BOT 4503
5 hours
Gas Exchange

Objectives

1. Know the entire scientific vocabulary used (e.g., ectodesmata, xerophytic, niche &c.).

2. Name and discuss the several adaptations that were required or coincided with the evolution of a terrestrial lifestyle for plants. How did each change confer fitness?

3. Make a plot. Let the x-axis be the wind speed (arbitrary units) across (parallel with) the leaf surface. Let the y-axis be the vapor pressure (arbitrary units). (This is not exactly the same plot as shown on the overhead.) Now, draw a family of curves, each at a different (arbitrary) distance from the leaf surface. [The purpose of this exercise is to have you mentally explore the concept—rigorous treatment (e.g., to assign real distances) would require data and concepts (e.g., as a first step, calculation of the Reynolds number) not in hand.]

4. What is the driving force for gaseous diffusion? What is the boundary layer? How does it affect the driving force, aforementioned? Explain how wind affects boundary-layer resistance.

5. What is the current CO_2 concentration in the atmosphere? Is it changing? Is it constant year-round? What was the concentration in the pre-Industrial times? Calculate the CO_2 concentration in molarity. (Hint: from the Ideal Gas Law, you know that a gas is ca. 50 mM, total species.) Expressed in molarity, what is the maximum difference in CO_2 concentrations between the inside and the outside of the leaf? For ease of calculation, let the saturating vapor pressure of H_2O be 20 mm Hg, its value near 20° C. Expressed in molarity, what is the concentration of water inside the leaf and outside the leaf at 50% RH? Let the distance over which these two concentrations operate be 1.0 (i.e., 100% of anything). Now, what is the ratio of the driving forces of water/carbon dioxide?

6. Discuss quantitatively the parallel pathways of water loss from leaves, viz., cuticular transpiration, and stomatal transpiration. How much does cuticular transpiration vary among species? How does cuticular transpiration relate quantitatively to stomatal transpiration? Describe how the basis for expression of transpiration will affect an interpretation.

7. Describe various morphological features of leaves that serve to reduce water loss. Be sure to include stomatal position, epidermal protrusions (e.g., hairs), size, and distribution of stomata, size of leaf, shape of leaf.

8. What is the paradox of pores?

9. Discuss the CO_2 response of stomata. Do guard cells themselves detect depressed CO_2 concentration?. What is transmittance? . . . Optical density? . . . Absorbance? In brief, describe

how the concentration of a light-absorbing substance can be determined? What are the wavelengths of blue, green, and red light? Which is more, or less, energetic, on a photon basis? What is an absorption spectrum? Roughly draw the absorption spectrum for chlorophyll. What is an action spectrum? Discuss the consonance of the action spectrum for photosynthesis (which is based largely on the harvesting of light energy by chlorophyll) and the absorption spectrum for chlorophyll.

10. Do stomata respond to light? . . . if so, how? Is the light signal perceived by chlorophyll? How do you know? What is a nominal value for full-sun photon-flux density? How much light is required to cause stomatal opening? Is chlorophyll involved to any extent in mediating a light response? If so, how do you know? Is this a direct response, or an expected indirect response owing to the lowering of the CO_2 concentration by photosynthesis?

11. Name other factors that promote stomatal opening.

12. What is ABA? Describe several physiological roles of ABA. What is the relationship between [ABA] and the water status of the plant?

13. Why is the internal RH not quite as high as 100%?

14. What is water-use efficiency? Describe why you would expect water-use efficiency to increase in droughted plants.

15. Using the concept of water potential, explain the basis for stomatal movements. Is water potential constant? . . . the components of water potential? What does the distention of guard cells have to do the molecular structure of the cell walls?

16. What is the Classical Starch⇔Sugar Hypothesis for stomatal movements? Name contemporary objections (hint: consistency of observations, quantitation). What requisite proof was lacking? Has this theory stood the test of time?

17. Write a brief essay based on stomatal "history" to support the contention that data not communicated of little value.

18. Write a detailed description of how—at the molecular level—stomatal opening is initiated and proceeds. Restrict you description to events at the plasmalemma, but describe the general potassium concentrations in the cytosol and in the vacuole. Voltage changes and regulation of voltage gated channels are required parts of the description.

19. Describe the pH-stat mechanism as it works in guard cells. Recall, you should know the basics of glycolysis. Discuss PEPC. How is it regulated?

20. Repeat #18, except for closure.

21. ABA effects stomatal closure and inhibition of stomatal opening. Describe the ways by which ABA causes the elevation of Ca^{2+}_{f}. Describe the effects of Ca^{2+}_{f} on channels.

22. Describe logically one technique for measuring CO_2 uptake. . . . for measurement of CO_2 concentration inside the leaf.

