

# Anisotropic Expansion of the Plant Cell Wall

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## Key Words

cellulose microfibrils, cortical microtubules, morphogenesis, elongation, radial expansion

## Abstract

Plants shape their organs with a precision demanded by optimal function; organ shaping requires control over cell wall expansion anisotropy. Focusing on multicellular organs, I survey the occurrence of expansion anisotropy and discuss its causes and proposed controls. Expansion anisotropy of a unit area of cell wall is characterized by the direction and degree of anisotropy. The direction of maximal expansion rate is usually regulated by the direction of net alignment among cellulose microfibrils, which overcomes the prevailing stress anisotropy. In some stems, the directionality of expansion of epidermal cells is controlled by that of the inner tissue. The degree of anisotropy can vary widely as a function of position and of treatment. The degree of anisotropy is probably controlled by factors in addition to the direction of microfibril alignment. I hypothesize that rates of expansion in maximal and minimal directions are regulated by distinct molecular mechanisms that regulate interactions between matrix and microfibrils.

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## INTRODUCTION

Plants shape their organs with a precision demanded by optimal function, from the thin, flat solar panels of leaves to the coiled grappling hooks of tendrils. Thompson (1917) realized that adaptive advantage is insufficient to explain form; he argued that additionally the process of construction plays a role. The only construction process used to shape plant organs is expansion of cell walls. The cell wall surrounds neighboring cells in a continuous sheet and stretching it requires a large force. These features preclude plants from

driving morphogenesis with cell migration or motility, processes used routinely by animals to build organs. The cell wall also seems to preclude plants from using programmed cell death in morphogenesis, as used, for example, to shape the human hand, because when a plant cell dies, programmatically or otherwise, the cell wall remains. The shape of a plant organ thus reflects the history of the expansion undergone by its cell walls.

When an area of cell wall expands at the same rate in all directions, expansion rate is isotropic, whereas when the rate in one direction differs from the rate in another, expansion rate is anisotropic. Integration of all the local expansion behavior throughout the growing regions gives the organ its shape and for this reason understanding the anisotropic expansion of the cell wall is pivotal for understanding plant development.

I review the patterns of anisotropic expansion that have been documented in plants and discuss the causes and controls of such expansion. My review focuses on multicellular organs. To my knowledge, anisotropic expansion per se has not been reviewed, but certain aspects are treated by Green (1980) and Taiz (1984). Cosgrove (1999) provides an authoritative treatment of cell wall yielding. Readers interested in understanding how anisotropic growth fits into the overall problem of morphogenesis will enjoy reading the paper by Coen and colleagues (2004). Finally, Harold (1990, 2002) masterfully explores shape generation in single cells of all kingdoms.

## Defining Terms

**Two-dimensional expansion of a unit area of cell wall.** This review focuses on a unit area of cell wall and its expansion. Cell walls are thin, and changes in thickness do not contribute directly to changes in cell or organ size; therefore, I treat cell wall expansion as a two-dimensional problem. Even though the third dimension (i.e., thickness) is of undoubted relevance to the behavior of the wall (e.g., Dumais et al. 2004), this simplification is

unavoidable because there are almost no data from which this dimension can be assessed. Except where noted, I refer to expansion in the maximal direction as elongation and in the minimal direction as radial.

**Strain rate.** This review focuses on anisotropy of the rates of expansion for a unit area of cell wall, and not on the anisotropy of shape. These expansion rates are best treated relatively because cell walls expand throughout their area, making the absolute amount of expansion proportional to area. Engineers refer to relative expansion rates as strain rates; botanists often refer to them as relative elemental expansion rates. For brevity, I use engineering nomenclature.

**Direction and degree of anisotropy.** For any material that expands anisotropically, fundamental laws of mechanics dictate that the direction in which the maximal rate occurs is perpendicular to the direction of the minimal rate (**Figure 1**). Therefore, anisotropy is characterized by two parameters: direction and degree. Direction specifies the direction in which the maximal strain rate occurs, and degree specifies the relationship between the maximal and minimal strain rates. A widespread convention for showing anisotropy diagrammatically is the so-called strain ellipse in which the major and minor axes represent the magnitudes of maximal and minimal expansion rate, respectively.

The orientation of the ellipse shows the direction and the ellipticity shows the degree. The greater the difference between maximal and minimal strain rates, the closer the ellipse is to a line and the greater the degree of anisotropy. Mathematically, there are various ways to represent the degree of anisotropy; the simplest of these is the ratio of maximal to minimal rates, as used here. There are more complex representations; for example, one may calculate the eccentricity of the ellipse or use the difference between maximal and minimal rates divided by the sum.

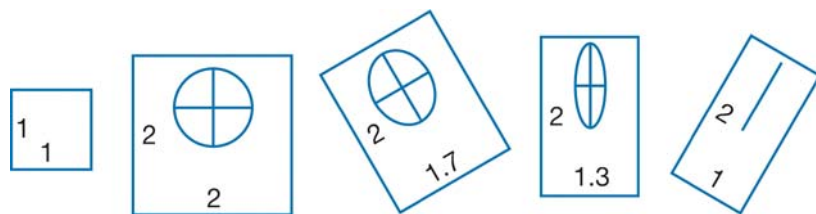
### Historical Foundation

The foundation for the modern understanding of anisotropic expansion was built on studies of the Brobdingnagian internodal cells of *Nitella*. Three influential results emerged from these studies. First, the degree of growth anisotropy is constant; the rate of elongation is invariably four to five times greater than the rate of radial expansion (Probine & Preston 1961, Green 1965). Second, the anisotropic expansion of the cell wall originates from its anisotropic mechanical construction, specifically in the deposition of aligned cellulose microfibrils (Probine & Preston 1961, 1962). Third, the ability of a cell to expand anisotropically requires an array of microtubules called cortical microtubules, which are beneath the plasma membrane and thought to determine the deposition direction of the microfibrils

**Anisotropy:** the state where a property differs as a function of direction, in contrast to isotropy where the property is the same in all directions

**Strain rate:** Strain is a relative deformation, typically defined as the ratio of final size to initial size; strain rate is the temporal rate change of this quantity.

