Determination of volumetric elastic moduli of plant leaf cells based on pressure-volume curves

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Abstract Volumetric elastic modulus (VEM) is an important parameter in biophysics and biomechanics of plants for in particular understanding cell growth. This paper proposes a new relation that can be used for precisely determining VEM. With the aid of this relation, it shows that the exponential approximation of the pressure-volume relationship adopted in most of the literatures in this field may lead to serious errors on VEM. © 2012 The Chinese Society of Theoretical and Applied Mechanics. [doi:10.1063/2.1201403]

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As schematically illustrated in Fig. 1, a plant leaf cell comprises a shell or cell wall which is a solid material but has plenty of porous or inter-fibrillar space, the symplastic or solute water which occupies the inner space of the shell and mainly stays in the vacuole, the apoplastic water which fulfills the inter-fibrillar space of shell, and the semi-membrane which separates the symplastic water and the apoplastic water. To balance the difference of the usually lower chemical potential ($\Psi_w$) of the apoplastic water, the semi-membrane develops an inward hydrostatic pressure—the turgor pressure ($\Psi_T$) —applied on the symplastic water. At the same time cell wall is subjected to the same magnitude outward hydrostatic pressure provided by turgor pressure (Fig. 1), and thus experiences a deformation of cell wall which results in a change of the volume ($V$) of water including symplastic and apoplastic water inside the cell wall. The volumetric elastic modulus (VEM)

\[
\varepsilon = V \frac{d\Psi_p}{dV},
\]

which is the improved form from the original definition of VEM in Tyree’s study. Since $\Psi_p$ is responsible for driving the growth of leaf cells, $\varepsilon$ is thus a key parameter in establishing the water relation inside cells.

The pressure-volume (PV) curve technique, proposed by Scholander et al. in 1965 using pressure-chamber devices schematically illustrated in Fig. 2(a), has been the most widely used method to measure the basic water status parameters $\Psi_w$ and $\Psi_T$, as well as turgor pressure $\Psi_p$ through the relationship as follows

\[
\Psi_w + \Psi_p = \Psi_T.
\]

During constructing PV curve using pressure-chamber in Fig. 2(a) test device, a leafy twig is completely enclosed inside the chamber except for the cut end of the stem which protrudes through an air-tight seal into the open air. The nitrogen pressure of the bomb applied on leaf tissues is increased until fluid flows out of the twig; after a measured fluid quantity has been expressed from tissues under this pressure until fluid neither flows in nor out. This pressure is noted and called the balance pressure $P$. Then the applied pressure is increased by an increment of 0.1–0.2 MPa. The above process is repeated, and each time the expressed water volume increment and the new balancing pressure reached. At each balance state, negative pressure is noted and positive pressure is noted, namely the linear dependence of $1/P$ upon $V_{ex}/V_0$ after $P$ reaches a certain value $P_{cr}$, namely

\[
\frac{1}{P} = \alpha - \beta \frac{V_{ex}}{V_0},
\]

where $\alpha$ and $\beta$ are the coefficients of fitted linear function. The parameter $V_0$ denotes the total water volume inside the cell before extrusion, which can be experimentally measured from the difference between the dry weight and the fresh weight of the sample. Hereinafter
change of the cell volume. Substituting the relations
\[ V = V_{\text{sym}} + V_{\text{apo}} + V_{\text{ex}} \]
into Eq. (4) yields
\[ \frac{1}{\Psi} = \frac{1}{\Psi_{\text{sym}}} = \frac{V_{\text{apo}} + V_{\text{ex}}}{C} = \frac{1}{\Psi_{\text{apo}}} + \frac{1}{\Psi_{\text{ex}}} \]

Comparing Eq. (5) with Eq. (3), we find that the linearity in Eq. (3) would correspond to \( \Psi_{\text{sym}} = 0 \). This observation has been widely interpreted as a proof of the osmotic phenomenon of the protoplasts, leading to disappearance of the turgor pressure.  

