10

Photosynthesis and Respiration

INTRODUCTION

For most organisms, photosynthesis makes life possible. Plants use its products for their respiration and for building all the macromolecules and organic cofactors necessary for their metabolism and structure. Animals harvest plants and use the cell contents for their own metabolism and structure. As a consequence, animals have a simpler biochemistry than plants. They cannot synthesize many necessary metabolites and obtain them instead from plants. Their existence depends on the existence of plants.

Life began in the oceans, and photosynthesis appeared a little later. Water surrounded the cells and thus was abundant at the beginning. The earliest photosynthesis used light simply to move protons and electrons and to generate ATP. However, at a later time, light began to be used to oxidize water which generated a reductant for metabolic reactions, and water became a substrate. Oxygen was the byproduct and its release turned the atmosphere from a reducing one to an oxidizing one. Eventually, the oxygen reached the atmospheric levels of the present day (20.9%).

One of the main roles of the hydrogen obtained in the oxidation of water was the reduction of carbon in CO_2, which became an additional substrate and was abundant in the ocean mostly in the form of dissolved bicarbonate. The accumulated mass of hydrogen, carbon, and oxygen was thus a measure of the amount of photosynthesis. The cells used the photosynthetic products in part for respiration, which consumed the biomass and returned water and CO_2 to the environment, and in part to build new plant structure.

Table 10.1 Global Net Productivity by Photosynthesis in Terrestrial and Marine Plants[a]

Community	Production (10^9 tons dry mass · year^{-1})	Chlorophyll (10^7 tons)	Standing biomass (10^9 tons dry mass)	Area (% of surface)
Marine				70.78
Open Ocean	42	1.0	1.0	
Coastal[b]	13	0.8	2.9	
Terrestrial	118	23	1840	29.22

Note. The quantity of chlorophyll and standing plant biomass are also shown.
[a] After Whittaker and Likens (1975).
[b] Continental shelves, estuaries, seaweed beds, and reefs.

Rather late in this chain of evolutionary events, plants began to inhabit the land (Chapter 12). The availability of radiation appears to have been the primary force for this move but the plants risked dehydration, extreme temperatures, and wide variations in the availability of inorganic nutrients. Table 10.1 shows that the global production of biomass on land is now almost three times that in the ocean even though the area of the land is less than half that of the ocean. Thus, photosynthetic activity is greater on land than in the ocean mostly because of the higher radiation levels. Radiation is so abundant on land that stems, branches, and roots can be built even though they often do not carry on photosynthesis and instead consume photosynthetic products. The standing biomass of these structures is large compared to that in the ocean (Table 10.1) and serves to support the large photosynthetic surface. The amount of chlorophyll is higher (Table 10.1), and thus there is more radiation harvesting capability on the land than in the ocean.

The large amount of photosynthesis probably compensates somewhat for the extremes in environment that plants face on land. The extremes limit productivity more than in the ocean, and water can be particularly limiting, with large global effects on agriculture (see Chapter 12). The causes are complex because water is not only a substrate for photosynthesis, but also a solvent for the other substrate, CO_2, and is the medium in which all the reactions take place. Leaves are covered with an epidermis containing stomata that admit CO_2 from the atmosphere according to the availability of water. Therefore, the way in which water affects photosynthesis is not immediately apparent and has been the subject of several reviews (Boyer in Kozlowski, 1976; Boyer, 1990; Kaiser, 1987; Kriedemann and Downton, 1981; Ort and Boyer, 1985). This chapter gives an overview of the area with special emphasis on the role played by water. The reader is referred to texts by Foyer (1984) and Lawlor (1993) for detailed accounts of photosynthesis.

PHOTOSYNTHESIS AND WATER AVAILABILITY

For most plants, the overall reaction for photosynthesis can be written as

$$6CO_2 + 12H_2O = C_6H_{12}O_6 + 6O_2 + 6H_2O, \qquad (10.1)$$

which shows that carbon dioxide is the preferred substrate and that water also is required. All of the water is split into hydrogen and oxygen and the O_2 product is lost as a gas. The hydrogen reduces CO_2 to form the carbohydrate product ($C_6H_{12}O_6$). The C and O in the carbohydrate thus come entirely from CO_2. The remainder of the O in CO_2 is reduced to water in later carbon metabolism ($6H_2O$). The C and O are relatively heavy atoms compared to H, and most of the mass of the $C_6H_{12}O_6$ comes from the CO_2 molecule and thus most of the land biomass comes from CO_2. In the ocean, the supply of CO_2 and bicarbonate is quite stable but on land, the supply can be variable. The concentration of CO_2 is low in the atmosphere (0.035%) or, in terms of partial pressure, 35 Pa $\cdot$ $(10^5 \text{ Pa})^{-1}$. Inside the leaf the CO_2 partial pressure is regulated by the stomata and the rate of consumption in photosynthesis. Because the stomatal pores change diameter according to a number of factors, including water availability (see Chapter 8), the supply of CO_2 inside the leaves varies through the day.

Similarly for O_2, aquatic environments are generally stirred and the O_2 released by photosynthesis is mixed with surrounding water. The O_2 provides a substrate for respiratory activity during the night. On land, photosynthesis cannot occur below the soil surface because of the absence of light, and the soil solution is unstirred. The O_2 consumed in respiration of roots and microorganisms must be restored by diffusion from the atmosphere. The high O_2 content of the atmosphere readily supplies shoot tissues but diffusion into the soil depends on the amount of water in the soil pores (see Chapters 4 and 5).

Flooding and Dehydration of Soil

It was pointed out in Chapter 5 that flooding can decrease the diffusion of O_2 to roots which respond by losing some of their ability to conduct water to the shoot, leading to dehydration of the shoot. Flooding forces roots to obtain most of their O_2 from the shoot by diffusion through intervening tissues. Air spaces exist between the cells (intercellular spaces) and allow O_2 to diffuse (for example, see D. A. Barber *et al.*, 1962), but the movement is restricted by the amount and tortuosity of the spaces. In most land species, the intercellular spaces are small and so tortuous that flooding tends to decrease the O_2 available to the roots but in aquatic species there may be large tissue channels (aerenchyma) that facilitate O_2 diffusion to the roots.

Dehydrating the soil results first in improved gas diffusion as water is replaced by air in the soil pores. The effect can be seen in a study of soybean

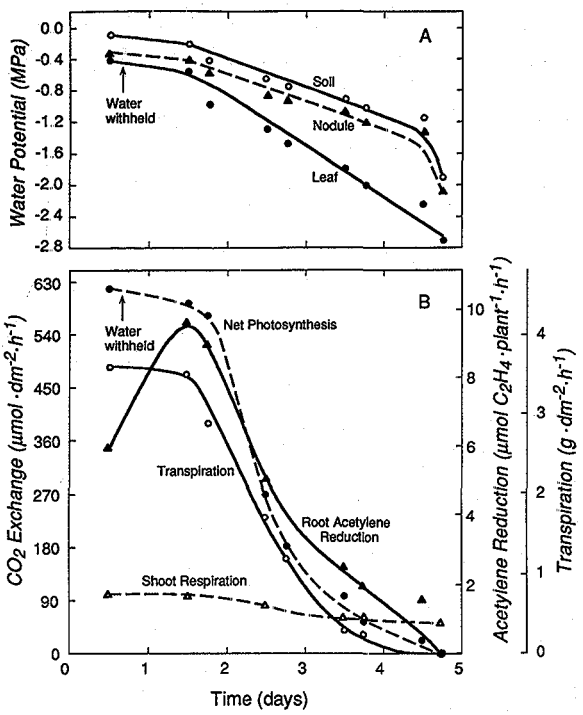

Figure 10.1 Water potential of leaves, nodules, and soil (A), and shoot photosynthesis, transpiration and respiration, and root acetylene reduction (B) in soybean after water was withheld from the soil for various times. All activities were measured simultaneously in the same intact plants in soil. Shoot activities are for the whole shoot and root activities are for the whole root system. Acetylene was supplied to the atmosphere around the soil–root system and the reduced product ethylene was measured in the same atmosphere after it diffused out of the soil. Acetylene reduction was a measure of nitrogenase activity in the roots *in situ* and also the ability of gas to diffuse through the soil and into the root nodules. After Huang *et al.* (1975a).

conducted by Huang *et al.* (1975a) and shown in Fig. 10.1. Soybean is a legume that fixes N_2 gas in the roots. As discussed in Chapter 9, the activity depends on a supply of N_2 and can be measured by supplying acetylene gas that diffuses through the soil to the roots where it is reduced to ethylene that diffuses out of the soil and is measured. The ethylene production measures not only the activity of nitrogenase but also how readily gas diffuses through the soil. Figure 10.1 shows that acetylene reducing activity was depressed in overwet soil and, as the soil drained, the activity increased. Photosynthesis and respiration were unaffected in the shoots. This suggests that nitrogen fixation had been curtailed by limited gas diffusion in the wet soil.

However, with further soil dehydration, acetylene reducing activity de-

creased. The dehydrated soil allowed very rapid gas diffusion, and the decrease thus resulted from unfavorable factors in the plant. The plants had only enough stored photosynthetic products to support acetylene reducing activity for a few hours and the dehydration required several days. Photosynthesis decreased as acetylene reducing activity declined (Fig. 10.1), suggesting that the supply of photosynthetic products may have become limiting (also see Chapter 9).

When severely dehydrated, net photosynthesis (the amount by which photosynthesis exceeds respiration) fell to zero in soybean (Fig. 10.1). Cell death was not an important factor because leaf respiration was only moderately inhibited. This indicates that photosynthesis was more labile than respiration and that the plant was deprived of its normal source of high energy compounds while the demand remained high. Note that the stomata closed as shown by the inhibition of transpiration (Fig. 10.1). The closure restricted the loss of water vapor and thus delayed the dehydration of the shoot. Dehydration was delayed for several days and this delaying effect is observed in nearly all leaves when they dehydrate.

