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Determination of Plant Leaves Water Status using Air-Coupled Ultrasounds

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Abstract— Water in plants is studied by analyzing the magnitude and the phase spectra of the first thickness resonance of their leaves. These resonances appear at ultrasonic frequencies and have been excited and sensed using air-coupled ultrasounds. In spite of the complex leaf microstructure, the resonances of the leaves can be well described by the resonances of either a homogeneous and isotropic solid plate or a four-layered composite. Results reveal that these resonances are strongly sensitive to leaf microstructure, water content and water status in the leaf. As the technique is completely non-contact and non-invasive, it offers a unique possibility to study the complex dynamic behaviour of water in plants and the water exchange between the plant and the atmosphere across the leaves.

Keywords-component; plant leaves; air-coupled ultrasound, spectral analysis, thickness resonance.

I. INTRODUCTION.

Plant leaves are key elements of the structure of life as it is there where the photosynthesis takes place; through the leaves the plants interchange gases, water vapour and energy with the atmosphere. In this sense, they are the last stage of an important and intriguing plant mechanism that permits to lift water from the soil up to the canopy. The cohesion-tension theory commonly accepted to describe this mechanism is based on the reduction of pressure in the leaf due to evaporation and the appearance of large negative pressures that stretches the water in the capillaries. This mechanism has recently been tested in synthetic trees [1]. Recently, a synthetic leaf has been proposed to harvest energy from evaporation-driven flows. [2]

Figure 1 shows a schematic cross section of the structure of a leaf made up of four layers: upper epidermis (densely packed cells with very thick walls and a waxy cuticle), pallisade parenchyma, spongy mesophyll and lower epidermis. In addition, leaves also contain veins. In the spongy mesophyll there are large spaces between cells that contain moist air and allow diffusion of carbon dioxide). Stomata in the lower epidermis permit the exchange of moisture and carbon dioxide between the leaf and the atmosphere. Unlike stretched water in the plant capillaries, water contained on leaf cells (vacuoles) is subjected to positive pressures. This is due to the elastic energy stored in the vacuole membrane. This mechanism is responsible for the turgor pressure which maintains the leaf (and in some cases the whole plant) rigid.

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In the context of plant physiology it is extremely interesting to develop non-invasive procedures to monitor the dynamic of water movement through the plant and from the plant into the atmosphere, in the context of the plant-soil-atmosphere continuum model. This is also interesting for the optimization of water supplies. These applications demand sensors capable of monitoring water content of the plant directly and online. Available techniques present serious difficulties as they are either too laborious or the equipment too complex or automation is not possible or they are not fully non-invasive.

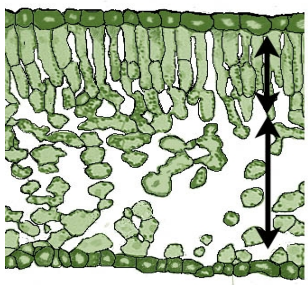

Figure 1. Cross-section of the leaf

Recently, Fukuhara [3] measured velocity and attenuation of ultrasounds in plant leaves. However, this technique presents the drawback of using water as coupling medium. This water penetrates in the leaves and modifies their acoustic properties (see Ref. [4]). More recently [4], we have used air-coupled ultrasounds to excite and sense the first order thickness resonance of leaves of different plants. The spectral response of the transmission coefficient in the vicinity of the first thickness resonance (both magnitude and phase) is well described by the theory of sound transmission through a homogeneous solid plate. Solution of the inverse problem permitted the authors to obtain, simultaneously, velocity and attenuation of ultrasounds in the leaf and the thickness of the leaf. Finally, it was experimentally demonstrated that small variation of water content in the leaf give rise to a measurable frequency shift of the leaf thickness-resonance.

In this paper, we investigate the possibility of using a more detailed model based on a four layered representation of the cross-section of the leaf. These layers correspond to the leaf structure shown in Fig. 1.

II. II. EXPERIMENTAL SET-UP AND MATERIALS.

Ultrasonic transmitter and a receiver specially designed for efficient transmission/reception to/from the air, were positioned in opposition. Transmitter is driven by a negative square semi cycle tuned to the centre frequency of the transducers. So it launches an ultrasonic signal that travels across the air-gap between transmitter and receiver transducer and is eventually received by the receiver. This converts the ultrasonic signal into electrical, it is then amplified and filtered. Afterwards it is displayed and digitized in a digital oscilloscope.

