \tag{11.1}$$

where G is the relative growth rate (rate of change in volume per unit of cell volume in units of $m \cdot m^{-3} \cdot sec^{-1}$ or sec^{-1}), Ψ_p is the turgor pressure (MPa), Y is the yield threshold turgor (MPa), and M is the wall extensibility ($m \cdot m^{-3} \cdot sec^{-1} \cdot MPa^{-1}$ or $sec^{-1} \cdot MPa^{-1}$). This equation describes a straight line of slope M as shown in Fig. 11.2A. It considers the wall to act as a deformable polymer having ideal linear properties. Turgor is the driving force, and the amount above the yield threshold is the growth-active turgor ($\Psi_p - Y$) for the deformation of the wall. According to this equation, growth occurs more rapidly when Ψ_p increases.

However, when Green *et al.* (1971) decreased Ψ_p in *Nitella,* elongation ceased and then resumed at the original rate. Ortega *et al.* (1989) observed a similar behavior when Ψ_p was increased in single cells of a fungus. Although this behavior disagrees with Eq. (11.1), Green *et al.* (1971) thought that Y could change, thus maintaining growth when turgor changed. However, Green *et al.* (1971) altered the turgor with external osmotica that necessarily changed the composition of the wall solution. All elongation was considered to be irreversible growth and no elastic responses were reported, but Kamiya *et al.* (1963) observed elastic responses in mature *Nitella* cells when turgor was changed.

These differences between theory and observation caused Zhu and Boyer (1992) to do additional experiments with *Chara.* They were able to repeat the results of Green *et al.* (1971) when external osmotica were supplied but by using a microcapillary filled with cell solution, they also could inject cell solution. Thus for the first time, turgor alone could be changed without using osmotica in the external medium. Enlargement was monitored in the same cell, and the

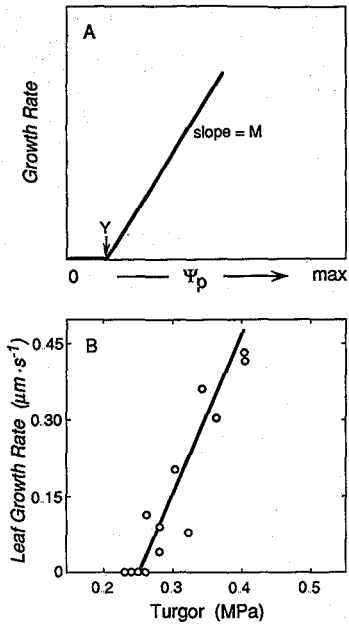

Figure 11.2 Relationship between turgor and growth. (A) Lockhart relationship showing yield threshold Y and wall extensibility M (slope of the line) obtained when the growth rate G is plotted as a function of the turgor Ψ_p. (B) Experiment showing growth of leaves at various turgor pressures in sunflower (from Matthews *et al.*, 1984). The experiment was done by withholding water from the soil and measuring growth for several days.

relationship among turgor, growth, and elastic responses could be investigated while avoiding the complications of previous experiments.

Figure 11.3 shows that when turgor was above a threshold of about 0.4 MPa, growth occurred in *Chara*. Below the threshold only elastic effects were present (Fig. 11.3, steps 1 and 2). Above the threshold, elastic changes were apparent initially but steady growth was seen after the elastic effects were completed (Fig. 11.3, steps 0, 3, and 4). Except for the threshold, turgor had no other effect on growth rate (Fig. 11.4). Since only the turgor was varied in these cells, this interpretation seems very strong. Zhu and Boyer (1992) also used inhibitors of energy metabolism and observed that growth was immediately inhibited without affecting the turgor, suggesting that growth was highly dependent on metabolism. A plastic-like wall deformation was observed only when turgor was forced to a higher level than would be possible by osmosis in the cells. In this situation, increased growth occurred for a short time but was soon irretrievably lost, indicating wounding. Therefore at normal turgors, Zhu and Boyer (1992) proposed that the growth rate may be controlled by enzymes involved in alter-

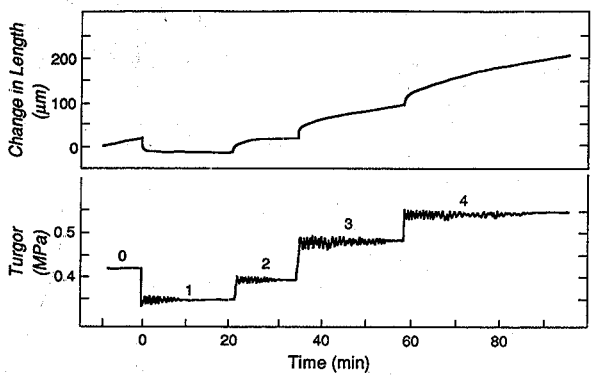

Figure 11.3 Relationship between enlargement and turgor pressure in *Chara*. Note that growth was zero at turgors 1 and 2 but growth occurred at turgors above a threshold (turgors 0, 3, 4). However, above the threshold, variations in turgor did not affect the growth rate. The initial shrinkage or swelling at each turgor step was caused by rapid elastic effects of the pressure change not related to growth. The turgor was changed by injecting the cell solution into the cell or removing the solution from the cell. After Zhu and Boyer (1992).

ing wall structure and synthesizing a new wall (Fry, 1989a,b; Hayashi, 1989) but above normal turgors, there may be a transient extensibility response like that observed by Probine and Preston (1962). The transient response would have little role to play because turgor could not reach the levels required. Why

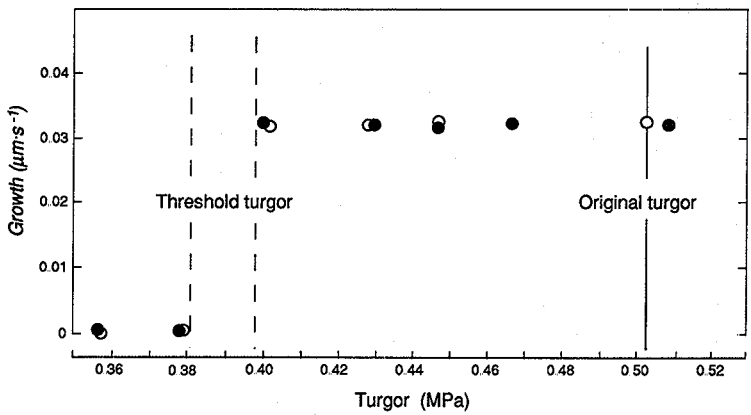

Figure 11.4 Growth at various turgor pressures in a *Chara* cell in an experiment similar to that in Fig. 11.3. The turgor pressure extends from zero to the original pressure in the cell, which was the maximum that could develop because of the cell osmotic potential. The turgor pressure was varied by injecting or withdrawing the cell solution as in Fig. 11.3. Solid points, injections; open points, withdrawals. Note that there is no growth at low pressures and that growth begins abruptly at a threshold pressure of 0.38 to 0.40 MPa. The growth rate remains constant at any pressure between this threshold and the original (maximum) pressure (adapted from Zhu and Boyer, 1992).

a threshold turgor is required in the normal turgor range is a mystery unless turgor is needed for the incorporation of intermediates into the wall as suggested by Robinson and Cummins (1976) or for straining wall polymers to a suitable conformation for enzyme action as proposed for bacterial growth (Koch, 1990).

These observations taken together indicate that the wall is complex and functions more dynamically than might at first be thought, especially when growth occurs. In order to explore the process, elastic responses and growth need to be carefully distinguished, and the role of new wall synthesis needs investigation. It seems that detailed studies of wall deposition may be helpful, and mutants of wall composition might be used such as those recently described by Kokubo *et al.* (1989, 1991). It is particularly curious that a threshold turgor is involved in growth, a fact about which all experiments are in unanimous agreement. It is likely that any theory of growth will need to include not only the role of synthesis but also how the threshold is involved. From work with these cells, it is possible to build a concept of growth from basic principles but the extent to which they apply to more complex plants needs to be explored.

GROWTH IN COMPLEX TISSUES

There is a crucial difference between single cells bathed in water and cells in a tissue competing with each other for water. In a complex tissue, the cells closest to the supply must conduct water fast enough to provide for all the other cells. This requirement adds new constraints to the growth process and is treated later.

In other respects, many of the principles observed with single cells carry over to complex plants, and Cleland (1971) and Taiz (1984) provide reviews of this area. The structure limiting the expansion of the cell is the wall, but epidermal tissues also appear to influence the rate and direction of growth (Kutschera, 1989; Kutschera and Briggs, 1988) and sometimes vascular tissue can have an effect. Cells packed inside a plant organ can create a tension in the epidermis much as pressure in a cell creates turgor in the wall. The enlargement of the epidermis then contributes to the shape and size of the organ.

