8

Stomata and Gas Exchange

INTRODUCTION

Stomata (singular, stoma), sometimes anglicized as stomates, provide an essential connection between the internal air spaces of plants and the external atmosphere. The external surfaces of most herbaceous plants and the leaves of woody plants are covered with a waxy layer of cutin (see Fig. 7.7) which is relatively impermeable to water vapor and carbon dioxide. This enables plants to conserve water in dry air, but it also hinders the entrance of the carbon dioxide essential for photosynthesis. Stomata are pores in the epidermis and associated cuticle bordered by pairs of structurally and physiologically specialized guard cells and adjacent epidermal cells termed subsidiary cells. This group of cells forms the stomatal complex and facilitates gas movement through the epidermis. Stomatal development and structure are discussed in Jarvis and Mansfield (1981), in Weyers and Meidner (1990), and in anatomy texts. In the absence of stomata the supply of carbon dioxide for photosynthesis would be inadequate for survival of most plants, but at the same time the unavoidable loss of water vapor through them creates the danger of dehydration. Thus, the ability of stomata to adjust their aperture is extremely important to the success of plants (Cowan, 1982; Raschke, 1976).

Historical Review

Malpighi observed the presence of pores in leaves in 1674 and in 1682 Grew pictured them in his plant anatomy. Apparently A. de Candolle applied the term

"stomata" in 1827 and the study of stomatal behavior began with von Mohl about the middle of the 19th century. The history of early research on stomata was reviewed briefly by Meidner (1986). The study of diffusion through small pores such as stomata was placed on a sound physical basis by the research of Brown and Escombe (1900). This was followed by the work of Stålfelt (1932, 1956a) and Bange (1953) who showed that in moving air, where the boundary layer resistance is low, transpiration is closely correlated with stomatal aperture. Various investigations (see Mansfield, 1986, p. 202) showed that ABA increases in water-deficient leaves and that an external application of ABA usually causes stomatal closure. This led to the concept that stomatal closure in water-deficient plants often is caused by chemical signals from the roots. This was reviewed by Davies and Zhang (1991) and Davies *et al.* (1994), and is discussed later in this chapter and in Chapter 5 in the section on roots as sensors of water deficits, also in Chapter 10.

Occurrence and Frequency

Stomata occur on stems, leaves, flowers, and fruits, but not on aerial roots. They occur on both surfaces of many leaves (amphistomatous) or on only one surface, usually the lower (hypostomatous), especially in woody plants. Common exceptions among woody plants are poplar and willow which are amphistomatous. The adaptive importance of this is not clear (Parkhurst, 1978). Stomata vary widely in size and frequency, as shown in Table 8.1, and species with smaller stomata usually have a higher frequency. The frequency ranges from 60 to 80 per mm^2 in corn to 150 in alfalfa and clover, 300 in apple, and over 1000 in scarlet oak. There often are variations in number in various parts of a leaf (Smith *et al.*, 1989) and among genotypes of a species (Muchow and Sinclair, 1989). Additional data on frequency can be found in Meyer *et al.* (1973, pp. 74–75), Miller (1938, p. 422), and Weyers and Meidner (1990). In monocots, conifers, and some dicots, stomata occur in parallel rows, but in leaves with netted venation they are scattered. They sometimes are sunken below the surface but occasionally are raised, and usually they open into substomatal cavities in the mesophyll tissue. They are easily visible on leaf surfaces under magnification because of the peculiar shape of the guard cells (Figs. 7.8, 8.1, and 8.2) and the fact that guard cells, unlike other epidermal cells, usually contain chloroplasts. When wide open, stomatal pores usually are 3–12 μm wide and 10–30 μm or more in length. Usually, specialized epidermal cells, called subsidiary cells, are associated with the guard cells and play a role in guard cell functioning. According to Meidner (1990) and Stålfelt (1956a), the full opening of stomata is associated with a slight decrease in turgor of epidermal cells.

Table 8.1 Representative Dimensions and Frequencies of Guard Cells

Plant type	Representative species	Comments	Stomatal frequency (pores · mm^{-2})		Guard cell dimensions (μm)				Dimensions of stomatal pore (lower surface, μm)		Pore area as proportion of total leaf area if open to 6 μm (%)
					Upper surface		Lower surface				
			Upper	Lower	Length	Width	Length	Width	Length of pore	Depth of whole pore	
Moss	*Polytrichum commune*	Stomata present on sporophyte only	16	16	46	15	46	15	15	12	—
Fern	*Osmunda regalis*	Many chloroplasts in guard cells	0	67	—	—	56	19	30	15	0.5
Gymnosperm tree	*Pinus sylvestris*	Sunken stomata, needle-like leaf	120	120	28	7	28	7	20	6	1.2
Dicot tree	*Tilia europea*	Hypostomatous leaf	0	370	—	—	25	9	10	8	0.9
Dicot herb	*Helianthus annuus*	Typical mesophyte	120	175	35	13	32	14	17	—	1.1
Dicot herb (xerophyte)	*Sedum spectabilis*	Succulent leaves	35	56	32	10	33	10	20	18	0.4
Monocot herb	*Allium cepa*	Cylindrical leaves, elliptical guard cells	175	175	42	19	42	19	24	18	2.0
Monocot C_3 grass	*Avena sativa*	Graminaceous guard cells	50	45	52	15	56	13	20	10	0.5
Monocot C_4 grass	*Zea mays*	Graminaceous guard cells	98	108	38	10	43	12	20	10	0.7

Note. The mean values quoted are derived from Meidner and Mansfield (1968) with additional data estimated by the authors. "Upper" and "lower" surfaces refer to leaves except in *Polytrichum;* note that there is no differentiation of surfaces in *Allium, Pinus,* and *Polytrichum.* Dashes indicate that category is not applicable. It should be appreciated that the values of parameter shown depend on cultivars, growth conditions, insertion level of leaf, and other factors. From Weyers and Meidner (1990).

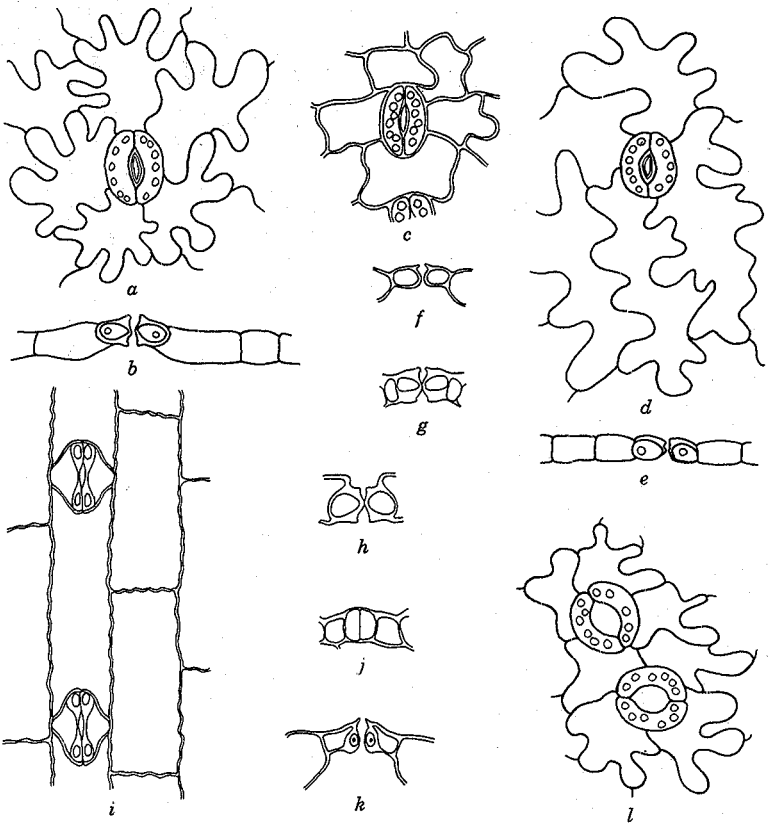

Figure 8.1 Various types of stomata: (a,b) *Solanum tuberosum* in face view and in cross section; (c) apple; (d,e) *Lactuca sativa;* (f) *Medeola virginica;* (g) *Aplectrum hyemale;* (h) *Polygonatum biflorum;* (i,j,k) *Zea mays.* Part (i) is a face view; (j) is a cross section near the ends of guard cells; (k) is a cross section through the center of a stoma; and (l) is a face view of *Cucumis sativus.* From Kramer (1983), after Eames and MacDaniels (1947), by permission of McGraw-Hill.