Lecture

Progenitors to terrestrial plants evolved in an aqueous milieu. The transition to land required several adaptations. First, a strong support system is necessary. The evolved solution was lignin, which we covered during the lectures on cell walls. Second, anchorage is required; roots evolved, although the first land plants, which probably resembled *Psilotum*, probably did not have roots. Third, competition for sunlight is strong; thus, shoots evolved for the support of light-harvesting leaves. Indeed, long stretches of shoot—take a look at the Strozier oak—may exist "simply" to position the leaves. Fourth, as some parts of the plant will be quite removed from the source of nutrition, a vascular system evolved to redistribute the acquired minerals and photosynthate. Fifth, the terrestrial environment is ever changing; it can be scorching hot or bitterly cold, or wet or dry. Thus, plants evolved mechanisms to survive periods of unfavorable conditions—the seed is a prime example, but there are many others such as tubers. Especially cantankerous in these parts is *Smilax,* which you may recognize by the sometimes-common name, "bullis."[1] *Smilax* has rhizomes (≡underground stems) that swell to the size of a tennis ball. Almost regardless of what happens to the aerial portion of the plants, the tuber, or swollen portion, stores sufficient energy and sprouts. Sixth, plants evolved a dominant diploid generation, whereas the aqueous evolutionary progenitors almost certainly exhibited a dominant gametophytic, or haploid, generation. The possible presence of an alternative allele could provide the plant with a "genetic escape" from a lethal situation. Seventh, in the general sense of the word, mobility was lost. Thus, angiosperms lack flagella, as do fungi. You are aware, of course, that plants do move. Morning glory flowers open in the morning and close at night. Sunflowers and many other plants track the sun, hence their name. Some plants, e.g., soybean, turn toward or turn away from the sun, depending on conditions. Other plants change leaf shapes, e.g., when maize leaves dry out, they roll, which is a water-conserving "strategy." In other instances, plants move by growing! As an example, the pollen grain itself is immobile, and therefore the gametic nuclei can not be delivered, as they would be for a flagellated gamete. However, pollen—delivered by an organic vector or wind—germinates on the stigma, and a pollen tube grows down and penetrates the egg. The sperm nuclei descend the tube. (This "motility by growth," as I dub it, can cover quite a distance—consider the pollen tube that grows

[1] As an aside, I suspect that "bullis" is a degenerate "country" form of "bullace," which is a synonym of plum. *Smilax* is a vine and superficially resembles the muscadine, which is called bullace grape, or, in Gullah, simply bull grape.)

down the silk (stigma + style) of maize.) Finally, and most important for our present purposes, plants had to evolve a mechanism to avoid desiccation. In brief, two simultaneous adaptations arose: (a) The aerial portion of terrestrial plants became covered with a waxy cuticle, which—as a beginning—we will say is impermeable to the passage of water. However, this cuticle is also impermeable to CO_2. Klaus Raschke once asserted that there was no natural substance that could provide a barrier to water, and not to CO_2. In the several years that have transpired since his assertion, I have not thought of an exception. (b) Pores of adjustable aperture size punctuate the epidermis, the substratum for the cuticle. These stomata, having a "diameter" in the micrometer range, are completely non-selective. When the pores are open, they admit CO_2, but water is also lost from within the leaf intercellular spaces. On the other hand, CO_2—required for photosynthetic reduction—is only admitted to the leaf interior through stomata. Again, to borrow non-verbatim from Klaus, "Plants adjust the stomatal aperture size from moment-to-moment in an effort to satisfy the opposing priorities of admitting CO_2 and avoiding desiccation."

In an introductory course, I explain that a leaf is a photosynthetic factory. I say that the photosynthetic parenchyma is sandwiched between two water-impermeable epidermes, except where these epidermes have stomata. This statement is only approximate.

Overhead: Cuticular transpiration

As this overhead shows, some water does escape through the cuticle. The amount depends very much on the species. The xerophytic succulent *Opuntia* (prickly pear—a weed commonly found on the thin soil overlying granite in North Georgia (USA), the pad also being found as a gourmet item in Publix from time to time) has a very low rate of cuticular transpiration. Judging from its ecological niche, you would "expect" that this plant would exhibit water-conserving characteristics. At the other extreme is *Impatiens*, generally a shade-loving plant, one that Geo. Parks has proclaimed as America's No.1 flower. In brief summary, as expressed ($mg\ H_2O \cdot hr^{-1} \cdot gm_{fresh\ mass}^{-1}$), the range of cuticular transpiration varies by two orders of magnitude. This comparison is not really a good one, however, because *Opuntia* has a thick photosynthetic organ, whereas the leaf of *Impatiens* is thin, almost ephemeral. A more valid comparison would be on surface-area basis. When the data are thus transformed, a large difference, albeit diminished to nominally 20x, remains.

The preceding data illustrate well the variation, as a function of species, of cuticular transpiration. However, a comparison of the rate of cuticular transpiration to the rate of stomatal transpiration is of the essence:

Overhead: Resistance to water permeation

A comparison of three species reveals that the cuticle offers a much, much higher resistance to water loss than does passage through stomata. As an overall "perspective" statement, we conclude that the vast majority of water (>95 %) molecules lost from a plant passes as gaseous water through the stomata. (You need to know that resistance is the reciprocal of conductance, but you do not need to know the units for conductance (or resistance) —just appreciate the relative values.) To finalize our knowledge of cuticular transpiration, note that some cuticles have ectodesmata, aqueous channels that seem to connect the epidermal cell wall with the environs.

