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New Evidence for the Role of Mechanical Forces in the Shoot Apical Meristem

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Abstract

The mechanism for initiation of lateral organs in the shoot apical meristem is still unknown. In this article we investigate one critical component of a buckling mechanism of organ initiation (that is, the presence and distribution of compressive stresses in the meristem). Direct evidence for compression in the sunflower capitulum was obtained from the gaping pattern of shallow cuts and the propagation of fractures. Cuts gaped widely in the central region of the capitulum but remained closed, or nearly so, in the generative and differentiation regions, suggesting the presence of circumferential compression at these locations. Fractures were initiated in the generative region and propagated circumferentially over most of their length. They did not cross the generative region perpendicularly, suggesting again the presence of compressive stresses in the circumferential direction. This conclusion was confirmed by the stress distribution computed from the geometry of the capitulum at three stages of development. One interpretation of these results is that the generative region corresponds to a zone of compression that could control the initiation of new primordia by means of buckling of the tunica layer.

Key words: Mechanical buckling; Meristem; Primordium initiation; Pressurized shell; Sunflower; Tissue stresses

Introduction

To say that organisms experience mechanical forces is as much a truism as to say that they are made of chemicals. However, the significance of these forces remains, for the most part, to be established. This situation reflects not so much the fact that mechanical forces have a minor role in biology but rather that they have received much less attention compared with, for example, biochemistry and molecular biology, where phenomenal advances have been made. Nevertheless, there is growing evidence for the importance of mechanical factors in many aspects of biology (see, for example, Beloussov 1998; Chen and others 1997; Hejnowicz and others 2000; Lintilhac and Vesecky 1984).

The role of mechanical factors in plants has been considered from a variety of standpoints. One important standpoint deals with the mechanical constraints imposed on plant growth, especially those pertaining to the stability of supporting structures (stem, petiole, and so on) (Niklas 1992; Romberger and others 1993). These considerations show how the laws of physics and mechanics set the limits within which plant structure can evolve and remain mechanically stable. A second standpoint considers how plants, during their evolutionary history, have

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"discovered" and made use of some engineering principles to achieve specific purposes. A familiar example is the use of turgor pressure for the opening and closing of stomata, a direct application to guard cells of the principles dictating the deformation of pressure vessels (Aylor and others 1973; Cooke and others 1976).

Of special interest here is how plants may have evolved to make use of mechanical factors during development. This has been a major theme in Paul B. Green's career (see, for example, Green 1994, 1999) and places his work in line with the tradition established by S. Schwendener (1874, 1878) and D'Arcy W. Thompson (1942). One application of continuum mechanics to plant development concerns the initiation of lateral organs in the shoot apical meristem. We consider in this article how mechanical buckling can explain primordium initiation in the sunflower capitulum. As background to our work, we briefly review the development of the sunflower capitulum and the buckling model for primordium initiation.

Development of the Sunflower Capitulum

The development of the sunflower inflorescence (capitulum) has been subdivided into 10 floral stages (Marc and Palmer 1981) during which the shape of the meristem changes substantially (Figure 1). Between floral stage 1 (FS1) and floral stage 2 (FS2), the meristem starts enlarging and is dome-shaped. At floral stage 3 (FS3), the first involucral bracts are initiated at the periphery while the meristem dome becomes more shallow (flatter). Starting with floral stage 4 (FS4) the meristem takes on a saucer-like shape and keeps this characteristic shape for the remaining stages. The initiation of the first florets marks the beginning of floral stage 5 (FS5), and by the end of floral stage 7 (FS7) the whole capitulum is covered with floret primordia. The florets are initiated in an annular region, the *generative region*, that moves slowly toward the center of the meristem. At the same time, expansion of the *central region* pushes cells toward the periphery. For a period of several days, encompassing FS5 to FS7, these two processes balance each other and the size of the generative region remains nearly constant (Palmer and Steer 1985). Finally, at the end of FS7, surface expansion is reduced and the generative region moves inward until the capitulum is fully covered with floret primordia. The remaining stages (FS8–FS10) account for the differentiation of florets, but no new primordia are initiated.

One significant feature of the initiation process is that the distance between neighboring floret primordia is fixed. Consequently, the pattern must adjust to fit the changing size of the generative region. This results in the decreasing sets of intersecting spirals characteristic of the mature sunflower head. Typically, the numbers of spirals running clockwise and counterclockwise are members of the Fibonacci sequence. This mysterious feature has attracted attention from botanists for more than two centuries and is one of the long-standing questions of structural biology (see Jean 1994). In this article we are not so much concerned with explaining the pattern as to find evidence for the mechanism of primordium initiation. To date, no consensus exists on the nature of the initiation mechanism let alone the details of the process. The mechanisms proposed have ranged from purely chemical ones to purely mechanical ones. We describe in the following the basic features of the buckling model for primordium initiation. Reviews of other models can be found in Schwabe (1984) and Adler and others (1997).

Buckling Model for Primordium Initiation

Engineers define mechanical buckling as the out-of-plane deflection of a surface caused by in-plane compression. A familiar example dear to Paul Green is the potato chip (Green 1996). When a flat disk of potato is cooked in oil, the center of the disk shrinks more than the periphery so that compressive stresses develop in the outer region. To accommodate these stresses the disk must undulate, leading to the characteristic saddle shape of potato chips. Ideas of this nature were used to explain the local bulging of the meristem during primordium initiation.

The buckling model of phyllotaxis has taken various forms. Schüepp (1914) and Priestley (1928) maintained that the outer layer of the meristem (tunica) is expanding faster than the inner layers (corpus). More recently, Green and coworkers (1996, 1998) postulated variations in growth intensity within the tunica layer. In both cases, excess growth would force some regions of the tunica to undulate, each hump specifying the location of a new primordium. This is the *differential growth* version of the buckling model. A second proposal draws a parallel between plant meristems and pressure vessels (Selker and others 1992; Steele 2000; Steucek and others 1992). In this case, the tunica (mostly the thicker outer wall) would resist the pressure generated by the corpus and thus control the growth of the meristem. From this, one would predict that the tunica is under tension. If the meristem were a perfect hemisphere, the tension would be the same in

