CHAPTER

24

Stomatal Anatomy and Stomatal Resistance

The two main parts of a plant that control its water status are the roots, where water enters, and the stomata on the leaves, where water exits. We considered roots in Chapters 15 and 16. Here we consider stomata.

24.1 DEFINITION OF STOMATA AND THEIR DISTRIBUTION

The stomata are apertures in the epidermis, each bounded by two guard cells. In Greek, *stoma* means "mouth", and the term is often used with reference to the stomatal pore only. Esau (1965, p. 158) uses the term stoma to include the guard cells and the pore between them, and we will use her definition. The plural of stoma is *stomata*. There is no such word as "stomates".

Stomata occur in vascular plants. Vascular plants include the lower vascular plants such as horsetails (*Equisetum*), ferns (class Filicinae), gymnosperms, and angiosperms. As noted before, the angiosperms are the flowering plants and this group consists of the two large classes: Monocotyledoneae (monocotyledons) and Dicotyledoneae (dicotyledons) (Fernald, 1950).

By changing their shape, the guard cells control the size of the stomatal aperture. The aperture leads into a substomatal intercellular space, the substomatal chamber, which is continuous with the intercellular spaces in the mesophyll. In many plants, two or more cells adjacent to the guard cells appear to be associated functionally with them and are morphologically distinct from the other epidermal cells. Such cells are called *subsidiary*, or *accessory*, cells (Esau, 1965, p. 158).

The stomata are most common on green aerial parts of plants, particularly the leaves. They can also occur on stems, but less commonly than on leaves. The aerial parts of some chlorophyll-free land plants

(*Monotropa*, *Neottia*) and roots have no stomata as a rule, but rhizomes have such structures (Esau, 1965, p. 158). Stomata occur on some submerged aquatic plants and not on others. The variously colored petals of flowers often have stomata, sometimes nonfunctional. Fruits also can have stomata. Stomata are found on stamens and gynoecia.

Stomata can be distributed in the following ways on the two sides of a leaf:

- An *amphistomatous* leaf has stomata on both surfaces. Most plants have such a distribution.
- A *hypostomatous* leaf has stomata only on the lower surface. Many tree species are characterized by having hypostomatous leaves, such as horse chestnut (*Aesculus hippocastanum*) and basswood (*Tilia europaea*) (Meidner and Mansfield, 1968; see their Table 1.1). The leaf of poplar (*Populus* sp.) is an exception. It has stomata on both surfaces and a petiole that allows the leaf to turn readily in the wind. These adaptations may allow its fast growth rate. The fast growth rate of poplar is one reason it is widely used in phytoremediation (use of plants to remove pollutants from soil).
- An *epistomatous* leaf has stomata only on the upper surface of the leaf. Some floating plants are epistomatous.
- A *heterostomatous* leaf has stomata that occur with more than twice the frequency on the abaxial surface than on the adaxial surface. An *isostomatous* leaf has stomata that occur with approximately equal frequencies on both surfaces.

The *stomatal ratio* is the ratio of stomatal frequency on the adaxial surface to that on the abaxial surface.

24.2 STOMATAL ANATOMY OF DICOTS AND MONOCOTS

Figure 24.1 shows how the stomata develop differently in broad-leaved plants (mainly dicotyledons), which have elliptical shapes, compared to grass species (monocotyledons), which have dumbbell shapes. The most commonly occurring stomata are elliptical in shape and differentiate from a protodermal cell by division into two guard cells, which soon assume their typical shape—like a bean in surface view (Figure 24.1(A)). By separating slightly in the center, the guard cells form the stomatal pore between them. There is no radical change in the shape of the guard cells as they grow in size except that the early rounded shape changes into a more elongated, elliptical one. Adjacent epidermal cells may or may not be distinctive in appearance, but they usually function as subsidiary cells (Meidner and Mansfield, 1968, p. 6).

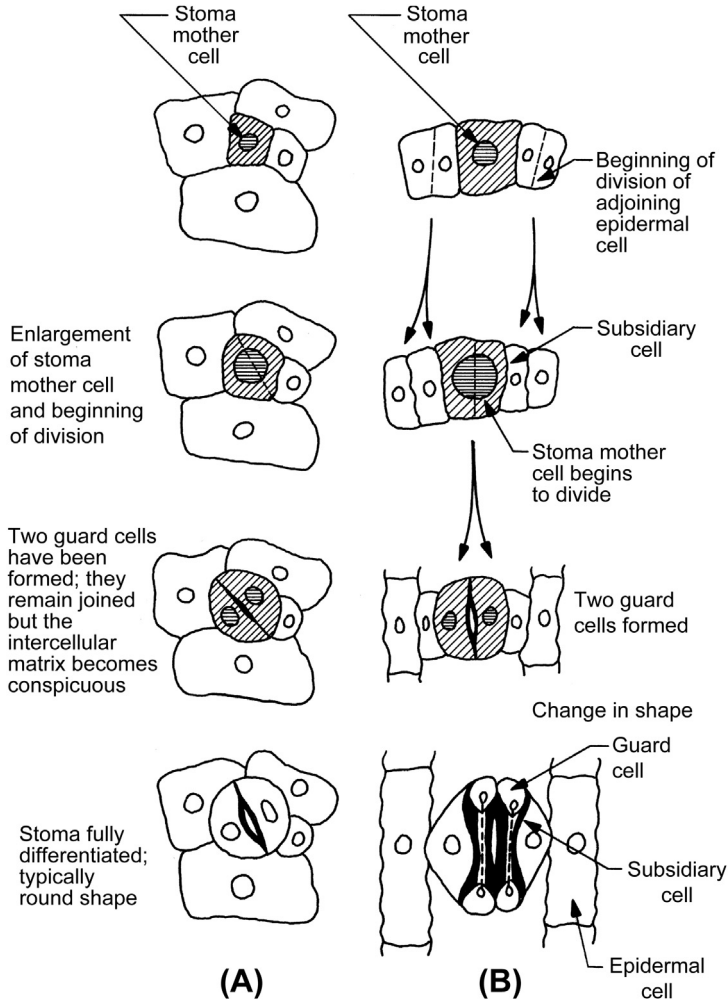

FIGURE 24.1 Four stages in the differentiation of (A) elliptically shaped and (B) graminaceous stomata. *From Meidner and Mansfield (1968), p. 7. This material is reproduced with permission of The McGraw-Hill Companies.*