The two pathways for water movement discussed above are parallel pathways: either a particular water molecule moves from the leaf through stomata, or through the epidermis (cuticle, *per se*). Other resistances to CO_2 uptake occur in series. The first of these resistances is the so-called boundary layer. Consider any surface—immediately in contact with the surface is a relatively unstirred layer of the medium. In the case of leaves, this boundary layer is of air. As the air is unstirred, it is not at the same concentration as the bulk air phase. In short, the driving force, recall, for the diffusion of gaseous molecules is the **concentration gradient**. The more sharply the concentration difference is applied, the greater the driving force. This is an important principle that you see applied daily in your life—e.g., your automobile radiator is supplied with a fan, which, when "called for" by your engine temperature sensors, will blow across the radiator. Thus, your fan reduces the unstirred layer of air around the vanes, and heat is dissipated more efficiently. Clothes are tumbled in a dryer, for the same reason—it is to keep to a minimum the unstirred layer. Speaking broadly, the resistance of the boundary layer is in the same range as open stomata, but much lower (by an order of magnitude) than the resistance of stomata that are not open. Perhaps more important than simple recognition of the presence of a boundary layer is the knowledge that (a) the boundary layer is regulated by the environment, and (b) the boundary layer is under morphological and developmental control. The next overhead will demonstrate both of these important points.

Overhead: Boundary layer—effects of morphology and environment

The top portion of the overhead shows that a wind will make a big difference in the size of the boundary layer. (This is a complicated graph; focus only on wind.) Consider the top situation, where the air is only slowly moving. At the origin (0 distance from the leaf surface), the wind speed will be zero. As one follows the trace from the leaf surface (read the y-axis as the independent variable), he notes that the wind speed increases, but it increases slowly—the wind speed does not become constant until one traces the

graph up 3 units of distance from the leaf surface. Contrast that curve with that at the bottom, which depicts the effect of a fast wind across the leaf surface. In this case, when the distance from the leaf surface is traced up the y-axis, it is apparent that only after less than two units of distance, the wind is up to the maximum speed. The important point is, of course, that the gradient (for water, or for CO_2) is increased by the wind. (To reveal the obvious, by lessening the distance, a denominator term in the gradient (dC/dX), increases the magnitude of the gradient, which, said again, is the driving force for gaseous diffusion.) The summary conclusion for this section on environmental regulation of the boundary layer is: high-speed wind across an exposed leaf surface facilitates gas exchange. Please, in your mind, be certain to register by qualification—an exposed leaf surface. Exposed leaf surfaces are well known in the laboratory and exist in nature on the periphery of the canopy, and no place else. In an average productive soybean field, the leaf-surface area is about six times the ground-surface area. Thus, many leaves are buried inside the canopy, where wind does not touch them. Obviously, these leaves have a huge boundary layer. This is a practical consideration—not one restricted to some esoteric research interest. Within a maize canopy, the CO_2 concentration is a great deal lower than that of ambient. I do not have published data to cite, but Larry Christy (who then worked at Monsanto) told me that the concentration may drop to around 100 ppm (ppm ≡ parts per million, or $\mu l\ l^{-1}$—as we refer to gases, which equally occupy a volume proportional to the molarity, such an expression is sound). Current CO_2 concentration is ca. 360 ppm in the atmosphere; the concentration is slightly more in the winter and spring (in the relevant hemisphere), when less is tied up in organic material, and the value is increasing by about 2 ppm per annum because of our utilization of fossil fuels. (The pre-Industrial Revolution concentration was about 280 ppm.)

In addition to this environmental regulation of the boundary layer (point (a), above), there is morphological regulation of the boundary layer, which is portrayed in the bottom of this overhead. In the upper left hand of the bottom panel, one sees the “normal” situation—there are guard cells on both leaf surfaces, perhaps a 50% higher distribution on the abaxial (bottom) epidermis, compared with the density of the adaxial surface. (This is a “temporary” summary conclusion, and we will return with more detail in a subsequent section of this lecture series.) In this normal situation, the guard cells are flush with the epidermal surface. Pine (lower left of bottom panel) is a xeric species, i.e., it is adapted to a dry environment. At first, this statement is counter-intuitive; we know that pine grows well (competes well) here in Tallahassee, which receives abundant precipitation. However, our soil is sandy, and does not hold water well, so even soon after a rain, the soil is dry. On the other hand, our water table is high, so roots do not penetrate deeply, because oxygen is only poorly soluble in water; roots drown in water. In this X-section, the dark arrows again indicate the stomata. Note that the stomata are sunken; they are not flush with the surface. Obviously, the increased distance from the region of high water concentration (low CO_2

concentration) to the region of low water concentration (outside, "high" CO_2 concentration) diminishes the gradient, the driving force. On the lower right, one sees a X-section of maize leaf. The surface of maize leaf is decorated with trichomes, outgrowths of the epidermis. These extending structures obviously interfere with the free movement of air and diminish gas exchange. In the upper right, oleander exhibits both of the aforementioned morphological features, sunken stomata and a hairy vestibule. Maize leaves (bottom right) exhibit a different type of morphological adaptation. The bulliform cells in the upper epidermis are turgid when the leaf is well watered. When water stressed, the leaf loses water first from the bulliform cells; in effect, the shrinkage of these cells shortens the upper portion of the leaf, whereas the bottom of the leaf is unchanged in dimensions. The result is that the leaf rolls, or as farmers say, "The corn is twisted." By rolling back on itself, the leaf exposes less surface area. This is a description of moderate to severe water stress. As a final point, these morphological adaptations are not simply of interest in esoteric research areas. Plant shape is important for many agronomic reasons, *viz*. light interception (have you noticed that modern corn hybrids have more-or-less erect leaves?), amenability to mechanical harvest, &c.