As in large-celled algae, cellulose microfibrils are oriented to strengthen the cell walls in certain directions in plant organs but growth can occur in other directions by spreading the microfibrils apart, and the embedding wall matrix appears to determine growth rates (Frey-Wyssling, 1976; Preston, 1974; Taiz, 1984). When turgor is high enough to allow growth to occur (Cleland, 1959), the cell walls and cytoplasm maintain their thickness despite the 10-fold enlargement that typical cells will undergo (Bonner, 1934; Bret-Harte *et al.*, 1991) and thus a new wall and cytoplasm are synthesized at about the rate the cells enlarge (Ray, 1967). Sugars supplied to growing cells and tissues are rapidly incorporated into the walls (Gibeaut and Carpita, 1991; Kutschera and Briggs,

1987) and some wall polymers show evidence of turnover (Gibeaut and Carpita, 1991; Labavitch and Ray, 1974). If sugars are not available, the walls become thinner (Bret-Harte *et al.,* 1991) and the content of some wall polymers decreases (Loescher and Nevins, 1972, 1973). There is a rapid decrease in enlargement if inhibitors of biosynthetic metabolism are supplied to the cells (Brummell and Hall, 1985; Masuda, 1985; Robinson and Ray, 1977). If the cells are prevented from enlarging by exposure to osmotica, the walls become thicker for at least a few hours as synthesis continues in the absence of enlargement (Bret-Harte *et al.,* 1991; Loescher and Nevins, 1973).

In both large-celled algae and more complex multicellular plants, the ionic environment of the wall can influence growth rates. A low pH of the wall solution enhances rates and high Ca^{2+} diminishes them (Cleland, 1973, 1975, 1977, 1983, 1986; Cleland and Rayle, 1978; Taiz *et al.,* 1981). There is evidence that the plasmalemma secretes protons (H^+) as a result of ion accumulation by the cell and it was suggested that the wall solution could become acidified to pH's as low as 4 to 5 (Cleland, 1973, 1975, 1983). Vanderhoef *et al.* (1976, 1977b) point out that sustained growth associated with these low pH's would still require the synthesis of new wall polymers and recent studies have been conflicting, some claiming that the role for protons was an artifact of the experimental technique (Schopfer, 1989; Vanderhoef *et al.,* 1977a) and others that it was not (Lüthen *et al.,* 1990). There is a large buffering capacity of cell walls in the pH range between 5 and 6 (Vanderhoef *et al.,* 1977a) and the deposition of new wall material adds new buffering capacity. Thus, while it is likely that proton extrusion plays a role, its contribution to growth is still debated.

The role of turgor also is uncertain. Equation (11.1) has been applied extensively to plants of many kinds, and Fig. 11.2B shows a typical result with a land plant growing in gradually dehydrating soil. The growth fits the linear relationship predicted by Eq. (11.1) and exhibits a threshold turgor at about 0.25 MPa. Nevertheless, Nonami and Boyer (1989) observed decreased growth without a turgor decrease under similar conditions in soybean stems, and Shackel *et al.* (1987) found little relationship between turgor and growth in grape leaves. Soybean seedlings whose stem growth was slowed by low temperature had increased rather than decreased turgor and the ($\Psi_p - Y$) did not change (Boyer, 1993). Seedlings also showed increased turgor despite slower stem growth when deprived of auxin (Maruyama and Boyer, 1994) and little change in turgor as the tissue matured (Cavalieri and Boyer, 1982), which are counter to the theory of turgor-driven growth by plastic extension or "creep." It may be possible to reconcile these results by postulating a change in the wall extensibility M or the yield threshold Y in Eq. (11.1), as suggested by Serpe and Matthews (1992), but it is also possible that some other turgor relationship applies such as that of Zhu and Boyer (1992) observed in *Chara.* Thus far, it has not been possible to do experiments in multicellular tissues like those in *Chara* because of technical difficulties.

Table 11.2 Potentials Measured in the Cell Interior and in the Cell Exterior (Apoplast)

		Interior (MPa)		Apoplast (MPa)	
		Ψ_s	Ψ_P	Ψ_s	Ψ_P
Chara[a]	Growing	−0.60 ± 0.05	0.58 ± 0.06	−0.01 ± 0.00	0.0
	Mature	−0.62 ± 0.05	0.61 ± 0.05	−0.01 ± 0.00	0.0
Soybean[b,c]	Growing	−0.68 ± 0.05	0.42 ± 0.02	−0.04 ± 0.01	−0.24 ± 0.04
	Mature	−0.56 ± 0.02	0.50 ± 0.02	−0.04 ± 0.01	0.00 ± 0.00

Note. Measurements are in individual cells of intact plants: internode cells in *Chara* and stem cells in soybean. *Chara* cells were bathed in growth medium whereas soybean seedlings had roots in wet vermiculite and shoots in saturated air that prevented transpiration. For *Chara*, the cell apoplast was in growth medium under atmospheric pressure which is defined to have Ψ_P of zero. For soybean, apoplast Ψ_P was measured and was lower than zero (Nonami and Boyer, 1987).

[a]Zhu and Boyer (1992).

[b]Nonami and Boyer (1989).

[c]Nonami and Boyer (1987).

Growth-Induced Water Potentials

The competition for water between growing cells in a tissue can be demonstrated by raising the humidity sufficiently to prevent transpiration, thus bringing the tissue to the same condition as *Chara* surrounded by water. In this situation, the only water transport is for growth and metabolism of the cells. Table 11.2 compares the osmotic potential and turgor pressure in *Chara* with the same potentials in stems of soybean seedlings treated this way. In *Chara* growing in a medium whose water potential is near zero, the turgor pressure essentially balances the osmotic potential in the protoplasts (Zhu and Boyer, 1992). In soybean, the turgor pressure of the growing region does not balance the osmotic potential even though it nearly does in the mature part of the same stems (Nonami and Boyer, 1987, 1989). This lack of balance indicates that the cells in the growing tissue have a water potential associated with the growth process, termed a growth-induced water potential, that is scarcely detectable in cells surrounded by water or in mature cells.

It was proposed that growth-induced water potentials arise from the enlargement of the cell walls which prevents turgor pressure from becoming as high as it otherwise would if the walls were rigid (Boyer, 1968). The low pressure would result in a low water potential that would favor water uptake by the cells because the potential would be transmitted to the cell wall solution as a tension (negative pressure) that would move water out of the xylem and into the apoplast from which it would enter the cells. Tensions of an appropriate magnitude can be detected in the apoplast of growing tissues using a pressure chamber and are near zero in the adjacent mature tissues (Nonami and Boyer, 1987). The tensions are able to mobilize water from mature tissues to supply the growing cells when water uptake by the roots is prevented (Matyssek *et al.*, 1991a,b).

Another theory is that growth-induced water potentials are generated by high concentrations of solute in the apoplast of growing tissues, and Cosgrove and Cleland (1983a) detected high concentrations of solute in extracts of the apoplast. Meshcheryakov *et al.* (1992) similarly proposed that large and rapid changes in solute concentrations could occur in the apoplast but did not measure them. However, repeating the Cosgrove and Cleland (1983a) experiments, Nonami and Boyer (1987) found solutes released by phloem and cell membranes disturbed by the experimental procedures. When these problems were avoided, concentrations were too small to account for growth-induced water potentials (Nonami and Boyer, 1987). Others also detected only low solute concentrations in the apoplast of complex tissues (Boyer, 1967a; Jachetta *et al.*, 1986; Klepper and Kaufmann, 1966; Scholander *et al.*, 1965), with the only exception being in the placental tissues of developing seeds where concentrations can be significant (Bradford, 1994; Maness and McBee, 1986).

It was also proposed that growth-induced water potentials are artifacts of excision (Cosgrove *et al.*, 1984) that might cause the cell walls to relax because growth would continue without water entry and turgor would decrease (Boyer *et al.*, 1985; Cosgrove *et al.*, 1984). Cosgrove *et al.* (1984) and Cosgrove (1985, 1987) found large decreases in water potential after excision and considered them to account for the growth-induced potential. However, the Cosgrove experiments (Cosgrove, 1985, 1987; Cosgrove *et al.*, 1984) were done with excised tissues having some mature or slowly growing tissue attached. Matyssek *et al.* (1988) showed that water was moved to the growing cells in this situation and the water potential of the whole tissue decreased substantially so that relaxation was delayed and appeared much larger than it actually was. When water movement from mature tissue was prevented by excising only the rapidly growing tissue, relaxation was much faster and smaller than 0.1 MPa. Also, growth-induced water potentials were detected in plants that were completely intact where no excision artifacts were possible (Boyer, 1968, 1974; Boyer *et al.*, 1985; Cavalieri and Boyer, 1982; Nonami and Boyer, 1987, 1989, 1990a).

Thus, growth-induced potentials appear to arise from the growth activity of the cells in complex tissues where there is competition for water from a distant vascular system. The enlargement of walls keeps turgor from rising to a level that would be achieved if the walls were rigid, and a growth-induced water potential results. Water is pulled into the growing cells by this potential, indicating that the growth of the wall is the cause and the underlying mechanism is thus tied to the metabolism of the wall. Metabolism determines the wall composition and, as pointed out earlier for *Chara*, is involved in the growth of the wall when cells enlarge irreversibly.