In most members of the Poaceae (formerly Gramineae) (grass family) and Cyperaceae (sedge family), differentiation of a stoma begins with the division of two protoderm cells on either side of a stoma mother cell. The two daughter cells resulting from these divisions, which lie adjacent to the stoma mother cell, are the two future subsidiary cells. They are clearly distinguishable in shape from the other epidermal cells. The stoma mother cell divides next to form the guard cells, between which the stomatal pore appears. At this stage, the graminaceous stoma resembles the

elliptical one in shape, but a further stage in its development results in an elongation of the guard cells which finally assume the characteristic dumbbell shape (Figure 24.1(B)) (Meidner and Mansfield, 1968; pp. 6–8).

In leaves with parallel veins, such as those of monocotyledons and some dicotyledons, and in the needles of conifers, the stomata are arranged in parallel rows. In netted-veined leaves, which include most dicotyledons and a few monocotyledons, the stomata are scattered (Esau, 1965; p. 158). In leaves with parallel veins, which have the stomata in longitudinal rows, the developmental stages of the stomata are observable in sequence in the successively more differentiated portions of the leaf. This sequence is basipetal, that is, from the tip of the leaf downward. In the netted-veined leaves, the different developmental stages are mixed in mosaic fashion so that mature stomata occur side by side with immature ones (Esau, 1965; p. 166).

24.3 STOMATAL DENSITY

Esau (1965, p. 158) gives the density of stomata as between 100 and 300/mm^2 for leaves of many species. The number of stomata depends on the species. Meidner and Mansfield (1968; see their Table 1.1) give the frequency of stomata on leaves of different species, including the lower vascular plants (ferns), gymnosperms, and angiosperms. Most plants have more stomata on the lower (abaxial) surface than on the upper (adaxial) surface, but wheat (*Triticum* sp.) is an exception. It has more stomata on the upper surface than on the lower surface. The number of stomata per unit area changes as a leaf grows. It tends to be higher in earlier stages of development than in later stages (Meidner and Mansfield, 1968; p. 6). Stomata may grow in size and change shape as the leaf blade expands.

At maturity of a leaf, the number of stomata per unit leaf area may not be constant. It can be affected by environmental factors. More stomata per unit area occur in sun leaves than in shade leaves. More stomata per unit area occur in leaves of plants growing in moist soil and high humidity than in those growing in dry conditions. Stomatal density can be affected by leaf position. Liang et al. (1975) measured stomatal density on the 15 uppermost leaves of six varieties of the grain sorghum [*Sorghum bicolor* (L.) Moench] and their 15 F_1 hybrids. The second leaf from the top had the highest density, and leaf no. 15 had the lowest. Distribution can also vary with distance from the leaf base. Liang et al. (1975) found in their study with sorghum that stomatal density on the abaxial surface (which had more stomata than the adaxial surface) was highest at the basal portion of the leaves.

24.4 DIFFUSION OF GASES THROUGH STOMATAL PORES

The distribution of stomata affects the diffusion of gases through them. Early important investigations on the diffusion of gases and liquids through small openings were carried out by Brown and Escombe (1900). These investigations proved that the rates of diffusion through small single apertures are proportional to the diameters and not to the areas of the openings. This agreed with results previously established by Stefan for the converse case of evaporation from circular surfaces of water (Maximov, 1929; p. 172). He compared evaporation from large surfaces (e.g., lakes) and small surfaces. For surfaces of small dimension, diffusion is more rapid at the edges than at the center, because at the margins the molecules of water vapor can diffuse fanwise in all directions instead of only perpendicularly to the surface at the center. It follows that, in still air, the smaller the area of the evaporating surface, the more rapid the rate of evaporation. But for areas of such small dimensions as leaves or small bowls of water, it appears, as has been mathematically calculated by Stefan (1881), that evaporation is proportional not to the area of these objects but to their periphery or radius (Maximov, 1929; p. 136).

Brown and Escombe (1900) found that their "diameter law" holds good also for the case of diffusion through a number of small openings (i.e., through a "multiperforate septum"). From this it follows that more water vapor will diffuse in unit time through several small apertures than through a single larger opening with an area equal to the combined areas of the smaller ones. If, however, the perforations in a septum separating two mixtures of gases of different composition (e.g., dry and moist air) are very close together, the rate of diffusion is modified. The "lines of flow" of the diffusing molecules, which normally tend to diverge fanwise as they issue from the apertures (Figure 24.2), now interfere with one another and mutually hinder the spread of the diffusing particles, thus slowing down the rate of diffusion. Brown and Escombe (1900) showed experimentally that such interference begins when the distance between the apertures is somewhat less than 10 times the diameter of the holes. The fact that the rate of diffusion through small openings is proportional not to the area, but to the diameter of the opening, greatly increases the possible amount of diffusion that can take place through a multiperforate septum (Maximov, 1929; pp. 172–173).

Molecular biology research has been done to understand stomatal spacing (Shpak et al., 2005). An enzyme in the flowering plant *Arabidopsis thaliana*, called *YODA* (*YDA*), is crucial to the formation and arrangement of stomata. *YODA* is the name given to a mitogen activated protein (MAP) kinase kinase kinase (MAPKK kinase gene), which is an important

