



Chapter 5

Leaf Anatomy and Function

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Summary	98
I. Introduction.....	98
II. Types of Leaves and Their Anatomy	99
III. Leaf Anatomy and Its Major Functions	104
A. Light Absorption – Leaf Optics	104
B. CO ₂ Diffusion and Assimilation	107
1. Diffusion Through Intercellular Airspaces	108
2. Diffusion Through the Mesophyll Cells	112
C. Temperature Modulation	112
D. Anatomy and Water Transport.....	114
E. Mechanical Function	116
F. Functions of the Leaf Surface – The Role of Trichomes.....	119
1. Trichome Morphology	119
2. Trichome Functions	119
IV. Acclimation and Adaptation	121
A. Responses of Leaf Anatomy to Light	121
B. Responses of Leaf Anatomy to Temperature	124
C. Responses of Leaf Anatomy to Water Stress.....	125
V. Conclusions.....	125
Glossary	126
Acknowledgments.....	128
References	128

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Summary

Plant leaves provide the following main functions: (1) light interception and utilization of light energy for photosynthesis. This includes efficient light absorption under low and moderate light, while reducing excess light absorption under high light. (2) Incorporating CO₂ as the substrate of photosynthesis, while limiting the amount of water lost. (3) Maintaining a stable internal environment for physiological processes by modulating leaf temperature. (4) Maintaining structural integrity that allows leaves to photosynthesize under various mechanical stresses such as gravity, wind, rainfall and herbivory. (5) Transporting water, photosynthates, and nutrients to realize efficient functioning of the plant and the leaf.

Subjected to both anatomical and environmental constraints, natural selection has resulted in an intricate leaf anatomy that balances the above functions and allows plants to grow and produce progeny. As a result, plants coordinate size, number, shape, and arrangement of cells, adjust the thickness and chemical composition of cell walls, and utilize physical phenomena such as water evaporation, refraction, and reflection of light.

In the present chapter, common features of leaf anatomy are described and the current knowledge related to its functions is summarized. Special emphasis will be given to leaf optical properties, gas diffusion, water transport, and mechanical properties. Acclimation and adaptation of leaf anatomy in response to environmental conditions will be reviewed. In addition, we will discuss the physiological mechanisms and ecological significance of these responses.

I. Introduction

Leaf morphology varies greatly across species, and a closer look, for example by using an optical microscope, reveals finer-scale differences in cell and tissue structure between different species and between plants grown in different environments (Fig. 5.1). Leaves are typically composed of an epidermis, parenchymatous tissue called mesophyll, and a vascular system (Fig. 5.1a). Many mesophyll cells contain small green organelles, the chloroplasts. These chloroplasts play a vital role in the process of photosynthesis, which sustains almost all life on earth. Leaf anatomy facilitates this primary function of plants: accommodating the biochemical and biophysical processes of photosynthesis that provide carbohydrates to the whole plant.

The photosynthetic machinery uses light energy to power the biochemical reactions leading to the fixation of CO₂. Chlorophyll and possibly other pigments in the chloroplasts absorb light energy that is utilized by

the photosystems to drive electron transfer in the photosynthetic machinery located in thylakoid membranes. Oxygen and chemical energy (adenosine triphosphate and nicotinamide adenine dinucleotide phosphate) are produced by this machinery, and the chemical energy is used to assimilate CO₂ by enzymatic reactions (Calvin-Benson cycle) in the stroma of chloroplasts. CO₂ molecules diffuse from the atmosphere into the chloroplasts through the stomata and the porous leaf structure. Assimilated carbon is partly stored as starch in the chloroplasts and the rest is transported to other parts of the plant in a form of sucrose (plus raffinose-family sugars and sugar alcohols) via the phloem (see Chap. 8 for details on the photosynthetic mechanism and Chap. 3 for photosynthate transport). The biochemical and photochemical reactions of the photosynthetic process are sensitive to environmental factors such as irradiance, CO₂ concentration, temperature, water availability, and nutrient availability. All these processes are facilitated, and sometimes even constrained by the structural

organization of plant leaves. For example, light absorption by the leaf depends on the anatomy because the structure of the leaf tissue affects the light path via reflection and refraction. The diffusion rate of CO₂ depends on the porosity and tortuosity within leaves as well as other anatomical and biochemical processes. The transport of water and photosynthates depend on the mesophyll anatomy and vascular system.

Leaves emerge from the shoot apical meristem of stems or branches. Cell divisions in many dicot leaves finish at an early stage of leaf expansion. After that, cell expansion is chiefly responsible for the growth of the leaf blade. Monocot leaves and some dicot leaves keep their elongation zone limited to the base of leaves even at later stages. In parallel with leaf expansion, the photosynthetic machinery in the chloroplasts develops and starts photosynthesizing. In most cases, the maturation of cell walls marks the end of the leaf expansion. Active photosynthesis continues until the end of the leaf lifespan unless environmental stresses limit the activity. Nutrients are then reallocated from the senescing leaf for recycling.

In this chapter, functional aspects of leaf anatomy will be reviewed, focusing on the plasticity of leaf anatomy in response to environmental variation and interspecific differences. We will see that the mesophyll anatomy affects light absorption, gas-exchange, and water transport. The vasculature provides mechanical support and a continuous water supply from the veins, which is indispensable for metabolic processes. A shortage of water may interfere with biochemical processes such as photosynthesis, but the loss of turgor will also affect the structure and morphology of a leaf and inhibit its ability to receive sunlight efficiently. The leaf surface not only controls and regulates the light environment inside the leaf, CO₂ exchange, and water evaporation, but also maintains leaf structure and acts as a barrier against physical damage and biotic stresses. The control of transpiration

by the stomata also plays a role in regulating leaf temperature and preventing over-heating under strong irradiance. As a consequence, the inner mesophyll tissues are protected, which allows for a more stable environment for biochemical processes such as photosynthesis.

II. Types of Leaves and Their Anatomy

Leaves can be described from an ontogenetic or anatomical perspective. The existing terminology for both fields is often confused and incorrectly or inconsistently applied (Augsten et al. 1971). Because this confusion persists even in recent literature, we will describe leaf types from both viewpoints, and give clear definitions of the most commonly used terms.

From an ontogenetic perspective, leaves and tissues are distinguished based on the meristematic origin of the cells. In an early stage of leaf formation, the leaf meristem closest to the shoot apical meristem (the adaxial side) differentiates from that on the other (abaxial) side (Nakata and Okada 2013; Fukushima and Hasebe 2014). Most flat leaves are so-called bifacial leaves, the adaxial side of the leaf primordium becomes the morphological upper side of the leaf, and the abaxial side forms the lower side (Fig. 5.1a). However, in rare cases (i.e., resupinate leaves: Fig. 5.2c, d), the petiole or leaf lamina may twist, and the abaxial side becomes the morphological upper side of the leaf. Layers of tightly linked cells, called epidermis, develop from the adaxial and abaxial meristems and form the two distinct sides of a bifacial leaf. Bifacial leaves are typically flat, but more or less cylindrical (terete), or even spherical leaves (e.g., *Senecio rowleyanus*) are also known (Fukushima and Hasebe 2014). Parenchyma cells located between the two epidermal layers are called mesophyll and this tissue develops from both the adaxial and abaxial meristems. Vascular bundles

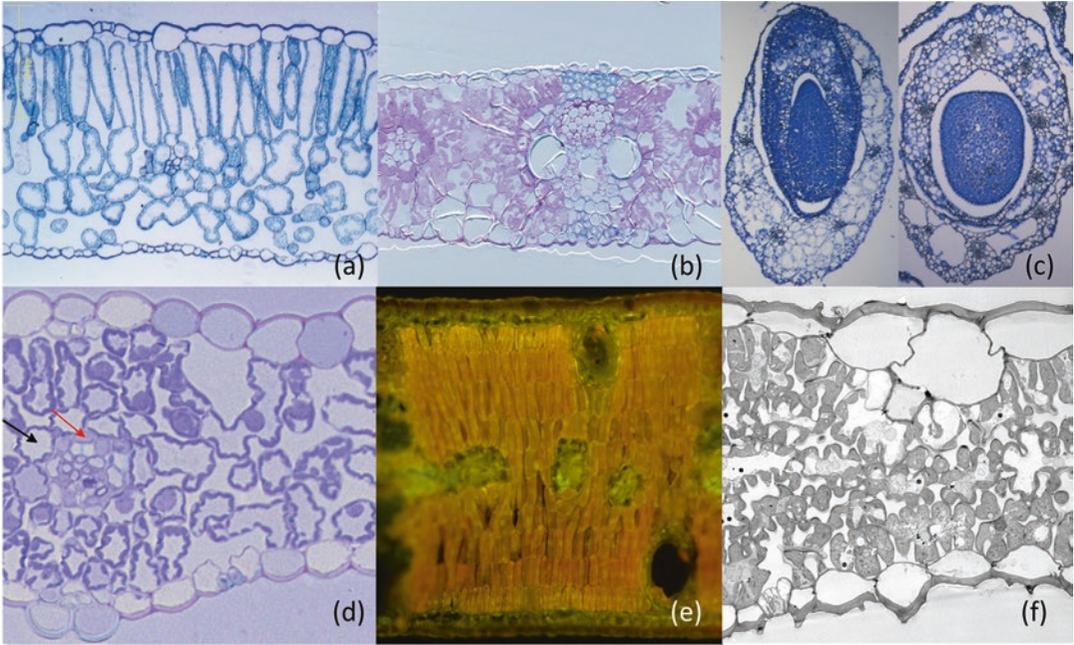


Fig. 5.1. Cross-sectional photographs of (a) a typical C₃ leaf (dorsiventral, bifacial, and homobaric) of *Nicotiana tabacum*, (b) a typical C₄ leaf of *Sorghum bicolor*, (c) unifacial leaves of *Juncus prismatocarpus* (left) and *Juncus torreyi* (right), (d) a homogenous isolateral leaf of *Triticum aestivum*, (e) an isolateral leaf from *Eucalyptus pauciflora*, and (f) a leaf with lobed mesophyll cells from *Oryza sativa*. In (d), red arrow indicates the mestome sheath and black arrow indicates the parenchymatous bundle sheath. Photographs were kindly provided by Dr. Shinichi Miyazawa (a), Dr. Chieko Saito and Dr. Youshi Tazoe (b), Dr. Xiaofeng Yin (c), Ms. Elinor Goodman and Dr. Margaret Barbour (d) and Dr. Shinya Wada (f)

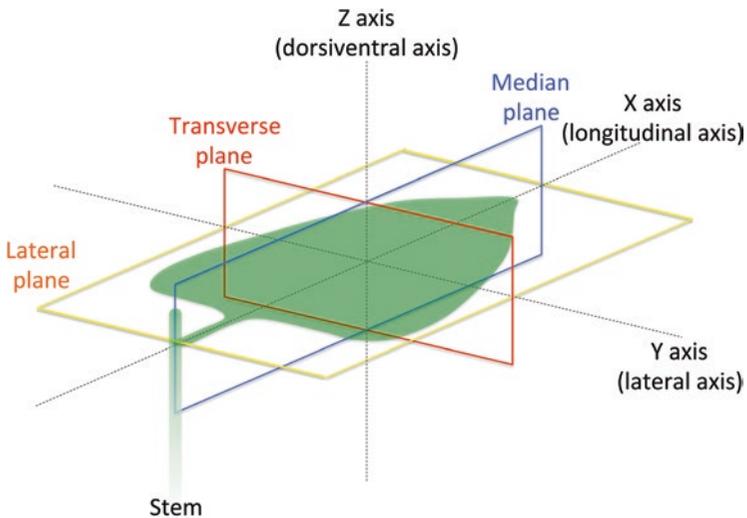


Fig. 5.2. Anatomical axes and planes in the leaf

are embedded in the mesophyll, with xylem developing adaxially and the phloem abaxially (Nakata and Okada 2013).

Some monocots develop so-called unifacial leaf blades (Fig. 5.1c), where the development of the adaxial meristem is suppressed, and the epidermis and underlying mesophyll are typically formed by the abaxial meristem alone (Kaplan 1975; Yamaguchi et al. 2010). Such leaves can be tubular or cylindrical (terete), with vascular bundles arranged in a ring (e.g., *Juncus* and *Allium* species with rounded leaves). Alternatively, the leaves are flattened, such as the ensiform (i.e., sword-shaped, medially flattened) leaves of *Acorus gramineus* and *Iris* spp. Such leaves often have two layers of vascular bundles (i.e., the vasculature remains radially symmetrical), with the phloem part of the vascular bundle located closest to the nearest leaf surface (Yamaguchi et al. 2010; Fukushima and Hasebe 2014; Mashayekhi and Columbus 2014). Occasionally the two layers cannot be clearly distinguished and the orientation of the vascular bundles appears alternating (Imamura and Hida 1956; Mashayekhi and Columbus 2014).

Leaf tissues and cells can also be distinguished based on their shapes and arrangement (i.e., from a purely anatomical perspective). In many species, cells in some or all layers of the mesophyll can differentiate into elongated, regularly distributed cells. This elongation can be in the longitudinal direction (e.g., *Elodea canadensis*, *Galanthus nivalis*, *Leucojum vernum*, see also Fig. 5.2), medio-laterally (members of the Iridaceae, *Erythronium dens-canis*), or more or less perpendicular to the leaf surface (dorsiventrally; Haberlandt 1914). Only in the latter case is this type of tissue typically called palisade tissue (Haberlandt 1914; Esau 1965; c.f. Fig. 5.1d). Cells that are more variably shaped and irregularly distributed are called spongy tissue cells (Haberlandt 1914; Esau 1965). Although these cells often appear to be isodiametric (roughly spherical) in 2D transverse sec-

tions, the shape can be variable, from spherical to multi-lobed.

If mesophyll cells have multiple protruding lobes or have a gear-like shape as a result of cell-wall invaginations (ingrowths of the cell wall), they are typically called armed mesophyll (Chatelet et al. 2013). Examples can be seen in the Ranunculaceae, Pooideae, Bambusoideae, *Oryza* spp., *Sambucus* spp., *Viburnum* spp., *Pinus* spp., and *Adiantum* spp. (Haberlandt 1914; Carolin et al. 1973; Chonan 1978; Sage and Sage 2009). Sometimes the individual lobes are predominantly arranged perpendicular to the leaf surface, just like regular palisade parenchyma, and these can be called “armed palisade” (Haberlandt 1914; Chatelet et al. 2013).

