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Wall extensibility: its nature, measurement and relationship to plant cell growth

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SUMMARY

Expansive growth of plant cells is controlled principally by processes that loosen the wall and enable it to expand irreversibly. The central role of wall relaxation for cell expansion is reviewed. The most common methods for assessing the extension properties of plant cell walls ('wall extensibility') are described, categorized and assessed critically. What emerges are three fundamentally different approaches which test growing cells for their ability (a) to enlarge at different values of turgor, (b) to induce wall relaxation, and (c) to deform elastically or plastically in response to an applied tensile force. Analogous methods with isolated walls are similarly reviewed. The results of these different assays are related to the nature of plant cell growth and pertinent biophysical theory. I argue that the 'extensibilities' measured by these assays are fundamentally different from one another and that some are more pertinent to growth than others.

Key words: Cell walls (viscoelasticity), growth biophysics, plant growth, stress relaxation, wall expansion, wall extensibility.

'That's a great deal to make one word mean,' Alice said in a thoughtful tone.

'When I make a word do a lot of work like that,' said Humpty Dumpty, 'I always pay it extra.'

'Oh!' said Alice. She was too much puzzled to make any other remark.

Lewis Carroll, *Through the Looking-glass*

I. INTRODUCTION

Wall extensibility is like love. It conveys different meanings in different situations and the attendant confusion can trip up the unwary. Growing cell walls are said to be extensible, by which we mean they

deform irreversibly in a time-dependent manner under the action of tensile forces in the wall, forces usually generated by cell turgor. This is the most common *general* meaning of 'wall extensibility' in the literature on plant growth. However, this word should be paid extra, by Humpty Dumpty's reason-

ing, because it has taken on many additional technical definitions as new methods for measuring wall extensibility have been devised. This has led to some confusion, in my view, because these techniques rely on different principles and assumptions about the nature of the wall properties that govern cell expansive growth. Because these various measures are all called wall extensibility, the distinction is often blurred, or obliterated entirely, between what these methods measure and what is really wanted in most cases, namely the wall yielding properties relevant for growth. This confusion arises because the wall is a complex polymeric material which may be experimentally extended by various means, but not all such extensions are relevant for growth.

This review attempts to clarify the meaning of wall extensibility in the context of plant growth, in so far as is possible at this time, and to assess the meaning, underlying assumptions and problems of the major techniques in current use to measure wall extensibility properties. My hope is that this analysis will help others select an appropriate technique and correctly interpret the results obtained.

There are many topics on plant cell expansion that have been omitted due to space limitations and the focused nature of this review. For a more general view of plant growth biophysics, see Cosgrove (1986, 1993) and Tomos, Malone & Pritchard (1989). For growth kinematics I refer the reader to Green (1976) Silk (1984), and Gandar & Hall (1988). Biochemical and hormonal aspects have been reviewed by Cleland (1981), Taiz (1984), Fry (1988) and Brett & Waldron (1990), whereas Roelofson (1965), Roland & Vian (1979), Vian & Roland (1987) and McCann, Wells & Roberts (1990) deal with the ultrastructural aspects of the primary wall.

II. THE NATURE OF WALL EXPANSIVE GROWTH

The fact that growing plant cells have a substantial turgor pressure, and therefore must have a wall strong enough to constrain the protoplast and to generate this hydrostatic force (Fig. 1), has shaped the thinking about the nature of plant cell expansion and its control. These ideas are briefly summarized below.

1. *The growing wall is a complex polymeric structure bearing a high tensile stress*

The primary (expanding) cell wall of vascular plants is a heterogeneous polymeric structure, in which crystalline microfibrils of cellulose are embedded in a complex matrix of pectins, heterogeneous mixed-linked glucans, structural proteins and other materials (Cassab & Varner, 1988; Fry, 1988; Talbot & Ray, 1992; Carpita & Gilbeaut, 1993). The exact structure of many of these matrix polymers is still uncertain, as is the nature of the interactions and

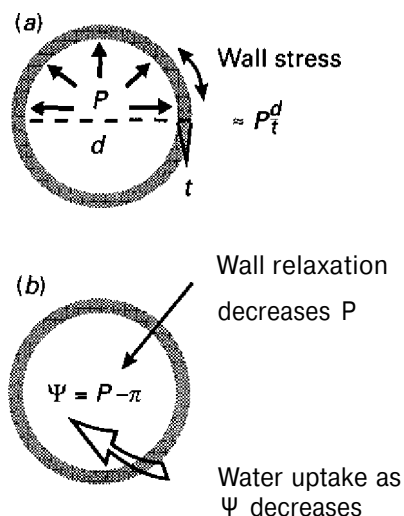


Figure 1. Mechanics and hydraulics of a turgid, growing plant cell, (a) Cell turgor pressure (P) is balanced by equal and opposite forces in the wall. Because the wall is thin (thickness t) and supports the pressure from a large cross section (diameter d), the wall supports large tensile forces. Cell expansion begins when wall stress dissipates as a result of rearrangements of load-bearing wall polymers. Cell turgor and water potential fall (b), thereby initiating water uptake and physical expansion of the wall.

bonds that hold the wall together. Recent advances have been made using genetic approaches to analyze wall proteins and immunochemical methods to identify the location of specific polymers within the wall. There may be three networks capable of bearing tensile stresses within the wall: (a) cellulose microfibrils glued together into a network by mixed-linked glucans and related matrix polysaccharides ('hemicelluloses'), which are thought to form a hydrogen-bonded surface coat over the microfibrils and which may bridge between microfibrils; (b) pectins gelled into a network by calcium crossbridging; and (c) structural proteins covalently linked by oxidative phenolic crossbridges and perhaps other linkages. These three networks are probably intertwined and may be covalently linked with each other.

Rapidly growing plant cells have turgor pressures which typically range from 3 to 10 bar (0.3 to 1 MPa), depending on species and growing conditions. Given that the plasma membrane and cell cytoskeleton are mechanically weak compared with the wall, it follows that the outward force generated by turgor pressure is borne by the wall, whose cross-sectional area is about one-hundredth that of the cell. This geometry means that the tensile stress (stress = force/area) borne by growing cells walls is very large, on the order of 1000 bar (100 MPa), and that at least one of the polymeric networks of the wall must be under a large mechanical load. It may be that each of the three polymeric networks bears a fraction of the wall stress, but there are no definitive data on this point.

The polymeric nature of the wall endows it with

viscoelastic properties. Viscoelasticity denotes the mechanical properties of materials that are intermediate between elastic solids and viscous liquids; it refers to intrinsic mechanical properties of materials, not properties mediated by chemical or enzymatic reactions. Classical elastic solids are those that obey Hooke's law: they are immediately and reversibly deformed in direct proportion to the forces acting on them. In contrast, classical viscous liquids flow at a rate proportional to the forces acting on them. Viscoelastic materials display a complex, time-dependent deformation in response to a force; the response usually includes an instantaneous, reversible deformation (elasticity), a time-dependent but fully reversible deformation (retarded elasticity) and a permanent deformation (viscous flow if time-dependent; plastic flow if otherwise). Growing walls behave like viscoelastic liquids (they do not return to their original shape and size after removal of all external forces), whereas mature walls act much more like viscoelastic solids (their size and shape is restored, or nearly so, upon removal of force).*

2. Wall relaxation initiates cell enlargement

How does the growing plant cell physically enlarge? Lockhart (1965) and Ray, Green & Cleland (1972) were among the first to recognize that cell enlargement begins with a reduction, or *relaxation*, of wall stress (see below). As a consequence, turgor pressure and water potential are reduced (turgor is the Newtonian counterforce to wall stress and is a major component of water potential), and water is drawn into the cell (see Fig. 1). The result is that the cell enlarges by uptake of water (a reversible process) initiated by yielding of the wall (an irreversible process). Note that wall synthesis, *per se*, is insufficient to cause cell enlargement because it does not induce water uptake. A weakening of the load-bearing network in the wall is needed to reduce turgor and thereby cause water uptake, according to these ideas.

This theory was confirmed with the recent development of practical techniques for measuring wall relaxation (reviewed in Cosgrove, 1987a-c). These relaxation methods showed that when growing cells are constrained to a constant size, turgor and wall stress decreased, as expected, in a time-dependent manner - quickly for rapidly-growing cells and more gradually for slowly-growing cells. These observations contradicted the alternative theory, which postulated that cell expansion begins

with an osmotic uptake of water (driven, say, by uptake of solute) and elastic expansion of the wall, followed secondarily by a breakdown and relaxation of the wall to reduce turgor and wall stress back to the initial conditions (e.g. see Hettiaratchi & O'Callaghan, 1974). With such a mechanism, turgor would remain high, or even increase, when cells were constrained to constant size.

Recent studies have proposed variations of this alternative mechanism for growth control, for example, Katou & Furumoto (1986) suggested that auxin might stimulate growth by activating a membrane pump to transport wall solutes into the cell, thereby enhancing water uptake into the cell (but see Cosgrove & Cleland, 1983 *b*, and Nakahori, Katou & Okamoto, 1991, for evidence against this hypothesis). Likewise, Meshcheryakov, Steudle & Komor (1992) suggested that growth processes may be driven by the coupling of water uptake with phloem solute unloading into the growing cells. These studies focus on the coupling of water uptake with solute uptake, which undoubtedly occurs so as to minimize dilution of cell osmotic pressure during cell expansion. However, these studies fail to appreciate that the unique and essential quality of growing cells ultimately lies in the yielding characteristics of the wall, not in their solute uptake properties. For example, some storage cells accumulate solutes (sugars, salts) when mature, but they do not grow when they take up solutes; rather they elastically swell as they take up water and increase their turgor. The irreversible nature of cell expansion in growing cells depends on the ability of the wall to become loosened and undergo stress relaxation, which then leads secondarily to water uptake. In the long term, many other processes, such as wall and protein synthesis, ion uptake, etc. are needed to keep the growing cell in a healthy state, but these processes seem to be secondarily coupled to cell expansion.

3. The biochemical nature of wall relaxation remains uncertain

Wall relaxation is the biophysical side of what has been termed 'wall loosening'. Wall loosening refers to the rearrangements of load-bearing bonds which must occur to relax (decrease) wall stress and to allow polymer slippage as water is taken up into the cell and the wall expands. The term is deliberately vague because we know rather little about the specific molecular details of wall loosening processes. Considerable speculation has been given- to the idea of wall loosening enzymes, e.g. enzymes which can break or transfer crosslinks within the wall. In this regard, glycanases and transglycosylases have attracted much recent attention (Fry, 1989; Hoson & Nevins, 1989; Hoson & Masuda, 1991; Fry *et al*, 1992; Nishitani & Tominaga, 1992). However, these

* The term 'viscoelastic' is used in two different senses in the materials science literature. I accept the dominant use of the word, as adopted by Ferry (1970), to denote all mechanical properties intermediate between elasticity and viscosity. A minority of authors use the term to denote materials showing only retarded elasticity, without permanent deformation; elastoviscous then denotes materials with permanent deformation. This is not my usage.

Table 1. List of various meanings of 'extensibility'

- (a) Strain/stress
- (b) Strain rate/stress
- (c) Strain at breaking point
- (d) Stress at breaking point
- (e) Δ Length after fixed time under fixed force

enzymatic activities have never been shown to induce prolonged wall extension in isolated walls, as occurs normally during cell growth. Such a demonstration, it seems to me, is essential before an activity is accepted as a wall loosening mechanism with developmental significance.

The vagueness of 'wall loosening' may be a source of confusion, because it is evidently possible to break various bonds in the wall without leading to prolonged wall expansion, as occurs during growth. For example, proteases can break down the structural protein network in the wall and cause a mechanical weakening of the wall, but they do not cause significant extension in living plant cells or isolated walls (Cosgrove, 19896). Likewise, treatments with cellulase (Ruesink, 1969) or supra-optimal auxin (Coartney & Morre, 1980) lead to mechanically weaker walls without increasing wall extension. At present we do not know which bonds are critical for wall expansion, so it may be premature or prejudicial to identify a wall-modifying enzyme as a physiologically significant wall-loosening enzyme without direct evidence that it can cause wall extension.

