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Air-Coupled Ultrasonic Resonant Spectroscopy for the Study of the Relationship Between Plant Leaves' Elasticity and Their Water Content

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***Abstract*—Air-coupled wideband ultrasonic piezoelectric transducers are used in the frequency range 0.3 to 1.3 MHz to excite and sense first-order thickness resonances in the leaves of four different tree species at different levels of hydration. The phase and magnitude spectra of these resonances are measured, and the inverse problem solved; that is, leaf thickness and density, ultrasound velocity, and the attenuation coefficient are obtained. The elastic constant in the thickness direction (c_{33}) is then determined from density and velocity data. The paper focuses on the study of c_{33}, which provides a unique, fast, and noninvasive ultrasonic method to determine leaf elasticity and leaf water content.**

I. Introduction

Ultrasonic spectroscopy was first suggested in the early 1960s as a method of obtaining information about size and orientation of discrete defects in metals [1]. Later, this technique was used in many different contexts. In 1978, Haines *et al.* used magnitude and phase spectral analysis, with a pulse-echo water immersion technique, to study reflection from multilayered materials in a frequency range in which thickness resonances appeared [2]. Sachse and Pao proposed a method to determine the phase velocity of dispersive materials in the absence of thickness resonances by using the phase spectrum and a water immersion and through-transmission technique [3]. Later, this method was extended to include the information of the magnitude spectra and to obtain both velocity and attenuation [4]. Pialucha *et al.* proposed a magnitude spectrum method to measure phase velocity [5]; they used water immersion, a through transmission technique, and the magnitude spectral analysis of the thickness resonances.

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Use of air-coupled ultrasound to obtain material properties is especially interesting for those materials that cannot be wetted, or those situations where avoiding the use of water or other coupling liquids may be an advantage. This technique was first used to study paper using through transmission and plate wave resonances by Luukkala *et al.* [6]. Thickness and plate resonances have been largely used as a means to enhance the amount of transmitted energy. For example, Hutchins *et al.* used this technique for poly(methyl methacrylate) and carbon fiber reinforced polymers (FRPs) [7]. Later, Schindel and Hutchins used air-coupled ultrasound to measure the ultrasound velocity in plates by comparing time of flight measurements with measurements of the frequency of the thickness resonance [8]. Air-coupled techniques in the time domain have also been used to measure velocities or attenuation coefficients and to calculate elastic constants or other material properties for a variety of materials including porous rocks [9], textiles [10], FRPs [11], polymers during polymerization [12], and wood [13].

A more detailed analysis of the spectrum of the thickness resonances permit the density of the plate and the velocity and the attenuation coefficient of ultrasound in the plate to be obtained if the thickness is known. This technique has been applied to silicone rubber plates, filtration membranes, and paper [14]–[16]. Later, this resonant technique in the frequency domain was extended for oblique incidence to obtain, in addition, the velocity and attenuation coefficient of shear waves and viscoelastic constants [17]. Analysis of more complex resonances of finite-sized materials has also been used to determine the elastic properties of drug tablets [18]. More recently, a method that uses both phase and magnitude spectra of thickness resonances has been proposed. It requires no independent determination of the thickness and has been applied to composite materials, membranes, and leaves [19]. Later, improvements of this technique were applied to determine plate thickness and density and velocity and attenuation of ultrasound in polypropylene ferroelectret films and vacuum-packaged cured ham [20], [21].

Development and use of non-invasive techniques to determine the status and properties of plants is a key issue in fields such as applied botany and plant physiology. In particular, there is a rising need for non-invasive sensing of plants' watering needs to control horticultural and agricultural water application and irrigation systems [22], [23].

Ultrasonic techniques represent a good alternative for this purpose; actually, ultrasonic water immersion techniques have already been used [24], [25]. However, these techniques are not useful to determine the leaf's water status. Noncontact ultrasonic spectroscopy has recently been used to measure the transmission coefficient of plant leaves over the frequency range 0.2 to 2.5 MHz at normal incidence [26]. Later, the frequency location of the first thickness resonance (f_{TR}) and the quality factor of the resonance (Q) were measured through the desiccation process of some leaves; these data were compared with relative water content (RWC) measurements, pressure-volume curves [27], and cryo-scanning electron microscopy images [28]. Very high correlation factors have been found between f_{TR} and the RWC, and between f_{TR} and the water potential. Moreover, it has been possible to establish that the point of null turgor pressure and the point of inflexion in the curve of f_{TR} versus RWC appear at the same RWC value.