Though both palisade and spongy tissues can be loosely arranged, with airspaces between individual cells, the relative amount of airspace and the size of the individual pores are larger in the spongy tissue (Terashima et al. 2001; Lehmeier et al. 2017). In addition to the typical elongated palisade cells and irregularly shaped spongy cells, a layer of trapezoid cells (funnel cells) can be observed in some species between the palisade and spongy tissues (e.g., *Ficus elastica*, *Pulmonaria officinalis*) or instead of the palisade layer (e.g., *Begonia* spp.) (Haberlandt 1914; Sheue et al. 2012).

A few types of special mesophyll cells deserve mention here. In many legumes (Fabaceae), one or occasionally two layers of the mesophyll are anatomically distinct, and consist of laterally stretched and lobed parenchyma cells between the minor veins of the leaf (Fig 5.3a, Metcalfe and Chalk 1950; Brubaker and Lersten 1995). The occurrence of this so-called paraveinal mesophyll is mostly restricted to members of the Fabaceae, but Brubaker and Lersten (1995) suggest that it may also be present in *Populus deltoides* and *Solidago canadensis*. A second type of unusual mesophyll cell is found in some grasses and many members of the Bambusoideae, where distinct colorless cells

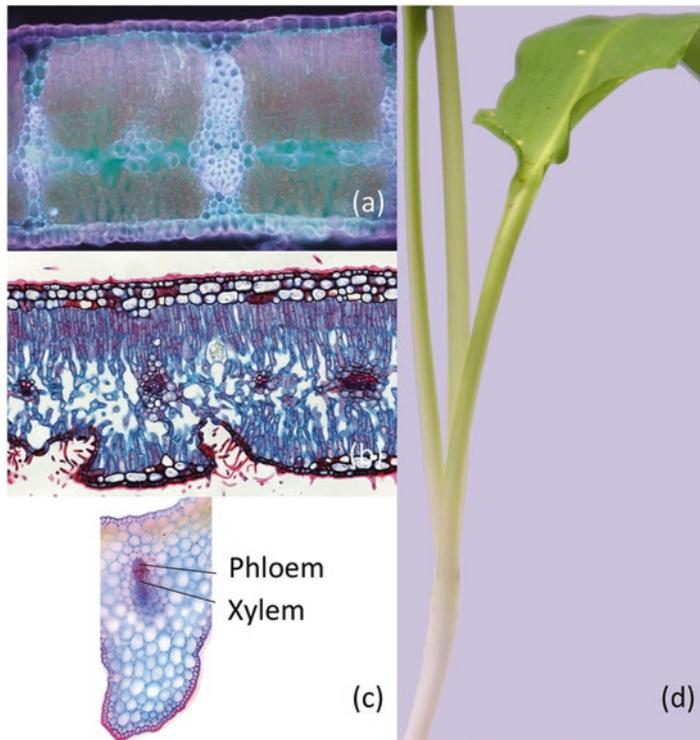


Fig. 5.3. Cross-sectional photographs of (a) a heterobaric leaf from *Erythrina crista-galli* and (b) a leaf with a multi-layered epidermis and crypts from *Nerium oleander*. (c) and (d) show the upside-down positioning of phloem and xylem and the twisting petiole of a resupinate leaf from *Allium ursinum*, respectively

are visible between the veins. These cells are called fusoid cells because they appear to have a pyriform or fusiform shape (i.e., pear or spindle-shaped) when viewed in transverse sections (Metcalf 1956; March and Clark 2011). The cells are elongated in medio-lateral and longitudinal directions (see Fig. 5.2) and thus have more plate-like shapes when viewed in three dimensions (Metcalf 1956). Large intercellular spaces, which may result from partial collapse of some of the cells, are often found between two consecutive fusoid cells (March and Clark 2011). The development of fusoid cells seems to be dependent on shade conditions (March and Clark 2011).

If a clear differentiation in cell shape can be observed between both sides of a leaf (e.g., palisade and spongy cells at the adaxial and abaxial side, respectively), a leaf is called dorsiventral. Although the develop-

mental origin of a cell may put some constraints on the function, it has been shown that the functional and morphological fate of a cell is mainly determined by the location of cells within the organism (Poethig 1987). Thus, both bifacial and unifacial leaves can develop a dorsiventral anatomy (e.g., Imamura 1931; Mashayekhi and Columbus 2014). In addition, some leaves develop palisade tissue both ad- and abaxially, giving rise to the so-called heterogeneous isolateral (or isobilateral) leaf structure (Fig. 5.1e, DeLucia et al. 1991; Smith et al. 1997). Often, in particular for many grasses, no clear differentiation of the mesophyll can be observed, and such leaves are called homogeneous, homogeneous isolateral or equifacial (Fig. 5.1d, Metcalf and Chalk 1950; Pyankov et al. 1999).

A typical dicotyledonous leaf is dorsiventral, with palisade tissue forming near the

light-exposed surface of the leaf (usually the adaxial side) and spongy tissue developing towards the shaded (usually abaxial) side (Metcalf and Chalk 1950). In resupinate leaves, or leaves that adhere closely to the stem with the adaxial surface, the abaxial side is exposed to light and these leaves often show an inverse dorsiventral anatomy, with palisade tissue developing on the abaxial side (Haberlandt 1914; Bredenkamp and Van Wyk 2001; Hofreiter and Lyshede 2006).

Not only the mesophyll, but also the epidermal cells often show a typical dorsiventrality, with differences in the density of stomata, cell sizes and trichome (hair) density (Metcalf and Chalk 1950). Although stomata can occur on both sides of leaves with a dorsiventral mesophyll (amphistomatic or amphistomatous), it is more common they are exclusively found on the abaxial (usually shaded) side (hypostomatic or hypostomatous; Wilkinson 1979; Muir 2015). In floating leaves of aquatic plants, resupinate leaves, or leaves that closely adhere to soil, stem, or other leaves (e.g., some *Saxifraga* species), stomata may develop only on the adaxial (usually light-exposed) side (epistomatic or epistomatous, sometimes also called hyperstomatic) (Metcalf and Chalk 1950; Bredenkamp and Van Wyk 2001; Muir 2018).

The epidermis is covered with a thin layer called cuticle. The cuticle consists of wax layers, a cuticular membrane composed of hydrophobic compounds, and cellulose encrusted with cutin (Martin and Juniper 1970). The cuticle is an important barrier that limits water loss from leaves (Burghardt and Riederer 2007), reflects or filters ultraviolet light (Krauss et al. 1997), and mechanically protects the leaf from external stresses such as rubbing, abrasion, and biotic threats (e.g., pathogens or herbivory) (Onoda et al. 2012).

When a leaf is sectioned paradermally (i.e., in a plane parallel to the epidermis), we can observe veins extending throughout the mesophyll. These veins include the xylem

and phloem, but only rarely a vascular cambium (Esau 1965). Water moves through the xylem from roots to the cells of the leaves (Chap. 4) and carbohydrates are transported by the phloem from mesophyll cells to the whole plant (Chap. 3).

These veins are usually separated from the mesophyll by a bundle sheath: a tightly connected, radial file of parenchymatous or sclerenchymatous cells, sometimes with a few airspaces, that wholly or partially surrounds the vascular bundle (Carolin et al. 1973; Leegood 2008). Cells not directly connected to the vasculature, but structurally similar have sometimes been included in this definition (see Carolin et al. 1973). Examples of the latter can be seen in some C_4 grasses (e.g., *Arundinella nepalensis*, *Triodia scariosa*), where bundle-sheath-like cells are not always immediately adjacent to the vasculature (Carolin et al. 1973; Dengler et al. 1994; Lundgren et al. 2014). Bundle-sheath cells are not lobed or dorsiventrally elongated like many mesophyll cells, but may be elongated centrifugally or in the direction parallel to the adjacent veins. Cell walls between the bundle-sheath cells are sometimes impregnated with lignin (and possibly suberin), and in these cases the bundle sheath has been referred to as an endodermis (Lersten 1997; Liesche et al. 2011). In many grasses, a suberized layer of cells called the mestome sheath surrounds the vasculature, but a second, parenchymatous bundle sheath is also present (Carolin et al. 1973; Canny 1990; Dengler et al. 1994; Dengler and Nelson 1999). Mestome and bundle-sheath cells generally contain few chloroplasts (Leegood 2008), except in leaves featuring the Kranz anatomy associated with C_4 -metabolism (Fig. 5.1b, Dengler et al. 1994; Dengler and Nelson 1999; Muhaidat et al. 2007). In gymnosperms, the bundles are embedded in a so-called transfusion tissue consisting of tracheids and parenchymatous cells. The transfusion tissue is separated from the mesophyll by an endodermis-like bundle sheath (Esau 1965; Liesche et al. 2011).

Bundle-sheath extensions (BSE) are groups of cells extending from the bundle sheath to the epidermis (Wylie 1952). These cells contain no chloroplasts and are often sclerified. If many veins possess such BSEs, the resulting compartmentalization may restrict gas diffusion in the leaf; such an anatomy is called heterobaric (Fig. 5.3a). On the other hand, if BSEs are absent, the leaves are called homobaric (Neger 1912, 1918; Terashima 1992).

III. Leaf Anatomy and Its Major Functions

A. Light Absorption – Leaf Optics

Chlorophyll pigments absorb light of wavelengths between 400 nm and 700 nm (the photosynthetically active radiation, PAR; McCree 1972; Clark and Lister 1975a,b), which is in the same range as the light visible to the human eye. A leaf typically absorbs ca. 90% of the available PAR (Evans and Poorter 2001). Figure 5.4a shows the absorbance spectrum of a spinach leaf, indicating that leaves absorb blue (400–500 nm) and red (600–700 nm) light well. The absorbance between 500 nm and 600 nm (green) is 10 to 15% lower. As a result, the reflectance and transmittance of leaves peak around 550 nm, leading to the perception that leaves are green.

Similar to the light gradients observed in a forest canopy, there is a light gradient inside a single leaf (Terashima and Saeki 1983; Vogelmann and Björn 1984). Under sunny conditions, chloroplasts located close to the light-exposed surface of a leaf receive strong collimated light while chloroplasts located close to the shaded side of a thick leaf experience lower light intensities (Fig. 5.4b). The photosynthetic rates of individual chloroplasts increase with light absorption, but this response levels off at high light intensity (light saturation). If the absorbed light energy is greater than that can

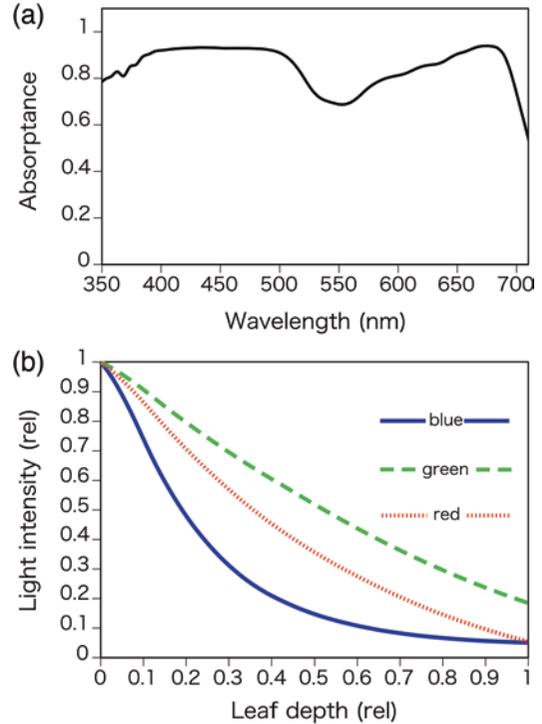


Fig. 5.4. (a) Absorbance spectrum of a *Spinacia oleracea* leaf and (b) light intensity gradients for different wavelengths of light (blue = 450 nm, half bandwidth of 50 nm; green = 550 nm, half bandwidth of 50 nm; red = 650 nm, half bandwidth of 10 nm) through a leaf (from the upper surface = 0 to the lower surface = 1.0) measured by chlorophyll fluorescence emission from cross-sections of spinach leaves. The data for (b) was kindly provided by Dr. John R. Evans. (Re-calculated from Vogelmann and Evans 2002)

be utilized by photosynthesis, photosynthetic light use efficiency decreases and such an excess of energy may result in chronic photodamage to the photosystems (Aro et al. 1993; Long et al. 1994). Therefore, for chloroplasts near the light-exposed side, reducing light absorption is important. On the other hand, for the chloroplasts near the shaded side, maximizing light absorption is important so that the carbon-gain by photosynthesis is sufficient to cover the cost of their construction and maintenance (Terashima and Hikosaka 1995). The light gradient within a leaf is affected not only by the amount of light-absorbing pigments in a leaf but also by its anatomy and distribution

of chloroplasts (Vogelmann 1993; Terashima et al. 2009).

The shape of mesophyll cells affects the light gradient in a leaf. When the light is collimated, the columnar and vertically-aligned palisade cells allow a considerable portion of the light to penetrate deeper into the leaf (Terashima and Saeki 1983; Vogelmann and Martin 1993; Vogelmann and Evans 2002), especially when chloroplasts are arranged along the anticlinal (vertical) cell walls (Gorton et al. 1999). The light intensity becomes weaker with increasing depth in the leaf, and the irregular shaped spongy cells alternating with air spaces increase reflection and refraction of light, which increases the optical path length through a leaf and thus increases light absorption. This increase in the optical path length through a leaf due to mesophyll anatomy is called the *détour* effect (Vogelmann 1993; Terashima et al. 2009). Reflection and refraction are caused by the difference in refractive indexes of leaf components (e.g., cells have a higher refractive index than air spaces), and thus the *détour* effect is influenced by cell shape. Terashima and Saeki (1983) sliced leaves of *Camellia* paradermally and showed a greater capacity for light absorption per unit chlorophyll in spongy tissue than in palisade tissue (Fig. 5.5). They also examined the effect of infiltration with olive oil (with a refractive index similar to that of the cell) into the intercellular air space on the apparent extinction coefficient of chlorophyll in order to examine the effect of the *détour* effect. Infiltration significantly reduced the extinction coefficient, especially in spongy tissue, which supported the hypothesis that the elongation of light path length increased light absorption. Similar results were obtained by Vogelmann and Evans (2002), who showed that the path-lengthening effect in the spongy tissue is especially strong for green light.