III. TECHNIQUES FOR MEASURING WALL EXTENSIBILITY

1. Meaning and measurement of 'extensibility'

In the literature on plant growth, wall extensibility denotes the ability of the wall to extend irreversibly during growth.* As described above, this ability ultimately arises from wall modifications that cause stress relaxation of the wall. Unfortunately, the discussion and quantitative measurement of wall extensibility is often complicated by multiple and contradictory meanings of the word 'extensibility'. At this point, I need to introduce the technical definitions for the terms *stress* and *strain*. Strain here specifically means the relative change in length of a material and is a dimensionless quantity (i.e. a strain of 0.1 is a 10% increase in length). Stress is defined as the force divided by the area across which the

* One can speak of elastic extensibility, which refers to the reversible and immediate wall extension that occurs in response to an applied force. Although elastic extensibility depends on the mechanical structure of the wall, especially of the wall matrix, the importance of various wall components and bonds remains largely conjectural and deserves greater attention. Elastic extensibility does *not* show a consistent relationship to the irreversible extensibility that is important for wall expansive growth, despite occasional claims to the contrary.

force is applied. It has units of N m^{-2} or Pa (pressure).

Table 1 lists several meanings of 'extensibility'. It is sometimes defined as (a) the ratio of wall strain to wall stress, that is, the amount of wall deformation in response to applied force. An example of this measure of extensibility is the Instron technique, described below. Sometimes extensibility refers to (b) the *rate* of extension when the wall is under constant stress. This is the sense used in various growth assays of wall extensibility and in 'creep' tests, both described below. Sometimes extensibility is defined as (c) the amount of strain a material can withstand before breaking (Wainwright *et al.*, 1976), or (d) the stress at this breaking point. Still another version of extensibility denotes (e) the amount of deformation occurring upon application of a given (arbitrary) force for a given (arbitrary) short period of time.

These measures of extensibility have different units and measure somewhat different properties of the wall. The relevant question for our purposes is, do they all serve as equivalent estimates of the wall yielding properties that govern growth? This review is my attempt to answer this question. My short answer is that these various techniques can contradict one another and in fact measure very different properties of the wall. This discrepancy points to an important issue often obscured, namely that wall extensibility is not determined exclusively, or even principally, by the intrinsic mechanical properties of the wall. There is considerable evidence to indicate that walls can change their extensibility (growth) properties without a significant change in mechanical (viscoelastic) properties. Thus, mechanical assays may be misleading if they are taken as simple measures of wall growth properties, but they do prove useful in assessing changes in wall structure.

In the section below I describe the theory, practice, interpretation, and caveats of various techniques in common use for measuring wall extensibility, I begin with methods employing living tissue - where the results can be related to the biophysical theory of plant cell expansion - and then follow with an assessment of various techniques using isolated walls, where the relationship to this theory is lacking.

Before describing these methods, it is important to emphasize that these methods only make sense when applied to tissues which are relatively homogeneous with regard to growth rate. Most tissues, e.g., coleoptiles, stems and roots, are spatially inhomogeneous in this regard and exhibit regions of maximal growth surrounded by more slowly growing cells. The experimentalist is usually faced with a compromise between working with a tiny piece of tissue, which encompasses a small growth gradient or working with a large piece of tissue which is usually easier to manipulate and measure but may contain

cells with different growth properties. For this reason a kinematic analysis (Silk, 1984) of the growth pattern is a prerequisite to all of the following techniques. Moreover, because these methods take minutes to hours to complete, the growing tissue should ideally be in steady-state growth (at least relative to the time scale of the measurement) before the onset of the measurement. Additional considerations that apply to multicellular tissue are reviewed by Cosgrove (1986).

2. Living tissues

(a) *Techniques used.* Three different techniques make use of living tissues to obtain estimates of wall yielding properties. The first, and classical, method (a) relates growth rate to turgor pressure, with the assumption that plant cell growth is turgor-dependent because the rate of wall yielding is a function of wall stress (generated by turgor). In other words, the turgor-sensitivity of growth lets us quantify wall yielding properties and relate them to the biophysical theory of plant cell growth (described below). The second, and newer, method (b) measures wall yielding properties from the kinetics of wall stress relaxation. Such relaxation can also be related to the biophysical theory of growth. The third method (c) assesses wall properties by measuring the growth response to mechanical forces (usually tensions) applied to the growing tissues. These three methods are based on different principles and make different assumptions about the growing tissue, yet they have two characteristics in common: they use living tissues in which the walls are still subject to modification by energy-dependent metabolic processes, and they gauge wall properties from the response of the growing tissue to some perturbation. The nature of the perturbation is different, however, in the three cases.

Analyses of wall properties are most useful when they are related to a quantitative model of plant growth. In this context, the most useful model of plant cell growth originated with Lockhart (1965) and has been extended in various ways in the intervening years (Molz & Boyer, 1978; Silk & Wagner, 1980; Cosgrove, 1981, 1985; Ortega, 1985; Meshcheryakov *et al.*, 1992). Despite its critics (e.g. Hohl & Schopfer, 1992a), this biophysical model remains a convenient framework for analysing plant growth. I will treat the simplest formulation of the model, which is adequate for this discussion, but readers should see the papers cited above for additional complexities, which are also reviewed in Cosgrove (1993). In this model, the growth rate (r) is given by:

$$r = \phi(P - Y) \quad (1a)$$

$$= \frac{\phi L}{\phi + L} (\Delta\pi - Y) \quad (1b)$$

(see Table 2 for units and for further details Cosgrove, 1986). The first expression (1a) says that growth rate (r) depends on the product of ϕ , the wall yielding coefficient, and $(P - Y)$, turgor (P) in excess of the yield threshold (Y) or minimum turgor required for growth. The parameter ϕ is often called wall extensibility, but it should be noted that this model dissects wall yielding properties into two parameters (ϕ and Y), each of which measures the ability of the wall to extend (yielding ability goes up as ϕ goes up and as Y goes down). The wall parameters in this equation are expressed in terms of sensitivity of growth to cell turgor rather than wall stress *per se* for practical reasons: turgor is readily measured and modified and can be directly related to relevant hydraulic parameters, whereas wall stress is a complex function of turgor, wall thickness and cell geometry, and is not easily measured. Indeed, it is probably not uniform across the thickness of the wall (Richmond, Metraux & Taiz, 1980; Richmond, 1983). Thus the turgor (P) term in (1a) is a substitute for wall stress, which is envisioned to be the real physical force driving wall yielding. Note also that although this equation asserts that turgor is the driving force for wall expansion, this does not mean that turgor is the major control factor; it simply states that cell expansion is sensitive to turgor (i.e. is a partial function of turgor). Thus we may modulate growth by modulating turgor, but it is not necessarily true that growth modulation is accomplished *in vivo* by turgor modulation (this, in fact, occurs seldom, if ever).

Expression (1b) is a more elaborate formulation which adds the process of water uptake into the model. The additional terms refer to the osmotic pressure difference across the plasma membrane ($\Delta\pi = \pi_i - \pi_o$) and the ease with which water enters the growing cell (L , hydraulic conductance). This formulation allows for restriction of growth rate by water transport processes. If L is much larger than ϕ , then (1b) reduces algebraically to (1a) (that is, $\phi L / (\phi + L)$ approaches ϕ and P approaches $\Delta\pi$).

This biophysical model of growth has served as a useful framework for analyzing growth responses of plants by allowing the dissection of growth into parameters governing wall expansion (ϕ , Y) and water uptake (L , $\Delta\pi$).

(b) *Growth rate versus turgor.* This technique measures wall extensibility by gauging the turgor sensitivity of growth and relating the results to eqn. (1a, b). In the conventional procedure (Cleland, 1976; Hohl & Schopfer, 1991), this is accomplished by incubating excised tissues in solutions of varying concentrations of impermeant osmoticum, such as mannitol or polyethylene glycol, to modulate turgor pressure. After a period of time - minutes to hours - growth is measured and plotted against cell turgor, or failing this, then against the water potential

Table 2. List of major symbols and their units

r	Relative growth rate; % h ⁻¹ or h ⁻¹
P	Turgor pressure; bar
ϕ	Wall yielding coefficient, or growth-specific extensibility; bar ⁻¹ hr ⁻¹
Y	Wall yield threshold; bar
$\Delta\pi$	Difference between inside and outside osmotic pressures; bar
L	Growth-specific hydraulic conductance, bar ⁻¹ h ⁻¹ . For single cells, this is equal to membrane area divided by cell volume times membrane hydraulic conductivity ($L_p A/V$); for tissues it incorporates the geometry of the pathway for water flow and is normalized to volume.
ϵ	Volumetric elastic modulus of the cell; bar
$\Delta\psi$	Difference between inside and outside water potentials; bar. For tissues, this value needs to be corrected for apoplastic solutes which may reduce water potential without inducing a concomitant water flow (Cosgrove & Cleland, 1983 a).
T_0	Minimum relaxation time; s

or osmotic pressure of the incubating solution. In some variants of this technique, turgor is modulated not by an osmoticum, but by letting plants dry down (Bunce, 1977; Eastham, Oosterhuis & Walker, 1984;

Matthews, Van Volkenburgh & Boyer, 1984). Then growth rate and turgor (or water potential) of the tissue are measured over short intervals during the dry-down period. In a third variant of this method, the xylem pressure is raised or lowered by controlling the pressure of a chamber enclosing the roots (Cosgrove & Green, 1981; Termaat, Passioura & Mums, 1985). Okamoto *et al.* (1989, 1990) used a variant of this procedure to modulate xylem pressure in excised segments and recorded growth responses to these pressure changes. In a fourth variation, the pressure is modulated in a chamber enclosing the growing tissue (Boyer, 1968; Cosgrove, 1988). With all of these methods, the principal idea is to measure the sensitivity of growth to changes in turgor.

If the growing tissue behaves according to the ideal, modelled in eqn (1 a, b), then growth rate should fall linearly as turgor pressure falls. When growth is measured as a relative rate (%/h) and turgor is directly measured, the slope of the line measures the wall yielding coefficient (ϕ), or 'extensibility', used in eqn (1a, b). The x-intercept gives the yield threshold (Y), the minimum turgor needed for growth.