By way of review, we have looked at the boundary layer resistance, which is in series with the parallel resistances of the cuticle and of stomata. Briefly, on its way to being reduced to carbohydrate, CO_2 encounters other resistances, which we mention (mesophyll-cell wall, "mesophyll" resistances) but do not else touch on.

Overhead: Other morphological attributes that affect transpiration rate.

In addition to the features on the previous overhead that show the effect of morphological attributes on transpiration, this overhead summarizes others. First, the overall leaf shape can affect the rate of transpiration. Small leaves—such as pine needles—have a small boundary layer. You may have inferred this relationship from the previous overhead; recall the forward surface of a leaf slowed the air relatively little. Intuitively, the longer the surface area that is wind—swept, the larger the diminution in the velocity of the wind. Another feature related to overall leaf shape is the ratio (internal surface area)/(external surface area). The range for this ratio that expresses the relationship between cell surface area in the leaf intercellular spaces to the overall surface area of the leaf is 5 — 30. The higher ratio—reflecting a "fat" leaf—is typical of water-conserving species, such as *Opuntia* that we just reviewed. A second morphological attribute that affects transpiration rate is combination of the number and size of stomata. The overhead shows, in a survey of 8 species, that the number of stomata can vary from 0 to 28000 per square centimeter! As exemplified, it is not uncommon to find species that lack stomata on the adaxial

("upper") leaf surface. As far as I am aware all terrestrial leaves have at least some stomata on the abaxial leaf surface. (Some leaves that float on the surface of water have no stomata on the abaxial side, but do have stomata on the adaxial side.) Because the variation is so large, it is perhaps of little value to try to generalize about the distribution of stomata; with that qualification, a suspect generalization is that leaves have more stomata on the lower surface than on the upper surface, but even this survey of only 8 species provides an exception (wheat). Stomatal size varies also. *Vicia faba*, the plant that we in our laboratory primarily investigate, has large stomata: the outside dimensions of the stomatal complex with a closed stoma are 40 x 15 μm. (Maximally open—not a condition typical of a photosynthesizing leaf on an intact plant—the stoma itself is nominally 15 micrometers across.) To stress the point, I note that I am only aware of one angiosperm that has similarly large stomata, *Diffenbachia*, the broad-leaf "dumb cane" used commonly as a house-plant. At the other extreme, some stomata are small, as the overhead shows in the case of bean (which is in the same family as *Vicia faba*, the common name of which is "bean," although it is a vetch.)

[Because of time constraints, we will not be able to discuss other features, particularly the different morphologies and distribution of stomata. This latter point will be briefly touched on as we discuss the basic mechanism of aperture size adjustment. We will also have to delete a discussion that pertains to the effect of size, shape, and distribution of stomata on the rate of transpiration. Finally, we will delete a discussion on the "paradox of pores," which, briefly, describes the phenomenon of equal rates of evaporation from an open surface and an impermeable surface that is frequently punctuated with pores.

Overhead: Stimuli that effect stomatal opening and closing.

Given the fact that stomata are non-selective (the pathway is the same for CO_2 uptake as for water loss), and the teleological assumption that plants want to conserve water, it is unsurprising that the factors that "indicate" a demand for CO_2 are those that open stomata. Said another way, you would "expect" that low CO_2 concentration would cause stomatal opening. As the overhead shows, low CO_2 concentration, say 150 ppm, does indeed cause stomatal opening. Low CO_2 concentration is sensed by the guard cells themselves, as a French scientist taught us more than four decades ago. In brief, he stripped away the epidermis of a leaf. On exposure of the epidermal peel to various CO_2 concentrations, a response was observed. As the epidermal peel is a monolayer of mostly damaged ordinary epidermal cells in which the guard cells are embedded, he concluded that the guard cells themselves sensed the CO_2 concentrations. (The alternative hypothesis is that other cells, such as the mesophyll cells, would sense the CO_2 concentration, and then—by way of a secondary messenger—would "alert" the stomata to open.) We have

not a single clue about the nature of the CO_2 sensor, but recently developed gene-tagging technologies suggest that this question in now amenable to experimental investigation.

The sensing of CO_2 is a complicated phenomenon. The efficacy with which a response is elicited varies depending on the growth history of the plants. We learned as early as the late 70's that greenhouse-grown plants performed differently in this regard than did growth-cabinet plants. We also know that there is an interaction of the CO_2 response and ABA exposure. (We will return to ABA later; presently, simply recognize it as an endogenous compound that prevents stomatal opening and causes stomatal closure.) As interpreted by some, I will again show no scruples and try to explain the situation as I see it through a teleological approach. First, given a constant environmental condition, the driving force for water loss is virtually constant. On theoretical basis and by data, we know that the intercellular air spaces have a relative humidity of almost 100%. (It is not exactly 100% for two reasons: (a) the source of the water vapor in the intercellular spaces is the tissue itself, which has a water potential of less than zero. (b) water is being lost from the leaf..) The rate of transpiration can thus be described as a function of a single variable, resistance, which, of course, is higher when stomata are narrower. The situation for CO_2 is not so simple, however; under the same constant environmental condition. The rate of CO_2 uptake is, of course, a function of stomatal-aperture size. However, the internal CO_2 concentration is variable. Consider an extreme case, one very favorable to photosynthesis, but with a water-insufficient plant. In this case, the stomata will be narrow (to diminish water loss); CO_2 uptake will not be affected proportionately because the intercellular CO_2 concentration will be lowered a lot (say, to 140 ppm), which means that there is a large driving force for CO_2 uptake (relative to a plant that has widely open stomata and in which the photosynthetic CO_2 uptake is modest). In summary, water-use efficiency is higher when plants have narrow stomata. On these bases, it is "expected" that there should be an interaction between regulation by CO_2 concentration and water stress.