STOMATAL FUNCTIONING

Guard Cells

The walls of guard cells bordering the pores usually are thickened and sometimes have ledges and projections that extend into the pores, as shown in Figure 7.8A and in Weyers and Meidner (1990, p. 8). Wax filaments also often extend into stomatal pores, especially in conifers (Gambles and Dengler, 1974; Jeffree *et al.,* 1971). The thickening of the inner walls was supposed to play an essential role in causing turgid guard cells to bulge and separate, opening the

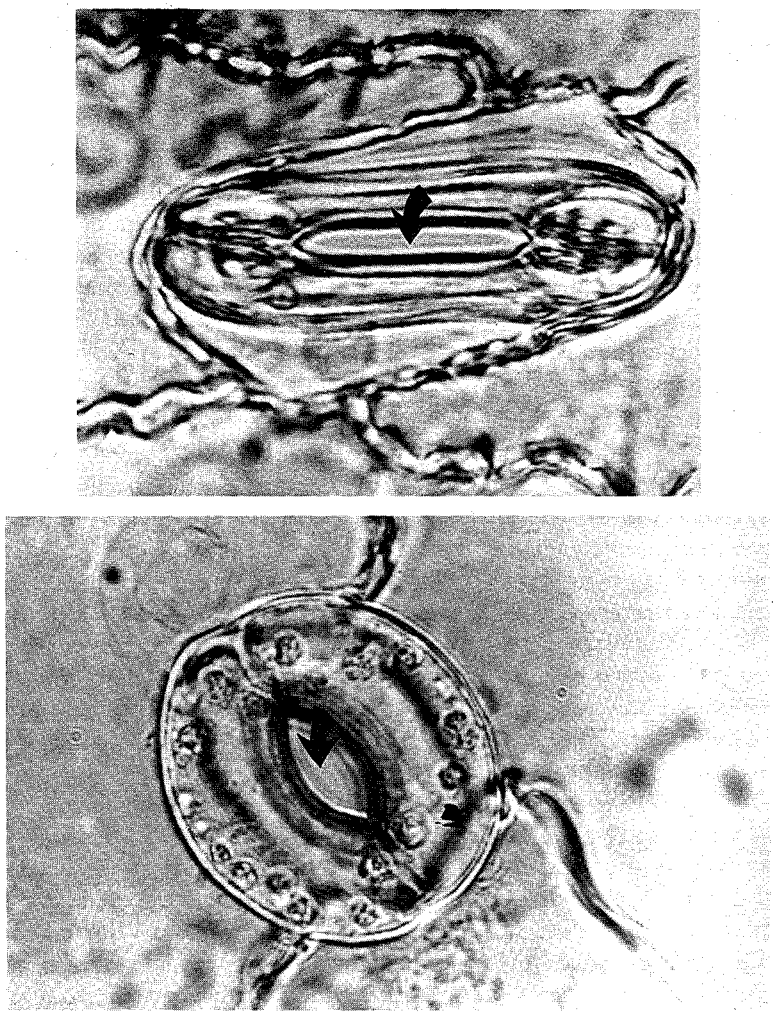

Figure 8.2 (Top) An open stoma of maize, typical of stomata of grasses. (Bottom) An open stoma of bean, typical of most dicots. From Kramer (1983). Courtesy of J. E. Pallas, U.S. Department of Agriculture.

stomatal pores, but Aylor *et al.* (1973) concluded that the micellar structure of the cell wall is more important than the thickening. This is discussed in Mansfield (1986, p. 160). Guard cells are often described as kidney or bean shaped, but those of grasses (Fig. 8.2) are elongated and the ends are enlarged, resembling dumbbells, and various other shapes occur. When wide open the stomatal pores occupy from less than 1 to 2% or more of the leaf surface. Inter-

esting scanning electron micrographs of leaf surfaces, stomata, and leaf interiors can be found in Troughton and Donaldson (1981).

Stomatal Behavior

The most important characteristic of stomata is that they open and close, and the change in size of their aperture regulates gas exchange. In general they are open in the light and closed in darkness, although the stomata of plants with Crassulacean acid metabolism (CAM plants) behave in the opposite manner, being largely closed during the day and open at night. CAM plants have the capacity to fix large amounts of CO_2 in darkness as malic acid. This is decarboxylated during the day, releasing CO_2 that is refixed into carbohydrates in the light by photosynthesis. A comparison of daily cycles of CO_2 exchange and transpiration of the C_3 plant, sunflower, and the CAM plant, *Agave americana*, is shown in Fig. 8.3. This behavior greatly reduces water loss without an equiva-

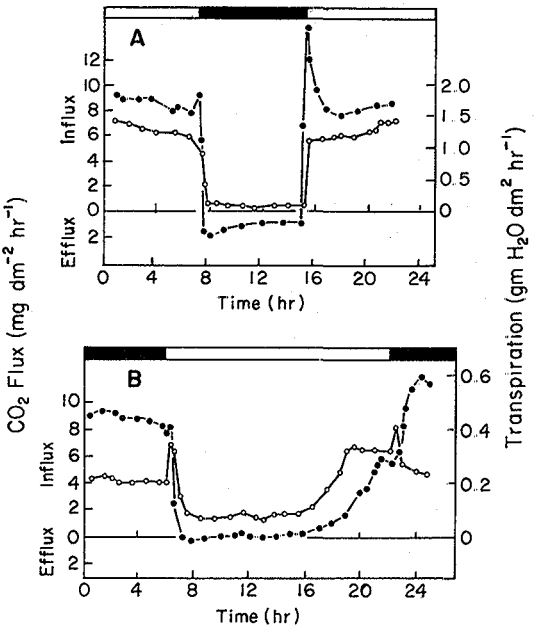

Figure 8.3 Comparison of daily cycles of carbon dioxide exchange (●) and transpiration (○) of the C_3 plant, sunflower (A), and the CAM plant, *Agave americana* (B). Both carbon dioxide uptake and transpiration of sunflower ceased in darkness, and there was some efflux of carbon dioxide released by respiration. The situation was reversed in *Agave* with little transpiration and no carbon dioxide uptake during most of the light period. Note that the units for transpiration are about four times greater for sunflowers than for *Agave* and that the transpiration rate of sunflower is proportionately greater. Adapted from Neales *et al.* (1968), from Kramer (1983).

lent decrease in dry matter production because the rate of transpiration is low at night. It is found in a number of succulents and other plants of dry habitats; pineapple is the best example among crop plants. Its leaves are heavily cutinized and its stomata are at the bottom of deep furrows covered with hairs and do not open until late afternoon or evening. As a result it has a very high water use efficiency, using only 50 or 55 g of water per gram of dry matter produced (Joshi *et al.*, 1965) compared with several hundred grams for most crop plants (Table 7.3). Crassulacean acid metabolism has been discussed in detail by Kluge and Ting (1978), Osmond (1978), and Ting (1985).