Figure 2 shows some examples from the literature. In many cases the growth curve indeed appears fairly linear with turgor (Fig. 2a, b) and estimation of a

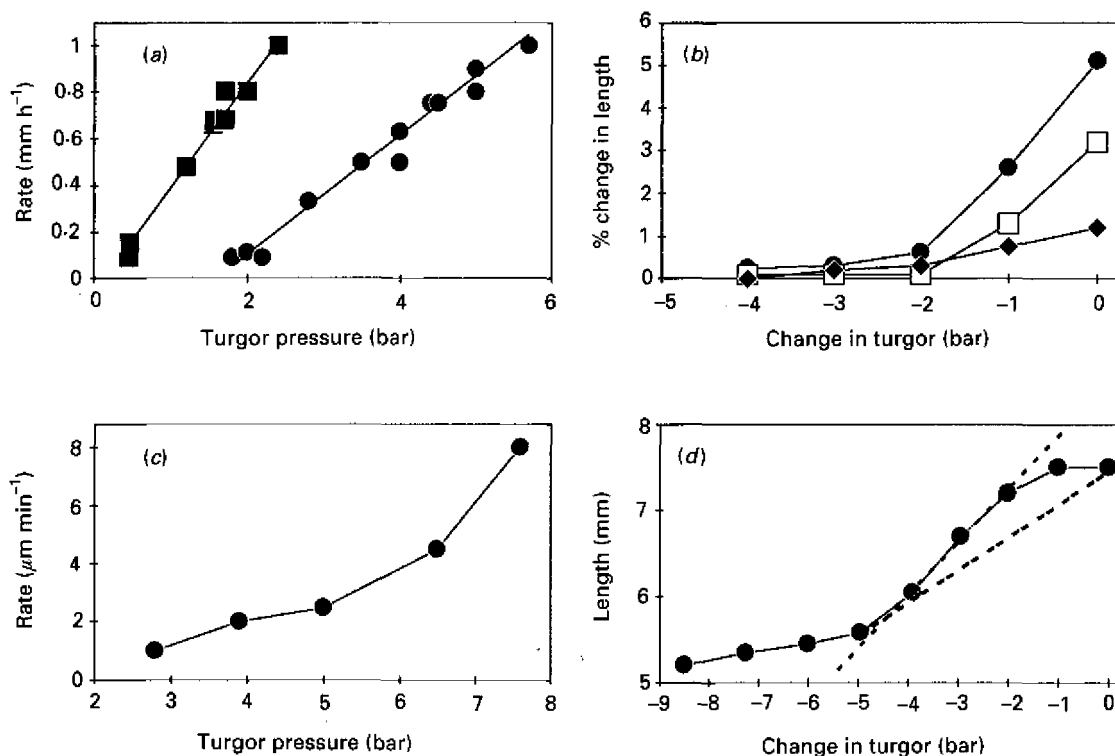


Figure 2. Some examples of turgor-dependent growth, (a) Data for soybean leaf elongation from Bunce (1977). The two lines represent plants grown under varying degrees of water stress in chambers (circles) and outdoors (squares), (b) Growth of excised oat coleptiles treated with different concentrations of auxin, as a function of external osmoticum [shown as nominal change in turgor; from Cleland, 1976]. (c) Nonlinear (increasing) dependence of wheat root growth at 15 °C on turgor (Pritchard *et al.*, 1990). (d) Nonlinear (decreasing) growth of excised pea epicotyls as a function of external osmoticum [shown as nominal change in turgor; from Cosgrove *et al.*, 1984]. The broken lines illustrate two different ways to estimate the slope of the line (wall 'extensibility').

slope presents little difficulty. In other instances marked nonlinearities appear in the growth-turgor curve (Fig. 2 c, d). These are of two principal types. Some curves become steeper as turgor is increased, whereas others become flatter at higher turgor. If we define ϕ as the local slope of this line, then it is evident that its value varies with turgor pressure. In these cases the notion of constant ϕ is inaccurate and needs to be expanded to include some measure of this nonlinearity. Green, Erikson & Buggy (1971) found that growth rate in *Nitella* inclined more steeply as turgor was increased, and they expressed this nonlinearity as a power function, i.e. $r = \phi(P - Y)^n$ where the data best fit a value of $n = 1.4$. Another way of treating this nonlinearity would be to consider ϕ to be an increasing function of turgor, rather than a constant. Lockhart (1965) suggested that auxin might stimulate growth by changing wall extension from a linear function of turgor to a nonlinear function. In conformity with this idea, Cleland (1959) found that auxin-treated coleoptiles exhibited a steeper, more nonlinear relationship to turgor pressure.

Nonlinearities of the second type (Fig. 2d), in which growth rate reaches a plateau at high turgor pressure, present another kind of difficulty. Here the problem is that the local slope of the line is zero; that is, growth at high turgor is not sensitive to small changes in turgor. Although one might then calculate that the yield coefficient ϕ is zero because the slope is zero, this contradicts our intuitive concept of extensibility. Perhaps a better interpretation is that turgor pressure is sufficiently high that it does not limit wall expansion. This might come about simply because of an upper limit on the amount of polymer shearing in response to any given loosening event in the wall or because of the action of a feedback mechanism limiting the maximum growth rate (Cosgrove, 1988, 1989a). This is not to say that growth is entirely independent of turgor, because as turgor is reduced beyond the plateau region, growth indeed falls. The measure of extensibility in this case might best be taken from the linear region of the curve. Another measure might be to take the 'chord slope' of the line, i.e. the slope of the line drawn between two points, at the yield threshold and at maximum turgor (see Fig. 1 d). This is in effect the method used by Nonami & Boyer (19906) to estimate ϕ from water potential data. The value obtained by this approximation is an average, as opposed to a local, measure of ϕ and underestimates the maximum ϕ .

One consequence of this second type of nonlinearity is that growth cannot be limited by hydraulic conductance because it is insensitive to small changes in water potential and turgor pressure. This type of nonlinearity probably accounts for occasional reports of turgor-independent growth (Shackel & Matthews, 1987; Termaat *et al.*, 1985; an extreme example in

Chara observed by Zhu & Boyer, 1992). Because plants often show a rather complicated growth response to step changes in turgor or wall stress (Marshall & Penny, 1976; Kuzmanoff & Evans, 1981; Cosgrove, 1988), it is likely that a complicated adaptation mechanism underlies this type of limited turgor independence.

Another expedient for dealing with nonlinearities of the types described above would be to abandon altogether a numerical estimate of ϕ and instead simply compare graphs of the growth-turgor relationships of various treatments. One loses some quantitative aspects of growth analysis with this expedient, but for many purposes it might be more revealing. Nonlinearities thus complicate our measurements of extensibility because a single number (slope) does not adequately capture the turgor dependence of growth.

It should also be noted that nonlinearities sometimes appear as artifacts. For example Pritchard, Wyn Jones & Tomos (1990) measured a nonlinear growth response of intact wheat roots to external mannitol, but when turgor pressure was measured with the pressure probe in parallel experiments, growth was quite linear with respect to turgor. The apparent discrepancy between the two data sets probably arose because of changes in the osmotic pressure of the root cells due to water loss (upon mannitol incubation) and solute accumulation. Similarly, Matthews *et al.* (1984) measured a nonlinear growth dependence on water potential in sunflower leaves, but growth was linear when plotted against turgor pressure. These results emphasize the importance of measuring turgor in the growing tissue and avoiding the assumption that turgor and water potential change in a 1:1 fashion.

The turgor-growth relation can also give estimates of the yield threshold (Y). In the ideal situation, Y is estimated as the turgor value where the growth rate attains a value of zero and there is a marked kink in the curve. In practice, the growth rate often does not stop abruptly as external osmoticum is increased, but exhibits a more gradual transition to lower rates (e.g. Fig. 2c, d). It is common practice to estimate Y in these instances as the point of intersection of two straight regions of the curve. The lack of a distinct kink is due at least in part to the fact that elastic shrinkages can be substantial in this part of the curve. Cleland (1959) showed that when elastic and irreversible changes in coleoptile length are separated, the yield threshold for growth becomes more distinct. He also showed that this yield threshold for growth occurred at a turgor well above zero bar.

Recent studies by Hohl & Schopfer (1992a) rediscovered a curious phenomenon, first reported by Cleland (1959). When the turgor of coleoptile segments is reduced to Y , there is a gradual loss of elastic extensibility. This may signify the continued operation of wall crosslinking processes in the

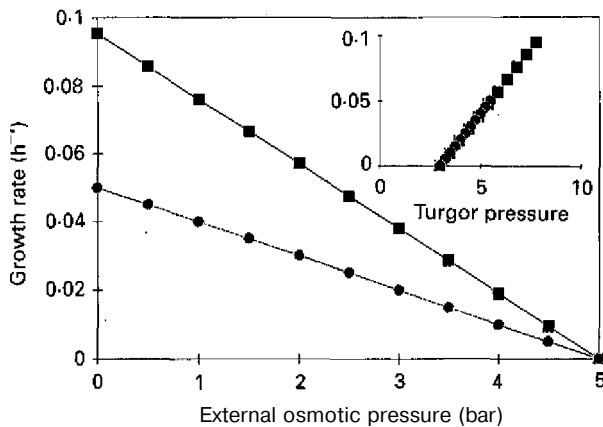


Figure 3. Theoretical example of growth rate as a function of external osmotic, assuming high hydraulic conductance (squares', $L = 20\phi$) or low conductance (circles; $L = \phi$). Note that the two cases exhibit identical turgor dependence (inset). The reason for the different sensitivities to external osmoticum is that in the low-conductance case a change in external osmotic pressure has a diminished effect on turgor pressure.

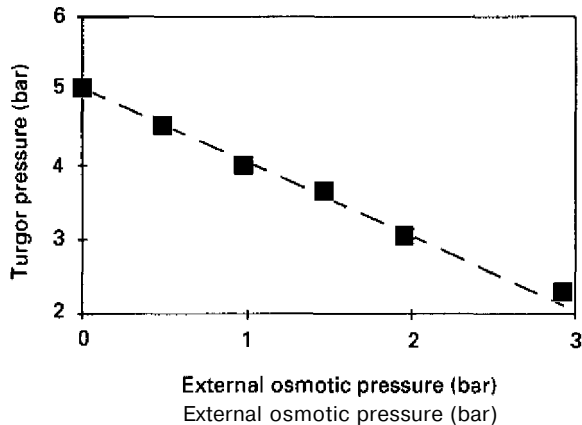


Figure 4. Relationship between turgor pressure and external osmotic pressure in excised pea epicotyls. Segments were incubated with $10 \mu\text{M}$ indole acetic acid to stimulate growth (as in Fig. 4-d) in a graded series of mannitol solutions. Turgor pressure was measured in 5-8 segments with the pressure probe (Cosgrove, 1985). The broken line shows an ideal slope of 1. The least-squares fit was 0.94. The deviation from unity is mostly due to elastic effects (i.e. $\epsilon/(\epsilon+\pi)$ is approximately 0.94).

absence of wall loosening at reduced turgor. Biochemical analyses of wall structure might prove useful for identifying the nature of this hypothetical wall stiffening process.

This method for measuring wall extensibility has the advantage that the results can be related to the biophysical theory of plant growth [eqn 1 (*a*, *b*)] and it is relatively easy to carry out. However, there are several limitations and cautions to be aware of. The techniques using excised segments work well only for tissues which continue to grow and exhibit responses after excision. Hence the method has been most useful in the study of hormones applied to excised tissues. Some responses, however, are lost or so attenuated after excision that the results are of

little value. Turgor can sometimes be manipulated without excising the tissue, e.g. roots can be exposed to an osmoticum (Pritchard *et al.*, 1990) or pressurized in a chamber (Termaat *et al.*, 1985). Pressure chamber methods have some advantages over the use of external osmoticum, e.g. fast action, no boundary layer problems, and no penetration of an osmoticum.

There is another complication associated with these procedures. Normally one assumes that changes in turgor are exactly equivalent to the changes in external osmotic pressure. Then the slope of the line relating growth rate to external osmotic pressure is used to estimate the yield coefficient (ϕ). However, this is valid only if the walls are relatively stiff so that the protoplast does not lose much water and become more concentrated as external osmotic pressure increases: in other words, the volumetric elastic modulus of the cell (ϵ) must be large, i.e. $\epsilon \gg \pi$.

Also it is important that the hydraulic conductance be large so that the water potential gradient within the tissue (needed to sustain water uptake by the growing cells) is small relative to the effective turgor ($P-Y$). There are several ways of assessing these conditions (reviewed in Cosgrove, 1986, 1987*a*); the easiest is to measure osmotically induced swelling or shrinkage. It should be fast (1-2 min half-time) and small if these conditions are met. Otherwise, changes in turgor may be less than the changes in external osmotic pressure, by the approximate factor $\epsilon L/[(\epsilon+\pi)(\phi+L)]$. I will simplify our discussion by assuming that $\epsilon \gg \pi$, so that $\epsilon/(\epsilon+\pi) = 1$; then $\epsilon L/[(\epsilon+\pi)(\phi+L)]$ reduces to $L/(\phi+L)$. If L and ϕ are equal, this factor is 1/2. In such a case, the slope of the line will not be ϕ but rather the ratio $(\phi L)/(\phi+L)$. Figure 3 shows a theoretical example of this situation.

