Does Growth Correlate with Turgor-Induced Elastic Strain in Stems? A Re-Evaluation of de Vries’ Classical Experiments

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The correlation between growth and turgor-induced elastic expansion was studied in hypocotyls of sunflower (Helianthus annuus) seedlings under various growth conditions. Turgor-induced elastic cell wall strain was greater in hypocotyls of faster growing seedlings, i.e. in etiolated versus light-grown ones. It also was higher in rapidly growing young seedlings as compared with nongrowing mature ones. However, analysis of the spatial distribution of elastic strain and growth demonstrated that their correspondence was only apparent. Profiles of elastic strain declined steadily from the top of the hypocotyls toward the basis, whereas the profiles of relative elemental growth rate along the hypocotyls showed maxima within the growing zones. In contrast to earlier hypotheses, we conclude that turgor-induced elastic cell wall strain and growth do not correlate precisely in growing hypocotyls.

Plant cell walls restrain significant intracellular hydrostatic pressure that ranges from 0.1 to more than 3 MPa (Peters et al., 2000). Thus, cell walls are exposed to considerable mechanic stresses, causing elastic wall expansion. Tensile elastic (i.e. reversible) cell wall strain generally is considered prerequisite for cellular elongation growth, which by definition includes irreversible wall expansion (Cosgrove, 1987, 1993; Passioura and Fry, 1992). The relation between the capacitance for irreversible cell elongation and elastic wall extensibility as defined by the reversible response to an exogenous load has frequently been studied (for reviews, see Taiz, 1984; Cosgrove, 1993; Pritchard, 1994). However, quantitative studies on the relation between growth rates in planta and elastic cell wall strain induced endogenously by turgor pressure are sparse (Cleland, 1959; Edelmann, 1995; Hohl et al., 1995; Proseus et al., 1999).

This is surprising, given the crucial role the correlation between growth and turgor-induced elastic wall strain played when the fundamentals of modern plant cell biomechanics were laid. In 1877, Hugo de Vries established the mechanism of plasmolysis, and devised methods to quantify turgor-induced elastic expansion. He demonstrated that elastic expansion generally was highest in growing tissues, and provided circumstantial but persuasive evidence that gradients of elastic strain along plant organs were due to differential cell wall properties rather than differential turgor. He eventually concluded that plant cell growth was controlled by the ability of the walls to undergo elastic expansion in response to turgor pressure.

In his time, de Vries’ conclusions were widely accepted. Decades later, the regulative role he had assigned to turgor-induced elastic cell wall strain became doubtful, as the focus of interest shifted to the “plastic extensibility” of growing walls (Heyn, 1940). However, in his original work de Vries (1877) had argued that the apparent “overstepping of the limit of elasticity” (plastic deformation, in modern terminology) of the cell walls reported by contemporary researchers was an artifact caused by viscoelastic wall properties. It is interesting that the concept of plastic cell wall deformation has been challenged again recently using the same argument (Nolte and Schopfer, 1997).

de Vries’ experimental approach seems surprisingly “modern.” Working on a variety of plant material, he attempted to establish the role of elastic wall strain in the regulation of growth rates by comparing spatial patterns of both parameters, thereby surpassing more recent studies in methodological adequacy and consistency. However, the validity of his results remains questionable; the soundness of spatial growth analyses depends critically on the adherence to methodological rules, which are far more rigorous than the 19th century’s botanists could have envisaged (Green, 1976; Silk, 1984; Peters and Bernstein, 1997; Peters et al., 1999).

A reevaluation of de Vries’ findings using state-of-the-art methods of kinematic growth analysis would seem a useful starting point for a clarification of the role endogenously induced elastic wall strain plays...
in cell growth. Therefore, here we scrutinize the correlation between gradients of growth and elastic strain along sunflower (Helianthus annuus) hypocotyls under varying growth conditions.

RESULTS

Hypocotyl Elongation Growth

Seedling shoots started to elongate rapidly on the 3rd d after germination. Hypocotyls of dark-grown plants became taller than those grown under day/night conditions on d 4, due to a higher velocity of whole organ elongation (Fig. 1). Whole organ elongation velocity reached its maximum 1 d later in etiolated hypocotyls than in light-grown ones, and decreased rapidly thereafter. No further elongation of the hypocotyls could be detected after d 11. We decided to perform experiments on seedlings 120 to 136 h after germination because plants of both groups exhibited near-maximum velocity of hypocotyl elongation at that time.