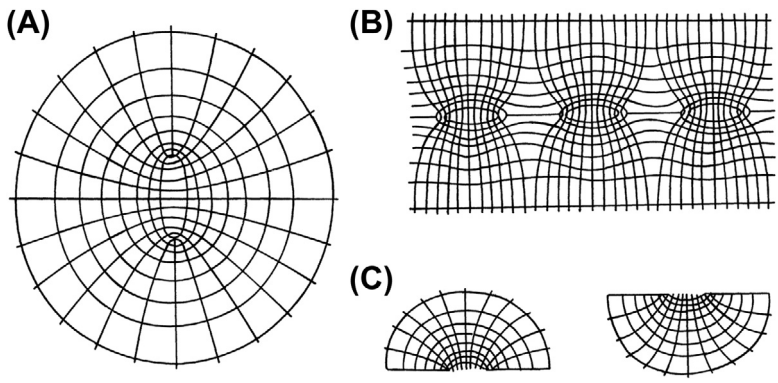

FIGURE 24.2 Diagrammatic representation of the diffusion of water vapor through small openings. (A) Diffusion through a single opening in a vertical septum; the fanlike, diverging lines show the courses of the diffusing particles; perpendicular to them are concentric lines of equal vapor density. (B) Diffusion through a horizontal multiperforate septum (three openings are represented); (C) Diffusion through single openings of the same size. *From Maximov (1929), p. 173.*

regulator of stomatal development. Mutations in the *YDA* gene result in a plant in which almost all the cells at the plant surface are guard cells. This overproduction of stomata has severe consequences, and many of the mutant seedlings die (Bergmann et al., 2004; Serna, 2004). Mutations in three other genes—named *Too Many Mouths* (*TMM*), *Stomatal Density and Distribution1* (*SDD1*), and *Four Lips* (*FLP*)—also affect stomata, not only producing stomatal clusters but also allowing the formation of single stomata. The single stomata ensure correct gas exchange and so these three mutants develop normally (Serna, 2004).

24.5 GUARD CELLS

Guard cells may occur at the same level as the adjacent epidermal cells, or they may protrude above or be sunken below the surface of the epidermis (Figure 24.3). In some plants, stomata are restricted to the epidermis that lines depressions in the leaf, the stomatal crypts. Epidermal hairs may also be prominently developed in such crypts. Stomata are level with the epidermal cells in most mesophytic plants and plants that grow in moist habitats. Plants that grow in dry habitats often have stomata that are situated below the level of the epidermal cells.

The guard cells are generally crescent-shaped with blunt ends (kidney shaped) in surface view (Figure 24.3(D)) and often have ledges of wall material on the upper and lower sides. In sectional views such ledges appear like horns (Figure 24.3(E), (F) and (H)). Sometimes a ledge occurs only on the upper side (Figure 24.3(A), (G) and (I)), or none is present. If

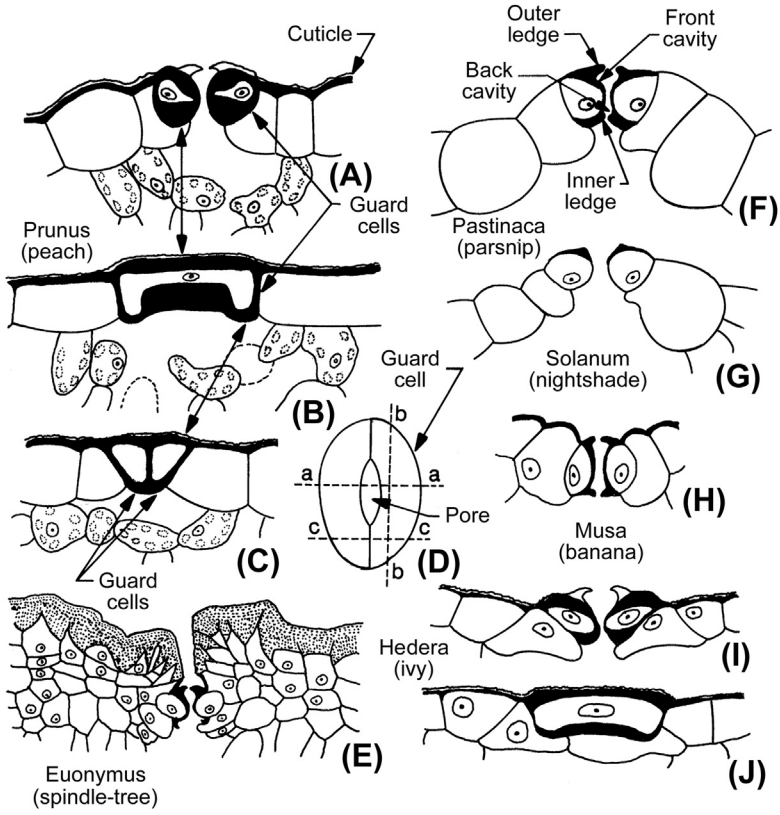

FIGURE 24.3 Stomata in abaxial epidermis of foliage leaves. (A–C) Stomata and some associated cells from each leaf sectioned along planes indicated in D by the broken lines *aa*, *bb*, and *cc*. (E–I) stomata from various leaves cut along the plane *aa*. (J) One guard cell of ivy cut along the plane *bb*. The stomata are raised in A, F, G. They are slightly raised in I, slightly sunken in H, and deeply sunken in E. The hornlike protrusions in the various guard cells are sectional views of ledges. Some stomata have two ledges (E, F, H); others only one (A, G, I). Ledges are cuticular in A, E, I. The *Euonymus* leaf has a thick cuticle; epidermal cells are partly occluded with cutin. *From Esau (1965), p. 159. This material is used by permission of John Wiley & Sons, Inc.*

two ledges are present, the upper delimits the front cavity above the stomatal pore, and the lower encloses the back cavity between the pore and the substomatal chamber (Figure 24.3(F)). The ledges are more or less heavily cutinized (Esau, 1965; p. 159).

The walls of the guard cells can be differentially thickened. The change in shape of the guard cells occurs because the wall that is turned away from the stomatal aperture, the so-called back wall, is thin and apparently elastic (Figure 24.3(A), (E–I)). When the turgor increases, the thin wall bulges away from the aperture, while the front wall (facing the pore)

becomes straight or concave. The whole cell appears to bend away from the aperture, and the aperture increases in size. Reversed changes occur under decreased turgor (Esau, 1965; p. 161).