Mechanism of Stomatal Opening and Closing

The opening of stomata requires an increase in turgor of guard cells while closing requires a decrease in turgor. Although explanation of the cause of turgor change has been drastically revised in recent years, many questions remain unanswered (Kearns and Assmann, 1993). Originally, changes in turgor were attributed to changes in proportions of starch and sugar in guard cells (Lloyd, 1908; Sayre, 1926). It was believed that in light when photosynthesis removed CO_2 the increase in pH resulted in hydrolysis of starch to sugar, causing a decrease in osmotic potential and an increase in turgor as water entered. Decreasing light intensity and photosynthesis resulted in an accumulation of CO_2, decreasing pH and causing conversion of sugar back to starch. This neat explanation was rendered obsolete by observations in Japan and by the work of Fischer (1968b), Fischer and Hsiao (1968), and Fischer (1971) showing that the transport of K^+ in and out of guard cells is chiefly responsible for changes in turgor (see Mansfield, 1986, p. 164). Actually, Macallum observed in 1905 that the K^+ concentration was much higher in guard cells of open stomata than in those of closed stomata, but the significance of this early observation was neglected for more than half a century in favor of Lloyds' explanation. It now seems to be well established that the concentration of K^+ in guard cells of open stomata is several times greater than that in the surrounding cells, and there appears to be a good correlation between the K^+ content of guard cells and stomatal aperture. In the cell, the K^+ is accompanied by various anions that balance the positive charge on K^+. Some guard cells take up Cl^- as a balancing anion but organic acids also can be synthesized internally and serve the same function. This brings back a possible role for starch as the source of organic compounds, including sugar and organic acids, chiefly malic (Outlaw and Manchester, 1979). There seems to be renewed interest in the carbohydrate metabolism of guard cells (Hite *et al.*, 1993). However, this is complicated by the fact that onion guard cells contain no starch, yet function normally (Schnabl and Ziegler, 1977).

The guard cell chloroplasts exhibit fluorescence transients resembling those of mesophyll chloroplasts (Ogawa *et al.*, 1982; Outlaw *et al.*, 1981; Zeiger

et al., 1980), and K^+ and abscisic acid affect the transient as though energy from guard cell chloroplasts is used to accumulate K^+ (Ogawa *et al.*, 1982). Evidence exists that photophosphorylation occurs in these chloroplasts (Grantz *et al.*, 1985a; Shimazaki and Zeiger, 1985) and that CO_2 fixation probably occurs as well, although this has been a controversial area (Outlaw, 1989). According to Cardon and Berry (1992), guard cell chloroplasts probably carry on photosynthesis that is similar to that occurring in mesophyll cells, although it may be slow (Reckmann *et al.*, 1990). It now seems most likely that CO_2 is fixed chiefly by the enzyme phosphoenolpyruvate carboxylase and that the oxaloacetate product is reduced to malate (Scheibe *et al.*, 1990) that balances some of the charge of the incoming K^+. The malate together with incoming Cl^- thus form osmoticum that adds substantially to the osmotic effect of the incoming K^+. It is likely that mitochondrial respiration can supply the energy for opening in the absence of guard cell photophosphorylation and photolysis since opening can occur in the dark under certain conditions, particularly low CO_2 (Fischer, 1968a; Raschke, 1972). The starch of guard cell chloroplasts probably serves as a store of carbon compounds that can be used for energy as well as for organic counterions for K^+ (Raschke, 1975; Zeiger, 1983). The K^+ available in fertile soils appears to be sufficient for guard cell function (Ishihara *et al.*, 1978).

The specialized CO_2 fixation of guard cells can sometimes be seen in the response to CO_2 concentration which would ordinarily be expected to enhance opening at high concentrations. However, the reverse often occurs and stomata open more fully at low CO_2 concentrations, indicating that CO_2 fixation is different from that in leaf mesophyll cells. So far, an explanation of this behavior has not been forthcoming, although Mansfield *et al.* (1990) suggest that there could be more than one process competing for CO_2 one of which is inhibitory and the other stimulatory for opening. Opening would be affected according to whichever process dominates.

The loss of K^+ that results in stomatal closure can be brought about by elevated levels of abscisic acid around the guard cells (Ehret and Boyer, 1979; Mansfield and Jones, 1971) and this probably is the main means of closure (Harris and Outlaw, 1991; Neill and Horgan, 1985). Because the guard cells can metabolize and thus inactivate abscisic acid (Grantz *et al.*, 1985b), they exert considerable local control over the opening and closing process. This suggests that there could be some variability in stomatal aperture across a leaf because of variable rates of local breakdown of the abscisic acid. It is commonly observed that leaves have a statistical distribution of openings as described by Laisk *et al.* (1980) and rarely have all their stomata at the same aperture. The loss of K^+ that also occurs during stomatal closure in water-deficient leaves is found whether the roots are present or not (Ehret and Boyer, 1979) and further indicates that local synthesis and metabolism of abscisic acid probably account for much of the opening and closing response during water deficits.

Readers who wish to learn more about guard cell metabolism are referred to Mansfield (1986), Zeiger *et al.* (1987), Cardon and Berry (1992), and the current literature. Metabolic inhibitors such as sodium azide and the absence of oxygen prevent stomatal opening (Walker and Zelitch, 1963), emphasizing the dependence of opening on metabolic processes. Kearns and Assmann (1993) point out that the process of stomatal closure is not exactly the reverse of stomatal opening.

FACTORS AFFECTING STOMATAL APERTURE

The changes in guard cell turgor that bring about stomatal opening and closing are dependent on a number of environmental factors, including light, carbon dioxide concentration, humidity, and temperature (Schulze and Hall, 1982), and on internal factors such as tissue water status and the level of such plant growth regulators as ABA and cytokinins. Complex interactions often exist among these factors which make it difficult to distinguish the relative importance of individual factors such as light and CO_2 or water status and ABA. Information about these interactions can be found in Burrows and Milthorpe (in Kozlowski, 1976) and Weyers and Meidner (1990, pp. 27–30). Ball and Berry (1982), Ball *et al.* (1987), and Collatz *et al.* (1992) have proposed a simple model to account for the interactions.

The relationship among stomatal conductance, transpiration, and photosynthesis of a larch tree and some environmental factors is shown in Fig. 8.4. However, it is not entirely clear how stomatal conductance responds to these environmental signals. Collatz *et al.* (1991), drawing on earlier work, suggested that responses of stomata to environmental factors be divided into two groups, those dependent on photosynthesis and those independent of photosynthesis, but there are important interactions between the two groups. The role of stomatal conductance with respect to photosynthesis has been discussed in detail by Cowan (1982) and by Farquhar and Sharkey (1982). The latter concluded that although stomatal conductance substantially limits transpiration, it rarely seriously limits photosynthesis because the latter is limited by other factors in addition to those contributing to stomatal closure. Collatz *et al.* (1991, p. 122) state that the primary factor causing a midday decrease in stomatal conductance is a decrease in net photosynthesis, related to rise in leaf temperature above the optimum for photosynthesis but this must be a special case. The temperature rise is said to be caused by low boundary layer conductance.

The Role of Light

Although it has been known for many years that stomata usually open in the light, it has been difficult to determine whether this is a direct effect of light or

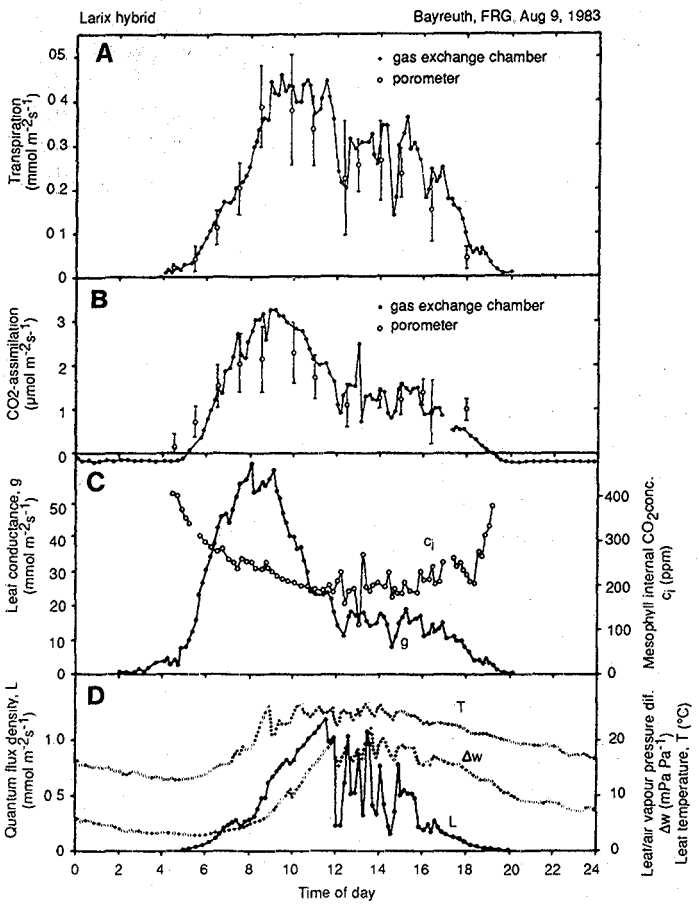

Figure 8.4 Relationships among (A) the daily course of transpiration, (B) CO_2 uptake during the day and efflux at night, and (C) leaf conductance (g) and internal CO_2 concentration (C_i) of a larch tree. Air temperature (T), vapor pressure deficit (Δw), and light (L). From Schulze *et al.* (1985), by permission of the author and *Oecologia*.