Growth Zone Properties

Relative elemental growth rate (REGR) profiles of etiolated hypocotyls (Fig. 2A) showed a single peak and were skewed toward the shoot apex, which is in agreement with previous reports (Berg et al., 1986; Peters and Tomos, 2000). The situation was more complicated in plants grown under a day/night regime. About three-fourths of the individuals had a more or less pronounced REGR peak between 3 and 15 mm below the insertion of the cotyledons. The growth profiles of the rest seemed to decline steadily in the proximal direction. However, such declining profiles might be artificial. When a peak is located close to the distal end of the profile, there might be only a few (sometimes only one) data points distal of the peak. Such a peak will be hidden by statistical variance of data in a fraction of the experiments. Average profiles based on pooled data (Fig. 2, B and C) possessed peaks near the distal end of the profile. Peaked and monotonously declining profiles are represented equally well by the modified four-parameter Weibull function (Eq. 2) that we fitted to the data (a detailed discussion of the function’s properties in the context of plant growth analysis will be given elsewhere). Therefore, fitting this function can be expected to result in the statistically most appropriate curve.

Plants kept under day/night conditions grew slightly faster during the night, but the difference was insignificant statistically (Fig. 2, B and C). The effect was probably due to growth inhibition by higher transpiration rates during the day; humidity was not controlled during the experiments. The slight decrease of whole organ elongation velocity during the day phase seemed due to a growth rate reduction along the entire growing zone (Fig. 2, B and C). In contrast, the higher velocity of whole organ elongation in etiolated plants was caused by an increase of growing zone length (compare Fig. 2A with Fig. 2, B and C).

Gradients of Elastic Strain

It has long been known that, following a short phase of rapid contraction, plasmolized tissues contract continuously at low rates for 1 d or more (de Vries, 1877). Although we found continued shrinkage along the whole hypocotyl in plants of all treatments, the effect was irrelevant for evaluating the correlation between gradients of growth and elastic wall strain for two reasons. First, the amount of shrinkage during the period between 0.1 and 20 h after freezing and thawing was only a fraction of the shrinkage that had occurred immediately after thawing (Fig. 3). Second, and more importantly, the kine-

![Figure 1. Hypocotyl growth in sunflower seedlings grown in darkness (■, ●) or under day/night conditions (□, ○). A, Hypocotyl length plotted against time after germination. B, Whole hypocotyl elongation velocity calculated from data shown in A.](https://plantphysiol.org)
tics of shrinkage were independent of the magnitude of elastic strain in the tissue. This was demonstrated by grouping segments of numerous hypocotyls according to the amount of shrinkage observed after 20 h, and comparing time courses of shrinkage between groups (Fig. 3). Because all time courses were similar, strain profiles along hypocotyls were of similar shape as well, regardless of the time elapsing before those measurements of plasmolized segment lengths that were used to calculate strain (see also Figs. 4 and 5).

Turgor-induced elastic strain always continuously declined from the tip of the hypocotyl toward its base (Figs. 4 and 5), with steeper gradients occurring in growing hypocotyls (Fig. 4A and Fig. 5, A and B) as compared with nongrowing mature ones (Figs. 4B and 5C). In general, elastic strain tended to be greater in faster growing hypocotyls; it was higher along the entire hypocotyl in etiolated plants than it was in day-/night-grown ones (compare Fig. 4A with Fig. 5, A and B), and it was higher in rapidly growing hypocotyls as compared with mature ones that had undergone the same treatment (compare Fig. 4A with Fig. 4B, and Fig. 5A and 5B with 5C). The latter effect was particularly evident along the distal portion of the growing zone. Proximal of the growing zones, strain values were statistically indistinguishable between growing and mature hypocotyls. No significant difference was observed between strain gradients of plants grown under day/night conditions measured either at night or day (Fig. 5, A and B).