The decrease of water loss reflects a general restriction of gas diffusion into and out of the leaf caused by stomatal closure. Respiration can continue under these circumstances because O_2 is so abundant in the atmosphere that internal consumption by respiration can generate a very large gradient in partial pressure in the inward direction, causing O_2 to enter fast enough to compensate for stomatal closure. Similarly, CO_2 can diffuse out during respiration. Leaves undergoing respiration in the dark build up concentrations of CO_2 inside the leaf large enough to cause outward diffusion of CO_2 even though stomata may be closed. This is not true for photosynthesis. CO_2 needs to be supplied by diffusion from outside the leaf, and the partial pressure of CO_2 is so low in the atmosphere that only a small gradient can develop as CO_2 is depleted inside the leaf. Therefore, the delaying effect of stomatal closure on leaf water loss has important implications for photosynthesis but not for respiration. This will be discussed later in more detail but it suffices to state that the more the stomata restrict water loss the more they restrict CO_2 entry for photosynthesis.

Plants differ in their photosynthetic response to dehydration (Boyer in Kozlowski, 1976; Kriedemann and Downton, 1981), even when photosynthesis occurs at similar rates in the hydrated plants. For example, leaves of vigorous sunflower plants display maximum rates of photosynthesis of about 60 μmol $\cdot$ $m^{-2} \cdot sec^{-1}$ when they are fully hydrated. *Fucus vesiculous,* an intertidal alga, displays a rate of about 40 μmol $\cdot$ $m^{-2} \cdot sec^{-1}$. In Fig. 10.2A, sunflower lost most of its activity when its water content fell to 40 to 65% of full hydration while *Fucus* displayed only slight losses at those contents. *Fucus* had to be dehydrated to relative water contents below 20% before most of its activity was lost.

Figure 10.2B shows even more extreme differences when the responses are

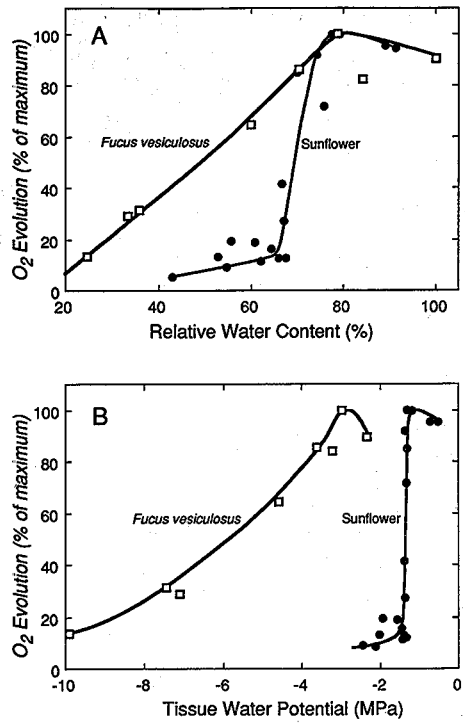

Figure 10.2 Photosynthesis during dehydration in *Fucus vesiculosus*, a marine plant, and sunflower, a land plant. (A) Oxygen evolution was measured at various tissue relative water contents (water content relative to fully turgid tissue). Full turgidity is achieved in seawater for *Fucus* but in wet soil for sunflower. (B) Oxygen evolution was measured at various tissue water potentials (note that, for *Fucus*, full hydration occurred in seawater having an osmotic potential of -2.4 MPa). Maximum photosynthesis was measured at 1% CO_2 in air and saturating radiation, and the rate at 100% of maximum was 40 and 60 μmol $\cdot$ m^{-2} $\cdot$ sec^{-1} for *Fucus* and sunflower, respectively. Y. Kawamitsu and J. S. Boyer, unpublished data.

expressed at various tissue water potentials. Sunflower photosynthesis is markedly inhibited at water potentials supporting maximum photosynthesis in *Fucus*. Of course, *Fucus* is fully hydrated in seawater, which has a low water potential (-2.4 MPa) because of its large salt content. Hydrated sunflower encounters much higher water potentials because the soil is wetted with rainwater with low salt content. This illustrates the problem of expressing plant water status in terms of relative water content, as discussed in Chapter 2. Water that fully rehydrates *Fucus* is damaging to sunflower, and the significance of the water content becomes unclear whereas the water potential has no such ambiguity. Nevertheless, as pointed out later in this chapter, decreases in water content may be the cause of losses in activity of photosynthetic metabolism. The large

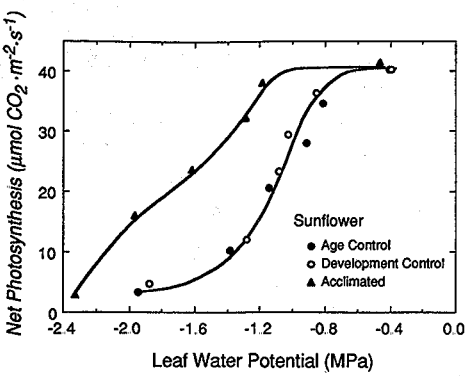

Figure 10.3 Photosynthesis at various leaf water potentials in sunflower acclimated for 2 weeks to partially dehydrated soil. The age control was given abundant water until the plants were the same age as the acclimated plants. The development control was given abundant water until the plants had developed to the same extent as the acclimated plants. After Matthews and Boyer (1984).

response of sunflower photosynthesis is curious when one considers that this land plant is probably dehydrated as often and occasionally as severely as the intertidal alga. Bewley (1979) reviews extreme desiccation tolerance in plants and points out that it is most common in primitive species such as *Fucus*.

Differences in photosynthetic response also can be found in a single species. Figure 10.3 shows that photosynthesis in sunflower decreased less than controls when the plants were exposed to moderately dehydrating conditions for long times before making the test dehydration (Matthews and Boyer, 1984). The pretreatment acclimated the plants, shifting the photosynthetic response. Growth conditions thus determine a significant part of the response of photosynthesis to dehydration.

MECHANISMS OF THE PHOTOSYNTHESIS RESPONSE

Respiration Changes

Under normal conditions, photosynthesis substantially exceeds respiration in plants. As a result, the surplus products of photosynthesis are available at times when photosynthesis cannot occur, as at night. It also provides substrate for building nonphotosynthetic parts such as roots. Photosynthesis produces products that can be moved and stored in various cells. These can be consumed at any time, thus preserving the viability of the cells. When photosynthesis is inhibited but respiration continues, photosynthetic reserves are depleted.

An important distinction between dehydration tolerant plants like *Fucus* and sensitive land plants like sunflower is the behavior of respiration. Boyer (1970, 1971a) showed that respiration continues in sunflower at water potentials low

enough to completely prevent net photosynthesis (photosynthesis equaled respiration). Because respiration occurs, the tissue consumes stored photosynthetic products, and respiration has progressively less substrate. This can decrease growth and even cause some plant parts to die, as pointed out for some reproductive structures of crops in Chapter 12. By contrast, Quadir *et al.* (1979) showed that as photosynthesis approaches zero in *Fucus* during dehydration, respiration approaches zero much as in desiccation tolerant seeds. Thus, when photosynthesis is unable to occur because of dehydration, *Fucus* does not consume stored photosynthetic products.

Eventually, respiration becomes inhibited in all plants as dehydration progresses. In land plants, part of the inhibition undoubtedly comes from the scarcity of photosynthetic products to be used. However, there also are other effects. For example, Fig. 10.4 shows that isolated maize mitochondria lose activity even though malate and pyruvate are supplied at the levels expected if photosynthesis is occurring and ADP is supplied for phosphorylation (Bell *et al.*, 1971). This inhibition is thus a loss in the intrinsic activity for respiration rather than a lack of substrate, and all activity disappears at water potentials around −3.5 MPa (Bell *et al.*, 1971) and is generally lethal. Typically, photosynthesis of land plants would have approached zero much earlier (at water potentials around −2.0 MPa, Boyer, 1970; McPherson and Boyer, 1977; Westgate and Boyer, 1985a).

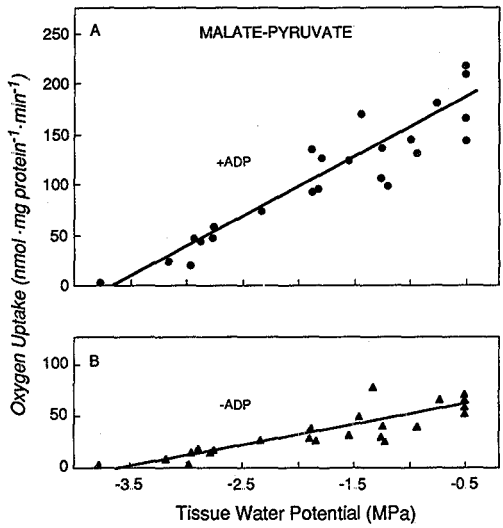

Figure 10.4 Mitochondrial respiration after isolation from maize mesocotyls having various tissue water potentials. Malate and pyruvate substrates were supplied at physiological concentrations. Rates were measured both with (A) and without (B) ADP in the medium. After Bell *et al.* (1971).

Although this inhibition of respiration occurs later than the loss of photosynthetic activity in land plants, the response is somewhat variable. Tomato, maize, soybean, and sunflower exhibit a simple decrease in respiration as the plants dehydrate (Boyer, 1970; Brix, 1962; Flowers and Hanson, 1969; Koeppe *et al.*, 1973) but young pine trees show an increase, then a decrease (Brix, 1962). The reason for this variation is unknown unless there is a temporary increase in substrate because of the action of increased amylase activity on starch as discussed later in this chapter.