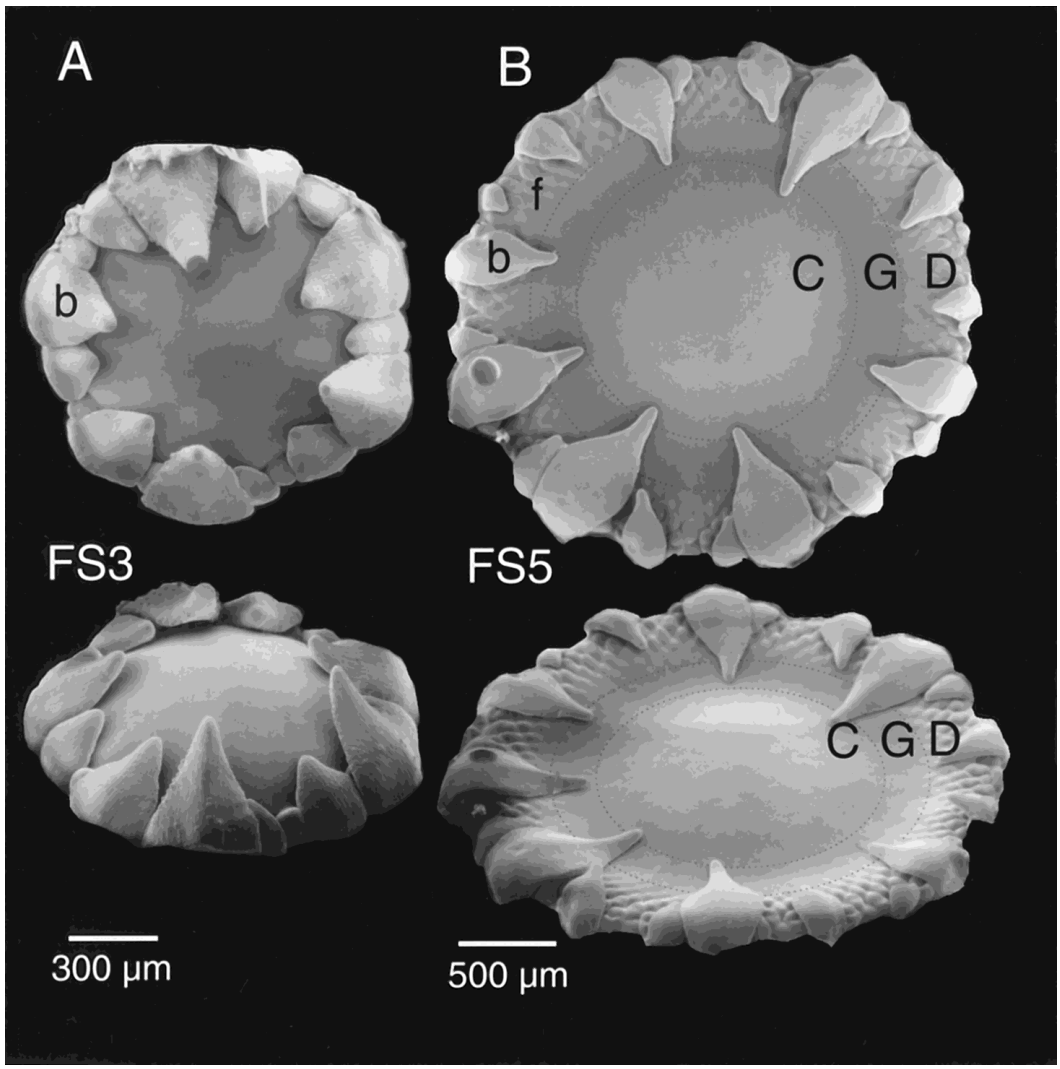

Figure 1. Scanning electron micrographs of capitula at floral stage 3 **A** and 5 **B.** Three regions can be distinguished: the central (C) and generative (G) regions that make up the meristem proper and the differentiation region (D) flanking the meristem. (Similar regions can be defined for earlier stages as in [A], but they are obscured by the overarching bracts). The central region is a zone of expansion without initiation of new primordia. It is surrounded by the generative region, which is the site of primordium initiation. Starting with floral stage 4, the generative region corresponds to a small annular concavity on the surface of the meristem. The location where the primordia become visible defines the inner margin of the differentiation region. The first primordia to be initiated differentiate into involucral bracts (b). Primordia initiated later differentiate into florets (f) and their subtending bracts.

all directions and over the whole surface. However, for a nonhemispheric meristem the stresses will vary, and although most of the surface should be under tension because of the internal pressure, some regions can develop compressive stresses. In other words, meristem geometry determines to a large extent the intensity and distribution of stresses. For the meristem shapes commonly seen in plant shoots (that is, shallow domes) circumferential compression is expected in the periphery where lateral organs are, in fact, initiated (see discussion). According to the model, the compression would cause a local undulation of the surface (buckling). The resulting periodic stress would give the necessary signals for primordium initiation. This is the *pressurized shell* version of the buckling model.

It is not easy to distinguish between these two proposals empirically. In this article we adopt the pressurized shell model. However, the interpretation of our experimental results does not depend on any given version of the buckling model. Moreover, the two versions lead to the same fundamental predictions: (i) compressive stresses should be present on the surface of the meristem at least some time

before primordium initiation and (ii) the wavelength of the undulation should depend on the flexural rigidity (resistance to bending) of the undulating layer. For example, a tunica with thick-walled cells would give a long wavelength; a tunica with thin, flexible walls would give a short wavelength. The amplitude of the undulation can be quite small (approximately one cell height), so although the stress pattern associated with buckling marks the location of new primordia, the buckling undulation should not be equated with the humps formed during primordium initiation. By the time a primordium is physically apparent on the surface of the meristem, the stresses that led to its initiation may very well have changed substantially.

Several earlier articles have presented evidence for buckling. Hernández and Green (1993) showed that the application of constraints limiting lateral growth of the sunflower capitulum modifies the pattern of floret initiation. This provides experimental evidence for the effect of stresses on primordium initiation. More recently, Green and coworkers (Green and others 1996,1998) showed that the buckling of circular plates can reproduce the patterns observed in plants, suggesting that this type of model offers at least a plausible mechanism for primordium initiation. However, the two most critical features of the buckling model (the presence of compressive stresses and the dependence of the undulation wavelength on the flexural rigidity of the tunica wall) have not been adequately tested. This is of special importance given earlier reports that in some plant species the meristem surface is under multidirectional tension (Hussey 1971,1973; Snow and Snow 1947,1951; Wardlaw 1948). Obviously, decisive evidence for the lack of compressive forces would rule out buckling as a potential mechanism. Therefore, the objective of this study was to test for the presence of compressive stresses in the sunflower capitulum. Our microsurgical manipulations and computer simulations provide direct and indirect evidence, respectively, for a zone of circumferential compression in the generative region of the capitulum. The dependence of spacing on material properties of the meristem is more difficult to assess. Some suggestive preliminary observations were published recently (Steele 2000). Additional experiments are underway.