Another distinct type of stomatal mechanism is illustrated by the guard cells of Poaceae and Cyperaceae. These cells are bulbous at two ends and straight in the middle (Figure 24.1, right). The middle part has a strongly but unevenly thickened wall; the bulbous ends have thin walls, and the wall between the bulbous ends of two adjacent cells may be incomplete so that the protoplasts of the two guard cells are partially confluent. Increase in turgor causes a swelling of the bulbous ends and the consequent separation of the straight median portions from each other. The nucleus in a gramineous guard cell is extended and simulates the shape of the cell lumen. It has two enlarged ends connected by a thin threadlike middle part.

In addition to the nucleus, guard cells contain chloroplasts, which are not present in other epidermal cells. These chloroplasts are considered to be photoreceptors involved in the light-induced opening of stomata. Mitochondria are also present in guard cells. The osmotic pressure of guard cell sap of open stomata is higher than that of sap in neighboring epidermal cells. The increase in osmotic pressure is thought to be due, in large part, to the influx of potassium (see Section 24.6). Anthocyanin is absent in guard cells, but occurs in epidermal cells. Proteinaceous crystals and calcium oxalate crystals are absent in guard cells, but occur in epidermal cells. Guard cells are, in general, more resistant to adverse conditions such as low temperatures and drought. They do not senesce as rapidly as other epidermal cells.

24.6 MECHANISM OF STOMATAL OPENING

The outstanding feature of stomata, the unevenly thickened walls of the guard cells, is related to the changes in shape and volume (and the concomitant changes in the size of stomatal aperture), which are operated by turgor changes in guard cells (Esau, 1965; p. 160). Many factors control guard cell turgor, including carbon dioxide concentration, light, temperature, endogenous rhythms, atmospheric vapor pressure deficit, and soil water potential (Heath and Mansfield, 1969; see their summarizing Figure 9.9).

No matter what causes the opening, the basic mechanism underlying stomatal opening in light, in most cases, is thought to be related to the uptake of potassium by guard cells in amounts sufficient to lower significantly the solute potential. It has been known for a long time that potassium accumulates in guard cells. As early as 1905, Macallum (1905) observed accumulation of potassium in the guard cells of tulip. Imamura (1943)

found an abundance of potassium in guard cells of open stomata, but little in closed ones. He also observed that the "suction force" of guard cells changed during opening and closing without any appreciable changes in their starch content. Imamura suggested that these changes in suction force were regulated by movement of solutes, particularly potassium, in and out of the guard cells. Two more Japanese workers, Yamashita (1952) and Fujino (1967), confirmed that the potassium content in guard cells is correlated with stomatal movement. Earlier papers by Fujino in Japanese, unknown in the United States, showed that guard cells contain large concentrations of potassium, but small quantities when closed in the dark. Fischer (1968) independently showed that potassium uptake was necessary for stomatal opening. When Fujino published his paper in English in 1967, his earlier work became known in America. Both Fujino and Fischer are attributed with proposing that stomatal opening and closing are the result of transport of potassium ions.

Many papers were published between the late 1960s and the 1990s confirming the role of potassium in the control of stomatal opening. The work was done at the leaf or cellular level. For example, Peaslee and Moss (1968) showed that K-deficient corn (*Zea mays* L.) leaves had smaller stomatal widths than control leaves. Stomata of normal corn leaves opened about 6.5 μm in diameter, while stomata in K-deficient leaves were less than 1 μm in diameter. Now, work focuses on the molecular biology of potassium transport into guard cells. Stomatal opening depends on blue and red light. Stomata open in response to weak blue light and the opening is enhanced by background red light. Blue light activates an enzyme (plasma membrane H^+-ATPase; ATPase stands for adenosine triphosphatase) and increases the inside negative electrical potential across the plasma membrane in guard cells. The potential drives K^+ uptake, and the accumulated positive K^+ charges are compensated mainly by malate^{2-} formed in guard cells. Red light induces stomatal opening at high intensity. Red light likely mediates stomatal opening via reduction of the intercellular concentration of carbon dioxide by mesophyll photosynthesis (Shimazaki et al., 2007).

Even though much is known about stomata, stomatal movements cannot yet be reliably predicted (Brodribb and McAdam, 2011). The complexity that characterizes stomatal control in seed plants is absent in early-diverging vascular plant lineages. Ziegler (1987) reviews the evolution of stomata.

24.7 BOUNDARY LAYER

Above all objects is a thin layer of still air, called the laminar sublayer (Rosenberg, 1974; p. 78), or the boundary layer, which adheres to their

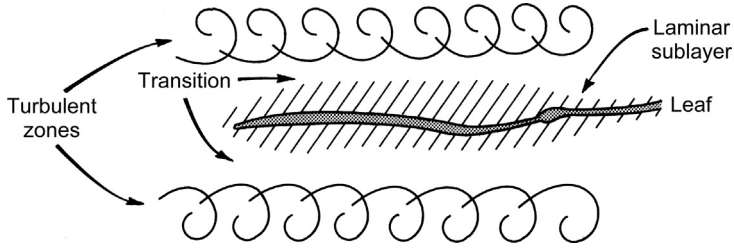

FIGURE 24.4 Air structure near a small object (like a leaf) in an air stream. *From Rosenberg (1974), p. 79. This material is used by permission of John Wiley & Sons, Inc.*

surfaces. A plane with only one surface exposed, such as the soil surface, will have such a layer on one side. An object, like a leaf, within an air stream will have the layer on all surfaces (Figure 24.4). The thickness of the layer depends on the roughness of the surface, on the wind speed, and on the leaf dimension. A leaf with a hairy surface is rougher than a leaf with a smooth wax. The boundary layer will be thicker the rougher the surface is. Roughness is nearly zero over very smooth surfaces, like open water on a calm day. Roughness increases with increasing height of objects sticking above the surface (Rosenberg, 1974; p. 104). The boundary layer is thinner the more windy the conditions. In growth chamber experiments, it is important to have fans circulating air in the closed chambers, so that the boundary layer (or more specifically, boundary-layer resistance) is reduced and gas exchange (in particular, carbon dioxide uptake) is similar to natural conditions in the open environment. The thickness of the boundary layer depends on the linear dimension of the leaf in the downwind direction (Nobel, 1974; p. 305). Both boundary-layer resistance and stomatal resistance are important in controlling gas transport through leaves (see next section).

