

If tolerance increases as a result of exposure to prior stress, the plant is said to be **acclimated** (or hardened). Acclimation can be distinguished from **adaptation**, which usually refers to a *genetically* determined level of resistance acquired by a process of selection over many generations. Unfortunately, the term *adaptation* is sometimes used in the literature to indicate acclimation. And to add to the complexity, we will see later that gene expression plays an important role in acclimation.

Adaptation and acclimation to environmental stresses result from integrated events occurring at all levels of organization, from the anatomical and morphological level to the cellular, biochemical, and molecular level. For example, the wilting of leaves in response to water deficit reduces both water loss from the leaf and exposure to incident light, thereby reducing heat stress on leaves.

Cellular responses to stress include changes in the cell cycle and cell division, changes in the endomembrane system and vacuolization of cells, and changes in cell wall architecture, all leading to enhanced stress tolerance of cells. At the biochemical level, plants alter metabolism in various ways to accommodate environmental stresses, including producing osmoregulatory compounds such as proline and glycine betaine. The molecular events linking the perception of a stress signal with the genomic responses leading to tolerance have been intensively investigated in recent years.

In this chapter we will examine these principles, and the ways in which plants adapt and acclimate to water deficit, salinity, chilling and freezing, heat, and oxygen deficiency in the root biosphere. Air pollution, an important source of plant stress, is discussed in [Web Essay 25.1](#). Although it is convenient to examine each of these stress factors separately, most are interrelated, and a common set of cellular, biochemical, and molecular responses accompanies many of the individual acclimation and adaptation processes.

For example, water deficit is often associated with salinity in the root biosphere and with heat stress in the leaves (resulting from decreased evaporative cooling due to low transpiration), and chilling and freezing lead to reductions in water activity and osmotic stress. We will also see that plants often display cross-tolerance—that is, tolerance to one stress induced by acclimation to another. This behavior implies that mechanisms of resistance to several stresses share many common features.

WATER DEFICIT AND DROUGHT RESISTANCE

In this section we will examine some drought resistance mechanisms, which are divided into several types. First we can distinguish between **desiccation postponement** (the ability to maintain tissue hydration) and **desiccation tolerance** (the ability to function while dehydrated), which are sometimes referred to as drought tolerance at high and low

water potentials, respectively. The older literature often uses the term *drought avoidance* (instead of *drought tolerance*), but this term is a misnomer because drought is a meteorological condition that is tolerated by all plants that survive it and avoided by none. A third category, **drought escape**, comprises plants that complete their life cycles during the wet season, before the onset of drought. These are the only true “drought avoiders.”

Among the desiccation postponers are water savers and water spenders. *Water savers* use water conservatively, preserving some in the soil for use late in their life cycle; *water spenders* aggressively consume water, often using prodigious quantities. The mesquite tree (*Prosopis* sp.) is an example of a water spender. This deeply rooted species has ravaged semiarid rangelands in the southwestern United States, and because of its prodigious water use, it has prevented the reestablishment of grasses that have agronomic value.

Drought Resistance Strategies Vary with Climatic or Soil Conditions

The water-limited productivity of plants (Table 25.1) depends on the total amount of water available and on the water-use efficiency of the plant (see Chapters 4 and 9). A plant that is capable of acquiring more water or that has higher water-use efficiency will resist drought better. Some plants possess adaptations, such as the C₄ and CAM modes of photosynthesis that allow them to exploit more arid environments. In addition, plants possess acclimation mechanisms that are activated in response to water stress.

Water deficit can be defined as any water content of a tissue or cell that is below the highest water content exhibited at the most hydrated state. When water deficit develops slowly enough to allow changes in developmental processes, water stress has several effects on growth, one of which is a limitation in leaf expansion. Leaf area is important because photosynthesis is usually proportional to it. However, rapid leaf expansion can adversely affect water availability.

TABLE 25.1
Yields of corn and soybean crops in the United States

Year	Crop yield (percentage of 10-year average)		
	Corn	Soybean	
1979	104	106	
1980	87	88	Severe drought
1981	104	100	
1982	108	104	
1983	77	87	Severe drought
1984	101	93	
1985	112	113	
1986	113	110	
1987	114	111	
1988	80	89	Severe drought

Source: U.S. Department of Agriculture 1989.

If precipitation occurs only during winter and spring, and summers are dry, accelerated early growth can lead to large leaf areas, rapid water depletion, and too little residual soil moisture for the plant to complete its life cycle. In this situation, only plants that have some water available for reproduction late in the season or that complete the life cycle quickly, before the onset of drought (exhibiting drought escape), will produce seeds for the next generation. Either strategy will allow some reproductive success.

The situation is different if summer rainfall is significant but erratic. In this case, a plant with large leaf area, or one capable of developing large leaf area very quickly, is better suited to take advantage of occasional wet summers. One acclimation strategy in these conditions is a capacity for both vegetative growth and flowering over an extended period. Such plants are said to be *indeterminate* in their growth habit, in contrast to *determinate* plants, which develop preset numbers of leaves and flower over only very short periods.

In the discussions that follow, we will examine several acclimation strategies, including inhibited leaf expansion, leaf abscission, enhanced root growth, and stomatal closure.

Decreased Leaf Area Is an Early Adaptive Response to Water Deficit

Typically, as the water content of the plant decreases, its cells shrink and the cell walls relax (see Chapter 3). This decrease in cell volume results in lower turgor pressure and the subsequent concentration of solutes in the cells. The plasma membrane becomes thicker and more compressed because it covers a smaller area than before. Because turgor reduction is the earliest significant biophysical effect of water stress, turgor-dependent activities such as leaf expansion and root elongation are the most sensitive to water deficits (Figure 25.1).

Cell expansion is a turgor-driven process and is extremely sensitive to water deficit. Cell expansion is described by the relationship

$$GR = m(\Psi_p - Y) \quad (25.1)$$

where GR is growth rate, Ψ_p is turgor, Y is the yield threshold (the pressure below which the cell wall resists plastic, or nonreversible, deformation), and m is the wall extensibility (the responsiveness of the wall to pressure).

This equation shows that a decrease in turgor causes a decrease in growth rate. Note also that besides showing that growth slows down when stress reduces Ψ_p , Equation 25.1 shows that Ψ_p need decrease only to the value of Y , not to zero, to eliminate expansion. In normal conditions, Y is usually only 0.1 to 0.2 MPa less than Ψ_p , so small decreases in water content and turgor can slow down or fully stop growth.