Materials and Methods

Plant Material and Treatments

Sunflower (*Helianthus annuus* L.) achenes were sown in potting soil and grown under a 14L:10D light cycle. Four to 5 weeks after sowing, involucral bracts were removed to expose the developing capitula of plants. Capitula were then subjected to three treatments: (i) In one treatment, a radial cut was made into the surface of the capitula using a fine scalpel. The depth of the cuts was about one half to one fourth the diameter of the capitulum. In some plants, a circular plug was isolated in the center of the capitulum with a flexible razor blade (Gillette, Techmatic, Boston) rolled into a cylinder of 1 mm in diameter. The capitulum was then cut radially as in the preceding. (ii) In a second treatment, cracks were induced in the meristem during dissection by purposely pulling a large number of bracts laterally. (iii) In a third treatment, the stem was cut 1 cm below the meristem and the excised apical segment (including the capitulum and 2–3 nonelongated internodes) was placed in a hypertonic solution (0.5 M mannitol) to induce plasmolysis of the meristem's cells. After 15 min, loss of turgor pressure was evident from the wilting of the leaves remaining on the apical segment. This last treatment was done in combination with the previous two.

Before and immediately after each treatment a replica of the surface was made using a nondestructive method (Green and Linstead 1990, Williams and Green 1988). A vinyl silicone impression polymer (GC EXAFLEX, G-C Dental Industrial Corp., Tokyo, Japan) was applied to exposed capitula and left to polymerize for approximately 10–15 min. The negative molds were removed with fine tweezers, affixed upside-down onto microscope slides with silicone sealant, and filled with slow-setting epoxy resin (Ace Hardware Corp., Illinois). The bubbles present in the epoxy were dislodged from the surface of the mold with a glass pipette pulled to a thin and flexible rod. The replicas were polymerized for at least 12 h.

Scanning Electron Microscopy

All microscopic observations and measurements were made on the epoxy replicas of meristems (prepared before and after treatment). The replicas were stuck to metal stubs and sputter-coated with gold to about 10–15 nm. The specimens were observed in a scanning electron microscope (Philips SEM505, The Netherlands) at a voltage of 10–15 kV and a spot size of 100–200 nm. Photographs were taken with Polaroid film (Polapan 55PN, Cambridge, MA).

Evaluation of Tissue Stresses Using Fast4

The stress distribution was inferred from the capitulum geometry and estimated values for internal pressure and material properties of plant tissue (Table 1). The capitulum outlines were derived by fitting spherical and toroidal shell elements to poly-

Table 1. Parameters Used for the Computation of Stresses in the Sunflower Capitulum

Parameter	Value
Tunica thickness	25 μm
Internal pressure	0.1 MPa
Young's modulus	1 GPa
Poisson's ratio	0.3–0.5

The internal pressure, Young's modulus, and Poisson's ratio are estimates based on published values for the sunflower hypocotyl and Nitella *wall (see materials and methods section).*

nomials computed from meristem cross-sections and published by Hernández (1991). One spherical element and two toroidal elements were sufficient to fit the outlines of the meristem well within the expected natural variation in shape. The outer edge of the capitulum was assumed to be clamped (that is, the edge could not move or rotate about its position). This could account for the clasping of the bracts at this location (see Figure 1). The material properties of the capitulum have not been measured, therefore simulations were performed with estimates of material properties on the basis of published values for other plant tissues. The turgor pressure inside sunflower hypocotyl cells is approximately 0.5 MPa (Hejnowicz and Sievers 1996, Kutschera and Köhler 1992). We used 0.1 MPa as a reasonable value for the pressure exerted on the tunica. Hejnowicz and Sievers (1995) measured Poisson's ratios in the epidermis of the sunflower hypocotyl. They obtained a mean Poisson's ratio of 0.15 for the effect of transverse stress on longitudinal stress and a mean Poisson's ratio of approximately 1 for the effect of the longitudinal stress on the transverse stress. We used Poisson's ratios between 0.3–0.5 in our simulations. Finally, Young's modulus of cellulose is 100 GPa, whereas that of *Nitella* wall is 1 GPa (Wainwright and others 1976). We have adopted a value of 1 GPa. By use of this information, solutions for the tissue stresses were obtained with the Fast4 software. Fast4 is designed for the analysis of stress and deformation in elastic shells of revolution. It uses both asymptotic and direct numeric methods for efficient solution (Steele and Shad 1995). The results were plotted using Matlab (The MathWorks, Natick, MA).

Results

We analyzed the stress distribution in the meristem in terms of two components, N_r and N_θ corresponding to the stress in the radial and circumferential directions. The stress at a point on the surface of the capitulum can be decomposed into these two orthogonal components. To a first approximation, N_r and N_θ are independent and can be any combination of tension (positive stress) and compression (negative stress). Throughout the dome, surface stress is determined by the local geometry (curvature) and material properties of the tunica.

The Capitulum Is Under Circumferential Compression: Evidence from Cuts

To get an estimate of the stress distribution in the sunflower capitulum we performed radial cuts into the surface of the meristem at different stages of development (Figure 2). All the cuts showed the same behavior: the two sides gaped widely in the center of the meristem, whereas they remained closely pressed near the generative region and beyond. The mean distance between the two edges of the cuts at the center of the meristem (W_c) and in the generative region (W_g) is reported in Table 2. The data show a gradual increase in W_c and W_g, although the ratio W_c/W_g remains relatively constant between FS3 and early FS7. A more substantial decrease in the width ratio is seen at late FS7. This corresponds to the time when the area of the central region starts decreasing.

The stress in the intact meristem can be inferred from the gaping pattern of the cut in the following way. Consider the plane along which the incision will be made. The stress along this plane has a normal component and a shear component. After the cut is made, the edge of the cut is a free surface with zero normal and shear stress components. The effect of the cut can therefore be represented as adding the negative of the normal and shear stresses while removing the displacement constraints. Where the normal stress is positive (tissue tension), adding the negative of the normal stress on the two edges will open the cut, and where the normal stress is negative (tissue compression), adding the negative of the normal stress will pull the edges tighter together. The shear component does not significantly open or close the cut. The gaping pattern observed suggests that the tunica is under tension in the central region while it is under circumferential compression near the generative region (Figure 2F). Note that the degree of opening and closing at a particular point depends on the total stress distribution; a large region of opening can overcome a small region of closing and vice versa.