In a leaf, absorption of PAR occurs in discrete units (the chloroplasts), whereas the rest of the leaf is relatively transparent. This

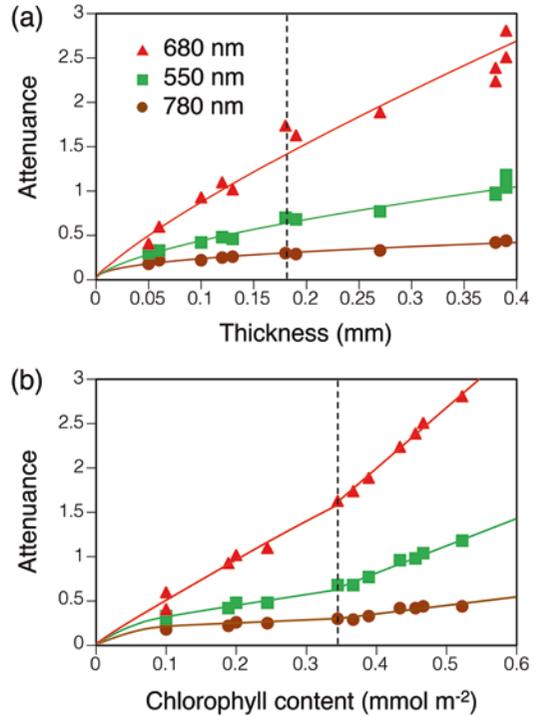


Fig. 5.5. Attenuance of monochromatic light in paradermal sections of a *Camellia japonica* leaf as a function of (a) thickness and (b) chlorophyll content. All paradermal sections had an upper epidermis and a collimated light source was used to illuminate the section. The dotted lines indicate the boundary between the palisade tissue and the spongy tissue. (The figure was redrawn from data originally published by Terashima and Saeki 1983)

non-uniform distribution of pigments decreases the possibility of photons encountering pigments and results in less light absorption compared to a hypothetical leaf with pigments homogeneously distributed. This is called the sieve effect, and this effect is larger for strongly absorbed light (e.g., blue versus green). The sieve effect may seem to be a disadvantage for leaves, but it allows for a better control of light absorption. For example, in many plant species, chloroplasts change their shape and position in response to environmental factors such as light intensity (Fig. 5.6; Wada 2013). Under high light, chloroplasts adhere along cell walls parallel to the light direction (*profile position*: Fig. 5.6b), whereas under low light

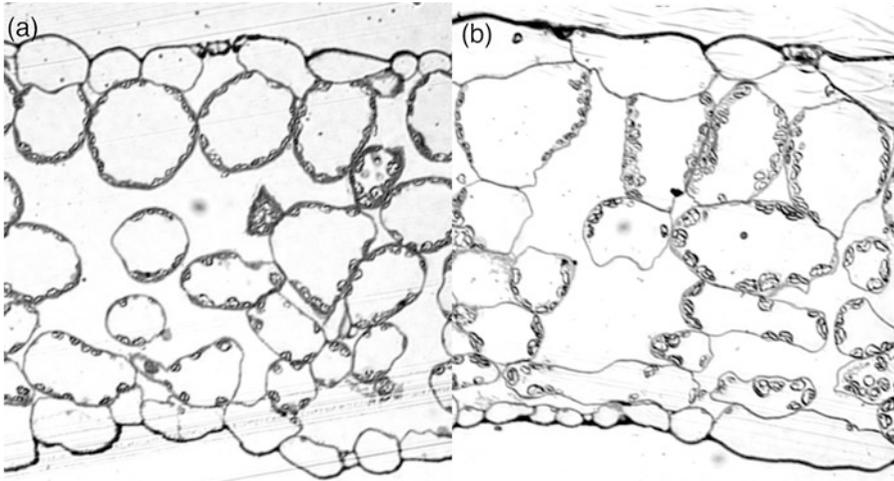


Fig. 5.6. Cross-sections of *Arabidopsis thaliana* leaves. Chloroplasts are arranged in (a) a typical face position under blue-filtered light and (b) in a profile position under high white light. Light micrographs of leaf sections were taken at 200 times magnification. (See Tholen et al. 2008 for experimental conditions)

they accumulate along walls perpendicular to the light (*face position*: Fig. 5.6a). The profile position allows a larger part of the light to pass through the leaf (Gorton et al. 1999) and limits the exposure to high light to those chloroplasts close to the illuminated side of the leaf. Thus, the profile position can reduce the amount of photodamage resulting from strong light (Kasahara et al. 2002; Sztatelman et al. 2010). In addition, the profile position allows more light to penetrate deeper in the leaf, resulting in a more uniform light gradient throughout the leaf, allowing chloroplasts deeper in the leaf to contribute efficiently to whole-leaf photosynthesis (Brugnoli and Björkman 1992; Terashima and Hikosaka 1995; Gorton et al. 1999). This would also allow photodamage to be shared over the leaf, which may prevent a decrease in the photosynthetic potential (Davis and Hangarter 2012). By contrast, the face position limits transmittance and may increase photosynthetic efficiency under low light intensities (Zurzycki 1955). In shade leaves, which often have large mesophyll cells, chloroplasts may move more freely, resulting in relatively large differences (>20%, Haupt and Scheuerlein 1990) in light absorption between low and high light envi-

ronments. Chloroplast movement may be more restricted in sun leaves, with narrower palisade cells, resulting in only small differences in optical properties (Davis et al. 2011; Higa and Wada 2016).

BSEs, which are composed of tightly packed transparent cells (containing few or no chloroplasts), would allow light to penetrate deeper into the leaf (Karabourniotis et al. 2000; Nikolopoulos et al. 2002; Xiao et al. 2016). Karabourniotis et al. (2000) showed that some BSEs behave as transparent windows and can decrease the slope of the light gradient in the leaf.

Biominerals such as calcium carbonate or oxalate crystals in the mesophyll or epidermis are frequently observed in many plant families (Metcalf and Chalk 1950). These biominerals also allow light to scatter and penetrate deeper, resulting in a reduced light gradient within a leaf (Gal et al. 2012). Under high light intensities, this allows more light to reach the deeper parts of the leaf, possibly increasing photosynthesis.

The fusoid cells of basal grasses and bamboo have also been related to light absorption. While such cells are always present in shade leaves of these species, March and Clark (2011) showed that sun leaves of three

bamboo species lacked fusoid cells. Infusion of leaves containing fusoid cells with mineral oil changed their optical properties, suggesting that such cells may play a role in absorbing light more efficiently in shade leaves (March and Clark 2011).

Because light absorption by chlorophyll pigments strongly depends on the color of the light, the light gradient through a leaf also changes dependent on color. Using a chlorophyll fluorescence imaging technique, originally developed by Takahashi et al. (1994), Vogelmann and colleagues showed significant differences in the light gradient through a leaf among blue, green, and red light (Fig. 5.4b, Vogelmann and Evans 2002; Brodersen and Vogelmann 2010). Since there is less attenuation of green than of blue or red light as light penetrates through a leaf (Fig. 5.4b), any additional green light absorbed by chloroplasts deep in the leaf increases leaf photosynthesis to a greater extent than additional red or blue light (Terashima et al. 2009). If leaves were black and absorbed all wavelengths equally, light absorption of chloroplasts near the irradiated surface would increase, but absorption of light by chloroplasts deeper in the leaf would decrease. Light use efficiency (photosynthetic rate per unit illuminated light) in such black leaves would be lower compared to that of green leaves (Terashima et al. 2009).

With the exception of the guard cells of the stomata, the epidermal cells of angiosperm plant species rarely contain chloroplasts (but see Joel and Gepstein 1985). Chloroplast-containing epidermal cells are much more common in ferns, bryophytes, and submerged or aquatic higher plants (Nasrulhaqboyce and Duckett 1991; Sakurai et al. 2005; Sheue et al. 2007; Mommer et al. 2006). Nevertheless, the epidermis may still play a role in light absorption. Many species, especially herbaceous plants, have a papillary or bumpy leaf surface consisting of convex epidermal cells that may affect the light intensity in the underlying mesophyll tissue (Haberlandt 1914). The leaves of some

understory species, such as *Begonia erythrophylla*, *Colocasia esculenta*, and *Impatiens velvetea*, have extremely bumpy surfaces (Brodersen and Vogelmann 2007). It has been suggested that such a rough leaf surface lowers reflectance and increases the absorptance of diffuse light (Haberlandt 1914; Bone et al. 1985; Vogelmann 1993) because a smooth cuticular layer has been shown to result in a more mirror-like (specular) reflectance of light (Woolley 1971). However, Brodersen and Vogelmann (2007) found no relation between the convexity of epidermal cells and the absorptance of diffuse light. An alternative view holds that convex shaped cells may focus light on specific chloroplasts in the mesophyll (Vogelmann 1993; Vogelmann et al. 1996). Interestingly, this focusing effect is greater for direct light compared to diffuse light and is also present in leaves of sun plants adapted to high light intensities such as *Trifolium repens* and *Medicago sativa* (Martin et al. 1989; Vogelmann 1994). It remains unclear whether such light focusing by epidermal cells has an advantage for photosynthesis. Turgor pressure of the epidermal cells may inevitably lead to some level of bumpiness at the leaf surface. Moreover, it has been argued that convex cells may also make the leaf surface more water repellent (Neinhuis and Barthlott 1997; Wagner et al. 2003; Bhushan and Jung 2006). Water droplets or films on the surface of leaves can induce stomatal closure and reduce photosynthesis (Ishibashi and Terashima 1995; Terashima et al. 1995). This would provide leaves with convex shaped epidermal cells with an advantage under wet conditions.

B. CO₂ Diffusion and Assimilation

The supply of carbon dioxide (CO₂) affects the rate of photosynthesis. Atmospheric CO₂ diffuses through stomata into intercellular airspaces, dissolves in apoplastic water layers, and subsequently diffuses through a number of cellular components, including

cell walls, membranes, cytosol, chloroplast envelopes, and chloroplast stroma (Evans et al. 2009). Finally, CO₂ reaches the site of carboxylation, where it is fixed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). The ease with which CO₂ diffuses through a leaf is called conductance (the reciprocal of resistance). The conductance of CO₂ through the leaf is determined not only by the physical and chemical properties of the conducting medium (air, cell material), but also by the arrangement of the tissues, cells, and cellular components, i.e., by the leaf anatomy (Parkhurst 1994; Evans et al. 2009). It is common to divide the leaf CO₂ diffusion conductances into two main components: the stomatal conductance (g_s) describing the conductance between the atmosphere and substomatal cavity and the mesophyll conductance (g_m) describing the conductance between the substomatal cavity and the site of carboxylation. When stomata are open, the magnitudes of these conductances are roughly comparable (Warren 2008; Flexas et al. 2008). Both g_s and g_m are sensitive to environmental conditions and other physiological processes, which are further explained in Chaps. 6 and 7. Here we focus on the relationship between leaf anatomy and mesophyll conductance.

1. Diffusion Through Intercellular Airspaces

Diffusion of CO₂ through the gas phase is about four orders of magnitude faster compared to diffusion through the liquid phase (Nobel 2009). However, the diffusion-path through airspaces inside leaves is often more than two orders of magnitude longer compared to the liquid-phase path through mesophyll cells into the chloroplasts, especially in thicker, hypostomatic leaves (Parkhurst 1994). For example, estimates of airspace resistance based on leaf anatomy in sun leaves are generally higher compared to those in shade leaves (Syvertsen et al. 1995; Piel et al. 2002; Ivanova et al. 2006). Direct measurements of gas space conductance are

rare and rely on comparing assimilation rates between leaves in air and in helox (air with nitrogen replaced by helium); since CO₂ diffuses faster in helox than in air, airspace resistance can be estimated from the difference in assimilation rates. Parkhurst and Mott (1990) found that assimilation rates increased significantly under helox in several hypostomatic leaves such as *Cissus rhombifolia*, *Nerium oleander*, *Brassica actinophylla*, and *Plectranthus australis*, calculating that diffusion through air accounted for about 20% of the total diffusion resistance. Other authors (Piel 2002; Piel et al. 2002) found no measurable resistance by airspace for hypostomatic leaves of *Rosa rubiginosa* and *Nerium oleander*, although in *Quercus ilex* resistance through air accounted for more than 30% of the total diffusion resistance. It must be noted that these measurements depend on an accurate estimate of the CO₂ concentration in the substomatal cavity, which is commonly determined based on the evaporation of water from the mesophyll. Thus these measurements also rely on assumptions with respect to the location of water evaporation from the mesophyll cells. If transpiration occurs deeper in the mesophyll (Boyer 1985; Parkhurst and Mott 1990; Buckley et al. 2015), it would result in an underestimation of the airspace conductance, because estimates of stomatal conductance would already include a sizable portion of conductance through the airspaces (Piel 2002).

In models, diffusion through the intercellular airspaces can be described using the porous medium approximation, allowing the effect of leaf anatomy on gas diffusion to be analyzed using a simple model based on a few leaf anatomical characteristics (Syvertsen et al. 1995; Piel 2002; Niinemets and Reichstein 2003). The conductance through intercellular airspaces (g_{ias} ; [mol m⁻² s⁻¹ bar⁻¹]) can be described by:

$$g_{ias} = sD(\rho / \tau) / (aL) \quad (5.1)$$

where s is the solubility of CO_2 in water [$\text{mol m}^{-3} \text{bar}^{-1}$], D is the diffusion coefficient of CO_2 through air [m s^{-1}], ρ is the porosity [$\text{m}^3 \text{m}^{-3}$] of the leaf and τ is the tortuosity factor [$\text{m}^2 \text{m}^{-2}$]. τ is defined as the squared ratio between the actual length of the diffusion path between two points and a straight-line distance between these points (Epstein 1989). L is the mesophyll thickness [m]. a is a dimensionless correction factor to account for the fact that the mesophyll thickness is not equal to the effective distance between stomata and chloroplasts because of the spacing of stomata on the leaf surface and the presence of CO_2 sinks along the diffusion path (Parkhurst 1994). These effects are often taken into account in reaction-diffusion models of the diffusion process (Parkhurst 1994; Terashima et al. 2001; Aalto and Juurola 2002; Ho et al. 2016). Terashima et al. (2001) accounted for the effect of sinks along the diffusion path in a one-dimensional model and showed that the diffusion path length becomes dependent on the assimilation rate of the leaf. From their results, it can be inferred that for many thinner hypostomatic leaves, the effective path length can be approximated by $a = 1/3$. Assuming the path length is halved when CO_2 can enter a flattened leaf from both sides, $a = 1/6$ may be used for amphistomatous leaves.