Substrate Starvation

Water. Just as respiration can be limited by the availability of products of photosynthesis that can be used as substrates, photosynthesis can be blocked by a lack of substrates. Water is a substrate whose availability often is altered by the water supply. As water is removed from the cell, the water potential decreases, the water content decreases, and the cell shrinks (Chapter 3). Enzyme-mediated reactions requiring water as substrate encounter less water in the cell. As pointed out in Chapter 9, enzymes require water for catalytic activity and sometimes also require water as substrate, as in the example urease. However, isolated urease required water potentials below -14 MPa before significant activity was lost, indicating that substrate water could not have been limiting at water potentials above -14 MPa. However, inside plant cells, metabolic activity is often affected at water potentials of -1.0 to -2.5 MPa (see Fig. 10.1 for photosynthesis). Moreover, respiration often occurs when photosynthesis is inhibited (see Fig. 10.1). Respiration requires considerable substrate water for some of the associated hydrolytic reactions, and water is a product of respiration. It would seem that if there is enough substrate water for respiration and its associated reactions, there must be enough for photosynthesis. Therefore, the loss in photosynthesis is unlikely to be caused by a lack of substrate water.

Carbon Dioxide. CO_2 is the other substrate of photosynthesis that could become limiting during dehydration. In seawater, bicarbonate is present at a concentration of about 2.5 m*M* and marine plants can use bicarbonate as a source of CO_2 according to the reaction

$$H_2O + CO_2 = H_2CO_3 = H^+ + HCO_3^- \qquad (10.2)$$

which can occur in reverse. H_2CO_3 forms rapidly from HCO_3^- and H^+ but it dissociates slowly into H_2O and CO_2. Because photosynthesis uses CO_2 as substrate, the slow dissociation could be a problem for marine plants, but virtually all photosynthetic cells possess an enzyme, carbonic anhydrase, that increases the rate of H_2CO_3 dissociation (Graham and Smillie, 1976; Hatch and Burnell, 1990; Spalding *et al.*, 1983; Thielmann *et al.*, 1990). As a consequence, marine

plants probably are able to obtain CO_2 readily under most conditions. For land plants, CO_2 is supplied by the atmosphere and diffuses through the epidermal barrier mostly through the stomatal pores. Once inside, it dissolves in the water in the walls of the cells of the leaf interior. The CO_2 probably is maintained inside the cell by carbonic anhydrase much as in marine plants (Hatch and Burnell, 1990).

It has long been clear that the stomata open and close according to the amount of light and the extent of plant dehydration, as discussed in Chapter 8. During dehydration, stomatal closure can decrease transpiration to less than 10% of the rate in hydrated plants (see Fig. 10.1), and transpiration may become almost undetectable in some desert species. While this slowdown is a major way of preserving the hydration of plant tissues, it also restricts CO_2 diffusion into leaves. The result is that if photosynthetic metabolism continues at substantial rates, CO_2 partial pressures will decrease in the intercellular space system of the leaf and photosynthetic metabolism will become limited by the low CO_2. On the other hand, if metabolism is also decreased, the demand for CO_2 may become less and CO_2 depletion may not occur.

Figure 10.5A shows the condition inside a hydrated leaf during active photosynthesis when CO_2 is diffusing in and being absorbed by the mesophyll cells. If stomata close but absorption continues, the CO_2 partial pressure will decrease as in Fig. 10.5B because the CO_2 is used faster than it enters. However, if photosynthetic metabolism also is blocked,CO_2 use decreases inside the leaf and CO_2 may accumulate, as shown in Fig. 10.5C. In this case, the CO_2 partial pressure can build inside the leaf until it equals the external partial pressure whereupon CO_2 entry ceases. The effect of stomatal closure thus depends on the response of photosynthetic metabolism to dehydration.

This important principle often was overlooked by early investigators. Pfeffer (1900) and Schneider and Childers (1941) understood that stomatal closure was correlated with losses in photosynthesis as leaves become dehydrated and they concluded that closure could *cause* the losses, as in Fig. 10.5B. A similar argument can occasionally be seen in recent papers (for example, Quick *et al.*, 1992). However, without knowing the response of photosynthetic metabolism or more particularly the partial pressure of CO_2 inside the leaf, such a conclusion cannot be made. It is likely that this problem accounts for much of the diversity in the literature on this subject. At present, it seems best for one to ignore those conclusions based solely on correlations between stomatal closure and photosynthesis and focus instead on results that take photosynthetic metabolism into account.

Various approaches have been used to explore the metabolic contribution. Correlations were noted between photosynthesis and decreased activities of isolated chloroplasts (see reviews by Boyer in Kozlowski, 1976; Farquhar and Sharkey, 1982; Ort and Boyer, 1985) and were considered evidence that the rate

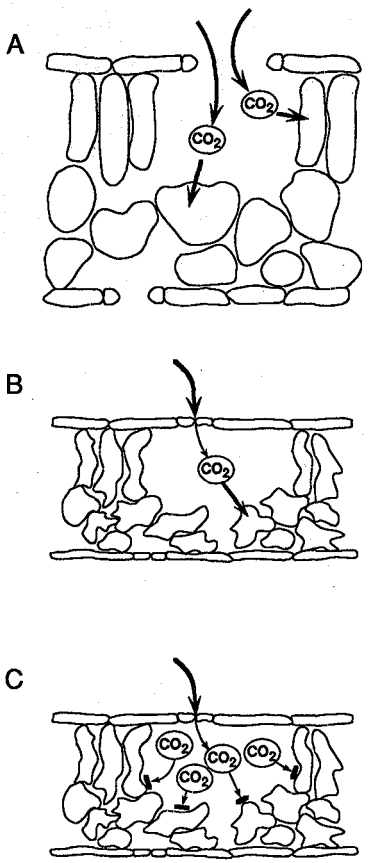

Figure 10.5 Hydrated leaf showing normal entry and use of CO_2 during active photosynthesis while stomata are open (A), dehydrated leaf showing depletion of CO_2 because of active photosynthetic metabolism while stomata are closed (B), and dehydrated leaf showing accumulation of CO_2 because of inhibited photosynthetic metabolism while stomata are closed (C). In (C), the use of CO_2 is limited more than the entry of CO_2 through the closed stomata and CO_2 builds up inside the leaf, indicating that photosynthesis is more affected by metabolism than by stomatal closure.

can be limited by metabolism and not stomatal closure. Calculations of the partial pressure of CO_2 inside leaves also indicated that CO_2 depletion was not occurring (Ehleringer and Cook, 1984; Forseth and Ehleringer, 1983; Matthews and Boyer, 1984; Radin and Ackerson, 1981). However, Terashima *et al.* (1988) and Downton *et al.* (1988a,b) found that the rate of photosynthesis may not be uniform throughout leaves and proposed that if patches of stomata close while others remain open, photosynthesis might appear to be inhibited by losses in metabolic activity whereas in reality the patchy closure was responsible

(Mansfield *et al.*, 1990; Terashima, 1992). However, Cheeseman (1991) argued against the importance of patchiness.

Patchy stomatal closure especially placed in doubt the calculation of the CO_2 partial pressures inside leaves. The calculations assume that H_2O molecules diffuse over the same path as CO_2 but in the opposite direction (Moss and Rawlins, 1963) and also that there is uniform stomatal opening across the leaf and uniform leaf temperatures. If temperatures are not uniform because of patchy closure, the partial pressure of CO_2 could be inaccurately calculated (Mansfield *et al.*, 1990; Terashima, 1992) and metabolism might be wrongly identified as the rate limitation.

The issue is further complicated by the hormonal control of stomatal aperture. As pointed out in Chapter 8, ABA is normally present at low levels in leaves; work with ABA mutants shows that stomatal opening is regulated by ABA (Imber and Tal, 1970; Neill and Horgan, 1985). In dehydrated plants, the leaf ABA normally rises (Beardsell and Cohen, 1975; Wright, 1969; Wright and Hiron, 1969). Figure 10.6 shows that leaf ABA content increased as leaf water potentials decreased, returning to normal after the plants were rewatered. The ABA rise was correlated with stomatal closure. Upon rewatering, the stomata reopened but somewhat more slowly than ABA disappeared so there was an

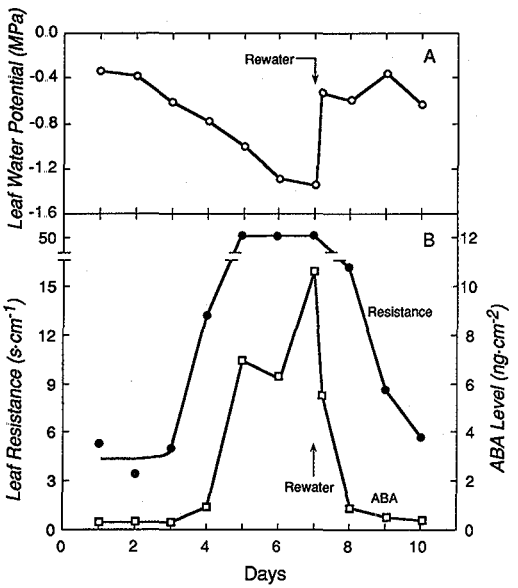

Figure 10.6 Leaf water potential (A) and abscisic acid level and leaf diffusive resistance (B) in maize from which water was withheld. The soil was rewatered on Day 7. After Beardsell and Cohen (1975).