After performing the incisions, several meristems were placed in hypertonic solution (0.5 M mannitol) to ascertain that the gaping pattern was due to turgor-induced tissue stresses and not a result of pos-

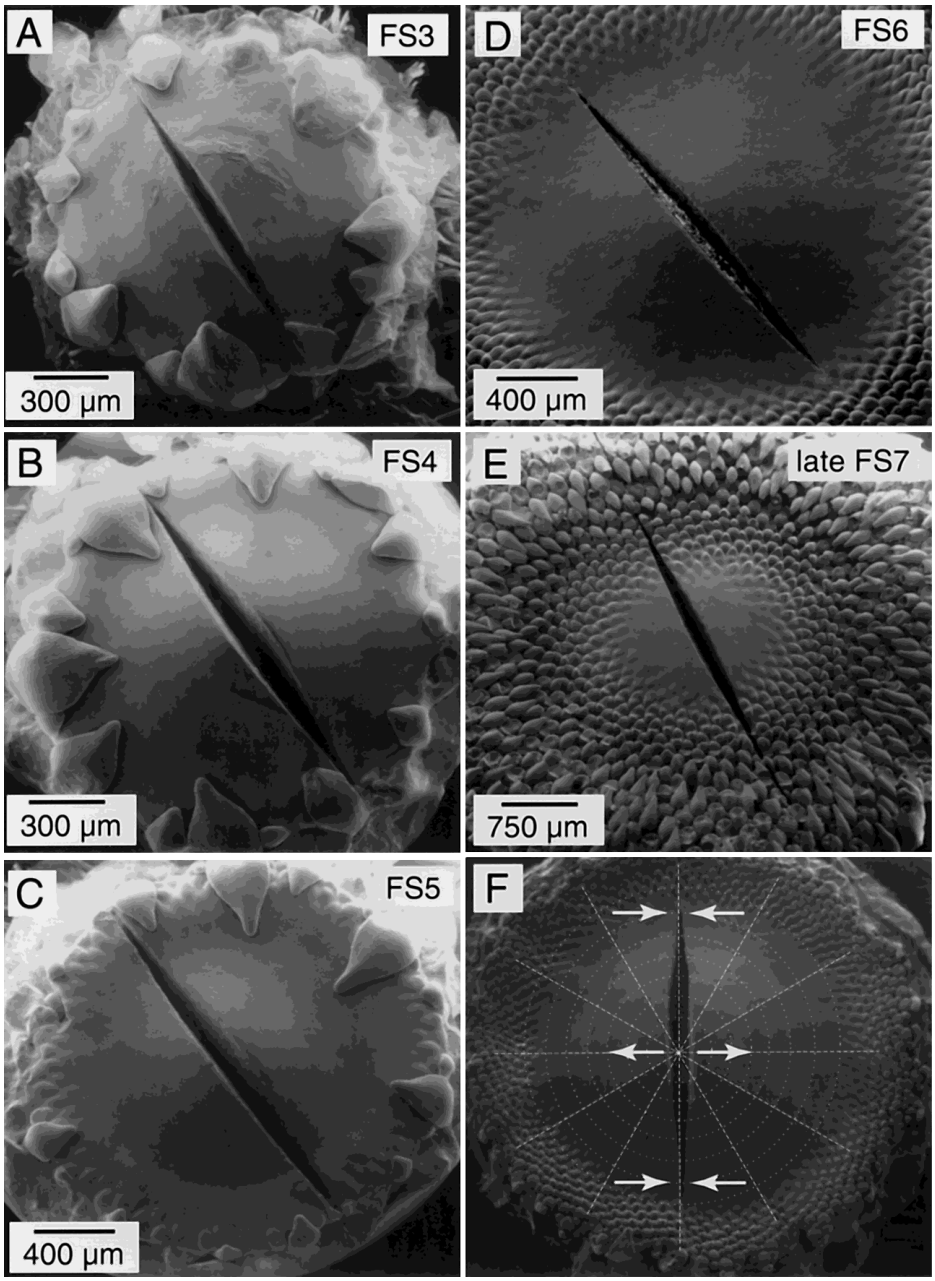

Figure 2. **A–E** Radial cuts of the sunflower capitulum at five floral stages. All cuts cover the whole diameter of the capitulum, although they vanish in regions of high circumferential compression. **F** Stress analysis for shallow meristem cuts. Arrows indicate the forces acting on the edges of the cut in the generative region and in the center of the capitulum. The dashed and dotted lines show the direction of radial stress (N_r) and circumferential stress (N_θ), respectively.

sible variations in the depth of the cuts. The uniform gaping of the cut once wilting was evident indicates that tissue stresses are for the most part responsible for the gaping pattern observed (Figure 3A,B). During capitulum development, the area of the central and generative regions progressively decreases relative to the area of the differentiation zone. A possible consequence of this is that the observed gaping pattern for the late stages of development (FS6 and FS7) is dominated by the forces present outside the zone of primordium initiation. For example, fast-growing florets in the differentiation region could lead to high levels of circumferential compression that would be solely responsible for closing the cut. To exclude this possibility, we made circular cuts to isolate the central and generative regions from the remainder of the meristem (Figure 3C). This operation did not affect the gaping pattern and thus establishes that the compressive stresses are active within the central and generative regions.

The Capitulum Is Under Circumferential Compression: Evidence from Fractures

Additional evidence for the presence of compressive stress in the generative region came from the fortu-

Table 2. Measurements of the Gaping Width at the Center of the Capitulum (W_c) and in the Generative Region (W_g) for Different Stages of Development

Floral Stage	Gaping Width (μm)		Width Ratio	*n*
	Center (W_c)	Generative Region (W_g)	(W_c/W_g)	
FS3	82 ± 36	23 ± 21	3.6	6
FS4	175 ± 13	38 ± 24	4.6	6
FS5	183 ± 38	52 ± 20	3.5	9
FS6	227 ± 26	58 ± 25	3.9	6
FS7 (early)	239 ± 64	72 ± 41	3.3	7
FS7 (late)	241 ± 61	140 ± 43	1.7	7

*Values are given as mean ± SD (*n = *number of meristems).*

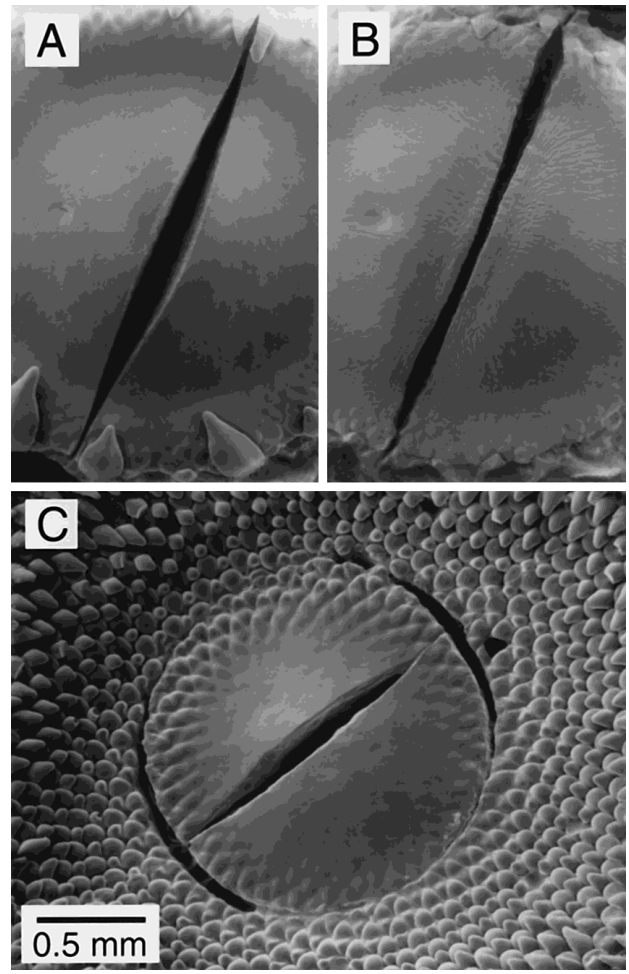

Figure 3. Gaping pattern of a transverse cut before **(A)** and after **(B)** a 15-min exposure to a hypertonic solution. The more uniform gaping of the cut in **(B)** suggests that the gaping is due for the most part to turgor-induced tissue stresses. **(C)** Gaping pattern of the capitulum when the central and generative regions are isolated from most of the differentiation region. It can be seen that the gaping pattern remains unaffected.