Gradients in Water Potential during Growth

The mechanism for generating growth-induced water potentials does not involve any particular specialization of the growing cells. As pointed out in Chap-

ter 3, each cell is nearly in water potential equilibrium with its own wall (Boyer, 1985, 1988; Molz and Ferrier, 1982) because the hydraulic conductivity of the cell membranes is large (see Table 3.1 in Chapter 3) and water for growth flows so slowly that there are minimal potential gradients just as in *Chara*. Over distances of several cells, however, significant gradients begin to appear (Boyer, 1985, 1988; Molz and Boyer, 1978; Molz and Ferrier, 1982; Silk and Wagner, 1980) because water must move from cell to cell across many wall-cytoplasm barriers and through the apoplast. Additionally, water must move through the cells next to the xylem to reach all the outlying cells. The gradients in water potential are thus steepest in cells close to the xylem where the flux of water is the greatest (Molz and Boyer, 1978).

Figure 11.5A shows the gradient predicted from the hydraulic characteristics of stem tissue of soybean seedlings (Molz and Boyer, 1978) and Fig. 11.5B gives

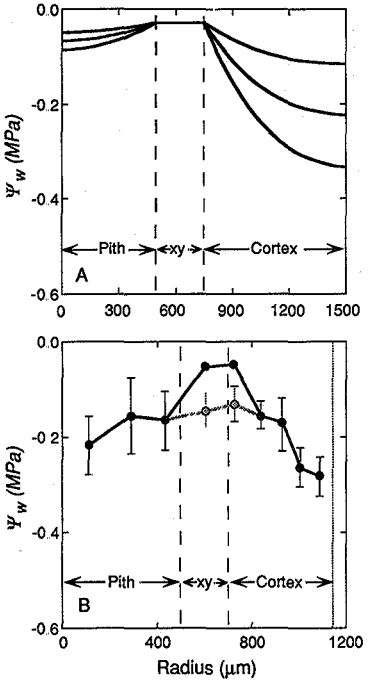

Figure 11.5 Water potential gradient in the growing region of soybean stems. The plants were grown in the dark in a water-saturated atmosphere so that the water potentials reflected only those for the growth process. The stem has a central pith in the center of a vascular cylinder and has cortical tissue covered by an epidermis on the outside, as shown. (A) Water potentials calculated at various positions along the radius of the stems assuming transport and anatomical properties characteristic of plant cells (adapted from Molz and Boyer, 1978). (B) Water potentials measured in cells at various positions along the radius of the stems by determining cell turgor and cell osmotic potential and summing the two. Values were corrected for mixing of solutions in the microcapillary used to sample for osmotic potential. Shaded values were uncorrected. From Nonami and Boyer (1993).

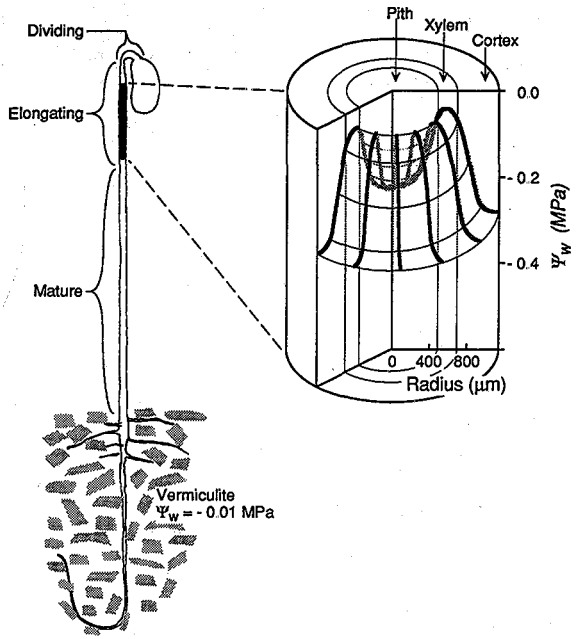

Figure 11.6 Three-dimensional water potential field induced by growth in the stem of soybean. The field is shown next to the stem in which it was measured, as in Fig. 11.5. The water potential is highest in the xylem and decreases in the surrounding pith and cortex tissues. Compare with the two-dimensional view of Fig. 11.5. After Nonami and Boyer (1993).

the gradient observed by direct measurements of water potentials in cells of the tissue (Nonami and Boyer, 1993). Water potentials were highest in the xylem (about -0.05 MPa, Fig. 11.5B) and lowest at the outermost layer of growing cells (-0.28 MPa, Fig. 11.5B). The three-dimensional shape of this gradient is shown in Fig. 11.6 next to the soybean plants in which it was measured. The three-dimensional nature of the gradient creates a potential field around the xylem that moves water radially inward and outward to the growing cells.

The field can be detected from measurements of the average water potential of whole tissues that are not transpiring. The average water potential is about -0.15 to -0.4 MPa (Barlow, 1986; Boyer, 1968; Cavalieri and Boyer, 1982; Molz and Boyer, 1978; Nonami and Boyer, 1989; Sharp and Davies, 1979; Westgate and Boyer, 1984) and can vary as conditions change around the plant. As temperatures decrease, for example, plant growth decreases and the field becomes less steep as expected from the smaller demand for water movement through the tissue (Boyer, 1993). Similarly, when growth decreases because the auxin supply is removed, the field becomes less steep (Maruyama and Boyer, 1994).

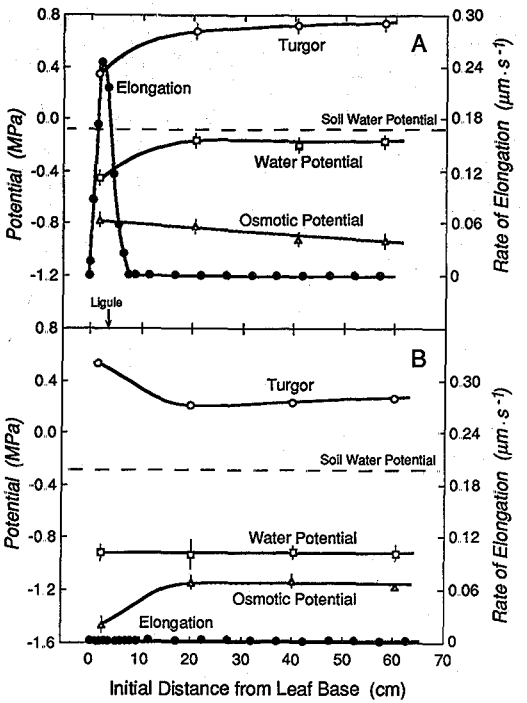

Figure 11.7 Water potential, turgor, osmotic potential, and elongation at various positions along a maize leaf at night. (A) High soil water potential. (B) Low soil water potential. Note in (A) that elongation occurred in basal tissue (left part of figure) but not in mature tissue far from the base (to the right in the figure). There was a low water potential in the basal tissue but not in the mature tissue, and a similar pattern was observed for the turgor pressure. The water potential of the mature tissue was similar to that of the soil (dashed line), indicating that the intervening xylem had a similar water potential. Thus, the low Ψ_w of the elongating tissue at the leaf base occurred in the tissues outside of the xylem. In (B), the decreased soil water potential prevented leaf elongation. Wilting was observed in the mature blade beyond 30 cm from the leaf base, and turgor pressure was low. There was a large decrease in the water potential of all leaf tissues compared to (A), and there was no difference in water potential between the elongating and mature tissue, indicating that the favorable water potential gradient for growth shown in (A) had collapsed. However, turgor pressure remained high in the elongating tissues. The collapse of the gradient thus appeared to inhibit leaf growth, and the turgor could not account for the effect. Adapted from Westgate and Boyer (1985b).

Westgate and Boyer (1985b) showed that these water potential gradients exist in all rapidly growing parts of maize plants. Roots, stems, leaves, and stigmas of developing florets exhibit substantial growth-induced water potentials compared with nearby mature tissues. For example, Fig. 11.7A shows that in a maize leaf, growth occurs at the leaf base and not in the exposed blade. The water potential of the blade is close to that of the soil at night when transpiration is negligible. The xylem connects the roots to the blade hydraulically, and

the similarity of the blade and soil water potentials shows that the connecting xylem has a similar water potential as well. The water potential in the growing basal part of the leaf is substantially below that of the soil and blade. Since the connecting xylem passes through the growing zone, the lower water potential in this zone is outside of the xylem in the growing cells. In this situation, the surrounding tissue forms a three-dimensional gradient with the xylem, and the presence of these gradients in each growing part of the plant (Westgate and Boyer, 1985b) indicates that the gradients probably are universal in growing tissues.

It is worth noting that the turgor pressure of the basal growing zone also is below that of the mature blade in Fig. 11.7A. The lower turgor is consistent with the concept that the growth-induced water potential originates from the growth of the walls that prevents turgor pressure from developing as much as it would if the walls were rigid. This pattern was observed in each of the organs of the maize plant (Westgate and Boyer, 1985b).

