A LAYERED ACOUSTIC MODEL FOR THE THICKNESS RESONANCES OF PLANT LEAVES

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In this work a layered acoustic model for the thickness resonances of plant leaves is presented based on a two layer approach, were one layer represents the upper epidermis and the palisade parenchyma and the other one the lower epidermis and the spongy mesophyll. The value of this model is that it permits to obtain information about mechanical properties and water relations of these different tissues from the ultrasonic measurements that are fully non-destructive and non invasive. Measurements of the magnitude and phase spectra of the transmission coefficient in the frequency range 0.1-2.0 MHz using air-coupled and wide band ultrasound are presented for 12 different dicot plant genus, this frequency range is enough to observe, at least, two orders of the thickness resonances. Experimental measurements are compared with the theoretical calculations obtained by a one-layer (effective medium) approach and the proposed two-layers approach in order to validate the proposal. Additional analysis of microscopic images of the tissues regarding the parameters obtained from the layered model is discussed, in order to clarify the relation between the ultrasonic measured parameters of the two layers of the leaves and their histological arrangement. Measured and calculated (one-layer and two-layers models) resonance spectra of the transmission coefficient, together with image analysis allows us to conclude that the simplified two-layers approach is a meaningful acoustic model of the thickness resonance of plant leaves.

1. Introduction

Leaves constitute a highly interesting system from a mechanical point of view (Dumais et al., 2012). Their organization in different tissues, the wide variety of adaptative solutions as consequence of their development under diverse abiotic factors and their ability to respond immediately to environmental stimulus, make the leaves an interesting object for study.

Multilayered materials are widespread in nature. The hierarchical structure in natural materials have many scales or levels, have highly specific interactions between these levels, and have the architecture to accommodate a complex spectrum of requirements (Gibson et al., 2010). Leaves are not an exception. Consequently, different layers can be distinguished: a compact layer where photosynthesis is carried out, a porous layer where gaseous interchange takes place, a surface area for receiving sunlight, etc. At the same time, the whole ensemble keeps mechanical strength as well as allows growth.

Air-coupled ultrasound techniques present several advantages in characterization of multi-layered materials. Thickness resonance analysis methods are especially well suited to this problems, when touch the sample is not allowed. Using a single layer model has made possible in the past to determine effective density, ultrasound velocity and attenuation of leaves of a large number of different plant species. However, when the experimental frequency range is expanded and several orders of the thickness resonances are observed in the transmission coefficient spectra, the effective approach, one layer model, fails to correctly clarify the measurements due to the appearance of a strong harmonic distortion. This harmonic distortion can be accounted for, from a theoretical point of view by using a layered model instead of the one layer model used so far.

2. Methods

2.1 Single layer model

From previous works (Álvarez-Arenas et al., 2009), we know that the measured spectra of the transmission coefficient at normal incidence of different plant leaves in the frequency range limited around the first order thickness resonance can be reproduced by a theoretical model that assumes the leaf as a homogeneous and flat plate. In this case a simple analytical expression (Brekhovskikh, 1960) can be derived for the transmission coefficient (γ), see Eq. (1), that depends on the acoustic impedance of the leaf and the surrounding medium (air in this case): Z_1 and Z_2, respectively, the thickness of the leaf (*t*), the ultrasound velocity (*c*) and the attenuation (α) in the leaf and the ultrasound frequency (*f*).

(1)
$$\gamma = \frac{-2Z_1Z_2}{2Z_1Z_2\cos kt \ + i[Z_1^2 + Z_2^2]\sin kt}$$

where $\omega=2\pi f$, and $k=\omega/c$.