whether it occurs because photosynthesis decreases the internal concentration of CO_2. However, the effect of light, independent of its role in photosynthesis, has now been demonstrated (see reviews by Mansfield, 1986, pp. 181–191; Zeiger, 1983, pp. 460–463). It is believed that two photoreceptors are involved; one that is sensitive to red and far red light and another that absorbs in the blue and ultraviolet (Hsiao *et al.*, 1973; Ogawa *et al.*, 1978). These seem similar, or possibly identical, to the systems controlling photomorphogenetic processes such as photoperiod and phototropism. They must operate by affecting the amount and direction of ion transport across guard cell membranes (Serrano

et al., 1988). Although the relative importance of red and blue light in stomatal opening is of physiological interest, plants are exposed to both wavelengths in the field. Adding to the complexity is the fact that plants often show rhythmic opening and closing of stomata after being transferred to continuous darkness.

A considerable difference seems to exist among plants in the response of stomata to light. Kitano and Eguchi (1992a) found that increasing irradiance from 135 to 540 w/m^2 caused oscillation in stomatal conductance, transpiration, water absorption, plant water balance, and leaf expansion of cucumber seedlings with a period of about 20 min. However, Schulz *et al.* (1993) reported that although *Impatiens pallida* wilted immediately in direct sunlight, stomatal conductance increased at first and only decreased after several hours. Wilting reduced the heat load on the leaves about 40% and reduced transpiration significantly, but caused little reduction in net photosynthesis. This is puzzling and deserves further investigation.

Most research on the effects of light on stomatal behavior has been done with relatively constant light intensity. However, leaves within canopies and on the forest floor are commonly exposed to rapid fluctuations in light intensity, known as sunflecks. Pearcy (1990) reviewed the literature on the effects of sunflecks on photosynthesis. Cardon *et al.* (1994) reported that oscillations in light intensity cause stomatal conductance to vary widely, either above or below that in steady light, depending on the frequency of the oscillations. They also reported that oscillations in the carbon dioxide concentration of the atmosphere, such as those caused by air turbulence, can cause oscillations in stomatal conductance. The ecological significance of such oscillations probably deserves more study.

Carbon Dioxide

The stomatal aperture of many kinds of plants is approximately inversely proportional to the CO_2 concentration in both light and darkness, increase in CO_2 in the intercellular or ambient air causing closure and decrease causing opening. According to Mott (1988), stomata respond only to changes in the intercellular CO_2 concentration, but this is affected by the external concentration. Ball and Berry (1982) suggested that the ratio of internal to external concentration of CO_2 is important in controlling stomatal aperture. Rogers *et al.* (1983) reported that the stomatal conductance of corn, soybean, and sweet gum decreased about 50% when the concentration of CO_2 in open topped outdoor chambers was increased from ambient to 910 ppm, but photosynthesis of soybean and sweet gum increased 65 to 70%. There was no increase in photosynthesis of corn which has the C_4 carbon pathway that is saturated at a low CO_2 concentration. The reaction to CO_2 seems to vary with light intensity, temperature, humidity, and presence of ABA (Raschke, 1986). However, the stomata of some conifers seem less responsive to CO_2 than those of most other plants (Jarvis in Turner and Kramer, 1980). The complex relations of stomatal

behavior to CO_2 are discussed in detail by Mansfield *et al.* (1990, pp. 61–67). The effects of CO_2 on photosynthesis and stomatal behavior also were discussed in Chapter 4 of Lemon (1983) and by Raschke (1986). As CO_2 concentration affects photosynthesis and the latter affects stomatal aperture, there may be indirect effects through photosynthesis on stomatal conductance. However, the exact mechanism by which CO_2 affects guard cells remains uncertain (Kearns and Assmann, 1993). Peñuelas and Matamala (1990) reported that the stomatal density and nitrogen content of leaves have decreased since the CO_2 concentration of the atmosphere has increased. This is based on a study of leaves of various ages preserved in herbaria.

Humidity

It was long assumed that the midday closure of stomata was caused by loss of leaf turgor, but it has now been demonstrated that exposure of the epidermis to dry air also causes closure in at least some kinds of plants (Hirasawa *et al.*, 1988; Lange *et al.*, 1971; Schulze *et al.*, 1972; Sheriff, 1977b). Hall and Kaufmann (1975) found closure of stomata of sesame and Kaufmann (1976) found closure of spruce stomata in dry air while Lawlor and Milford (1975) reported that stomata of sugar beets could be kept open in humid air even when a water deficit existed in the leaves. However, some plants show less response to humidity; for example, camellia and privet (Wilson, 1948) and *Atriplex halimus* and *Kochia* (Whiteman and Koller, 1964). It is uncertain how much of the difference in response reported by various investigators is intrinsic and how much is related to previous treatment and differences in experimental methods. Mansfield (1986, pp. 194–202) discussed the effects of humidity in detail and emphasized Meidner's idea that the effect of humidity on evaporation from guard cells is important. However, Nonami and Schulze (1989) found that the water potential of the mesophyll cells in transpiring leaves was lower than that of epidermal cells. This suggests that transpiration from epidermal and guard cells is less important than sometimes claimed.

Aphalo and Jarvis (1991) found that stomatal conductance is better correlated with a vapor pressure deficit than with humidity in *Hedera helix*, and Assmann and Grantz (1990) found a similar situation in sugarcane and sorghum. Of course the vapor pressure gradient is greatly affected by temperature, as shown in Fig. 7.3. Kaufmann (1982) found a good correlation between stomatal conductance and the difference in absolute humidity between air and leaves in subalpine forest trees. In fact, he concluded that temperature effects on stomata in those trees could be explained fully in terms of photosynthetic photon flux density and the difference in absolute humidity between leaves and air, except following freezing nights or during severe water deficiency. However, Mott and Parkhurst (1991) concluded that stomata do not respond

directly either to the absolute humidity at the leaf surface or to the difference in vapor pressure between the interior and exterior of leaves, but to the rate of transpiration.

Schulze (1986b) discussed the effects of dry soil and dry air on stomatal behavior; the role of signals from roots is discussed in the section on internal factors later in this chapter. Sojka and Stolzy (1980) found that oxygen deficiency in wet soil causes stomatal closure and speculated that closure caused by deficient soil aeration may be as important as that caused by deficient moisture.

Temperature

The effects of temperature on stomatal aperture and the rate of response to stimuli vary among different kinds of plants (Meyer and Anderson, 1952). It is difficult to separate direct effects of temperature from indirect effects caused by larger vapor pressure deficits associated with increasing temperature (see Chapter 7 and Fig. 7.3). Temperature effects were so small in Kaufmann's (1976, 1982) study of subalpine forest trees that he could disregard them except near freezing. In contrast, Wuenscher and Kozlowski (1971) found that in five Wisconsin tree species stomatal aperture decreased and stomatal resistance increased significantly as the temperature increased from 20 to 40°C; the change was the greatest for trees that normally grow on dry sites. However, the vapor pressure deficit also varied in this study. Pereira and Kozlowski (1977) found considerable interaction between light intensity and temperature on stomatal aperture of sugar maple, as shown in Fig. 8.5. Wilson (1948) found that the responses of

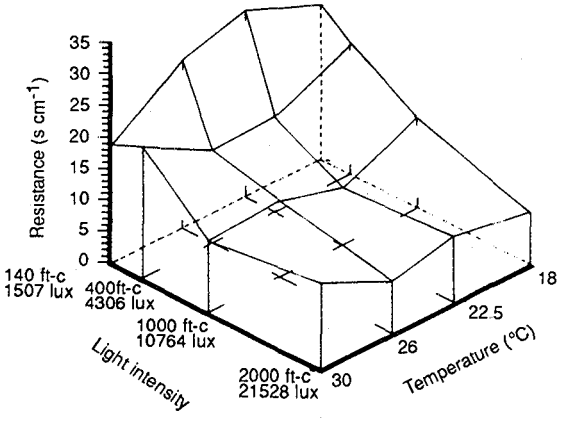

Figure 8.5 Interacting effects of light and temperature on stomatal resistance of sugar maple (*Acer saccharum*). From Pereira and Kozlowski (1977), by permission of the authors and the *Canadian Journal of Forest Research*.