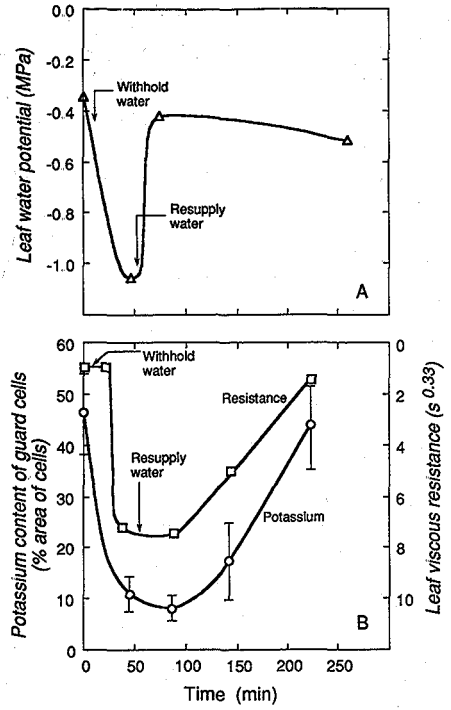

Figure 10.7 Leaf water potential (A) and guard cell K^+ content and stomatal resistance to the viscous flow of air (B) in a detached sunflower leaf from which water was withheld and resupplied. A numerically large resistance indicates stomatal closing. The authors also showed that high abscisic acid concentrations caused losses in K^+ and closure of stomata similar to those shown here for low water potentials. After Ehret and Boyer (1979).

after effect of the low water potentials not accounted for by ABA. However, high ABA can cause the loss of guard cell K^+ which is an essential osmoticum for stomatal opening (Mansfield and Jones, 1971), and guard cells of dehydrated leaves lose their K^+ (Ehret and Boyer, 1979). Figure 10.7 shows that K^+ was lost within a few minutes after leaf water potentials decreased. It reaccumulated slowly after rewatering. The stomatal opening was closely correlated with the amount of K^+ in the guard cells. Thus, it appears that high ABA causes stomatal closure because of a loss in K^+ and that the slow recovery after dehydration is caused by an inability of the guard cells to immediately recover K^+.

These findings show that ABA is an important regulator of stomata when the water supply varies. Leaves can be fed ABA through their petioles and will close their stomata, and Robinson *et al.* (1988), Downton *et al.* (1988a), and Terashima *et al.* (1988) used this approach to simulate the effects of low water potentials. Hydrated leaves fed ABA showed a patchy inhibition of photosynthesis

that could be reversed at very high CO_2. Robinson *et al.* (1988) concluded that dehydration affects photosynthesis similarly by causing patchy stomatal closure. In support of this idea, Terashima *et al.* (1988) peeled the epidermis from ABA-fed leaves and observed a recovery of photosynthesis at high CO_2. Thus, the ABA results confirmed that patchy closure might occur.

On the other hand, Graan and Boyer (1990) showed that dehydration was not fully simulated by feeding ABA. Stomatal closure occurred in both cases but the dehydrated leaves did not fully recover photosynthesis at high CO_2 whereas the ABA-fed leaves did (Graan and Boyer, 1990). Lauer and Boyer (1992) directly measured the CO_2 partial pressure inside dehydrated leaves and found no decrease, but there was a decrease in ABA-fed leaves. Figure 10.8A shows the typical day/night response of the internal CO_2 (p_i) and external CO_2 (p_e) in well-watered sunflower and indicates that p_i was lower than p_e during the day, establishing an inwardly directed CO_2 gradient, and higher during the night, establishing an outward gradient (some time had to elapse at night before the outward gradient could be seen because of the measurement apparatus). In Fig. 10.8B, this diurnal pattern was disturbed by dehydration and p_i initially remained stable, then rose as the stomata closed. The inwardly directed gradient did not form on the second day. Without the gradient, CO_2 uptake could not occur. Thus, there was no evidence of CO_2 depletion inside dehydrated leaves.

In contrast, stomatal closure in ABA-fed leaves caused p_i to decrease and the inwardly directed gradient was steepened, indicating that CO_2 depletion had occurred (shown in Lauer and Boyer, 1992). This experiment indicated that stomatal closure had opposing effects on CO_2 inside the leaf depending on whether dehydration or ABA feeding caused the closure. With ABA, closure was the only response and CO_2 levels became lower inside the leaf as in Fig. 10.5B. With dehydration, there were effects in addition to those caused by closure, and the CO_2 did not become lower inside the leaf, as in Fig. 10.5C. Eventually, the CO_2 rose in the dehydrated leaves, indicating that CO_2 became more available, not less available. The additional effects were thus attributable to decreased photosynthetic metabolism that decreased the demand for CO_2 even though the stomata closed.

The problems associated with stomatal patchiness are caused mainly by nonuniform leaf temperatures that are difficult to measure accurately and are needed for calculations of p_i. In the Lauer and Boyer (1992) experiments, measuring p_i avoided the problem because leaf temperature was not involved, and the p_i was an average for the measured area of the leaf. A number of methods have been used to assess stomatal patchiness and have been reviewed (Terashima, 1992). Starch accumulation (Terashima *et al.*, 1988), autoradiography of fixed $^{14}CO_2$ (Downton *et al.*, 1988a,b; Gunasekera and Berkowitz, 1992; Sharkey and Seemann, 1989; Wise *et al.*, 1992), and fluorescence transients (Cornic *et al.*, 1989) and imaging (Daley *et al.*, 1989) have shown patchy photosynthesis under some

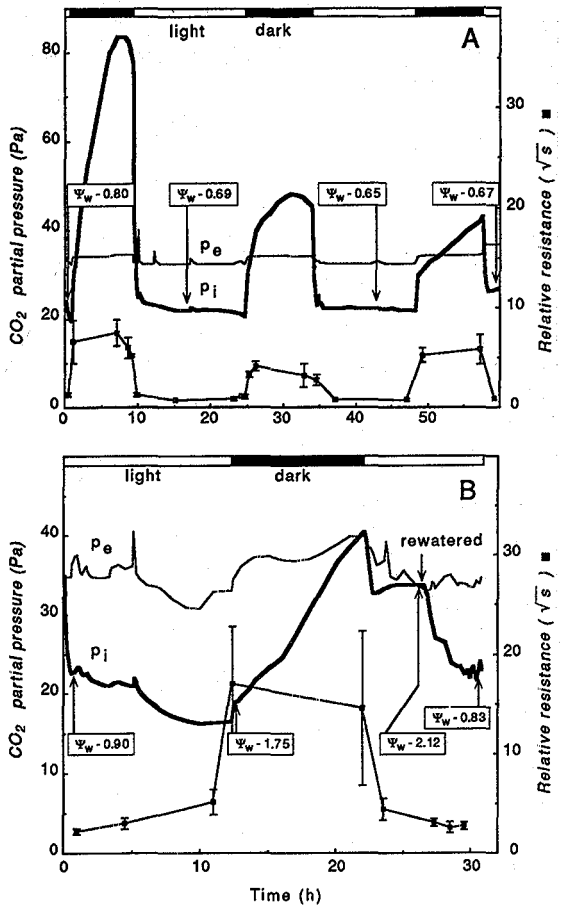

Figure 10.8 CO_2 partial pressure inside a sunflower leaf (p_i) and outside the same leaf (p_e), and stomatal resistance to the viscous flow of air through the same leaf in a hydrated control plant (A) and dehydrated plant (B). In (A), note the regular stomatal closure at night and the rise in p_i above p_e. During the day, p_i decreases below p_e because photosynthesis uses the CO_2 inside the leaf until an inward gradient forms that supplies CO_2 from the atmosphere as fast as it is used. In (B), water was withheld and p_i rises until it equals p_e. Water was resupplied as shown. Leaf water potentials are shown in boxes. The p_i was measured by equilibrating leaf CO_2 with the CO_2 in a cup attached to the underside of the leaf. After Lauer and Boyer (1992).

conditions. Although the results often have been attributed to patchy stomatal closure, the methods depend on photosynthetic metabolism and could as well reflect nonuniform metabolism (Lauer and Boyer, 1992; Wise *et al.*, 1992). Other methods are more specifically determined by stomatal aperture and include the infiltration of liquids (Alvim and Havis, 1954; Beyschlag and Pfanz,

1990; Beyschlag *et al.*, 1990, 1992; Molisch, 1912), stomatal impressions (Smith *et al.*, 1989; Weyers and Johansen, 1985), thermal imaging of leaves (Hashimoto *et al.*, 1984), and direct observations of stomata (Laisk *et al.*, 1980; Van Gardingen *et al.*, 1989). Of these, the direct observation of stomata gives the most unambiguous measure (Terashima, 1992). The infiltration of liquids is affected by patchy wetting of the stomatal pores, and stomatal impression materials may not uniformly enter the stomata. Thermal imaging usually does not have the required spatial resolution. The direct observation of stomata can indicate exactly which leaf areas might be deprived of CO_2 (Terashima *et al.*, 1988) but this has not been attempted on the scale necessary because closure must be demonstrated on a large number of stomata and on corresponding areas of the upper and lower leaf surfaces simultaneously if stomata occur on both surfaces. Several aspects of stomatal heterogeneity also are discussed in Chapter 8.