It was found that typical measured PV curves in their curvilinear sections (under pressures lower than \( P_{\text{cr}} \)) can be well fitted by the exponential form \( 1/P = aw^b \), where \( a \) and \( b \) are two fitting constants, as illustrated by an example shown in Fig. 2(b). The extension of the linear section to the lower region of pressure than \( P_{\text{cr}} \), as shown by the red dashed line in Fig. 2(b), presents the dependence of \( 1/\Psi_{\text{sym}} \) or \( 1/(P + \Psi_{\text{sym}}) \) upon \( w = V_{\text{ex}}/V_0 \). Comparing with the dependence of \( 1/P \) upon \( w \) as depicted by the solid line and dashed line in Fig. 2(b), we find the dependence of \( \Psi_{\text{sym}} \) upon \( w \).

Since 1980s, values of \( \varepsilon \) have been mostly determined through the exponential model \( 1/P = aw^b \) fitted to the measured PV curves, the above-mentioned progress determined functions of \( \Psi_{\text{sym}} \) upon \( w \) and the definition Eq. (1). Here we show that this progress of determining VEM \( \varepsilon \) may lead to serious errors.

During a PV curve experiment, researchers actually measure the volume \( V_{\text{ex}} \) of extruded water as a function of the applied chamber pressure \( P \). Therefore, we can rewrite Eq. (1) into the following alternative forms
\[ \varepsilon = \frac{V}{V_{\text{sym}}} \frac{\mathrm{d}\Psi_{\text{sym}}}{\mathrm{d}V} = -(V - V_{\text{sym}}) \frac{\mathrm{d}\Psi_{\text{sym}}}{\mathrm{d}V_{\text{sym}}} = -(1 - w) \frac{\mathrm{d}\Psi_{\text{sym}}}{\mathrm{d}w} \]

Replacing \( \Psi_{\text{sym}} \) with \( -\Psi_{\text{sym}} - P \) in Eq. (4) and making its differential yield
\[ \frac{\mathrm{d}\Psi_{\text{sym}}}{\mathrm{d}V_{\text{sym}}} = \frac{C}{(V_{\text{sym}})^2} \frac{\mathrm{d}V_{\text{sym}}}{\mathrm{d}w} \]

Substituting Eq. (7) and \( \frac{\mathrm{d}V_{\text{sym}}}{\mathrm{d}w} = -V_{\text{ex}} \) into Eq. (6) yields
\[ \frac{\mathrm{d}P}{\mathrm{d}w} = \frac{\varepsilon}{1 - w} - \frac{c}{(1 - w - w_{\text{apo}})^2} \]

where \( c = C/V_0 \) is a new constant and \( w_{\text{apo}} = V_{\text{apo}}/V_0 \) is the relative apoplastic water volume.

Particularly, we show that both \( c \) and \( w_{\text{apo}} \) can be directly determined through the linear relationship Eq. (3) after \( P \) exceeds \( P_{\text{cr}} \). Since \( \varepsilon = 0 \) in the linear section,\(^8\) from Eqs. (3) and (8) we have respectively
\[ \frac{\mathrm{d}P}{\mathrm{d}w} = \left(\frac{\alpha}{\beta}\right) \frac{\beta}{(1 - w - w_{\text{apo}})^2} \]

Comparing these two results in Eq. (9), we obtain
\[ c = \frac{1}{\beta}, \quad w_{\text{apo}} = 1 - \frac{1}{\beta}. \]
Substituting the above results into Eq. (8), we can finally express \( \varepsilon \) into the following form

\[
\varepsilon = (1 - w) \left[ P'(w) - \frac{\beta}{(\alpha - \beta w)^2} \right],
\]

(11)

where \( P'(w) \) denotes the derivative of \( P(w) \) with respect to \( w \). The above expression for determining \( \varepsilon \) is new in which all involved parameters and function are given from the measured relationship between \( P \) and \( w \).