24.8 LEAF RESISTANCES

The resistances to water vapor transport in a leaf are the epidermal resistance, made up of the stomatal resistance and the cuticular resistance, and the boundary-layer resistance. The resistances to carbon dioxide transport in a leaf are the same as for water vapor (stomatal, cuticular, and boundary-layer resistances); a fourth resistance called the mesophyll resistance is discussed later in this section.

Water vapor diffuses through two of the resistances in a leaf acting in *series*: the stomatal aperture resistance (stomatal resistance) (r_s) and the boundary-layer (air) resistance (r_a), which results from the lengthening of the diffusion path outside of the stomata and which is an inverse function

of wind and turbulence (Gale and Hagan, 1966). The resistance to cuticular water loss (r_c) is very large and is in *parallel* to r_s.

Let us now review our physics concerning resistors in series and parallel (Schaum, 1961; p. 156). In a series circuit (Figure 24.5, left), resistance is as follows:

$$R = R_1 + R_2 + R_3 + \ldots, \tag{24.1}$$

where R = equivalent resistance of a series combination of conductors having resistances R_1, R_2, R_3, … The total *potential difference* across several resistors connected in series is equal to the sum of the potential differences across the separate resistors. Current in every part of the series circuit is the same.

In a parallel circuit (Figure 24.5, right), resistance is as follows:

$$1/R = (1/R_1) + (1/R_2) + (1/R_3) + \ldots, \tag{24.2}$$

where R = equivalent resistance of a parallel combination of conductors having resistances R_1, R_2, R_3, … R is always less than the smallest of the individual resistances. Connecting additional resistors in parallel decreases the joint resistance of the combination. The *potential difference* across several resistors in parallel is the same as that across each of the resistors. The potential difference is the same across all branches. The sum of the currents in the branches is equal to the value of the line current. Current values in the different branches vary inversely as the resistances of the different branches (Figure 24.5, right) (Schaum, 1961).

The conductance via the cuticle r_c^{-1} is very small and may be neglected, unless r_s is large, as when the stomata close. As noted, the epidermal resistance (r_e) is made up of the two resistances r_c and r_s in parallel (Waggoner, 1966):

$$r_e = 1/[(1/r_c) + (1/r_s)] = (r_c r_s)/(r_s + r_c). \tag{24.3}$$

The stream of water, T (in units of g/cm^2/s, for example), transpired from a leaf is assumed in accordance with diffusion theory to be

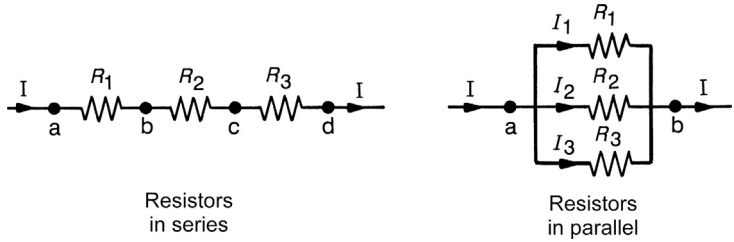

FIGURE 24.5 Left: Resistors in series. Right: Resistors in parallel. *From Schaum, D., ©1961. Theory and Problems of College Physics. Schaum Publishing Co, New York, p. 158. This material is reproduced with permission of The McGraw-Hill Companies.*

proportional to the difference ΔX in water concentration (g/cm^3) between the surfaces of the mesophyll cells and the free air outside (Waggoner, 1966):

$$T = \Delta X/[r_a + (r_s r_c)/(r_s + r_c)] = \Delta X/(r_a + r_e). \tag{24.4}$$

Or Eqn (24.4) may be shown as follows (Gale and Hagan, 1966):

$$T = \{[H_2O]_{int} - [H_2O]_{ext}\}/(r_s + r_a), \tag{24.5}$$

where T is transpiration, defined above; $[H_2O]_{int}$ is the water vapor concentration at the mesophyll surface and $[H_2O]_{ext}$ is the vapor concentration of the air (cm^3 vapor/cm^3 air); and r_s and r_a are the resistances as defined above (s/cm). We are neglecting r_c.

The diffusion theory, upon which Eqns (24.4) and (24.5) are based, is Fick's law. (For a biography of Fick, see the Appendix, Section 24.11.) In 1855, Adolf Fick discovered the linear flow law of diffusion, which is called Fick's law, to describe the diffusion of solutes in solution, and it is as follows (Kirkham and Powers, 1972; p. 75):

$$Q = DA(C_1 - C_2)/L, \tag{24.6}$$

where Q is the quantity of solute per unit time, D is the diffusion coefficient, L is the length of the element through which the diffusion is occurring, A is the cross-sectional area of the element, and $(C_1 - C_2)/L$ is the *concentration gradient*. We give Fick's law in Table 7.1, which lists linear flow laws used in soil–plant–water relationships.

Photosynthesis may be described similarly as a diffusion process of CO_2 from the outside air to the chloroplasts, but here a fourth resistance (in addition to r_s, r_c, and r_a) to diffusion of CO_2 is present in the liquid phase from the mesophyll wall to the chloroplast (r_m'). In addition to liquid phase CO_2 diffusion resistance, r_m' also includes all the metabolic factors that affect the photosynthetic rate. Thus, photosynthesis may be expressed as follows (Gale and Hagan, 1966):

$$P = \{[CO_2]_{ext} - [CO_2]_{int}\}/(r_s' + r_a' + r_m'), \tag{24.7}$$

where P is the photosynthetic rate ($cm^3\ CO_2/cm^2/s$); $[CO_2]_{ext}$ is the concentration of the carbon dioxide in the outside air and $[CO_2]_{int}$ is the CO_2 concentration at the site of the CO_2 sink, that is, the chloroplast ($cm^3\ CO_2/cm^3$ air); and r_s', r_a', and r_m' are the resistances to CO_2 diffusion as defined above (s/cm). The primes denote resistance to flow of carbon dioxide, and no primes are used to denote resistance to flow of water vapor. Using Waggoner's (1966) analysis, we get:

$$P = \Delta X'/[r_a' + (r_s' r_c')/(r_s' + r_c') + r_m'] = \Delta X'/(r_a' + r_e' + r_m'), \tag{24.8}$$

where P is the photosynthetic rate, as defined above, and $\Delta X'$ is the decrease in carbon dioxide concentration between the air and the site of chemical combination of carbon dioxide with a receptor.