Light also promotes stomatal opening. By which pigment is light sensed? Applying Ockham's razor, it would be reasonable to suppose that chlorophyll itself might be involved in the signal transduction pathway. (As we will learn later, two "places" in the photosynthetic electron transport "chain" do indeed provide for sensing of light absorbed.) A simple approach to disproving this hypothesis is to compare the action spectrum to the absorption spectrum of the pigment. See overhead. The independent variable for both these plots is light wavelength. Blue light (about 450 nm) and red light (about 660 nm) is absorbed by chlorophyll. Green light (about 550 nm, where your eyes are maximally sensitive) is only modestly absorbed by chlorophyll. (Plants are green because they reflect, and do not appreciably absorb, green light.) The absorption by a pigment is determined by placing a dilute solution of the pigment in a light path; a light sensing device—set at 100% transmittance with the solvent absent the pigment—is used to

measure the diminution of the light as it passes through the solution. (Although we can not develop the idea owing to the pressure of time, the transmittance data are transformed to optical density (or absorbance); absorbance, but not transmittance, is proportional to the concentration of the solution—e.g., an 80 μM solution of NADH will have an A_{340} (1-cm light path) of 0.5 and a 160 μM solution of NADH will have an A_{340} of 1. The "340" specifies that the absorbance value was obtained for light of 340 nm. Returning to the major point, blue light (more energetic) is absorbed on a photon-by-photon basis about equal to red light (less energetic) by chlorophyll. (A more in-depth discussion would include some disclaimers, e.g., the effect of the solvent.) Keep the shape of the absorption spectrum in mind. The action spectrum compares the efficacy of differently colored light with the relevant action, in this case, the rate of stomatal opening or the extent to which stomata are open. The shape of the curve for the action spectrum is in sharp contrast with that of the absorption spectrum of chlorophyll: on a photon-by-photon basis, blue light is much more effective than red light. This large difference implies that chlorophyll is not the pigment involved in the sensing of light that causes stomata to open, or, at least, that chlorophyll is not the sole or major pigment. In fact, today, we do not know the identity of the blue-light pigment, although there are some candidates—as you might expect, these candidates were nominated based on their absorption spectra. From an entirely different perspective, we would also be tempted to discount the idea that chlorophyll is a major factor in transducing light-stimulated stomatal opening: the blue-light response requires only low-light levels (say 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$, compared with a Florida full-sun summer value of 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Saturation of the photosynthetic response requires high light levels, from one-third full-sun to full-sun, depending on the type of photosynthetic metabolism exhibited by the particular plant. A low-fluence blue light response is not restricted to stomata; in the case of stomata, the signal is perceived by the guard cells themselves. With the foregoing background, the question that arises is whether chlorophyll is at all involved—might there be parallel pathways, a predominant one that does not involve chlorophyll, and a second smaller one that relies on the absorption of light by chlorophyll? (Some facts: guard cells have chloroplasts; these chloroplasts appear to harvest light like those of mesophyll cells.) This question was tackled rather straightforwardly several years ago. The investigators compared the action spectrum for stomatal opening in leaves that were fed a solution of the photosynthetic inhibitor DCMU, commercially known as diuron, to that of leaves fed a control solution. They did observe a slight effect, particularly in the red region. DCMU eliminated the slight red bump in the spectrum. A second conclusion is that chlorophyll does play a minor role in the adjustment of stomatal-aperture size, by a mechanism we have total ignorance of. As is the normal case, the answer to one question poses another question: Is the chlorophyll-mediated effect indirect (caused by a reduction in CO_2 concentration, which is secondarily sensed, or is the effect direct? To make a long story short, the effect is direct.

[Other factors, notably the plant-growth-regulator cytokinin and circadian rhythms, promote stomatal opening also.]

The overhead shows also that stomata close when water is insufficient. When plants are water stressed, they synthesize and accumulate the plant-growth-regulator abscisic acid (aka ABA), as discussed in the Water Potential Lectures. In the case of detached *Vicia faba* leaves that are rapidly and severely stressed by placing them in the path of a fan until they lose 10 % of the original fresh mass, the accumulation is nominally 20-30x that of control leaves, but a more typical accumulation factor is, say, 8-10 fold. Thus, water stress results in the accumulation of ABA and exogenously applied ABA causes stomatal closure at low levels (with a threshold in the range of 10^{-8} to 10^{-7} M). Whereas there is no question that ABA mediates water-stress-induced stomatal closure, we have only begun to find water-stress effects that are not mediated by ABA.