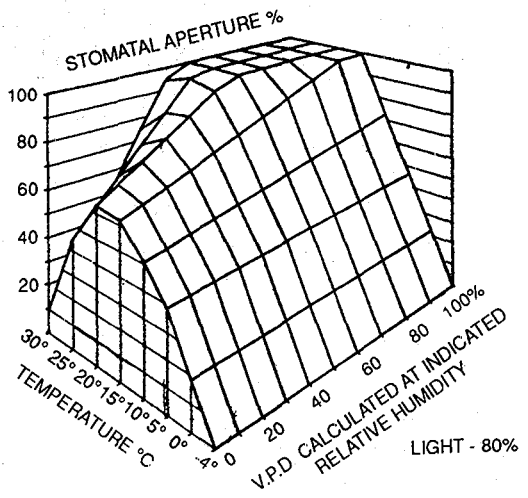

Figure 8.6 Effect of temperature and vapor pressure deficit on stomatal aperture of camellia at 60% of full sun. From Wilson (1948).

stomata of camellia and privet to light and humidity were slowed and that stomatal resistance increased at low temperatures, as shown in Fig. 8.6.

There are important differences in the stomatal reaction to low temperature among plants of various species; there is no increase in the stomatal resistance of collards (*Brassica oleracea,* var. *viridis*) at 5°C, but there is a large increase in cotton and bean (McWilliam *et al.,* 1982). A slow closure at low temperatures was observed in cotton and bean (McWilliam *et al.,* 1982), but not in soybean at 10°C (Musser *et al.,* 1983). An increased opening was observed at low temperatures in garden bean (*Phaseolus vulgaris*) but a pre-exposure to cool temperatures eliminated the effect (Wilson in Raper and Kramer, 1983), indicating that stomata can acclimate to low temperature conditions.

Wind

The effect of wind on transpiration was discussed in Chapter 7, and the varying effects on transpiration of plants of different species are shown in Fig. 7.5. This probably results at least in part from varying effects on stomata and in part from effects on the boundary layer conductance. It also affects the latent heat flux from leaves. Although there have been few studies on the effect of wind on stomatal conductance, it appears that it usually decreases with increasing wind velocity (Burrows and Milthorpe in Kozlowski, 1976, pp. 126–127). Davies *et al.* (1974) reported that stomata of American ash and sugar maple had lower conductances at wind speeds of 0.6 to 2.7 m sec^{-1} than in quiet air, and students sometimes find that when they turn a fan on plants to increase transpiration

it actually is decreased by stomatal closure. According to Kitano and Eguchi (1992b), a sudden increase in wind velocity caused strong cycling in absorption, transpiration, and stomatal conductance of cucumber plants in bright light, but less in low light and none in darkness. It also has been reported that shaking plants or branches causes stomatal closure, and both dehydration and shaking may be involved in decreased stomatal conductance in wind. Perhaps this problem deserves more research.

Mineral Nutrition

There seems to be some uncertainty concerning the role of mineral nutrition in respect to stomatal behavior, perhaps partly because of lack of research on the problem. Desai (1937) reported that deficiency of nitrogen, phosphorus, and potassium all reduced stomatal responsiveness in several kinds of plants, and Pleasants (1930) reported that nitrogen deficiency reduced stomatal response to water deficit and resulted in increased transpiration in bean seedlings. In contrast, Radin and Parker (1979b) found that nitrogen deficiency caused stomata of water-deficient cotton plants to close sooner. However, further research by Radin and Boyer (1982) indicated that nitrogen deficiency increases root resistance of cotton and the resulting leaf water deficit probably was the cause of early stomatal closure. Radin (1984) found that the stomata of phosphorus-deficient cotton plants closed before the leaf mesophyll cells lost their turgor and suggested that phosphorus deficiency might affect the balance between ABA and cytokinins. Calcium appears to be involved as a second messenger in signal transduction in various events triggered by red light and involving phytochrome in plant cells (Hepler and Wayne, 1985), and probably is involved in stomatal reactions (Mansfield *et al.*, 1990; McAinsh *et al.*, 1990). There is further discussion of the relationship between mineral nutrition and stomatal behavior in Chapter 10.

Stomata and Air Pollution

The effect of air pollutants such as SO_2, ozone, and fluorides on stomata is important because stomata are the pathway for the entrance of most gaseous pollutants into leaves. There is likely to be much less leaf injury from fumigation with SO_2 or ozone when stomata are closed than when they are open (see references in Omasa *et al.*, 1985a; Kozlowski *et al.*, 1991, pp. 365–366). Low concentrations of SO_2 are said to cause stomatal opening in moist air, but not in dry air (Mudd in Mudd and Kozlowski, 1975), but this may not be true for all species. Omasa *et al.* (1985a) found that 1.5 μl of SO_2 per liter of air caused patchy stomatal closure and tissue injury to sunflower leaves, and there was wide variation in the degree of closure among stomata in various parts of leaves. Olszyk and Tingey (1986) found ozone twice as effective as SO_2 in causing sto-

matal closure in pea and saw evidence of synergistic effects between the two. In preliminary experiments on young beech trees, Pearson and Mansfield (1993) found that ozone causes stomatal closure on well-watered trees, but keeps them open on water-deficient trees. According to Martin *et al.* (1988), data from both short- and long-term experiments suggest stomatal limitation of photosynthesis in polluted air.

Occasionally, stomata are plugged by particulate material such as dust and soot. This sometimes occurs on foliage near sources of dust such as the volcanic explosion of Mt. St. Helens, cement plants, and along dusty roads. Injurious accumulations of smoke and soot are said to occur on evergreen foliage in cities, but Rhine (1924) reported that stomata of at least some conifers are partially plugged by naturally occurring wax that is not related to smoke injury. This is corroborated by observations of Gambles and Dengler (1974) and Jeffree *et al.* (1971). Some conflicting evidence on the importance of particulate material is presented in Mudd and Kozlowski (1975).

Stomata and Fungi

Arntzen *et al.* (1973) reported that a toxin produced by *Helminthosporium maydis* causes rapid closure of stomata on leaves of corn plants containing the Texas male-sterile gene, possibly by inhibiting the uptake of K^+ by guard cells. However, fusicoccin slows stomatal closure of alfalfa, and Turner (1970) suggested that the drying of alfalfa hay might be hastened by spraying alfalfa with fusicoccin a few hours before cutting. Some effects of pathogenic organisms are discussed by Ayres in Jarvis and Mansfield (1981).

Internal Factors Affecting Stomata

Guard cell behavior and stomatal aperture are affected by internal factors such as leaf water status, internal CO_2 concentration, and growth regulators, especially ABA and cytokinins. As indicated earlier, changes in stomatal conduction seem to be correlated with changes in the rate of photosynthesis or at least in photosynthetic capacity (Wong *et al.*, 1979). According to McCain *et al.* (1988), NMR spectroscopy indicated that chloroplasts in sun leaves contain less water than those of shade leaves, but the significance of this is unknown.