The fact that there is a fourth resistance (mesophyll resistance) for carbon dioxide transport, which is not present for water vapor transport, has been the theoretical basis for the use of antitranspirants. When an antitranspirant is applied to a leaf, transpiration (T; Eqn (24.5)) should be reduced more than photosynthesis (P; Eqn (24.7)) is reduced. However, in practice, an antitranspirant reduces both T and P tremendously, so that photosynthesis is essentially stopped until the antitranspirant is removed. Figures 24.6 and 24.7 show circuits that illustrate resistances encountered in a leaf, as conceived by plant physiologists (Kramer, 1983; Baker, 1984).

The upper surface of a leaf (usually the adaxial surface) and the lower surface of a leaf (usually the abaxial surface) each have a resistance associated with them. If a leaf has no stomata on a surface, then there will be no stomatal resistance for that surface. Resistances of adaxial and abaxial stomata are assumed to act in parallel (Kramer, 1983; p. 302), or

$$1/R_{\text{total}} = (1/R_{\text{abaxial}}) + (1/R_{\text{adaxial}}). \tag{24.9}$$

However, this assumption means that the potential on the abaxial surface of the leaf is the same as the potential on the adaxial surface of the leaf (see the preceding paragraphs concerning resistance in a parallel

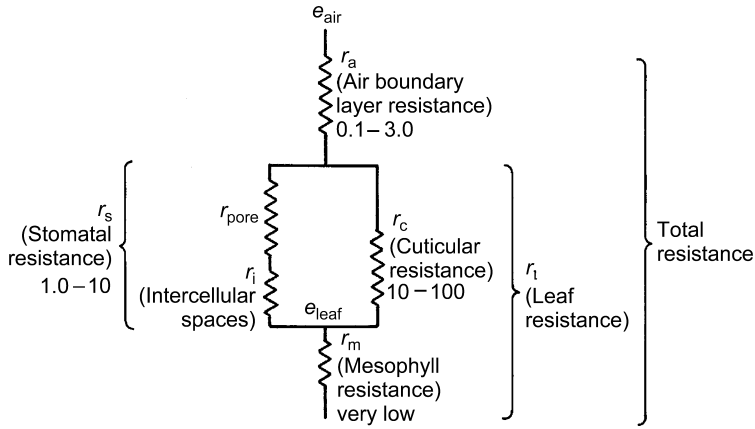

FIGURE 24.6 Diagram showing resistances in seconds per centimeter to diffusion of water vapor from a leaf. Stomata and cuticular resistances vary widely among species and with leaf hydration and atmospheric humidity. The rate of transpiration is proportional to Δe, the water vapor pressure gradient, e_{leaf} to e_{air}, and inversely proportional to the resistances in the pathway. *From Kramer (1983), p. 296. Reprinted by permission of Academic Press.*

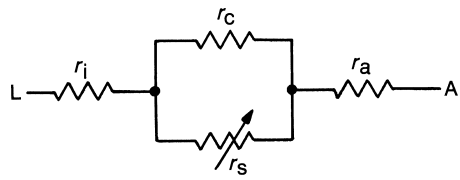

FIGURE 24.7 Resistances encountered by a water molecule diffusing from a leaf cell (L) into the surrounding air (A). r_i is the resistance of the intercellular spaces, r_c is the resistance of the cuticle, r_s is the variable resistance of the stomata, and r_a is the resistance of the boundary layer of unstirred air at the leaf surface through which water molecules must diffuse. *From Baker (1984). Reprinted by permission of Pearson Education Limited, Essex, United Kingdom and by permission of Dennis A. Baker.*

circuit). But this is not the case for plants (Kirkham, 1986). The adaxial surface of a leaf has a different water potential than the abaxial surface. So the limitation of Eqn (24.9), as applied to the total resistance of a plant leaf, should be recognized. However, it is the only equation we have to get the total resistance of a leaf, when the resistances on each surface are known. So we use it.

24.9 MEASUREMENT OF STOMATAL APERTURE AND STOMATAL RESISTANCE

Because water is lost mainly through the stomata on the surfaces of leaves, it is critical to know the extent of stomatal opening, to evaluate how much water a plant is losing. Kanemasu (1975a) enumerates different methods used to assess stomatal aperture. These methods include the following:

1. Observation under a microscope (Hsiao and Fischer, 1975a).
2. Use of cobalt–chloride paper (Teare et al., 1973; Kanemasu and Wiebe, 1975). Cobalt–chloride paper is prepared by dipping filter paper in a solution of $CoCl_2.6H_2O$ and then drying it. The paper is blue when dry, but pink when moist. The dry, blue paper, when placed on a leaf, covered with plexiglass, and held firmly by a small spring clamp, will turn pink from water vapor escaping from the leaf surface. This method can be used to compare the rates of transpiration from upper and lower leaf surfaces and from leaves of different plants under different environmental conditions.
3. Determination of resistance from leaf chamber (cuvette) transpiration, which can also incorporate the capability to monitor CO_2 assimilation for photosynthesis (Davenport, 1975). Work with the two models of the portable photosynthetic systems of Li-Cor, Inc (Lincoln, Nebraska) (Model LI-6200 and Model LI-6400) reports data

from cuvette measurements. In 1987, Model LI-6200 was put on the market and is a closed system; subsequently, Model LI-6400 was developed and it is an open system.