**Microfibril:** the most pronounced structural unit of the cell wall; formed by the lateral noncovalent association of many  $1 \rightarrow 4$   $\beta$ -linked glucose chains.



**Figure 1**

A unit area of cell wall (*left-most box*) expands, doubling in length (*right-hand boxes*). The numbers denote the length and width of the cell wall area, and the ellipses represent the anisotropy, which can vary in direction and degree. The direction of anisotropy is defined by the angle formed by the major ellipse axis and a given reference and the degree of anisotropy is defined conveniently by the length ratio between the major and minor axes of the ellipse.

(Green 1962). These three results permeate all subsequent experiments but, as discussed below, need modification as applied to multicellular organs.

## QUANTIFICATION AND OCCURRENCE OF EXPANSION ANISOTROPY

Before considering the causes and controls of anisotropic expansion in multicellular organs, one needs to know what the patterns of anisotropic cellular expansion actually are. I describe these patterns next, starting with a description of how to measure them.

### Measuring Expansion Anisotropy

**Theory.** In essence, plant growth is the movement of water, and to a first approximation, is described appropriately via the formalism of continuum mechanics (Silk & Erickson 1979, Silk 1992). In a moving continuum, each element has a velocity, which is a vector quantity with a magnitude and direction. For a moving body, the set of velocities of all elements fully characterizes the movement, including growth. If the velocities among a group of elements are all the same, then that group is moving but not otherwise changing. But if the velocities diverge, then new material has to be added, and if the velocities converge, then material has to be removed; otherwise the continuum would fail. Diverging velocities mean growth, whereas converging velocities mean shrinkage. Shrinkage is rare in plants and is not considered further in this review.

Quantitatively, the divergence of the velocity field represents the strain rates within the material. The problem of finding those strain rates amounts to quantifying the rates at which elements within a material move and then differentiating along a given reference to obtain the divergence. One may use either a spatial or a temporal reference (Silk 1984). A spatial reference, sometimes called a Eulerian reference, characterizes the behavior of

the movement for a set of spatial coordinates; in contrast, a temporal reference, sometimes called a material or Lagrangian reference, follows one element as it moves over time. The difference can be appreciated from a waterfall: Euler would measure the velocity at which water droplets are moving at various places in the fall, whereas Lagrange would choose a drop at the top and measure its velocity at different times as it fell.

Two complexities arise: time-dependent behavior and organ geometry. Only if the pattern of movement is constant in time is there a straightforward mapping between time and position. Movements due to growth are three dimensional but methods for observing growth, such as photography, are inherently two dimensional. Furthermore, growth within a volume can be spatially heterogeneous, but usually only the surface is accessible. Thus the velocities at which elements inside the organ move are obscure. Both of these problems are illustrated by a leaf: A photographic record of the lamina over time could provide information about the velocity vectors in the plane of the leaf but not about those in the perpendicular plane. But even were an accompanying map of changes in leaf thickness to be obtained, it would not be possible to know whether the observed increase in thickness occurred uniformly among leaf tissues or reflected instead a thickness increase in only a single tissue (e.g., palisade mesophyll). To assess how the mechanical properties of the cell wall regulate expansion anisotropy, the measured rates of expansion must be related to specific cell walls (Liang et al. 1997). In the case of a leaf, measurements of the lamina could be coupled to structural data for the outer epidermal wall, but leaf thickness measurements could not be coupled to data for the anticlinal walls unless the spatial distribution of expansion in thickness were resolved.

**Practice.** Embodying the essence of most later approaches, often termed kinematic, a method for handling one-dimensional

velocity fields was worked out in the 1950s for the plant root (Silk 1992). The root (or other organ whose expansion in one direction is of interest) is photographed as it grows, and from the photographs, the trajectories of marks on the surface are plotted and used to calculate velocities. The marks may be exogenous, such as ink or beads, or endogenous, such as cross walls. The plot of velocity versus distance from a suitable reference, for example the root tip, is differentiated, and this derivative plot gives the strain rate as a function of distance from the reference point. This approach can be extended to two dimensions although the mathematics becomes more complicated (Silk 1984). Recently, researchers have developed software that can recover velocity fields from image sequences algorithmically without requiring marking (Schmundt et al. 1998, van der Weele et al. 2003), greatly increasing measurement resolution and processing speed compared to manual methods.

An alternative approach for characterizing the velocity field was developed by Hejnowicz & Romberger (1984). The approach, called the growth tensor, is based on the strain rate tensor, which Silk & Erickson (1979), using the tools of continuum mechanics, introduced in their treatment of plant growth. A line can move through a growing continuum and retain its orientation only if it is parallel to one of the three principal directions of expansion rate. In a median longitudinal section of a growing plant organ, many such lines are present in the form of periclinal cell walls forming cell files, maintained through the growth zone. Starting with the assumption that these periclinal cell wall lines are parallel to one of the principal growth directions (likewise, the associated cross walls are parallel to another of the principal directions), researchers derived a complete velocity field for root apices (Hejnowicz 1989). Although these calculated velocity fields reproduce the main features of apices qualitatively, the fit has not been assessed quantitatively, nor is it clear that the solution is unique, meaning that there may

be a family of velocity fields that can give rise to the observed cell files. Perhaps the growth-tensor approach could be enhanced if it were constrained by direct velocity data for the surface.