Formerly it was supposed that loss of turgor in leaf cells was the principal cause of the midday closure of stomata observed on dry, sunny days. However, experiments involving split root systems and use of pressure to keep the shoots turgid, although part of the root system is water deficient (Davies and Zhang, 1991; Davies *et al.*, 1994; Gollan *et al.*, 1986), indicate that signals from drying roots can cause stomatal closure even in turgid shoots. The signals might include decreases in amino acids, ions, and cytokinins, but an increase in ABA probably is the chief signal. Generally the ABA concentration increases in roots

of water-deficient plants, and Davies and Zhang (1991) and Davies *et al.* (1994) discuss in detail the possible role of roots as detectors of increasing soil water deficit and sources of chemical signals to the shoots that can modify or override the effects of shoot water status. The failure of stomata to close in "wilty" tomatoes (Tal, 1966) was attributed by Livne and Vaadia (in Kozlowski, 1972) to their inability to synthesize ABA. Stomata often fail to reopen immediately after water-deficient plants are rewatered (Fischer, 1970) and this has been attributed to persistence of a high concentration of ABA, although experiments of Beardsell and Cohen (1975) and Harris and Outlaw (1991) led them to question this. On the other hand, cytokinins promote opening and interact with ABA.

It seems possible, as Trewavas (1981) suggested, that too much emphasis is being placed on the role of ABA. Closure of stomata is not well correlated with the ABA content of leaves in all plants (Ackerson, 1980) and stomata sometimes remain open in leaves high in ABA or stay closed or partly closed after the ABA concentration has decreased (Beardsell and Cohen, 1975). Trejo *et al.* (1993a) reported that ABA is rapidly metabolized in mesophyll tissue, and this probably has contributed to the uncertainty concerning the role of ABA in stomatal closure. Stomatal sensitivity to ABA also varies with the water status of the tissue. Munns and King (1988) suggest that some compound in the xylem sap in addition to ABA must be involved in stomatal closure in wheat. It also is difficult to reconcile the midday closure and late afternoon reopening of stomata of plants in moist soil on hot, sunny days with control by ABA from roots (Kramer, 1988).

In their review, Mansfield *et al.* (1990) suggest that calcium ions may also affect stomatal aperture, as Ca^{2+} ions reduce it on epidermal strips. It is even suggested that such varied stimuli as darkness, ABA, and cytokinins use calcium ions as second messengers. Ehret and Boyer (1979) reported that large losses of K^+ occur from guard cells of leaves on slowly dehydrated plants and suggested that this contributes to loss of guard cell turgor in water-deficient plants.

ANOMALOUS BEHAVIOR OF STOMATA

Stomata do not always behave as expected. It was mentioned earlier that opening and closing sometimes continue for several days after plants are placed in continuous darkness and they sometimes cycle during the day. Also, not all of the stomata on a leaf behave in the same manner. A few examples of anomalous behavior will be discussed.

Cycling

Under some circumstances stomata show cycling or oscillation between the open and closed condition, as shown in Fig. 8.7. Cycling occurs most commonly

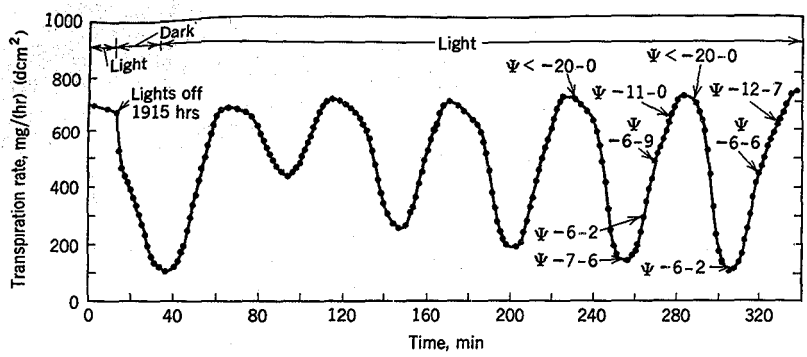

Figure 8.7 Cyclic variation in transpiration and water potential of cotton leaves, caused by stomatal cycling initiated by a 20-min dark period at 3:15 PM. The cycling was attributed to oscillations in water potential caused by high root resistance in rapidly transpiring plants. From Kramer (1983), after Barrs and Klepper (1968).

in water-stressed plants with relatively high root resistance and can be initiated by a sudden shock such as a short period of darkness, cooling the soil, or changes in humidity or temperature. The cycling has a periodicity ranging from minutes to hours, but most often occurs in the range of 15 to 120 min. It has been observed in several kinds of herbaceous plants (Barrs, 1971; Barrs and Klepper, 1968; Kitano and Eguchi, 1992b; Shiraishi *et al.*, 1978), and Levy and Kaufmann (1976) observed it in citrus trees in an orchard. Shiraishi *et al.* (1978) observed cyclic variation in tobacco leaves with a scanning electron microscope. Various leaves on a plant can be at different phases of the oscillation cycle at the same time. Raschke (1975) suggested that it might occur when a negative feedback signal is delayed and reaches the guard cells at a time such that it reenforces the initial response instead of counterbalancing it. Cowan (1972) proposed that cycling optimizes the conflicting requirements for carbon dioxide uptake and control of water loss.

As mentioned earlier, there also are endogenous rhythms that cause stomata to open and close for several days after plants are moved to continuous darkness. These anomalies increase the difficulty of developing a general theory of stomatal behavior. The possible occurrence of short-term cycling and daily and seasonal rhythms should be considered in research on stomatal behavior.

Heterogeneity in Stomatal Response

The stomata on the upper and lower surfaces of leaves sometimes behave differently, as shown in Fig. 8.8. One might expect most or all of the stomata on one surface of a leaf to respond similarly to a given stimulus, but this is not always true. For example, when ABA was supplied to detached leaves through

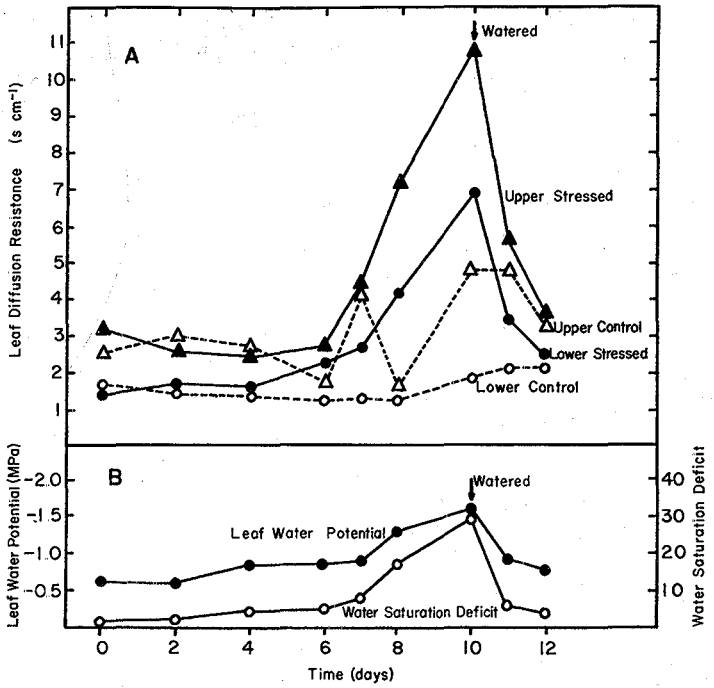

Figure 8.8 (A) Diffusion resistances of upper (adaxial) and lower (abaxial) surfaces of the seventh and eighth leaves of water-deficient and control maize, measured in the morning with a diffusion porometer. The plants were grown in a controlled environment chamber. (B) Leaf water potential and saturation deficit of water-deficient maize plants. From Kramer (1983), after Sanchez-Diaz and Kramer (1971).

the petioles, stomatal closure occurred in patches over the leaf surface and similar patchiness occurred in plants of other species when the endogenous ABA concentration was increased by water deficit (Downton *et al.*, 1988a; Terashima *et al.*, 1988). This heterogeneity in stomatal behavior might result either from an uneven distribution of ABA to different parts of the leaf or from differences in sensitivity of guard cells in various parts of the leaf. The margins of leaves tend to dry out sooner than the central regions (Cook *et al.*, 1964; Hashimoto *et al.*, 1984), causing early closure of stomata in the margins. The relationship between patchiness in stomatal opening and photosynthesis is discussed in more detail in Chapter 10.