However, the identification of the underlying physical mechanisms which permit explanation of this observed behavior is not straightforward. The main reason is that f_{TR} depends on several leaf properties that are affected, in different and complex ways, by the modifications produced in the leaves by the variation of the RWC, including thickness, density, elastic constant, and cell structure.

To avoid this problem, a more detailed analysis of the leaf resonances is presented in this paper. This analysis is based on the solution of the inverse problem, as explained in [19], and a subsequent improvement of the fitting of the theoretically calculated thickness resonance curve into the experimentally measured one, as presented in [20]. Toward this end, an effective model for the leaf has been used, based on the assumption that thickness resonances in leaves can be represented by the thickness resonances of a homogeneous plate with effective properties. For this analysis, we have developed a fast, efficient, and automatic processing of the spectra that is performed *in situ*, right after the measurement. This provides, almost in real time: leaf density and thickness, and velocity and attenuation coefficient of ultrasound in the leaves. *In situ* processing of the data is a key issue considering the large number of samples and measurements to be taken. This procedure is applied to study the desiccation process of leaves of four different tree species (two evergreen and two deciduous species): *Prunus laurocerasus, Ligustrum lucidum, Populus x euroamericana*, and *Platanus hispanica*.

II. Analysis of the Spectral Response of the Leaves' Thickness Resonance

Elastic response of plant leaves is largely determined by the water contained in them and by the air-filled pore space. Most of the water contained in plant leaves is enclosed within the leaf cells at a pressure greater than the atmospheric pressure because of the mechanical stress stored in the cell membrane. This membrane stress in the densely packed cell structure of the leaf is the main factor responsible for the leaf's elastic properties. When a fully hydrated leaf loses water, the volume of water contained by each cell is reduced and, consequently, the stress stored in the cell membranes is also reduced. On the macroscopic scale, these changes give rise to leaf shrinkage and a loss of rigidity. The elastic constants (c_{ij}) are expected to decrease as a result of both the loss of mechanical stress in the cells membrane and the deformation of the cell structure.

Leaves' first thickness resonance spectra are analyzed, considering the leaves as a homogeneous plate with effective properties. A multilayered model, closer to the real leaf structure, was proposed in [29]. However, this model is currently of little practical use because it introduces a much larger set of parameters which are difficult to determine. In addition, the one-layer model provides a reasonable fitting into the experimental data if the analysis is limited to the vicinity of the first-order thickness resonance and it is not extended to higher resonance orders. The transmission coefficient, T, at normal incidence for a single solid plate of homogeneous material embedded in a fluid is given by $T = |\xi|^2$, where

$$\xi = \frac{2r\Gamma}{2r\Gamma\cos(k_2 l) - i(1 + r^2\Gamma^2)\sin(k_2 l)}, \tag{1}$$

where l is the thickness of the plate; r is the ratio of wave numbers (fluid to solid), $r = k_1/k_2$; and $\Gamma = \rho_1/(r^2\rho_2)$, where ρ is the density [30]. Plate roughness can be introduced by considering a modification of the transmission and reflection coefficients [31]. The transmission coefficient is measured using a through-transmission technique, then the following relations between the measured modulus and phase of the transmission coefficient (T and $\Delta\phi$) and the calculated ξ [magnitude, $|\xi|$, and phase, $\phi(\xi)$] hold:

$$T = |(\xi\exp(\alpha_1 l))|^2 \cong |\xi|^2 \tag{2}$$

$$\Delta\phi = \phi(\xi) - lk_1, \tag{3}$$

where α_1 is the attenuation coefficient in air.