In reality, the diffusion through a leaf is not one-dimensional, and most CO_2 enters the leaf at discrete positions (through the stomata), which would increase the effective path length to some extent (Parkhurst 1994). It has been suggested that amphistomaty is an adaptive feature of thick leaves, allowing faster diffusion of CO_2 to chloroplasts in such leaves (Parkhurst 1978; Mott et al. 1982; Muir 2015). Amphistomaty is correlated with higher stomatal conductance (Beerling and Kelly 1996), but it is unclear whether the same is true for mesophyll conductance. If amphistomaty indeed allows for a higher g_{ias} , this may explain the greater water-use efficiency observed for amphistomatous species (Bucher et al. 2017) and the

scarcity of hypostomatic leaves in dryer habitats (Parkhurst 1978). Amphistomaty is indeed associated with thicker leaves across hundreds of species, but the correlation is weak (Muir 2015). Amphistomaty was most closely associated with herbaceous plants with short life cycles and with plants from low precipitation environments (Muir 2015). In addition, amphistomaty was linked to high light environments, suggesting that amphistomaty is favored when CO_2 limits photosynthesis (Muir 2018). The prevalence of hypostomy suggests, however, that a disadvantage of amphistomaty exists, perhaps related to increased pathogen susceptibility (Muir 2015, 2018). It is tempting to conclude from the above that diffusion through the intercellular airspace is limiting photosynthesis in fast growing herbs that have high photosynthetic rates. Since stomata on the adaxial side and the abaxial side respond differently to environmental stimuli (Pospíšilová and Solárová 1980; Driscoll et al. 2006; Wang et al. 2008), the diffusion path length through airspaces is variable, and may result in a dynamic g_{ias} (Morison and Lawson 2007). A change in the path length for CO_2 diffusion through the mesophyll as a result of inhomogeneous (i.e., depending on the leaf side or patchy) stomatal closure may contribute to the often observed covariation between g_{m} and g_{s} (Flexas et al. 2008; Warren 2008).

In leaves of many xeromorphic or sclerophyllous (hard leaf) species, stomata are located in epidermal depressions called crypts (e.g., *Nerium oleander*, Fig 5.3b, Metcalfe 1979). Such crypts have traditionally been thought to reduce water loss through increasing boundary layer resistance, but recent modeling suggested that crypts have negligible effects on the transpiration rate (Roth-Nebelsick et al. 2009). Crypts reduce the distance between stomata and chloroplasts, and this may lower the diffusion resistance through the gas spaces in the leaf (Piel 2002; Hassiotou et al. 2009).

Equation 5.1 suggests that mesophyll porosity and tortuosity are important factors

affecting airspace conductance. Porosity can be estimated by infiltration with a water volume or by measuring the air volume in microscopy sections of the leaf. Slaton and Smith (2002) analyzed a wide range of species showing variations in porosity between 4 and 51%. Generally, shade leaves are more porous compared to sun leaves (Slaton and Smith 2002; Piel et al. 2002; Sack et al. 2003; Ivanova et al. 2006; Onoda et al. 2008; but see Syvertsen et al. 1995). Tortuosity is more difficult to assess compared to porosity, and depends on the shape and size of the pores in the mesophyll. Using a 2D model that accounted for the effect of distributed CO₂ sinks throughout the leaf, Morison et al. (2005) estimated the effective diffusion through airspaces. Their model was parameterized by measurements of CO₂ gradients surrounding small patches of grease on the leaf surface. Assuming the reduction in the diffusion coefficient relative to that in air was due to the effective porosity (ρ / τ) of the mesophyll, a tortuosity factor of $\tau = 2.9$ for heterobaric *Phaseolus vulgaris*, and $\tau = 1.9$ for homobaric *Commelina communis* can be calculated. These results may suggest that the lack of bundle-sheath extensions lowers tortuosity and allows for more rapid lateral diffusion in a leaf, which may be beneficial under conditions where the light intensity on the leaf surface is heterogeneous (Morison et al. 2005; Morison and Lawson 2007).

Tortuosity can also be estimated by simulating a diffusion process using images of the anatomy of a porous medium (Nakashima and Kamiya 2007). However, to our knowledge, this method has not yet been applied for leaves (but see Table 5.1). A recent study investigating the effect of manipulating genes related to cell division patterning in *Arabidopsis* used a different image-analysis with a network theory and found that a reduced mesophyll tortuosity was linked to a lower g_m (Lehmeier et al. 2017).

Because the estimation methods of tortuosity described above require non-easily accessible techniques, an estimated factor of 1.57 has been used occasionally (Niinemets and Reichstein 2003; Peguero-Pina et al. 2012). However, since tortuosity generally scales negatively with porosity, it would be better to estimate tortuosity with estimations that take this into account. A commonly used method is based on the so-called Bruggeman relation: $\tau = \rho^{-\alpha}$, where α is a material specific constant that depends on the morphology of the porous material (Tjaden et al. 2016). It is important to note that the Bruggeman relation is valid only for materials in which the insulating phase is present in a low volume fraction and characterized by isotropically arranged shapes (Tjaden et al. 2016). Neither of these assumptions is valid for many leaves, which may have strongly anisotropic tissues (e.g., palisade paren-

Table 5.1. Comparison between the estimate of a mesophyll tortuosity factor (τ) based on porosity (ρ) alone ($\tau = \rho^{-0.55}$; Syvertsen et al. 1995) with experimental methods

Species	Porosity (%)	$\tau = \rho^{-0.55}$	Experimentally estimated τ	Method and citation
<i>Phaseolus vulgaris</i> (heterobaric)	36.4	1.7	2.9	Chlorophyll fluorescence imaging in combination with a lateral diffusion model (Morison et al. 2005)
<i>Commelina communis</i> (homobaric)	40.9	1.6	1.9	Chlorophyll fluorescence imaging in combination with a lateral diffusion model (Morison et al. 2005)
<i>Arabidopsis thaliana</i> (homobaric)	23.0	2.2	1.6	Network analysis of 3D images (Lehmeier et al. 2017)
<i>Arabidopsis thaliana</i> (homobaric)	23.0	2.2	2.3	Random walk method (Nakashima and Kamiya 2007) using microscope images (Tholen et al. 2008, D. Tholen, unpub. results)

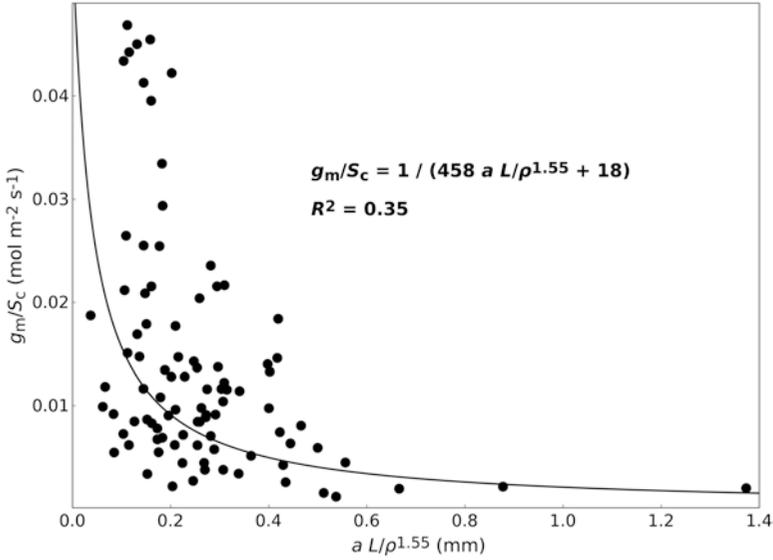


Fig. 5.7. Mesophyll conductance per unit chloroplast surface area (g_m/S_c) plotted against estimated effective distance of CO_2 diffusion in intercellular air space ($aL/\rho^{1.55}$). To account for the fact that the tortuosity estimates are inaccurate for leaves with very low amounts of airspace (Tjaden et al. 2016), leaves with $\rho < 0.2$ were excluded from the analysis. Data are from Evans et al. (1994), Syvertsen et al. (1995), Hanba et al. (2002), Kogami et al. (2001), Vyas et al. (2007), Giuliani et al. (2013), Tomás et al. (2013, 2014), Muir et al. (2014), Tosens et al. (2016), and Peguero-Pina et al. (2017). Leaf thickness was used as an estimate of mesophyll thickness (which was not reported in most cases). The line indicates the best fit through the data. Mesophyll conductance can be expressed as follows: $g_m = 1/(r_{ias} + r_{liq}) = 1/(\text{effective distance}/(Ds) + r_{liq})$, where r_{ias} is the resistance of CO_2 to diffusion in the intercellular air space, r_{liq} is the resistance of CO_2 to diffusion in mesophyll liquid phase, D is the diffusion coefficient of CO_2 in air, and s is CO_2 solubility in apoplastic water. Therefore, a line with the equation of $y = 1/(ax + b)$ was fitted to the data

chyma) and very low amounts of airspace (the dataset used for Fig. 5.7 had $0.07 < \rho < 0.68$). For isotropically arranged spheres, $\alpha = 0.5$, whereas for isotropically arranged cylinders $\alpha = 1$ should be used (Tjaden et al. 2016). Syvertsen et al. (1995) used $\alpha = 0.55$ to estimate the effective porosity of several hypostomatic leaves, simplifying Eq. 5.1 to:

$$g_{ias} = sD(\rho / \rho^{-0.55}) / L = sD\rho^{1.55} / (aL) \quad (5.2)$$

In Table 5.1 the relation by Syvertsen et al. (1995) is compared with the few available experimental estimates. The results suggest that this relation somewhat underestimates tortuosity, especially for heterobaric leaves.

If the gas-phase conductance is limiting the total mesophyll conductance, a correla-

tion between g_m and the anatomical features described above should be evident. We collected published data from several studies that investigated g_m and leaf anatomy and tested whether g_m showed negative correlation with the effective distance of CO_2 diffusion through intercellular air spaces: $(aL)/\rho^{1.55}$ (Fig. 5.7). Since thicker leaves generally possess more chloroplast surface area facing intercellular spaces per unit leaf area (S_c) and since a larger surface area for diffusion allows for more liquid phase diffusion (Evans and Loreto 2000; Terashima et al. 2006), we expressed g_m per unit S_c . The negative correlation between the effective distance and g_m ($P < 0.001$) suggests that g_{ias} explains a significant amount of the variation observed in g_m . However, the diffusion coefficient estimated from the fit in Fig. 5.7 is about 3 times higher than the expected value

(Nobel 2009). Therefore, we may have overestimated the length of the diffusion path in the calculations above, but it is also possible that g_{ias} covaries with the thickness of cell walls or other properties that affect g_{m} .

2. Diffusion Through the Mesophyll Cells

At the interface between intercellular air and apoplastic water, the gas and liquid phases are commonly assumed to be in equilibrium. The concentration of CO_2 in solution can then be calculated using Henry's law. CO_2 subsequently diffuses through apoplastic water and the cell wall into the mesophyll cells. Mesophyll conductance per unit of exposed chloroplast surface area correlates negatively with wall thickness (Evans et al. 2009; Terashima et al. 2011) and the cell wall is thought to be responsible for up to half of the total diffusion resistance (Evans et al. 2009; Terashima et al. 2011).

After diffusion through the plasmalemma, CO_2 enters the cytosol. In C_3 plants, chloroplasts are generally located immediately adjacent to cell walls facing the intercellular space, and S_{c} correlates well with g_{m} (Evans and Loreto 2000; Terashima et al. 2006, 2011). In C_4 plants, CO_2 is initially fixed by phosphoenolpyruvate carboxylase, which is located in the mesophyll cytosol. Chloroplasts in these cells may be located farther away from the cell wall than is the case in C_3 plants (Stata et al. 2014). Recent measurements found no large differences in mesophyll conductance between C_3 and C_4 species (Barbour et al. 2016).

An increase in chloroplast thickness or number without a corresponding increase in the exposed chloroplast surface area would increase the diffusion path through the liquid phase and lower g_{m} (Terashima et al. 2006, 2011). A high g_{m} can be achieved by increasing S_{c} , which can result from either increasing leaf thickness or surface-to-volume ratios. This in turn can be achieved by the construction of smaller cells or elongation of mesophyll cells. However, smaller cells

require more cell divisions, which could slow leaf growth, and thicker leaves may result in increased nitrogen costs, increased airspace resistance, and lower light-use efficiency. These issues may explain the occurrence of lobed mesophyll cells in many taxa (Haberlandt 1914). For example, rice mesophyll is heavily lobed, and chloroplasts with stroma-filled protrusions cover nearly the entire periphery of the small mesophyll cells (Fig. 5.1f, Chonan 1978; Sage and Sage 2009). Adachi et al. (2013) observed that the density of these mesophyll lobes was linked to a lower resistance to CO_2 diffusion and increased photosynthetic rates. However, Giuliani et al. (2013) found no strong effect of cell lobing on the photosynthetic rate in an analysis of structural leaf traits in rice and its wild relatives.

Mitochondria are typically located towards the center of mesophyll cells, with chloroplasts closer to the periphery (Sage and Sage 2009; Hatakeyama and Ueno 2017). This suggests increasing S_{c} may not only increase the surface area available for CO_2 diffusion, but may also partially block and re-assimilate the respiratory and photorespiratory CO_2 released by mitochondria that would otherwise escape to the atmosphere. More re-assimilation of (photo) respiratory CO_2 would increase the apparent g_{m} and potentially increase photosynthetic rates (Tholen et al. 2012b; Busch et al. 2013).

It has been hypothesized that chloroplast arrangement may also affect mesophyll conductance (Terashima and Hikosaka 1995). However, no significant effects on mesophyll conductance were found in *Alocasia brisbanensis* (Gorton et al. 2003). In *Arabidopsis thaliana*, the chloroplast avoidance response to high light resulted in a small decrease in S_{c} coupled to a corresponding decrease in g_{m} (Tholen et al. 2008).