In the growth-tensor approach, cell boundaries are used to derive an overarching function for the entire growing object, but cell boundaries can also be used to infer the local field (Hejnowicz & Brodzki 1960, Silk et al. 1989). For example, a cell in the elongation zone of a root moves away from the tip at a speed proportional to its length divided by its length at maturity, provided that elongation rate is constant over time and there is no cell division in the elongation zone. The proportionality constant is the total root elongation rate. This approach is ideal for situations where the organ cannot be photographed over time, such as when a root is growing in compact soil, but suffers from the facts that cell lengths are extremely variable and that cell division or time-dependent changes invalidate the results.

Because cell boundaries are customarily viewed in sections, they are difficult to use for organs with complex geometry, which are also nearly impossible to photograph. However, cell boundaries can be revealed when a cell undergoes a mutation, such as in a pigment biosynthetic pathway, that alters the cell's appearance compared with that of its neighbors. If the cell is dividing, then its progeny are likewise marked. Groups of marked cells are called clones; these have been used for many years in studies of fate determination. The shape of a clone carries information about the directional growth experienced by that boundary. Recently Rolland-Lagan et al. (2005) developed a painstaking method to extract that information: By following the shapes of clones induced at different times, these authors constructed the growth history of the organ. Although the method yielded measurements with relatively low precision, it provided the first assessment of local expansion anisotropy for an inaccessible organ (Rolland-Lagan et al. 2003).

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**Stress:** the force on a body divided by the cross-sectional area across which the force acts.

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## Tip-Growing Cells

Pollen tubes, root hairs, and fungal hyphae, as well as apical cells in some filamentous algae and lower plants, grow by tip growth. In this mode, expansion is confined to one end of the cell, the tip, in contrast with diffuse growth in which growth occurs throughout one or more faces of the cell. For a single tip-growing cell, the stress distribution can be calculated from geometric considerations (Hejnowicz et al. 1977). Unfortunately, because shape and growth rate change steeply as a function of position within the tip, observations are prone to measurement error.

Over the past half century, anisotropic expansion rates have been quantified for a small number of tip-growing cells, including the young sporangiophore of the fungus, *Phycomyces blakesleeanus* (Castle 1958); the shoot and leaf apical cells (Green 1965) and rhizoid (Chen 1973) of the characean green alga, *Nitella*, as well as the rhizoid of *Chara* (Hejnowicz et al. 1977); the shoot apical cell of the xanthophycean green alga, *Vaucheria geminata* (Kataoka 1982); and the root hair of the angiosperm, alfalfa (Dumais et al. 2004). Despite this taxonomic diversity and the somewhat different approaches used for calculations, a key regularity emerges: Near the apex, expansion is isotropic, but within the basal part of the growth zone, expansion rate in circumference is greater than the longitudinal rate.

Taking advantage of the well-understood mechanical behavior of a thin-walled, pressurized shell with rotational symmetry, Dumais and colleagues (2004) calculated the distribution of longitudinal and circumferential stresses as a function of position in the dome. Then, they modeled how those anisotropic stresses would deform the cell wall under three alternative cell wall reinforcements: complete isotropy (uniform material in three dimensions), transverse isotropy (a wall made in layers but with each layer isotropic), and full anisotropy (e.g., a wall with aligned microfibrils). A wall with full anisotropy matches the

observed expansion anisotropy, but so does a wall with transverse anisotropy, which indicates that directional wall reinforcement is not required to generate anisotropic expansion patterns in a tip-growing cell; instead, the anisotropic distribution of stresses suffices. Consistently, cell wall layers at the tips of tip-growing cells appear to be transversely isotropic when analyzed structurally (references in Dumais et al. 2004). Thus, tip growth strikingly contrasts diffuse growth, for which the degree of anisotropy is usually high, the cell wall layers are anisotropic structurally, and the maximal stress is probably parallel to the minimal expansion rate.

## Multicellular Cylindrical Organs: Stems and Roots

Cylindrical organs, such as stems and roots, are figures of revolution and thus share the rotational symmetry of single cells. I note in passing that whereas roots generally are circular in cross section, stems frequently have more complicated shapes, indicating that expansion around the stem circumference is not uniform. To my knowledge, the only attempt to consider this has been a theoretical derivation of growth fields required to sustain the helical form of twining vines (Silk 1989).

Only a handful of papers have measured, at an elemental level, expansion anisotropy of stems and roots, despite their relatively straightforward geometry. Silk & Abou Haidar (1986) measured longitudinal and circumferential strain for the stem of morning glory (*Pharbitis nil*). Strain rates in each direction are roughly constant through the growth zone, indicating that the degree of anisotropy is constant and the longitudinal rate exceeds the tangential rate by approximately a factor of two. The long growth zone (more than 10 cm), small strain rates ( $\sim 2\% \text{ h}^{-1}$ ), and twining habit all hindered the analysis; finer scale patterns may have been missed. On the other hand, Cavalieri & Boyer (1982) found that when the dark-grown soybean hypocotyl acclimates to water deficit, elongation and

radial expansion vary independently throughout the growth zone, although the authors did not calculate strain rates in this study.