[ABA was discovered independently as an agent in disparate physiological processes. The name itself derives from the fact that some plants respond dramatically to slow imposition of water stress by loss of leaves. This is a developmental process—not the throes of death. An abscission zone forms; cell walls are degraded because of the plant's production of certain enzymes. In mid century, endogenous compounds that prevent growth of the latent meristem of potato tubers were discovered. One of these, "dormin" (from dormant), turned out to be synonymous with ABA. Seed development is an exquisite desiccation-driven developmental process. ABA promotes seed dormancy; gibberellins (a class of plant-growth regulators) are antagonists. The involvement of ABA in seed production has been a model plant-growth system, started by Joe Varner, erstwhile of Michigan State University, and latterly of Washington University in St. Louis. It is only a small exaggeration to say that plant-molecular biology has its origins in the system studied by Varner, who died July 4, 1995, and his colleagues.]

Overhead: Simplified mechanics of stomatal aperture size regulation.

We boldly change directions now, and turn our attention to a description of how stomatal-aperture size is regulated at the simple biophysical and biochemical levels. Although water potential varies over the course of day, generally being lowest in mid-afternoon, which coincides with a period of stomatal-aperture-size depression, it is not necessary to invoke foliar-water-potential changes to explain stomatal movements. Over the short distances across a leaf, it is sufficient to suppose that there is not a large water-potential difference. Thus, regardless whether stomata are open or closed, the water potential of guard cells and of epidermal cells can be considered a constant. Recall that water potential comprises two terms, solute potential and hydrostatic potential, which we treat additively.

Nearly 150 years ago, it was possible to visualize starch in individual cells. The new branch of science was called histochemistry. As far as I am aware, the first histochemistry was conducted on guard cells. A water-soluble dye was applied to guard cells; the dye—still used—intercalates in the molecular structure. Heavy staining indicates abundant starch; less staining, less starch. (Starch, being water-insoluble, is easy to study by the indicated histochemical method, as there is no problem with its loss or redistribution during the experimental procedures.) As the overhead shows, starch content of guard cells declines during stomatal opening, which coincides with a volume increase. The interpretation was the so-called Classical Starch⇔Sugar Hypothesis, which reached its heyday in the 20's. The basic idea is simple. Starch is not osmotically active, so a high starch content in guard cells does not contribute to the solute potential. On stomatal opening, it was proposed that starch (an α 1,4 1,6 glucan) would be broken down to glucose. The resulting glucose would lower the solute potential, so that water would move in. The resulting movement of water would increase the volume (consistent with observation) and contribute to the hydrostatic pressure, so that the overall water potential would be unchanged (i.e., the decrease in solute potential would be exactly balanced by the change in the pressure potential). Unfortunately, it has only been within the past two decades easily possible to measure sugars in single cells. (At the expense of boring you, I remind you that guard cells exist as isolated pairs that are embedded in other cells—the valid measurements, therefore, must be made at the single-cell level.) From the beginning, there were three nagging questions with regard to the starch/sugar hypothesis: (a) as mentioned, sugars could not be measured, thus, one-half of the hypothesis was untestable; (b) the decline in starch was not consistent—sometimes it did not appear to decline; (c) the method of estimating starch was only semiquantitative (i.e., the starch content was scored by "+++++" or "++")—without the quantification, it was not possible to determine whether the hydrolysis of starch could provide enough sugar to lower the solute potential sufficiently. As you will soon learn, the starch/sugar hypothesis turned out to be wrong, but it was, incredibly, still being offered up as the explanation for stomatal movements in new editions of plant-physiology textbooks in the 80's. Some basic observations are correct, however. It is a change of solute and pressure potentials that drive stomatal movements; when water moves in, the volume increases. Because of the orientation of the wall polymers, the guard cells distend asymmetrically. A picture is worth a thousand words; see your text.

In 1905, a physician by the name of McCollum used a histochemical stain that was specific for potassium to survey a number of different types of cells in the animal and vegetable kingdoms. Among the surveyed cell types were guard cells, which he found to have an elevated potassium content. In 1943, a Japanese scientist (Imamura) published a "sleeper," a paper that did not have an impact on contemporary science, but later provided a focal point. The paper was too long (>100 pages), published in German,

published in the Japanese Journal of Botany, published during W.W.II. Other Japanese scientists also showed that the potassium content of guard cells increased when stomata opened, but their work was also not recognized by the western world. (One of the papers was published in Japanese (which ethnocentric Americans read none of) and in the Nagasaki Bulletin of Education, of all places!) Finally, Ted Hsiao and his student Bill Allaway independently rediscovered the role of potassium in stomatal movements. They published in *Science* and *Plant Physiology*; the rest is history. In summary, by a variety of techniques, in a variety of species, with different opening stimuli, stomatal movements are driven by potassium influx. A nominal value for the change in potassium concentration is 300 mM, but much higher values—to 0.8 M have been reported. On your own, plug that concentration into the van't Hoff equation to arrive at the change in osmotic potential; then, multiply that value by a factor, say 2, to account for an associated anion. Although your calculation should be "scientific," and result in MPa units, convert it into psi, which you have a better "feel" for.

