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Leaves as Shell Structures: Double Curvature, Auto-Stresses, and Minimal Mechanical Energy Constraints on Leaf Rolling in Grasses

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ABSTRACT

Grass leaves are natural examples of shell structures because they are thin and display a double curvature. An important mechanical property of shells is that changes in longitudinal and transverse curvatures are not independent. The basis of this mechanical coupling is presented using simple diagrams. The relevance of the structural constraints for the processes of hydronastic rolling and developmental unrolling in grass leaves is then reviewed. I show that mechanical constraints can explain a large part of the genetic and developmental variability of hydronastic rolling in grasses, without reference to specific anatomic features such as bulliform cells. Mechanical analysis of a rolled maize mutant also revealed that developmental unrolling is not limited to a pure transverse expansion of hinge cells and involves both longitudinal and transverse dimensional changes in the upper epidermis. Interest in using mechanical models as a tool to reveal structural interactions at the tissue and organ level is discussed, and the importance of Paul Green's biophysical approach to the study of plant morphogenesis is emphasized.

Key words: Biomechanics; Biophysics; Leaf rolling; Grasses; Poacae.

INTRODUCTION

Paul Green had a long history of studying the involvement of structural mechanics in plant morphogenesis. As a young scientist, Green, Lockhart, and others belonged to the second generation of plant scientists who, in the 60s and 70s, revisited the connection of internal mechanical forces and turgor in the process of cell expansion (for example, Green and others 1971) and pioneered the use of quantitative physical modeling as an analytical tool in the growth process (see Green 1999). Equipped with such tools, they envisioned the role of cell shape, cell wall ultrastructure assembly and biorheology, and the role of other regulations involved in the process of growth and anisotropy control. Another point was also crucial. The stress field involved in cell wall expansion is not related to any external load but to an internal mechanical interaction between the liquid phase and the solid phase (turgor). When an isolated cell is considered, turgor and cell wall

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stresses in the cell are equilibrated, and no external force is required to balance their action. Similar internally balanced stresses also exist in organs (tissue tensions, see for example Hejnowicz and Sievers 1995; Kutchera 1989). These stresses arise because the internal structure and physical states of the system are not compatible. You cannot break the system into pieces and fit them back together. Some compression or tension of parts is required to rebuild the original structure. In mechanics, this is called an auto-stress field (Moulia 1993).

Auto-stress fields are an important matter for biomechanics, and an example will be presented in this article. The larger context is evident from Paul Green's work on the biophysics of pattern formation and morphogenesis in plants. Green was aware of the importance of auto-stress fields in biomechanics and also of the kinematic description of growth (Erickson and Sax 1956 a,b; Silk and Erickson 1979, see Silk 1984 for a review). His hypothesis was that the expanding tunica (which can be considered as a plate or as a shell) is constrained in its growth by its boundaries, thus generating an auto-stress field. Given the general principle of minimal total mechanical energy that rules the equilibrium shape of any physical body, a bifurcation in the minimal energy configurations (buckling) may occur. This bifurcation brings a rupture of the initial symmetry and thus leads to pattern formation, that is, to a shell with a complex curvature landscape (bulging). Some emphasis should be given to the fact that because energy is a global, integral quantity, it is the overall system that is involved in the bifurcation, and not particular cells (Green 1994, 1996, 1999). This gives an alternative view to the role of cells in morphogenesis and brings a novel perspective on genetic control of shape, based on a differential-integral model embodying structural constraints and auto-stress fields. In this article my goal is to give another example of auto-stress and minimal energy involvement. Energy considerations have significance for the cell versus organ dialectic and for genetic variation in shape in another context, namely, the process of leaf rolling and unrolling in grass leaves.

LEAF ROLLING AND UNROLLING IN GRASS LEAVES

Gramineous leaves have a slender flattened lamina (also called the leaf blade). In many species the young leaf is transversely rolled, with an adaxial concavity (involuted ptyxis, Ellis 1976; Shield 1951). As the blade emerges from the enclosing sheaths (Figure 1), unrolling occurs, starting from the leaf tip. In the primary leaves of wheat, this unrolling is a light-induced growth process, involving phytochrome (at least) as the photoreceptor (Virgin 1990). However, the processes linking phytochromic perception to unrolling remained unclear.

Figure 1. A young maize plant illustrating the general morphology of a grass shoot and the process of developmental unrolling (modified from Ledent and others 1990). The grass shoot has a very short stem axis such that the node-bearing leaves are very close together. Each leaf is composed of a tubular part, called the leaf sheath, which surrounds the next youngest leaf, and a flattened lamina called the leaf blade. The intercallary meristem responsible for leaf expansion sits at the base of the leaf (and is thus hidden in the enclosing sheaths). The lamina is initially rolled and progressively unrolls while emerging from the previous leaf sheaths (see the three shaded upper youngest leaves).

In mature unfolded leaves, water stress induces an hydronastic transverse rolling of the blade (Hsiao and others 1984; Shield 1951; Sobrado 1987). Several authors have argued that leaf rolling has adaptive value, reducing light interception, transpiration, and protecting the leaf from dehydration and overheating. It has been demonstrated that this rolling results from a differential top-bottom elastic shrinkage in the leaf cross section. Indeed, water stress rolling can be fully reversed through rehydration of the leaf and restoration of leaf turgor, provided that no changes in cell osmolarity occur during the process (Moulia 1994).