TRANSPIRATION AND GROWTH

Land plants differ from single cells of algae not only in their multicellular tissues but also in the large amounts of water that flow through the tissues for transpiration. A sunflower plant can transpire enough to completely replace all of the water in the leaves every 20 min (Boyer, 1974, 1977), but in the same time rapid leaf growth will import only about 0.2 or 0.3% of that amount. What happens to water uptake for growth when xylem water potentials decrease because of transpiration?

As discussed in Chapter 7, the water potential of the xylem decreases as transpiration becomes rapid mostly because dehydration of the leaf creates a tension in the apoplast water that is transmitted to the xylem and ultimately through the roots into the soil. The tension develops until water enters the leaves at the rate it is lost, preventing further dehydration. Since water for shoot growth must be extracted from the same xylem, the increased xylem tension affects the growth of surrounding tissues. In effect, water obtained for growth is obtained in competition with transpiration.

The competition is decided by the water potentials that can be exerted on water in the xylem. One theory (Boyer, 1974, 1985) is that water to be used for transpiration evaporates close to the xylem vessels and thus bypasses many of the cells outside of the xylem. There is increasing evidence that this occurs (Boyer, 1985; Nonami and Schulze, 1989; Nonami *et al.*, 1991) and that water for growth follows a much longer path since it must enter all the cells of a growing tissue. As a consequence, water lost by transpiration would encounter a low resistance path (Boyer, 1974, 1977; Raney and Vaadia, 1965a,b; Rayan and Matsuda, 1988) and water for growth would encounter a high resistance

path. The low resistance for transpiration would allow large flows to occur without developing xylem tensions that would inhibit growth (Boyer, 1974, 1977, 1985).

Another theory is that water evaporates from surfaces close to the stomatal pores. Meidner (1975, 1976a,b) showed convincingly that water will evaporate from surfaces close to a pore if all the surfaces are uniformly wet, and he proposed that water moves from the veins along bundle sheath extensions to the epidermis where it can pass to the guard cells and evaporate, which is also supported by Maercker (1965) and Maier-Maercker (1979a,b, 1983). However, the internal surfaces of leaves probably are not uniformly wet. There is a waxy cuticle covering cell walls inside leaves and it is particularly thick close to the stomatal pores (Boyer, 1985; Leon and Bukovac, 1978; Nonami *et al.*, 1991; Norris and Bukovac, 1968; Scott, 1964, 1966). Because the inner cuticle undoubtedly decreases evaporation from guard cells and the cells near them, most evaporation probably occurs from deeper surfaces close to the xylem within the leaf (Boyer, 1985; Nonami and Schulze, 1989; Nonami *et al.*, 1991). In this situation, evaporation occurs before water moves far from the xylem and most of the leaf cells are bypassed by water for transpiration. Therefore, the accumulating evidence argues against near-stomatal transpiration. This is also discussed in Chapter 7.

Despite the low resistance of the transpiration path, there is an impact on growth-induced water potentials as transpiration-induced tensions develop. For example, leaf growth occurs both during the day and night in maize, and Fig. 11.8A shows that the water potential remains lower in the growing basal leaf tissue than in the exposed mature blade at both times (Westgate and Boyer, 1984), indicating that the water potential of the growing tissue is below that in the nearby xylem. Figure 11.8B shows that the turgor pressure also is much lower in the growing tissue than in the mature tissue, but the reverse tends to be true for the osmotic potential, especially during the day, as shown in Fig. 11.8C. Nevertheless, the osmotic potential during the day is somewhat lower than at night in the growing tissue and together the lower turgor pressure and osmotic potential in the daytime decrease the water potential in the growing tissues enough to compensate for the decreased water potential in the xylem. The water potential in the growing cells thus is favorable for water uptake from the xylem regardless of transpiration.

Growth can occur only if transpiration develops slowly enough to allow sufficient change in osmotic potential and turgor to generate the growth-induced water potentials needed to maintain water movement into the cells. If xylem water potentials change rapidly, as during sudden illumination or shading of the plant, it is unlikely that the osmotic potential of the enlarging cells will keep pace (McNeil, 1976; Meyer and Boyer, 1981; Schmalstig and Cosgrove, 1990), and gradients in water potential might rapidly become unfavorable. This may ex-

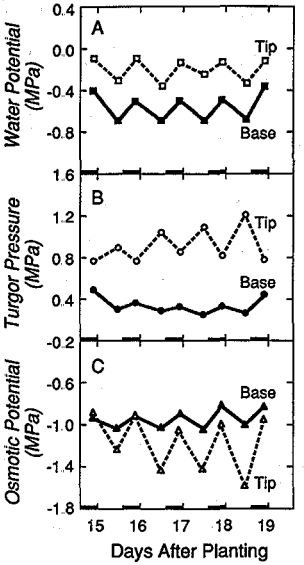

Figure 11.8 Night and day water potential, turgor pressure, and osmotic potential in the elongating tissue (leaf base, closed symbols) and mature tissue (leaf tip, open symbols) of a maize leaf. Night is shown by black bars on the *X* axis, day by open intervals between black bars. In (A), there was a lower water potential in the growing base than in the mature tip at night when transpiration was negligible and during the day when transpiration occurred. Assuming the mature tip indicated the xylem water potential (see text), the gradient between the xylem and growing base favored water uptake from the xylem during night and day. In (B), there was less turgor pressure in the growing base than in the mature tip during night and day. In (C), the osmotic potential tended to be lower (more negative) in the tip than in the base during the day, presumably because solute was produced by photosynthesis in the tip but not the base. However, the osmotic potential also was lower during the day than at night in the base, reflecting osmotic adjustment. Adapted from Westgate and Boyer (1984).

plain why growth can show rapid fluctuations under these conditions (McIntyre and Boyer, 1984; Milligan and Dale, 1988a,b; Smith and Dale, 1988).

GROWTH AT LOW WATER POTENTIALS

The variations in xylem water potential brought about by transpiration are similar to those caused by the depletion of soil water, but the latter often is of long duration and can cause very low water potentials. Several approaches have been used to study the phenomenon. One shown in Fig. 11.9 has been to grow seedlings in vermiculite in the dark at constant temperature and saturating humidities (Meyer and Boyer, 1972). Transpiration is negligible and soil water potentials remain stable for days while the seedlings consume photosynthetic products stored in the cotyledons. Water limitation is imposed by carefully

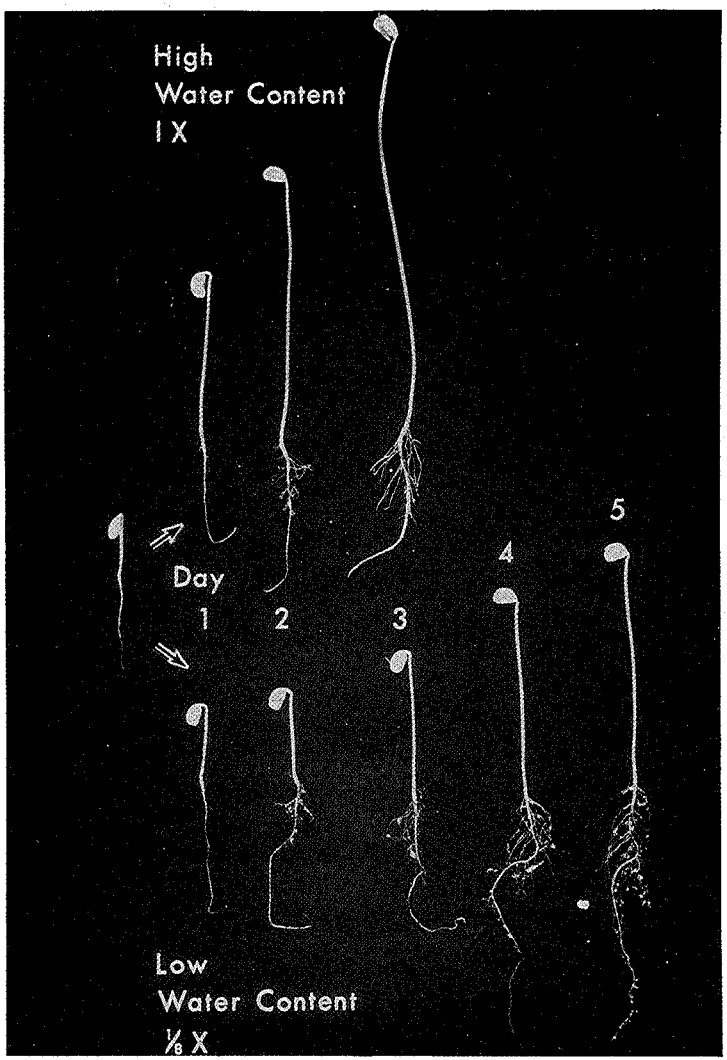

Figure 11.9 Soybean seedlings germinated in the dark and transplanted either to vermiculite containing adequate water (1×) or limited water (1/8×). The 1/8× vermiculite contained one-eighth of the water in the 1× vermiculite and had a water potential of −0.3 MPa compared to −0.01 MPa in the 1× control. Note the marked inhibition of stem (hypocotyl) growth for the first 2 days in 1/8× vermiculite followed by a modest resumption of growth. Roots continued to develop as fast at 1/8× as at 1×.