Overhead: Increase in K^+ Salts Causes Stomata to Open

We turn now to a rather brief overview of potassium movements at the molecular level. (We will not cover all the details shown on the overhead.) As we have covered *ad nauseam* membrane transport, I will defer to your general sophistication. (Lacking that, go back to your notes!) (a) The initial event is activation of the plasmalemma ATPase. Proton extrusion hyperpolarizes the membrane, which increases the driving force for the influx of any cation. (b) Hyperpolarization also causes the opening of a voltage-gated K^+ inward-rectifier. Potassium flows inward, down its electrochemical potential gradient. (c) Conventional wisdom is that the ionic environment of cytosol is more-or-less fixed. A large change in the concentration of ions would be expected to be disruptive, e.g., by causing changes in protein conformation. By mechanisms that are only being currently elucidated, potassium accumulates in the vacuole, which squares with the observation that guard cells of open stomata have a large central vacuole, whereas guard cells of closed stomata have small "vacuolules," which in 3-D probably comprise a network.

The foregoing provides only a partial explanation, however. Recall that the ionic asymmetry across membranes is exceedingly small. Thus, one K^+ will be taken up for each H^+ extruded. However, the change in potassium is approximately 0.3 M, whereas the proton concentration is nominally 10^{-7} M. Obviously, there must be a mechanism to "replenish" protons, to account one-to-one for the potassium taken up. One possibility is that the cytosol is sufficiently buffered. (For this class, and for your career, you need to refamiliarize yourself with the Henderson-Hasselbalch equation.) Although conceptually sound, buffering is not an explanation, as the buffering capacity of cytosol is low, say 10 mM $\cdot$ (pH unit)$^{-1}$.

A second possibility, realized, is that Cl^- is taken up concomitantly with K^+. The uptake of chloride via a proton symport would restore "the" proton to the cytoplasm. (Recall that uptake of chloride, an anion, to any significant extent would be energetically uphill. Proton influx is energetically downhill, due both to the membrane potential and to the higher external proton concentration.) *In planta*, the chloride influx is equal to the potassium influx for some species, those having specialized subsidiary cells (see textbook). In these cases, potassium and chloride concentrations are high in the subsidiary cells when stomata are closed; these concentrations decline when stomata open. Thus, potassium and chloride are shuttled from subsidiary cell to guard cell when stomata open. In most species, the potassium influx exceeds (by, say, 3x) the chloride uptake: There is still a problem with pH.

Overhead: pH-stat Mechanism

The pH-stat mechanism, proposed more than two decades ago (but not in the context of stomata), provides for a general mechanism for controlling cytosolic pH within tolerable limits, say 7.4 ± 0.3. Starch, recall, is at higher concentration when stomata are closed. Starch is stored in chloroplasts in higher plants without credible exception. Guard—cell chloroplasts are atypical in a number of ways (*viz*., small and pale [having in *V. Faba* about 0.1x the chlorophyll content of mesophyll-cell chloroplasts], packed with starch independently of the photosynthetic history of the plant, abundant peripheral reticulum [which is associated with high rates of metabolite transport], being traversed by microtubule-like structures, having transport systems that resemble those of amyloplasts). When proton dearth is sensed, starch is degraded to hexose-P, which I believe is the export species. In the cytosol, the hexose is oxidized glycolytically. (Please, if you have not done so as suggested, you must stop now and go back and review glycolysis, as this discussion is predicated on the assumption that you will recognize the rudiments.) At the oxidative step of glycolysis—where the aldehyde (*viz*., phosphoglyceraldehyde, PGal) is oxidized to the acid (*viz*., phosphoglyceric acid , PGA)—a proton is released to solution. The end-product of this sequence is phospho*enol*pyruvate (PEP); i.e., the whole glycolytic sequence is not "used." PEP is carboxylated through the agency of the ubiquitous plant enzyme, PEP carboxylase (PEPC, which, in guard cells, is elevated in activity nominally 10x). There are two substrates for PEPC, namely PEP (as the Mg chelate) and bicarbonate. This latter substrate is formed by the hydration of CO_2, during which a proton is released. The product of the reaction catalyzed by PEPC is a divalent anion, which is reduced to the more stable malate. In summary, each anhydroglucosyl moiety of starch is converted to two divalent organic anions, during which four protons are released to solution. The accumulated malate is the counter ion for potassium, represents a proton "debt," and serves a modest role as an osmolyte.

PEP, as enunciated by Davies, plays a central role in plant metabolism. It may proceed glycolytically to form pyruvate. Else, it may be dephosphorylated, also to form pyruvate, by a specific plant enzyme. It may be formed in glycolysis, as described, or by a carboxykinase, which is reversible. And, of course, as we discussed, it is also the substrate for PEPC. How is this traffic controlled? Cellular metabolism must be "directed" from moment-to-moment to meet the particular temporal demand. The present interpretation is that two mechanisms account for the siphoning of PEP toward malate accumulation in guard cells. The first mechanism (for which we have obtained the first proof) is that PEPC is postranslationally modified in a reversible manner. This alteration manifests itself as a diminution of sensitivity to malate. The second mechanism involves the properties of PEPC and alteration in the chemical environment of this enzyme. (Indeed, this explanation is the standard pH-stat theory.) PEPC is very sensitive to pH. When assayed *in vitro*, the K_M is nominally 4x the cytosolic substrate concentration at pH 7.0. At pH > 8, however, the affinity of substrate and enzyme is dramatically increased, so that the K_M is perhaps 12x lower than the substrate concentration. Go back to the earlier objectives, and make a v vs. S plot. You will recognize that the velocity of PEPC increases many-fold, which is supposed to account for diversion of PEP to malate.