The sedate strain rates of the morning glory stem contrast with those of the root, where maximal longitudinal strain rates can reach  $50\% \text{ h}^{-1}$ . Data from roots confirm that longitudinal and radial strain rates can vary independently. This can be inferred from cell-length data for the maize root swelling under the influence of a microtubule inhibitor (Bystrova 1984), although rates were not quantified explicitly. Likewise, tomato roots, both wild type and gibberellin deficient, have bell-shaped spatial profiles of elongation and circumferential strain rates that peak at different locations (Barlow et al. 1991).

Expansion rates in length and width have been thoroughly quantified for maize roots growing at low water potential (Liang et al. 1997). The roots of many species, when exposed to water deficit in a substrate that avoids compaction, acclimate by thinning. In maize roots, circumferential strain in the stele and cortex and radial strain in the cortex vary independently of longitudinal strain. In particular, water stress decreases longitudinal strain in the basal half of the growth zone while leaving radial and circumferential strain rates unaffected, but in the apical half, water stress inhibits radial and circumferential strain rates without affecting the longitudinal rate. The degree of anisotropy varies from 3 to more than 30. Similarly, longitudinal and circumferential strain rates vary independently in arabidopsis roots exposed to low concentrations of the microtubule inhibitor oryzalin (Baskin et al. 2004). These roots become thicker, and because the oryzalin concentration is low enough to permit cell division to continue, they grow at approximately steady state.

In sum, whereas the morning glory stem grows with a constant degree of anisotropy in accord with the classic picture from *Nitella*, the soybean hypocotyl, as well as the maize and arabidopsis roots, grow with a variable degree of expansion anisotropy. This suggests

that distinct mechanisms exist to control expansion rates in different directions.

### Multicellular Lamellar Organs: Leaves and Petals

Although leaves are found in many shapes and sizes, there have been few attempts to quantify the anisotropy of local expansion rates. In contrast, leaf shape has long been characterized allometrically (Tsukaya 2003), with logarithmic plots of length and width during development often remaining constant (e.g., Haber & Foard 1963). For *Nitella* internodal cells, the linearity of logarithmic plots of cell length versus width allows one to conclude that the cell wall expands with a constant degree of anisotropy, but a log-linear relation for the length and width of leaves allows no similar conclusion. This is because the leaf has thousands of growing cells in each dimension and the allometric relation pertains to the integrated output of them all. Allometric constancy requires only that the total growth in each direction stay steady in time (or change in both directions proportionally) and does not preclude cell walls at different leaf regions from expanding with various degrees of anisotropy. Indeed, anisotropic leaf shape can arise from purely isotropic local expansion rates when the magnitude of area expansion rate differs in different regions of the leaf (Green 1965).

Perhaps the earliest quantification of expansion anisotropy of any material was made for the tobacco leaf (Richards & Kavanagh 1943); later, similar measurements were made for cocklebur (*Xanthium pennsylvanicum*) (Erickson 1966). Both data sets show that expansion in the lamellar plane of these broad-leaf plants is essentially isotropic. There are small changes in the degree of anisotropy, but these may reflect measurement errors, although a few marginal regions appear to have significant expansion anisotropy. These photographic records were made rather late in development when the leaf could be reasonably flat for photography, so earlier patterns

are unknown. To my knowledge, spatial profiles of thickness have never been mapped for the dicot leaf. Plots of average thickness over time for the cocklebur leaf suggest that growth in thickness follows a trajectory that is independent of growth in area (Maksymowych 1990), but spatially resolved measurements are required before conclusions can be extended to the level of cell wall expansion anisotropy.

Despite major interest in the growth of grass leaves, there is apparently only one published characterization of local expansion anisotropy. Maurice et al. (1997) combined spatial and material methods to quantify the spatial profiles of strain in length, width, and thickness for tall fescue (*Festuca arundinacea*). Leaves continue to thicken for several centimeters past the terminus of the elongation zone; this behavior may relate to the differentiation of epidermal cell types or to de novo cell thickening (Macadam & Nelson 2002). In the elongation zone, longitudinal strain has a bell-shaped profile, typical of grass leaves, which remains steady over time with a peak ( $6\% \text{ h}^{-1}$ ) about one third of the way through the growth zone. In contrast, strain rates in width and thickness are maximal ( $6\% \text{ h}^{-1}$ ) at the very base of the growth zone, decrease steeply with position, and change over time. Although strain rates in width and thickness were obtained for the entire leaf width or thickness and hence do not apply directly at the cellular scale, it is clear that cell walls in the grass leaf expand with considerable anisotropy, where the degree and even sign change during development.

In the papers above on leaves, researchers used material that is far past the primordium stage because of the requirements for marking and of photography. But many of the growth transformations required to bring about the complex forms of plant organs presumably happen soon after the primordium bulges up and away from the shoot meristem. Recently, Rolland-Lagan et al. (2003, 2005) used clonal analysis to analyze the anisotropic expansion in the laminar plane of the snap-

dragon flower's dorsal petal. Like the dicot leaf, expansion in the petal lamina is only slightly anisotropic and variations in the degree of anisotropy in different regions of the petal were not large enough to be resolved. Interestingly, the direction of maximal expansion rate did change in a programmatic way, which the authors argued is essential for the development of the petal's shape.

### **Multicellular Organs: Shoot Apical Meristem**

Attempts to characterize the expansion of the shoot apical meristem (Kwiatkowska 2004) either have been highly theoretical (e.g., Nakielski 1987, Hejnowicz et al. 1988) or have failed to account for the curvature of the surface, which limits their precision (Hernández et al. 1991). In a tour de force, Kwiatkowska & Dumais (2003) quantified the expansion behavior of the surface of the shoot apical meristem (including early primordia) of *Anagallis arvensis*. This was accomplished by viewing successive replicas of the same meristem in the scanning electron microscope at two angles and then calculating the elevations of points on the surface (Dumais & Kwiatkowska 2001). As may be expected for a meristem initiating leaves with spiral phyllotaxis, expansion patterns are complex. Cell walls at the center of the dome expand more or less isotropically as do cells at the distal tips of primordia, but cell walls at other locations can expand with considerable anisotropy, especially walls at the meristem flank or those forming an axil. These data for the meristem further confirm that plants readily modify both the direction and the degree of cell wall expansion anisotropy.