C. Temperature Modulation

Photosynthesis is a chemical reaction catalyzed by enzymes and the rate of photosyn-

thesis therefore depends on leaf temperature. As a result of increased membrane fluidity and higher enzymatic activities at warmer temperatures, photosynthetic rate increases up to an optimum temperature (Lambers et al. 1998). When the temperature exceeds this optimum, the photosynthetic rate decreases due to 1) deactivation of enzymes, especially Rubisco and Rubisco activase and 2) an increase in the rate of photorespiration for C_3 plants (Salvucci and Crafts-Brandner 2004; Sage and Kubien 2007) (see Chap. 8 for details). Leaf temperature is influenced by atmospheric temperature, irradiance, water/humidity, wind, air pressure, and leaf angle, shape, and transpiration (Medina et al. 1978; He et al. 1996). Light energy increases leaf temperature because of light absorption by leaf pigments, as part of the light energy is converted into thermal energy (Gates 1980; Jones 1983). Leaf temperature can be decreased by transpirational cooling: latent heat is taken from the leaf when the vapor pressure difference between intercellular space and atmosphere causes evaporation of apoplastic water. The control of stomatal aperture is thus an important mechanism to control leaf temperature (Vogel 2009). Because the details of stomatal control are reviewed in Chap. 6, we focus on the anatomical aspects of leaf temperature homeostasis here.

Leaf size can affect temperature homeostasis. An increase in leaf area increases the thickness of the boundary layer, a thin layer of stagnant air surrounding the leaf (Nobel 2009): The boundary layer can be estimated as $\delta = c\sqrt{(vd/u)}$, where δ is boundary layer thickness [m], u is wind speed [$m\ s^{-1}$], d is leaf width [m] measured in the direction of the wind, v is the kinematic viscosity of air ($1.53 \times 10^{-5}\ m^2\ s^{-1}$ at $25\ ^\circ C$), and c is a dimensionless empirical coefficient, which varies from 1.03 to 1.72 (Nobel 2009; Monteith and Unsworth 2013). If the thickness of the boundary layer increases, the resistance to water and CO_2 diffusion also increases (Nobel 2009). For example, for

20 cm wide leaves (e.g., *Aesculus* and *Platanus* leaves), the water vapor conductance of the boundary layer ($= D_v/\delta$, where D_v is the diffusion coefficient of vapor) at $25\ ^\circ C$ becomes $0.6\text{--}1.0\ mmol\ m^{-2}\ s^{-1}$ at a wind speed of $3\ m\ s^{-1}$ and $0.3\text{--}0.6\ mmol\ m^{-2}\ s^{-1}$ at a wind speed of $1\ m\ s^{-1}$. In the case of plants that possess gigantic leaves larger than 1 m, like banana (*Musa* spp.) and *Alocasia*, the boundary layer conductance becomes $0.3\text{--}0.4\ mmol\ m^{-2}\ s^{-1}$ and $0.2\text{--}0.3\ mmol\ m^{-2}\ s^{-1}$ at wind speeds of $3\ m\ s^{-1}$ and $1\ m\ s^{-1}$, respectively. These values are comparable with the typical stomatal conductance of tree species and herbal species ($0.25\ mmol\ m^{-2}\ s^{-1}$ and $0.5\ mmol\ m^{-2}\ s^{-1}$; Nobel 2009). Therefore, a larger leaf area may result in slower evaporation rates and CO_2 exchange between leaf and atmosphere (Parkhurst and Loucks 1972), especially when the wind speed is low. A decrease in evaporation increases leaf temperature, and a decrease in CO_2 diffusion results in a lower CO_2 concentration inside the leaf (C_i).

Okajima et al. (2011) developed a model that estimates the photosynthetic rate of leaves in relation to leaf size, temperature, and wind speeds. The model showed that an increase in leaf area increases leaf temperature in the daytime, which could lead to a higher photosynthetic rate below the optimum temperature for photosynthesis. However, a larger leaf area also lowers C_i due to slower diffusion of CO_2 through the boundary layer, which in turn limits the rate of photosynthesis. Accordingly, at cooler temperatures, there is an optimum leaf area that balances the effects of boundary layer resistance and leaf temperature on the rate of CO_2 exchange. On the other hand, at warmer temperatures, the increase in leaf temperature does not lead to an increase in photosynthetic rates, and a smaller leaf size therefore seems more advantageous in terms of efficient photosynthesis. However, when temperatures fluctuate during the day, the larger surface area may allow the leaves to heat up more quickly to temperatures suitable for

photosynthesis, leading to higher photosynthetic returns during cool mornings (Michaletz et al. 2016). At the shoot level, large leaved species require less investment in stems and the reduced supporting cost per unit leaf area may lead to a growth advantage (Pickup et al. 2005). These considerations may explain why many tropical species have large leaves (Wright et al. 2017). It is also suggested that, in cold regions, larger leaves may experience greater negative consequences from freezing temperatures at nighttime. Radiative cooling leads to leaf temperatures that are lower than air temperature and slower heat diffusion from the surrounding air may increase the possibility of freezing (Wright et al. 2017).

Increases in leaf thickness and/or leaf water content increases the heat capacity, which can prevent a rapid rise in leaf temperature under high light conditions especially at slow wind speeds ($< 0.1 \text{ m s}^{-1}$). Thus, increased leaf thickness and/or increased leaf water content are likely to be important for decreasing the incidence of extreme heat stress (Leigh et al. 2012; Schymanski et al. 2013). For example, Li et al. (2011) reported that higher heat tolerance in polyploid plants of *Lonicera japonica* compared to diploid plants was a result of the greater epidermal and palisade tissue thickness in the former. It is also reported that plants adapted to an active geothermal field (e.g., *Cistus salviifolius* and *Agrostis castellana*) develop a thicker epidermis and cuticle, higher palisade and spongy tissue density, and longer palisade cells (Bartoli et al. 2014, 2015).

D. Anatomy and Water Transport

Stomata provide an opening in the cuticle and epidermis through which CO_2 can diffuse into the leaf, but they also allow water to escape to the atmosphere. The transpiration of water has an important function in regulating leaf temperature (Vogel 2009), but also carries with it a risk of dehydration of the mesophyll cells, which could impair leaf

biochemistry (Cornic and Massacci 1996). Without an efficient pathway for water to reach the site of evapotranspiration, stomata would need to close rapidly under conditions where transpiration rates suddenly increase, and this will negatively affect CO_2 assimilation rates. Leaves have developed an extensive vascular system for water transport and the hydraulic conductance through a leaf is affected by structural properties of the vasculature, such as minor and major vein length per area, conduit numbers and size, and vein topology (Cochard et al. 2004; Brodribb et al. 2007; Caringella et al. 2015; Sack et al. 2015; Xiong et al. 2015). For example, veins with large diameters can transport disproportionately greater amounts of water than those with small diameters (see Chap. 4, Hagen-Poiseuille flow), but larger veins are more susceptible to cavitation (formation of air bubbles, which interfere with water transport). Finer networks of leaf veins are associated with higher photosynthetic capacity across phylogenetically diverse species (Brodribb et al. 2007).

In many species, a layer of bundle-sheath cells surrounds at least a part of the vascular bundle and may even enclose the terminal tracheids (Leegood 2008). This bundle sheath conducts the flow of water between xylem and mesophyll. Water and dissolved substances may move easily through cell walls, but suberin or lignin deposits inside the wall could block this pathway, forcing the transpiration stream through the symplast (Sack and Holbrook 2006; Leegood 2008). In *Rhododendron* species sampled along an altitudinal gradient, lignified primary walls in the bundle sheath were associated with a lower leaf hydraulic conductance (Taneda et al. 2016). Ohtsuka et al. (2018), in a study with 11 angiosperm tree species, also showed that species with a lignified bundle sheath surrounding minor veins have higher resistance to apoplastic water movement. Such an impediment to the apoplastic pathway across the bundle sheath would allow for regulation of the transpiration

stream through water transport across cells by transporting membrane proteins such as aquaporins. Despite the observation that extensive suberin deposits are uncommon in leaves (Lersten 1997), experiments with fluorescent dye tracers suggested that the water stream indeed enters the symplast close to the xylem elements (Canny 1990). More recently, Shatil-Cohen et al. (2011) showed that the bundle sheath in *Arabidopsis thaliana* had decreased water permeability in response to drought or abscisic acid treatment. A similar and reversible effect was seen after treatment with an inhibitor of aquaporin action. Knocking down aquaporin expression in the vein or bundle sheath results in reduced leaf hydraulic conductance (Prado et al. 2013; Secchi and Zwieniecki 2014). These results support the view that the bundle sheath plays an important role in regulating water transport. A recent model of water transport in the leaf suggested that the surface area of the bundle sheath per unit leaf area (which mainly depends on vein length per area) is a strong determinant of the outside-xylem hydraulic conductance, but that the contribution of the bundle sheath to the total resistance is relatively small (Buckley et al. 2015).

The BSEs of heterobaric leaves connect the vasculature with the epidermis. Wylie (1952) hypothesized that the well-connected BSE cells would act as a more efficient water pathway compared to the sparsely connected mesophyll cells. Some earlier experiments with fluorescent dyes supported this view, although the use of dyes for tracing water transport beyond the bundle sheath is questionable (Canny 1990). More recently, Scoffoni et al. (2008) found that changes in hydraulic conductance in response to short-term differences in light intensity are stronger in heterobaric species, suggesting BSEs do play a role in facilitating water transport. Moreover, a tomato mutant without BSEs has reduced leaf hydraulic conductance (Zsögön et al. 2015). Additional evidence for a role of BSEs in water transport came from

studying the response of stomata to changes in water availability. Before stomata close as a result of a rapid increase in evaporative demand or reduced water supply, they tend to open briefly as a result of a loss of turgor in the surrounding tissues that normally provide back pressure to the guard cells. Buckley et al. (2011) showed that such “wrong-way responses” are much stronger in leaves with BSEs, and further modeling suggested that hydraulic conductance was between 4 and 16 times higher in heterobaric leaves. The higher hydraulic conductance in heterobaric leaves could also be a result of other anatomical traits influencing hydraulic conductance, such as the size of mesophyll cells, which covary with presence of BSEs (Buckley et al. 2015).

The hydraulic resistance of the pathway through the mesophyll is substantial, but the effect of anatomy on this part of the pathway is still poorly understood (Sack et al. 2015). If most of the water evaporates from mesophyll cells that are close to the stomata, connectivity between mesophyll cells would be an important factor affecting hydraulic conductance, as the lost water is replenished by well-connected cells. It can also be expected that hydraulic conductance correlates negatively with the distance between veins and the epidermis. On the other hand, if most of the water evaporates from mesophyll cells that are close to the bundle sheath, water transport through the gaseous space would be more significant and hydraulic conductance would be expected to also correlate with leaf porosity (Buckley 2015; Buckley et al. 2015). Some studies have indeed found a negative correlation between leaf thickness and hydraulic conductance (Brodribb et al. 2007), but the opposite has also been reported (Aasamaa et al. 2001; Sack et al. 2003; Sack and Frole 2006). Buckley (2015) and Buckley et al. (2015) developed an analytical framework to study water transport outside of the bundle sheath and highlighted the importance of the gas phase for water transport. Mesophyll porosity and cell connectiv-

ity were key parameters influencing water conductance (Buckley et al. 2015). In addition to water transport via BSEs and between parenchymatous mesophyll cells, lignified cells outside the xylem occur in many species and are hypothesized to play an additional role in water transport between veins and epidermis (Brodribb et al. 2010). The paraveinal mesophyll of the Fabaceae (legumes) could also play a role in water transport. This tissue consists mainly of mesophyll cells, but sometimes partially or even completely of bundle-sheath cells (Kevekordes et al. 1988; Brubaker and Lersten 1995; Leegood 2008). In addition, at least in *Glycine max*, there is substantial evidence that paraveinal mesophyll plays a role in photosynthate transport, nitrogen metabolism, and protein storage (Brubaker and Lersten 1995).

Recent work suggests that the leaf water status itself serves as a feedback on hydraulic conductance (Scoffoni et al. 2014). Leaf dehydration is known to result in tissue shrinkage and mesophyll cells in dehydrated leaves are often deformed (Fellows and Boyer 1978; Canny and Huang 2006; Sancho-Knapik et al. 2011; Canny et al. 2012); such changes are likely to alter mesophyll porosity and cell connectivity resulting in an effect on leaf hydraulic conductance (Scoffoni et al. 2014).

E. Mechanical Function

Given the primary function of many leaves, it may seem that leaf anatomy has been exclusively optimized for photosynthesis. However, leaves allocate significant mass to non-photosynthetic structural components, such as cell walls, that account for between 10 and 70% of the leaf dry mass (see Chap. 17; Onoda et al. 2011). Such high allocation of dry mass to structural components indicates that mechanical stability is an important function of leaves. In this section, we describe how leaf anatomy is important for leaf mechanical functions.

Leaves are typically thin, flat, and often maintained horizontally, which is ideal to maximize light interception per unit leaf mass and facilitates the diffusion of CO₂ to the chloroplasts as discussed previously. However, such a structure increases the risk of mechanical failure because the horizontal alignment maximizes the bending moment of a leaf with its own weight and also because thin leaves are more vulnerable to external forces, such as wind, rainfall, abrasion, pathogen attacks, herbivory, and trampling. Leaves must therefore be mechanically “tough” in order to survive physical disturbance. Long-lived leaves (e.g., evergreen leaves) often have a tough structure and allocate relatively more leaf mass to structural components. Such an increase in allocation to structural components would increase leaf longevity, which in turn allows for more carbon gained per unit of carbon originally invested in the leaf over the leaf lifespan. However, higher investment in structural components often constitutes a trade-off with instantaneous photosynthetic efficiency because of lower resource allocation to the photosynthetic apparatus and lower CO₂ diffusion conductance due to thicker cell walls (See section IIIB2, Chap. 16 and Onoda et al. 2017).

Different types of mechanical properties are important to withstand or avoid various types of mechanical stresses (Niklas 1992; Wright and Vincent 1996). For example, bending stiffness is important to maintain leaf structure against gravity or under moderate wind (e.g., Onoda et al. 2015), shear strength is important to protect against insect herbivores that feed on leaf tissues by chewing (e.g., Sanson 2006), tensile strength may avoid damage resulting from mammalian grazers who forage leaves by pulling (e.g., Vincent 1982), and surface hardness is important for defense against the initial attacks of herbivores (e.g., Grubb 1986) and to avoid damage related to rubbing between leaves (Wilson 1984). Finally, the cellular bulk modulus of elasticity determines to

what extent cells are deformed under desiccation stress (e.g., Tyree and Hammel 1972). These different mechanical properties may be achieved by differences in the amount and quality of cell walls, leaf thickness, density, cell size, the amount and arrangement of particular tissues (sclerenchyma), and so on (Grubb 1986; Wright and Illius 1995; Wright and Vincent 1996). There are also leaves that have special mechanical properties, such as spines, thorns, prickles, and silica accumulation, which have been reviewed elsewhere (Raven 1983; Read and Stokes 2006).