Interestingly, both leaf hydronastic rolling and leaf developmental unrolling show significant genetic variability in grasses (Shield 1951). Ecophysiologists quantify this variability by characterizing the response-curve of individual blades to leaf water potential (the internal state of the water in the leaf) or more precisely to turgor (Hsiao and others 1984;

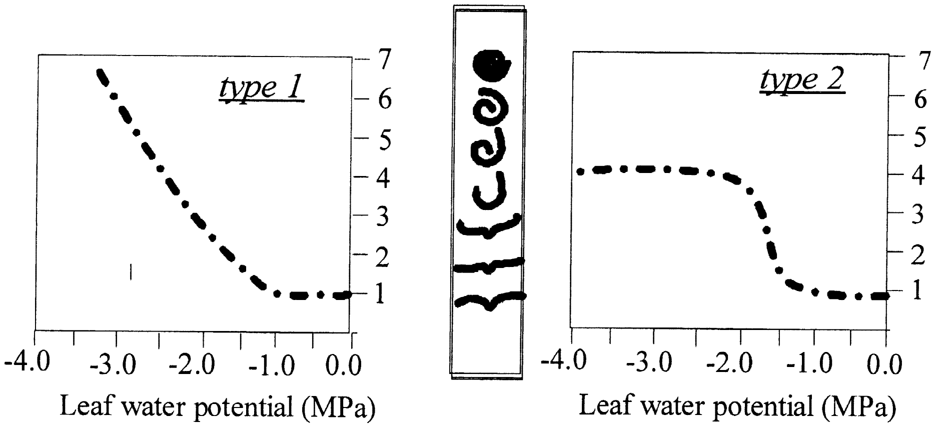

Figure 2. The two types of hydronastic leaf rolling in grasses (modified from Moulia 1994). During water stress, the grass leaf rolls (that is, it develops transverse curvature). Seven stages of increasing transverse curvature with increasing water stress are shown in the vertical rectangle where each image represents the cross-sectional shape of the intact leaf blade at defined water potential. The type 1 leaf rolls progressively more with decreasing water potential and achieves the high transverse curvature of stage seven, whereas the type 2 leaf rolls only to the stage four shape at −2 MPa and more negative water potentials.

Moulia 1994; Price and others 1997). Two types of relationships between internal water status of the leaf and transverse rolling can be described (Figure 2, see Moulia 1994 for more details and a more complete review of the bibliography). Some species, such as rice or tall fescue, display an almost complete rolling (type 1), whereas others, such as maize or sorghum, roll much less (type 2). Moreover, both the range of water potential over which the leaf rolls and the shape of the rolling curve are clearly distinct. Type 1 leaves display a monotonic increase in rolling over a large range of water potential, spanning approximately 2 MPa, after a threshold dependent on the osmolarity of the leaft (Hsiao and others 1984). In type 2 leaves, rolling is limited to a much smaller range (approximately 0.6 MPa). This difference does not seem to be related to differences in the regulation of internal turgor pressure through osmotic adjustment because type 1 leaves also present conspicuous adjustment (Hsiao and others 1984). Differences in the amount of rolling also exist within a given species. As a consequence, leaf rolling scores were used by plant breeders to screen the intraspecies genetic variability and to find quantitative trait loci for drought resistance (e.g., O'Toole and Moya 1978; Price and others 1997).

Genetic variability in the unrolling process has been much less studied (apart from descriptive classifications of the types of unrolling in different species, see Bell 1991). Concerning the intraspecific genetic control of unrolling, however, an interesting mutant Rld1-O has been described in maize (Bird and Neuffer 1985; see Hay and others 2000 for more details). The blades of this mutant fail to unroll, despite a normal morphology and an apparently normal photomorphogenesis (at least in terms of deetiolation and greening).

The fact that both unrolling and rolling involve a differential expansion of the tissues within the leaf cross section suggests an anatomic basis for the movements. Indeed, the role of the bulliform cells have often been argued (Price and others 1997; Salisbury and Ross 1985). These large cells, with a thin wall and a large vacuole, are usually found in the adaxial side of the lamina. Their role as motor cells during changes in transverse rolling has been argued in both developmental unrolling and hydronastic rolling. Indeed, changes in hydronastic rolling in rice correlate with changes in the internal turgor pressure of bulliform cells (Price and others 1997). However, their role has been disputed from two points of view. Concerning hydronastic rolling, it has been argued that no interspecific correlation can be found between the amount of bulliform cells and the ability to roll in a large sample of gramineous species (Ellis 1976; Shield 1951). Concerning the developmental unrolling, two detailed quantitative studies of differential expansion within leaf cross sections (Burström 1942; Shield 1951) revealed that bulliform cells do not seem to have a special role. In her comprehensive study of both developmental unrolling and hydronastic rolling, Shield (1951) concluded, "any explanation of involution in grass blades cannot be applied universally because of the histological variations of different genera."

What, then, causes the genetic variability in leaf rolling and unrolling? I hypothesize that auto-stress fields and minimal energy shape are important. In structural mechanics, shells are surfaces with double curvatures and are thin compared with the other dimensions of the shell. Rolled grass leaves are thus a natural example of shells because they are thin, and both longitudinally and transversely curved (Moulia 1993, 1994). One characteristic of the mechanical behavior of shells is that the changes in curvature in the two directions are coupled through plane strains and stresses within the lamina. This mechanical shell design has consequences for the rolling-unrolling processes.

MECHANICAL COUPLING OF CURVATURES WITHIN A SHELL (A SCHEMATIC AND QUALITATIVE ILLUSTRATION)

I illustrate the coupling of the curvature property of shells by a schematic drawing to familiarize readers with the equations of the thin shell theory. A more precise mathematical specification can be found in Moulia (1993, 1994), and a general presentation of shell theory can be found in many mechanical engineering textbooks. To avoid an overly general statement, I will concentrate on two simple examples referred to as the problem of leaf rolling and unrolling in grass leaves. I will then briefly discuss more complicated cases. The important starting point here is to deal with transverse curvature change and auto-stress fields. A crucial point is the "assembly" of leaf structural tissues and parts as a result of the primary growth process and differentiation. To make it simple, I will consider the following two hypothetical (and hopefully pedagogic) examples of leaf mechanical design and detail the consequences of these designs for the rolling-unrolling processes.