The analysis of the experimentally measured resonance spectra follows the procedure explained in [18] and [19]. Using (4) and (5), this procedure permits us to get a first estimation of the ultrasonic velocity (v_2) and the thickness of the plate (l) from the measured values of the frequency location of the first thickness resonance (f_{TR}) and the value of the phase spectra $\Delta\phi$ at this frequency $\Delta\phi(f_{\mathrm{TR}}) = \Delta\phi_1$.

$$v_2(f_{\mathrm{TR}}) = 2f_{\mathrm{TR}}l \tag{4}$$

$$v_2(f_{\mathrm{TR}}) = l/(l/v_1 - \Delta\phi_1/(2\pi f_{\mathrm{TR}})), \tag{5}$$

where v_1 is the velocity of ultrasound in the fluid in which the plate is immersed (air in this case). In this work, a first estimation of the density of the leaves $\rho_2^0 = 950$ g/m^3 was considered. The attenuation of the ultrasonic waves in the plate at f_{TR} ($\alpha_2^0(f_{\mathrm{TR}})$) can be estimated from the leaf impedance and the Q-factor of the thickness resonance (see [15] and [16]). The variation of the attenuation with the frequency ($\alpha(f)$) can be described by a power law, as has been done in the past for a large number of porous materials and biological tissues (see [17] and [20]):

$$\alpha'_2(f) = \alpha_2^0\left(\frac{f}{f_{\mathrm{TR}}}\right)^{1.5}. \tag{6}$$

Velocity is assumed to be constant, which is a reasonable assumption considering the frequency range in which measurements are performed.

The values of v_2^0, $\alpha'_2(f)$, ρ_2^0, and l^0 so obtained are considered a first approach. We then calculate k_2 according to

$$k_2(f) = \frac{\omega}{v_2} + i\alpha_2(f), \tag{7}$$

where, in this case, $v_2 = v_2^0$ and $\alpha_2(f) = \alpha'_2(f)$.

These values are introduced in (1), and then ξ is calculated. From ξ, we get $T(f)^{\text{theo}} = /\xi|^2$ and $\Delta\phi(f)^{\text{theo}} = \phi(\xi) - lk_1$. The estimation of the quality of the agreement is given by τ^T and τ^ϕ, defined as:

$$\tau^T = \frac{1}{N}\sum_{n=1}^{N}((T(f_n)^{\exp} - T(f_n)^{\text{theo}})/T(f_n)^{\exp})^2 \tag{8a}$$

$$\tau^\phi = \frac{1}{N}\sum_{n=1}^{N}((\Delta\phi(f_n)^{\exp} - \Delta\phi(f_n)^{\text{theo}})/\Delta\phi(f_n)^{\exp})^2, \tag{8b}$$

where f_n are the frequencies at which T and $\Delta\phi f$ are measured and N is the number of measurements (length of vector f_n).

To improve this agreement, we changed the values of velocity, attenuation, thickness, and density; for each set of these values, we calculated T and then we estimated the quality of the agreement (8). The set of input parameters that maximizes this agreement is taken as the final result. To avoid having to change the four parameters simultaneously, which is a very time-consuming approach, we have designed a procedure based on the knowledge of the effect that each one of these parameters produces on T. So, this procedure has two main steps, in the first one only attenuation and density are changed, and (8a) is taken as the optimization criterion. In the second step, velocity and thickness are changed in such a way that we kept the location of the resonant frequency constant, and (8b) is taken as the optimization criterion. The procedure is shown in Fig. 1.

These operations are implemented in a Matlab function (The MathWorks, Natick, MA) loaded in the Windows-based (Microsoft Corp., Redmond, WA) oscilloscope used for the measurements; the function runs right after the measurement is taken, permitting us to obtain, practically in real time (run time about 1.0 s), the estimation of the leaf's effective parameters. The value of this simple fitting routine is that it runs fast enough so that the full resonance analysis can be performed *in situ* and almost in real time, and it is efficient enough so that it provides a satisfactory curve fitting for all measurements performed in this work. Our experience has shown that final results do not depend on the initial guess values, but that the time for the calculations increases when the initial guess is less accurate or when the interval for the variation of the magnitudes is enlarged.