The measuring procedure is as follows. First the signal received without any sample is acquired; FFT (magnitude and phase) is calculated and stored as reference. Then the sample is put in between the transducers at normal incidence, and the procedure is repeated, hence we calculate the phase shift ($\Delta\phi$) and the insertion loss. Two pairs of special air-coupled transducers with centre frequency of 0.25 and 0.75 MHz were employed. Thickness was measured using a micrometer (error ± 5 μm).

Leaves from *Prunus laurocerasus*, *Ligustrum lucidum*, *Platanus x hispanica* and *Populus x euroamericana* were studied; for each species ten different leaves were measured.

III. III. MODEL FOR SOUND TRANSMISSION THROUGH A PLANT LEAVE.

Thickness resonances of solid plates have been used in the context of air-coupled ultrasound with the purpose to increase the amount of transmitted energy [5]. For a homogeneous and isotropic plate the amplitude ratio of transmitted to incident wave potentials for normal incidence is given by:

$$\xi = \frac{-2Z_1Z_2}{2Z_1Z_2\cos(kh) + i\left(Z_1^2 + Z_2^2\right)\sin(kh)} \tag{1}$$

where Z_1 and Z_2 are the acoustic impedances of the plate and the surrounding fluid, h the thickness of the plate and k the wave number. The first thickness resonance appears at a frequency given by the following relation:

$$v(\omega_o) = 2\omega_o h / 2\pi \tag{2}$$

where v is the velocity of sound in the plate and ω_0 the angular frequency of the first thickness resonance. So if the proper combination of transducer frequency band, ultrasound velocity in the material and plate thickness is given, thickness resonances are observed.

Later, these resonances have been used to study the properties of the solid material. The most basic approach is to get an independent measurement of the thickness, and then work out the velocity of the ultrasounds in the solid from the relation given above. A more complete characterization of the solid can be obtained from the analysis of the whole resonance vs. frequency curve. This procedure is explained in detail and used in [6] and [7]. An important limitation of this method is that the thickness of the plate must be independently measured. In some cases, this is either not possible or not accurate enough. Plant leaves is one of these cases.

However, if both magnitude and phase spectra of the transmission coefficient are simultaneously measured it is possible to overcome this problem and to obtain, simultaneously, both ultrasound velocity and thickness of the plate. This method was developed and tested for different materials and plates by Gómez Álvarez-Arenas [8] and it is based on the fact that at resonance and when attenuation is negligible the following relation is verified:

$$v(\omega_0) = \frac{h}{h / v_f - \Delta\phi / \omega_0} \tag{3}$$

where v_f is the velocity in the coupling fluid (in this case 340 m/s) and $\Delta\phi$ is the phase shift. Equation 3 is the relation normally used to work out velocity from the measurement of phase spectrum in a through transmission experiment, [9] which is similar to the Sachse and Pao method [10]. Then, using equations 2 and 3, it is possible to simultaneously obtain both v and h from measured ω_0 and $\Delta\phi(\omega_0)$.

Finally, v and h so calculated are then used as initial guess to fit the theoretical value of $T=|\xi|^2$ and $\Delta\phi$ into the experimentally measured ones. A power law for the variation of the attenuation coefficient with the frequency is assumed: $\alpha=\alpha_0 f^{\,m}$, where $1<m<2$. It must be noted that this non-negligible attenuation displaces the resonant frequency away from the condition given by Eq. 1. Further details of the technique can be seen in Refs [4] and [8]

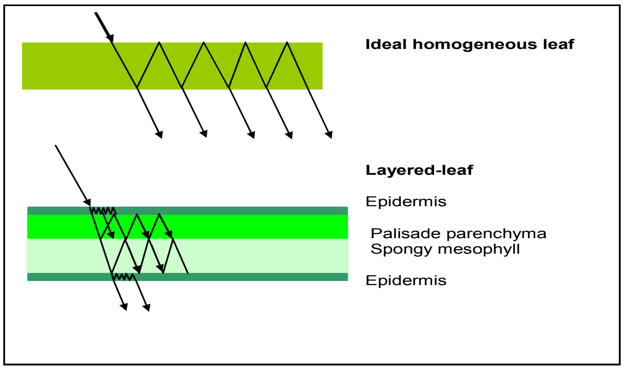

Figure 2. 1-layer and 4-layers models for plant leaves cross-section structure.