Metabolic Inhibition

Chloroplasts isolated from leaves having low water potentials display lower photosynthetic activity than chloroplasts isolated from the same leaves at high water potentials (Boyer and Bowen, 1970; Keck and Boyer, 1974; Potter and Boyer, 1973). The change reflects either a lowered photosynthetic activity *in vivo* or an increased susceptibility to chloroplast damage from isolation, but in either case fundamental metabolic change has occurred. Thylakoid membranes show less photosystem II, photosystem I, and photophosphorylating activity (Keck and Boyer, 1974; Mayoral *et al.*, 1981), and extracts of stromal enzymes display lower activities (Antolin and Sanchez-Diaz, 1993; Gunasekera and Berkowitz, 1993; Huffaker *et al.*, 1970; Johnson *et al.*, 1974; Mayoral *et al.*, 1981; O'Toole *et al.*, 1976). Crystalline structures sometimes are seen in the stroma and appear to be precipitated ribulose bisphosphate carboxylase (Fellows and Boyer, 1976; Freeman and Duysen, 1975; Gunning *et al.*, 1968; Shumway *et al.*, 1967).

Figure 10.9 shows an example in water-deficient wheat which lost activity for electron transport, photophosphorylation, and carboxylation of ribulose bisphosphate and phosphoenolpyruvate (Mayoral *et al.*, 1981). Of these activities, photophosphorylation was most inhibited. Keck and Boyer (1974) similarly found a large inhibition of photophosphorylation. An important aspect was that the extracts and assays were standard ones that would rehydrate membranes and enzymes. Thus, the inhibitions were not reversible after isolating the enzymes. This persistence of the inhibition after isolation is a central feature of losses in chloroplast activity caused by dehydration *in vivo*.

Electron transport and photophosphorylating activities are important for the photochemical activity of the chloroplasts and one would expect that if so much

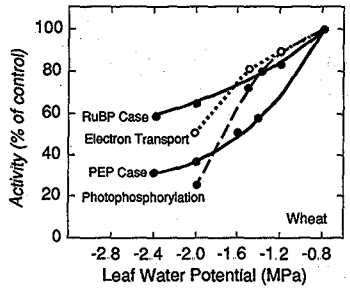

Figure 10.9 Activities for electron transport, photophosphorylation, ribulose bisphosphate carboxylase (RuBP Case), and phosphoenolpyruvate carboxylase (PEP Case) in extracts from wheat leaves having various water potentials. Electron transport, photophosphorylation, and RuBP Case are chloroplast activities. PEP Case is a cytoplasmic enzyme in this species. Activities were measured in standard media. After Mayoral *et al.* (1981).

inhibition was present in isolated chloroplasts, the overall photochemical activity also should be less in the intact leaves. Photochemical activity can be determined in leaves by measuring the number of CO_2 molecules fixed per quantum of radiation, termed the quantum yield of photosynthesis. In sunflower and soybean, quantum yields were reduced as the plants dehydrated (Matthews and Boyer, 1984; Mohanty and Boyer, 1976; Sharp and Boyer, 1986). Losses in photochemical activity also decreased the maximum rate of photosynthesis measured by saturating the leaf with CO_2 and radiation. In sunflower, maximum rates became only a small fraction of the control rates as plants dehydrated (Graan and Boyer, 1990; Matthews and Boyer, 1984; Sharp and Boyer, 1986). Measurements of photophosphorylation in the intact leaf (Ortiz-Lopez *et al.*, 1991) also confirmed the losses in photochemical activity but were not so severe when the leaves were pretreated in light. Light pretreatment activates ATP synthetase, the terminal phosphorylating enzyme sometimes called coupling factor (Hangarter *et al.*, 1987) which suggests that leaf dehydration may act in part on the activation of certain photosynthetic enzymes.

The electron microscope shows some of these dehydration-induced changes (see Chapter 3) when the dehydration is preserved during fixation (see Appendix 3.1). Fellows and Boyer (1976) showed that osmoticum can be added to the fixative to give the same water potential as the leaf tissue and thus preserve low water potentials during fixation. With this treatment, the chloroplast thylakoids did not show damage but instead displayed changes in conformation in response to the leaf water potential (Fellows and Boyer, 1976). Figure 10.10B shows that the illuminated thylakoid lamellae were 150 Å thick in the controls but only 120 to 130 Å thick in the dehydrated leaves. Because thinning normally occurs as part of electron transport and photophosphorylation when thylakoid

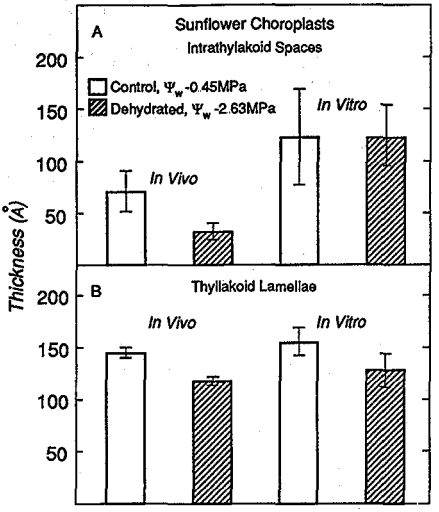

Figure 10.10 Thickness of intrathylakoid spaces (A) and thylakoid lamellae (B) measured with the electron microscope in chloroplasts of sunflower leaves (*in vivo*) or isolated from sunflower leaves (*in vitro*) having high or low water potentials. Open bars are from the control leaves and shaded bars are from the dehydrated leaves. The isolated membranes were suspended in identical medium and were exposed to light before and during fixation. The fixative had the same water potential as the leaves or isolation medium. Note that spaces (A) and lamellae (B) were thinner in dehydrated cells, but after isolation the spaces (A) swelled whereas the lamellae (B) remained thinner. The thickness of the spaces between lamellae responds to the osmotic potential significantly but the thickness of the lamellae themselves does not. After Fellows and Boyer (1976).

membranes are energized by light, the excessive thinning may be unfavorable. The excessive thinning persisted after the membranes were isolated which is consistent with the persistence in losses of chloroplast activities described previously.

The intrathylakoid spaces between the membranes also were thinner *in vivo*, but the differences did not persist after isolation (Fig. 10.10A). The thylakoid spaces respond to changes in osmotic potential across the membranes and the thinning *in vivo* indicates that dehydration had increased the potential difference. In effect, the intrathylakoid spaces acted as ultrastructural osmometers (Fellows and Boyer, 1976). In the cell, they showed how much water was removed. In the rehydrating assay medium, they swelled and showed how much rehydration occurred after isolation.

The persistence of dehydration effects in the thylakoid membranes could be seen in certain protein components of the membranes. Figure 10.11A shows that the membrane enzyme, coupling factor, changed its conformation in dehydrated leaves (Younis *et al.*, 1979), and the altered conformation was associated with a decreased binding affinity for the substrate ADP at the active site of

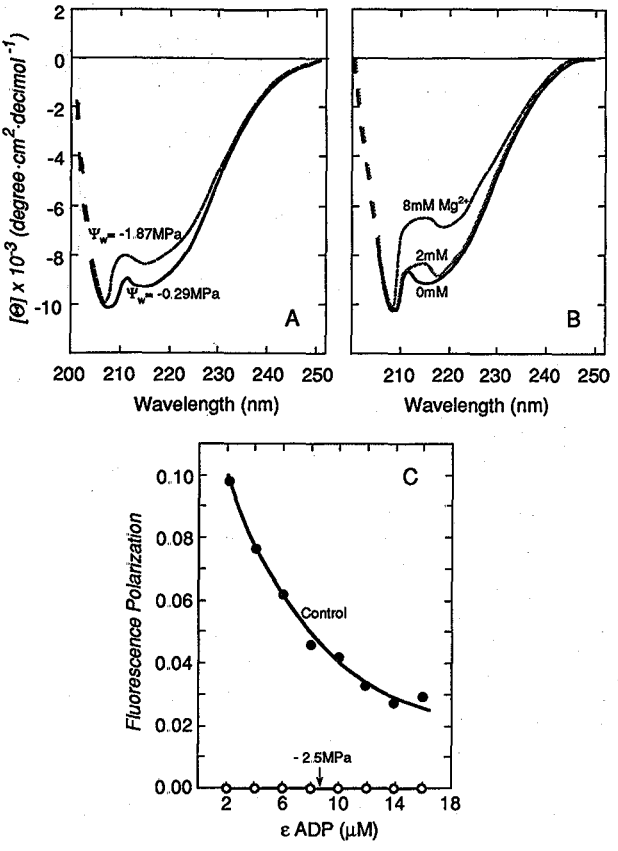

Figure 10.11 Conformation of chloroplast coupling factor (ATP synthetase) isolated from spinach leaves having high or low water potentials (A), or isolated from solutions having various Mg^{2+} concentrations (B). Isolation from Mg^{2+} solution simulated isolation from leaf. The 2 m*M* Mg^{2+} represents the concentration in control chloroplasts and 8 m*M* the possible concentration in dehydrated chloroplasts. Conformation was determined from differences in circular dichroism spectrum shown (θ). Note the similar conformation change caused by a low water potential and high Mg^{2+} concentration. (C) Binding of ADP analog (ϵADP) to coupling factor isolated from spinach leaves having high or low water potentials. The ϵADP binds to the active site of the enzyme. High binding is shown by high polarization at left that decreases with saturation of sites in control. Binding was undetectable when the coupling factor was isolated from leaves having a water potential of −2.5 MPa. After Younis *et al.* (1979) and Younis *et al.* (1983).

the enzyme (Fig. 10.11C). Thus, it seems that the altered conformation of the membranes seen in the electron microscope was traceable to an altered conformation of specific molecular components of the membranes. The changed conformation of the components might physically block access to the active site of some membrane enzymes such as coupling factor where the lack of ADP bind-

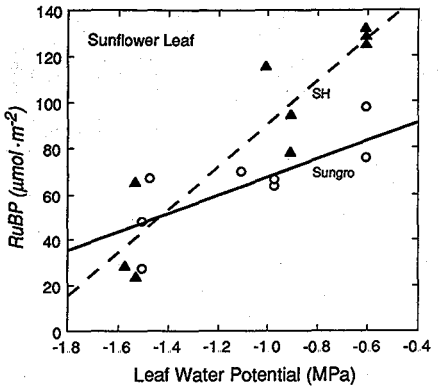

Figure 10.12 Ribulose bisphosphate (RuBP) concentrations in leaves of two sunflower cultivars (SH and Sungro) from which water was withheld. RuBP is regenerated in the carbon reduction cycle (Calvin cycle) with an input of products of the photochemical reactions. Decreased RuBP levels indicate a block may be present in the photochemical reactions or carbon reduction cycle. From Gimenez *et al.* (1992).

ing was observed (Fig. 10.11C). Such a block will also decrease the regeneration of ribulose bisphosphate (RuBP), which is the CO_2 acceptor in the carbon reduction cycle (Calvin cycle) of photosynthesis (Farquhar and Sharkey, 1982). RuBP regeneration was observed to be diminished in dehydrated sunflower leaves (Fig. 10.12) and was sufficient to account for the loss in activity for CO_2 fixation (Gimenez *et al.*, 1992). This supports the idea that the chloroplasts could lose important photochemical activities in dehydrated cells although other changes could alter RuBP regeneration as well (Boyer in Kozlowski, 1976; Farquhar and Sharkey, 1982; Ort and Boyer, 1985).