Two Examples of Leaf Mechanical Design

Let us consider a segment of the blade (typically 1/10th of the total blade length) between two transverse cuts. When this segment is rolled, it has both a longitudinal and a transverse curvature (Figure 3). From a mechanical point of view, it is thus a shell. For simplicity, let us assume its longitudinal curvature to be homogenous (that is, its midrib lies approximately on an arc of a circle of radius R_l and central angle θ; see Figure 4 a2). Now suppose that we cut this blade segment longitudinally into very thin parallel strips (for example, files of cells) in such a way that their internal state is not changed except that they are now free from any mechanical interaction. Then we consider (i) how to "re-build" the leaf segment from these isolated strips and (ii) the consequences in terms of the mechanical state of the leaf segment. We will consider two hypothetical scenarios on different assumptions concerning the rest-length of the strips.

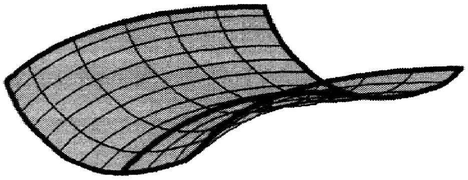

Figure 3. A leaf segment showing a shell structure with double curvature. The leaf bends (curves longitudinally downward) and also rolls (curves transversely).

Figure 4 illutrates diagrammatically the consequences of these two scenarios. It depicts the small segment of the blade. The left column shows a front view of the blade segment and the right column shows a side view. Each line in Figure 4 corresponds to one mechanical state of this shell. To facilitate understanding, we will focus on three particular strips on the drawing, strip 1 along the centroid line of the midrib, strip 2 halfway between the midrib and the edge of the blade, and strip 3 close to the edge of the leaf blade. The behavior of the other strips is intermediate. For simplicity, rolling is supposed to be less than half a gyre (which corresponds to a moderate rolling).

In the first scenario (Figure 4, scenario 1, a1, a2), we assumed that the strips have their rest shape (length) in the unrolled situation. Thus, the unrolled blade segment could be "restored" simply by gluing the strips together, without any tension or compression (the pieces of this puzzle will just fit). In the second scenario, we assume that the strips have their rest shape in the rolled situation (Figure 4, scenario 2, c1, c2). In both cases the perturbation is a change in the transverse shape of the blade (from unrolled to rolled in the first scenario, from rolled to unrolled in the second scenario), with no other change in the other directions. This could correspond, for example, to an anisotropic transverse shrinkage of the adaxial epidermis in the case of rolling, and an anistotropic transverse swelling of the adaxial epidermis in the case of unrolling. Because both unrolling and rolling occur after the differentiation of the hypodermic sclerenchyma fibers along the veins, making the lamina much stiffer in the longitudinal direction, this assumption of anisotropy in shrinkage or swelling is a priori likely to be realistic for the situation in the grass leaf (see Moulia 1993, 1994 for a more mechanical discussion of this assumption). Note, incidentally, that the origin of the swelling/shrinkage as related either to growth or to turgor changes has no influence on this problem;