### **CAUSES AND CONTROLS OF ANISOTROPIC EXPANSION RATES**

A unit area of material may expand anisotropically because of two (not exclusive) causes: It may be acted on by forces whose distribution



is anisotropic, or its ability to resist force may be anisotropic (Harold 2002).

### Causes of Growth Anisotropy: Force and Resistance

**Force.** The forces acting on the plant cell wall originate from hydrostatic pressure, which is strictly isotropic. The force must act over a cross section of cell wall, thus creating a stress. The shape of the cell impinges on the distribution of stresses so that, for example in a single cylindrical cell, the stress acting circumferentially is twice that acting longitudinally (Probine & Preston 1961). Given that single cylindrical cells expand faster longitudinally than radially, the resistance of the cell wall to stress must be anisotropic.

However, even for a single cell with an anisotropic cell wall, the distribution of stresses and its relation to wall reinforcement is complex and can give rise to unexpected behavior, such as helical twisting (Sellen 1983). For a cell in a tissue, and especially for growing tissues, the theoretical problems are formidable (Bruce 2003). A major complexity arises for shoots from the thick inextensible epidermal layer, which sheathes more compliant inner tissue (Hejnowicz & Sievers 1996a, Nicklas & Paolillo 1998). The epidermis and inner layers are connected so that forces generated in one may be borne by the other. This force-sharing is called tissue tension (Hejnowicz & Sievers 1995a, Pas-sioura & Boyer 2003).

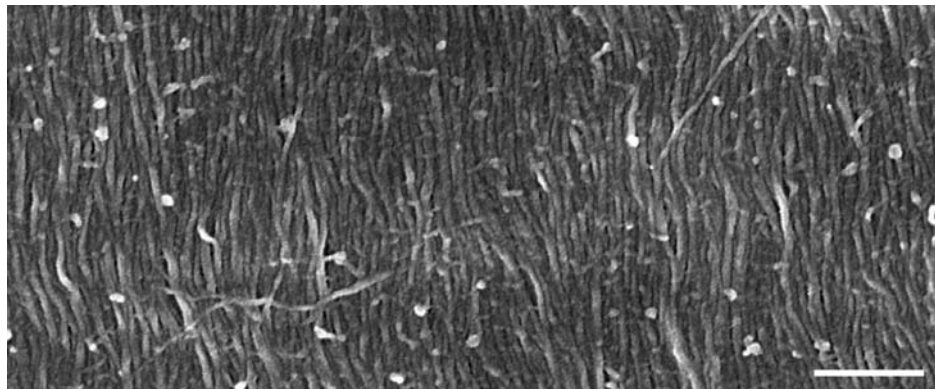
In the seedling stems of sunflower and tulip, which are subject to tissue tensions, the longitudinal stress was reported to be larger than the transverse stress by threefold for the inner tissue and by sixfold for the epidermis (Hejnowicz & Sievers 1995b, Hejnowicz et al. 2000). The direction of this anisotropy is opposite to the classic twofold excess of transverse stress for single, cylindrical cells and implies that anisotropic expansion rates in a stem could occur without anisotropic reinforcement of the cell wall. This surprising stress anisotropy was discovered by means of

measuring the mechanical properties of mechanically isolated inner and outer tissue layers; excising tissue alters hydraulic relations and may confound analysis (Peters & Tomos 2000). Furthermore, when stems are treated so as to remove or reduce the anisotropic mechanical reinforcement of the cell wall, they often expand faster radially than longitudinally. For example, in maize coleoptiles, colchicine treatment converts a sixfold strain rate anisotropy favoring length to a fourfold anisotropy favoring girth (Schopfer 2000), a result hardly in accord with a longitudinally dominant stress anisotropy and underscoring the importance of mechanical resistance of the cell walls.

An unexpected stress anisotropy has also been reported for the flattened dome of the sunflower capitulum, only in this case the model agreed with the qualitative stress distribution obtained experimentally from the gapping of cuts made on the surface (Dumais & Steele 2000). It would be worthwhile to obtain directly the stress distribution for growing stems.

**Resistance.** For multicellular organs, the mechanical anisotropy of the cell wall, as compared with the anisotropy of stress, is well described. Principally, cell walls are reinforced anisotropically by cellulose microfibrils (Brett 2000), which are made from long polymers of 1 → 4, β-linked glucose that associate laterally to form a partly crystalline lattice (Doblin et al. 2002). The word microfibril applies to any group of glucose chains, whether it be the “elementary” microfibril whose number of chains is a matter of debate or to higher-order assemblies of already crystalline domains. Neighboring microfibrils tend to be roughly parallel, giving the cell wall a mat-like appearance and a distinct structural anisotropy (**Figure 2**).

Microfibril alignment is sometimes equated to resistance. However, resistance emerges from the way in which microfibrils interact with each other and with other wall components; all these interactions sum



**Figure 2**

Micrograph of the innermost cell wall layer of a cucumber hypocotyl imaged with field-emission scanning electron microscopy (Marga et al. 2005). The long axis of the stem runs left to right. The cell wall is structurally anisotropic. Scale bar = 250 nm.