Water stress not only decreases turgor, but also decreases m and increases Y . Wall extensibility (m) is nor-

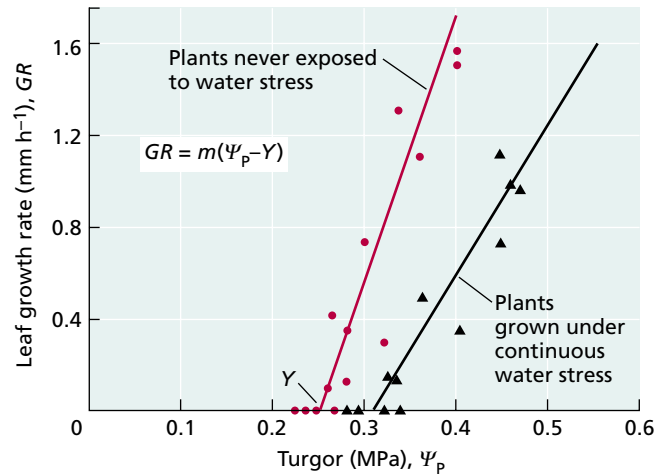


FIGURE 25.1 Dependence of leaf expansion on leaf turgor. Sunflower (*Helianthus annuus*) plants were grown either with ample water or with limited soil water to produce mild water stress. After rewatering, plants of both treatment groups were stressed by the withholding of water, and leaf growth rates (GR) and turgor (Ψ_p) were periodically measured. Both decreased extensibility (m) and increased threshold turgor for growth (Y) limit the leaf's capacity to grow after exposure to stress. (After Matthews et al. 1984.)

mally greatest when the cell wall solution is slightly acidic. In part, stress decreases m because cell wall pH typically rises during stress. The effects of stress on Y are not well understood, but presumably they involve complex structural changes of the cell wall (see Chapter 15) that may not be readily reversed after relief of stress. Water-deficient plants tend to become rehydrated at night, and as a result substantial leaf growth occurs at that time. Nonetheless, because of changes in m and Y , the growth rate is still lower than that of unstressed plants having the same turgor (see Figure 25.1).

Because leaf expansion depends mostly on cell expansion, the principles that underlie the two processes are similar. Inhibition of cell expansion results in a slowing of leaf expansion early in the development of water deficits. The smaller leaf area transpires less water, effectively conserving a limited water supply in the soil over a longer period. Reduction in leaf area can thus be considered a first line of defense against drought.

In indeterminate plants, water stress limits not only leaf size, but also leaf number, because it decreases both the number and the growth rate of branches. Stem growth has been studied less than leaf expansion, but stem growth is probably affected by the same forces that limit leaf growth during stress.

Keep in mind, too, that cell and leaf expansion also depend on biochemical and molecular factors beyond those that control water flux. Much evidence supports the view that plants change their growth rates in response to

stress by coordinately controlling many other important processes such as cell wall and membrane biosynthesis, cell division, and protein synthesis (Bursens et al. 2000).

Water Deficit Stimulates Leaf Abscission

The total leaf area of a plant (number of leaves \times surface area of each leaf) does not remain constant after all the leaves have matured. If plants become water stressed after a substantial leaf area has developed, leaves will senesce and eventually fall off (Figure 25.2). Such a leaf area adjustment is an important long-term change that improves the plant's fitness in a water-limited environment. Indeed, many drought-deciduous, desert plants drop all their leaves during a drought and sprout new ones after a rain. This cycle can occur two or more times in a single season. Abscission during water stress results largely from enhanced synthesis of and responsiveness to the endogenous plant hormone ethylene (see Chapter 22).

Water Deficit Enhances Root Extension into Deeper, Moist Soil

Mild water deficits also affect the development of the root system. Root-to-shoot biomass ratio appears to be governed by a functional balance between water uptake by the root and photosynthesis by the shoot (see Figure 23.6). Simply stated, *a shoot will grow until it is so large that water uptake by the roots becomes limiting to further growth; conversely, roots will grow until their demand for photosynthate from the shoot equals the supply.* This functional balance is shifted if the water supply decreases.

As discussed already, leaf expansion is affected very early when water uptake is curtailed, but photosynthetic activity is much less affected. Inhibition of leaf expansion

reduces the consumption of carbon and energy, and a greater proportion of the plant's assimilates can be distributed to the root system, where they can support further root growth. At the same time, the root apices in dry soil lose turgor.

All these factors lead to a preferential root growth into the soil zones that remain moist. As water deficits progress, the upper layers of the soil usually dry first. Thus, plants commonly show a mainly shallow root system when all soil layers are wetted, and a loss of shallow roots and proliferation of deep roots as water in top layers of the soil is depleted. Deeper root growth into wet soil can be considered a second line of defense against drought.

Enhanced root growth into moist soil zones during stress requires allocation of assimilates to the growing root tips. During water deficit, assimilates are directed to the fruits and away from the roots (see Chapter 10). For this reason the enhanced water uptake resulting from root growth is less pronounced in reproductive plants than in vegetative plants. Competition for assimilates between roots and fruits is one explanation for the fact that plants are generally more sensitive to water stress during reproduction.

Stomata Close during Water Deficit in Response to Abscisic Acid

The preceding sections focused on changes in plant development during slow, long-term dehydration. When the onset of stress is more rapid or the plant has reached its full leaf area before initiation of stress, other responses protect the plant against immediate desiccation. Under these conditions, stomata closure reduces evaporation from the existing leaf area. Thus, stomatal closure can be considered a third line of defense against drought.

Uptake and loss of water in guard cells changes their turgor and modulates stomatal opening and closing (see Chapters 4 and 18). Because guard cells are located in the leaf epidermis, they can lose turgor as a result of a direct loss of water by evaporation to the atmosphere. The decrease in turgor causes stomatal closure by **hydropassive closure**. This closing mechanism is likely to operate in air of low humidity, when direct water loss from the guard cells is too rapid to be balanced by water movement into the guard cells from adjacent epidermal cells.

A second mechanism, called **hydroactive closure**, closes the stomata when the whole leaf or the roots are dehydrated and depends on metabolic processes in the guard cells. A reduction in the solute content of the guard cells results in water loss and decreased turgor, causing the stomata to close; thus the hydraulic mechanism of hydroactive closure is a reversal of the mechanism of stomatal opening. However, the control of hydroactive closure differs in subtle but important ways from stomatal opening.