**Figure 2.** Kinematic description of the sunflower hypocotyl growing zone. Profiles of REGR along the hypocotyl are given; position 0 refers to the insertion of the cotyledons. Profiles are shown for dark-grown etiolated seedlings (A) and for plants grown under day/night conditions, measured either during the night (B) or the day (C) phase. Experiments took place between 120 and 136 h after germination. Curves are four-parameter Weibull functions fitted to pooled data from seven or more plants (see “Materials and Methods” for details). Maximal growth rates are similar in all plants; greater velocity of organ growth in etiolated plants (compare with Fig. 1) is due to the greater length of the growing zone.

**Figure 3.** Time courses of plasmolytic shrinkage in segments marked on growing sunflower hypocotyls. Segmental shrinkage data from 15 etiolated and 24 light-grown plants were divided into classes defined by the magnitude of turgor-induced strain (see “Materials and Methods” for details). Expressed by shrinkage after 20 h, the classes were defined as: ●, shrinkage between 0% and 95.2% of initial length, n = 168; □, 95.1% to 90.9%, n = 208; ▲, 90.8% to 87.0%, n = 129; ○, 86.9% to 83.3%, n = 56; and ■, <83.3%, n = 25. Segment length (means ± se) was plotted class-wise versus time after plasmolysis. Kinetics of shrinkage are similar in all classes, and thus are independent of the amount of elastic strain prevailing before plasmolysis.
DISCUSSION

de Vries (1877) had postulated that the location of maximum growth coincided with maximum turgor-induced strain, implying that the profile of elastic strain along growing organs had a peak at the location of the growth rate maximum. Our findings are not in line with this claim. One reason for the discrepancy might be that de Vries measured growth profiles in excised organs incubated in water, whereas we determined growth profiles in situ. On the other hand, an evaluation of the significance of older studies is not always simple because in the 19th century, plant physiologists did not usually analyze their data statistically. de Vries (1877) listed data from 30 exemplary individual organs; not more than...
10 of them seem consistent with his interpretation. Moreover, significant errors in the determination of the spatial gradient of growth must have occurred. Using the methodology available at his time, de Vries (1877) subdivided growing stems into a few large segments (not more than five, each 20 mm or more long), and measured segmental growth increments over relatively long periods (usually 12 h). In analyzing his data, he did not account for the movement of the segments along the growth rate gradient during the experiment. Therefore, it is hardly surprising that his results differ from our findings (for a discussion of analytical errors caused by excessive segment size and duration of experiments, see Silk, 1984; Peters and Bernstein, 1997). Based on our results produced with state-of-the-art methods of spatial growth analysis, we conclude that there is no precise correlation between the monotonously declining strain gradients and the peaked REGR profiles in sunflower hypocotyls.

In a recent study on sunflower seedlings, good correlation of whole hypocotyl elongation velocity and elastic strain measured in segments excised from the organ was found (Edelmann, 1995). It is unfortunate that no sound conclusions follow from comparisons of whole organ growth and growth-relevant parameters measured in excised segments, if the spatial distribution of growth is unknown (Silk, 1984). For clarification, consider segments cut from hypocotyls 5 to 15 mm below the cotyledons of either etiolated or light-grown plants at 130 h after germination. Turgor-induced elastic strain is clearly greater in segments from etiolated plants (mean value about 0.14; Fig. 4A) than in segments from light-grown ones (mean value approximately 0.09; Fig. 5, A and B). Because at that time hypocotyls elongate three times faster in etiolated plants than in light-grown ones (Fig. 1B), one might jump to the conclusion that elastic strain correlates well with growth. But unlike whole organs, segments of both groups grow at practically identical rates, as the growth rates are not generally regulated by the extent to which the cells are elastically strained. Profiles of elastic strain decline steadily along growth zones also in roots and leaves of maize (Zea mays; W.S. Peters, unpublished data). Growth profiles of these organs (for roots, see Erickson and Sax, 1956; Peters and Felle, 1999; for leaves, see Meiri et al., 1992; Ben-Haj-Salah and Tardieu, 1995) usually possess maxima near the centers of the growing zones. Studies on the bending modulus in maize roots also have indicated that the profile of elastic properties along the growing zone does not correlate with the gradient of growth (Beusmans and Silk, 1988). Therefore, our conclusion appears to be of general validity.