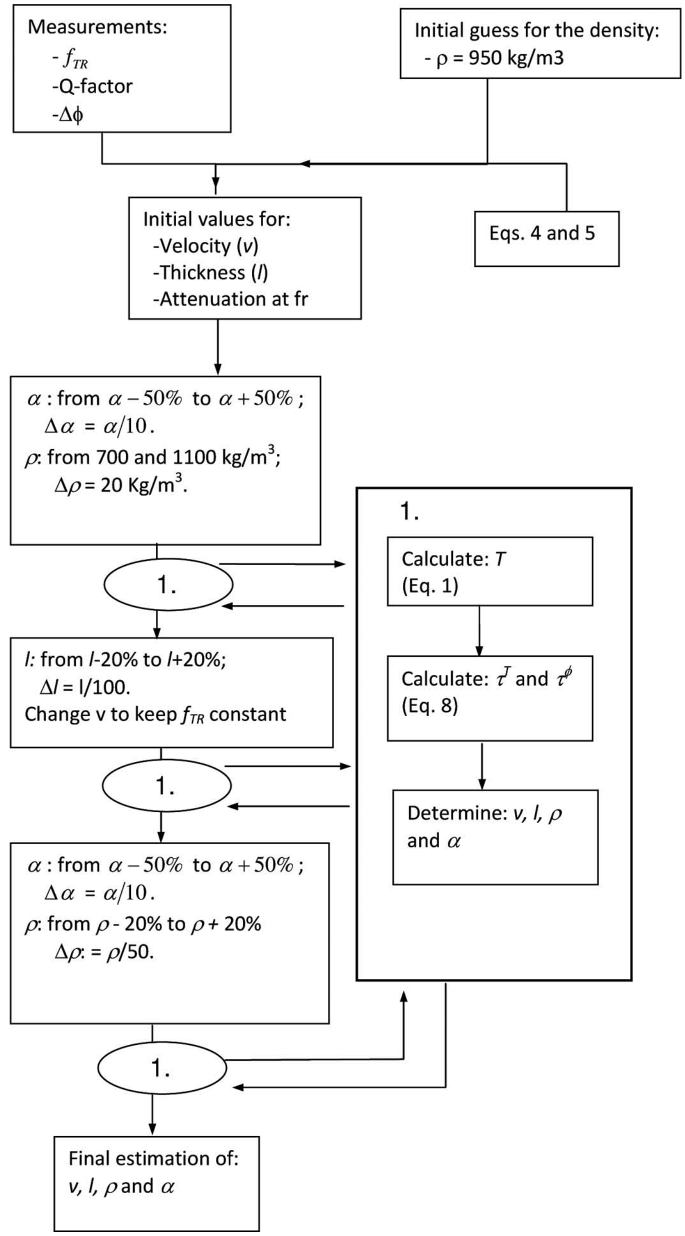

Fig. 1. Procedure to calculate the value of the velocity, the thickness, the density, and the attenuation coefficient of the leaf from the measured magnitude and phase of the transmission coefficient and the theoretical expression of the transmission coefficient of a homogeneous plate.

III. Plant Material and Experimental Setup and Procedure

A. Plant Material and Conventional Measurements

The study was carried out using four different tree species: *Populus x euramericana*, *Prunus laurocerasus*, *Platanus hispanica*, and *Ligustrum lucidum.* A set of eight leaves per species was used for the measurements. Leaves were allowed to dry under environmental conditions; they were weighed and ultrasonically measured at constant time intervals and for a time period between 4 and 6 h. The measurements in this paper are represented in leaf RWC intervals. Within each interval, we only represent the mean value and the error bars correspond to the standard deviation. RWC was measured as explained in [28].

The thickness of the leaves was measured through the desiccation process using a conventional micrometer and also by using a digital contact sensor GT-H10L coupled to an amplifier GT-75AP (GT Series, Keyence Corp., Osaka, Japan). To determine the density, 9 circular samples of each species (30 mm diameter) were cut. Thickness, diameter, and weight were measured throughout the desiccation process (12 to 15 measurements for each sample).