Thick cell walls are often found in the epidermis, vascular tissues, and BSEs, whereas palisade and spongy tissues have relatively thin cell walls. Cell wall thicknesses of these thick-walled tissues are often 5 to 10 times thicker than those in mesophyll tissues, e.g., 0.5–10 μm for epidermal cell walls (Onoda et al. 2015) compared to 0.1–1 μm for mesophyll cell walls (Terashima et al. 2011; Tosens et al. 2016). The external cell wall of the epidermis is thicker than the internal wall. Cuticle layers are deposited on top of the external wall, thereby hardening the leaf surface (Wiedemann and Neinhuis 1998; Onoda et al. 2012).

Vascular tissues need to have rigid cell walls to resist negative water potentials. Cell wall thickness per unit conduit diameter is tightly associated with embolism resistance (Hacke et al. 2001). Furthermore, although the main function of veins is related to water and nutrient transport, major veins (and petioles) also play a role as scaffold in maintaining leaf orientation and structure (Roth-Nebelsick et al. 2001). In addition, the veins provide a higher shear resistance than leaf lamina, which reduces insect herbivory often targeting soft leaf lamina tissues (Lucas et al. 1991). The thin cell wall of the spongy and palisade tissue facilitates diffusion of CO_2 into the cells (see section IIIB). However, given the substantial variation in mesophyll cell wall thickness, thicker walls may have advantages under some conditions even at the cost of lower CO_2 diffusion rates.

For example, thicker walls are thought to prevent excessive shrinkage of leaves under drought that would negatively affect hydraulic conductance (Scoffoni et al. 2014). In addition, thicker walls could serve as a better defense against herbivores, especially leaf miners that selectively feed on mesophyll tissue.

In addition to the mechanical function of each individual tissue, the combination of multiple tissues affects the mechanical properties of the whole leaf. As described earlier, in flattened leaves, the leaf lamina is composed of two epidermal layers with mesophyll tissue in between. This anatomy is analogous to the “sandwich structure” used in engineering applications, such as airplane wings and surfboards (Gibson et al. 1988). A typical sandwich structure is composed of stiff outer surfaces and a lightweight core, which greatly increases specific stiffness (stiffness per unit mass) for bending. High bending stiffness with a minimum leaf mass would be ideal to increase the capacity to maintain large flat leaves horizontally and thus maximize light capture per unit dry mass (Gibson et al. 1988; Niinemets and Fleck 2002). A recent study showed that leaves across a range of species have indeed stiff epidermises and soft mesophyll tissues, forming efficient sandwich structures (Fig. 5.8, Onoda et al. 2015).

BSEs in heterobaric leaves and turgor pressure in homobaric leaves keep the two epidermal layers some distance apart, helping to maintain the lamina bending stiffness and preventing local buckling, because bending stiffness is proportional to the third power of lamina thickness (Gere and Timoschenko 1999). In addition, turgor pressure also serves to “control” lamina thickness such that a 20% reduction in leaf water content resulted in similar (~20%) or even greater (35%) reduction in lamina thickness in a deciduous woody species (Sancho-Knapik et al. 2011). If leaf thickness decreases by 20%, bending stiffness would decrease by 49% ($(1 - 0.2)^3 = 0.51$). Therefore, a small

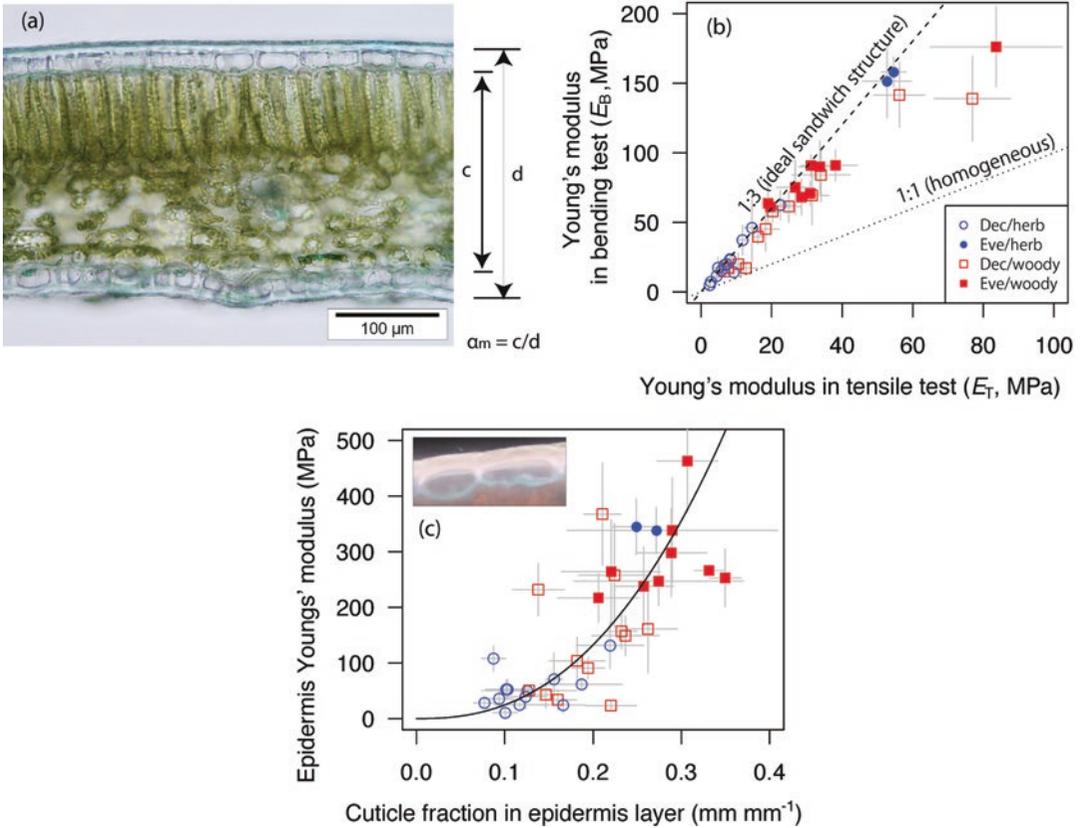


Fig. 5.8. The leaf “sandwich” structure and its importance to mechanical functions. **(a)** Typical leaf cross-section. The leaf lamina consists of an upper and lower epidermis with mesophyll tissue in-between. This resembles a composite material used in engineering construction (e.g., in airplane wings), where high bending stiffness in combination with minimum weight is important. The Young’s modulus of the lamina can be expressed as the products of the Young’s modulus of the epidermis (E_f) and that of the mesophyll (E_c) weighted by the thickness fraction of the mesophyll (α_m). The Young’s modulus measured by a tensile test (E_T) can be expressed as $E_T = (1 - \alpha_m)E_f + \alpha_mE_c$ and that measured by a bending test (E_B) can be expressed as $E_B = (1 - \alpha_m^3)E_f + \alpha_m^3E_c$.

From these two equations, E_B/E_T can be expressed as $\frac{E_B}{E_T} = \frac{1 - \alpha_m^3(1 - \beta)}{1 - \alpha_m(1 - \beta)}$ where β is E_c/E_f . If the epidermis

is much stiffer than the mesophyll (i.e., $\beta \sim 0$), $E_B/E_T \sim 1 + \alpha_m + \alpha_m^2$, which approaches 3 for leaves with a thin epidermis. **(b)** The relationship between E_B and E_T was examined for 36 angiosperm species. Many species aligned closely to the 1:3 line, indicating that many leaves have an efficient sandwich structure. **(c)** The variation in epidermis stiffness correlates with the fraction of the leaf comprised of cuticle and outer cell walls of the epidermis, suggesting that leaf cuticles are made of stiff material. (Figures are redrawn from Onoda et al. 2015)

change in leaf water content greatly affects the leaf bending stiffness when leaf thickness is controlled by turgor pressure. Such a reduced stiffness allows leaves to wilt and avoid strong light under water stressed conditions (Zhang et al. 2010).

Intercellular airspace can also contribute to bending stiffness by increasing the lamina thickness per unit dry mass, i.e., leaves with

more airspace can be thicker for a given amount of leaf mass (Onoda et al. 2008). A higher volume fraction of intercellular airspace in shade leaves compared to sun leaves can be explained by mechanical requirements rather than by photosynthesis requirements as described above, because improved CO_2 diffusion conductance is not a limiting factor in shade leaves (Parkhurst 1977;

Terashima et al. 2001). The volume fraction of intercellular airspace in the mesophyll of shade leaves can be 10–30% more than in sun leaves (Piel et al. 2002; Onoda et al. 2008). Following a similar logic as for turgor-controlled leaf thickness, a 20% greater lamina thickness due to increased intercellular space can translate into a 73% higher lamina bending stiffness without adding biomass ($1.2^3 = 1.73$). This rough quantification suggests the importance of intercellular airspace in maintaining a larger light interception area for a given mass in shaded environments.

F. Functions of the Leaf Surface – The Role of Trichomes

1. Trichome Morphology

Outward growths of the shoot epidermis are often referred to as trichomes (Werker 2000). Trichomes are either unicellular or multicellular appendages, and can be found on various plant organs. Here, we will focus on leaf trichomes. There is an enormous diversity in trichome morphology, dimension, position, microstructure and capacity to secrete (Fig. 5.9). Trichomes can be unicellular and unbranched (a simple one-celled hair), while uni/multicellular branched trichomes are also common, such as the star-shaped hairs of *Arabidopsis thaliana*. Other common trichomes are the scale and peltate hairs, which are shield-shaped and usually attached to the plant surface by a short stalk. Some trichomes are capable of secretion (Wagner et al. 2004). These so-called glandular trichomes may secrete various kinds of chemicals such as terpenoids and phenylpropanoids depending on the species, and are important for plant-animal interactions (Wagner et al. 2004).

The density of trichomes varies extensively across and also within species and even within a single individual or organ. Plants growing in arid or sunny environments often show higher trichome densities,

but many other environmental factors are thought to play a role (Johnson 1975; Pérez-Estrada et al. 2000; Gregoriou et al. 2007). It is important to note that such plastic changes in the density of trichomes could be strongly associated with an ontogenetic change in leaf area (Roy et al. 1999).

2. Trichome Functions

Many functions of leaf trichomes have been proposed, including but not limited to a role in increasing water use efficiency (Nobel 2009), light reflection (Ehleringer and Björkman 1978), temperature control (Ehleringer and Mooney 1978), water absorption (Schmitt et al. 1989), as water or dust repellent (Barthlott et al. 2010), as a defense against herbivory (Levin 1973; Dalin et al. 2008), or providing a more suitable environment for leaf-inhabiting arthropods (Voigt et al. 2007).

Trichomes, especially on the abaxial side of hypostomatic leaves, can increase the thickness of the leaf boundary layer, thus reducing the rate of water loss in water-limited habitats (Nobel 2009). For example, Ehleringer and Mooney (1978) estimated that a trichome layer thickness of 0.35 mm can increase the boundary layer thickness by up to 50% in a desert plant (*Encelia farinosa*). However, Benz and Martin (2006) analyzed 12 *Tillandsia* species whose trichome layer thickness varied between 0.052 and 0.5 mm and concluded that the contribution of trichomes to the leaf boundary layer thickness was at most 9.7%. The relative contribution of leaf trichomes to the total resistance for H₂O diffusion may be small or negligible because the boundary layer resistance is usually much smaller than stomatal resistance (Amada et al. 2017). Nonetheless, there are a number of studies that reported positive correlations between trichomes and water use efficiency (e.g., Vitousek et al. 1992; Ichie et al. 2016). It is likely that several leaf characteristics such as low stomatal conductance, high nitrogen content, and

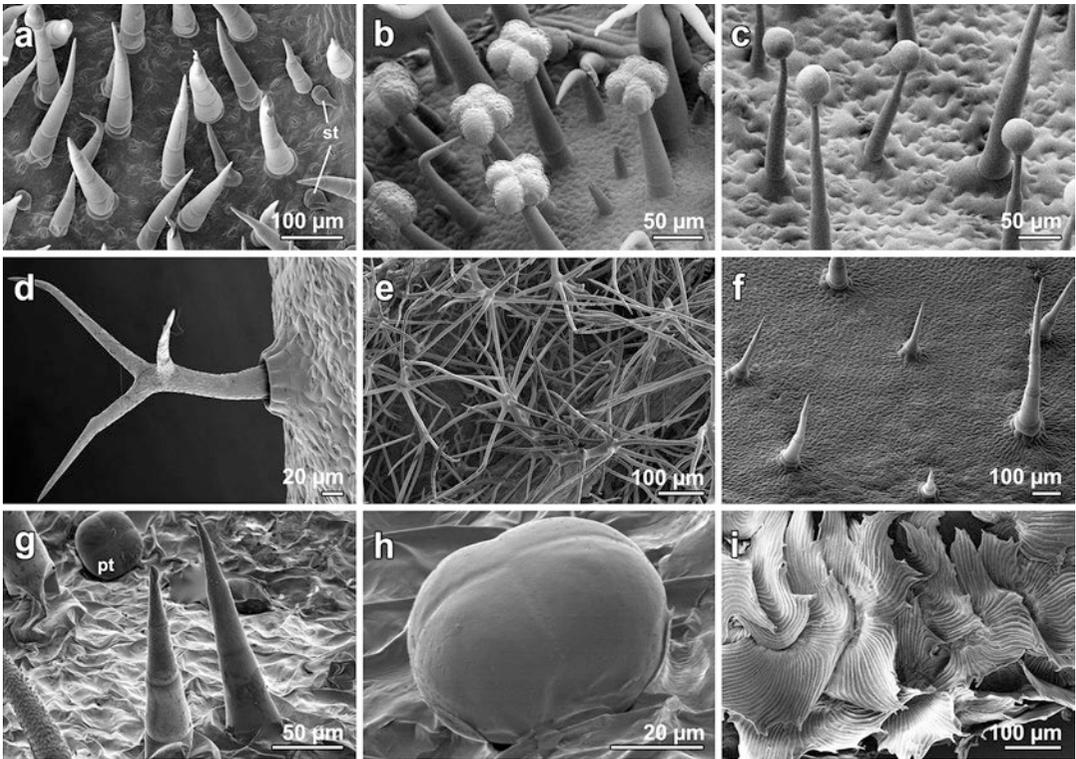


Fig. 5.9. Cryo-scanning electron micrograph images of pubescent abaxial leaf surfaces. (a) *Lageneria siceraria* (Molina) Standl. densely covered with multicellular, uniseriate, tapered trichomes. (b) Capitate and cone-shaped trichomes on *Solanum lycopersicon* L. (c) Capitate, multicellular, uniseriate trichomes on *Nicotiana sylvestris* Speg. & Comes. (d) Unicellular, branched trichome of *Arabidopsis thaliana* L. (e) *Verbascum thapsus* L., densely covered with multicellular, branched trichomes. (f) *Cucumis sativus* L. bears multicellular, uniseriate, tapered trichomes with multicellular socket and corrugated surface. (g, h) *Columnnea sanguinea* (Pers.) Hanst. is equipped with cone-shaped, tapered, multicellular and peltate trichomes; detail of a four-cellular peltate trichome in h. (i) A dense coverage of peltate trichomes found on *Tillandsia usneoides* L. (Courtesy of Dr. Dagmar Voigt, Technische Universität Dresden, Institute for Botany, Dresden, Germany)

thicker leaves may be coordinated with trichome density to increase water use efficiency in water-limited environments (see Cordell et al. 1999; Wright et al. 2003).