**MATERIALS AND METHODS**

**Plant Material and Growth Conditions**

Sunflower (Helianthus annuus L. cv Frankasol) seeds were sterilized in 150 mM NaClO, soaked in tap water for 6 h, and sown on moist vermiculite each in one plastic tube (3-cm diameter). Plants either were kept in the dark at 24°C, or were light-grown, i.e. under day/night conditions (14 h light at 26°C, 10 h dark at 22°C). Hypocotyl length was measured with a ruler to the nearest millimeter twice a day.

**Kinematic Analysis of the Growing Zone**

Plants were selected for experiments between 120 and 136 h after germination. Marks were made with Indian ink along hypocotyls at 3- to 4-mm distance from each other. Hypocotyl lengths were measured to the nearest 0.5 mm, and photographs of each plant were taken on a custom-built stage with integrated scale. Hypocotyl lengths were measured and plants were photographed again after 8 h (Δt). Initial and final length of marked segments (L<sub>i</sub> and L<sub>f</sub>, respectively) were determined on photographs to the nearest 20 μm, and the relative rate of segmental elongation growth (R<sub>S</sub>) was calculated as (Radford, 1967):

\[
R_S = \frac{\ln L_f - \ln L_i}{\Delta t}
\]

R<sub>S</sub> was plotted versus average segment position (Peters and Bernstein, 1997). Derivatives of various asymptotic functions (for a useful collection, see Hunt, 1982) were fitted to individual data sets to yield profiles of REGRs, using the curve-fitting routines of the software package SigmaPlot (Version 4.0, SPSS, Chicago). In most cases, a modified four-parameter Weibull function (where x refers to position):

\[
REGR = a(1 - c)^{-1} (b(x - d) + (1 - c)^{1 - d}) \left[ \frac{1}{x - d + (1 - c)^{1 - d} - c + 1} \right]^{1 - e} e^{-\left[ b(x - d) + (1 - c)^{1 - d} - c + 1 \right] (1 - c)^{-1}}
\]

provided the most satisfying result (as defined by standard goodness-of-fit criteria evaluated automatically by the software). Whole hypocotyl elongation velocity was calculated as the integral of the REGR profiles, and compared with the value measured directly on the individual plant. Plants were discarded if the difference was more than the maximum error of ruler measurements expressed in percentage of the average growth increment during Δt (i.e. 17% and...
21% for experiments on light-grown plants during the night or day phase, respectively, and 9% in etiolated plants; compare with Peters and Felle, 1999; Peters and Tomos, 2000. Remaining data were pooled and an average REGR profile was determined by fitting Equation 2.

**Determination of Profiles of Turgor-Induced Elastic Strain (ε)**

Profiles of turgor-induced elastic strain were determined in three groups of rapidly growing plants (light-grown ones during either the day or night phase, and etiolated ones, all 120–136 h old), and in two mature, nongrowing groups (etiolated and light grown at 260 h after germination). Hypocotyls were marked at 5-mm intervals, and segment lengths were measured (stressed length, \( L_\text{S} \)) to the nearest 20 \( \mu \text{m} \) under a stereomicroscope. Plants were plasmolized by freezing at \(-20^\circ\text{C}\). After 15 min they were thawed and stored in a 1 M mannitol solution. Segment lengths were measured again (unstressed length, \( L_\text{U} \)) at different times following thawing (0.1, 3, 5, 10, and 20 h), and turgor-dependent relative elastic expansion was calculated for each segment as conventional strain, \( ε \):

\[
ε = \frac{L_\text{S}}{L_\text{U}} - 1
\]

Because tissue shrinkage slowly continued for at least 20 h, different values of \( ε \) were obtained depending on which value of \( L_\text{U} \) (measured at different times after thawing) was used in the calculation. Datasets were pooled for every time of measurement of \( L_\text{U} \), and segmental \( ε \) was plotted versus segment position on the turgescent hypocotyl. Second-order polynomials were fitted to yield elastic strain profiles that could be compared to the corresponding profiles of REGR.

To analyze the dependency of the kinetics of continuing plasmolysis-induced shrinkage on the extent of turgor-induced strain, all segmental strain data were divided into five classes according to the magnitude of turgor-induced elastic strain, as determined using measurements of \( L_\text{U} \) at 20 h after freezing (classes were defined by strains of \( 0-0.05, >0.05-0.1, >0.1-0.15, >0.15-0.2; >0.2 \)). Data were normalized (initial length equaling 100%), and time courses of shrinkage were compared between the classes.

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