The occurrence of heterogeneity or "patchiness" in stomatal behavior suggests the need for observation of leaves under a microscope to supplement porometer measurements of average leaf conductance (Omasa *et al.*, 1985a) to determine if patchiness occurs. Laisk (1983), Terashima *et al.* (1988), and

Mansfield *et al.* (1990, pp. 67–70) suggested that heterogeneity in stomatal closure causes errors in estimating internal CO_2 concentration, explaining observations that carbon fixation sometimes decreases, although the average internal CO_2 concentration (c_i) does not appear to be limiting. However, Cheeseman (1991) claims models show this explanation to be untenable. Also, Wise *et al.* (1992) found that although "patchiness" occurs in unstressed, chamber-grown cotton and sunflower plants, it is uncommon under field conditions. Apparently the importance of stomatal heterogeneity deserves further study.

The reaction of stomata to environmental factors varies with the age of leaves and their past treatment and it often is difficult to determine how much of the difference among experimental results is intrinsic and how much is caused by differences in methods, age, and previous treatment of the experimental plants. As leaves grow older the stomata often become less responsive and may open only partly, even at midday (Brown and Pratt, 1965; Slatyer and Bierhuizen, 1964a), and Tazaki *et al.* (in Turner and Kramer, 1980) reported that stomata on older leaves of mulberry do not close. According to Ackerson and Kreig (1977), although stomata of corn and sorghum close when they are water deficient during the vegetative stage, they do not close in similar conditions in the reproductive stage. However, Longstreth and Kramer (1980) found no significant change in leaf conductance during flower induction or flowering of cocklebur or soybean. It also has been reported that stomata of cotton grown in greenhouses and controlled environment chambers close at higher leaf water potentials than those of field-grown plants (Davies, 1977; Jordan and Ritchie, 1971). Schulze and Hall (1982) reviewed the effects of environmental factors on stomatal behavior and urged that short- and long-term effects be differentiated. Meinzer and Grantz (1991) discussed the coordination between stomatal conductance in sugarcane and the capacity of soil, roots, and stems to supply water, resulting in a relatively constant leaf water status over a wide range of plant sizes and growing conditions. They observed a decrease in stomatal conductance that compensated for the increase in leaf area, in their opinion probably controlled by signals from the roots. The effects of age and past treatment probably account for many of the differences in behavior found in the literature and increase the difficulty in generalizing about stomatal behavior.

OPTIMIZATION

There have been several attempts to develop theories explaining stomatal behavior in terms of optimization of cost to benefit, cost being a reduction in the uptake of CO_2 used in photosynthesis and benefit being a reduction in water loss. Optimum efficiency should occur when the stomatal aperture varies during a day in such a manner that there is minimum transpiration (E) for maximum

photosynthesis (A) and dE/dA is constant. Wong *et al.* (1979) claim that in at least some plants there is a feedback mechanism relating stomatal aperture to photosynthetic capacity of the mesophyll tissue and maintaining a fairly constant ratio of internal to external concentration of CO_2. The optimization principle was discussed in detail by Cowan (1982), by Farquhar and Sharkey (1982), and in Givnish (1986). However, Sandford and Jarvis (1986) cited some contradictory findings and reported that dE/dA is not constant in all woody plants. Bunce *et al.* (1977) question if an optimal stomatal response saves much water for plants in stands.

DIFFUSIVE CAPACITY OF STOMATA

The research of Brown and Escombe (1900), Sayre (1926), and Ting and Loomis (1965) establish that *in quiet air* diffusion through small pores such as stomata is better related to their circumference or perimeter than to their area. Because of their more or less elliptical shape, stomata have a high diffusive capacity even when only partly open. This situation is largely academic, however, because it applies only in quiet air where the boundary layer resistance is greater than the stomatal resistance. In nature, leaves usually are exposed to wind, decreasing the boundary layer resistance relative to the stomatal resistance and transpiration increases rapidly with increasing pore area, as shown in Fig. 8.9. Nobel (1991, Chapter 8) has a discussion of stomatal diffusion.

BULK FLOW IN LEAVES

Thus far we have assumed that all movement of gas in and out through stomata is by diffusion, but this may not be true. It is claimed that the bending of leaves in the wind and temperature changes due to passing clouds and intermittent shading cause bulk flow of gas through the stomata. Shive and Brown (1978) reported that the fluttering of eastern cottonwood leaves increases gas exchange. However, Rushin and Anderson (1981) reported that leaf fluttering has little effect on stomatal conductance. It seems possible that wind flowing over the surfaces of leaves or sudden changes in temperature can cause gas flow through the intercellular spaces of amphistomatous leaves. Vogel (1981, p. 83) observed such flow in water-filled leaves injected with a dye solution and immersed in a flow tank.

MEASUREMENT OF STOMATAL APERTURE AND CONDUCTANCE

Because of the importance of stomata in controlling water loss and CO_2 uptake, there has been much interest in the measurement of stomatal aperture or

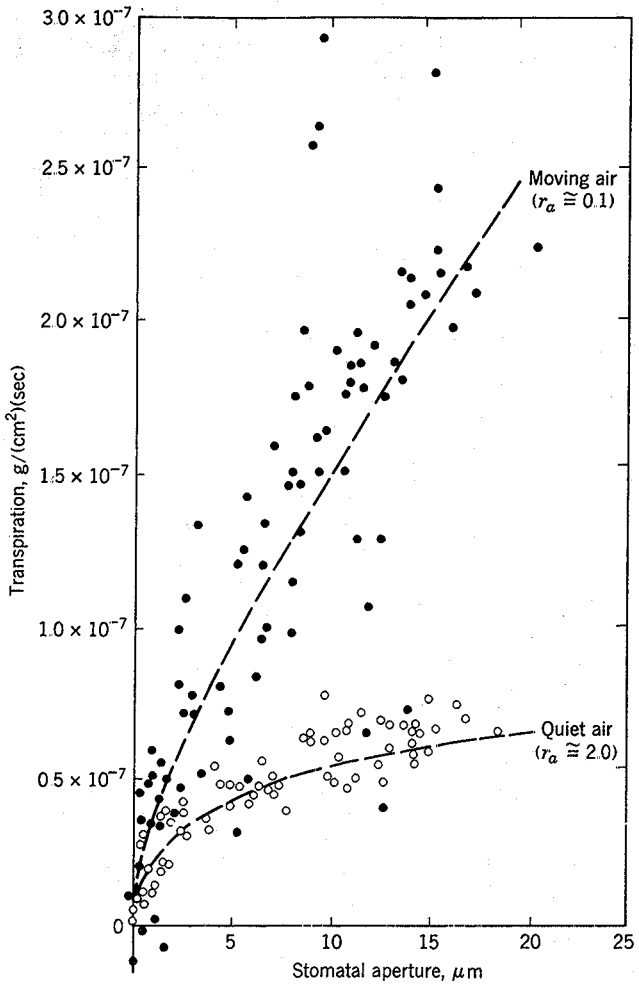

Figure 8.9 The effect of increasing stomatal aperture on transpiration rate of Zebrina leaves in quiet and moving air. The effect of increasing aperture is very large in moving air, but small in quiet air where the boundary layer resistance is relatively large compared to stomatal resistance. From Kramer (1983), after Bange (1953).

the conductance of stomata. Much relevant literature has been reviewed by Weyers and Meidner (1990) and Smith and Hollinger (in Lassoie and Hinckley, 1991), and only a few methods are discussed briefly.

Some investigators have expressed the stomatal opening in terms of conductance, others as resistance which is the reciprocal of conductance, resulting in

hyperbolic curves. Burrows and Milthorpe (in Kozlowskki, 1976, pp. 106–107) discussed the problems resulting from the use of resistance, but agreed that it has advantages in dealing with the complex pathway between plants and the atmosphere. The use of resistances and conductances also is discussed in Nobel (1991, Chapter 8).