transplanting the young seedlings to vermiculite having a low water content (Fig. 11.9). Methods also have proven useful that slowly dehydrate the root medium (Jones and Turner, 1978) or expose only a few roots to water by split-

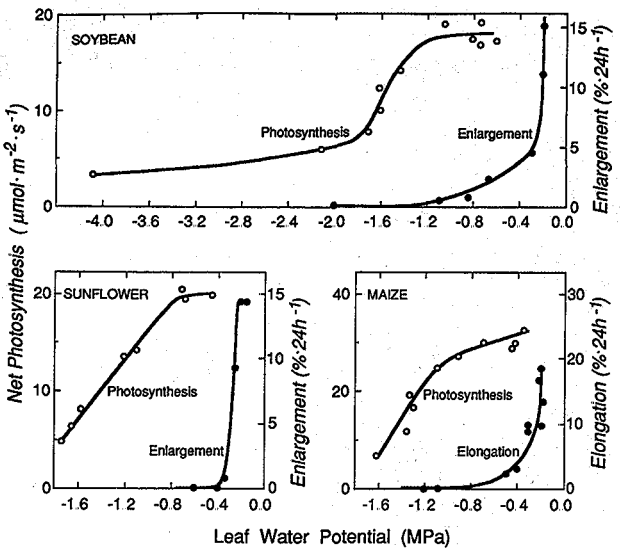

Figure 11.10 Leaf enlargement and photosynthesis at various leaf water potentials in soybean, sunflower, and maize plants. After Boyer (1970).

ting the root system into two or more volumes of soil (Blackman and Davies, 1985; Gowing *et al.*, 1990). Also, water can be supplied at a rate less than the plant requires (Matthews *et al.*, 1984; McPherson and Boyer, 1977). Each approach is a compromise that depends on the objectives of the experiments. Osmotica have sometimes been used to create water deficits but the solutes can directly inhibit growth (e.g., Zhu and Boyer, 1992) and this approach should be avoided. This problem is discussed in Chapter 4.

When soil water is gradually depleted, a number of plant functions are inhibited. Leaf growth is one of the first to diminish and Fig. 11.10 shows that it is nearly abolished before photosynthesis is affected except in maize where photosynthesis also is slightly inhibited (Boyer, 1970). Respiration is even less affected (Boyer, 1970), which illustrates that growth is inhibited by some factor other than the availability of photosynthetic products or the ability to use them in respiration. Figure 11.11 shows that growth is affected to varying degrees depending on the organ (Westgate and Boyer, 1985b). Leaves ceased growing whereas roots continued to grow rapidly on the same plants at the same tissue water potential, which probably served to reduce the development of a new transpirational surface and increase the access to soil water. The large inhibition of stigma (silk) and stem growth disrupts floral development and pollination (Herrero and Johnson, 1981; Westgate and Boyer, 1985b), a common problem in agriculture that is treated more fully in Chapter 12. Westgate and Boyer

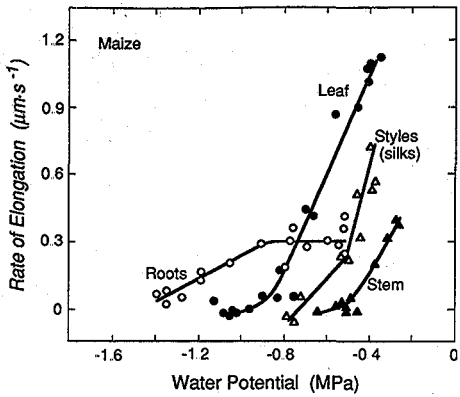

Figure 11.11 Elongation of various maize organs at various water potentials of the elongating tissues. At low water potentials, elongation often became negative, indicating that the tissue shrank. Adapted from Westgate and Boyer (1985b).

(1985b) conclude that there must be an internal control of growth that could depend on different metabolic and/or physical mechanisms in different tissues.

Primary Signals

During development, the amount of water passing through plants vastly exceeds the amount needed for growth. Fully developed maize plants normally consume about 1500 cm^3 of water every day and during a drought might absorb as little as 150 $cm^3 \cdot day^{-1}$ but only 14 $cm^3 \cdot day^{-1}$ are required for rapid growth (Aparicio-Tejo and Boyer, 1983; Boyer, 1970). Some factor other than water quantity must be responsible and for many years it was thought that loss in turgor was the problem (e.g., Hsiao, 1973; Pfeffer, 1900). Wilted leaves indicate an obvious loss of turgor, and growth generally is not observed during wilting (Boyer, 1970; Davies and Van Volkenburgh, 1983; Matthews *et al.*, 1984; Radin and Boyer, 1982). However, in leaves of grasses growth occurs at the leaf base which is hidden from view, and Michelena and Boyer (1982) and Westgate and Boyer (1985b) found high turgor in the basal growth zone when growth was completely prevented and the exposed blade was markedly wilted as shown in Fig. 11.7B. Barlow (1986), Cutler *et al.* (1980b), and Passioura (1988a) also noted that turgor loss often could not explain growth losses.

Another theory is that changes in the growth-induced water potential prevent growth. Figure 11.12C shows that stem growth immediately slowed when soybean seedlings were transplanted to vermiculite having one-eighth the usual amount of water (as shown in Fig. 11.9), which has also been seen by others in leaves (Hsiao *et al.*, 1970). Because of the transplanting, the water potential of

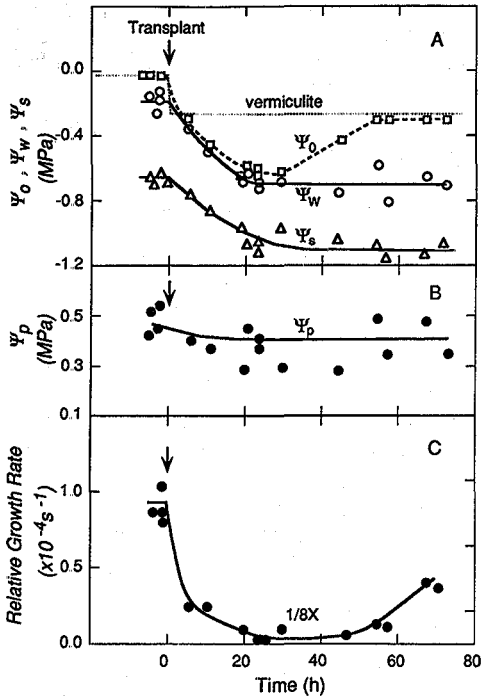

Figure 11.12 Tissue water potentials (Ψ_w), osmotic potentials (Ψ_s), turgor pressures (Ψ_p), and relative growth rates in the elongating region of soybean stems after transplanting to 1/8× vermiculite as in Fig. 11.9. Also shown are water potentials of the vermiculite (dotted line) and the basal nongrowing stem tissue (Ψ_o, dashed line). In (A), note that the basal tissue had Ψ_o close to that of the vermiculite before transplanting and at the end of the experiment, indicating that the Ψ_o monitored the xylem water potential. Before transplanting, the Ψ_w was below Ψ_o, indicating that a favorable water potential gradient existed for water uptake for growth. After transplanting, Ψ_o became similar to Ψ_w and the favorable water potential gradient collapsed. Growth was inhibited (C). Later, Ψ_o increased, reestablishing a favorable gradient and allowing growth to resume at a moderate rate. However, the lack of full recovery indicates that the growth limitation had shifted from the collapsed water potential gradient to a blockage of metabolism. (B) The tissue turgor pressure was virtually unchanged throughout the experiment. After Nonami and Boyer (1990a).

the vermiculite rapidly went from −0.01 to −0.3 MPa (Fig. 11.12A, dotted line) and the xylem water potential Ψ_o decreased faster than the water potential of the growing region Ψ_w. The potential difference ($\Psi_o - \Psi_w$) representing the growth-induced water potential collapsed as a result, indicating that the gradients in water potential became unfavorable for water extraction from the xylem, depriving the enlarging cells of water (Nonami and Boyer, 1990a). Growth ceased at the same time (Fig. 11.12C). After a few hours, osmotic adjustment occurred (Ψ_s decreased, while Ψ_p remained essentially stable as discussed in

Chapter 3) and the ($\Psi_o - \Psi_w$) recovered (seen as a rise in Ψ_o above Ψ_w after about 40 hr in Fig. 11.12A). Growth resumed slowly (Fig. 11.12C). The average tissue turgor was virtually unaffected throughout this time (Fig. 11.12B). This collapse and recovery of the growth-induced water potential indicates that there can be rapidly transmitted hydraulic signals between roots and shoots that can affect growth. Others (Boyer, 1969, 1971c; Cutler *et al.*, 1980b; Raschke, 1970) also have seen very rapid transmission of these signals, and simply opening the xylem by cutting at the apical end inhibits growth within a few seconds (Matyssek *et al.*, 1991a).