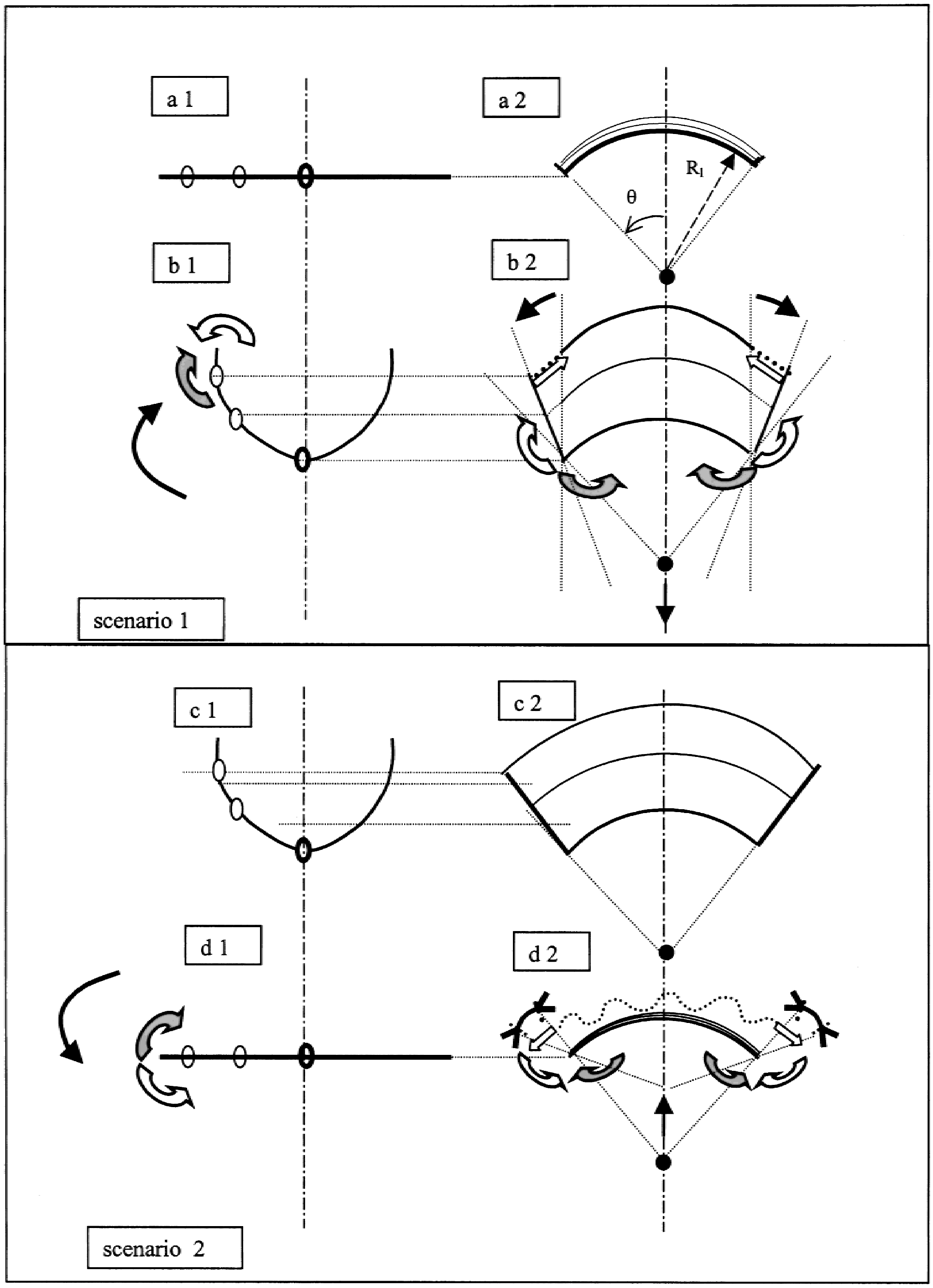

Figure 4. Diagram illustrating curvature coupling and auto stresses in the leaf segment in Figure 3. (*Left*) Cross-sectional and (*right*) side views of the leaf segment submitted to changes in transverse curvature. The location of longitudinal strips in the leaf lamina and midrib are shown as ovals in cross section and as curved lines in side view. *Scenario 1,* zero auto-stress state in the unrolled configuration (a), and consequences of rolling (b). *Scenario 2,* Zero auto-stress state in the rolled configuration (c), and consequences of unrolling (d). R_1, radius of longitudinal curvature of the midrib in its rest state, θ, angle defining the boundaries of the segment in its zero stress state. Black arrows represent movements. Empty arrows represent internal forces transmitted by the lateral strips to the lamina cross section. Gray arrows represent mechanical reactions caused by the longitudinal bending rigidity of the midrib and the transverse bending rigidity of the lamina. Thus in b2, for example, the diagram indicates tensile stresses in the lateral strips are transmitted as a pulling force on the cross section (*straight empty arrows*). These stresses act longitudinally to straighten the midrib (by means of a lever arm, curved empty arrows in b2) and transversely to unroll it (curved empty arrows in b1). Both the midrib and the lamina resist this bending through their bending rigidity (*curved gray arrows*).

the important point here is that some process induces a change in the transverse curvature of the lamina.

Scenario 1: Rolling of a Shell with an Unrolled Rest State

In this first case, the question is what happens if there is a transverse rolling. It can be seen in Figure 4 that the lateral strips 2 and 3 will tend to be displaced away from the center of the longitudinal curvature (compare a to b). However, their length is no longer compatible with the curvature of the midrib line (see b2). If the links between strips are to be maintained, it is necessary that the strips get extended (by a quantity $\Delta R_1.\theta$), and thus be put into tension. As a consequence, longitudinal tensile stresses would occur within the lamina (because the elongation is different for the different strips, some shear can also occur between the strips but can be neglected in this discussion). In return, the tensile stresses in the lateral strips are acting on the cross section as a “pulling force.” Because of its lever arm (see Figure 4 b2), this pulling force tends to rotate the cross section, inducing a bending up of the midrib strip (and bending stresses in the midrib). Thus, there is competition between the stretching of the lamina strips and the straightening (bending up) of the leaf. However, there is another way to decrease the elongation of the lamina strips: by a transverse bending back. These three processes (lamina tension, longitudinal bending up, and transverse bending down) are thus in competition, generating autostresses. The equilibrium shape meets the principle of minimal mechanical energy (Moulia 1994) and thus tends to decrease the sum of them. Depending on the relative stiffnesses related to each of these processes and on the longitudinal and transverse rest-curvatures of the leaf segment, their contribution to the global deformation of the shell will change and hence the equilibrium shape will be different. But there will necessarily be some lamina tension, some longitudinal straightening, and some transverse unbending. As a consequence no part of the shell is in its rest-state.

Scenario 2: Unrolling of a Shell with a Rolled Rest State

In this second case (illustrated by Figure 4 c,d), the mechanical rationale is the same as in case 1, that is, the problem of geometrical compatibility during the change in transverse curvature. But, now the zero-stress state is assumed to be the rolled state, and we consider unrolling. If unrolling occurs, the external strips will tend to be brought closer to their center of curvature and will thus undergo longitudinal compression to fit to their new location (Figure 4 d2). This is just the reverse of scenario 1. By the same token, the alternative processes are a longitudinal bending down and a transverse rolling. Here again the configuration of minimal energy (equilibrium state) tends to decrease the sum of the three processes. According to the relative rigidities and on the rest-shapes of the corresponding leaf parts, their contribution to the global deformation of the shell will change and hence the equilibrium shape will be different. But there will necessarily be some lamina compression, some longitudinal bending down, and some transverse bending up.

There are two small differences that keep scenario 2 from perfectly paralleling scenario 1. These differences involve (i) the longitudinal bending of the midrib and (ii) the longitudinal “compression” of the lateral strips.