B. Ultrasonic Measurements

The experimental procedure is based on a conventional through-transmission technique at normal incidence to measure the amplitude and the phase of the transmission coefficient in the frequency domain; toward this end, Fourier analysis is performed.

Ultrasonic measurements were performed using a pair of air-coupled piezoelectric transducers [32]. Two pairs of transducers with center frequencies of 0.25 and 1.0 MHz and circular aperture of 25 and 20 mm, respectively, were used for the evergreen and deciduous species, respectively. For a few measurements in some *Platanus hispanica* leaves at low RWC, it was necessary to use an additional pair of transducers with an intermediate center frequency at 0.5 MHz and 20 mm aperture diameter. For the study of plant leaves, the available frequency band for each pair of transducers is the band that fulfills that the SNR > 20 dB. This is, approximately, 0.19 to 0.34 MHz, 0.3 to 0.7 MHz, and 0.5 to 1.3 MHz for the 0.25-, 0.5-, and 1.0-MHz pairs of transducers, respectively. Transmitter and receiver transducers were located facing each other along the vertical at a distance of 2 to 4 cm (1 and 0.5 MHz) and 4 to 6 cm (0.25 MHz). A Panametrics P/R 5077 (Panametrics, Waltham, MA) was used to drive the transmitter and to amplify the received signal. A Tektronix 5052 oscilloscope (Beaverton, OR) was used to digitize the received signal. Scheme and further details of the experimental set-up can be found in [27] and [28]. Fig. 2 shows the through-transmitted signal (time and frequency domain) for the 1.0-MHz pair of transducers.

Air temperature and pressure were simultaneously measured. The velocity of sound in air (v_1), the attenuation coefficient (α_1), and the air density (ρ_1) were determined according to

$$v_1 = \left(331.3 + \frac{T(^\circ\mathrm{C})}{1.6502(^\circ\mathrm{C})}\right)\ (\mathrm{m/s}) \tag{9}$$

$$\alpha_1\ (\mathrm{dB/m}) = 1.6 \times 10^{-10}(f\ (\mathrm{Hz}))^2 \tag{10}$$

$$\rho_1 = \frac{P}{RT}, \tag{11}$$

where P is the pressure, R is the gas constant, and T is the absolute temperature

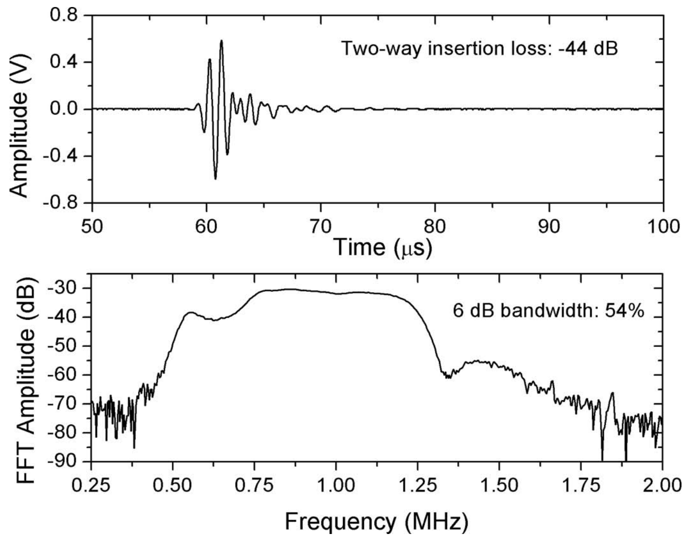

Fig. 2. Response (top: time domain; bottom: frequency domain) of the pair of transducers with center frequency at 1 MHz, separation 20 mm, excitation 200 V, 1 semicycle of a square wave, gain at reception 0 dB. Insertion loss is calculated from the ratio of the peak-to-peak voltage measured at the receiver transducer to the peak-to-peak voltage applied to the transmitter transducer.