Plant Signals That Trigger the Metabolic Response

These changes in photosynthetic metabolism indicate that certain features of the cell are inhibitory for photosynthesis during dehydration but, since photosynthesis responds differently between species and within the same species, the factors must be variable and somehow under cell control. One possibility is that the removal of photosynthetic products is disrupted, and photosynthesis becomes inhibited by their accumulation. However, this seems unlikely in view of the lack of an immediate effect on photosynthesis when the phloem is disrupted (Huang *et al.*, 1975b) or when the leaves are detached. Photoinhibition also has been proposed as another cause of inhibited photosynthetic metabolism during dehydration (Björkman, 1981; Kriedemann and Downton, 1981; Osmond *et al.* in Turner and Kramer, 1980) and according to this hypothesis, stomatal closure would deprive the chloroplasts of the substrate CO_2 that normally ac-

cepts most of the hydrogen atoms from the photolysis of water. Continued photolysis would result in damage to the thylakoid membranes. There is evidence that photoinhibition can be detected in some species at low water potentials (Björkman and Powles, 1984; Ludlow and Björkman, 1984; Ögren and Öquist, 1985) but the effects on gas exchange appear to be negligibly small (Sharp and Boyer, 1986) and thus unimportant for CO_2 fixation.

Another hypothesis is that the low chemical potential of water may alter enzyme structure and thus inhibit metabolic reactions. Enzyme structure and activity rely on an ordered shell of water around proteins (Klotz, 1958; Rupley *et al.,* 1983) and it seems possible that decreasing the potential of water might alter the order of that water. However, as pointed out earlier and in Chapter 9, dehydration must be extreme before an effect is seen. Indeed, Boyer and Potter (1973) and Potter and Boyer (1973) showed that varying the chemical potential around the chloroplast thylakoid membranes by altering the turgor or osmotic potential had only small effects on their photochemical activity. However, the kind of solute causing the osmotic potential has a large effect on photochemical activity. Santarius (1969) and Santarius and Giersch (1984) convincingly showed that increasing concentrations of sucrose and glucose had little effect on electron transport or cyclic photophosphorylation whereas increasing NaCl was markedly inhibitory. Thus, direct ion effects are more important than the osmotic potential they create.

For a time it was thought that high ABA might alter photosynthetic metabolism (Raschke and Hedrich, 1985) but applying ABA to isolated chloroplasts and intact cells did not cause altered photophosphorylating activity (Boyer, 1973) and removing the epidermis removed the effects of ABA on photosynthesis (Terashima *et al.,* 1988). Thus, although leaf ABA content increases during dehydration and contributes to stomatal closure, no important alterations in photosynthetic metabolism have been substantiated.

As dehydration occurs, the solute environment around enzymes changes because water is removed but the solutes remain in the cell (Boyer, 1983). In sunflower leaves, photosynthesis is markedly inhibited when half the cell water is lost (Fig. 10.2). Solute concentrations would double in this situation, particularly for solutes that cannot be metabolized. Figure 10.13A shows that the photophosphorylating activity was lost in thylakoid membranes from dehydrated spinach leaves (Younis *et al.,* 1979) and that doubling the Mg^{2+} concentration around normal membranes similarly inhibited photophosphorylation (Fig. 10.13B; Younis *et al.,* 1983). Mg^{2+} is a known regulator of photophosphorylating activity (Anthon and Jagendorf, 1983; Pick and Bassilian, 1982; Racker, 1977; Tiefert *et al.,* 1977) and is markedly inhibitory when concentrations increase.

It is noteworthy that this effect persisted after isolating the membranes from the high Mg^{2+} concentrations just as the dehydration effect persists after isolat-

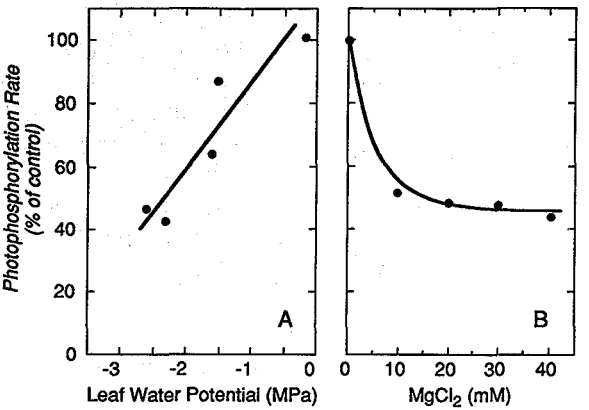

Figure 10.13 Photophosphorylation in thylakoid membranes isolated from spinach leaves having various water potentials (A) or isolated from solutions having various Mg^{2+} concentrations (B). Isolation from Mg^{2+} solutions simulated isolation from the leaf. Higher Mg^{2+} concentrations are expected at lower leaf water potentials. Concentrations of Mg^{2+} are normally about 3 m*M* around the thylakoid membranes in the light and could be significantly higher during dehydration. After Younis *et al.* (1979) and Younis *et al.* (1983).

ing the membranes from dehydrated cells (Fig. 10.13). Moreover, the chloroplast stromal concentration of Mg^{2+} is normally around 3m*M* in the light (Rao *et al.*, 1987) and could readily increase to inhibitory levels of 6 m*M* or higher as the cell loses half its water. Perhaps most significant, there is a change in conformation of coupling factor in high Mg^{2+} that is similar to the one caused by dehydration in the cell (Fig. 10.11B, cf. Fig. 10.11A).

Figure 10.14 shows the likely ionic environment around the chloroplast thylakoids where these effects occur. The change in enzyme conformation for the coupling factor may make the membrane appear thinner in the electron microscope and be associated with the lack of access of ADP to the active site for photophosphorylation. The Mg^{2+} may increase in concentration as dehydration occurs and bind increasingly to the coupling factor protein (Younis *et al.*, 1983). If Mg^{2+} remains bound during isolation of the enzyme, the large amount of bound Mg^{2+} could result in decreased activity *in vitro*. Thus, this conception of the membrane would account for both the *in vivo* and *in vitro* inhibition of photophosphorylation and the persistence of the effects *in vitro*.

In support of this concept of inhibitory solute concentrations, the K^+ in cells is an essential regulator for metabolism (Evans and Sorger, 1966; Evans and Wildes, 1971; Wilson and Evans, 1968), and Berkowitz and Whalen (1985) and Pier and Berkowitz (1987) observed an altered photosynthetic response when leaf tissue of differing K^+ status was exposed to low water potentials. Leaf tissue that was becoming depleted of K^+ appeared to be more inhibited than high K^+

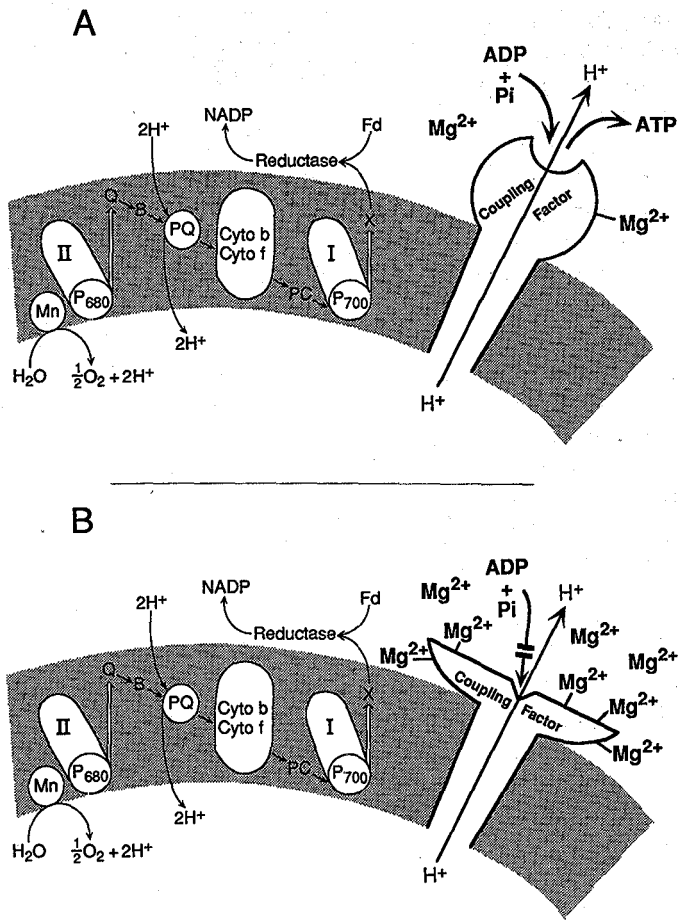

Figure 10.14 Ionic environment around chloroplast thylakoid membrane in a hydrated cell (A, high water potential) or a dehydrated cell (B, low water potential). Note the higher concentration of solutes in (B) than in (A) leading to the binding of more solute (Mg^{2+}) to the coupling factor protein required for photophosphorylation and the changed conformation of the coupling protein associated with a blocked access of ADP to the active site in (B). The binding of Mg^{2+} may account for the persistence of chloroplast inhibition after the membranes are isolated from the cell and for the changes in conformation of the membrane observed *in vivo* and *in vitro*.