With regard to the longitudinal bending of the midrib, we noted that the longitudinal stresses in the lateral strips act on the midrib through a lever arm. The length of this lever arm is related to the height of the lateral strips above the midrib (see Figure 4 b2). The higher the lever arm and/or the higher the longitudinal stresses in the lateral strips, the higher their efficiency in bending the midrib (what mechanical engineers call the bending moment). This is true in both scenarios. However, in scenario 1 as the lateral strips are pushed up from the midrib, they gain both longitudinal tension and also lever arm. In contrast, in scenario 2, as the lateral strips are displaced down during unrolling, they develop longitudinal expansive force (reaction to compression) but lose lever arm, leading to a partial compensation in terms of the bending moment they apply on the midrib. Therefore, for pure geometric reasons, the coupling between transverse unrolling and longitudinal bending down in scenario 2 is less “strong” than that of rolling and bending up in scenario 1.

Concerning the “compression” of the lateral strips, it should be noted that thin plates subjected to compression may easily buckle (as depicted in Figure 4, d2). Therefore, as we hypothesized for scenario 2 the strips in the rolled blade are not pretensioned; they will probably buckle sometime during the unrolling. Therefore, they will resist the load through bending, with presumably a decreased rigidity compared with the tensional rigidity they have in scenario 1.

Conclusions on Mechanical Coupling in Shells

The general conclusion is that in a shell structure, there is a mechanical coupling between changes in longitudinal and transverse curvature through tensile/compressive auto-stressing of the lamina (what mechanical engineers call membrane stresses). This coupling is a general mechanical property of shells and thus should apply to any shell-like structure and thus to the rolled grass blades. In this aspect the two previous scenarios are similar. Rolling is coupled with a longitudinal straightening (compare a2 to b2, but also d2 to c2), and unrolling is coupled with a longitudinal curving (compare c2 to d2, but also b2 to a2). This means that a rolling blade should straighten up longitudinally, whereas an unrolling blade should droop down longitudinally (irrespective of associated changes in the longitudinal bending rigidity or on the weight of the blade). The major difference between the 2 scenarios deals with energy states. In scenario 1, the unrolled configuration (configuration a) is a lower energy state than the rolled one (configuration b), and thus rolling is resisted (or unrolling is favored). Opposite in scenario 2, the rolled configuration (configuration c) has a lower energy level than the unrolled one, and thus unrolling is resisted (or rolling is favored).

The second conclusion is that despite the relative simplicity of the geometrical and mechanical rationale of curvature coupling in shell leaves (as illustrated by the drawings in Figure 4), dealing with its quantitative consequences on leaf habits is a much more complex matter that cannot be treated with simple rules of thumb. Indeed, the result will depend on the rest-shapes and on the stiffness of all the tissues within the segment of leaf blade. Moreover, loads can be transmitted from one element to another along the leaf blade (once again, energy minimization applies to the whole organ and not to some of its parts), and there may be heterogeneity in the anatomy of the different segments (because of gradients in tissue differentiation). We therefore need tools to help us in tackling such problems, and these tools are biomechanical mathematical models (we will see an example of such models applied to the case of rolling leaves later on in this article).

Last, it should be noted that more complicated designs are also possible for grass leaves. For example, strip 2 may have its rest length as in scenario 1, whereas strip 3 may have it as in scenario 2 (and the intermediate strips could be intermediate). This is not completely unlikely. Wavy files of cells within a flat unrolled blade are often seen in maize leaves for example (see for example, Poethig and Szymkowiak 1995). However, it is impossible to assess the possible designs of a leaf just by looking at it. An experimental study of the shapes and sizes of mechanically isolated parts is required. The "splitting method" of cutting up the lamina into segments and longitudinal strips, as presented earlier, is one such study, but although it is useful to explain the phenomenon of curvature coupling, it turns out to be impractical. A more convenient method was proposed by Moulia (1993, 1994), in which the lamina is split into the midrib (allowing characterization of the rest-state longitudinal curvature of the blade) and in narrow transverse strips (Figure 5). Because (i) the transverse curvature is about two orders of magnitude higher than the longitudinal curvature in rolled grass leaves and (ii) a significant part of the longitudinal bending rigidity is due to the midrib, if the transverse strips are narrow enough, the restraint caused by curvature coupling is very low, and the rest-state transverse curvature can be almost completely expressed (Moulia 1994). The water status of the midrib and the transverse strips can be controlled experimentally either by controlling evaporation or by bathing them in nonpermeant osmotic solution (Hay and others 2000; Moulia 1994). This method has been applied for hydronastic rolling (Moulia 1994) and for developmental unrolling (Hay and others 2000).

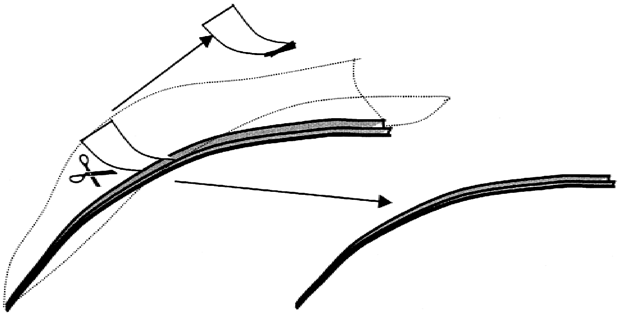

Figure 5. Diagram of excision experiments to assess internal auto-stresses caused by curvature coupling. Isolated transverse segments are observed for possible changes in rolling tendency, and isolated midribs are observed for possible changes in longitudinal curvature.