Solute loss from guard cells can be triggered by a decrease in the water content of the leaf, and abscisic acid (ABA) (see Chapter 23) plays an important role in this



FIGURE 25.2 The leaves of young cotton (*Gossypium hirsutum*) plants abscise in response to water stress. The plants at left were watered throughout the experiment; those in the middle and at right were subjected to moderate stress and severe stress, respectively, before being watered again. Only a tuft of leaves at the top of the stem is left on the severely stressed plants. (Courtesy of B. L. McMichael.)

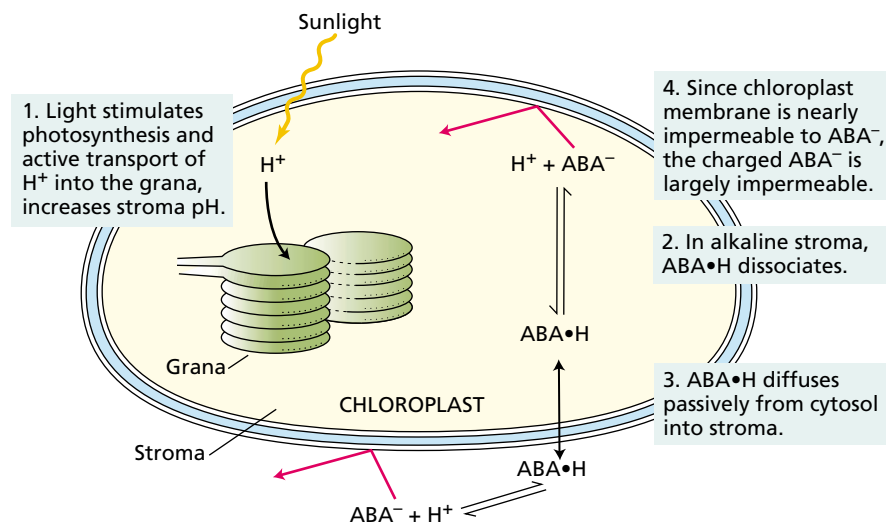


FIGURE 25.3 Accumulation of ABA by chloroplasts in the light. Light stimulates proton uptake into the grana, making the stroma more alkaline. The increased alkalinity causes the weak acid $\text{ABA}\cdot\text{H}$ to dissociate into H^+ and the ABA^- anion. The concentration of $\text{ABA}\cdot\text{H}$ in the stroma is lowered below the concentration in the cytosol, and the concentration difference drives the passive diffusion of $\text{ABA}\cdot\text{H}$ across the chloroplast membrane. At the same time, the concentration of ABA^- in the stroma increases, but the chloroplast membrane is almost impermeable to the anion (red arrows), which thus remains trapped. This process continues until the $\text{ABA}\cdot\text{H}$ concentrations in the stroma and the cytosol are equal. But as long as the stroma remains more alkaline, the total ABA concentration ($\text{ABA}\cdot\text{H} + \text{ABA}^-$) in the stroma greatly exceeds the concentration in the cytosol.

process. Abscisic acid is synthesized continuously at a low rate in mesophyll cells and tends to accumulate in the chloroplasts. When the mesophyll becomes mildly dehydrated, two things happen:

1. Some of the ABA stored in the chloroplasts is released to the apoplast (the cell wall space) of the mesophyll cell (Hartung et al. 1998). The redistribution of ABA depends on pH gradients within the leaf, on the weak-acid properties of the ABA molecule, and on the permeability properties of cell membranes (Figure 25.3). The redistribution of ABA makes it possible for the transpiration stream to carry some of the ABA to the guard cells.
2. ABA is synthesized at a higher rate, and more ABA accumulates in the leaf apoplast. The higher ABA concentrations resulting from the higher rates of ABA synthesis appear to enhance or prolong the initial closing effect of the stored ABA. The mechanism of ABA-induced stomatal closure is discussed in Chapter 23.

Stomatal responses to leaf dehydration can vary widely both within and across species. The stomata of some dehydration-postponing species, such as cowpea (*Vigna unguiculata*) and cassava (*Manihot esculenta*), are unusually responsive to decreasing water availability, and stomatal

conductance and transpiration decrease so much that leaf water potential (Ψ_w ; see Chapters 3 and 4) may remain nearly constant during drought.

Chemical signals from the root system may affect the stomatal responses to water stress (Davies et al. 2002). Stomatal conductance is often much more closely related to soil water status than to leaf water status, and the only plant part that can be directly affected by soil water status is the root system. In fact, dehydrating only part of the root system may cause stomatal closure even if the well-watered portion of the root system still delivers ample water to the shoots.

When corn (*Zea mays*) plants were grown with roots trained into two separate pots and water was withheld from only one of the pots, the stomata closed partially, and the leaf water potential increased, just as in the dehydration postponers already described. These results show that stomata can respond to conditions sensed in the roots.

Besides ABA (Sauter et al. 2001), other signals, such as pH and inorganic ion redistribution, appear to play a role in long-distance signaling between the roots and the shoots (Davies et al. 2002).

Water Deficit Limits Photosynthesis within the Chloroplast

The photosynthetic rate of the leaf (expressed per unit leaf area) is seldom as responsive to mild water stress as leaf expansion is (Figure 25.4) because photosynthesis is much less sensitive to turgor than is leaf expansion. However, mild water stress does usually affect both leaf photosynthesis and stomatal conductance. As stomata close during early stages of water stress, water-use efficiency (see Chapters 4 and 9) may increase (i.e., more CO_2 may be taken up per unit of water transpired) because stomatal closure inhibits transpiration more than it decreases intercellular CO_2 concentrations.