I have attempted to assess this problem by using the pressure probe to measure the turgor pressure of excised pea epicotyls which were incubated with auxin and various mannitol solutions. Although mannitol has been reported to penetrate oat coleoptile segments (Hohl & Schopfer, 1991) this is not the case with pea epicotyls, at least over short term exposures (Cosgrove & Cleland, 1983*a*). As shown in Figure 4, there is a linear relationship between external osmotic pressure and cell turgor pressure. By least square fit, the slope is 0.94, which is close to the ideal of unity. By equating the slope with $(\phi L)/(\phi+L)$, we calculate that L is at least 16 times larger than ϕ . In fact, this is an underestimate because we ignored the term $\epsilon/(\epsilon+\pi)$, which can account for most, if not all, the deviation from unity. This result is consistent with earlier estimates of L/ϕ in intact pea seedlings (Cosgrove, 1985; Cosgrove & Cleland, 1983*b*). Thus the assumption of near equilibrium in water potential is verified in this case.

This last problem is avoided in a variant of the technique, in which the pressure probe is used to regulate cell turgor pressure directly, and growth

responses are recorded (Ortega, Zehr & Keanini, 1989; Ortega *et al.*, 1991; Zhu & Boyer, 1992). So far this method is only feasible with large-celled organisms such as *Phycomyces* and *Chara*, where hydraulic limitations can be shown to be minimal (Cosgrove, 1986), so this advantage is not significant *vis-a-vis* hydraulic limitations. *Phycomyces* has the additional complication of a 'stretch' response in which sudden increases in wall stresses cause a reactive inhibition of cell growth (Dennison & Roth, 1967; Ortega *et al.* 1991).

A last issue that needs to be addressed concerns the time for the growth measurement following the change in turgor. With pressure-chamber and pressure-probe methods, the change in turgor is accomplished quickly and so the growth change may also be recorded quickly, within a few minutes typically. With methods employing external osmotic, somewhat longer times are required to reach steady state in multicellular tissues, and often growth rates are assessed over rather long periods, typically several hours. This improves the signal-to-noise ratio for the growth measurements, but limits the temporal resolution of the method. Moreover, many growing tissues show a time-dependent adjustment in growth rate, such that the turgor-growth curve is much steeper (growth more sensitive to turgor) when growth is assayed quickly (minutes) than when assayed over hours or days (Green *et al.*, 1971; Green, Bauer & Cummins, 1977; Cosgrove, 1989a; Serpe & Matthews, 1992). This behavior is also evident in the stress-relaxation time courses of some plants (see below) and seems to be more prevalent in intact plants than in excised sections (Cosgrove, unpublished observations). In *Chara*, adaptation can fully compensate for turgor changes until turgor is reduced below the yield threshold (Zhu & Boyer, 1992). One way of dealing with such adaptations has been to distinguish between 'instantaneous' (short term) and ultimate (long term) yield thresholds and yield coefficients (Green *et al.*, 1977; Taiz, 1984; Taiz & Richmond, 1984). The conversion between instantaneous and ultimate values for these parameters remains to be clarified, but is probably a widespread mechanism of growth adaptation and compensation for perturbations. The significant point for our purposes is that different values for these parameters may be obtained, depending on the time scale of the measurement, so caution is needed in comparing parameters obtained with different time measurements.

(c) *In vivo relaxation.* With the previous technique, one estimates wall yielding properties from the sensitivity of growth to changes in turgor pressure. *In vivo* stress relaxation techniques depend on a different principle, namely that in the absence of water uptake and cell expansion, wall yielding inevitably causes wall stress to decay, or to relax.

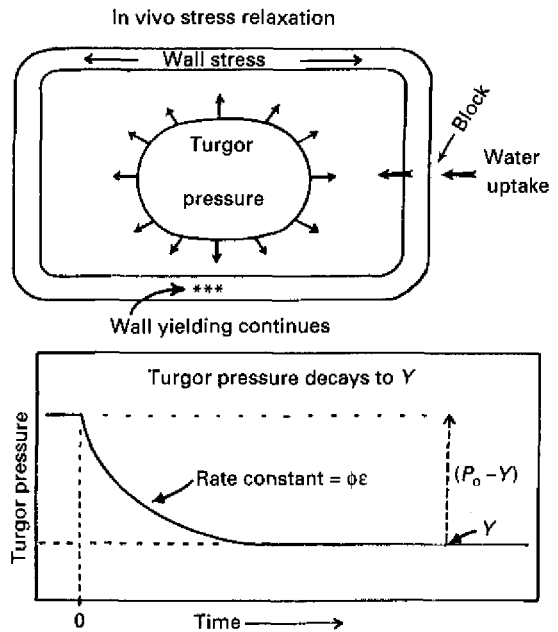


Figure 5. Procedure and dynamics of *in vivo* stress relaxation. The top panel shows the conditions for stress relaxation, namely a blockage of water uptake at the same time that wall yielding continues. The bottom panel shows the expected time course for relaxation, measured as a decrease in turgor pressure. Turgor will relax down to the yield threshold (Y) with a rate constant determined by the product of the elastic coefficient (ϵ) and the yielding coefficient (ϕ , 'extensibility'). If these two parameters are not constants, the time course will deviate from an exponential decay.

Because wall stress is the counterforce to turgor, wall stress relaxation under these conditions leads to reduction in cell turgor pressure and water potential (Cosgrove, 1985, 1987c). Thus, without any formal analysis, we should expect the magnitude and time course of stress relaxation to provide some measure of the yielding properties of the wall. If wall yielding is accurately described by eqn (1a, b), we would expect an exponential decay of turgor pressure to the yield threshold (Y), with a rate constant given by $\phi\epsilon$, where ϵ is the cell volumetric elastic modulus (Cosgrove, 1985, 1986). Figure 5 depicts this relaxation. The initial rate of relaxation, and indeed the relaxation rate at every point along the curve, should ideally be given by the expression $\epsilon\phi(P - Y)$. As relaxation proceeds, turgor (P) decreases and thus the rate of relaxation slows and eventually stops as P asymptotically approaches Y .

Several relaxation methods have been devised in recent years. If a piece of growing tissue is excised from the plant and sealed into a psychrometer chamber to measure water vapour concentration, then one can measure the time-dependent decrease in water potential which ensues as a result of wall relaxation of the tissue inside the chamber. Cosgrove, Van Volkenburgh & Cleland (1984) first used this method to verify the prediction that wall relaxation would substantially reduce cell water potential and

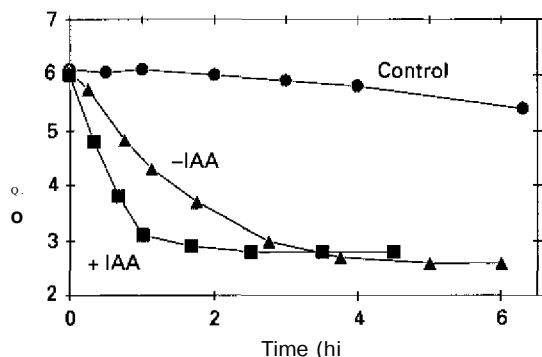


Figure 6. Relaxation of excised pea epicotyls treated with and without auxin (from Cosgrove, 1985). Segments were preincubated on solution without auxin, then given a brief exposure to solution+ indole acetic acid, blotted dry, and isolated from water inside a sealed chamber. Turgor pressure was measured with the pressure probe. Control segments were treated in the same way, except a drop of water was placed on the end of the segment to prevent relaxation.

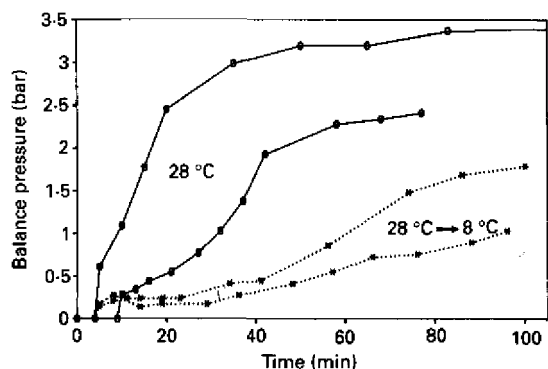


Figure 7. Relaxation of excised soybean hypocotyls at warm and cold temperatures (from Cosgrove, 19876). The apical ends of seedlings were sealed into an oil-filled pressure chamber, excised and the balance pressure was measured as relaxation proceeded at 28 °C (solid lines). The dotted lines show greatly diminished relaxations when the temperature of the tissue was dropped to 8 °C after excision. Note that this experiment also rules out a large value for growth-sustaining $\Delta\psi$ in the intact plants, because a high balance pressure would have been recorded shortly after excision and cooling.

reduce cell turgor to the yield threshold. They found that pea stem tissue stimulated to grow with auxin or with fusicoccin exhibited a fast relaxation to a steady turgor pressure of about 3 bar (0.3 MPa), whereas slowly growing tissues (pre-incubated on water to inhibit growth) showed much slower relaxation. This was also confirmed with parallel turgor pressure measurements with the pressure probe, as shown in Figure 6, using excised tissue sealed against evaporation. Significantly, tissues treated with and without auxin and fusicoccin relaxed to the same steady turgor pressure, indicating that these treatments did not alter the yield threshold. Moreover, the tissues relaxed to the same turgor pressure (3 bar), even when this was attained at different water potentials.

This provided direct experimental evidence that it was turgor pressure, not water potential *per se*, which governed the rate of relaxation of the wall.

Wall relaxation has also been observed with an adaptation of the standard pressure chamber technique. Milburn (1979) gave a preliminary description of an oil-filled pressure chamber used to observe a gradual decrease in the water potential of excised castor bean leaves, a decrease he attributed to growth. Unfortunately, the detailed results have never been published, to my knowledge, Cosgrove (19876) measured the time-dependent decrease in water potential of excised soybean hypocotyls and showed that cold treatment prevented this reduction in water potential (i.e. wall relaxation), just as it prevented growth. These results are shown in Figure 7.

The stress relaxation methods described above provided direct and independent confirmation of the basic tenets of the growth model by Lockhart, namely, that water uptake and wall yielding are separable processes which are linked by their common element, turgor pressure. These relaxation methods provided the first practical method for separating or unlinking the two processes and observing the predicted consequences. They also provided the first practical methods for studying wall yielding without concomitant complications from water uptake. However, these methods rely upon the simple expedient of tissue excision to prevent water uptake and growth. This is fine as long as excised tissues are suitable for experimental study, but excision interferes with many growth responses. We discovered just such a problem in a study of gibberellin action on pea stems (Cosgrove & Sovonick-Dunford, 1989). In some particularly sensitive tissues, growth may cease entirely upon excision because wall yielding ceases. This problem can be circumvented by a newer relaxation method, termed the pressure-block technique (Cosgrove, 19876), which has the important advantage of measuring relaxation in intact tissues.

The pressure-block instrument is shown in Figure 8. By applying gas pressure to the growing region, one can modulate the driving forces for water uptake and thereby modulate growth. Stress relaxation is induced by applying the minimum pressure to the growing tissue necessary to keep the tissue from enlarging. The instrument consists of a custom-made pressure chamber, inside of which a position transducer is installed to monitor growth. So far, we have used the instrument for studying stem growth, but its use with other growing tissues is also feasible. Typically, we seal the growing region of the stem into the chamber and attach the position transducer to the apical part of the stem. Relaxation is initiated by applying a small pressure to the chamber, so as to cancel the internal water potential gradients (xylem to epidermis) that draw in water for growth. For

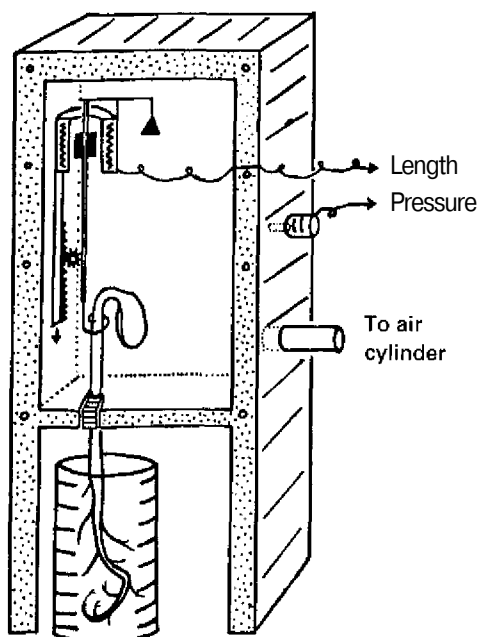


Figure 8. Diagram of the pressure-block instrument. The growing region of the stem is sealed into the chamber (usually with a thin film of epoxy as sealant) and attached to an internal position transducer to monitor stem length. Relaxation is induced and monitored by applying the minimum pressure required to keep the stem from elongating (from Cosgrove, 1987b).