itous initiation of cracks on the surface of the meristem during the removal of the surrounding bracts (Figure 4A). These cracks were rare under normal conditions (when the meristem was dissected by a well-rested experimenter!) but could also be induced more reliably by pulling a large number of bracts simultaneously. The capitulum surface would fracture before the bracts were pulled off in approximately 20% of the meristems. Rupture of the meristem was accompanied by a snapping sound, suggesting a sudden release of a substantial level of stress. Interestingly, the meristem material is brittle rather than ductile, rupturing before the stress had induced any permanent deformation of the tissue (Figure 4B).

All fractures were similar in their location and geometry (9 meristems). Their mean angular span was 135 ±16 degrees and mean maximal width was 221 ± 42 μm. The stereotypical response of the meristem despite the nondiscriminating experimental treatment establishes this mode of failure as a fundamental feature of the capitulum. Two aspects of these fractures must be explained: the position where they are initiated and the direction of propagation. The initiation in the generative region suggests that this region is already under high radial tension or that it has a lower fracture toughness. In either case, the additional stress imposed by pulling the bracts was sufficient to induce failure at this location. A more important observation is that the direction of propagation is such that cracks do not cross the generative region at a right angle. Rather they follow the generative region over most of their length and then cross it at an acute angle (Figure 4A). According to Griffith's fracture criterion (Anderson 1995; Gordon 1978), a crack can form and develop as long as the tensile energy released by its propagation is greater than the energy required to create new surfaces (the energy required to break cell walls). Consequently, fractures tend to follow lines of greatest tension. Circumferential compression (or reduced circumferential tension) in the generative region would explain why cracks do not readily cross this region.

The Generative Region Is a Zone of Circumferential Compression

Because gaping of the cut in the central region causes some stress redistribution, the region where the two edges of the cut remain closely pressed will

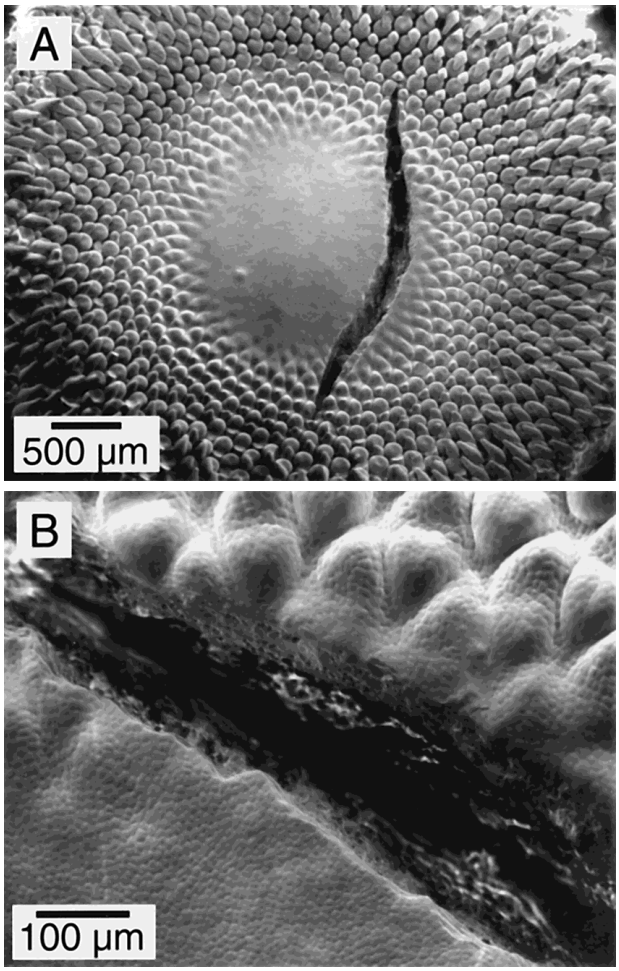

Figure 4. Fracture of the sunflower capitulum under tensile stress. **(A)** The tensile stress was applied by pulling the involucral bracts at the periphery of the capitulum in the region corresponding to the righthand side of the figure. Note that the fracture was initiated in the generative region. Its propagation was mostly circumferential until the generative region was crossed. **(B)** Close-up of the crack, showing that the surface of the meristem has sustained little permanent deformation. For example, the tunica cells are still isodiametric even in the vicinity of the crack.

not in general coincide perfectly with the region of circumferential compression. Similarly, the propagation of fractures induced in the capitulum can suggest that the generative region is under reduced circumferential stress, although the sign (compression vs tension) and exact location of this stress are not known precisely.

To establish a correspondence between the zone of circumferential compression and the generative region, a noninvasive approach is needed. As yet, no technique allows direct stress measurements in plant meristems. We have therefore adopted a computational approach on the basis of the pressurized shell model of the meristem presented in the introduction. This is an extension of the analysis presented by Wu (1993). The input for the computation included the cross-sectional geometry of the capitulum as published by Hernández (1991) and expected values for Young's modulus and Poisson's ratio of the tunica and the internal pressure (Table 1). From these the tissue stresses N_r and N_θ were computed.

The results of this analysis are shown in Figure 5A–C. It can be seen for the three developmental stages modeled that the radial stress is mostly positive (tissue tension) and relatively uniform in the capitulum. The circumferential stress is more variable spatially and becomes negative (tissue compression) either at the periphery of the dome (FS3 and FS4) or at an intermediate distance between the center of the capitulum and the periphery (FS5). If the intensity of the circumferential stress is overlaid onto the surface of the capitulum, it can be seen that the location of compressive stress corresponds to the generative region (Figure 5D–F). We conclude that for the developmental stages investigated, the generative region corresponds to a zone of circumferential compression. The compression could lead to primordium initiation by means of buckling of the tunica layer.