4. Mass-flow porometry: when stomata close, the permeability of the leaf to various gases (porosity) is greatly reduced. In mass-flow porometry, air is forced under pressure through the leaf, and the rate of flow or leaf resistance to flow is indicative of porosity (Figure 24.8) (Hsiao and Fischer, 1975b). The mass-flow porometer developed by Gregory and Pearse (1934) is the basis for most mass-flow porometers. Amphistomatous leaves are needed to use mass-flow porometers, because air must enter one side of the leaf and exit from the other side. If the resistance of one epidermis is high, the reading obtained with the porometer reflects mainly the opening of that epidermis. When using mass-flow porometers, it is assumed that the mesophyll resistance is constant and small compared to the resistance offered by the epidermis of a leaf with closed stomata. Mass-flow porometers are not available commercially.
5. Diffusion porometry: diffusion porometers measure diffusion of water vapor from the substomatal cavities through the stomata. Diffusion porometry includes both transient (dynamic)-state and steady-state methods (Kanemasu, 1975a). In the steady-state

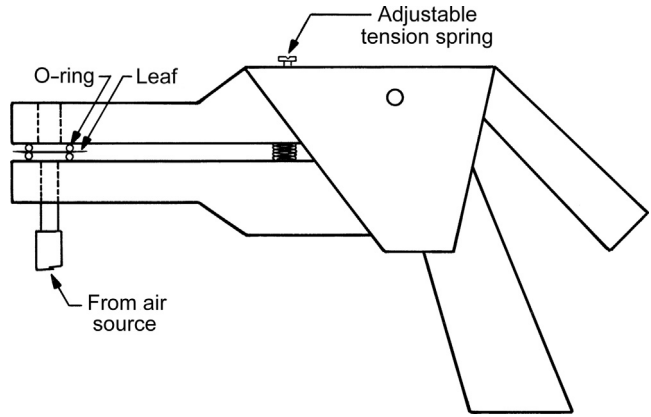

FIGURE 24.8 A mass-flow porometer. The basic structural material consists of plexiglass cemented together. The critical aspect in construction is alignment of the two O-rings, both horizontally and vertically. Alignment of the two arms of the cup (cut from 1.3-cm-thick plexiglass sheets) is ensured by fixing, with a close-fitting metal pin, the upper arm snugly between the two large parallel trapezoidal plates glued to the lower arm. One O-ring is glued to an arm first. The other arm is then sanded to ensure good horizontal alignment of the O-rings. Vertical alignment is effected when the second O-ring is glued onto the arm. *From Hsiao and Fischer (1975b). Reprinted by permission of the Director of the Washington State University Agricultural Research Center, Pullman, Washington.*

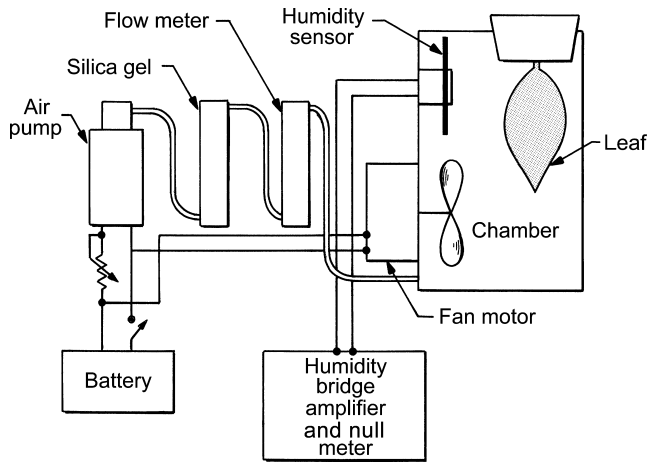

FIGURE 24.9 Block diagram of steady-state porometer showing components and interconnections. *From Campbell (1975). Reprinted by permission of the Director of the Washington State University Agricultural Research Center, Pullman, Washington, and Gaylon S. Campbell.*

porometer, dry gas is passed over an enclosed leaf at a known flow rate and the humidity of the exhaust gas is measured (Figure 24.9) (Campbell, 1975). In the transient-state porometer, a sensor responsive to a change in humidity is clamped to a leaf (van Bavel et al., 1965; Kanemasu et al., 1969; Ehrler, 1975; Kanemasu, 1975b). Tan and Black (1978) describe a diffusion porometer for use on conifer needles.

Day (1977) and Parkinson and Day (1980) give theory associated with the steady-state porometer, and Chapman and Parker (1981) supply theory for the transient-state porometer. Of all the methods used to measure stomatal resistance only (i.e., photosynthetic rate is not measured), diffusion porometers (transient- and steady-state types) are most widely used for quantitative measurements (Livingston et al., 1984). They are commercially available. The transient-state one is made in Cambridge, England, by Delta-T Devices, Ltd, and imported for sale in the United States by Dynamax, Inc (Houston, Texas) (Figure 24.10). The only commercially available steady-state diffusion porometer is made by Decagon Devices (Model SC-1; Figure 24.11). An attached leaf is put into its sensor head, which contains two humidity sensors: one close to the leaf and one farther away from the leaf. Temperature is also recorded at these two locations. The readout gives four values (relative humidity at two locations; temperature at two locations), and the software of the porometer calculates stomatal resistance from these measured values. The porometer can be adjusted so it reads out stomatal conductance instead of

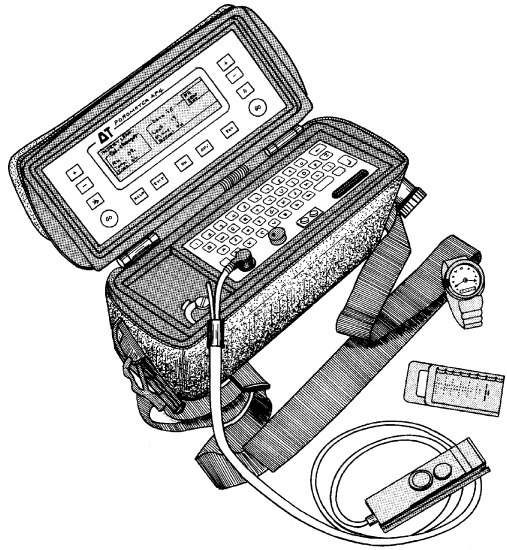

FIGURE 24.10 A commercially available transient porometer. Automatic cycling ensures consistent results by repeating the measurement cycle (in typically 3–10 s) so that as soon as a repeatable value has been reached—usually after about four or five cycles—the next leaf can be sampled. The relative humidity level at which the instrument cycles can be set between 20% and 70% to match the ambient relative humidity as closely as possible, to avoid upsetting the stomata. The sensor head, shown in the lower right of the figure, weighs 80 g and incorporates a window for checking the alignment of the leaf with the sampling area—a slot 2.5 × 22.5 mm. The calibration plate, shown above the sensor head in the figure, has six values of diffusion resistance in the range 0–30 s/cm. The porometer comes with a rechargeable battery, padded carrying case, and a strap so one can put it around the neck while taking measurements in the field. The size of the porometer is 350 × 200 × 100 mm and it weighs 3.2 kg. *From a Dynamax, Inc, Houston, Texas, brochure. Reprinted by permission of Dynamax, Inc., Houston, Texas.*