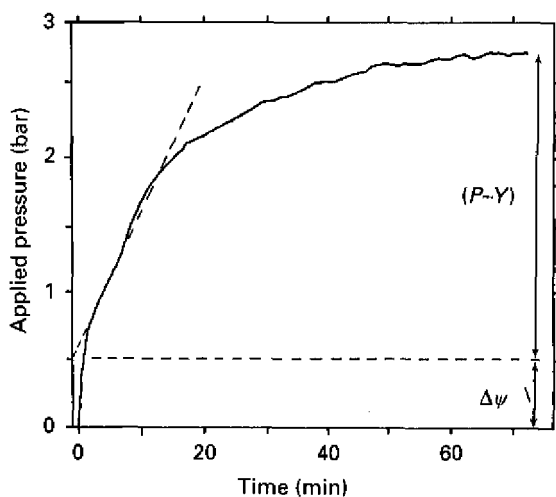


Figure 9. An example of stress relaxation in a slowly-growing pea epicotyl, measured with the pressure block technique. The water potential difference which drives water uptake ($\Delta\psi$) is estimated by extrapolating the initial rate of relaxation (at 2-4 min, after $\Delta\psi$ has collapsed) back to zero time (dashed line). The remainder of the chamber pressure indicates the degree of relaxation and gives an estimate for $(P-Y)$.

most seedlings, the size of this gradient appears to be a fraction of a bar and is cancelled in 1-2 min (Cosgrove, 1987c; Kigel & Cosgrove, 1990). Thereafter, water does not enter the tissue to cause expansion except by further loosening and relaxation of the wall. When the chamber pressure is finely

adjusted so as to keep stem length constant, one observes a gradual increase in the chamber pressure required to block growth (Fig. 9). The rate of pressure increase is at first rather high ($1-10 \text{ bar h}^{-1}$ is typical in rapidly growing etiolated seedlings), but slows down and eventually stabilizes (or nearly stabilizes), typically in less than 1 h for rapidly growing seedlings.

With the pressure-block technique, the final chamber pressure attained ought to be equal to $(P-Y)$ plus $\Delta\psi$ (the internal water potential gradient sustaining growth). As described above, $\Delta\psi$ is usually small ($< 10\%$ of the final chamber pressure) and dissipates within a couple of minutes of the start of the growth blockage. It can be estimated (dashed line in Fig. 9) by extrapolating the relaxation rate (usually at the 3-5 min interval, when $\Delta\psi$ has collapsed) back to time zero (Cosgrove, 1987b). If P is known from other measurements, e.g. with the pressure probe technique, then the value for Y can be directly estimated. Alternatively, the tissue may be removed from the pressure block instrument, submerged in oil to prevent evaporation and quickly measured with the pressure probe to get a direct value for the yield threshold (Cosgrove, unpublished).

Relaxations induced with the pressure-block technique have generally been similar to those measured with the other relaxation techniques, except that the relaxations are often faster and larger than those attained using excised tissues (Cosgrove, 1987b; Kigel & Cosgrove, 1990). We have seen this difference enough times now that I believe it is a general and significant difference between intact and excised tissues. With the pressure-block method, one can attain better temporal resolution of relaxation than is possible with other relaxation methods, and the kinetics are often found to be more complicated than a simple exponential decay. Rather, the relaxation rate accelerates transiently a few minutes after the start of relaxation, evidently as a response to the growth blockage. There are some indications that this acceleration might be due to a rapid decrease in the value of Y , analogous to that reported by Green *et al.* (1977) and Green & Cummins (1974). This phenomenon seems to be reduced or retarded in excised tissues. When excised tissues are measured with the pressure-block technique, relaxation is attenuated to a similar extent as observed with the psychrometric and pressure-probe methods (Cosgrove, unpublished). Thus, excision reduces the ability of some growing tissues to relax their walls.

This discovery may open the door for further study of the characteristics and mechanism of relaxation. At present, we can say that the wall parameters ϕ and Y are not simple, time-invariant parameters, but exhibit signs of a more complicated, underlying process. Interestingly, some genetic lines

of dwarf peas lack this complexity (Behringer *et al.*, 1990) and may prove useful in dissecting the process.

In-vivo stress relaxation methods have thus proved important in analyzing the basic mechanisms of growth and in characterizing the yielding properties of walls. In some cases, the methods have given informative results where other techniques proved unfeasible or even misleading (Cosgrove, 1988; Behringer *et al.*, 1990). For example, in cucumber seedlings blue light causes a rapid inhibition of growth, but this is largely lost upon excision of the hypocotyl. Thus the standard technique of segment incubation on different osmotic solutions was not feasible. Measurements of the mechanical extensibilities of the wall by the Instron technique (see below) indicated negligible effect of blue light, yet the pressure-block method revealed a substantial reduction in the rate of wall relaxation, corresponding to a large reduction in ϕ .

Relaxation methods are limited by problems associated with tissue excision (for all but the pressure-block methods) and the technical sophistication needed for some of the techniques. The pressure-probe method requires tissue with relatively large cells that do not leak and that do not plug the capillary tip of the probe. Complete wall relaxation may require 15 min to more than 1 h, which puts limits on the temporal resolution, e.g. fast growth responses cannot be followed in full detail by these methods. Furthermore, some plants apparently react to the blockage of growth (a precondition for all relaxation methods) by accelerating wall relaxation processes. Because we do not fully understand the processes involved in this reaction and their relation to unperturbed growth, it is possible that these reactions may complicate or hide the underlying process. Further elucidation of this growth adaptation response is needed. This complexity notwithstanding, the wall relaxation method provides an important assay of wall yielding properties, a method which is fundamentally different from other techniques described here and one which assays the ability of a cell to loosen its wall.

(d) *Applied force.* This method is based on the notion that growing walls extend irreversibly in response to the forces acting on them. When tensile forces are applied to growing tissues, the wall expands at a faster rate, at least temporarily until the tissue adapts with compensatory responses. Therefore, wall yielding is assayed as the ratio of extension response to applied tension.

The method makes the tacit assumption that hydraulic conductance is large and not limiting for growth. If this is true, then the extension response to applied force is indeed determined by wall properties. Otherwise, extension may be partially limited by water uptake into the extending cells and the extension rate is then a function of both water

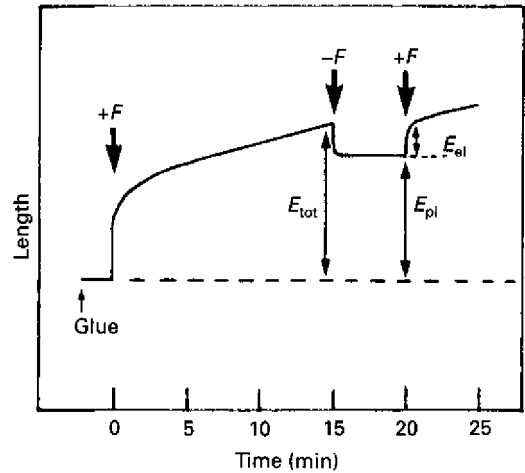


Figure 10. Illustration of elastic and plastic deformations by the applied stress technique (after Kutschera & Schopfer, 1986a). Maize coleoptile segments were glued into an extensometer and the deformation in response to applied tension was broken down into a reversible (E_{el}) and an irreversible (E_{pl}) component as shown. Note that E_{pl} depends on the duration of force application.

transport and wall properties. This assumption has not always been recognized in studies which employed this method, and has led to some inconsistencies between results and conclusions (see below).

The applied force technique has been invented several times, with minor variations. In its most primitive form, Heyn (1932) and later Lockhart (1960) placed a growing stem or coleoptile horizontally and hung a weight on the end. The tissue bent downwards under the action of this weight and after a short time this deformation was measured as the angle made by the stem; then the weight was removed and the residual, or irreversible, deformation was measured. These deformations provided an *ad-hoc* estimate of the elastic (reversible) and irreversible 'extensibilities' of the whole tissue, which are assumed to be closely related to the analogous wall properties. The results of these assays led Heyn to argue that auxin altered wall plasticity and Lockhart to conclude a similar action by light.

Kutschera & Briggs (1987, 1988) and Kutschera & Schopfer (1986a, b) used a more sophisticated version of this method. They attached growing stems or coleoptiles to an electronic displacement transducer and measured the time course for extension upon application and removal of a tensile force to the tissue (Figure 10). From the extension responses, they estimated an elastic and an irreversible ('plastic') change in length, which they took as measures of wall properties. The results confirmed that changes in wall 'plasticity' were associated with the action of various growth hormones. Hohl & Schopfer (19926) have examined more closely the 'plasticity' measured with this technique and have concluded that it is not a true

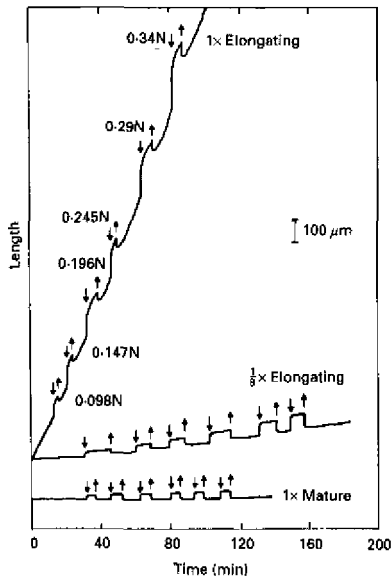


Figure 11. Deformations in response to a series of applied forces in soybean hypocotyls in a wall-watered ('1 x Elongating'), water-stressed ('1/8 x Elongating') or mature state (after Nonami & Boyer, 1990 a). Elastic and plastic components were dissected in a manner similar to that shown in Figure 10. Plastic deformations were found to be very sensitive to the growth state of the hypocotyl. Note that steady state elongation rate was negligibly affected by the applied force.

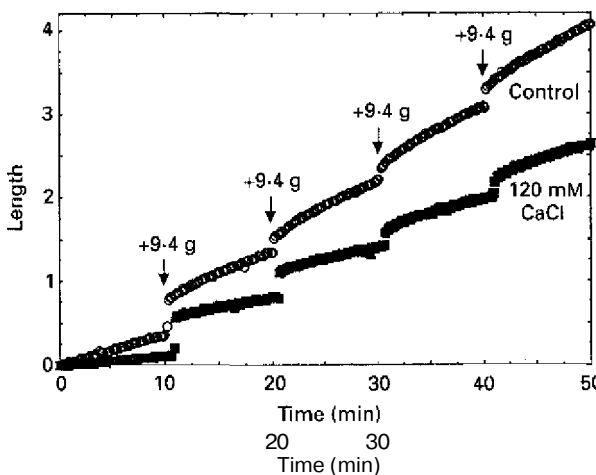


Figure 12. Deformations in response to a series of applied forces in maize leaves under low and high salinity (after Cramer & Bowman, 1991). Note that, as the forces are increased (in units of 9.4 gram force), the rate of extension increases,

plastic deformation, but a type of reversible viscoelastic deformation which shows retarded elasticity (delayed reversible deformations) and a hysteresis loop upon loading. Hysteresis in this case means that the load-extension curve obtained with increasing loads is different from that obtained with decreasing loads. Retarded elasticity and hysteresis are quite common in polymeric materials. Their results, showing wall viscoelasticity changes after auxin treatment, give additional evidence that auxin leads to a significant alteration in the bonding arrangements in the wall.