Let us now consider how useful what we have learned from the mechanics of shells can be. What is the importance of structural effects in the processes of leaf rolling-unrolling in grasses, and how helpful can they be in understanding the genetic control of leaf habit?

BIOMECHANICS OF HYDRONASTIC ROLLING

As previously stated, grass leaf rolling can be categorized into two types, one displaying a complete hydronastic rolling (type 1) and the other a poor rolling (type 2). Our approach was to assess whether shell structural effects could explain the poor rolling behavior of some species (Moulia 1993, 1994). This study was conducted in maize (*Zea mays* L.), which is

Figure 6. Transverse straightening of leaf blades during hydronastic rolling in maize **(a).** Fully turgid maize shoot with leaves that are unrolled, downward curving and somewhat horizontal. **(b)** Wilted plant with leaves that are rolled, straight and more vertically oriented (redrawn from photographs in Downey and Miller 1971).

a typical type 2 species. A maize leaf is characterized by a rather thick midrib, shown to be the major stiffening element influencing the longitudinal bending rigidity of the leaf (Moulia 1993; Moulia and others 1993) and acting as a composite beam (Moulia and Fournier 1997). In mature leaves, this midrib is curved downward, even when the instantaneous bending under self-weight is removed (Moulia and others 1993). When subjected to water depletion, the maize leaf displays, as stated, type 2 rolling (Figure 2). However, as shown in Figure 6, the amount of rolling at a given water status varies among the leaves along the stem. The upper straighter leaves usually roll more than the more longitudinally curved basal leaves. Furthermore, within the leaf the tip of the blade usually rolls more than the middle part of the lamina. Finally, leaf rolling in maize is associated with a trend toward longitudinal straightening (seen mostly in the upper leaves). Figure 7 shows isolated transverse strips at various water potentials, covering the typical rolling range for type 1 leaves (Moulia 1994). It is clear that unrestrained transverse rolling in isolated strips increases smoothly to a point that is never realized in intact maize leaves and can only be seen in type 1 species. Last, no particular difference in the amount of rolling of isolated transverse specimens could be found along the lamina or among leaves along the stem. This clearly demonstrates that rolling is restrained in the whole leaf structure (see Moulia 1994, for a more quantitative analysis of hydronastic rest-curvatures). This coupling was confirmed by observing that the midrib underwent an increase in longitudinal curvature when isolated from a rolled leaf.

The next step was then to determine whether shell curvature coupling could explain rolling suppression and the global phenomenology of leaf rolling in maize. This requires mechanical modeling. Figure 8 depicts a schematic drawing of the model by Moulia (1994), and Figure 9 shows typical results of simulation. In this simulation, two initial (observed) longitudinal habits were compared. The first one corresponds to a typical basal leaf, whereas the second corresponds to a typical younger leaf higher on the stem with a straighter rest-shape of the midrib. Loading corresponds to a change in water potential from −1.4 to −2.5 MPa (which is the range in which rolling has been reported to occur in maize). During this change of water status, the transverse rest-curvature of the blade changes (by the same amount all along the blade and in the two leaves). The hypothesis concerning the assembly of the blade and the rest-shapes of its parts was that of scenario 1. Clearly, the structural curvature coupling can explain most of the phenomenology of poor rolling, including longitudinal straightening of the leaf and heterogeneity of rolling within the leaf and among leaves (Moulia 1994). Analysis of the model revealed that the parameters that have the greatest control on the amount of rolling are the midrib rest-shape (its rest longitudinal curving) and midrib bending rigidity (Moulia 1993).

It should also be noted that in these leaves, there is no external mechanical loading, only a change in the internal water status. However, during rolling all the parts of the blade experience stress. This is typical of an auto-stress field.

In conclusion, it has been demonstrated experimentally and through a mechanical model that the

Figure 7. Transverse rest-shape of isolated transverse strips of maize at defined water status. The photographs here illustrate the part of the rolling response curve in which rolling type 2 leaves usually make a plateau, whereas rolling is continued in type 1 leaves (compare with Figure 2 and see Moulia 1993, 1994 for more details).

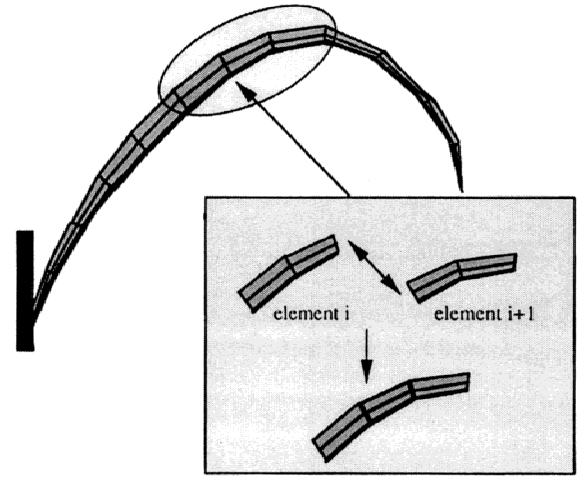

Figure 8. The mechanical model of the grass leaf as a set of leaf segments (adapted from Moulia 1994).