In general, we assume that the attenuation coefficient (α) varies with the frequency (f) following a power law:

(2)
$$\alpha = \alpha_0 (f / f_0)^n$$

As the acoustic impedance of a material is the product of the density and the acoustic wave velocity, then γ of a leaf is a function of the frequency of the wave that depends on the following leaf properties: thickness (t), density (ρ), ultrasound velocity (ν) and attenuation (α). All these leaf parameters can be obtained by fitting the calculated γ according to Eq. (1) into the experimentally measured transmission coefficient spectra (magnitude and phase) without any additional input parameter. First, as proposed in Álvarez-Arenas (2010) the measurement of the resonant frequency, the magnitude and the phase of the transmission coefficient at resonance and the Q-factor of the resonance peak are used to get an analytical estimation of the leaf thickness, density, and ultrasound velocity and attenuation coefficient at the resonant frequency. Then, these values are used as initial guess for a fitting routine based on the Gradient Descent method to find the set of leaf parameters (t, ρ, c, α and n) that minimize the error between the calculated transmission coefficient spectra and the measured one. This routine is written in Python 2.7 and is available at the group web page (http://www.us-biomat.com). The ultrasonic estimation of leaf thickness and density are compared with direct measurements obtained with the micrometer and by weighing the circles excised from the leaf with the punch holder. This comparison provides an independent verification of the accuracy of the ultrasonically estimated effective leaf parameters.

2.2 Layered model

A layered approach has been used before in a few cases where the analysis of the thickness resonances of plant leaves in a wide frequency range was necessary (e.g. study of shear waves (Fariñas et al., 2013), and relationship between ultrasonic properties and tissue functional design, in the case of Phormium tenax (Fariñas et al., 2014a), however, a comprehensive analysis and test of the layered using a more complete set of experimental data was still to be done.

In this case, one additional interface is added to the single layer model explained above. Consequently, the number of variables involved unfolds: thickness, density, ultrasound velocity and attenuation (also its variation with the frequency) of each layer is required, increasing the complexity of the system.

The reason to select a two layers model is two-fold. First, it is reasonable to consider that the complex layered structure of dicot leaves can be simplified in a two layered acoustic model as it seems reasonable that the upper epidermis (a thin layer of closely packed cells) and the palisade parenchyma (a densely packed layer of tissue composed of elongated cylindrical cells normal to the leaf plane) can be considered, from the acoustic point of view, as one layer (with density close to 1000 kg/m^3) and the spongy mesophyll (a layer of tissue that present a large and open-pore porosity) and the lower epidermis (a thin layer of closely packed cells with apertures -stomata- that connects the inner porosity with the external air) can be considered as another layer, with quite different acoustic properties (and density clearly below 1000 kg/m^3). And, second, increasing the number of layers may make the model closer to the real leaf, but also puts the complexity of the problem out of our reach.

The method used varies with respect to a single layer one: effective values considering a homogenous plate are used as initial guess for a fitting routine based on the Gradient Descent (GD) method to find the set of material parameters that minimize the error between the calculated transmission coefficient spectra and the measured one, assuming that every layer in the model are equal. The algorithm is structured in two main loops: the first one varies velocities and densities of the layers involved in the model (this is related to the location of the resonance peaks along the frequency); the second one varies the parameters related to the attenuation (this is related to the variation of energy with frequency). The explained structure of the algorithm can resemble the biological process where tissue differentiates functionally from one point.

There are infinite solutions for the selected error minimization requirements. All solutions turn out equivalent from the point of view of significant parameters: the acoustic impedance and the wavenumber and thickness product.

3. Experimental Set up

3.1 Experimental set up

Three pairs of air-coupled transducers were used. They are wide-band transducers developed, designed and built at the Spanish National Research Council and have frequency bands of 0.1–0.35, 0.35–0.95 and 0.5-1.3 MHz, peak sensitivities of –25, –30 and -32 dB, electrical impedances between 100 and 200 Ω and active area diameters of 20, 15 and 10 mm, respectively (see Gómez Álvarez-Arenas 2003a and 2003b for further details). Transducers were embedded in a U-shaped holder that maintained them facing each other at distances of 30–50 mm. The holder also had a slot in which the samples could be easily positioned between the transducers for measurements. This holder provides the necessary robustness for the system so that it can be easily manipulated without affecting the integrity of the signal. Samples are located approximately at the middle point and at normal incidence.