Recent studies have advanced this method to examine the effect of water stress on wall properties. Nonami & Boyer (1990a) applied an increasing series of tensions to the hypocotyl of soybean seedlings and recorded the elastic and plastic extensions, as illustrated in Figure 11. They found that water-stressed seedlings exhibited substantially smaller plastic (irreversible) extensions in response to applied tensions than did well-watered controls. Curiously, the applied force did not increase the steady-state rate of elongation. Cramer & Bowman (1991) used the same technique to examine the action of salinity on maize leaves, but observed little effect of water stress on either plastic or elastic extensions. Instead they found that applied force increased the steady-state extension rate of the leaf, as shown in Figure 12. Water stress had negligible effect on the extensions induced by applied tensions (Fig. 13a). The authors attributed their results to a salinity-induced decrease in $(P-Y)$, with little effect on ϕ (Fig. 13b).

The applied stress technique gives a measure of the viscoelastic properties of the wall (so long as water transport does not restrict growth) and has the advantage that values may be obtained rapidly, at least when single forces (and not a series of forces) are applied. The disadvantage of this single-force method is the difficulty in relating the viscoelastic values obtained to ϕ and $(P-Y)$. The problem is that there are many wall components which deform viscoelastically upon change in stress, but only a subset of these participate in the continuous, long-term creep that constitutes the surface expansion of growing walls (see below). Moreover, water flows are inevitably involved in any tissue deformations, and these may confound the analysis.

Cramer & Bowman (1991) attempted to relate force-induced extensions to eqn 1 (*a*, *b*) by applying a series of forces to a tissue and measuring the change in extension rate. The slope of this curve, they proposed, is proportional to the quantity $\phi L / (\phi + L)$; moreover, they suggested that if wall stress from turgor is also taken into consideration, then the *x*-intercept of the line gives an estimate of the yield threshold (*Y*). The units must be different from $\phi L / (\phi + L)$ and *Y*, however, because external unilateral forces are used instead of turgor pressure. One might attempt to convert the applied force into a pressure (stress) by dividing the force by the cross-sectional area of the tissue, as done by Nonami & Boyer (1990a) and Cramer & Bowman (1991), but there remains an intractable problem, namely that unilateral stress is not mechanically equivalent to the multilateral stress generated by turgor. Stress directionality can significantly affect the extension response of the wall because of mechanical coupling between deformations in the three dimensions, i.e. when a wall is stretched in one direction, it tends to retract in the other two directions (Metrax,

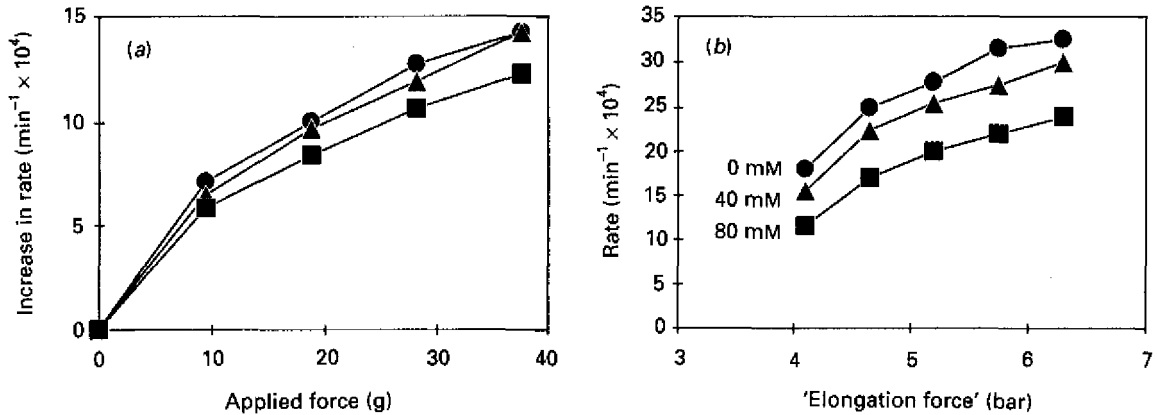


Figure 13. Extension rate as a function of applied force in maize leaves treated with 0, 40 and 80 mM NaCl (after Cramer & Bowman, 1991). (a) The *increase* in extension rate is shown as a function of applied force, (b) The rate is shown as a function of 'elongation force', a hypothetical force estimated by combining values for turgor pressure with the applied force normalized to cross-sectional area of the leaf. Note that the offset in these curves is consistent with a change in Y ,

Richmond & Taiz, 1980; Richmond *et al.* 1980). This difference between unilateral and hydrostatic stresses means that the method cannot yield values exactly corresponding to the parameters in eqn (1 a, b), as assumed by Cramer & Bowman (1991) and Nonami & Boyer (1990a). Sellen (1983) suggested that unilateral stress is three times as effective as multilateral stress, but this factor is sensitive to several assumptions about wall structure. Nevertheless, his analysis might provide a way of relating the extension responses to applied stresses more closely to ϕ and $(P-Y)$. It merits further attention.

Nonami & Boyer (1990a) also attempted to relate the force-extension behaviour to ϕ and $(P-Y)$, but their analysis contains two errors. First, they failed to appreciate that the extension response depends on hydraulic conductance if water transport partially limits growth. This is particularly relevant in their case because they concluded that water uptake substantially limits soybean hypocotyl growth. This conclusion invalidates their estimates of wall properties. A second error is in their estimate of wall extensibility (in their terms, M) from the transient plastic extension (see Figs 10, 11). They correctly define M as the strain rate divided by the stress (in their terms, strain rate $dZ/[Zdt]$ divided by the stress), but their use of the plastic deformation (E_{pi} in Fig. 10) to calculate strain rate is inappropriate. They calculate strain rate as the total plastic deformation that occurs upon adding a load to the stem (which they take as dZ/Z), divided by the total decay time for the plastic deformation (which they take as dt). It is evident from Figs. 10 and 11 that the strain rate during the plastic deformation falls off continuously and asymptotically; it is not constant until the steady-state rate of extension is attained a few minutes after addition of the weight. It is this constant value that should be used as the strain rate (see Cramer & Bowman, 1991). This value should

increase with applied force, yet their tissue did not behave in this way; rather, the extension rate quickly returned to the pre-stressed value. This anomalous behaviour of soybean hypocotyls was unexplained and differed from that of maize leaves, where extension rates increased with load (Cramer & Bowman, 1991).

These problems notwithstanding, the results of Nonami & Boyer (1990a) clearly showed that water stress had a large effect on the transient plastic deformation. To use this transient deformation to estimate wall extensibility, one might follow the analysis taken by Cosgrove (1981, 1985) or Ortega (1985) to identify the deformation as an exponential decay with a rate constant given by $(\epsilon + \pi)(\phi + L)$. This identification assumes that there is no viscoelastic deformation in response to the added load, other than that accounted for by the parameters ϵ and ϕ . This assumption is unlikely to prove true because many wall components are undoubtedly recruited into a load-bearing state in the initial response to abrupt changes in applied force. However, components with relaxation times faster than the components controlling ϕ would have little or no impact on growth, yet they would contribute to (contaminate) E_{pi} .

To sum up, the applied-force method has been used to indicate that hormones and water stress alter wall extension properties, but the theoretical foundation for relating the results to eqn (1a, b) remains problematical, despite recent attempts to unite them. Because the method relies on the rapid response to an applied force, its results are intermediate between the preceding physiological ones based on growth or wall relaxation behaviour and the following purely mechanical methods characterizing the viscoelastic behaviour of dead cell walls.

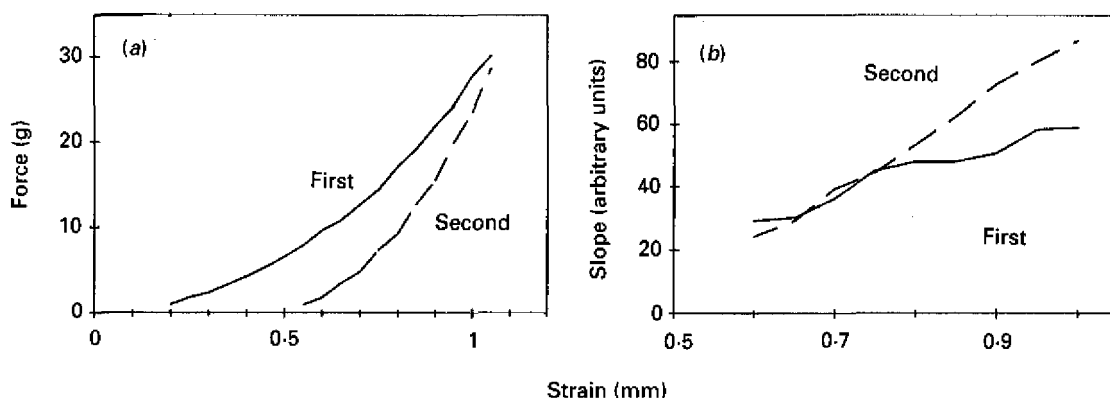


Figure 14. Stress/strain (Instron) measurements of cucumber hypocotyls. (a) Force is plotted as a function of wall extension in two consecutive extensions, (b) The slopes of the curves continue to increase as the walls are stretched. Hypocotyls were frozen, thawed and pressed to remove cell sap. The middle 5 mm region from a segment cut from the apical 12 mm of the hypocotyl was clamped in the extensometer and extended at a rate of 10 mm min^{-1} .

3. Isolated walls

(a) *Technique used.* Several methods have been used to assay the viscoelastic (stress/strain) properties of isolated cell walls, that is, walls in which the protoplasts are no longer living and contributing to wall metabolism and synthesis. Because wall viscoelasticity depends intrinsically upon wall structure (size and types of polymers, frequency and strength of crosslinks between polymers, etc.) and geometry, these methods can help to determine whether the structure of the wall has been altered by a treatment. In combination with viscoelasticity theory and biochemical analysis, it is sometimes possible to formulate specific molecular models to relate biochemical changes with viscoelastic changes of the wall (for example see Masuda, 1978). Because of the complexity and heterogeneity of the angiosperm wall (Fry, 1988) and our limited ability to alter single components of the wall in controlled ways, this more ambitious goal remains largely beyond our reach at present. Perhaps in the near future the power of highly specific wall enzymes ('wall restriction enzymes'), genetic mutants, wall biosynthesis inhibitors (Shedletsky *et al.*, 1990) and more powerful biochemical techniques will advance this important goal.

Viscoelastic assays have also been used in attempts to define and measure the wall yielding properties that control growth. This idea stems back to the Heyn's thesis that auxin alters wall 'plasticity' and was put on a more quantitative footing in the 1960s and 1970s when methods from material science were applied to wall viscoelasticity (Cleland, 1971 *a*, 1981; Yamamoto & Masuda, 1971; Taiz, 1984). This approach presupposes that wall expansion is inherently a viscoelastic phenomenon, in which the wall yielding properties depend only on wall structure and wall stress patterns. Given this, one would expect to find general agreement between the growth

rate of a tissue and the viscoelastic properties of the cell walls. In some specific cases (e.g. Kutschera & Schopfer, 1986 *a*), this idea has proved to have some merit, but there are numerous instances where wall yielding properties and wall viscoelastic properties do not correspond (Ruesink, 1969; Coartney & Morre, 1980; Beusmans & Silk, 1988; Cosgrove, 1988; Pritchard, Wyn Jones & Tomos, 1988; Iraki *et al.*, 1989). Thus it must be admitted that one of the aims of measuring the viscoelastic properties of isolated walls has not proved its general usefulness, though the techniques evidently can gauge structural alterations of the wall, which in some cases correlate with altered wall yielding properties.

These assays use walls in which the protoplasts are physically disrupted, often by freeze/thaw cycle. This is not to say that the walls are metabolically inactive, since many wall hydrolytic enzymes are found associated with such walls. These enzymes may alter the isolated wall sufficiently to affect the viscoelastic assay, although I know of no documented instance of this effect. Enzymatic action is usually minimized by storing the walls on ice or by boiling the walls in methanol and rehydrating prior to assay. The latter procedure destroys most enzymatic activities of the wall, but it also may change non-covalent bonding between wall polymers. Cleland (1984) found that methanol-boiled coleoptile walls have different viscoelastic characteristics than unboiled walls; nevertheless, the relative effects of auxin on these properties were similar in the two treatments.