Discussion

Our microsurgical manipulations (cuts and fractures) give direct evidence for the presence of circumferential compression near the generative region of the sunflower capitulum. However, in most meristems the observed cuts are not completely closed in the generative region (see Table 2). This should *not* be construed to mean that the generative region is under tension. The cuts remain closed only in those locations where the compressive stresses are sufficiently great to overcome the pulling forces resulting from the stress redistribution. Moreover, by comparing the surface of the meristem before and after the cut is made, we have estimated that the blade damages three or four cells, which represents a width of 45–55 µm (data not shown). Therefore, the width of the cuts in the generative region for FS3–FS6 does not exceed that expected for a similar treatment on an unstressed surface. The late FS7 shows a significant increase in the gaping width (Table 2 and Figure 2E). This corresponds to the time when the generative region starts moving toward the center of the meristem. Because of these factors, the microsurgical manipulations are not sufficient to ascertain that the region of circumferential

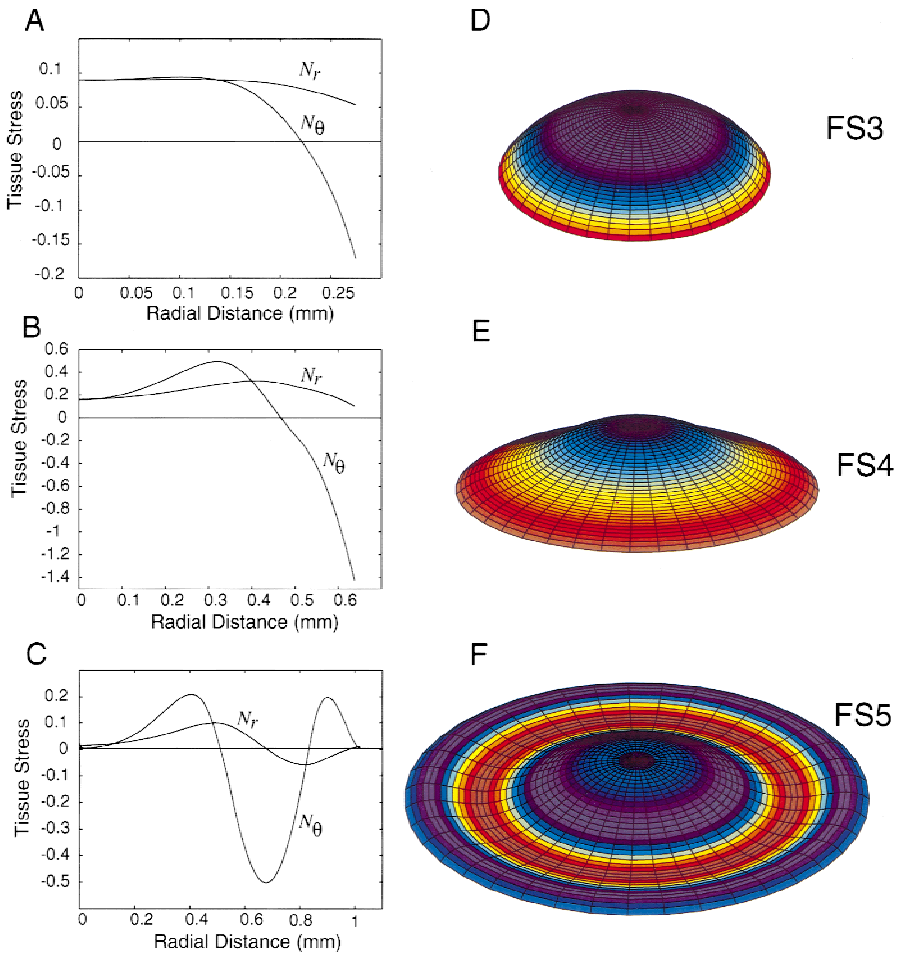

Figure 5. Fast4 computations of radial (N_r) and circumferential (N_θ) tissue stresses for three stages of capitulum development (FS3–FS5). **(A–C)** Plots of N_r and N_θ as a function of distance from the center of the meristem (the plots stop at the radial distance corresponding to the approximate location of the youngest involucral bracts). For all three stages, the radial stress is positive (tension) over most of the capitulum surface. The region of negative circumferential stress (compression) moves from the periphery *(FS3, FS4)* to an intermediate position along the radius *(FS5)*. **(D–F)** Color maps of the intensity of circumferential stress (N_θ) on the surface of the capitulum. Colors ranging from yellow to red indicate compressive circumferential stresses. The location of the compressive zone corresponds to the generative region (compare Figures 2A–C and 5D–F).

compression does, in fact, correspond to the generative region as postulated by the buckling model.

A more precise assessment of the stress distribution was obtained from computer simulations based on the cross-sectional geometry of the capitulum. They indicate that the compression co-localizes with the generative region throughout the extensive remodeling of the meristem accompanying the transition between FS3 and FS5. This is consistent with the buckling theory of primordium initiation. The accuracy of the stress values obtained with our approach depends on the quality and amount of information available about the system modeled. In this case, we expect good accuracy for the distribution of stresses because the geometry is known precisely. Simulations using a wide range of parameters have confirmed the robustness of the qualitative features reported here and, specifically, the presence of compressive stresses in the generative region. The exact amplitude of the stresses depends largely on material properties that have not been measured directly. Therefore, we will not draw any conclusions from the quantitative aspect of the stress distribution.

The computed stress distribution is consistent with that estimated from our microsurgical manipulations. For example, the calculated radial and circumferential tension in the central region of the capitulum explains the gaping of the cuts at this location. However, the predicted circumferential tension in the differentiation region (Figure 5C,F) has not been observed. This is not a major concern because the simulation assumes a simple surface geometry, whereas the corresponding surface of the capitulum is quite complex (compare Figure 5F and Figure 1B). Interestingly, the computed stresses also predict the site of initiation and the propagation of cracks. According to our simulations, the leading edge of the generative annulus where fractures are formed is also the region of highest radial tension (compare Figure 4 and Figure 5C). Consequently, this is the first location where the radial tension induced by pulling the bracts combined with the intrinsic radial tension can exceed the fracture toughness. An alternative explanation would be that the generative region has a lower fracture toughness and is therefore more prone to failure. A casual inspection of the

cellular details at the meristem surface has not revealed any striking differences that could account for a lower fracture toughness.