[Return to overhead, Increase in K^+ Salts . . . .]

Having provided a sketch of stomatal opening, now we turn to stomatal closure. The result of stomatal closure, though effected differently, is a reversal of opening. I.e., the accumulated solutes dissipate. There is an outward-rectifying channel that is voltage gated. The inward channel, recall, opens when the membrane potential is hyperpolarized, which coincides with an inward driving force for K^+ in a Nernstian fashion. The outward-rectifying channel opens when the membrane is depolarized, a condition that should shift the driving force to the outside. In addition, channels permit exit of anions. Clearly, Cl^- leaves. The situation concerning malate is somewhat unresolved. Based on efflux data and enzyme distribution, the conservative conclusion is that malate may be metabolized by guard cells, or malate may be lost from guard cells. I would think that this last mechanism would come into play when stomata close rapidly. Whereas the primary event in stomatal opening is activation of the ATPase, the current thinking is that the primary event is closure is the opening of the anion channel. It is thought that efflux of Cl^- would depolarize the membrane, sufficient to open the outward channel and provide the driving force for cation efflux. Despite encouragement, the authors of this idea have not provided a quantitative assessment (e.g., if $\Delta[Cl^-] << \Delta[K^+]$, what happens? It may be that malate must be lost!)

Guard cells have become a model system not only for the study of ion channels, but also for the study of cellular signal transduction. Time being limited, I will be brief and somewhat fanciful (in other words, my explanation will be based on some unproved entities that I opine will be discovered). Looking inside the cell, we see various effects of Ca^{2+}_f. This ion is well known to be involved in controlling myriad processes across taxa. Resting levels are ca. 100 nM and transiently excitatory levels reach as high as 1 μM. Focus your attention simply on the fact that this ion stimulates the anion efflux channel (postulated to be the primary event in closure) and inhibits the inward K^+ channel (and thereby prevents solute influx, inextricably linked to opening.) The question arising-what causes Ca^{2+}_f concentration elevation?—has multiple answers. First, ABA is a ligand that stimulates influx of this ion through an effect on a plasmalemma channel, as shown. (Opening of this channel would elevate Ca^{2+}_f and depolarize the membrane [backing up one step, note that the anion efflux channel is voltage gated, and only opens at depolarizing potentials.) A second means by which ABA causes the elevation of Ca^{2+}_f is through a signal-transduction cascade pathway. In brief, ABA interaction with an external face of a membrane-embedded receptor causes—through an intermediary step not here revealed—the hydrolysis of a membrane lipid, which yields the membrane-localized signal substance diacylglycerol (DAG) and inositol trisphosphate (IP_3), which is released to cytosol. IP_3 causes the release of Ca^{2+}_f from "internal stores"—in animals, the ER; in plants, the vacuole (wherein the $[Ca^{2+}_f] \sim 10$ mM), and the ER. To complicate further the situation, there is a Ca^{2+}_f-independent H^+-dependent means by which ABA effects stomatal closure.

As we whirred through this series of lectures on gas exchange, we were unable to have the luxury of discussing experimental approaches and techniques, the true underpinning of our understanding and, indeed, contemporary culture. Though hurried, we can not forego a brief explanation of (one) measurement technique for gas exchange itself, which is intended to supplement, not supplant, the more complete description in the required text. A leaf is placed in a chamber, closed except for ports that permit forced air turnover of the chamber. The inlet port provides for air of specified composition particularly of CO_2 (which is blended from commercial tanks) and of H_2O (which is set by passage of humidified air across cooling coils). The outlet port provides for measurement of CO_2 (the basis for which is IR absorption) and of water. Stomatal aperture size changes may be elicited by light (or various colors, or darkness), of increased humidity or decreased humidity (stomata measure humidity changes across the throat of the pore), CO_2 concentration . . . . Since the driving force for water is known (the internal RH ~ 100 % and the external RH is a parameter under experimental control), a comparison of the $[H_2O]_{in}$ and $[H_2O]_{out}$ and the flow rate permits calculation of the conductance of the stomata. As we have mentioned so many times before, stomata are non-selective and permit with equal ease the permeation of H_2O and of CO_2. Thus, the conductance (or its reciprocal, the resistance) for water and for carbon dioxide are proportional; the

proportionality factor is the ratio of the velocity of CO_2 molecules to the velocity of H_2O molecules—faster molecules will "collide" with the pore more often, and thus enhance the effusion of the gas. The proportionality factor is easy to calculate: In a mixture of gases, the kinetic energies of the different types of molecules are equal. I.e., KE (H_2O) = KE(CO_2); KE = ½ mv^2. Rearrangement of this equation will show that the velocity ratio =1.6 (i.e., [44/16]). As the CO_2 conductance is known, and the external [CO_2] is known, it then possible to calculate the internal [CO_2]. Of course, often the most sought after value is simply the rate of CO_2 uptake, which is a direct measure of photosynthetic carbon metabolism ("net photosynthesis" in this case, as it is uncorrected for CO_2 evolution that results from respiratory processes).

I had fun; hope that you did, too.