Nonami and Boyer (1989, 1990a) found that hydraulic signals could be rapidly transmitted to cells next to the xylem, detected as a change in turgor pressure, and Fig. 11.13 diagrams this dynamic system in elongating stem tissues. Decreasing the water potential of the xylem inverts the water potential gradient next to the xylem, rapidly decreasing the turgor pressure Ψ_p of the nearby cells. Growth is inhibited because water can only move down a potential gradient and it is blocked by the inverted gradient, depriving the outlying cells of the water they need for growth. This type of rapid inhibition occurred even though the

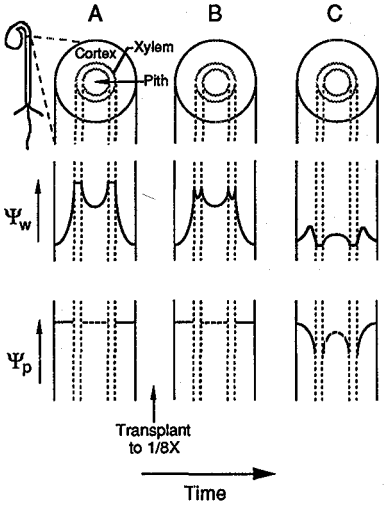

Figure 11.13 Diagrammatic representation of dynamics of water potential (Ψ_w) and turgor pressure (Ψ_p) at various radial positions in the elongating region of soybean stems before and after transplanting to dehydrated (1/8×) vermiculite as in Figs. 11.9 and 11.12. (A) Water potential gradient before transplanting when growth is rapid. In this case, turgor is uniform in the tissue. (B) Decreased xylem water potential immediately after transplanting to dehydrated vermiculite. Growth is inhibited because the gradient is inverted next to xylem and prevents water from being extracted. (C) Decreased water potentials after transplanting for several hours. Turgor is decreased next to xylem. Subsequent to (C), the gradient reestablishes and growth resumes slowly. Adapted from Nonami and Boyer (1989).

outlying cells did not change in water potential or turgor pressure, and it probably is common when transpiration rates increase or soil water is depleted.

Another factor that possibly is important is the production of plant growth regulators as dehydration signals (Gowing *et al.,* 1990; Saab and Sharp, 1989; Saab *et al.,* 1990; Zhang and Davies, 1989a,b). As discussed in Chapters 5 and 9, some molecular signals are transmitted from roots to shoots, and some may involve plant growth regulators but evidence for the latter is difficult because they may be produced in many plant tissues and their mode of detection and regulation in target tissues is uncertain. As discussed next, responses to plant growth regulators sometimes resemble those to water limitation but for different molecular reasons.

Metabolic Changes

It is difficult to identify which changes among the many in the cell may be important for growth when the water supply is limited. Obvious factors such as changes in turgor pressure or supply of photosynthetic products often fail to account for growth losses, and molecular changes such as osmotic adjustment, polyribosome alteration, and hormone accumulation tend to occur simultaneously as water is slowly depleted in the soil. The most fruitful approach has been to subject plants to realistic water limitation rapidly and observe the sequence of molecular events that follows. The idea is to decrease water availability just enough to inhibit shoot growth in a plant system like that of Fig. 11.9. Because the roots continue to grow in this condition, they can be used as a control on the same plant.

From these types of studies, it was found that cell division and cell enlargement decreased simultaneously and to about the same extent when soybean stem growth was inhibited in this way (Meyer and Boyer, 1972). Others found that cell division and enlargement were both reduced (Kirkham *et al.,* 1972) or division was reduced more than enlargement in leaves (Terry *et al.,* 1971) or the elongating region became shorter and narrower during a severe water limitation in roots (Sharp *et al.,* 1988) and the cells became smaller (Fraser *et al.,* 1990). One report of changes in cell size in stems (Paolillo, 1989) appears to have been confounded by transplant shock that itself decreased growth, making the results difficult to interpret.

Cell division and enlargement consume sugars and amino acids for biosynthesis and respiration, and new molecules of low molecular weight must be acquired to maintain both processes (McNeil, 1976; Schmalstig and Cosgrove, 1990; Sharp *et al.,* 1990). Under favorable growth conditions, there is evidence (Meyer and Boyer, 1981; McNeil, 1976) that import generally balances use and that the concentration of solutes remains practically constant, making the cell osmotic potential fairly stable. As growth slows with water limitation, however,

biosynthesis slows but import remains high (Meyer and Boyer, 1981; Sharp *et al.*, 1988, 1990). This causes import to exceed use, and the imported solute accumulates. The osmotic potential becomes more negative (osmotic adjustment, see Chapter 3 and also Fig. 11.12), but after a few hours it stabilizes as import decreases and comes back into balance with use (Meyer and Boyer, 1981). The osmotic adjustment gives an increased ability of the cells to extract water from the xylem and ultimately the soil (Barlow, 1986; Meyer and Boyer, 1972; Morgan, 1984). Wilson and Ludlow (1984) observed maximum osmotic adjustment of 0.8 to 1 MPa in grass leaves.

Osmotic adjustment allows growth to continue at a faster rate than it would without adjustment, albeit still at a slow rate (Meyer and Boyer, 1972; Michelena and Boyer, 1982). Figure 11.14 shows that maize leaves that adjusted osmotically (Control) grew faster at the same water potential than leaves whose adjustment was diminished (Dark 48 hr). In other instances, osmotic adjustment was not as extensive and the effect on growth was less apparent (Acevedo *et al.*, 1971; Cutler and Rains, 1977a,b; Cutler *et al.*, 1977, 1980a; Hsiao *et al.*, 1976; Jones and Turner, 1978; Turner *et al.*, 1978). Because osmotic adjustment involves a decreased use of imported solutes like sugars and amino acids (Meyer and Boyer, 1981; Morgan, 1984; Munns and Weir, 1981) but their import often continues at high rates, it follows that there must be a metabolic block in their use for biosynthesis of new cells. Barlow *et al.* (1976) present

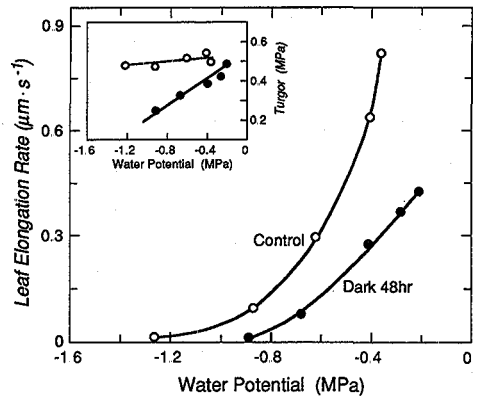

Figure 11.14 Rates of maize leaf elongation at various water potentials measured in the elongating tissues. (Inset) Turgor at various water potentials in the same elongating tissues. Darkening the plants for 48 hr (darkened points) prevented photosynthesis and decreased the transport of photosynthetic products to the elongating tissues. Osmotic adjustment was diminished and turgor was not maintained as much as in the undarkened controls (open points). After the dark treatment, leaf growth was slower and ceased at higher water potentials than in the controls. Adapted from Michelena and Boyer (1982).

evidence that ATP concentrations decrease in leaf growing regions about this time, which suggests that metabolic energy may be less available for biosynthesis. Thus, the cells appear to lose some biosynthesis but gain increased osmoticum and water that permit slow growth where otherwise none would occur.

Additional evidence for decreased biosynthesis comes from observations of decreased rates of deposition of dry matter (Meyer and Boyer, 1981), reduced DNA synthesis (Meyer and Boyer, 1972), and fewer polyribosomes in the enlarging stem tissues of water-deficient plants (Hsiao, 1970; Morilla *et al.*, 1973; Mason and Matsuda, 1985; Mason *et al.*, 1988a). The decreased biosynthesis is correlated with changes in physical characteristics of the cell walls. Using a psychrometer method to monitor the relaxation of cell walls (Nonami and Boyer, 1990a) and an extensiometer method to stretch the walls (Nonami and Boyer, 1990b), wall deformability was monitored in stems of soybean seedlings grown as in Fig. 11.9. Figure 11.15 shows that there was a gradual decrease in deformability that reached a minimum at 24 hr and showed a slow recovery thereafter. Also, there was a decreased rate of proton extrusion into the apoplast

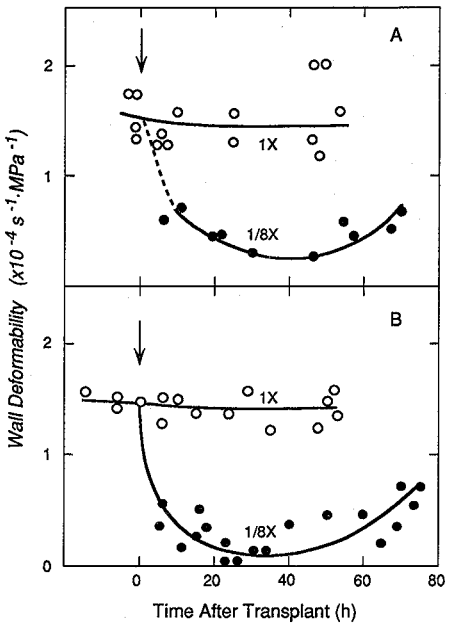

Figure 11.15 Wall deformability in elongating stem tissues at various times after transplanting soybean seedlings to vermiculite of low water content. (A) Wall deformability measured from wall relaxation after removing the water supply by excising the rapidly elongating tissues. (B) Wall deformability measured by pulling the stems in the direction of growth. Deformation was measured after subtracting the elastic component. Adapted from Nonami and Boyer (1990a,b).

of growing maize tissues (Van Volkenburgh and Boyer, 1985). Since the walls control the size of the cell, the decrease in deformability and proton extrusion may have been related to the inhibition of growth.