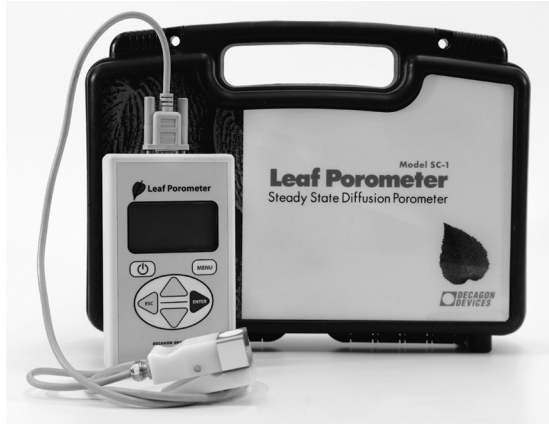

FIGURE 24.11 The steady-state diffusion porometer sold by Decagon Devices, Pullman, Washington. *Photograph taken by Marsha K. Landis, Graphic Designer and Web Manager, Departments of Agronomy; Plant Pathology; and Horticulture, Forestry, and Recreation Resources, Kansas State University, Manhattan, Kansas. Used by permission of Marsha K. Landis.*

stomatal resistance. Kirkham (2007) gives further details about the operation of the porometer.

When taking measurements of stomatal resistance with a porometer, one must be aware of leaf angle because light intensity strongly affects stomatal opening. (We discuss interception of solar radiation by leaves at different angles in Chapter 30.) For example, stomatal resistance on corn (maize) can vary from less than 1 s/cm when stomatal are wide open and perpendicular to the sun's rays to more than 40 s/cm when a leaf is shaded (John M. Norman, Department of Soil Science, University of Wisconsin, personal communication, February, 1982).

24.10 THEORY OF MASS-FLOW AND DIFFUSION POROMETERS

As noted in Section 24.4, if stomata are spaced so that diffusion from one does not interfere with another, stomata can be considered to conduct water vapor more or less independently of each other. Hence, stomatal mass-flow and diffusive resistances per unit leaf area are inversely proportional to the number of stomata in that area (i.e., to stomatal frequency) (Hsiao, 1975).

The simplest physical model of a stomatal pore is that of a cylinder (Hsiao, 1975). Therefore, the relation between mass-flow resistance (in the mass-flow porometer) and stomatal opening tends to take on a form similar to that of the Poiseuille equation: resistance to flow is inversely proportional to the fourth power of the radius of the opening. This approximation becomes invalid, however, in the case of nearly closed stomata, because the term for interaction with the path wall becomes large and must be considered. In most cases, the stomatal pore is not circular and the length of the pore (normal to the conducting path) does not necessarily vary with the width of the pore. The mass-flow resistance then becomes inversely proportional to the third or even lower power of the width (Hsiao, 1975).

For diffusive resistance, the simplest approach is to apply Fick's law of diffusion to an assumed simple pore geometry. The result is that, to the first approximation, stomatal diffusive resistance is inversely proportional to the total pore area. For circular stomatal pores (Hsiao, 1975),

$$r_s = (A/nD)\left(4L_s/\pi d^2\right), \tag{24.10}$$

where A is the leaf area being studied, n is the number of stomata, D is the diffusivity of water vapor in air, L_s is the depth of the stomatal tube (i.e., of the stomatal pore), and d is the stomatal pore diameter. If the so-called end correction (Monteith, 1973; p. 145) is applied to one end (outer end) of the

stomatal tube, a factor, $1/2\,d$, is added, and the resulting equation is (Kanemasu, 1975b):

$$r_p = (A/nD)\left[4t/\left(\pi d^2 + 1/2d\right)\right] \tag{24.11}$$

where r_p is the resistance of a calibration plate, t is the thickness of the plate, A is the aperture area, n is the number of holes, D is the diffusivity of water vapor in air, and d is the diameter of the holes. Equation (24.11) is identical to the equation used for calculating the resistance of the calibration plate for the transient-state diffusion porometer (Kanemasu, 1975b).

24.11 APPENDIX: BIOGRAPHY OF ADOLF FICK

Adolf Eugen Fick (1829–1901), a physiologist, was born in Kassel, Hesse, Germany, on September 3, 1829, the son of Friedrich Fick and Marianne (Spousel) Fick. He got his MD at the University of Marburg in 1851 and married Emile von Cölln in 1862. He was an assistant to Carl Ludwig (1816–1895; German physiologist) in Zurich in 1852. Fick was a professor of physiology in Zurich beginning in 1862 and was a professor at the University of Würzburg from 1868. He was the author of *Die medizinische Physik* (1856) and *Untersuchungen über elektrischen nervenreizung* (1864).

He made important discoveries in every branch of physiology. He proved that carbohydrates rather than albumin are the source of muscle energy. He constructed the first plethysmography, which measured the pulse rate. In about 1864, he invented the myotonograph for measuring and recording muscle tension. In 1870, he developed a method to determine cardiac output by gasometry (Marquis Who's Who, 1968). He discovered the linear flow law of diffusion, named after him, to describe diffusion of solutes in solution, as in animal tissue (Fick, 1855). (He was a prosector; Kirkham and Powers, 1972; p. 429.) He died in Blankenberge, Belgium, on August 21, 1901.

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