IV. Experimental Results

A. Thickness and Density

Thickness and density of the leaves were obtained using the proposed ultrasonic technique and also the conventional methods explained in Section II-A. The comparison is used as a test of the accuracy of the proposed ultrasonic technique. The results of both techniques are in agreement within the experimental error range. For one measurement on one particular leaf, typical deviation of ultrasonic and conventional thickness measurements is about 5 to 10 μm, whereas typical deviation of ultrasonic and conventional density measurements is about 50 to 100 kg/m^3 (see [26]). For all the leaves of a given species at a given value of RWC, maximum observed standard deviation for the ultrasonic thickness and density measurements is ±50 μm and ±100 kg/m^3, respectively. The large thickness standard variation reveals the fact that different leaves of a given species may have quite different thicknesses; however, variability of density measurements is much smaller. For all the species, the loss of water produces a reduction of the thickness. For a reduction of RWC from 1 to 0.75, the averaged thickness of *Prunus laurocerasus* leaves is reduced from 405 to 310 μm; for *Ligustum lucidum* leaves, an RWC reduction from 1 to 0.7 corresponds to a thickness reduction from 320 to 205 μm; for *Platanus hispanica* and *Populus x euroamericana* leaves, an RWC reduction from 1 to 0.6 produces a thickness reduction from 260 to 190 μm and from 230 to 175 μm, respectively.

This thickness reduction compensates the loss of mass of the leaf (resulting from the loss of water), hence the density remains rather constant with some local variations. Density of the *Prunus laurocerasus* leaves slightly

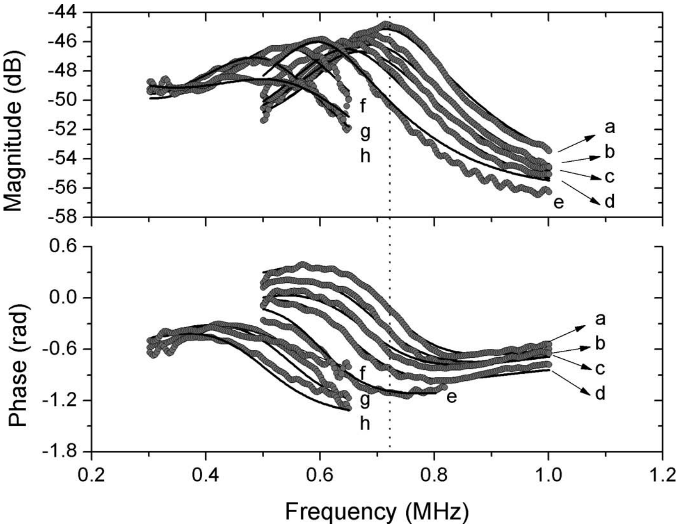

Fig. 3. Spectral response (top: magnitude; bottom: phase) of the first thickness resonance of a *Platanus hispanica* leaf at different values of the relative water content (RWC); a: 1.00, b: 0.97, c: 0.95, d: 0.94, e: 0.92, f: 0.85, g: 0.77, and h: 0.72.

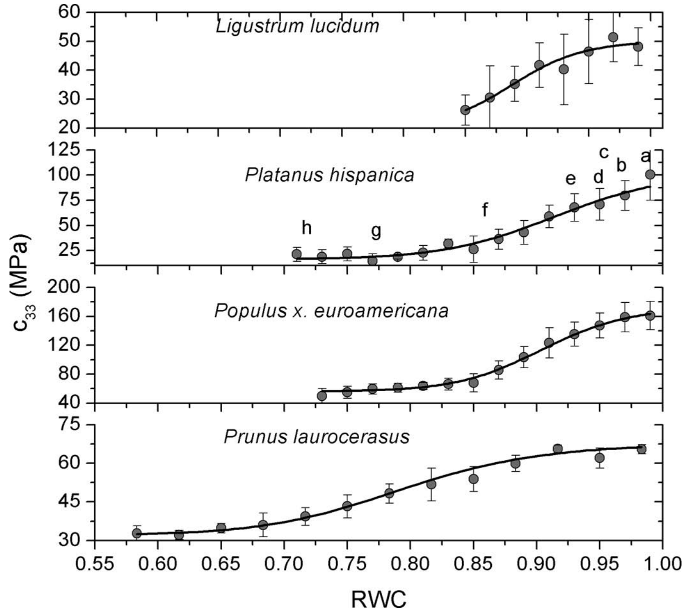

Fig. 4. Variation in the leaf's elastic constant in the thickness direction versus the relative water content (RWC). In the case of the *Platanus hispanica*, the letters a–h indicate the RWC location of the resonances shown in Fig 3.