A commercial pulser/receiver (5077PR, Olympus, Houston, TX, USA) was used to drive the transmitter transducer (200-V-amplitude semi-cycle of square wave tuned to the transducer centre frequency) and to amplify and filter the electrical signal provided by the receiver transducer (up to 40 dB and low pass filtered: 10 MHz). The signal was then sent to a digital oscilloscope (TDS5054, Tektronix, Beaverton, OR, USA) with the impedance set at 1 MΩ and the bandwidth set at 20 MHz and averaged (between 80 and 120 samples). Samples were digitized at 2 MS/s and 8 bit. The result was then transferred to the oscilloscope PC for further signal analysis. First, a rectangular time window was applied to the transmitted waveform to filter out the reverberations within the air cavities. The signal was padded with zeroes up to 4K to increase frequency resolution, and then the Fourier transform was extracted using the fast Fourier Transform (FFT) algorithm. Real and imaginary parts of the FFT are used to compute the magnitude and phase spectra of the transmitted signal. Further calculations to obtain the frequency location of the maximum transmission (resonant frequency) or the Q-factor of the resonance were performed in MATLAB (The MathWorks, Natick, MA, USA) (see Sancho-Knapik et al. 2012).

3.2 Experimental procedures

For each species of plant leaves, branches were collected from a single tree at down taken with the whole petiole; they were kept under water in order to avoid embolism. Thereafter, leaves were rapidly introduced to plastic containers with water in order to ensure a water-vapour saturated atmosphere while they were carried to the laboratory. Ultrasonic measurements were taken at full turgor (water saturation). Then, all this data is processed as above. After that, circles were excised from the leaves using a punch holder (14 mm diameter). Disk thickness was measured with a micrometer, weighed using the precision balance (Precisa XT220A) and finally, density was worked out. Then, the excised leaf circles were put in an oven at 80 ºC for 48 hours to remove the water; finally, they were removed from the oven and weighed again to get the dry matter content (Sancho-Knapik et al., 2010).

An Allmikro (Haga company, Nuremberg, Germany) hand microtome was used to get thin slices of the cross section in order to can be seen through a Leica DM 750 (Wetzlar, Germany) optical microscope. Then, several images of the leaves at full turgor were taken and recorded by a ICC50 HD camera connected to the microscope.

4. Materials

Leaves of dicot and evergreen species were collected: *Abelia edward goucher*, *Acer* (*campestre*, *platanoides*, *rubrum* and *campestre*), *Prunus* (*lusitanica* and *laurocerasus*), *Photinia* (*serrata*, *x fraseri* and *robusta*), *Camellia japonica*, *Rhododendron* (*clivianum*, *purple splendour* and *virginia richards*), *Ligustrum* (*japonicum*, *lucidum* and *laurocerasus*), *Bougainvillea*, *Nerium oleander*, *Viburnum tinus*, *Citrus x sinensis* and *Clematis armandii*.

The species were selected with a view to show a set of differences between forming tissue layers (pallisade parenchyma and spongy mesophyll mainly) which could prove the potential of the acoustic model presented in this work.