**Polarized-light microscopy:**

an optical technique whereby contrast is generated from molecular alignment, as in crystals. Modes can use absorption, fluorescence, or phase. Useful for cell walls because of the crystallinity of microfibrils.

to give the resistance of the wall to stress. Maximal resistance in a composite material with anisotropic fibrillar reinforcement is predictably parallel to the long axis of the fibers (Sellen 1983); accordingly, the alignment of microfibrils commonly does reflect the direction of strain rate anisotropy. However, the magnitude of the resistances and hence the degree of anisotropy depend on the interactions between microfibrils.

**Controls on Growth Anisotropy: Microfibril Synthesis**

Cellulose microfibrils are probably necessary for anisotropic expansion to take place. Several herbicides target the synthesis of cellulose and reduce the anisotropy of expansion (Sabba & Vaughn 1999, Scheible et al. 2003). Similarly, numerous mutants that either directly or indirectly reduce the rate of cellulose synthesis are known, and organ growth in these mutants is invariably less anisotropic (Robert et al. 2004). Cellulose synthesis continues, albeit at a lower rate, in these treatments and mutants, and the new microfibrils are poorly aligned in some cases (Wasteneys 2004) but not in others (Refrégier et al. 2004). Surprisingly, several laboratories have selected tissue culture cell lines that grow well despite hav-

ing scant microfibrillar cellulose (Shedletzky et al. 1992, Sabba et al. 1999); also, pollen tubes have little if any cellulose, particularly at the tip (Ferguson et al. 1998). However, these cellulose-deficient cells grow isotropically: The herbicide-adapted cultures grow as cell clumps without a major axis of expansion, and any anisotropy in the expansion of the pollen tube tip, as in root hairs, probably occurs without mechanical anisotropy within the plane of the cell wall (Dumais et al. 2004).

**Controls on Growth Anisotropy: The Direction of Anisotropy**

**The paradoxical behavior of the stem epidermis.** For multicellular organs, the direction of maximal expansion rate is generally perpendicular to the net orientation among microfibrils. This generalization applies to microfibril order throughout the cell wall, as assayed with polarized-light microscopy, and has been reported widely (Roelofsen 1965). Recent examples include roots (Green 1984, Baskin et al. 1999), rice internodal parenchyma (Sauter et al. 1993), grass leaves for both cortical parenchyma and epidermis (Hogetsu 1989, Paolillo 1995), and a variety of

expansion planes and tissues within the shoot apical meristem (Green 1988, Sakaguchi et al. 1990). Additionally, the outer epidermis in dicot leaves is isotropic in both microfibril alignment and expansion (Hogetsu 1989, Kerstens et al. 2001). The generalization has been strengthened experimentally by finding that, when the direction of maximal expansion changes from longitudinal to radial, the net orientation of microfibrils in parenchyma also changes (e.g., pea epicotyls exposed to hormones) (Probine 1965, Veen 1970, Ridge 1973). However, as noted by Roelofsen (1965) and recently confirmed by others for many species (Iwata & Hogetsu 1989, Paolillo 2000, Verbelen & Karstens 2000), in stems (and coleoptiles) the net alignment of microfibrils in the epidermis is longitudinal, even in rapidly elongating regions. Note that an exception is the rice coleoptile, which has transverse microfibrils throughout its epidermis (Paolillo 2000).

Microfibril alignment is also widely assessed with electron microscopy. Cell walls viewed in cross sections often have many layers of microfibrils, taking on complex three-dimensional patterns (Roland et al. 1987). Furthermore, cellulose in conventional ultrathin sections is erratically stained by heavy metals, and researchers have resorted to extraction regimes to enhance microfibrillar contrast (Roland et al. 1982). However, extraction even with relatively gentle reagents can disorganize microfibrils (Crow & Murphy 2000). For these reasons, assessing the direction of microfibril alignment in electron microscopy is problematic.

A partial solution is offered by the innermost cell wall layer. This layer can be imaged reliably with scanning or transmission electron microscope images of the cell wall surface and is the layer of microfibrils whose orientation is directly controlled by the cell. Some researchers have further justified examining the innermost wall layer by arguing that the innermost layer in a multicellular organ bears most of the load. This argument is based on results from *Nitella* indicating that

only the inner quarter of the wall is load bearing (Richmond et al. 1980) but this extrapolation is probably invalid (Baskin et al. 1999, Hejnowicz & Borowska-Wykręt 2005).

Studies on the innermost cell wall layer support the generalization that microfibrils are perpendicular to the direction of maximal expansion, for example, in cortical parenchyma of roots (Hogetsu 1986, Baskin et al. 1999), bamboo shoots (Crow & Murphy 2000), and the cambium of a conifer (Abe et al. 1995). In elongating stems with polylamellate cell walls, inner microfibril orientation can be longitudinal, transverse, or oblique, but it is transverse in a majority of parenchymatous cells (Takeda & Shibaoka 1981a,b, Iwata & Hogetsu 1989).

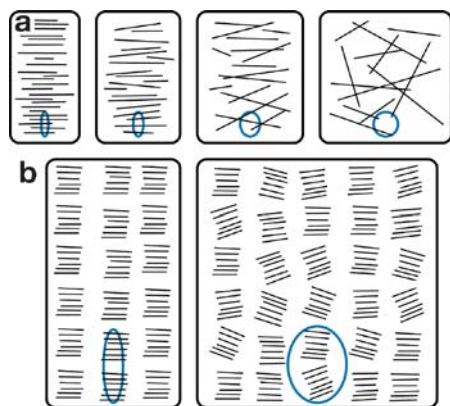
Again, electron microscopy confirms that stem (and coleoptile) epidermis is exceptional. Microfibrils of the innermost epidermal wall in lettuce hypocotyls elongating anisotropically are consistently longitudinal (Sawhney & Srivastava 1975), as in epidermis of intact, elongating maize coleoptiles (Bergfeld et al. 1988). Furthermore, microfibril alignment in the innermost layer of the azuki bean epidermal cell wall is predominantly transverse only in short, apical cells, whereas in longer but still anisotropically elongating cells, alignments among cells are mixed between transverse, oblique, and longitudinal, mirroring alignments in cortical parenchyma of epicotyls treated to induce more or less isotropic expansion (Takeda & Shibaoka 1981a,b).