As stress becomes severe, however, the dehydration of mesophyll cells inhibits photosynthesis, mesophyll metabolism is impaired, and water-use efficiency usually decreases. Results from many studies have shown that the relative effect of water stress on stomatal conductance is significantly larger than that on photosynthesis. The response of photosynthesis and stomatal conductance to water stress can be partitioned by exposure of stressed

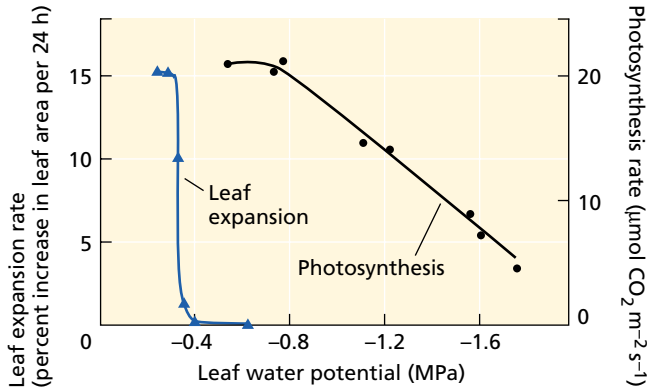
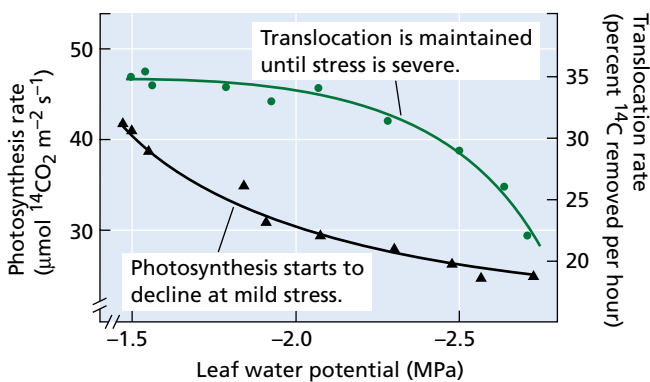


FIGURE 25.4 Effects of water stress on photosynthesis and leaf expansion of sunflower (*Helianthus annuus*). This species is typical of many plants in which leaf expansion is very sensitive to water stress, and it is completely inhibited under mild stress levels that hardly affect photosynthetic rates. (After Boyer 1970.)

leaves to air containing high concentrations of CO_2 . Any effect of the stress on stomatal conductance is eliminated by the high CO_2 supply, and differences between photosynthetic rates of stressed and unstressed plants can be directly attributed to damage from the water stress to photosynthesis.

Does water stress directly affect translocation? Water stress decreases both photosynthesis and the consumption of assimilates in the expanding leaves. As a consequence, water stress indirectly decreases the amount of photosynthate exported from leaves. Because phloem transport depends on turgor (see Chapter 10), decreased water potential in the phloem during stress may inhibit the movement of assimilates. However, experiments have shown that translocation is unaffected until late in the stress period, when other processes, such as photosynthesis, have already been strongly inhibited (Figure 25.5).

This relative insensitivity of translocation to stress allows plants to mobilize and use reserves where they are needed (e.g., in seed growth), even when stress is extremely severe. The ability to continue translocating



assimilates is a key factor in almost all aspects of plant resistance to drought.

Osmotic Adjustment of Cells Helps Maintain Plant Water Balance

As the soil dries, its matric potential (see [Web Topic 3.3](#)) becomes more negative. Plants can continue to absorb water only as long as their water potential (Ψ_w) is lower (more negative) than that of the soil water. Osmotic adjustment, or accumulation of solutes by cells, is a process by which water potential can be decreased without an accompanying decrease in turgor or decrease in cell volume. Recall Equation 3.6 from Chapter 3: $\Psi_w = \Psi_s + \Psi_p$. The change in cell water potential results simply from changes in solute potential (Ψ_s), the osmotic component of Ψ_w .

Osmotic adjustment is a net increase in solute content per cell that is independent of the volume changes that result from loss of water. The decrease in Ψ_s is typically limited to about 0.2 to 0.8 MPa, except in plants adapted to extremely dry conditions. Most of the adjustment can usually be accounted for by increases in concentration of a variety of common solutes, including sugars, organic acids, amino acids, and inorganic ions (especially K^+).

Cytosolic enzymes of plant cells can be severely inhibited by high concentrations of ions. The accumulation of ions during osmotic adjustment appears to be restricted to the vacuoles, where the ions are kept out of contact with enzymes in the cytosol or subcellular organelles. Because of this compartmentation of ions, other solutes must accumulate in the cytoplasm to maintain water potential equilibrium within the cell.

These other solutes, called **compatible solutes** (or compatible osmolytes), are organic compounds that do not interfere with enzyme functions. Commonly accumulated compatible solutes include the amino acid proline, sugar alcohols (e.g., sorbitol and mannitol), and a quaternary amine called glycine betaine. Synthesis of compatible solutes helps plants adjust to increased salinity in the rooting zone, as discussed later in this chapter.

Osmotic adjustment develops slowly in response to tissue dehydration. Over a time course of several days, other changes (such as growth or photosynthesis) are also taking place. Thus it can be argued that osmotic adjustment is not an independent and direct response to water deficit, but a result of another factor, such as decreased growth rate.

FIGURE 25.5 Relative effects of water stress on photosynthesis and translocation in sorghum (*Sorghum bicolor*). Plants were exposed to $^{14}\text{CO}_2$ for a short time interval. The radioactivity fixed in the leaf was taken as a measure of photosynthesis, and the loss of radioactivity after removal of the $^{14}\text{CO}_2$ source was taken as a measure of the rate of assimilate translocation. Photosynthesis was affected by mild stress, whereas, translocation was unaffected until stress was severe. (After Sung and Krieg 1979.)

Nonetheless, leaves that are capable of osmotic adjustment clearly can maintain turgor at lower water potentials than nonadjusted leaves. Maintaining turgor enables the continuation of cell elongation and facilitates higher stomatal conductances at lower water potentials. This suggests that osmotic adjustment is an acclimation that enhances dehydration tolerance.

How much extra water can be acquired by the plant because of osmotic adjustment in the leaf cells? Most of the extractable soil water is held in spaces (filled with water and air) from which it is readily removed by roots (see Chapter 4). As the soil dries, this water is used first, leaving behind the small amount of water that is held more tightly in small pores.

Osmotic adjustment enables the plant to extract more of this tightly held water, but the increase in total available water is small. Thus the cost of osmotic adjustment in the leaf is offset by rapidly diminishing returns in terms of water availability to the plant, as can be seen by a comparison of the water relations of adjusting and nonadjusting species (Figure 25.6). These results show that osmotic adjustment promotes dehydration tolerance but does not have a major effect on productivity (McCree and Richardson 1987).

Osmotic adjustment also occurs in roots, although the process in roots has not been studied so extensively as in leaves. The absolute magnitude of the adjustment is less in roots than in leaves, but as a percentage of the original tis-

sue solute potential (Ψ_s), it can be larger in roots than in leaves. As with leaves, these changes may in many cases increase water extraction from the previously explored soil only slightly. However, osmotic adjustment can occur in the root meristems, enhancing turgor and maintaining root growth. This is an important component of the changes in root growth patterns as water is depleted from the soil.