5. Experimental Results

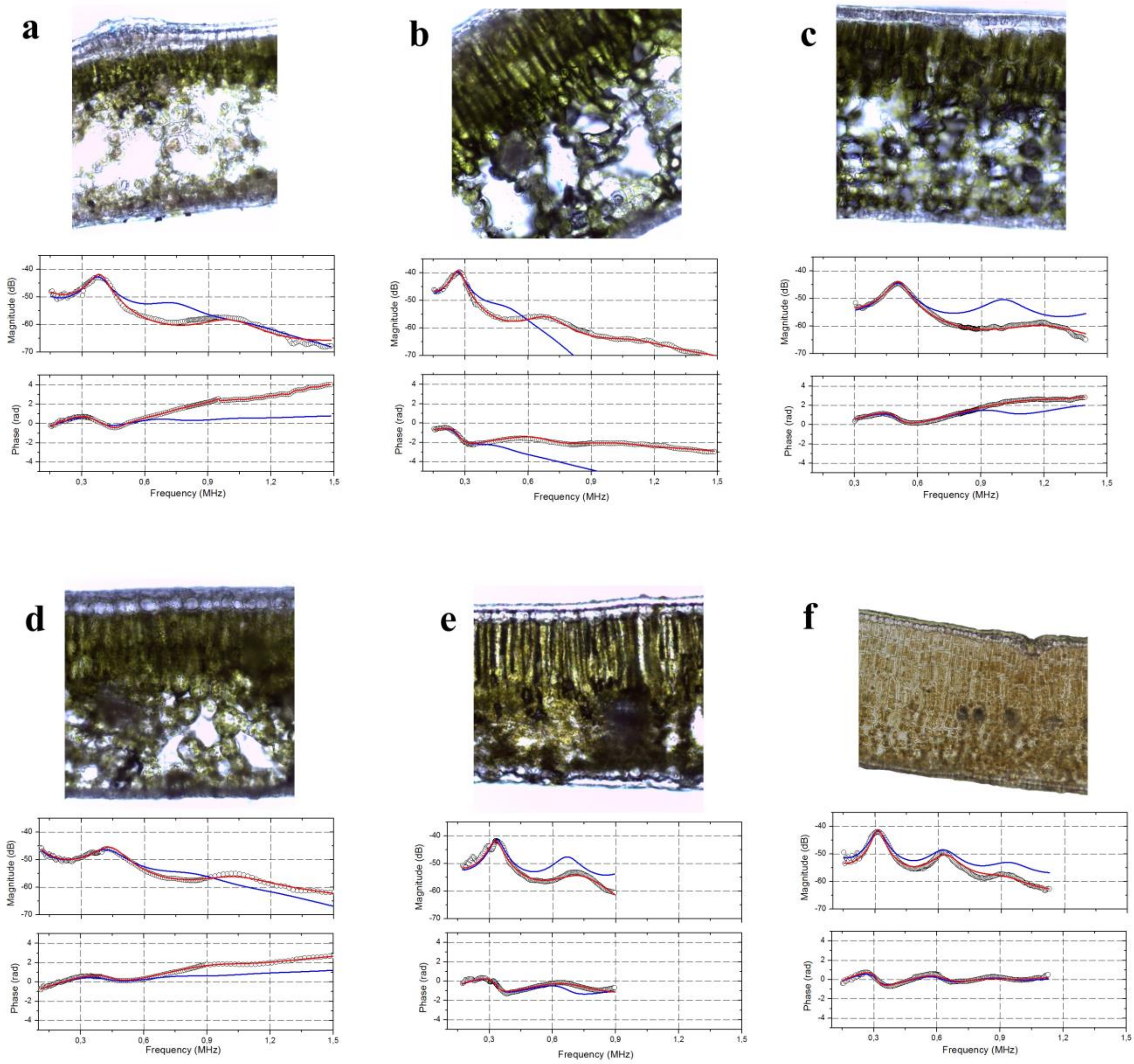

Figure 1. Cross section images and magnitude and phase spectra of transmission coefficient (*circles*: experimental data; *blue line*: one-layer-model fitting; *red line*: two-layer-model fitting) in leaves of different plant genera: (*a*) *Abelia*, (*b*) *Prunus*, (*c*) *Camellia*, (*d*) *Rhododendron*, (*e*) *Clematis*, (*f*) *Ligustrum*.

The histological structure of dicots leaves can be formed up to four layers: adaxial epidermis, palisade parenchyma, spongy mesophyll and abaxial epidermis. From the acoustic point of view not each of these histological layers is different, this means that the acoustic impedance (product of density and ultrasonic velocity) between layers can agree.

Figure 1 shows six different leaf cross section images as well as their transmission coefficient spectra. The ultrasound response is different between them; this can be clearly seen in terms of the position of the resonance peaks, the level of energy not only in the maximum of magnitude spectra but also in the minimums or the slope between resonance frequencies position and in the value and the slope of the phase measurements.