If microfibril alignment determines the directionality of expansion, then why do stem epidermal cells not swell? The most likely answer is that epidermal cells in stems require tissue tensions to expand. Apparently, epidermal cell turgor is weaker than the stiffness of the outer cell wall and cannot drive expansion without help from the inner tissues (e.g., Hejnowicz & Sievers 1996b). That epidermal reinforcement direction can be overridden is supported by findings that developmental swelling in onion bulbs occurs despite transverse epidermal microtubules

(Mita & Shibaoka 1983) and microfibrils (Verbelen & Kerstens 2000). If the coherent anisotropic mechanical reinforcement in cortical parenchyma transmits to the epidermis a large axial force but a small transverse force, then this could be sufficient to determine the yielding behavior of the epidermis. The possibility that the inner tissue plays a dominant role in regulating the directionality of organ expansion suggests that this neglected tissue requires further study.

### Controls on Growth Anisotropy: The Degree of Anisotropy

**Green's degree of alignment hypothesis.** Green (1964) noticed that *Nitella* internodal cell walls have better-aligned microfibrils than those of another, green algal species, which expand less anisotropically. He hypothesized that the degree of expansion anisotropy is proportional to the degree of alignment among microfibrils (**Figure 3a**).



**Figure 3**

Models for controlling the degree of anisotropy by microfibril alignment. The degree of anisotropy is shown by the blue ellipses. (a) Green's (1964) hypothesis, where the degree of anisotropy is proportional to the degree of local alignment among microfibrils. (b) Modified hypothesis for a tissue (black rectangle with rounded corners) in which the degree of anisotropy is proportional to the uniformity of alignment in different cells or cell regions (cells are not shown). Figure redrawn from Baskin et al. (2004).

Green's hypothesis is economical because it allows the microfibrils to control the direction of anisotropy by means of their net orientation and the degree of anisotropy by means of how well they are aligned.

To test this hypothesis, my collaborators and I quantified the alignment of microfibrils throughout the cell wall with polarized-light microscopy and on the innermost layer with surface views for the maize root growing more anisotropically in response to water stress (Baskin et al. 1999) as well as for the arabidopsis root growing less anisotropically in response to low doses of a microtubule inhibitor (Baskin et al. 2004). In both systems, we found that, contrary to Green's hypothesis, the degree of orientation among microfibrils is uncorrelated to the degree of anisotropic expansion experienced by the wall. However, the imaging method used for arabidopsis allowed the angle of net orientation of each wall area's microfibrils to be measured, and this revealed that, where the degree of expansion anisotropy is less, the alignment among neighboring cell walls is less uniform. Recently, polarized-light microscopy revealed that a similar patchiness in microfibril alignment accompanies a decreased degree of expansion anisotropy in the root of the arabidopsis *cobra-1* mutant (Roudier et al. 2005). Green's hypothesis could be revised by invoking microfibrillar alignment among cells (global order) rather than among individual microfibrils (local order) in the regulation of the degree of expansion anisotropy (**Figure 3b**).

In this view, a uniform microfibril alignment in cells across a tissue promotes a high degree of expansion anisotropy, whereas the tissue expands less anisotropically when the mechanical reinforcement within the tissue becomes patchy, even though local regions of cell wall contain well-aligned cellulose. This view is consistent with experiments where the induction of more or less isotropic expansion is associated with organs losing a preferential alignment direction among constituent cells but with each cell having perfectly

well-aligned microfibrils (Itoh 1976, Takeda & Shibaoka 1981b, Iwata & Hogetsu 1989). It is also consistent with models in which the loss of uniform reinforcement around the periphery of the shoot apical meristem is associated with bulge formation to begin a leaf primordium (Green 1988).

**Generalizing Green's hypothesis.** The economy of invoking microfibril alignments to explain both the direction and the degree of anisotropy is no guarantee of validity. The uniformity of microfibril alignment may be one mechanism among many that the plant uses to regulate radial expansion. Several lines of evidence suggest that microfibril alignment, although necessary, is insufficient to explain strain rate anisotropy. Studies have shown that several root morphology mutants of *Arabidopsis* have stimulated radial expansion without any worsening of the alignment among microfibrils (Wiedemeier et al. 2002, Sugimoto et al. 2003) and that no change in microfibril alignment occurs to explain the increased strain rate anisotropy of maize roots growing at low water potential (Baskin et al. 1999), although none of these studies checked explicitly for patchiness of reinforcement direction among cells. In *Nitella*, Richmond et al. (1980), who prepared cell wall ghosts from growing cells and pressurized them to mimic turgor-driven expansion, found that the ghosts expanded more anisotropically than did the living cells, an observation that implies there is more to anisotropic yielding than the structural disposition of the cell wall.

I propose as a general hypothesis that expansion rates in length and width are regulated by independent molecular mechanisms. A similar proposal has been made for leaves (Tsuge et al. 1996, Tsukaya 2003), although it was based on leaf allometry rather than cellular expansion rates. Recently, Hejnowicz and Borowska-Wykręt (2005) obtained evidence that the longitudinal stress in stems is borne principally by the outer layers of the outer epidermal cell wall, and they proposed that the

outer layers of the cell wall limit elongation while the inner layers of the wall limit radial expansion.