Does osmotic adjustment increase plant productivity? Researchers have engineered the accumulation of osmo-protective solutes by conventional plant breeding, by physiological methods (inducing adjustment with controlled water deficits), and through the use of transgenic plants expressing genes for solute synthesis and accumulation. However, the engineered plants grow more slowly, and they are only slightly more tolerant to osmotic stresses. Thus the use of osmotic adjustment to improve agricultural performance is yet to be perfected.

Water Deficit Increases Resistance to Liquid-Phase Water Flow

When a soil dries, its resistance to the flow of water increases very sharply, particularly near the *permanent wilting point*. Recall from Chapter 4 that at the permanent wilting point (usually about -1.5 MPa), plants cannot regain turgor pressure even if all transpiration stops (for more details on the relationship between soil hydraulic conductivity and soil water potential, see [Figure 4.2.A in Web Topic 4.2](#)). Because of the very large soil resistance to water flow, water delivery to the roots at the permanent wilting point is too slow to allow the overnight rehydration of plants that have wilted during the day.

Rehydration is further hindered by the resistance within the plant, which has been found to be larger than the resistance within the soil over a wide range of water deficits (Blizzard and Boyer 1980). Several factors may contribute to the increased plant resistance to water flow during drying. As plant cells lose water, they shrink. When roots shrink, the root surface can move away from the soil particles that hold the water, and the delicate root hairs may be damaged. In addition, as root extension slows during soil drying, the outer layer of the root cortex (the hypodermis) often becomes more extensively covered with suberin,

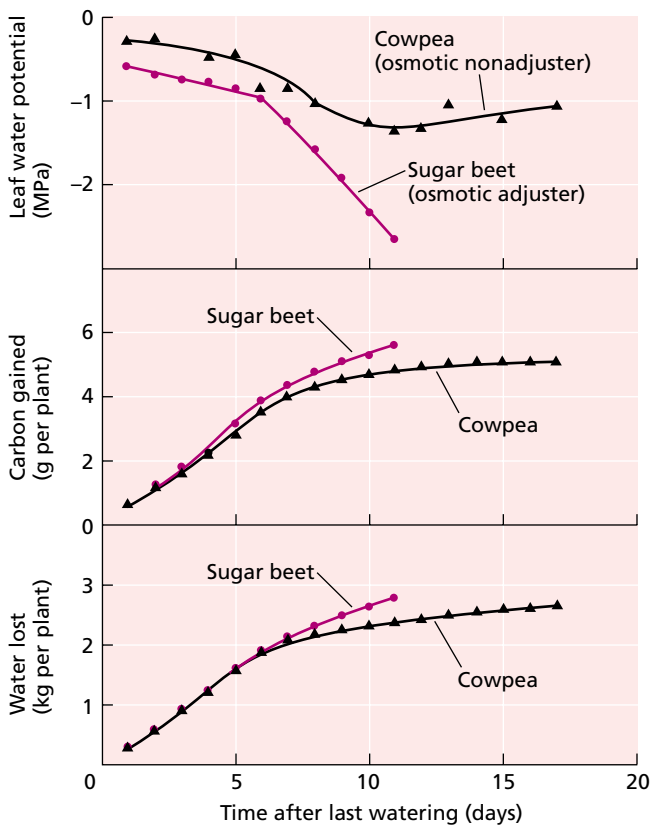


FIGURE 25.6 Water loss and carbon gain by sugar beet (*Beta vulgaris*), an osmotically adjusting species, and cowpea (*Vigna unguiculata*), a nonadjusting species that conserves water during stress by stomatal closure. Plants were grown in pots and subjected to water stress. On any given day after the last watering, the sugar beet leaves maintained a lower water potential than the cowpea leaves, but photosynthesis and transpiration during stress were only slightly greater in the sugar beet. The major difference between the two plants was the leaf water potential. These results show that osmotic adjustment promotes dehydration tolerance but does not have a major effect on productivity. (After McCree and Richardson 1987.)

a water-impermeable lipid (see Figure 4.4), increasing the resistance to water flow.

Another important factor that increases resistance to water flow is *cavitation*, or the breakage of water columns under tension within the xylem. As we saw in Chapter 4, transpiration from leaves “pulls” water through the plant by creating a tension on the water column. The cohesive forces that are required to support large tensions are present only in very narrow columns in which the water adheres to the walls.

Cavitation begins in most plants at moderate water potentials (–1 to –2 MPa), and the largest vessels cavitate first. For example, in trees such as oak (*Quercus*), the large-diameter vessels that are laid down in the spring function as a low-resistance pathway early in the growing season, when ample water is available. As the soil dries out during the summer, these large vessels cease functioning, leaving the small-diameter vessels produced during the stress period to carry the transpiration stream. This shift has long-lasting consequences: Even if water becomes available, the original low-resistance pathway remains nonfunctional, reducing the efficiency of water flow.

Water Deficit Increases Wax Deposition on the Leaf Surface

A common developmental response to water stress is the production of a thicker cuticle that reduces water loss from the epidermis (cuticular transpiration). Although waxes are deposited in response to water deficit both on the surface and within the cuticle inner layer, the inner layer may be more important in controlling the rate of water loss in ways that are more complex than by just increasing the amount of wax present (Jenks et al. in press).

A thicker cuticle also decreases CO₂ permeability, but leaf photosynthesis remains unaffected because the epidermal cells underneath the cuticle are nonphotosynthetic. Cuticular transpiration, however, accounts for only 5 to 10% of the total leaf transpiration, so it becomes significant only if stress is extremely severe or if the cuticle has been damaged (e.g., by wind-driven sand).

Water Deficit Alters Energy Dissipation from Leaves

Recall from Chapter 9 that evaporative heat loss lowers leaf temperature. This cooling effect can be remarkable: In Death Valley, California—one of the hottest places in the world—leaf temperatures of plants with access to ample water were measured to be 8°C below air temperatures. In warm, dry climates, an experienced farmer can decide whether plants need water simply by touching the leaves because a rapidly transpiring leaf is distinctly cool to the touch. When water stress limits transpiration, the leaf heats up unless another process offsets the lack of cooling. Because of these effects of transpiration on leaf temperature, water stress and heat stress are closely interrelated (see the discussion of heat stress later in this chapter).