With these mechanical methods, it is important that residual water and cell sap in the tissue be removed (e.g. by pressing under a constant weight for a few minutes before the assay). Otherwise a large component of the 'plastic' deformation may result from fluid movement out of the tissue. This effect is readily observed after clamping a frozen/thawed piece of tissue: within minutes after clamping under

tension tissue flattens as cell sap is expressed from the specimen, and this thinning is associated with a lengthening of the specimen as the cell chambers collapse. This simple precaution to avoid the confounding effects of sap movement is often neglected.

Wall viscoelasticity can be assayed by many techniques. Of greatest use are stress/strain (Instron) methods, creep methods, and relaxation methods, described below.

(b) *Stress/strain (Instron)*. When viscoelastic material such as a plant cell wall is extended at a rapid and constant rate, wall stress builds up in a curvilinear fashion, as illustrated in Figure 14a. If the material is now returned to its original length and extended a second time, a different stress/strain curve is obtained. Further repetitions of these extensions give curves nearly identical to the second curve, so this second curve represents the elastic (reversible) behaviour of the material. The difference between the first and second curves represents the plastic (irreversible) behaviour. This procedure is often called the Instron technique, after the name of an equipment manufacturer.

One may characterize the slope of each line to define a *modulus* - a measure of wall stiffness. A high modulus means high stiffness. The slope of the second curve gives a measure of the elastic modulus; the difference between the slopes of the first and second curves gives the plastic modulus. One may also express wall properties as the reciprocal of the modulus. This is known as a *compliance*. A high compliance means high deformability. Because the modulus and the compliance are calculated from the stress (the total force applied to the material divided by the area across which this force is exerted), these parameters measure the inherent viscoelasticity of the material, regardless of thickness of the material. Thus it is possible to compare the compliance of a thick object with that of a thin object and still make valid conclusions about the relative strengths of the materials.

With plant walls, it is easy to measure the force on the sample, but knowing the load-bearing area is more difficult. The usual procedure has been to make the stress/strain measurement, then cut out the wall material between the clamps, dry it and weigh it. This weight approximates the total wall mass in the sample, assuming negligible mass* from other cell constituents. In some case starch is removed by enzymatic digestion before drying, but this may also entail loss of wall material by autolysis. The area is then calculated (Cleland, 1967) as the dry weight per unit length of the wall sample times the density (assumed to be 1 g cm^{-3}).

In many instances, this correction for cross-sectional area has not been made. Instead, wall properties are expressed as strain/force, usually normalized as % extension per 100 g force. When

expressed in this fashion, these parameters have been called elastic and plastic extensibilities. Provided the thickness of the plant sample is the same from sample to sample, these extensibilities are proportional to the corresponding compliances.

Difficulties arise when the tissue thickness varies with treatment because wall cross-sectional areas usually scale with tissue cross-sectional area. In such a case, the thinner samples typically show larger extensibilities, but it may be erroneous to attribute these differences to altered cell wall viscoelasticity. If the thin and thick tissues are stretched to the same load limit, then the thin sample is put under a higher stress and will naturally deform to a greater extent. This problem is further aggravated by arguments that the epidermis is the load-bearing wall (Masuda & Yamamoto, 1972; Kutschera, 1989).

These difficulties are illustrated in a study by Cosgrove & Sovonick-Dunford (1989). They found that a gibberellin-biosynthesis inhibitor caused an increase in stem girth as well as inhibition of stem elongation in pea seedlings. When Instron extension curves were made on these samples, the dwarfed stems exhibited lower extensibilities, but when these extensibilities were corrected for wall cross-sectional area, there was no significant difference in the plastic compliances between dwarfed and control plants. Thus the inhibition of stem elongation could not be attributed to a 'stiffening' of the wall. In contrast, pressure-block relaxation demonstrated a substantial reduction in wall yielding properties.

I believe the Instron technique gives interpretable results when tissue thickness (more correctly, wall cross-sectional area) is constant, but when thickness is variable the results must be corrected for this variable and they thereby become more difficult to interpret. This problem is inherent in any method where an external force is applied to the wall; this includes all of the methods in this section and the applied force method in the previous section. Methods which use pressure — either hydrostatic pressure or osmotic pressure - to manipulate wall stresses do not suffer from this disadvantage, because pressure is equivalent to stress (force/area).

It is also important to note that the numerical values one obtains with this technique depend strongly on the assay conditions - specifically the rate of extension, the maximum force of extension, and wall pretreatment. A change in these conditions results in different numerical values. Furthermore, our trials with cucumber and pea stems have usually found that the force-extension curves do not really have a constant slope at high extensions, as usually assumed. Rather, the slope typically increases as the specimen is extended (Fig. 14 b). In some plant material with very 'stretchy' walls such as *Brassica* hypocotyls, we found the slope to decrease markedly at higher extensions (Wang, Cosgrove & Arteca, 1993). Thus one must arbitrarily select a point on the

curve to estimate the slope. We have usually chosen the point at the end of the extension.

These dependencies on conditions show that the parameters obtained by this analysis are not inherent properties of the wall, but rather empirical and *ad hoc* estimates of wall viscoelasticity. They allow one to determine quickly whether wall structure has been modified sufficiently that viscoelasticity is affected. These methods, however, do not measure ϕ or Y , as a general principle.

Based on experiments with auxin action on oat coleoptiles, Cleland (1984) suggested that the Instron plastic extensibility is related to the value of (ϕ over the preceding 90 min, but this is not a general phenomenon. For example, light inhibition of cucumber stem growth occurs by a decrease in ϕ but without a significant change in Instron parameters (Cosgrove, 1988). In roots there was poor correlation between growth rates at various positions along the root and mechanical properties (Beusmans & Silk, 1988; Silk & Beusmans, 1988), and when root growth was inhibited by excision, changes in wall viscoelastic properties lagged far behind growth rate changes (Pritchard *et al.*, 1988). Coartney & Morre (1980) found that supra-optimal auxin made the wall weaker viscoelastically, but inhibited growth. Rue-sink (1969) found that incubation of oat coleoptiles in wall hydrolases caused an increase in Instron extensibilities, but did not increase growth. Thus it is best to consider the Instron assay, as well as the others in this section, as valuable estimates of altered wall structure, but not necessarily as reliable indicators of wall yielding properties.

(c) *Wall creep.* A second method to measure wall viscoelasticity is a 'creep' assay. This technique measures the time-dependent extension of a sample under constant load. For a viscoelastic liquid, extension continues indefinitely, as long as the load is maintained, whereas a viscoelastic solid deforms only a limited extent. Simple creep assays have been used occasionally in assaying wall properties (Probine & Preston, 1962; Cleland, 1971b; Richmond *et al.*, 1980). Interestingly, some walls appear to be viscoelastic solids at neutral pH and viscoelastic liquids at pH <6 (Cleland, Cosgrove & Tepfer, 1987; Cosgrove, 19896). This suggests that major load-bearing crosslinks are broken, at least intermittently, at low pH. This observation is clearly related to 'acid-growth' phenomena (Cleland & Rayle, 1992).

An abridged variant of the creep assay has sometimes been used, in which the wall is put under constant load for a short time and then released. The irreversible and reversible changes in length are then used as measures of wall properties (Richmond *et al.*, 1980; Behringer *et al.*, 1990). This technique is equivalent to the 'applied force' method (above), except that isolated walls are used instead of living tissue. The data obtained by this method are similar

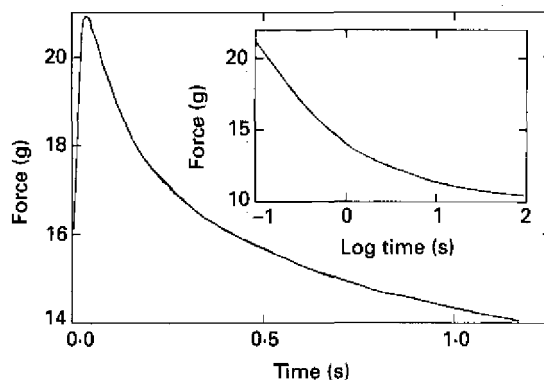


Figure 15. Stress relaxation of isolated cell walls from cucumber hypocotyls. After clamping the walls in the extensometer, they are extended rapidly until a force exceeding 20 g is attained, they they are held at constant strain and the decay in force is recorded. The inset shows the relaxation in log time. Hypocotyls were prepared as in Figure 14, except they were extended at a faster rate.

to those obtained by the Instron technique. The method requires somewhat more time per sample than the Instron method, but the method is easier to implement.

Like the Instron method, creep assays show a variable correspondence to growth behaviour. Short term creep behaviour (e.g. 0–5 min) is dominated by the inherent viscoelasticity of the walls; longer term behaviour (5 min to several h) may also depend on enzymatic activities in the wall (Cosgrove, 1989b, and refs. cited therein).

(d) *Stress relaxation.* This method has a long standing in the material science field and has been used for more than two decades to analyze plant cell walls, primarily by Y. Masuda, R. Yamamoto, and their colleagues in Osaka and Nara. The principle of the method is simple. A wall sample is held tightly between two clamps and is rapidly extended, then held to a constant length. The rapid extension increases the tensile force (stress) on the sample. After stretching has stopped, any molecular rearrangements of the wall's polymers will cause a reduction, or relaxation, of the wall stress, which is gauged by a force transducer attached to one of the clamps. A typical stress relaxation time course is shown in Figure 15. Stress (force) decays very rapidly at first, then more slowly.

This technique gives information about the time scale for relaxation and about the magnitude of relaxation for any given time scale. For example, one can distinguish relaxations that occur in, say, the first 50 ms from those occurring in the second half-second. The time scale for relaxation can give some clues about the size and nature of the relaxation event and may distinguish different relaxing elements in the wall, if their relaxation times are sufficiently different.

This idea may be further explained by considering

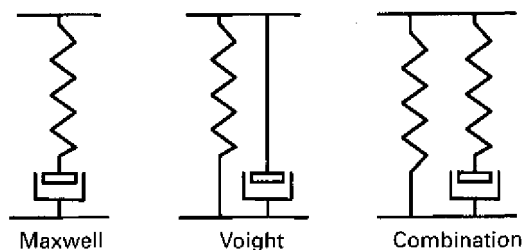


Figure 16. Three simple combinations of springs and dashpots give different responses to applied forces. A spring and dashpot in series acts like a viscoelastic liquid (it creeps forever) and is known as a Maxwell element (left). A spring and dashpot in parallel acts like a viscoelastic solid (creep decays with time) and is known as a Voigt or Kelvin element (middle). The combination of these two also acts like a viscoelastic solid and is sometimes known as the standard linear solid (right; see Vincent, 1982). Complex viscoelastic behaviour is often modelled by combining numbers of Maxwell or Voigt elements (with different spring constants and dashpot viscosities) to obtain a spectrum of time constants.

the classical model of viscoelastic materials as a set of mechanical ideals, known as springs and dashpots (Fig. 16). Springs are elastic elements which instantly stretch in response to a tensile force and are characterized by a spring constant (spring stiffness). Biochemically, springs correspond to wall polymers which deform elastically (e.g. coil and uncoil) in response to a mechanical force. Dashpots (pistons) correspond to viscous elements (e.g. sliding or slipping polymers) which 'creep' at a rate proportional to the stress and are characterized by a viscosity. Springs and dashpots, of varying properties and in various arrangements, may be combined to model complex relaxations, as exhibited by plant walls. These models let one dissect relaxations into (abstract) relaxing elements with specific viscosities and elastic constants. The details of such models go far beyond the domain of this review; some accessible treatments of polymer viscoelasticity may be found in Wainwright *et al.* (1976), Vincent (1982), and more detailed treatment in Ferry (1970).

The information contained in a stress relaxation is usually presented as a relaxation spectrum, shown in Figure 17. This spectrum can be viewed as a concise way of plotting the relaxation data in a way that indicates the magnitude of relaxation at different time scales. The x-axis is time, expressed on a log scale and the y-axis is rate of relaxation, expressed as change in force per log time unit.* This graphical form is perhaps somewhat foreign to most biologists, but it carries the advantage that one can see at a glance the minimum number and relative abundance of different relaxing elements needed to account for the relaxation. This is so because the relaxation caused by a single relaxing element (spring-and-

dashpot in series) exhibits a decay which, to a close approximation, starts and stops over about two log units of time. For instance, an element with a decay time constant of 2 s shows little relaxation in the first 0.1 s and is nearly completely relaxed at 10 s.