The presence of several sub-layers within the plate, having different acoustic properties, modifies the resonances of the plate. This situation is shown in Fig. 2. The main problem of modelling sound transmission through a leaf that presents a four-layered structure is that detailed information about each layer is needed. Unfortunately almost no information about the acoustic properties of these organic tissues is available. What we do know is the typical thickness of each layer and its microstructure or composition. The epidermis contains a very thin layer of a waxy cuticle and the epidermis itself: a layer of densely packed and rigid flattened cells. The pallisade parenchyma is a less dense packing of elongated cells oriented along the normal direction to the leaf plane. This could be considered a 1-3 connectivity composite of pillar-like cells in a matrix of water and air. The next layer, the spongy mesophyll, presents a foam or spongy-like structure and can be considered a 0-3/3-3 connectivity composite. [11] This is made of less densely packed rounded cells and large spaces between them to allow diffusion of carbon dioxide, oxygen and water vapour. The elastic stiffness of the cells in these two layers are largely determined by the pressure of water inside them.

Determination of the properties of each of these layers lies out of the scope of this work; however, it is possible to propose a rough estimation of the properties of each of them. Let us consider a typical *Prunus laurocerasus* leaf: h=450 µm, v=255 m/s, ρ =785 Kg/m^3, $\alpha(f_0)$=740 Np/m, f_0 =0.30 MHz and a linear variation of the attenuation coefficient with the frequency (ref. 4). The calculated transmission coefficient (magnitude and phase) is shown in Fig. 3. Now we assumed a four-layered composite leaf, and that attenuation in all layers varies linearly with the frequency. For the upper layer and the lower layers (wax and epidermis) we considered a h = 12 µm, ρ= 900 Kg/m^3, (in between density of water and density of wax) and a velocity of sound similar to the velocity in water: 1500 m/s, finally, $\alpha(f_0)$=50 Np/m. For the pallisade parenchyma, thickness is 200 µm, now we are using velocity and density values similar to those observed in microporous polymer films with similar structure: v= 405 m/s, $\alpha(f_0)$=600 Np/m and ρ = 900 Kg/m^3, Finally, for the spongy mesophyll, the most complex layer from the acoustic point of view, we worked out its properties so that the global density of the four-layered model be equal to the density of the single layer model. A similar procedure was followed to determine the velocity and the attenuation coefficient, obtained result for the spongy mesophyll layer are: h=226 µm, v=173 m/s, ρ = 624 Kg/m^3, $\alpha(f_0)$=929 Np/m.

Calculated transmission coefficient for this composite structure is shown in Fig. 3. There are three main differences. First, there is a frequency shift. Second, the insertion loss predicted by the multilayered model is larger. Third, the regular resonance pattern observed in the one-layer model is distorted in the four layered model: periodicity is lost and the resonance pattern completely disappears at high frequencies. All these effects are the result of the multilayered structure.

It is interesting to try to fit the resonance peak calculated with the one-layered model into the calculated values with the four-layered model. In some sense, this is what we do when we try to fit the one-layered model into the experimental data (of a four layered leaf). Result is shown in the inset in Fig 3. Best fitting is obtained for the following material parameters in the 1-layer model: h=492 µm, v=212 m/s, ρ = 785 Kg/m^3, $\alpha(f_0)$=1400 Np/m and a quadratic variation of the attenuation coefficient with the frequency. That is, the result of fitting the 1-layer model first thickness resonance to the 4-layered composite first thickness resonance is that estimated attenuation coefficient in the 1 layer model is much larger than the actual value in the four-layered model, velocity somewhat lower and thickness slightly larger. In addition, the 1-layer model fails to reproduce the shape of the resonance curve at high frequencies. This deviation of the parameters estimated with the 1-layer model related to the more precise parameter estimation of the 4-layered model has to be considered if leaf properties are going to be measured. However, this is not so important when relative variations of the leaf properties are to be studied; one example is the study of the variations of the leaf water content as it is shown below.

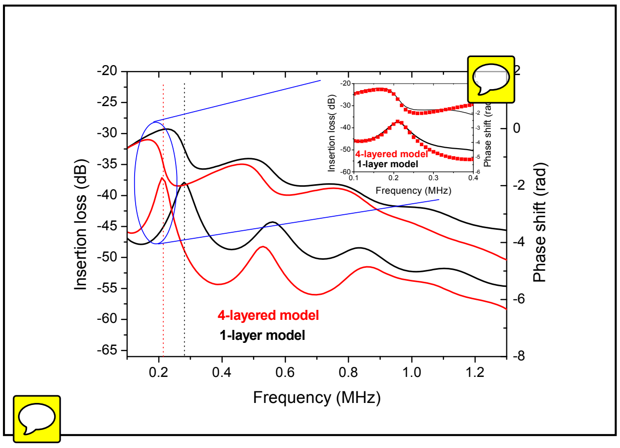

Figure 3. Coefficient of transmission (magnitude and phase) vs. frequency of a Prunus leaf.