Maintaining a leaf temperature that is much lower than the air temperature requires evaporation of vast quantities of water. This is why adaptations that cool leaves by means other than evaporation (e.g., changes in leaf size and leaf orientation) are very effective in conserving water. When transpiration decreases and leaf temperature becomes warmer than the air temperature, some of the extra energy in the leaf is dissipated as sensible heat loss (see Chapter 9). Many arid-zone plants have very small leaves, which minimize the resistance of the boundary layer to the transfer of heat from the leaf to the air (see Figure 9.14).

Because of their low boundary layer resistance, small leaves tend to remain close to air temperature even when transpiration is greatly slowed. In contrast, large leaves have higher boundary layer resistance and dissipate less thermal energy (per unit leaf area) by direct transfer of heat to the air.

In larger leaves, leaf movement can provide additional protection against heating during water stress. Leaves that orient themselves away from the sun are called *paraheliotropic*; leaves that gain energy by orienting themselves normal (perpendicular) to the sunlight are referred to as *diaheliotropic* (see Chapter 9). Figure 25.7 shows the strong effect of water stress on leaf position in soybean. Other factors that can alter the interception of radiation include wilting, which changes the angle of the leaf, and leaf rolling in grasses, which minimizes the profile of tissue exposed to the sun.

Absorption of energy can also be decreased by hairs on the leaf surface or by layers of reflective wax outside the cuticle. Leaves of some plants have a gray-white appearance because densely packed hairs reflect a large amount of light. This hairiness, or **pubescence**, keeps leaves cooler by reflecting radiation, but it also reflects the visible wavelengths that are active in photosynthesis and thus it decreases carbon assimilation. Because of this problem, attempts to breed pubescence into crops to improve their water-use efficiency have been generally unsuccessful.

Osmotic Stress Induces Crassulacean Acid Metabolism in Some Plants

Crassulacean acid metabolism (CAM) is a plant adaptation in which stomata open at night and close during the day (see Chapters 8 and 9). The leaf-to-air vapor pressure difference that drives transpiration is much reduced at night, when both leaf and air are cool. As a result, the water-use efficiencies of CAM plants are among the highest measured. A CAM plant may gain 1 g of dry matter for only 125 g of water used—a ratio that is three to five times greater than the ratio for a typical C₃ plant (see Chapter 4).

CAM is very prevalent in succulent plants such as cacti. Some succulent species display facultative CAM, switching to CAM when subjected to water deficits or saline conditions (see Chapter 8). This switch in metabolism is a remarkable adaptation to stress, involving accumulation of the enzymes phosphoenolpyruvate (PEP) carboxylase (Figure 25.8), pyruvate–orthophosphate dikinase, and NADP malic enzyme, among others.

(A) Well-watered



(B) Mild water stress



(C) Severe water stress



As discussed in Chapters 8 and 9, CAM metabolism involves many structural, physiological, and biochemical features, including changes in carboxylation and decarboxylation patterns, transport of large quantities of malate into and out of the vacuoles, and reversal of the periodicity of stomatal movements. Thus, CAM induction is a remarkable adaptation to water deficit that occurs at many levels of organization.

FIGURE 25.7 Orientation of leaflets of field-grown soybean (*Glycine max*) plants in the normal, unstressed, position (A); during mild water stress (B); and during severe water stress (C). The large leaf movements induced by mild stress are quite different from wilting, which occurs during severe stress. Note that during mild stress (B), the terminal leaflet has been raised, whereas the two lateral leaflets have been lowered; each is almost vertical. (Courtesy of D. M. Oosterhuis.)

Osmotic Stress Changes Gene Expression

As noted earlier, the accumulation of compatible solutes in response to osmotic stress requires the activation of the metabolic pathways that biosynthesize these solutes. Several genes coding for enzymes associated with osmotic adjustment are turned on (up-regulated) by osmotic stress and/or salinity, and cold stress. These genes encode enzymes such as the following (Buchanan et al. 2000):

- Δ^1 -Pyrroline-5-carboxylate synthase, a key enzyme in the proline biosynthetic pathway
- Betaine aldehyde dehydrogenase, an enzyme involved in glycine betaine accumulation
- *myo*-Inositol 6-*O*-methyltransferase, a rate-limiting enzyme in the accumulation of the cyclic sugar alcohol called pinitol

Several other genes that encode well-known enzymes are induced by osmotic stress. The expression of glyceraldehyde-3-phosphate dehydrogenase increases during osmotic stress, perhaps to allow an increase of carbon flow into organic solutes for osmotic adjustment. Enzymes involved in lignin biosynthesis are also controlled by osmotic stress.

Reduction in the activities of key enzymes also takes place. The accumulation of the sugar alcohol mannitol in response to osmotic stress appears not to be brought about by the up-regulation of genes producing enzymes involved in mannitol biosynthesis, but rather by the down-regulation of genes associated with sucrose production and mannitol degradation. In this way mannitol accumulation is enhanced during episodes of osmotic stress.

Other genes regulated by osmotic stress encode proteins associated with membrane transport, including ATPases

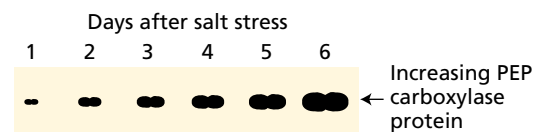


FIGURE 25.8 Increases in the content of phosphoenolpyruvate (PEP) carboxylase in ice plant, *Mesembryanthemum crystallinum*, during the salt-induced shift from C_3 metabolism to CAM. Salt stress was induced by the addition of 500 mM NaCl to the irrigation water. The PEP carboxylase protein was revealed in the gels by the use of antibodies and a stain. (After Bohnert et al. 1989.)