Relaxation spectra for oat coleoptile walls are said to have a 'box-like' distribution, as shown in Figure 17. This distribution means that essentially all of the relaxation occurred within the period between $10^{0.2}$ and 10^7 sec and that the relaxation times for the wall polymers are evenly distributed over this interval. From this box-like distribution, Yamamoto, Kawamura & Masuda (1974) devised an *ad hoc* equation to describe stress relaxation stress relaxation in this tissue, of the following form:

$$\text{stress}(t) = \frac{b \log(t + T_m)}{t + T_0} + c, \quad (2)$$

where stress (t) is the stress at time t . The parameters in this equation correspond directly with the characteristics of the box-like distribution in Figure 17. Specifically, T_0 is the relaxation time for the fastest relaxing element in the wall and it corresponds to the time at the left edge of the box. T_m is the maximum relaxation time, the time corresponding to the right edge of the box. The parameter b gives the rate of relaxation within the box and corresponds to the height of the box, whereas c is the residual stress remaining when relaxation is complete. It follows that, as long as the relaxation spectrum remains rectangular, the parameters in eqn (2) can be used to describe changes in the relaxation spectrum.

Masuda and co-workers have documented changes in b , T_m and T_0 in a number of situations (Masuda, 1978). The parameter drawing perhaps the greatest interest is the minimum relaxation time, T_0 . Work in Masuda's laboratory has shown repeatedly that auxin and some other growth agents alter T_0 . For example, treatment of oat coleoptiles with $10 \mu\text{M}$ auxin caused a reduction in T_0 , in various experiments, from 27 ms to 19 ms (Yamamoto & Masuda, 1971), or from 61 ms to 35 ms (Yamamoto, Shinozaki & Masuda, 1970), or from 280 ms to 100 ms (Sakurai *et al.*, 1982). Auxin causes either no change in b or, curiously, a decrease in b (i.e. auxin-treated walls relax more slowly; see Sakurai *et al.*, 1982). The large differences in T_0 values for the same plant material among reports from Masuda's laboratory is not explained, but probably reflects differences in specimen handling (length of methanol boiling, pretreatment with protease, etc.). It should be noted that T_0 is not an inherent property of the wall, but depends on rate of extension prior to start of relaxation (Fujihara, Yamamoto & Masuda, 1978) as well as on specimen handling. When one compares the value for T_0 (from 20 to 100 ms) with the time taken in these experiments to extend the wall specimen (on the order of 1 s), it is clear that a

* This explanation of the y-axis is only approximately correct. A more exact, though more complicated, explanation may be found in Ferry (1970).

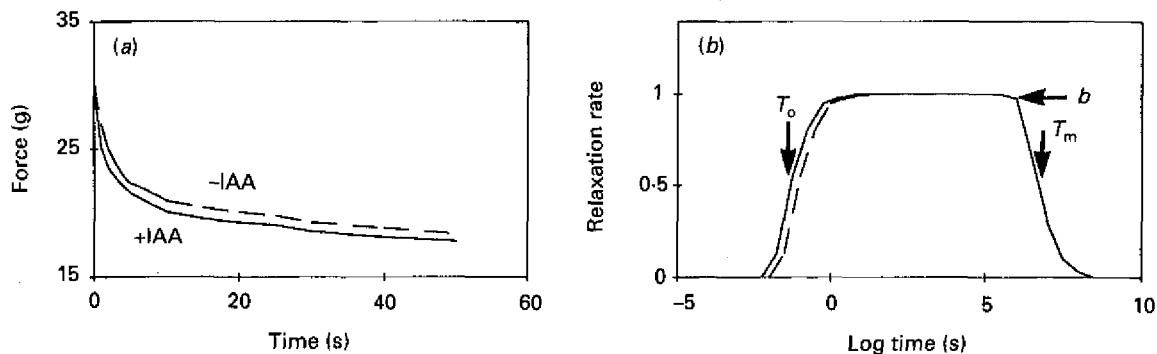


Figure 17. Stress relaxation time course (a) and spectrum (b) for oat coleoptile segments (after Yamamoto *et al.*, 1970). (a) The decay in force with time of methanol-boiled walls from coleoptile segments pretreated with or without indole acetic acid (IAA) for 1 h. (b) The corresponding relaxation spectrum, which is calculated, to a first approximation, as the slope of the line relating force to log time. Note the very slight difference in relaxation induced by auxin pretreatment. The values for the empirical parameters T_o , T_m and b are shown graphically in (b).

substantial amount of relaxation must, perforce, occur before extension of the wall is stopped and relaxation is recorded. Thus it is surprising that T_o values as short as 20 ms can be recorded at all. The answer to this minor mystery may lie in the fact that T_o is often estimated by extrapolating the wall stress (plotted on a log time scale) back to the initial stress (Sakurai *et al.*, 1982).

Masuda and co-workers (reviewed in Masuda, 1978, 1990) have suggested that the decrease in T_o by auxin is due to a reduction in the average molecular length of wall matrix polysaccharides. This is one of the few attempts to assign a structural interpretation to wall viscoelastic parameters. If this interpretation is correct, it is odd that auxin should decrease b , the rate of relaxation, as reported by Sakurai *et al.* (1982). This implies that walls from auxin-treated tissue relax less than walls from control tissue - which is counterintuitive to other evidence that auxin loosens walls. Much work remains to be done before wall viscoelastic properties are understood in terms of wall structure; nevertheless this technique is a useful complement to biochemical studies of cell walls.

The 25–50% reductions in minimum relaxation time (T_o) by auxin may at first sound like a large change in wall viscoelasticity, but this is not quite right. Recall that the spectrum is plotted on a log time scale. The major auxin effect shows up as a slight shift in the left edge of the box (see Fig. 17). I find it remarkable that auxin can have such a marked effect on the rate of wall extension in living cells, yet such a minor effect on wall viscoelasticity. This observation is also consistent with results from Instron assays, in which the viscoelastic parameters change much less than does growth. One explanation for this difference might have to do with the fact that in both of these assays stress is applied quickly to the cell wall samples and the subsequent polymer shear and stress relaxation are then observed. In this

situation many polymer movements can occur which are of limited extent and irrelevant for normal sustained expansion of walls. In contrast, growing plant walls have a long prehistory of sustained stress, in which all viscoelastic elements which could have relaxed will have done so. Thus it is essential to keep in mind that these mechanical assays inevitably detect many polymer movements which may not be connected to normal wall shear during growth.

From the foregoing discussion, the reader may get the impression that a rectangular relaxation spectrum is a general phenomenon for plant cell walls. Masuda and co-workers have carried out stress relaxation assays on several different plant materials (Masuda, 1978), but, to my knowledge, the box-like spectrum has been published only for oat coleoptiles. Subsequent studies used the empirical relation in eqn (2) as a convenient means for characterizing the relaxation behaviour of walls. Most often the relaxations were measured only for 60 s, so only two parameters (T_o and b) were possible to estimate. In contrast to the simple spectrum for oat coleoptiles, a more complicated spectrum is needed to account for the relaxation of pea epidermal walls (Yamamoto, Maki & Masuda, 1974). Similarly, Cosgrove (1989) obtained relaxation spectra for cucumber hypocotyl walls which were much more irregular (and more typical of complex polymeric materials). Thus it is not safe to assume that the rectangular relaxation Spectrum is typical, or even common. Nevertheless, the empirical fitting method devised by Yamamoto and co-workers can serve as a useful expedient for characterizing wall relaxation, so long as the data fit is reasonable and a deeper meaning of the parameters in eqn (2) is not implied.

It is worth noting that stress relaxation of isolated walls is quite different from *in vivo* stress relaxation of growing tissue, for example with the pressure-block technique. This difference is due in part to the difference in stress type (multilateral turgor versus

unilateral tension) and stress history (long-term prestress versus sudden onset of stress) as well as potentially many differences in wall metabolism. It might be informative to alter the normal protocol for relaxation assays of isolated walls to make the results more comparable with *in vivo* relaxation. This might be done by pre-equilibrating the isolated walls under a constant stress, as in the creep assay, and upon attainment of steady state creep to start stress relaxation by preventing further expansion. The difference between *in vivo* and *in vitro* relaxations under these conditions should indicate the aspects of wall relaxation which are lost upon cell death and wall isolation. This experiment appears not to have been done.

It is surprising that this stress relaxation method has been so seldom used, outside the laboratories of Masuda, Yamamoto and co-workers. The technique provides a wealth of information about the time scale for polymer rearrangements and certainly merits wider exploitation and study by cell wall researchers.

4. Summary

The preceding sections describe the major classes of techniques used to measure cell wall extension properties. They are based on different principles, measure different characteristics of the plant wall, and in some respects complement one another.

Which technique is most useful? The answer depends on the nature of the question of interest. For growth analyses, the first two methods (turgor-dependence of growth and *in vivo* stress relaxation) yield results which can be directly related to the biophysical theory of growth and can be used for quantitative assessments of growth alterations. For analyses of wall structure, the last three methods (Instron, creep, and *in vitro* relaxation methods) measure viscoelastic properties of walls and can detect alterations in the structure of the wall, but there is no theoretical relationship between these viscoelastic properties and the growth parameters (ϕ , Y) that are used to quantify wall expansion in growing cells. Because, as a general principle, the viscoelastic properties need not correlate with the growth properties, they should not be viewed as reliable indicators of growth properties. The third method (force applied to living tissues) represents a mixture between these two classes and has some of the advantages and disadvantages of each. Comparative studies of the relative merits of these various techniques are nearly lacking, so this assessment is based largely on comparing the results of different studies with one another — obviously not the best basis for a judgement. Probably the most informative approach at present for growth studies would be to use a combination of two techniques one to evaluate wall growth properties (ϕ and Y , by turgor-growth assays or by stress relaxation assays) and

another method using isolated walls to assess changes in wall viscoelasticity and by implication changes in the structure of the wall.

IV. CONCLUDING REMARKS AND PROSPECTUS

As this review illustrates, wall extensibility has many shades of meaning, which have not been delineated clearly in much of the past literature on walls and plant growth. It would probably be futile to insist on only one 'correct' meaning for wall extensibility. Rather, it is more important that we recognize the multifaceted meaning of this term and take care to identify the particular definition used in a particular study. I am afraid that all too often there has been a 'sleight of hand' with the term: it is measured by one method but discussed as if all measures and meanings of wall extensibility were equivalent.

In the context of plant growth, what we mean by wall extensibility is largely shaped by our thinking on the mechanism of plant cell expansion. I have summarized the view that wall expansion in growing cells arises from wall stress relaxation, which lowers the cell water potential and thereby draws water into the cell to physically extend the wall. It follows from this view that what we mean by wall extensibility is probably best assayed as the stress relaxation abilities of the walls. In my experience, *in vivo* relaxation assays gives the most reliable measure of the ability of growing walls to extend.

Another issue of major significance is the identification of wall relaxation processes and the growth parameters (ϕ , Y) with the biochemical processes that loosen the wall and thus start the whole process of cell expansion. Here we are limited largely by our ignorance of the structure of the wall and the biochemical mechanisms responsible for inducing wall relaxation. Because walls can become much more extensible or much less extensible very quickly (a few seconds) and with only a minor, and in some case no, change in wall viscoelastic properties, the change in structure induced by wall loosening processes must be very minor or perhaps ephemeral. *In vitro* systems for analyzing wall expansion (i.e. creep of isolated walls) have decided advantages over more complex living systems and merit further attention. McQueen-Mason, Durachko & Cosgrove (1992) used such a wall extension assay to identify proteins which catalyze wall extension. It may be feasible to use similar assays to assess the significance of many enzymes and biochemical processes speculated to be important for wall expansion.

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