tissue when low water potentials occurred. Berkowitz and Kroll (1988) and Gupta and Berkowitz (1987) showed that there may be a differential regulation of chloroplast water content compared to the surrounding cytoplasm. This could alter the ion concentrations in the chloroplast compartment. The solutes might be distributed differently between organelles as water is removed from

the cell and might change the susceptibility to dehydration. Kaiser *et al.* (1986) found evidence that ionic constituents affect the photosynthetic response to low water potentials in an artificial stroma. The activity of ribulose bisphosphate carboxylase was decreased by slightly elevated concentrations of sulfate, phosphate, and occasionally Mg^{2+} in the medium. Such elevations might occur during dehydration. Santarius (1969) and Santarius and Giersch (1984) found that high concentrations of NaCl could inhibit electron transport and cyclic photophosphorylation, but similar concentrations of sucrose or glucose were not inhibitory and partially prevented the effect of NaCl.

These results indicate that the most likely cause of inhibited photosynthetic metabolism in dehydrated plants is the changed solute environment around the enzymes, particularly the ionic environment. Many of these solutes can change concentrations passively as a simple result of the removal of water. Some cannot be metabolized and others may be moved to different compartments. Changes in enzyme activity probably occur because the ions play regulatory roles, and changing the concentration increases or decreases the activity, as also discussed in Chapter 9.

ACCLIMATION

The fact that photosynthesis can acclimate to dehydrating conditions implies that some characteristic of the cell can be altered to put photosynthesis at less risk. Because altered stomatal behavior or cell ion status or compartmentation could be important contributors, the nutritional history of the plants could play a role. In agreement with this concept, Radin and his co-workers (Radin and Ackerson, 1981; Radin and Parker, 1979a,b) showed that plants grown in differing nitrogen regimes had altered stomatal closure at low water potentials. Plants having a low nitrogen status closed their stomata earlier (Radin and Parker, 1979a,b) and had higher ABA levels (Radin and Ackerson, 1981) than plants having a high nitrogen status. This delayed the onset of severe dehydration and represented a form of acclimation for the plant.

Matthews and Boyer (1984) showed that acclimation did not depend solely on stomatal closure but could be observed in photosynthetic metabolism of sunflower leaves. Rao *et al.* (1987) grew sunflower plants at differing Mg^{2+} levels and found that their leaves differed by fourfold in Mg^{2+} content. When the plants were dehydrated, the maximum rate of photosynthetic metabolism was more severely reduced in the high Mg^{2+} leaves than in the low Mg^{2+} leaves. Thus, it was possible to acclimate photosynthetic metabolism to dehydration by altering the Mg^{2+} concentration of the leaves.

Various enzymes were shown to be much less inhibited by photosynthetic products and metabolites such as sugars and amino acids than by inorganic ions like Mg^{2+}, K^+, or Na^+ (Hanson and Hitz, 1982; Yancey *et al.*, 1982; Wyn

Jones, 1980). Cells packed with sugars and amino acids are commonly seen during dehydration (Crowe and Crowe, 1986; Crowe *et al.*, 1987; Meyer and Boyer, 1981; Munns *et al.*, 1979; Yancey *et al.*, 1982). These compounds can accumulate apparently without inhibiting photosynthetic metabolism, and thus their contribution to acclimation is mostly through osmotic adjustment (see Chapter 3) in a way that allows photosynthesis to continue.

These results indicate that there are two features of photosynthetic acclimation that are important. One is a dehydration avoidance that results from delaying the onset of dehydration as seen with nitrogen or osmotic adjustment. Another is a dehydration tolerance that affects photosynthesis less at a particular dehydration as seen with magnesium. The two factors contribute by affecting stomatal closure and enzyme responsiveness to dehydration. With delays in dehydration and less enzyme inhibition, photosynthetic products can continue to be produced and contribute to osmotic adjustment, which maintains hydration levels and further delays the onset of metabolic inhibition. As a consequence, acclimation develops most fully when plants are dehydrated slowly enough to allow the adjustment of regulator pools and the production of noninhibitory solute from photosynthesis.

RECOVERY

The ability to recover photosynthetic capacity is important for the resumption of plant growth when water is resupplied. Plants differ genetically in this respect, and the extent and duration of the preceding dehydration have marked effects. An example is apparent when *Fucus* recovery is compared to sunflower (Fig. 10.15). *Fucus* readily recovers photosynthetic activity after dehydration to 5% relative water content and sunflower recovers readily from 40% water content. However, dehydrating sunflower to a 5% content is lethal.

In *Fucus*, water enters through the surface of the plant but in sunflower, water enters through the roots. The roots and vascular system must function in sunflower in order for rehydration to occur. Boyer (1971b) showed that part of the ability to rehydrate depended on the extent of vascular blockage. Dehydration puts the xylem water under tension and breaks often occur due to cavitations, forming gas embolisms that block water movement (Tyree and Sperry, 1989; also see Chapter 7). The more severe the dehydration, the larger the tension and more frequent the blockage. Leaf water potentials of only -2 MPa were sufficient to cause a major blockage in sunflower stems (Boyer, 1971b). Excising the leaf with its petiole under water removed the blocked part of the vascular system in the lower part of the plant and allowed rehydration and resumption of photosynthesis to occur (Boyer, 1971b).

Even if full rehydration occurred, the ability of photosynthesis to recover depended on the severity of dehydration. Fellows and Boyer (1978) found that

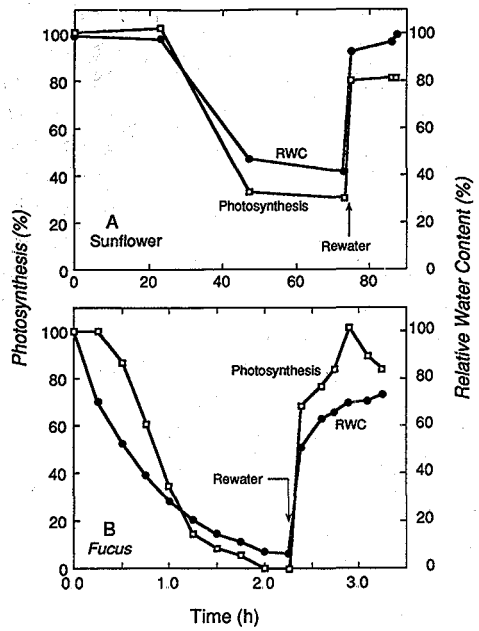

Figure 10.15 Photosynthesis and relative water content of sunflower (A) or *Fucus vesiculosus* (B) at various times after withholding and resupplying water. Sunflower was rehydrated by watering the soil whereas *Fucus* was rehydrated by submerging in seawater. Recovery was not observed in sunflower if relative water contents became as low as in *Fucus*. After Boyer (1971b) and T. Driscoll and J. S. Boyer (unpublished data).

sunflower leaf cells increasingly had breaks in the plasmalemma and/or tonoplast membranes as dehydration became more severe. Figure 10.16 shows that more breaks appeared as the water potentials became lower. The breaks resulted in lysis of the cells which limited the number of living cells that could recover when water was resupplied. Thus, even though the leaf recovered in water potential and appeared normal, the death of some of the cells limited the final extent of photosynthesis recovery. Interestingly, cell death appeared random but under severe conditions, clusters of cells were affected. Patchy photosynthesis, when it occurs during dehydration, may be attributable in part to this patchy cell death. Such patchy death would also cause patchy recovery.

Leopold *et al.* (1981) observed that leaf cells leaked internal solutes when dehydrated (see Fig. 3.3). The leakiness was a measure of the loss of plasmalemma function and was inversely correlated with the ability to recover from severe desiccation. If there was little leakage, recovery was complete but with significant leakage recovery was prevented. The leakage reported by these in-

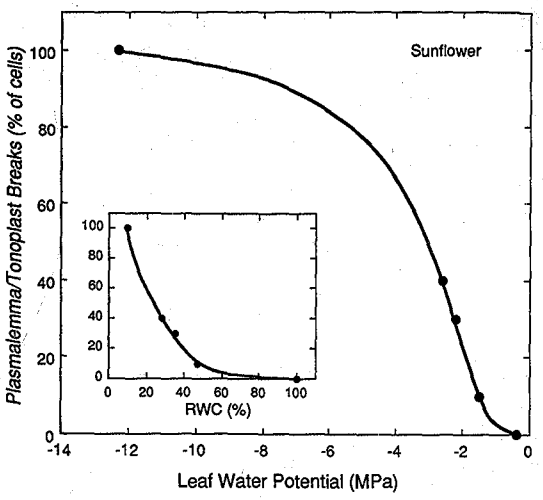

Figure 10.16 Plasmalemma and tonoplast breaks in cells of sunflower leaves having various water potentials (inset: various relative water contents). Breaks were observed under an electron microscope in tissue fixed at the same water potential as in the leaf. After Fellows and Boyer (1978).

vestigators could have resulted from the membrane breakage observed by Fellows and Boyer (1978).