According to the cross section images (see Fig. 1), the histological difference between palisade parenchyma and spongy mesophyll can be seen at first sight. Additionally, this difference decreases from *Abelia* (a) to *Ligustrum* (f) in fact, *Abelia* leaves present a large and highly porous spongy mesophyll layer with very large pores, while in the *Ligustrum* leaves palisade parenchyma and spongy mesophyll are not so different and porosity of the spongy mesophyll is very low and pores much smaller. Due to this fact, the location of the first and second resonances in the transmission coefficient spectra shifts: while in *Ligustrum* (Fig.1.f) the position of the second peak is the double of the first one (as it was expected by the single layer model), in *Abelia* measurements (Fig.1.a.) the frequency of the second maximum of magnitude spectra is about three times the frequency of the first resonance, that is, the harmonic distortion is maximal in the *Abelia* leaves, where the differences between the two layer of tissues are also maximal. This behaviour can be explain as follows: given the value of resonance frequency in every material characterized as a single layer the first peak is located at λ/2. This relation is given by the following equation:

$$n(w_0) = 2w_0 t / 2p \tag{3}$$

Table 1. Mean and standard deviation of acoustic properties of measured plant species classified by genus.

SPECIES	ACOUSTIC IMPEDANCE (MRayl)	SURFACE DENSITY (kg/m²)	ATTENUATION (Np/m)	VELOCITY / THICKNESS (MHz)
Abelia	0.864 ± 0.130	0.196 ± 0.015	28.8 ± 27.1	4.421 ± 0.732
	0.119 ± 0.017	0.088 ± 0.012	18.7 ± 34	1.351 ± 0.047
Photinia	0.653 ± 0.127	0.214 ± 0.026	52.8 ± 30.6	3.079 ± 0.637
	0.076 ± 0.019	0.059 ± 0.010	20.2 ± 3.2	1.270 ± 0.175
Prunus	0.544 ± 0.134	0.173 ± 0.035	62.1 ± 51.1	3.129 ± 3.155
	0.079 ± 0.022	0.062 ± 0.013	15.2 ± 25.3	1.265 ± 1.334
Nerium	0.756 ± 0.075	0.245 ± 0.05	51.2 ± 11.7	3.155 ± 0.465
	0.150 ± 0.013	0.113 ± 0.012	25.3 ± 3.4	1.334 ± 0.036
Camellia	0.864 ± 0.096	0.275 ± 0.040	15.4 ± 13.1	3.180 ± 0.469
	0.199 ± 0.059	0.114 ± 0.035	12.3 ± 2.2	1.761 ± 0.144
Citrus	0.704 ± 0.057	0.197 ± 0.023	18.9 ± 11.9	3.635 ± 0.623
	0.122 ± 0.031	0.064 ± 0.018	17.8 ± 1.2	1.935 ± 0.096
Viburnum	0.587 ± 0.084	0.167 ± 0.025	22.0 ± 14.1	3.588 ± 0.821
	0.140 ± 0.036	0.078 ± 0.024	15.6 ± 4.7	1.856 ± 0.419
Rhododendron	0.571 ± 0.106	0.172 ± 0.047	42.9 ± 28.2	3.452 ± 0.685
	0.133 ± 0.052	0.087 ± 0.039	25.9 ± 4.9	1.578 ± 0.171
Acer	0.494 ± 0.132	0.148 ± 0.049	374.9 ± 24.06	3.749 ± 2.406
	0.147 ± 0.051	0.071 ± 0.026	211.5 ± 24.9	2.115 ± 0.249
Bougainvillea	0.353 ± 0.041	0.216 ± 0.055	0.298 ± 11.7	1.690 ± 0.316
	0.109 ± 0.044	0.093 ± 0.044	15.1 ± 4.4	1.206 ± 0.115
Clematis	0.442 ± 0.066	0.294 ± 0.059	30.9 ± 14.6	1.517 ± 0.146
	0.175 ± 0.051	0.163 ± 0.060	13.0 ± 2.6	1.108 ± 0.152
Ligustrum	0.419 ± 0.081	0.525 ± 0.198	12.8 ± 7.3	1.403 ± 0.654
	0.239 ± 0.077	0.156 ± 0.061	12.2 ± 2.6	1.119 ± 0.139

In the particular case when the layer is attached to a plate made of another material, first resonance frequency of the lower impedance layer shifts to λ/4 while the resonance originated by the higher acoustic impedance keeps at λ/2. Due to the fact, that there is a tight link between the characteristics of the cells forming every different biological tissue in the leaf and their mechanical response, we can assert that as consequence, there is also a direct relationship between the distortion of the frequency pattern measured respect to the theoretical one layer model and how acoustically different are the constituent layers of the leaf.