Theoretically, if we can have a precise relation \( P(w) \), then from Eq. (11) we can obtain exact relation of \( \varepsilon(w) \) upon \( w \). Here we show that the previously most used approximation using the exponential-law \( aw^b \) in the region of \( P < P_{cr} \) may lead to serious errors for determining \( \varepsilon \). For this sake and by noting \( w < 1 \), we first approach \( P(w) \) in terms of polynomial functions

\[
P(w) \approx P_n(w) = a_0 + a_1 w + a_2 w^2 + \ldots + a_n w^n.
\]

(12)

The coefficients \( a_0, a_1, \ldots, a_n \) as well as those in the exponential-law relation \( P_e(w) = aw^{-b} \) are determined through the least-square fittings

\[
\begin{align*}
\sigma_n^2 &= \frac{1}{N - 2} \min_{a_0,a_1,\ldots,a_n} \sum_{i=1}^{N} |P(w_i) - P_n(w_i)|^2, \\
\sigma_\varepsilon^2 &= \frac{1}{N - 2} \min_{a,b} \sum_{i=1}^{N} |P(w_i) - P_e(w_i)|^2.
\end{align*}
\]

(13)

Where \( w_i \) \((i = 1, 2, \ldots, N)\) denote the measured points and \( N \) is the number of data. Figure 3(a) shows the 4-order polynomial approximations to the same data as those shown in Fig. 2(b). Table 1 shows the standardized errors \( \sigma_n^2 \) and \( \sigma_\varepsilon^2 \) for six pressure-volume curve datasets of conifer species Metasequoia glyptostroboides seedlings measured in the green-house experiments, indicating that the most widely used exponential-law approximation are not satisfied, while a simple polynomial up to the fourth orders of \( w \) provides sufficiently good results. Figure 3(b) shows a comparison between the standardized residual errors obtained from the function \([P(w_i) - P_e(w_i)]/\sigma_\varepsilon\) and \([P(w_i) - P_e(w_i)]/\sigma_\varepsilon\) respectively.

Based on the previous and current studies, VEM \( \varepsilon \) can be obtained from definition Eq. (1) and new determination Eq. (11) separately. An example of \( \varepsilon \) calculation in Fig. 4 is performed by using No.1 PV curve dataset in Table 1. The \( \varepsilon \) calculated from the new determination relation Eq. (11) smoothly decreases with \( w \) shown as \( \varepsilon_1 \) in Fig. 4, whose trend upon \( w \) is consistent with previous studies. Using long-term used method of Eq. (1) to obtain \( \varepsilon \), we must calculate \( \Psi_p \) from the difference of \( \Psi_\varepsilon \) and \( \Psi_{\text{u}} \). Big difference generates between \( \varepsilon \) calculated from 4th-order polynomial function and exponential function based on definition.
Fig. 4. The VEM ε calculated by different methods at $w_{apo} = 1\%$. $\varepsilon_1$ was calculated from Eq. (11) by using polynomial function of applied pressure $P$ vs. $w$; $\varepsilon_2$ was calculated from Eq. (1) by using polynomial function; $\varepsilon_3$ was calculated from Eq. (1) by using exponential function of applied pressure $1/P$ vs. $w$.

Eq. (1) respectively, shown as $\varepsilon_2$ and $\varepsilon_3$ in Fig. 4, which indicates that exponential fitted function of $P$ upon $w$ results in big error in $\varepsilon$. Comparison of $\varepsilon_1$ and $\varepsilon_2$ in Fig. 4 demonstrates that $\varepsilon$ calculated from definition method exists more error than that from our new developed calculation method.

In summary, this study presented a new determination method for $\varepsilon$ and also proved that long time used exponential fitted function of applied pressure $P$ upon $w$ may result in much error in $\varepsilon$ calculation.

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