increased from 835 kg/m^3 (RWC = 1) to 890 kg/m^3 (RWC = 0.75). Density of *Ligustrum lucidum* leaves increased from 850 kg/m^3 (RWC = 1) to 950 kg/m^3 (RWC = 0.7). Density of *Platanus hispanica* leaves first slightly decreased from 820 kg/m^3 (RWC = 1) to 780 kg/m^3 (RWC = 0.9) and then to 670 kg/m^3 (RWC = 0.66). Finally, density of *Populus x euroamericana* slightly decreased from 960 kg/m^3 (RWC = 1) to 815 kg/m^3 (RWC = 0.63). These two parameters (thickness and density) can be hardly used to determine RWC because of the large variability of the thickness between different leaves of the same species and because of the very small variations in the density with the RWC.

B. Variation of the Spectra of the First Thickness Resonance

Magnitude and phase spectra of the transmission coefficient were measured along the desiccation process. Fig. 3 shows the theoretical (solid line) and experimental (dots) phase and magnitude spectra of the ultrasonic transmission coefficient versus frequency in the vicinity of the first thickness resonance at several values of RWC for one of the *Platanus hispanica* leaves measured. A similar behavior was observed for the other species. As the leaves lose water, the resonant frequency shifts toward lower frequencies and the damping increases. It is interesting to observe the low frequency location of the first thickness resonance, especially when considering the typical leaf thicknesses. This is due, as we will show, to a very low value of the propagation velocity, which originates from a very low value of the elastic constant resulting from the leaf's porosity and internal structure.

These spectra are processed according to the procedure previously outlined to obtain the variation with RWC in: 1) the relative displacement of the resonant frequency ($\Delta f_{\mathrm{TR}}/f_{\mathrm{TR}}^0$); 2) the leaf thickness; 3) the leaf density; 4) the velocity of ultrasound in the leaf; and 5) the attenuation coefficient of ultrasound in the leaf. From velocity and density data, the elastic constant is determined. The subsequent analysis of the results focuses on the elastic constant.

C. Variation in the Leaves Elastic Constant (c_{33}) With RWC

Calculated c_{33} are shown in Fig. 4. A sigmoid-like behavior is observed, which is typical of a transition between two states (wet and dry in this case). This sigmoid evolution has also been previously observed in $\Delta f_{\mathrm{TR}}/f_{\mathrm{TR}}^0$ versus RWC graphs ([27], [28]).

The observed total variations in c_{33} with RWC (that is, from wet to dry states) are larger than the variations observed in $\Delta f_{\mathrm{TR}}/f_{\mathrm{TR}}^0$ (14% to 37%) or in the ultrasound velocity (35% to 49%) because the influence of the thickness and the density is eliminated for the c_{33} measurements. This result suggests that this parameter can provide a better way to estimate the RWC of the leaves.

In addition, a common trend of the variation in c_{33} depending on the leaf phenology is observed: the largest relative variation in c_{33} is observed in the deciduous species (85% and 68%, for *Platanus hispanica* and *Populus x. euroamericana*, respectively, and 53% and 59% for the evergreen species *Prunus laurocerasus* and *Ligustrum lucidum*). This difference could be explained by the more resistant leaves required by the evergreen species.