Comparison with Previous Experiments

Microsurgical manipulations similar to those reported here were performed by Snow and Snow (1947, 1951), Wardlaw (1948), Gulline and Walker (1957), and Hussey (1971, 1973). In *Euphorbia lathyris*, Snow and Snow (1951) reported complete gaping of their cuts. Their results were later repeated by Hussey (1973). The same authors observed no gaping in *Lupinus albus*. In *Pisum sativum*, Gulline and Walker (1957) and later Hussey (1973) reported no gaping. Mixed results were obtained for *Dahlia variabilis* (Snow and Snow 1951) and *Dryopteris aristata* (Wardlaw 1948), although the gaping tended to be reduced or absent at the presumptive site of leaf initiation. The discrepancies between these experiments can be explained in a number of ways. First, none of these authors distinguished explicitly between radial and circumferential stresses. Without a clear understanding of this difference, results such as ours could be interpreted to mean that the meristem's surface is under tension only. Because the articles did not include detailed illustrations of the gaping cuts, we have not been able to investigate this question further. Second, Snow and Snow (1951) reported that initially closed cuts could open quickly under certain conditions. They attributed the opening to loss of water by the meristem and were then able to show that under conditions of high humidity a greater number of cuts remained closed or nearly so. This may explain some of the variation observed. Third, we have already suggested in the introduction that compressive stresses, if present in the meristem, are likely to vanish soon after primordium initiation. It is therefore critical that the cuts be made at the location of presumptive primordium initiation not on the young primordia themselves. In this sense, the sunflower capitulum differs from other meristems in having continuous initiation of primordia and, accordingly, continuous circumferential compression.

Conditions for the Presence of Compressive Stresses

Given the range of meristem morphology in flowering plants and the ambiguous results in the few species that have been tested for the presence of compressive stresses, it is useful to consider conditions that would allow compressive stresses to develop. These conditions will differ whether compression results from differential growth of adjacent tissues layers or whether it arises from geometric instability, as for the pressurized shell model. Differential growth depends on clearly defined tissue layers, such as the tunica-corpus construction of many meristems. The geometric instability is obviously controlled by the overall shape of the meristem. We prefer the pressurized shell model over the differential growth model for a number of reasons. First, the excess tunica growth postulated by the differential growth model would most likely lead to multidirectional compression on the surface of the meristem. Our data and that of others (Hussey 1971, 1973; Snow and Snow 1947, 1951) show that the meristem is at least under radial tension, whereas compressive stresses tend to be transient and localized to the region of primordium initiation. Second, the close correspondence between the stress inferred from the gaping pattern of our cuts and the stress computed from the meristem geometry show the predictive power of the shell model. Finally, the assumptions of the shell model are compatible with recent models of stem elongation (Hejnowicz and Sievers 1995a,b, 1996; Kutschera 1991). Both are based on the induction of tissue stresses in the epidermal layer because of pressure exerted by interior cells (the pith and cortex in the stem and the corpus in the meristem). Given the physical continuity between the meristem and the stem, similar proposals for their underlying mode of development are rather appealing.

The dependence of the pressurized shell model on meristem geometry raises the question of how the observations presented in this article apply to other meristems. The morphology of intermediate and late stages of capitulum development (FS4–FS7) is unusual among flowering plants, although the Asteraceae include other examples (Harris 1995). Nonetheless, our cuts show that compression is present also in the early stages of development (FS3) when meristem morphology in sunflowers is not significantly different from that of other meristems. As a rule, circumferential compression is expected if the radius/height ratio for the meristem is greater than $\sqrt{2}$ (Timoshenko and Woinowsky-Krieger 1959). Again, the location of the predicted compression (the periphery of the dome) corresponds to the region where primordia are initiated. This rule encompasses a large number of meristems, although the meristems of monocots such as wheat and maize, and some dicots (*Hippuris*) are conspicuous exceptions. It is possible that a slightly different buckling mechanism is at work in these plants. A detailed

study of these species would be necessary to assess the generality of the compression observed.

Model of the Sunflower Capitulum

Our observations lead to the following model of the sunflower capitulum. The meristem proper can be divided into two regions (Figure 1): the central region and the generative region. These regions correspond approximately to the central and peripheral zones often recognized in the vegetative meristem (Steeves and Sussex 1989). We propose that the differences between these regions are caused by differences in stress distribution. The central region is morphogenetically inactive, although cell division and expansion occur. From the results shown in Figure 5, this region is under both radial and circumferential tension, which, according to the buckling model, could explain the absence of organ initiation. We define the inner boundary of the generative region as the location where compressive stresses first appear. This region is under circumferential compression and mostly radial tension (some radial compression is seen at FS5). It is competent for organ initiation in the sense that the driving force for tissue buckling is present. At some location within the generative region, the compressive stresses are sufficiently great to cause tissue buckling, thus leading to a periodic stress distribution that serves as a signal for primordium initiation.

If, indeed, periodic stress provides the signal for primordium initiation, how is this signal transduced? The findings by Lintilhac and Vesecky (1984) that compressive and tensile stresses can orient the plane of cell division suggest one possible transduction mechanism. Interestingly, it has been claimed that the first structural evidence indicating primordium initiation is the occurrence of periclinal cell divisions in the tissue layers below the tunica (see Lyndon 1994). We can therefore propose that the local stress variations created by buckling of the tunica layer trigger the periclinal divisions in the subjacent layers. An alternative explanation would be that cell-cell communication within the meristem is used to specify groups of cells to divide and form new primordia (see, for example, Meyerowitz 1997). However, there is experimental evidence that cell division is not a necessary step for the initiation. Gamma irradiated plantlets initiate leaves without concomitant cell divisions (Foard 1971), whereas local application of expansin on the surface of the meristem induces leaf initiation, presumably by affecting the wall material properties (Fleming and others 1997, 1999). These results suggest that the signal for primordium initiation is acting at the tissue level and is biophysical in nature. The pressurized shell model described in this article is a tissue-level phenomenon that can account for primordium initiation with or without concomitant cell divisions.

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REFERENCES

Adler I, Barabé D, Jean RV.1997. A history of the study of phyllotaxis. Ann Bot 80:231–244

Anderson TL.1995. Fracture mechanics. Fundamentals and applications. CRC Press, Boca Raton

Aylor DE, Parlange J-Y, Krikorian AD.1973. Stomatal mechanics. Am J Bot 60:163–171

Beloussov LV.1998. The dynamic architecture of a developing organism. An interdisciplinary approach to the development of organisms. Kluwer Academic Publishers, Dordrecht

Chen CS, Mrksich M, Huang S, Whitesides GM, Ingberg DE.1997. Geometric control of cell life and death. Science 276:1425–1428

Cooke JR, Debaer Demaeker JG, Rand RH, Mang HA.1976. A finite element shell analysis of guard cell deformations. Am Soc Ag Eng 76–5526:1–43

Fleming AJ, Caderas D, Wehrli E, McQueen-Mason S, Kuhlemeier C.1999. Analysis of expansin-induced morphogenesis on the apical meristem of tomato. Planta 208:166–174

Fleming AJ, McQueen-Mason S, Mandel T, Kuhlemeier, C.1997. Induction of leaf primordia by the cell wall protein expansin. Science 276:1415–1418

Foard DE.1971. The initial protrusion of a leaf primordium can form without concurrent periclinal cell divisions. Can J Bot 49:1601–1603