Visual Observations

Most of the early observations were made by stripping off bits of epidermis and fixing them in absolute alcohol before observing them under the microscope. This method seems to have been introduced by Lloyd (1908) and was used for the classical studies of Loftfield (1921), but it is difficult to strip epidermis from some leaves and stripping sometimes causes change in stomatal aperture. Another method is to make impressions of the epidermis of attached leaves in collodion (Clements and Long, 1934), silicone rubber (Zelitch, 1961), or dental paste (Kuraishi *et al.* in Hashimoto *et al.*, 1990; Weyers and Meidner, 1990, p. 111), or even Duco cement or nail polish, strip them off, and examine them under the microscope. However, Pallardy and Kozlowski (1980) warned that epidermal impressions can be unreliable if cuticular ledges develop on the inner surfaces of guard cells, as shown in Fig. 7.8A. Shiraishi *et al.* (1978) studied variations in stomatal aperture under the microscope and reported that the aperture of ledges often changes independently of the apparent pore aperture, leading to misinterpretation of effective pore aperture.

Modern technology has made better visual observations of stomata possible. For example, scanning electron microscopy reveals details of stomatal structure not previously observed (Shiraishi *et al.*, 1978). Hashimoto (in Hashimoto *et al.*, 1990), Hashimoto *et al.* (1984), and Omasa *et al.* (1985a; and in Hashimoto *et al.*, 1990) described use of a remote-controlled light microscope, television camera, and image processing system to observe the effects of stresses such as water deficit and air pollutants on stomata in different parts of a leaf. This method showed that stomata in different parts of a leaf sometimes responded differently, as mentioned in the section on Heterogeneity in Stomatal Response. They concluded that visual observation under a microscope is desirable to observe the effects of air pollutants on stomata in different parts of a leaf. Chlorophyll fluorescence also is useful in the study of guard cell metabolism (Cardon and Berry, 1992; Ogawa *et al.*, 1982; Outlaw *et al.*, 1981).

Infiltration

This method was popularized by Molisch (1912). It depends on measuring the time required for the infiltration of leaves by liquids of various viscosities. Alvim and Havis (1954) used mixtures of paraffin oil and *n*-dodecane whereas investigators in Israel used mixtures of paraffin oil and turpentine or benzol, or

paraffin oil (kerosene) alone. Fry and Walker (1967) described a pressure infiltration method for use on pine needles. This method clearly indicates whether stomata are open or closed, but the toxic fluids used kill the tissue infiltrated, the infiltration depends on wettability by the solvent, and the method does not permit calculation of stomatal resistances (Lassoie *et al.*, 1977a).

Porometers

Visual methods have been largely supplanted by porometers that measure the movement of gas out of leaves in such a manner that readings can be converted into diffusion resistance of the leaf in sec · m^{-1} or its reciprocal conductance in $m \cdot sec^{-1}$. This method measures total leaf resistance, but if cuticular resistance is high it gives a good approximation of stomatal resistance. There are two types of porometers: pressure or bulk flow, and diffusion.

Pressure or Bulk Flow Porometers. Francis Darwin, the plant physiologist son of Charles Darwin, is credited with developing the porometer and Darwin and Pertz (1911) described a simple pressure flow porometer. Numerous modifications have been made, including recording porometers (Gregory and Pearse, 1934; Wilson, 1947) and portable models such as that developed by Alvim (1965). Fiscus *et al.* (1984) and Fiscus (in Hashimoto *et al.*, 1990) described a viscous flow porometer that is computer controlled and can be used to schedule irrigation of a crop. Viscous flow porometers are not suitable for use on leaves with stomata on only one surface (many trees) and with bundle extensions that extend to the epidermis and prevent free lateral movement of gases through the intercellular spaces. Also a pressure greater than 10 cm of water can cause alterations in stomatal aperture (Raschke, 1975). Meidner (1992) discussed some problems with bulk flow porometers. However, these types of porometers have the advantage of being highly responsive to changes in aperture and indicate aperture effects rather than diffusion through the leaf.

Diffusion Porometers. These usually are small cuvettes that can be attached to leaves that measure the time required for a predetermined change in humidity to occur. They have undergone many modifications, but most of the earlier ones used a cuvette containing a humidity sensor connected to a meter to read humidity and a timing device. Many are based on the instrument described by Kanemasu *et al.* (1969) with various modifications to increase speed and accuracy. Beardsell *et al.* (1972) introduced the null point porometer that measures the steady-state rate of transpiration of a leaf enclosed in a cuvette into which dry air is blown to maintain a constant humidity. This eliminates some calibration problems and the lag caused by adsorption of water vapor on cuvette walls. Kaufmann (1981; and in Hashimoto *et al.*, 1990) used ventilated cuvettes, ap-

proximately 15 liters in volume and large enough to enclose leafy twigs. They were closed from time to time long enough to measure transpiration. A computer controlled opening and closing and data acquisition and processing. With this apparatus Kaufmann was able to estimate canopy transpiration from measurements of leaf conductance. The use of diffusion porometers has been reviewed by Smith and Hollinger (in Lassoie and Hinckley, 1991).

One of the limitations on use of diffusion porometers is that they average the behavior of stomata in a relatively large area of leaf surface, but provide no information concerning the behavior of individual stomata (Omasa *et al.*, 1985a) or differences in different parts of leaves (Hashimoto *et al.*, 1984). Thus, for some purposes visual observations may be desirable (Omasa *et al.*, 1985a; and in Hashimoto *et al.*, 1990). However, the data provided by larger samples generally are most useful. Diffusion porometers are affected by any factor controlling water loss by leaves; stomatal aperture is only one. Differences in cuticle thickness, leaf thickness, intercellular space dimensions, and other factors can contribute to diffusion differences but do not indicate differences in stomatal aperture. Care needs to be used when interpreting data from diffusion porometers.

SUMMARY

Stomata are pores in the epidermis whose aperture is controlled by pairs of specialized epidermal cells called guard cells. They provide passageways between the ambient air and the air spaces in photosynthetic tissue essential for the entrance of the CO_2 used in photosynthesis. Unfortunately, they also provide pathways for the exit of water vapor, subjecting plants to the danger of excessive dehydration in sunny, dry weather. Thus stomatal aperture plays an important role in controlling both transpiration and the CO_2 supply for photosynthesis.

Stomata usually open in the light and close in darkness. However, there are exceptions to this generalization. For example, the stomata of plants with CAM tend to remain closed in light and open in darkness. Opening of stomata is caused by an increase in turgor of guard cells caused largely by the uptake of K^+ and accumulation of its counterions, and closing results from the loss of K^+ and the consequent loss of turgor. The accumulation of K^+ is driven by energy from metabolism often involving photosynthesis or respiration, but direct effects of particular wavelengths of light indicate that photosynthesis is not the sole source of light effects on the process. Guard cells usually contain chloroplasts and most of the metabolic apparatus of photosynthesis but they can also fix CO_2 into malate which acts as a counterion for K^+. Abscisic acid exerts important control over stomatal opening by controlling the ability of the guard cells to accumulate K^+, and high abscisic acid appears to cause stomatal closure in water-deficient plants by this mechanism.

Changes in guard cell turgor are affected by environmental factors such as

light intensity, carbon dioxide concentration, humidity, and temperature and by internal factors such as internal carbon dioxide concentration, leaf water status, and the concentration of growth regulators, especially abscisic acid. The complex interactions among the factors often make it difficult to determine their relative importance. There also is evidence that chemical signals from roots affect stomatal behavior, especially in drying soil.

Occasionally, stomata exhibit anomalous behavior such as cycling in a constant environment. They sometimes show a "patchy" response, with stomata in some areas on a leaf closing while those in other areas remain open. The stomata on the upper and lower surfaces of leaves often behave differently.

The number of stomata per mm^2 of leaf area varies from 60 to 80 on corn leaves to over 1000 on scarlet oak. The size tends to decrease with increasing frequency, but the area of pore space when wide open usually amounts to 1% or more of the leaf area. The stomatal aperture can be observed visually on epidermal strips fixed in alcohol or by applying collodion or silicone rubber to leaves, stripping it off, and examining the impressions under a microscope. Direct visual observations can be made with special cameras and imaging equipment. Rapid estimates of stomatal aperture can be made from the rate of infiltration of liquids of various viscosities. However, most observations are now made with porometers which permit estimates of stomatal conductance.

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