Significant parameters were worked out applying the layered model for every plant species measurements. More than 10 different measurements of each species were taken. The ultrasound parameters obtained were classified according to the 12 different genera measured. Results suggest that the resonances are very sensitive to leaf microstructure (see Fariñas et al., 2013 and 2014b), as a consequence of the wide range of different leaf morphologies considered within each genus, high values of standard deviation are shown in Table 1.

According to the behavior of the layered structure of the leaf commented above, the measured ratio of the acoustic impedances of the two layers in *Abelia* leaves (see Table 1) is up to 10 while the range of this ratio obtained for *Ligustrum* leaves begins about 1. At the same time, values of attenuation and velocity and thickness ratio in each forming layer are similar, revealing that in the particular case of *Ligustrum*, the acoustic behavior can be explained as a single layer (which can be also seen in the transmission coefficient plotted in Fig.1).

Attenuation coefficient values are typical of porous materials (see Álvarez-Arenas, 2003b), however, it may appear strange that obtained values are larger in the palisade parenchyma than in the porous spongy mesophyll, where scattering effects must be significant. The reason for this behaviour can be that in the palisade parenchyma layer, the cell walls act as wave guide, and ultrasound propagation takes place preferentially along this path. However, as the cell wall only occupies a small volume fraction (φ), wave amplitude is reduced in the same proportion, so there is a large apparent energy loss due to this effect, and this is taken into account by the model as if it were due to the attenuation coefficient:

$$A = j\, e^{-at} \qquad (4)$$

Finally, the values of surface density shown in Table 1 are typical of leaves. This parameter indicates the distribution of mass between the considered layers. In case of *Abelia* leaves, the surface density ratio between layers is lower than the one observed in acoustic impedances. According to the optical image (Fig.1.a) this can be explained in terms of the palisade parenchyma, which is much thinner than the spongy mesophyll. On the contrary, in *Ligustrum* leaves the surface density ratio between layers is higher: palisade parenchyma (densely packed cells) in this case is thicker than spongy mesophyll one (high porosity).

6. Conclusions

An acoustic layered model for plant leaves was proposed in this work. The validity of this model was tested with experimental data coming from up to 12 different dicot leaf genera collected for this purpose. In order to make the selection, the histological differences between different layers forming the tissue of the plant leaves were taken into account.

Considering the one-layer-model, which assumes the whole leaf as a homogenous plate, effective parameters can be obtained: density, thickness, ultrasound velocity and attenuation. The layered model proposed mainly comprises two layers for the species under study: one accounts for the palisade parenchyma and the upper epidermis, while the other one corresponds to the spongy mesophyll and the lower epidermis. From a mechanical point of view, there is a link between the form, rigidity and density of cells on each layer and the acoustic impedance of the material.

According to the magnitude and phase spectra measured at normal incidence using air-coupled ultrasound, the fitting between one layer model and the measurements is not accurate anytime. This can be interpreted as a measure of how acoustically different are the leaf constituent layers. Therefore, in these cases a layered model is needed as long as properties of each layer are required.

In addition, it is well known that leaf tissue can be modified by abiotic factors as sunlight intensity, water irrigation, air current, dissolved oxygen, mineral content of the soil, etc. Consequently, the ultrasonic measurements taken as well as mechanical parameters worked out using the layered model change. This precise determination of the properties of the leaf tissues suggests that the technique and the acoustic model presented in this work can be used to find out the plant status, its health and possible needs in term of water or nutrients.

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