Previous works have demonstrated that the point of inflection of $\Delta f_{\mathrm{TR}}/f_{\mathrm{TR}}^0$ versus RWC graphs corresponds to the point of null turgor pressure obtained from pressure-

TABLE I. Location of the Inflection Point, Standard Error, and Coefficient of Determination of the Fitting (R^2) of the c_{33} Versus Relative Water Content (RWC) and $\Delta f_{TR}/f_{TR}^0$ Versus RWC Curves.

	x_0 = RWC$_0$.	R^2
Prunus laurocerasus		
c_{33}	0.790 ± 0.013	0.99
$\Delta f_r/f_r^0$	0.828 ± 0.011	0.93
Platanus hispanica		
c_{33}	0.918 ± 0.034	0.92
$\Delta f_r/f_r^0$	0.896 ± 0.004	0.99
Ligustrum lucidum		
c_{33}	0.890 ± 0.019	0.96
$\Delta f_r/f_r^0$	0.905 ± 0.008	0.99
Populus x euroamericana		
c_{33}	0.905 ± 0.008	0.99
$\Delta f_r/f_r^0$	0.895 ± 0.003	0.99

volume relationships determined by the free-transpiration method [27]; given the special significance of this point in terms of leaf physiology, it is of interest to obtain a measurement of the location of this point in the c_{33} versus RWC curves. Toward this end, the sigmoid-growth function

$$y = y_U + \frac{y_L - y_U}{1 + (x/x_0)^p} \tag{12}$$

is used, where x_0 corresponds to the point of inflection and y_U and y_L are the upper and the lower bounds of y, respectively.

Table I shows the obtained parameters from these fittings. For purposes of comparison, the parameters obtained for the $\Delta f_{TR}/f_{TR}^0$ versus RWC curves are also shown. The obtained location of RWC$_0$ according to both measurements is similar, which is an expected result because the dominant factor in the variation in $\Delta f_{TR}/f_{TR}^0$ with RWC is the variation of the leaf elastic constants ($f_{TR} = 1/2l\sqrt{c_{33}/\rho}$). The advantage of using c_{33} instead of $\Delta f_{TR}/f_{TR}^0$ is two-fold: c_{33} measurements are more sensitive and they do provide a correlation between the absolute value of the two magnitudes (RWC and c_{33}), and not a correlation between relative variations. RWC$_0$ data in Table I also show a good agreement with RWC$_0$ data for *P. laurocerasus* and *P. x euroamericana* obtained from pressure-volume relationships determined by the free-transpiration method [27].

V. Conclusions

This paper demonstrates that magnitude and phase spectra of the thickness resonances of plant leaves, excited and sensed using air-coupled piezoelectric transducers, can be well described by an effective medium approach, and that this can be used to obtain, among other leaf properties, the elastic constant in the thickness direction (c_{33}), which provides a very good indicator of the leaf status.

The known low values of ultrasound velocity in plant leaves (from 150 to 300 m/s) are explained by the very low value of the elastic constant (c_{33}) measured in this work. This is due to both the porosity and the microstructure of the leaves. The overall or macroscopic deformation of this microstructure is made of local bending and twisting of the microscopic components (fibers and cells), which are similar to those found in cellular solids; this gives rise to very low values of the c_{33}, even for intermediate porosity values [33].

The elastic constant, c_{33}, decreases when the leaves lose water, following a sigmoid shape; this can be attributed to the loss of tension in the cell membranes and by the deformation of the cell structure. Observed relative variations in c_{33} are larger than the observed variations in the resonant frequency ($\Delta f_{TR}/f_{TR}^0$), the velocity, the density, or the thickness, so monitoring of c_{33}-variations can be a better procedure to determine the RWC of leaves.

The sigmoid-like variation in c_{33} with RWC is very similar to that observed for the variation in $\Delta f_{TR}/f_{TR}^0$ with RWC and the point of inflection of both curves is located at approximately the same RWC value. This is an expected result because eventually $\Delta f_{TR}/f_{TR}^0$ depend on c_{33} and this is the dominant factor in the variation in $\Delta f_{TR}/f_{TR}^0$ with RWC. Considering the intrinsic complexity in the biological nature of plant leaves, each species should be treated as a different problem and should be carefully checked; however, for the cases studied thus far, the point of turgor loss can be ultrasonically determined and the best technique, because it provides better sensitivity and absolute values, is the one based on the measurement of the point of inflection in the variation of the elastic constant (c_{33}) versus RWC curve.

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