Gordon JE.1978. Structures, or why things don't fall down. Plenum Press, New York

Green PB.1994. Connecting gene and hormone action to form, pattern and organogenesis: biophysical transductions. J Exp Bot 45:1775–1788

Green PB.1996. Expression of form and pattern in plants. A role for biophysical fields. Cell Dev Biol 7:903–911

Green PB.1999. Expression of pattern in plants: combining molecular and calculus-based biophysical paradigms. Am J Bot 86:1059–1076

Green PB, Linstead P.1990. A procedure for SEM of complex shoot structures applied to the inflorescence of snapdragon (*Antirrhinum*). Protoplasma 158:33–38

Green PB, Steele CR, Rennich SC.1996. Phyllotactic patterns: a biophysical mechanism for their origin. Ann Bot 77:515–527

Green PB, Steele CR, Rennich SC.1998. How plants produce pattern. A review and a proposal that undulating field behavior is the mechanism. In: Jean RV, Barabé D (eds) Symmetry in plants. World Scientific, Singapore, pp 359–392

Gulline HF, Walker R.1957. The regeneration of severed pea apices. Aust J Bot 5:129–136

Harris EM.1995. Inflorescence and floral ontogeny in Asteraceae: a synthesis of historical and current concepts. Bot Rev 61:93–278

Hejnowicz Z, Rusin A, Rusin T.2000. Tensile tissue stress affects the orientation of cortical microtubules in the epidermis of sunflower hypocotyl. J Plant Growth Reg (this issue)

Hejnowicz Z, Sievers A.1995a. Tissue stresses in organs of herbaceous plants I. Poisson ratios of tissues and their role in determination of the stresses. J Exp Bot 46:1035–1043

Hejnowicz Z, Sievers A.1995b. Tissue stresses in organs of herbaceous plants II. Determination in three dimensions in the hypocotyl of sunflower. J Exp Bot 46:1045–1053

Hejnowicz Z, Sievers A.1996. Tissue stresses in organs of herbaceous plants III. Elastic properties of the tissues of sunflower hypocotyl and origin of tissue stresses. J Exp Bot 47:519–528

Hernández LF.1991. Morphometry and surface growth dynamics of the sunflower (*Helianthus annuus*) receptacle. Its importance in the determination of yield. Suelo y Planta 1:91–103

Hernández LF, Green PB.1993. Transductions for the expression of structural pattern: analysis in sunflower. Plant Cell 5:1725–1738

Hussey G.1971. Cell division and expansion and resultant tissue tensions in the shoot apex during the formation of a leaf primordium in the tomato. J Exp Bot 22:702–714

Hussey G.1973. Mechanical stress in the shoot apices of *Euphorbia, Lycopersicon,* and *Pisum* under controlled turgor. Ann Bot 37:57–64

Jean RV.1994. Phyllotaxis. A systemic study in plant morphogenesis. Cambridge University Press, Cambridge

Kutschera U.1991. Determination of the longitudinal tissue stresses in the growing and non-growing regions of sunflower hypocotyls. J Plant Physiol 138:460–465

Kutschera U, Köhler K.1992. Turgor and longitudinal tissue pressure in hypocotyls of *Helianthus annuus* L. J Exp Bot 43:1577–1581

Lintilhac PM, Vesecky TB.1984. Stress-induced alignment of division plane in plant tissues grown *in vitro*. Nature 307:363–364

Lyndon RF.1994. Control of organogenesis at the shoot apex. Tansley review no. 74. New Phytol 128:1–18

Marc J, Palmer JH.1981. Photoperiodic sensitivity of inflorescence initiation and development in sunflower. Field Crops Res 4:155–164

Meyerowitz EM.1997. Genetic control of cell division patterns in developing plants. Cell 88:299–308

Niklas KJ.1992. Plant biomechanics. An engineering approach to plant form and function. The University of Chicago Press, Chicago

Palmer JH, Steer BT.1985. The generative area as the site of floret initiation in the sunflower capitulum and its integration to predict floret number. Field Crops Res. 11:1–12

Priestley JH.1928. The meristematic tissues of the plant. Ann Bot 3:1–20

Reinhardt D, Wittwer F, Mandel T, Kuhlemeier C.1998. Localized upregulation of a new expansin gene predicts the site of leaf formation in the tomato meristem. Plant Cell 10:1427–1437

Romberger JA, Hejnowicz Z, Hill JF.1993. Plant structure, function and development: a treatise on anatomy and vegetative development, with special reference to woody plants. Springer-Verlag, Berlin

Schüepp O.1914. Wachstum und Formwechsel des Sprossvegetationspunktes der Angiospermen. Ber dtsch bot Ges 32:328–339

Schwabe WW.1984. Phyllotaxis. In: Barlow PW , Carr DJ (eds) Positional controls in plant development. Cambridge University Press, Cambridge, pp 403–440

Schwendener S.1874. Das mechanische Prinzip im anatomischen Bau der Monocotylen mit vergleichenden Ausblicken auf die übrigen Pflanzenklassen. Engelmann, Leipzig

Schwendener S.1878. Mechanische Theorie der Blattstellungen. Engelmann, Leipzig

Selker JML, Steucek GL, Green PB.1992. Biophysical mechanisms for morphogenetic progressions at the shoot apex. Dev Biol 153:29–43

Snow M, Snow R.1947. On the determination of leaves. New Phytol 46:5–19

Snow M, Snow R.1951. On the question of tissue tensions in stem apices. New Phytol 50:184–185

Steele CR.2000. Shell stability related to pattern formation in plants. J Appl Mech (in press).

Steele CR, Shad Ku-R.1995. Asymptotic-numeric solution for shells of revolution. Appl Mech Rev 48:S44–S51

Steeves TA, Sussex IM.1989. Patterns in plant development. 2nd edn. Cambridge University Press, Cambridge

Steucek GL, Selker JL, Reif WE.1992. Architecture of the plant shoot apex. In: Reiner R (ed). Natürliche Konstruktionen. Kurz & Co., Stuttgart

Thompson DW.1942. On growth and form, 1992 republication. Dover, New York

Timoshenko S, Woinowsky-Krieger S.1959. Theory of plates and shells. McGraw-Hill, New York

Wainwright SA, Biggs WD, Currey JD, Gosline JM.1976. Mechanical design in organisms. Princeton University Press, Princeton

Wardlaw CW.1948. Experimental and analytical studies of Pteridophytes XI. Preliminary observations on tensile stress as a factor in fern phyllotaxis. Ann Bot 12:97–108

Williams MH, Green PB.1988. Sequential scanning electron microscopy of a growing plant meristem. Protoplasma 147:77–79

Wu C-H.1993. Application of shallow shell stability to plant morphogenesis by Fourier series. PhD Thesis, Department of Mechanical Engineering, Stanford University.