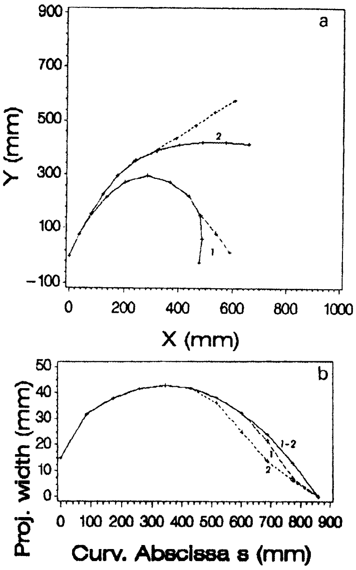

Figure 9. Effect of the initial longitudinal curvature of the leaf on its hydronastic transverse rolling and longitudinal straightening. **(a)** Leaf longitudinal profiles; **(b)** projected width of the lamina perpendicular to the midrib. In both figures, *curve 1* is a lower leaf with initial longitudinal curvature, and *curve 2* is an upper leaf with a straighter longitudinal midrib rest-shape (Moulia 1994).

structural "mechanical constraints" related to curvature coupling and auto-stressing and the existence of a rigid, downward curving midrib are the main causes of poor hydronastic rolling in maize. It is noteworthy that type 1 rolling species all share flexible blades with very small midribs (for example, rice, fescue), whereas type 2 rolling species have much thicker midribs (maize, sorghum). Moreover, shell structural effects also explain the variability within the plant. This has interesting applications to the genetic control of the rolling traits. At the species level, there was clearly a trade-off between designing large leaves (and thus stiff midribs) and retaining an ability for complete hydronastic rolling. The variation in rolling could be a side effect of changes in midrib design. At the developmental level, we have seen that a clear phenotypic heterogeneity along the successive leaves of the plant is not linked to a particular set of "rolling" genes whose expression would be developmentally regulated but an indirect consequence of the general heteroblasty of the maize leaf structure. This brings us to a consideration of the developmental aspects of leaf rolling.

BIOMECHANICS OF DEVELOPMENTAL UNROLLING: THE ROLLED MUTANT IN MAIZE

As described previously, the grass leaf undergoes developmental unrolling. In maize, a specific semi-dominant mutation, the *Rolled* mutation (Rldl-O/+)

causes a suppression of this developmental unrolling, leading to transversely rolled laminae in mature leaves of the mutant (Bird and Neuffer 1985; Hay and others 2000). However, this rolling remains moderate, far less than that found in the growing part of young leaves. Thus, some unrolling has taken place. A closer study of the phenotype revealed pleiotropic effects of this mutation: the leaves have a straighter longitudinal shape, a thinner midrib (while the lamina length is not significantly affected), a reverse top/bottom polarity between the leaf epidermal surfaces, and a decrease in the density of hypodermal sclerenchyma fibers in the midrib (Hay and others 2000). The immediate questions are: Are these anatomic and morphologic traits related? Can we explain part of this pleiotropy? What traits are really involved in unrolling suppression?

From the shell theory, an obvious hypothesis was that the straighter longitudinal habit was just a mechanical consequence of suppressed unrolling caused by a change in epidermal polarity. This hypothesis is just a transposition of what we have found in hydronastic rolling, another example of scenario 1. An alternative hypothesis could be that the type of assembly would correspond to scenario 2, but with the rolled mutant having a straighter and/or more rigid midrib. As a consequence, unrolling would be prevented through curvature coupling, the midrib resisting the unrolling (as in Figure 4 d2). This alternative could only be solved through a study of the rest-shape of mechanically isolated parts of the leaf. If hypothesis 1 holds true, it could be predicted that the mutant midrib would be bent up in the rolled lamina and would curve downward when isolated. Some transverse rolling would occur in the transverse strip on isolation. If hypothesis 2 holds true, the midrib should straighten on isolation and should have a straighter rest-shape than its wild-type siblings. Moreover, transverse strips should unroll when isolated. Figure 10 shows the results of the isolation experiments (see Hay and others 2000 for more quantitative results). The mutant midrib had a much straighter shape than its wild-type counterpart (compare Figure 10b and d), but displayed no significant change in curvature on isolation (compare Figure 10c and d). Moreover, the mutant transverse strips did not roll or unroll significantly when isolated at full turgor (Figure 10e). Last, the unrolled wild-type leaf (Figure 10 a,b) did not reveal any change in shape upon isolation. The conclusion here is that both the unrolled wild-type leaf and the mutant rolled leaf are in their almost-zero auto-stress configuration (with respect to curvature coupling). In other words, the mutation has shifted the state of the mature leaf from scenario 1 (zero auto-stress in the unrolled configuration see Figure 4a) in the mature wild-type leaf to scenario 2 (zero auto-stress in the rolled configuration see Figure 4c). We know that the mutation blocks developmental unrolling that normally takes place in the wild-type leaf. Therefore, the rolled mutation acts by preventing both the transverse developmental unrolling of the lamina and the developmental downward curving of the midrib in such a way that both curvatures remain geometrically compatible in the shell structure. No auto-stress field is generated. Furthermore, it means that the developmental process of unrolling in normal leaves involves a coordinated longitudinal and transverse expansion of the upper epidermis. Because we know that bulliform cells are not present in the upper epidermis of the midrib in maize, their existence is inadequate to explain developmental leaf unrolling. There is necessarily a coordinated differential straining of the overall cell layers of the lamina involved in the process. Moreover, because of the changes in longitudinal and transverse curvatures along the lamina in connection with changes in midrib thickness, a simple isotropic growth of the upper epidermis is unlikely to be involved, and a more complex growth regulation is required. Are the auto-stresses generated by temporary incompatible growth anisotropy involved in this coordination? Does the curvature field itself affect the localization and orientation of cell wall microfibrils (and thus of growth) as argued by Green (1999) in the case of apical meristems? Do auto-stresses act through their direct effect on the cell wall or through mechanoperceptive intercellular signaling (Green 1999; Trewavas and Malho 1997)? Does differentiation of sclerenchyma fibers also play a role in this process? Dealing with morphogenesis at the apex, Paul Green's proposal was that some morphogenetic gene products may act by modifying the biorheologic properties of the cell wall that are parameters for a differential-integral biomechanical transduction. Could this format also apply to wild-type genes of the *Rolled* mutant? This is a matter for future research. Meanwhile, we are back to our introduction and to the relationship between topographic events and the growth process.