Table 25.2
The five groups of late embryogenesis abundant (LEA) proteins found in plants

Group (family name) ^a	Protein(s) in the group	Structural characteristics and motifs	Functional information/proposed function
Group 1 (D-19 family)	Cotton D-19 Wheat Em (early methionine-labeled protein) Sunflower Ha ds10 Barley B19	Conformation is predominantly random coil with some predicted short α helices Charged amino acids and glycine are abundant	Contains more water of hydration than typical globular proteins Overexpression confers water deficit tolerance on yeast cells
Group 2 (D-11 family) (also referred to as dehydrins)	Maize DHN1, M3, RAB17 Cotton D-11 <i>Arabidopsis</i> pRABAT1, ERD10, ERD14 <i>Craterostigma</i> pcC 27-04, pcC 6-19 Tomato pLE4, TAS14 Barley B8, B9, B17 Rice pRAB16A Carrot pcEP40	Variable structure includes α helix-forming lysine-rich regions The consensus sequence for group 2 dehydrins is EKKGIMDKIKELPG The number of times this consensus repeats per protein varies Often contains a poly(serine) region Often contains regions of variable length rich in polar residues and either Gly or Ala., and Pro	Often localized to the cytoplasm or nucleus More acidic members of the family are associated with the plasma membrane May act to stabilize macromolecules at low water potential
Group 3 (D-7 family)	Barley HVA1 (ABA-induced) Cotton D-7 Wheat pMA2005, pMA1949 <i>Craterostigma</i> pcC3-06	Eleven amino-acid consensus sequence motif TAQAAKEKAXE is repeated in the protein Contains apparent amphipathic α helices Dimeric protein	Transgenic plants expressing HVA1 demonstrate enhanced water deficit stress tolerance D-7 is an abundant protein in cotton embryos (estimated concentration 0.25 mM) Each putative dimer of D-7 may bind as many as ten inorganic phosphates and their counterions
Group 4 (D-95 family)	Soybean D-95 <i>Craterostigma</i> pcC27-45	Slightly hydrophobic N-terminal region is predicted to form amphipathic α helices	In tomato, a gene encoding a similar protein is expressed in response to nematode feeding
Group 5 (D-113 family)	Tomato LE25 Sunflower Hads11 Cotton D-113	Family members share sequence homology at the conserved N terminus N-terminal region is predicted to form α helices C-terminal domain is predicted to be a random coil of variable length and sequence Ala, Gly, and Thr are abundant in the sequence	Binds to membranes and/or proteins to maintain structure during stress Possibly functions in ion sequestration to protect cytosolic metabolism When LE25 is expressed in yeast, it confers salt and freezing tolerance D-113 is abundant in cottonseeds (up to 0.3 mM)

^aThe protein family names are derived from the cotton seed proteins that are most similar to the family.

Source: After Bray et al. 2000.

(Niu et al. 1995) and the water channel proteins, *aquaporins* (see Chapter 3) (Maggio and Joly 1995). Several protease genes are also induced by stress, and these enzymes may degrade (remove and recycle) other proteins that are dena-

tured by stress episodes. The protein *ubiquitin* tags proteins that are targeted for proteolytic degradation. Synthesis of the mRNA for ubiquitin increases in *Arabidopsis* upon desiccation stress. In addition, some *heat shock proteins* are

osmotically induced and may protect or renature proteins inactivated by desiccation.

The sensitivity of cell expansion to osmotic stress (see Figure 25.1) has stimulated studies of various genes that encode proteins involved in the structural composition and integrity of cell walls. Genes coding for enzymes such as *S*-adenosylmethionine synthase and peroxidases, which may be involved in lignin biosynthesis, have been shown to be controlled by stress.

A large group of genes that are regulated by osmotic stress was discovered by examination of naturally desiccating embryos during seed maturation. These genes code for so-called **LEA proteins** (named for *late embryogenesis abundant*), and they are suspected to play a role in cellular membrane protection. Although the function of LEA proteins is not well understood (Table 25.2), they accumulate in vegetative tissues during episodes of osmotic stress. The proteins encoded by these genes are typically hydrophilic and strongly bind water. Their protective role might be associated with an ability to retain water and to prevent crystallization of important cellular proteins and other molecules during desiccation. They might also contribute to membrane stabilization.

More recently, microarray techniques have been used to examine the expression of whole genomes of some plants in response to stress. Such studies have revealed that large numbers of genes display changes in expression after plants are exposed to stress. Stress-controlled genes reflect up to 10% of the total number of rice genes examined (Kawasaki et al. 2001)

Osmotic stress typically leads to the accumulation of ABA (see Chapter 23), so it is not surprising that products of ABA-responsive genes accumulate during osmotic stresses. Studies of ABA-insensitive and ABA-deficient mutants have shown that numerous genes that are induced by osmotic stress are in fact induced by the ABA accumulated during the stress episode. However, not all genes that are up-regulated by osmotic stresses are ABA regulated. As discussed in the next section, other mechanisms for regulating gene expression of osmotic stress-regulated genes have been uncovered.

Stress-Responsive Genes Are Regulated by ABA-Dependent and ABA-Independent Processes

Gene transcription is controlled through the interaction of regulatory proteins (transcription factors) with specific regulatory sequences in the promoters of the genes they regulate (Chapter 14 on the web site discusses these processes in detail). Different genes that are induced by the same signal (desiccation or salinity, for example) are controlled by a signaling pathway leading to the activation of these specific transcription factors.

Studies of the promoters of several stress-induced genes have led to the identification of specific regulatory sequences for genes involved in different stresses. For example, the *RD29* gene contains DNA sequences that can be activated by

osmotic stress, by cold, and by ABA (Yamaguchi-Shinozaki and Shinozaki 1994; Stockinger et al. 1997).

The promoters of ABA-regulated genes contain a six-nucleotide sequence element referred to as the **ABA response element (ABRE)**, which probably binds transcriptional factors involved in ABA-regulated gene activation (see Chapter 23). The promoters of these genes, which are regulated by osmotic stress in an ABA-dependent manner, contain an alternative nine-nucleotide regulatory sequence element, the **dehydration response element (DRE)** which is recognized by an alternative set of proteins regulating transcription. Thus the genes that are regulated by osmotic stresses appear to be regulated either by signal transduction pathways mediated by the action of ABA (**ABA-dependent genes**), or by an **ABA-independent**, osmotic stress-responsive signal transduction pathway.