When the cells of the soybean stems were disrupted and the wall fraction was isolated (Bozarth *et al.*, 1987), a 28-kDa protein normally present in small amounts was found in large quantities in the walls from the elongating tissues (Fig. 11.16). It also was present in the cytoplasm and its mRNA was present among the polyribosomes, indicating that it was being synthesized in the enlarging tissues (Mason *et al.*, 1988b). Further work indicated that the protein was one of the acid phosphatases (DeWald *et al.*, 1992; Williamson and Colwell, 1991) which are common in plant tissues and release phosphate from a range of phosphorylated intermediates involved in biosynthesis and respiratory me-

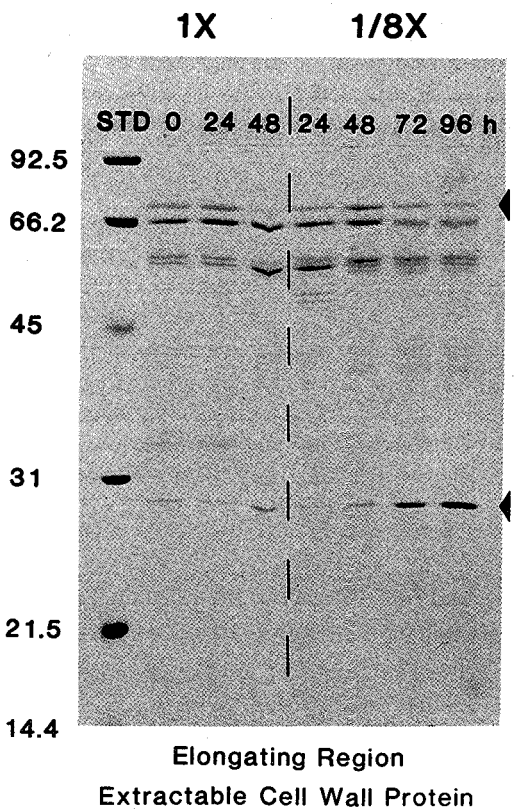

Figure 11.16 Accumulation of protein in cell walls of elongating stem tissues of soybean seedlings at various times after transplanting to vermiculite of low water content. Note the prevalence of a 28-kDa protein after several days in the drier vermiculite. From Bozarth *et al.* (1987).

tabolism. Their specific role is not well understood, however, and their presence in the cell walls raises many as yet unanswered questions.

Nevertheless, certain features of the accumulation may be significant. The 28-kDa protein and its mRNA accumulated in the enlarging tissues of the stem at low water potentials but not in the root (Surowy and Boyer, 1991). The antibodies to the 28-kDa protein also identified an antigenically related protein of about 31-kDa that did not accumulate in cell walls of shoot tissues exposed to low water potentials. The mRNA for the 31-kDa protein accumulated in the root tissues but not in the stem (Surowy and Boyer, 1991). The growth of the stem was inhibited but not the roots (Meyer and Boyer, 1981; Surowy and Boyer, 1991). The appearance of the mRNAs was thus specific for parts of the plant showing different growth responses and different protein accumulation in the cell wall fraction during water limitation. Moreover, the accumulation of the mRNA for the 28-kDa protein was particularly apparent in the epidermal cells of the stem (Mason and Mullet, 1990). Epidermal tissues are thought to contribute to the rate and form of development of plant organs (Kutschera, 1989; Kutschera and Briggs, 1988). The 31-kDa protein appears to be another acid phosphatase or part of a dimer between the 28- and 31-kDa forms (DeWald *et al.,* 1992; Mason *et al.,* 1988b).

These molecular changes were so specific that they were likely to be closely regulated. As pointed out in Chapters 8 and 9, abscisic acid is a plant growth regulator that accumulates in plants at low water potentials (Beardsell and Cohen, 1975; Neill and Horgan, 1985; Wright and Hiron, 1969; Zabadal, 1974) and it accumulates as well in the enlarging tissues of seedlings exposed to low water potentials as described earlier (Bensen *et al.,* 1988). Supplying ABA to the roots or shoots raised internal concentrations to those occurring at low water potentials and caused similar growth effects (Creelman *et al.,* 1990) but the mRNA for the 28-kDa protein showed only modest accumulation (Mason and Mullet, 1990) and, importantly, the polyribosome content of the tissue did not decrease in the ABA-treated tissue (Creelman *et al.,* 1990). The mRNA population from the polyribosomes responded differently to ABA than to low water potentials both in the shoot and in the root growing tissues (Creelman *et al.,* 1990), although Bray (1988) found a similar change in the mRNA population for the two treatments, perhaps because she used relatively mature tomato leaves that may not have been comparable to growing tissues. Others (Davies and Zhang, 1991; Davies *et al.,* 1990; Saab *et al.,* 1990) also noted similarities between growth effects of high ABA and low water potentials but failed to measure mRNA changes. Therefore, despite the similarity in growth responses, the molecular responses to ABA may be different from those occurring at low water potentials.

Another plant growth regulator, jasmonic acid, was much more effective than ABA in enhancing the mRNA content of the tissue for the 28-kDa protein

(Mason and Mullet, 1990). Jasmonic acid is released when epidermal cells are wounded. The 28-kDa protein was observed to accumulate in the vacuoles of soybean leaves when pods were removed from the reproductive plants (Staswick, 1988, 1989a,b,c) and was termed a vegetative storage protein. The jasmonic acid results make it likely that the protein was accumulating in response to the wound created by the pod removal.

Figure 11.17 shows the sequence of molecular events that have been observed in stems of soybean seedlings after transplanting to water-deficient vermiculite. The earliest hydraulic inhibition sets in motion a biosynthetic block that leads to osmotic adjustment and other metabolic changes eventually causing alterations in the cell walls. Upon rewatering, the growth inhibition is rapidly reversed, which indicates that the metabolic changes are reversible.

It is intriguing that growth regulators should give growth responses in many ways similar to those at low water potential but often not involving the same

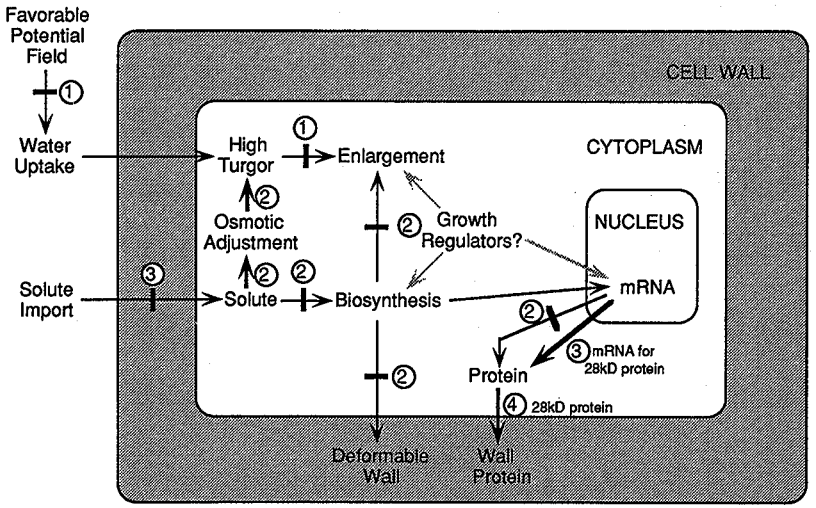

Figure 11.17 Sequence of events in elongating stem tissues after transplanting soybean seedlings to vermiculite of low water content. The order of events is shown by numbers 1–4. A block is shown by a heavy bar and enhancement is shown by increased arrow thickness. (1) Inversion of the potential field occurs first because of a decrease in water potential of the xylem which deprives the elongating cells of water and slows elongation. (2) Biosynthesis is blocked and osmotic adjustment occurs, maintaining turgor. Cell walls become less deformable. Less protein synthesis occurs. (3) Despite a decreased protein synthesis, an increased mRNA content occurs for a 28-kDa protein, a phosphatase, in soybean stems. Solute import slows, bringing import back into balance with solute use. (4) Phosphatase accumulates in cell walls. In roots, 1–3 occur rapidly but growth recovers to the control rate. The increased mRNA is for a 31-kDa protein and not the 28-kDa protein of the stem.

molecular responses. Also, it is curious that two proteins (28 and 31 kDa) should be so closely tied to the growth controlling tissues and growth responses of the plant, but have an enzymatic activity that so far has not been related to growth. Perhaps further investigation will show other aspects of regulation or wall biosynthesis that will make the mechanism clearer.