Some leaves have trichomes that reflect a certain fraction of solar irradiance. Reflecting strong irradiance may be important to prevent overexcitation of the photosynthetic apparatus, avoid over-heating of leaves, and maintain favorable leaf temperature for photosynthesis (Ehleringer and Björkman 1978; Skelton et al. 2012). Some trichomes can also effectively reflect or filter ultraviolet light (Liakopoulos et al. 2006).

Certain trichomes, particularly scale hairs, can absorb water. A notable example

is found in *Tillandsia* species (and other related members of the Bromeliaceae) that utilize elaborate, peltate trichomes that transfer water from the leaf surface into the mesophyll tissue, while their root structures function solely as holdfasts incapable of water absorption (Schmitt et al. 1989). Similarly, it is known that trichomes can absorb nutrients as well as water (e.g., pineapple, Sakai and Sanford 1980). Konrad et al. (2015) proposed that trichomes can promote water condensation and increase humidity at the leaf surface. This would allow for stomata to remain open for longer periods during the day, thereby increasing

the length of the effective photosynthetic period.

The role of trichomes as a defense mechanism against herbivory and pathogen attacks is well-known (reviewed by Levin 1973; Dalin et al. 2008). Many studies found that an increased trichome density is related to a decrease in herbivory or tissue damage (Dalin et al. 2008).

IV. Acclimation and Adaptation

A. Responses of Leaf Anatomy to Light

As explained in the previous sections, leaves develop under a certain condition can have various anatomical functions. However, plants experience a constantly changing environment. Because plants are immobile, the ability to adjust their leaf anatomy to environmental differences is vital for survival and successful competition with other plants (e.g., Oguchi et al. 2006, 2017). The light environment in particular varies significantly: for example, the light intensity above the leaf canopy of a tropical rain forest can be more than 100 times higher than that in the understory (Chazdon and Fetcher 1984; Onoda et al. 2014). Sun-acclimated leaves are generally thicker and have a higher nitrogen content, photosynthetic protein content, and photosynthetic capacity per unit area compared to shade leaves. A simplified explanation of these differences is that chloroplasts, as the main site of the photosynthetic process, require large amounts of nitrogen for their construction (Terashima and Hikosaka 1995). Under low light conditions, it would be inefficient to have too many chloroplasts because this would result in many chloroplasts (especially those on the shaded side of the leaf) unable to receive enough light to cover their cost of construction and maintenance by photosynthesis. On the other hand, under high light conditions, if the number of chloroplasts per unit leaf area is insufficient, the leaves lose some propor-

tion of the available energy. Sun leaves need to be thicker to achieve a large chloroplast surface area facing intercellular spaces per unit leaf area (S_c), high mesophyll conductance (g_m), and high photosynthetic capacity (see Terashima et al. 2001 and 2006 for details).

The formation of palisade tissue strongly depends on the light environment (Haberlandt 1914). Modeling has indicated that maximal photosynthetic rates at a given light intensity can be achieved with a specific ratio between palisade and spongy tissues (Tholen et al. 2012a). Yano and Terashima (2001) showed that the anatomy of developing leaves is not controlled by their own light environment, but by the light environment of already expanded leaves (Fig. 5.10). This long-distance control of leaf anatomy was also seen in C_4 plants (Jiang et al. 2011). Munekage et al. (2015) recently showed that locally perceived blue light influenced palisade elongation, but a long-distance signal (induced by high light and oxidative stress) in mature leaves increased palisade tissue cell densities in *Arabidopsis thaliana*. In isolateral and resupinate leaves, the development of palisade tissue does not depend on the adaxial origin of the tissue as it can also develop from the abaxial meristem (Hofreiter and Lyshede 2006, e.g., Fig. 5.1e). In addition, in the scale leaves of plagiotropic *Thuja plicata* shoots, palisade tissue can develop on any light-exposed side of the leaves irrespective of the ab- or adaxial origin of the tissue (Dörken 2013). Although the direction of light seems to be the most important signal for the elongation of mesophyll cells, temperature, CO_2 concentration, and gravity may also play a role (Imamura 1931; Leadley et al. 1987; Pino et al. 2008; Dumlao et al. 2012). In addition, genetic factors are also important in acclimation. For example, leaves of shade-tolerant plants generally have only a single layer of palisade tissue, exhibiting no propensity to develop multi-layered palisade parenchyma even under

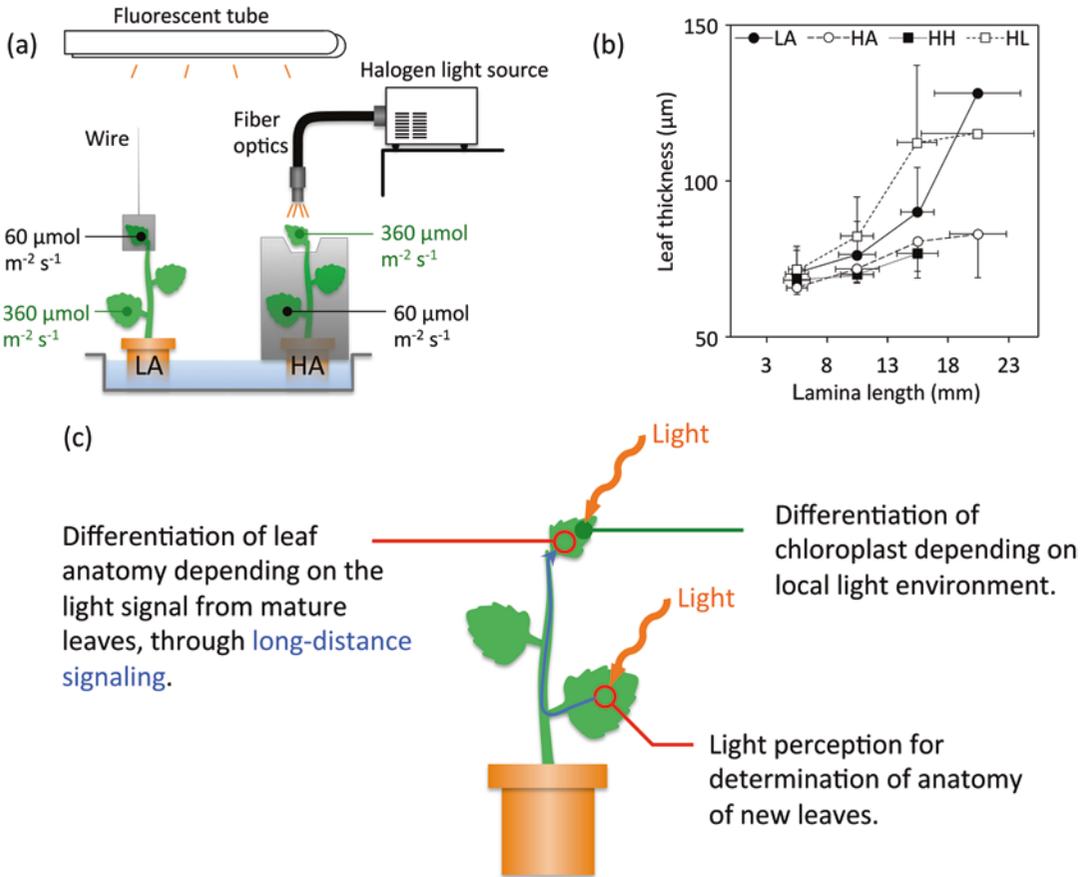


Fig. 5.10. (a) Light treatment design, (b) responses of leaf thickness to different light treatments, and (c) a model scheme of light sensing mechanisms for developing leaves following Yano and Terashima (2001). Low-light apex (LA) treatment and high-light apex (HA) treatment are left and right, respectively, in panel (a). In the LA treatment, only the shoot apex was covered by a shading screen. In the HA treatment, the shoot apex was illuminated with a halogen lamp through fiber optics, but the other parts of the plant were covered by a shading screen. In the HH treatment, whole plants were grown under high-light conditions ($360 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). In the HL treatment, whole plants were transferred to low-light conditions ($60 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). One month old high light grown plants were transferred to the above conditions and studied after 6 days. In panel (b), the vertical and horizontal bars indicate standard deviations ($n = 1$ to 8)

sunny conditions (Murchie and Horton 1997; Pons and Poorter 2014).

Chloroplasts also show sun/shade acclimation (Ballantine and Forde 1970; Skene 1974). Chloroplasts that developed under high light conditions had smaller granal stacks, larger stromal space, higher chlorophyll a/b ratios, higher Rubisco/chlorophyll ratios, and thus higher photosynthetic rates per unit chlorophyll at light saturation com-

pared to chloroplasts that developed under low light conditions (Terashima and Inoue 1985; Pearcy and Seemann 1990; Nishio et al. 1993). On the other hand, shade-acclimated chloroplasts have a higher amount of chlorophyll per photosystem. These differences are observed even within a single leaf dependent on the internal light gradient (Terashima and Inoue 1984). Although some of the leaf anatomical traits described in sec-

tion IIIA reduce the steepness of the light gradient, a difference in light intensity within the leaf is inevitable. Therefore, chloroplasts near the light-exposed side and near the shaded side show sun and shade traits, respectively, matching their photosynthetic metabolism with light availability (Evans 1999).

Plant responses to light availability can be considered at different time-scales; seconds (sunflecks), hours (daily changes), months (seasonal changes), and years (gap formation in the canopy). For understory plants, disturbances of the canopy as a result of wind-damage, disease, herbivory, or logging cause a sudden increase in irradiance. In response to such changes in light intensity, various biochemical and anatomical traits are adjusted both in the existing fully developed and newly expanding leaves (Björkman 1981; Pearcy and Sims 1994). The acclimation mechanism of newly expanding leaves is almost the same as the plasticity between sun and shade leaves described above; however, the acclimation mechanism of already expanded leaves can be restricted by anatomical constraints. It has been shown that once leaves have developed in shade, transfer to a higher growth light environment does not generally result in an increase in their photosynthetic capacity to the level of sun leaves that developed under sunny conditions (Jurik et al. 1979; Sims and Pearcy 1992; Frak et al. 2001; but see Amiard et al. 2005). This restriction is mainly caused by the inability of mature leaves to change leaf thickness (Milthorpe and Newton 1963; Wilson 1966; Sims and Pearcy 1992). As described in section IIIB, chloroplasts are located near mesophyll cell walls to efficiently receive CO_2 from the intercellular airspace. When the mesophyll cell surface is fully occupied by chloroplasts, leaves exposed to higher light conditions do not increase the volume of chloroplasts and photosynthetic capacity does not increase (Fig. 5.11a, b; Oguchi et al. 2005). In contrast, when the mesophyll cell surface is not fully occupied by chloroplasts,

leaves of some species are able to increase the volume of chloroplasts under high light and correspondingly achieve higher photosynthetic capacities (Fig. 5.11c, d, Oguchi

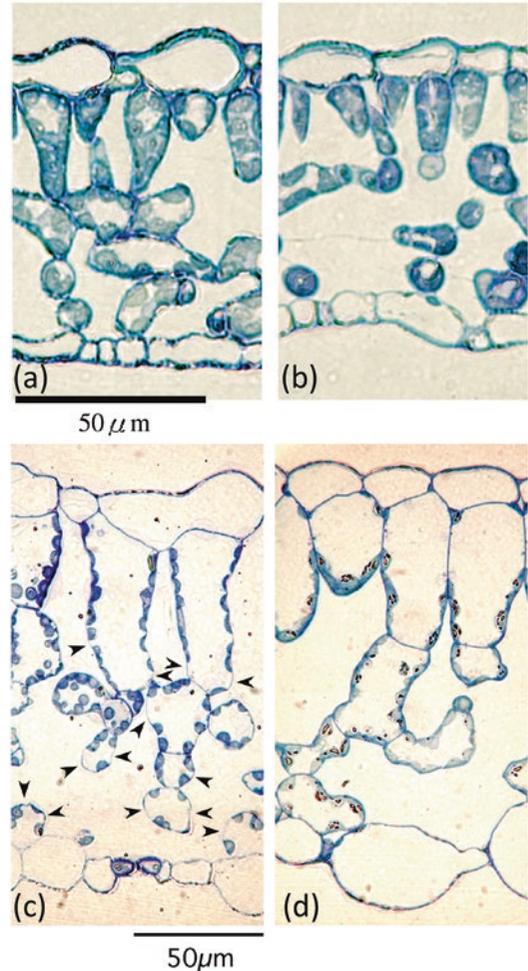


Fig. 5.11. Leaf cross-sections of mature leaves of *Fagus crenata* (a, b) and *Chenopodium album* (c, d) grown continuously in low light ($\text{LL} = 70 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) (a, c) and transferred from low to high light conditions ($\text{LH} = 350 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for *F. crenata* and $700 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for *C. album*) (b, d). Light micrograph magnification is 400 times. The depth of the cross-section was $0.8 \mu\text{m}$. In *F. crenata* (a, b), because there was no vacant space near the mesophyll cell surface, the leaves that were transferred from low to high light were unable to increase the chloroplast volume per leaf area (Oguchi et al. 2005). In *C. album*, arrowheads show the available open space for chloroplasts, which allowed for an increase in chloroplasts per leaf area in response to an increase in irradiance. (Oguchi et al. 2003)

et al. 2003). Interestingly, in a small number of species, fully expanded leaves can still change leaf thickness or mesophyll cell length, resulting in an increased mesophyll surface area in response to a change in irradiance (Gauhl 1976; Bunce et al. 1977; Bauer and Thoni 1988; Kamaluddin and Grace 1992; Oguchi et al. 2005, 2006). Such species may sacrifice some traits, such as leaf toughness and thereby herbivory tolerance, but retain more flexible cell walls that allow for growth after maturation.