At least two signaling pathways have been implicated in the regulation of gene expression in an ABA-independent manner (Figure 25.9). Transacting *transcription factors* (called DREB1 and DREB2) that bind to the DRE elements in the promoters of osmotic stress-responsive genes are apparently activated by an ABA-independent signaling cascade. Other ABA-independent, osmotic stress-respon-

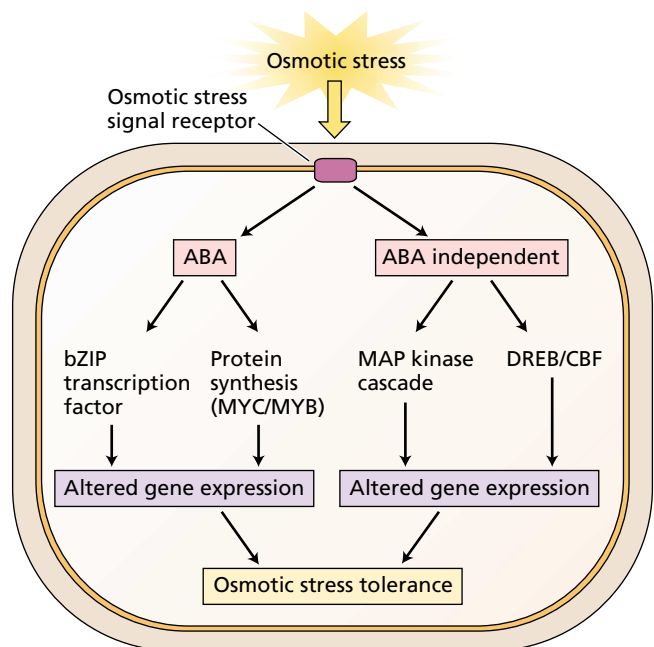


FIGURE 25.9 Signal transduction pathways for osmotic stress in plant cells. Osmotic stress is perceived by an as yet unknown receptor in the plasma membrane activating ABA-independent and an ABA-dependent signal transduction pathways. Protein synthesis participates in one of the ABA-dependent pathways involving MYC/MYB. The bZIP ABA-dependent pathway involves recognition of ABA-responsive elements in gene promoters. Two ABA-independent pathways, one involving the MAP kinase signaling cascade and the other involving DREBP/CBF-related transcription factors have also been demonstrated. (After Shinozaki and Yamaguchi-Shinozaki, 2000.)

sive genes appear to be directly controlled by the so-called MAP kinase signaling cascade of protein kinases (discussed in detail in Chapter 14 on the web site). Other changes in gene expression appear to be mediated via other mechanisms not involving DREBs.

This complexity and “cross-talk” found in signaling cascades, exemplified here by both ABA-dependent and ABA-independent pathways, is typical of eukaryotic signaling. Such complexity reflects the wealth of interaction between gene expression and the physiological processes mediating adaptation to osmotic stress.

HEAT STRESS AND HEAT SHOCK

Most tissues of higher plants are unable to survive extended exposure to temperatures above 45°C. Non-growing cells or dehydrated tissues (e.g., seeds and pollen) can survive much higher temperatures than hydrated, vegetative, growing cells (Table 25.3). Actively growing tissues rarely survive temperatures above 45°C, but dry seeds can endure 120°C, and pollen grains of some species can endure 70°C. In general, only single-celled eukaryotes can complete their life cycle at temperatures above 50°C, and only prokaryotes can divide and grow above 60°C.

Periodic brief exposure to sublethal heat stresses often induces tolerance to otherwise lethal temperatures, a phenomenon referred to as **induced thermotolerance**. The mechanisms mediating induced thermotolerance will be discussed later in the chapter. As mentioned earlier, water and temperature stress are interrelated; shoots of most C₃

and C₄ plants with access to abundant water supply are maintained below 45°C by evaporative cooling; if water becomes limiting, evaporative cooling decreases and tissue temperatures increase. Emerging seedlings in moist soil may constitute an exception to this general rule. These seedlings may be exposed to greater heat stress than those in drier soils because wet, bare soil is typically darker and absorbs more solar radiation than drier soil.

High Leaf Temperature and Water Deficit Lead to Heat Stress

Many CAM, succulent higher plants, such as *Opuntia* and *Sempervivum*, are adapted to high temperatures and can tolerate tissue temperatures of 60 to 65°C under conditions of intense solar radiation in summer (see Table 25.3). Because CAM plants keep their stomata closed during the day, they cannot cool by transpiration. Instead, they dissipate the heat from incident solar radiation by re-emission of long-wave (infrared) radiation and loss of heat by conduction and convection (see Chapter 9).

On the other hand, typical, nonirrigated C₃ and C₄ plants rely on transpirational cooling to lower leaf temperature. In these plants, leaf temperature can readily rise 4 to 5°C above ambient air temperature in bright sunlight near midday, when soil water deficit causes partial stomatal closure or when high relative humidity reduces the potential for evaporative cooling. The physiological consequences of these increases in tissue temperature are discussed in the next section.

Increases in leaf temperature during the day can be pronounced in plants from arid and semiarid regions experiencing drought and high irradiance from sunshine. Heat stress is also a potential danger in greenhouses, where low air speed and high humidity decrease the rate of leaf cooling. A moderate degree of heat stress slows growth of the whole plant. Some irrigated crops, such as cotton, use transpirational cooling to dissipate heat. In irrigated cotton, enhanced transpirational cooling is associated with higher agronomic yields (see [Web Topic 25.1](#)).

At High Temperatures, Photosynthesis Is Inhibited before Respiration

Both photosynthesis and respiration are inhibited at high temperatures, but as temperature increases, photosynthetic rates drop before respiratory rates (Figure 25.10A and B). The temperature at which the amount of CO₂ fixed by photosynthesis, equals the amount of CO₂ released by respiration, in a given time interval is called the **temperature compensation point**.

At temperatures above the temperature compensation point, photosynthesis cannot replace the carbon used as a substrate for respiration. As a result, carbohydrate reserves decline, and fruits and vegetables lose sweetness. This imbalance between photosynthesis and respiration is one of the main reasons for the deleterious effects of high temperatures.

TABLE 25.3
Heat-killing temperatures for plants

Plant	Heat-killing temperature (°C)	Time of exposure
<i>Nicotiana rustica</i> (wild tobacco)	49–51	10 min
<i>Cucurbita pepo</i> (squash)	49–51	10 min
<i>Zea mays</i> (corn)	49–51	10 min
<i>Brassica napus</i> (rape)	49–51	10 min
<i>Citrus aurantium</i> (sour orange)	50.5	15–30 min
<i>Opuntia</i> (cactus)	>65	—
<i>Sempervivum arachnoideum</i> (succulent)	57–61	—
Potato leaves	42.5	1 hour
Pine and spruce seedlings	54–55	5 min
<i>Medicago</i> seeds (alfalfa)	120	30 min
Grape (ripe fruit)	63	—
Tomato fruit	45	—
Red pine pollen	70	1 hour
Various mosses		
Hydrated	42–51	—
Dehydrated	85–110	—

Source: After Table 11.2 in Levitt 1980.