ECOLOGICAL AND AGRICULTURAL SIGNIFICANCE

Plants respond to dehydrating soils by continuing to grow roots but decreasing the growth of shoots. This causes the quantity of roots to increase in comparison to the shoots (Bennett and Doss, 1960; El Nadi *et al.,* 1969; Gales, 1979; Malik *et al.,* 1979). In these circumstances, roots tend to extend to deeper layers of soil and proliferate there (Klepper in Stewart and Nielsen, 1990; Klepper *et al.,* 1973). Figure 11.18 shows that cotton root systems in soils with and without irrigation are markedly different in root distribution. Lower soil layers had more roots in the nonirrigated treatment than in the irrigated treatment. The proliferation occurred between July 8 and July 29 while some roots disappeared from the upper layers, indicating that roots grew rapidly deep in the soil but died in shallower layers. Figure 5.13 also illustrates the rapid proliferation and death that can occur in root systems.

There is evidence that soil becomes more difficult to penetrate as it dries and that root growth is favored in wetter regions (Greacen and Oh, 1972). Thus, as the upper soil layers dehydrate, root growth slows because of increased fric-

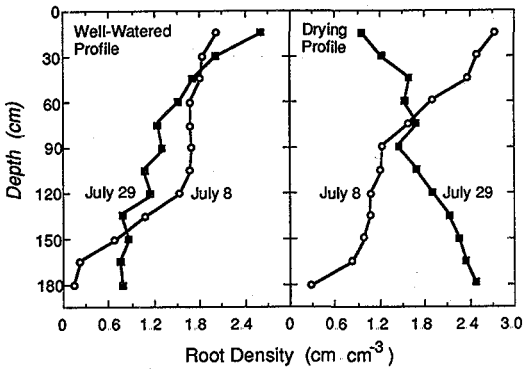

Figure 11.18 Change in root density profile upon withholding water from cotton plants. Root densities showed a typical declining density with increased depth when water was supplied (left graph) but showed an inverted profile after water was withheld (right graph). Note that there was a proliferation of roots in the deep layers but a loss of roots in the shallow layers, which dehydrated first. The changes occurred in only 3 weeks, indicating that root growth and death were occurring rapidly at this stage of development. Adapted from Klepper (in Stewart and Nielsen, 1990).

tional resistance and shifts to those roots in the wetter deeper soil at the periphery of the root system.

However, although this effect exists, there also appears to be an internal regulation of root growth. Because of the presence of growth-induced water potentials, one might expect that growing regions could obtain water from nongrowing tissues. Such a situation exists when stored onions or potatoes grow stems or cut tree stems sprout leaves even though there is no external supply of water. Figure 11.19 shows that when the roots of soybean seedlings were removed from the medium and stored in humid air, stem growth was immediately inhibited but continued slowly for many hours (Matyssek *et al.*, 1991a). The roots also grew relatively rapidly (Matyssek *et al.*, 1991a). The plants did not gain water from the humid air (Matyssek *et al.*, 1991a) and all the water for growth came from nongrowing parts of the plant (Matyssek *et al.*, 1991a).

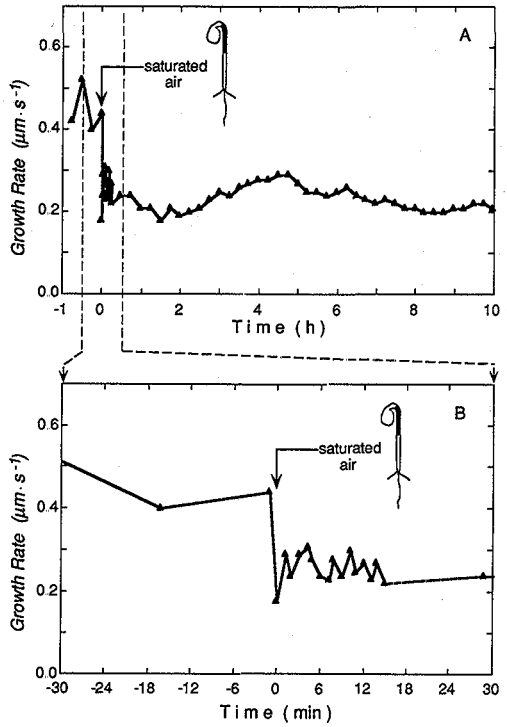

Figure 11.19 Stem growth of soybean seedling removed from the water supply and exposed to saturated air (arrow), preventing virtually all water exchange with the seedling. Continued stem growth relied on mobilization of internal water from nongrowing tissues mostly in lower parts of the stem. Expanded scale (B) shows that growth diminished immediately when the external water was removed. Adapted from Matyssek *et al.* (1991a).

Growth on internal water appears to depend not only on the ability of the enlarging cells to generate the growth-induced water potential but also on the volume of water stored in the surrounding tissue (Matyssek *et al.*, 1991a). This has practical application during transplanting where the external supply of water is disrupted and reestablishment depends on root growth to reconnect the roots to the external supply. The roots grow with water extracted from the mature tissues and reconnection is favored. It follows that plants having larger volumes of mature tissue will have greater success reestablishing after transplanting.

A similar situation applies to plants from which soil water is merely withheld. Internal water can provide a reservoir for continued growth activity. Tree stems and fleshy fruits often shrink during times of water shortage as water is withdrawn by other parts of the plant (Kozlowski in Kozlowski, 1972), and growing tissues sometimes appear to be the beneficiaries.

In this respect, it is important to note that roots can transport water not only to the shoot but also to other roots located in dry layers of soil. Several investigators observed the hydration of dry soil with water transported from roots in wet soil (Baker and Van Bavel, 1986; Caldwell and Richards, 1989; Mooney *et al.*, 1980). The net effect is to allow roots to grow where otherwise little growth would occur, and the wetting of the dry soil creates a small reservoir for the next day. This is discussed in Chapter 5 as hydraulic lift.

There are genetic differences in the ability of roots to penetrate deep layers of soil (Boyer *et al.*, 1980; Jordan *et al.*, 1979; O'Toole and Bland, 1987; Taylor *et al.*, 1978). In some instances, the differences are inherited simply. Ekanayake *et al.* (1985) and Armenta-Soto *et al.* (1983) showed that in rice the difference in depth of rooting was controlled by only a few genes. Boyer *et al.* (1980) found evidence that the high yields of modern soybean cultivars appeared traceable to less midday dehydration of the leaves resulting from deeper rooting than in older cultivars. Although this was contradicted by Frederick *et al.* (1990), there seem to be questions about the technology used by these authors, as described in Chapter 5. These experiments suggest that root systems can be genetically modified for improved performance in dehydrating soils. Because it is becoming increasingly possible to map genes, it eventually may be possible to determine the characteristics of root systems by looking at the genetic map without looking at the roots, which could enhance progress in this area.

SUMMARY

Growth is an irreversible increase in size and is the most central feature of plant activity because the plant must grow sufficiently to reproduce itself and thus ensure its representation in the next generation. Most of the increased size and weight that we consider growth consists of increased water content. However, the increased water content must be carefully distinguished from the re-

versible effects of hydration and dehydration of the tissues, which can affect the size of the cells but is not growth.

The bulk of the increased water content associated with growth is caused by the enlargement of individual cells. Water uptake is fundamentally driven by the high concentration of cellular solutes, and the turgor that develops plays a role. There is general agreement that a certain amount of turgor must be present before enlargement begins but there is debate about whether turgor also determines the growth rate. The evidence seems to favor a control of the rate by energy metabolism rather than turgor.

In multicellular tissues, water uptake must occur in competition with other cells and significant gradients in water potential can build up that extract water from the xylem fast enough to meet the demand. The faster the growth rate, the larger the gradient. When the xylem water potential falls, the gradient is reversed locally and inhibits growth, sometimes very rapidly. The evidence indicates that the gradient builds up because the enlargement of the cell compartment prevents turgor from becoming as high as it otherwise would and the water potential of the cell interior is lowered. This is transmitted mostly as a tension to the water in the cell walls and eventually the xylem. Provided the tension is greater than in the xylem, water moves out and into the growing cells. The competition for xylem water also involves transpiration which can change xylem tensions. When transpiration is rapid, it appears that the tension must increase in the cell walls of the growing cells if growth is to continue.

When water becomes in short supply around the roots, growth responds in a few seconds probably because of changes in these water potential gradients. Shoot tissues decrease in rate whereas root tissues tend to maintain their rate with obvious advantages for the plant. Subsequently, metabolic changes take place that inhibit the incorporation of small substrate molecules into the polymers needed to grow new cells, and a metabolic inhibition prevails after a few hours. The substrates accumulate, leading to osmotic adjustment, and turgor is maintained. Growth occurs more rapidly than if the accumulation had not occurred but the growth is slow.

At about this time, the molecular nature of the cell walls changes and the walls become less deformable. In particular, a phosphatase present in the cytoplasm appears to accumulate in the cell walls. A related but different protein appears to accumulate in the roots. These changes are accompanied by changes in growth regulators such as abscisic acid but, although the growth response can be mimicked by abscisic acid, the molecular changes appear different and the role of growth regulators remains unclear.

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