Whether controls on expansion in different directions reside in different layers of the cell wall, in different tissues, or within the same cell wall, reactions needed to shear microfibrils parallel to their lengths can be expected to differ from reactions needed to separate them. Elongation rate has been modeled as depending on the tension developed in a system of xyloglucan tethers between parallel microfibrils (Passioura 1994, Veytsman & Cosgrove 1998), and experimental evidence suggests that elongation involves the separation of parallel microfibrils (Marga et al. 2005); however, in principle, longitudinally taut tethers weakly resist shear between microfibrils. Biochemical influences on transverse expansion do differ from those on longitudinal expansion in *Nitella* cell walls assayed *in vitro*—for example, the threshold for acid-induced deformation is more than a whole pH unit more acidic for radial expansion compared with elongation (Métraux & Taiz 1979, Richmond et al. 1980)—but few comparable data exist for higher plants. A more explicit hypothesis must await improved understanding of the molecular interactions among cell wall polymers and the development of engineering models that handle anisotropic deformation.

### Controls on Growth Anisotropy: Microtubules

That microtubules participate in the regulation of anisotropic expansion is indisputable. Whether microtubules are inhibited chemically (Vaughn & Lehen 1991) or genetically (Wasteneys 2004), growing organs become swollen, indicating the loss of anisotropy. This has been interpreted as indicating that microtubules are required to control microfibril alignment (Green 1980, Baskin 2001). In the absence of microtubules, expansion tends toward isotropy and cellulose alignment often becomes more isotropic in the statistical sense

(averaged among cells in a tissue or through several cell wall layers or regions) (Takeda & Shibaoka 1981b, Iwata & Hogetsu 1989, Hogetsu 1989, Inada et al. 2002, Sugimoto et al. 2003).

This interpretation has been questioned pointedly on the basis of studies of the *mor1* mutant, which has disrupted cortical microtubules, reduced expansion anisotropy, and well-aligned microfibrils (Himmelspach et al. 2003, Sugimoto et al. 2003). The *mor1* mutant retains some cortical microtubules, which may be sufficient to align microfibrils insofar as a depleted cortical array is sufficient to align microfibrils and prevent swelling in other cell types (Baskin et al. 1994, Inada et al. 2002). But because the *MOR1* gene encodes a microtubule-associated protein, the swelling in the mutant, despite aligned microfibrils, plausibly results from the loss of some microtubule function other than that of aligning cellulose. Wasteneys (2004) has suggested the microtubules govern the effective functional length of the microfibril: When microtubules are disrupted, were the average microfibril length to decrease, then adjacent microfibrils would become easier to shear and radial expansion would be stimulated. This mechanism offers a straightforward way for the cell to regulate rates of radial expansion independently of elongation, as hypothesized above.

## SUMMARY AND PERSPECTIVES FOR FUTURE RESEARCH

Anisotropic expansion in plant cells depends on the alignment among cellulose microfibrils, but this dependence is incomplete. In general, in multicellular organs, expansion rates in maximal and minimal directions change independently, and a means for controlling expansion rate in the minimal direction needs to be discovered. Cortical microtubules may be part of a cellular mechanism that conditions cell wall resistance in the direction parallel to the microfibrils. This role could be in addition to that of specifying the direction of anisotropy by aligning microfibrils. Knowledge is limited as there have been few attempts to quantify strain rate anisotropy at a cell wall level, even in studies where cell wall or cellular ultrastructure is examined. Future research should involve testing the biochemical properties of radial expansion, as compared with those of elongation, and developing a mechanical analysis of stress and compliance anisotropy in growing, multicellular plant organs. Then we can begin to comprehend how the shape of the organ and its cells, the movement of water between cells, the architecture of the constituent cell walls, and the biochemical reactions modifying those cell walls are all united to generate the predictable and beautiful forms of higher plant organs.

### SUMMARY POINTS

1. The expansion anisotropy of a unit area of cell wall is characterized by the direction and degree of anisotropy; the plant must control both direction and degree to build organs with specific and heritable shapes.
2. The anisotropy of expansion rate throughout a growth zone has been quantified for only a few multicellular organs.
3. In multicellular organs, the degree of anisotropy can vary widely across a growth zone and as a function of treatment.
4. The direction of maximal expansion rate is usually regulated by the direction of net alignment among cellulose microfibrils.

5. In stems, the directionality of expansion of epidermal cells is apparently controlled by that of the inner tissue.
6. The degree of anisotropy is probably controlled by factors in addition to the direction of microfibril alignment.
7. The rates of expansion in maximal and minimal directions are hypothesized to be regulated by distinct molecular mechanisms.

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This is a readable and authoritative summary of mathematical models for cell wall biomechanics, including growing tissue.

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This is a clear and cogent essay on the interplay between molecular biology and mechanics.

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This study explains the observed anisotropic growth field of the root hair in terms of the stresses and mechanical properties of the cell wall.

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This is perhaps the only study for a growing organ where the calculated stress distribution (itself a challenge) was confirmed experimentally.

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This is a Michelin Red Book for cellular morphogenesis.

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These three papers (Hejnowicz & Sievers 1995b, 1996a,b) represent a pioneering theoretical and experimental analysis of the anisotropy of stress and compliance for a growing stem.

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This study quantifies the local expansion rates of a grass leaf in all three dimensions.

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This is an essential read for anyone who thinks the epidermis “controls” growth.

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