The light environment is perceived by a number of photoreceptors (light-receptors). So far, the following three photoreceptor categories have been discovered in plants: phytochrome is a pigment-protein activated by red light and deactivated by far-red light (Hendricks 1960; Smith 2000). Because sunlight transmitted through canopies is enriched with far-red light compared to red light, the phytochrome activation state enables plants to sense the level of shading. Cryptochrome is a blue light receptor identified by Cashmore and colleagues (Ahmad and Cashmore 1993; Cashmore et al. 1999) involved in the control of blue light inhibition of stem elongation and anthocyanin synthesis. Phototropins, blue-light receptors identified by Briggs and colleagues (Briggs and Huala 1999; Christie et al. 1999), are involved in the control of chloroplast movement (Wada 2013) and stomatal opening (Shimazaki et al. 2007) and may be also involved in the leaf positioning and flattening (Ohgishi et al. 2004; Harada et al. 2013). Recently ZEITLUPE and rhodopsins were also suggested to be active as photoreceptors in higher plants (Kim et al. 2007; Möglich et al. 2010; Atamna-Ismaeel et al. 2012). The signal transduction pathways directly related to these receptors are well studied (Kami et al. 2010; Casal 2013). However, the relationships between these receptors and leaf anatomy, especially with regard to long distance signaling from already expanded leaves to the newly expanding leaves (Fig. 5.10), remain little understood. Thus

far, it has been suggested that hormones, ROS, sugar levels, miRNA, or expansin might be the factors responsible for such long distance signaling (Karpinski et al. 1999; Pien et al. 2001; Stessman et al. 2002; Palatnik et al. 2003).

B. Responses of Leaf Anatomy to Temperature

Compared to the acclimatory adjustments in foliar anatomy that have been documented in response to the light environment, there have been fewer reports with regard to responses to temperature. Most of the available studies report that leaves grown at lower temperatures are thicker than leaves grown in higher temperature (Boese and Huner 1990; Gorsuch et al. 2010; Dumlao et al. 2012; Stewart et al. 2016), though leaf thickness may increase when temperature is extremely high (Ben Salem-Fnayou et al. 2011, see also section IIIC). In addition, the amount of palisade tissue increased in winter-active mesophytes in response to lower temperatures (Dumlao et al. 2012). The fact that these differences parallel those between sun and shade leaves may not be coincidental because the photosystems in the chloroplast detect low temperature and high light by a comparable mechanism (Huner et al. 1998). Huner et al. (1998) proposed that both low temperature and high light cause an imbalance in the energy absorbed versus energy utilized, increasing the reduction state of plastoquinone (excitation pressure), the pH gradient across the thylakoid membrane, the production of reactive oxygen species, and depletion of phosphate. This altered chloroplastic redox balance can initiate a signal transduction pathway. Similar responses to cold temperatures and high light can be also seen at the chloroplast level. For example, Muller et al. (2009) showed that a seasonal decrease in temperature is linked to an increase in the volume of chloroplasts in evergreen species, which is similar to the previously discussed increase in the number of chloroplasts in

response to an increase in irradiance. Temperature can change the fluidity of membranes and affects protein folding, which have been suggested to act as cues for temperature-induced changes in plants (Ruelland and Zachowski 2010). Calcium is one suggested candidate as a signaling molecule involved in the transduction from perception of temperature and low water availability to plant responses to these environmental factors (Shao et al. 2008).

C. Responses of Leaf Anatomy to Water Stress

Plants from dry habitats often show a particular leaf anatomy called xeromorphy. The most obvious characteristic of xeromorphic leaves is a lower surface-to-volume ratio to reduce transpiration (Shields 1950), such as seen in small leaves with inwardly-rolled margins (e.g., *Erica* spp.; Metcalfe 1979). Sometimes the morphologically adaxial surface is reduced to a groove or completely eliminated, resulting in a centric leaf (Metcalfe 1979). Other typical traits are a decreased cell size and thicker cell walls, in particular the outer periclinal walls of the epidermis. Hairy leaves may sometimes increase boundary layer resistance, but ecological studies have found limited support for this notion (see section IIIF). Sclereid idioblast and lignified BSEs often occur in xeromorphic leaves, possibly playing a role in preventing tissue damage under drought (Metcalfe 1979). A meta-analysis of variation in leaf dry mass per area (LMA) showed that an increase in LMA is weakly linked to a decreased water availability in a wide range of plant species from various functional groups and habitats (Wright et al. 2004, Poorter et al. 2009). Higher mesophyll thickness in plants adapted to Mediterranean-type climates is also considered to be a water stress response (Peguero-Pina et al. 2012, 2017). This negative correlation between leaf thickness or LMA and annual precipitation can also be observed within species

(Castro-Diez et al. 1997; Wang et al. 2011). The increase in LMA responding to water stress is often reported to be associated with an increase in leaf density (Groom and Lamont 1997; Bosabalidis and Kofidis 2002; Bussotti et al. 2002; Bacelar et al. 2006, 2007). However, avocado cultivars exhibited a decrease in mesophyll thickness and LMA in response to water stress (Chartzoulakis et al. 2002) and, in the case of tomato and grape cultivars, the response of mesophyll thickness and leaf density to water stress differed among accessions (Scippa et al. 2004, Galmés et al. 2013, Tomás et al. 2014). More detailed analyses of acclimation of mesophyll cell anatomy, such as changes in the thickness of cell walls and anatomy of the vascular system, are required. See the Anatomy and water transport section (section IIIB) for the contribution of the vascular system to water demand.

V. Conclusions

In the present chapter, we discussed relationships between leaf anatomy and various functions, including light absorption, CO₂ diffusion, water transport, transpiration, and mechanical strength. We also reviewed how leaf anatomy varies depending on species and environments. This suggests that leaf anatomical functions cannot all be optimized simultaneously, because (a) there are trade-offs between the associated anatomical features that are caused by physical, chemical, and biological constraints, (b) specific anatomical traits may affect multiple functions with contrasting requirements, and (c) plants continuously experience a changing environment.

Some anatomical traits may covary. For example, thinner cell walls and greater leaf areas per unit leaf dry mass are often accompanied by a higher porosity and lower tortuosity of leaves, all of which increases mesophyll conductance. Traits that have been primarily selected for one function

could also find use for additional purposes in some species. For example, though BSEs may have evolved primarily as a structure to provide mechanical stability, they may later have been adopted as more efficient water conduits or as transparent light-guides to improve the light gradient in leaves. These complicated relationships often make it difficult to observe or analyze the function of single anatomical traits.

Elucidation of each relationship, especially covariation and trade-offs, among anatomical and physiological functions is required. Knowledge about the developmental mechanisms controlling leaf anatomy in response to the environment is still limited. Progress in these fields will be a key step to understand the factors underlying global scale correlations among leaf traits, plant strategies, and environments, as shown in the leaf economic spectrum studies. This information is also indispensable for breeding or producing plants that have certain human-desired properties. We expect the recent rapid development of bio-imaging technology to help provide some of this information and this will allow further progress in the field of leaf functional anatomy.

Glossary

- abaxial** Facing away from an axis (i.e., from the stem), often in reference to the lower side of a leaf.
- adaxial** Facing towards an axis (i.e., toward the stem), often in reference to the upper side of a leaf.
- amphistomatous, amphistomatic** Stomata are found on both the adaxial and abaxial sides of the leaf (often in different densities).
- armed mesophyll** Leaf mesophyll characterized by parenchyma cells that have protrusions (lobes) or with cell walls that have invaginations.
- armed palisade** Armed mesophyll characterized by lobes that are arranged perpendicular to the leaf surface, similar to regular palisade mesophyll.
- bifacial leaf** A leaf that develops from both adaxial and abaxial meristems.
- bundle-sheath extension (BSE)** Groups of cells that extend from the bundle sheath towards the upper and lower epidermis. These cells contain no chloroplasts and have cell walls that are typically thickened or lignified.
- bundle sheath** A tightly connected file of cells, that wholly or partially surround the vascular tissue. Bundle sheath cells are typically parenchymatous, but may sometimes become lignified (see: endodermis).
- C₃ plants** Plants in which the first organic acid that is produced from CO₂ by photosynthetic metabolism (3-phosphoglyceric acid) has 3 carbon atoms.
- C₄ plants** Plants in which the first organic acid that is produced from CO₂ by photosynthetic metabolism (oxaloacetic acid) has 4 carbon atoms.
- capitate** Having a distinct globular end.
- centric leaf** See terete leaf.
- dorsiventral** Along the axis connecting dorsal and ventral sides (e.g., from adaxial to the abaxial side of a leaf).
- dorsiventral mesophyll** The mesophyll has dissimilar dorsal (i.e., facing towards the stem) and ventral (i.e., facing away from the stem) sides.
- endodermis** Bundle-sheath consisting of cells with primary walls that are characterized by localized impregnation with lignin.
- ensiform leaves** Sword-shaped leaves; typically with a lamina that is flattened in the median plane.
- epidermis** One (or sometimes several) layers of tightly-connected cells that cover the surface of a plant organ. Originating from the primary meristem.
- epistomatous, epistomatic** Stomata are exclusively found on the adaxial side of the leaf.
- fusoid cells** Distinct, colorless cells located between the veins. The cells are fusiform or pyriform when viewed in transverse sections, but elongated in mediolateral and longitudinal directions and thus have more plate-like

shapes when viewed in three dimensions. There are typically large intercellular spaces between two consecutive fusoid cells.

fusiform Spindle-shaped; tapering towards each end.

heterobaric A leaf property indicating that a large fraction of the veins have bundle sheath-extensions that extend to the epidermis.

homobaric A leaf property indicating that no or only a few veins in the leaf have bundle-sheath extensions.

hyperstomatous, hyperstomatic See epistomatous.

hypostomatous, hypostomatic Stomata are exclusively found on the abaxial side of the leaf.

idioblast A single cell that is conspicuously different from the surrounding tissue.

isobilateral mesophyll See isolateral mesophyll.

isodiametric Having a nearly equal diameter in all directions.

isolateral mesophyll Mesophyll that has similar dorsal and ventral sides.

lateral Along the lateral (Y-X) plane; a plane through a body or organ parallel to the longitudinal and mediolateral axes. Divides the body in a ventral and dorsal part. Compare: paradermal.

longitudinal Proximal to distal, from base to tip.

medial Along the median (X-Z) plane; a plane through the middle of a body or organ, parallel to the longitudinal and dorsiventral axes. Divides the body in a left and right half.

medio-lateral From side to side (or from midrib to margin).

meristem Tissue consisting out of small, thin-walled living cells with typically a large nucleus and a small vacuole. Produces new cells by cell division.

mesophyte Terrestrial plants that are not specifically adapted to a very wet or very dry environment.

mesophyll Parenchyma tissues between the epidermis and vascular tissues of a leaf. Excluding the bundle-sheath and bundle-sheath extensions. If the mesophyll cells contain chloroplasts, it is also called chlorenchyma.

mestome sheath The inner sheath of a two-layered bundle sheath, with cells that are smaller in diameter and with thicker, cell walls impregnated with lignin and possibly suberin.

ontogenetic Related to the development of an organism or part of an organism.

palisade mesophyll Leaf mesophyll characterized by parenchyma cells that are elongated in a dorsiventral direction and have no obvious lobing or cell wall invaginations.

paradermal Along a plane parallel to the leaf surface, compare: lateral, medial.

paraveinal mesophyll One or more anatomically distinct layers of mesophyll tissue that consists of laterally stretched and laterally lobed parenchyma cells. Located between the minor veins of the leaf, typically between palisade and spongy paranchyma.

parenchyma Tissue characterized by living cells with thin, non-lignified cell walls. These cells are typically isodiametric and retain their ability to divide.

parenchymatous Related to parenchyma.

peltate Shield-like; a more or less flat and circular structure, with a stalk attached to the lower surface.

periclinal In a direction parallel to the surface of an organ or tissue.

plagiotropic Having a longer axis that is oblique to the vertical.

primordium An organ or tissue in its earliest recognizable stage of development.

proximal-distal See longitudinal.

pyriform Pear-shaped.

radial Arranged like the radii of a circle.

resupinate leaves Twisting of the petiole or lamina. The result is that tissue with an adaxial developmental origin becomes located facing away from the stem.

sclereid A type of sclerenchyma cell that is not very elongated (cf. fibers) and typically has many branched pits.

scleireidal cell See sclereid.

sclerenchyma Tissue characterized by dead cells that have thick, lignified secondary walls.

sclerophyllous Related to plants with thickened and hardened foliage as a result of the devel-

opment of large amounts of sclerenchyma in the leaves.

spongy mesophyll Leaf mesophyll characterized by parenchyma cells that are not elongated in a specific direction and are not closely packed.

substomatal cavity Cavity located proximal to the stoma, which is connected to intercellular air spaces.

terete leaf Tubular or cylindrical leaf.

tortuosity A measure for the geometric complexity of a porous medium. The ratio of the effective path between two points through a pore medium relative to the straight-line distance.

tortuosity factor (τ) Square of the tortuosity.

transverse Along the transverse (Y-Z) plane; a plane through a body parallel to the mediolateral and dorsiventral axes. Divides the body in a distal (e.g., towards the leaf apex) and proximal (e.g., towards the leaf base) segment.

unifacial leaf A leaf that develops mainly from either the adaxial or abaxial meristem, leading to an absence of cells derived from one of these meristems in the greater part of the mature leaf.

uniserial Arranged in a single file, row, or layer.

vascular tissue Plant tissue that consist of tracheary elements, sieve elements, companion cells, and associated fiber, parenchyma, and meristematic cells. Typically surrounded by a bundle sheath.

vasculature See vascular tissue.

xeromorphic With morphological features that are thought to be an adaptation to dry conditions.

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