It is clear from ultrastructural studies (Bewley and Pacey, 1978; Fellows and Boyer, 1978; Hallam and Gaff, 1978a,b) that dehydrated cells become severely deformed by their decrease in volume (see Chapter 3). The deformation of the plasmalemma is extreme and is forced on the cell by wall folding as the cell shrinks. The folding may become so severe that the plasmalemma and/or tonoplast become pressed inside the folds (Fellows and Boyer, 1978) which undoubtedly creates large local stresses on the membranes. Perhaps these stresses lead to membrane disruption. However, the chloroplasts generally are less deformed than the plasmalemma because they can move inside the cells.

It has been proposed that much of the ability of cells to survive severe dehydration depends on the ability to maintain the membranes intact (Crowe and Crowe, 1986; Crowe *et al.*, 1987; Leopold *et al.*, 1981). In addition to evidence of physical deformation of membranes in dehydrating cells, evidence has been presented that certain sugars such as trehalose or sucrose can replace water in maintaining membrane structure (Crowe and Crowe, 1986; Crowe *et al.*, 1987; also see Chapter 12). If so, the removal of water would disrupt the plasmalemma and/or tonoplast less if the sugars were abundant. It would be interesting to determine whether the ability of photosynthesis to recover from severe dehydration is related to the tissue content of sugars.

Another common effect of dehydration is an accelerated leaf senescence. Maize leaves senesce prematurely when the plants have insufficient water (Boyer and McPherson, 1975), and leaf senescence appears to be under genetic control through hydrolytic enzymes that increase in activity after the onset of dehydration (Todd in Kozlowski, 1972). Soon afterwards, leaf yellowing and death are observed. Jacobsen *et al.* (1986) found that the activity of α-amylase increased as leaf water potentials became low in seedlings of barley from which water was withheld. Because this activity appeared to be located in the cytoplasm, it was thought to be one of the general class of hydrolytic enzymes involved in the early phases of leaf senescence. The mRNA increased for the enzyme in the wilted leaves which indicated that the activity increase was regulated by gene action rather than by activation of pre-existing enzyme. The new activity appeared to result from the synthesis of new enzyme.

Thus, the genetic basis of accelerated leaf senescence can be altered at least in principle. In plant breeding, it is possible to select for genotypes whose leaves remain green during a water deficiency. The "stay green" character allows photosynthesis a better chance to quickly recover after rain than in genotypes whose leaves senesce. Aparicio-Tejo and Boyer (1983) concluded that premature leaf senescence is undesirable and should be minimized by selecting for genotypes whose leaves remain viable.

TRANSLOCATION

Leaf senescence is associated with the hydrolysis of leaf constituents and the transport of the hydrolytic products from the senescing leaves to viable plant parts. This has the effect of conserving dry mass and mineral nutrients for the plant although the effect is small. However, the ability to transport hydrolyzed products illustrates that the phloem remains viable. Fellows and Boyer (1978) observed that the ultrastructure of leaf phloem remained normal in appearance while that of the surrounding cells showed major disruptions when the plants were exposed to water deficits that caused leaf senescence. The phloem is thus one of the last parts of the leaf to senesce and it is often observed that veins remain green while interveinal tissues show signs of senescence. In general, translocation is one of the most stable plant activities (Munns and Pearson, 1974).

Several studies showed that, when $^{14}CO_2$ was supplied to dehydrated leaves and fixed photosynthetically, the photosynthetic products were translocated more slowly than in controls (Brevedan and Hodges, 1973; Johnson and Moss, 1976; Munns and Pearson, 1974; Sung and Krieg, 1979; Wardlaw, 1967; Watson and Wardlaw, 1981). Comparisons of losses in photosynthetic activity and translocating activity showed inhibitions for both processes (Brevedan and Hodges, 1973; Johnson and Moss, 1976; Munns and Pearson, 1974; Sung and

Krieg, 1979; Wardlaw, 1967). On the other hand, measurements of dry matter transport in dehydrated plants indicated that translocation occurs readily (Jurgens *et al.*, 1978; McPherson and Boyer, 1977; Westgate and Boyer, 1985a); Chapter 12 describes experiments in which the products of photosynthesis are fed to stems and maintain embryo viability in distant reproductive tissues, indicating that translocation is active. Jurgens *et al.* (1978) showed that for the first 24 hr after the onset of dehydration severe enough to inhibit photosynthesis, the translocation of fixed ^{14}C was markedly inhibited but recovered moderately several days later. Thus, much of the severe inhibition of ^{14}C translocation appeared to be transient. Many $^{14}CO_2$ measurements have been conducted for only a few hours after plant dehydration (Brevedan and Hodges, 1973; Johnson and Moss, 1976; Wardlaw, 1967) but the translocation of dry matter generally requires several days to measure (Jurgens *et al.*, 1978; McPherson and Boyer, 1977; Westgate and Boyer, 1985a). Therefore, many differences between results probably can be attributed to differences in times employed for the measurements, and it may be concluded that translocation generally remains active in water-deficient plants except for a few hours after the onset of the deficiency when severe inhibition may be observed.

SUMMARY

Plant productivity is determined mostly by photosynthetic CO_2 fixation, and water is a major limiting factor for photosynthesis in many environments. In land plants, photosynthesis is much more susceptible than respiration to dehydration effects, which implies that there are reactions or features specific to photosynthesis that are the cause. Large differences exist in susceptibility among species, and growth conditions can change the susceptibility within a species, leading to acclimation.

While respiration is active, stored reserves of photosynthetic products are drawn upon whenever photosynthesis is sufficiently inhibited, with some deleterious effects. The continued respiration indicates that water is adequately available as a substrate for metabolism, and photosynthesis probably fails for other reasons. With severe dehydration, respiration also can decrease and eventually will cease.

CO_2 is another substrate for photosynthesis and enters the leaf primarily through stomata, and much work centers on the role of stomatal closure. The stomata lose osmotic quantities of K^+ when they close, probably because abscisic acid levels increase as the leaf dehydrates, and the loss appears to cause the closure. Closure may or may not inhibit photosynthesis depending on the demand for CO_2 by photosynthetic metabolism. If metabolism remains active, CO_2 partial pressures decrease in the leaf as the stomata close, and the decrease can limit photosynthesis. If metabolism is inhibited, the demand for CO_2 dimin-

ishes and the partial pressure of CO_2 can rise even though stomata close. The inhibition is then attributed to the inhibited metabolism because the CO_2 has actually become more available through disuse. This indicates that the stomatal limitation of photosynthesis cannot be decided simply from the stomatal closure that accompanies losses in photosynthesis. Recent methods of measuring CO_2 levels inside the leaf promise to provide a way to test effects of stomatal closure.

Most metabolic stages in the photosynthetic process are inhibited by dehydration, and the inhibition can be seen in the intact leaf. Photophosphorylation appears to be heavily involved, and there are molecular changes in enzyme conformation that correlate with the decreased access of substrates to the photophosphorylating enzymes. Certain causes can be ruled out such as reduced translocation of products, losses in ultrastructural integrity, damage by photoinhibition, and effects of the chemical potential of water directly on the photosynthetic enzymes. Instead, altered concentrations of regulatory solutes, particularly inorganic ions, appear most likely to be responsible. Concentrations of these ions change because of the passive concentrating effects of dehydration and perhaps because of altered compartmentation of the solutes inside the cells. Sugars and amino acids accumulate in dehydrated leaves but do not have the inhibitory action seen with high concentrations of inorganic ions. The accumulation of sugars and amino acids depends on photosynthesis to supply the appropriate substrates and in turn protects photosynthetic metabolism.

This probably explains why photosynthetic metabolism is affected by the water content of cells. Factors that delay the loss of water such as early stomatal closure also delay the concentrating of the cell solution and thus diminish the inhibition of metabolism. Delays in dehydration and changes in cell composition appear to be major mechanisms of leaf acclimation to dehydrating conditions.

During dehydration, leaf cells undergo lysis that becomes pervasive as dehydration becomes more severe. The extent of lysis determines in part how much recovery of photosynthesis will occur when water is resupplied to the leaves. Vascular emboli form during dehydration and can hinder the recovery from dehydration because of inhibited water transport, and photosynthesis may be suppressed for several days after a dehydration/recovery cycle because of this effect or because of incomplete stomatal opening due to inability of the guard cells to accumulate K^+. Leaves also undergo accelerated senescence during dehydration, at least some of which appears to be under genetic control. Translocation continues to be active in dehydrated plants, although there is evidence of transient inhibition for a few hours after dehydration occurs.

SUPPLEMENTARY READING

Boyer, J. S. (1976). Water deficits and photosynthesis. *In* "Water Deficits and Plant Growth" (T. T. Kozlowski, ed.), Vol. 4, pp. 153–190. Academic Press, New York.

Foyer, C. H. (1984). "Photosynthesis." Wiley, New York.
Lawlor, D. W. (1993). "Photosynthesis: Molecular, Physiological and Environmental Processes," 2nd Ed. Longman Scientific & Technical, Essex.
Ort, D. R., and Boyer, J. S. (1985). Plant productivity, photosynthesis and environmental stress. *In* "Changes in Eukaryotic Gene Expression in Response to Environmental Stress' (B. G. Atkinson and D. B. Walden, eds.), pp. 279–313. Academic Press, New York.
Yancey, P. H., Clark, M. E., Hand, S. C., Bowlus, R. D., and Somero, G. N. (1982). Living with water stress: Evolution of osmolyte systems. *Science* **217**, 1214–1222.