CONCLUSION

Our starting point was the influence of structural biomechanical aspects of shape generation and their possible involvement in morphogenesis and in the genetic control of shape. We have seen that curvature coupling within the shell structure made by the grass lamina and the related auto-stress fields are

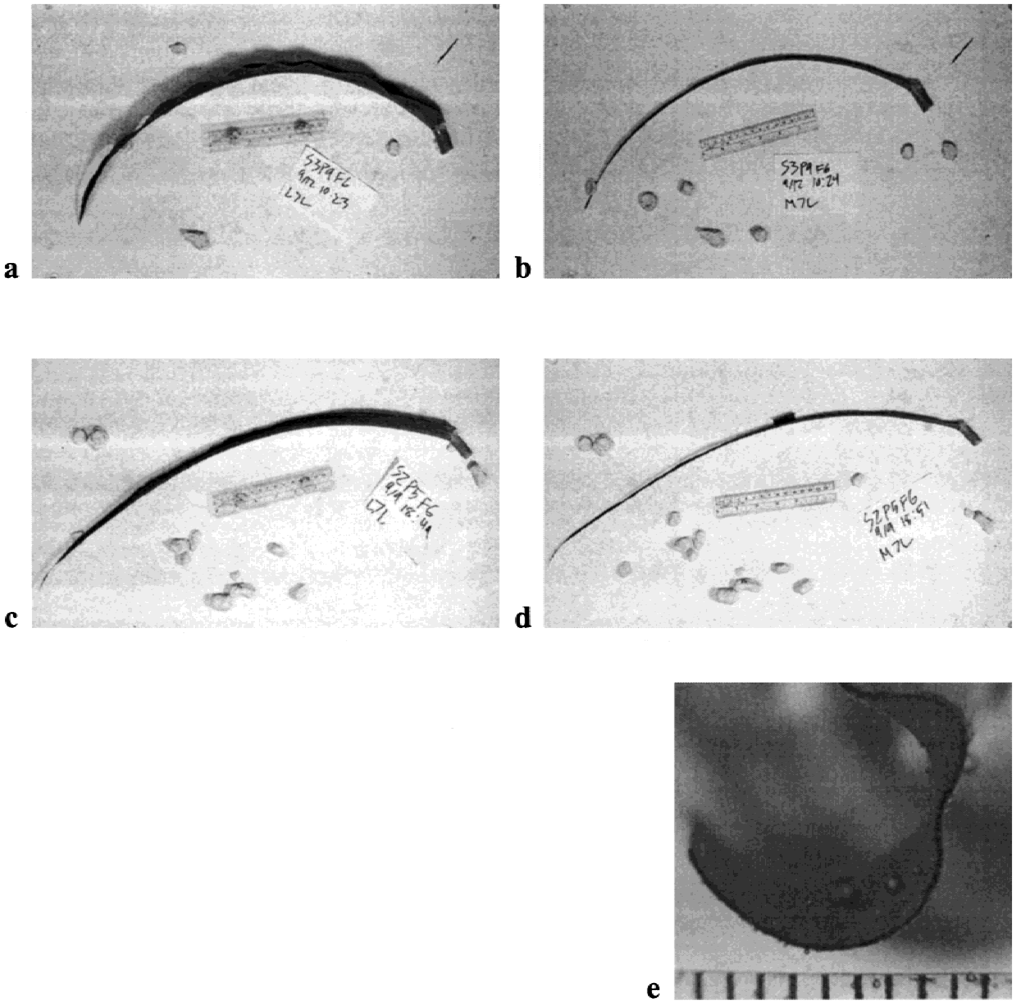

Figure 10. Excision experiments to observe possible changes in transverse and longitudinal curvatures of wild type and *Rolled* maize leaves (modified from Hay and others 2000). **(a)** Intact wild type leaf; **(b)** dissected wild type midrib; **(c)** intact mutant leaf; **(d)** dissected mutant midrib; **(e)** dissected transverse mutant strip at full turgor.

likely to explain much of the interspecies, intraspecies, and even developmental (that is, intraplant) variability of grass leaf rolling. Possible genetic control of this variability in leaf hydronastic shaping can thus apply to any of the parameters of the differential-integral mechanical model of a rolling leaf.

For developmental unrolling, like hydronastic rolling, genetic regulation is not by means of single hinge cells. The *Rolled* gene could prevent leaf unrolling by decreased surface growth of the upper epidermis in both the longitudinal or transverse directions or by increased shrinking in both directions on the lower side of the leaf, perhaps during sclerenchyma differentiation (Hay and others 2000).

When I first visited Paul Green at Stanford in 1996, I was struck by the fact that despite a very different initial focus and background in our French Plant Biomechanics Group (one could find few citations of Paul Green's work in our early articles), our findings could find a place as an example in the very large and thought-provoking framework he envisioned for plant biology (and obviously for plant biomechanics). Green has written, "Emphasis will be on the two dimensional structures . . . Tissue scale processes can influence details of subsequent cell behavior . . . The signal, in plane stress, is not localized within the formative area . . . Activities at the two levels interact to produce coherence across scales." (Green 1999). Thus in his posthumous article Green argued in favor of a biophysical differential-integral format embodying shell constraints and auto-stress fields to analyze plant morphogenesis. I hope I have shown that a similar approach must also hold for other aspects of plant biology, including the ecologically important phenomenon of leaf nastic rolling and unrolling in grass leaves.

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