IV. EXPERIMENTAL RESULTS.

Figure 4 shows measured and calculated T and $\Delta\phi$ for two cases. One interesting feature is that the 1-layer model provides a good fitting into the experimental results, at least in the vicinity of the first thickness resonance.

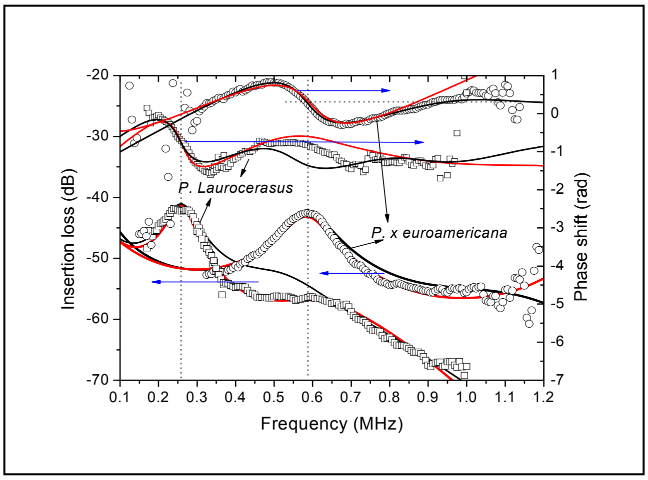

Figure 4. Experimental (dots) transmission coeffient vs. frequency of P. Laurocerasus and P. x euroamericana leaves. Solid line represents the theoretical predictions: Black 1-layer model, Red: 4-layers model.

Obtained ultrasound velocity and thickness from the 1-layer model for all the studied cases appear in Table I. Two cases for each species are show: the thickest and the thinnest leaves. Relationship between ultrasonic properties and thickness can be interpreted as the result of a close link between the ultrasonic properties and the leaf development stage. Ultrasonically measured thickness is very close to the thickness measured with a micrometer, observed differences can be accounted for either by the fact that the leave deforms under the pressure of the micrometer or by the error of using of a 1-layer model (in comparison with the real 4-layered leaf structure). Four-layers model parameters are summarized in Tables II and III. Calculated IL and phase appear in Fig. 4. As frequency increases the 4-layers model fits better into the experimentally observed behaviour.

TABLE I. MEASURED LEAF PROPERTIES.

Specie	*h* (micrometer) (μm)	*h* (ultrasound) (μm)	*v* (m/s)
P. laurocerasus	425	464 ± 10	255 ± 5
	445	475 ± 25	270 ± 15
L. lucidum	345	370 ± 5	196 ± 3
	540	552 ± 6	287 ± 3
P. x euroamericana	250	250 ± 3	365 ± 5
	280	295 ± 2	351 ± 5
P. x hispanica	190	214 ± 2	275 ± 5
	235	232 ± 8	369 ± 9

TABLE II. *P. LAUROCERASUS* PARAMETERS FOR THE 4-LAYER MODEL

Layer	*h* (μm)	ρ (Kg/m^3)	*v* (m/s)	α_0 (Np/m)
Epidermis	12	900	1500	50
Palisade p.	251	950	450	350
Spongy m.	200	555	210	900

TABLE III. *P. X EUROAMERIC*. PARAMETERS FOR THE 4-LAYER MODEL.

Layer	*h* (μm)	ρ (Kg/m^3)	*v* (m/s)	α_0 (Np/m)
Epidermis	12	900	1500	50
Palisade p.	157	950	550	800
Spongy m.	121	600	300	1460

Finally, location and shape of the first thickness resonance spectral response change as water evaporates. Fig. 5 shows IL and phase for the first thickness resonance of a *Prunus* and a *Populus* leaves left to dry at ambient conditions. Loss of water is measured by weighing the sample with a high precision balance. As water evaporates the resonance frequency shifts towards lower values. A similar behaviour was found in the other species. For cases shown in Fig. 5, velocity is 218, 210, 202 and 193 m/s, respectively, while thickness remains constant. This velocity decrease can be explained by an increase of the compressibility of the arrangement of vacuoles that build up the leaf structure due to a loss of pressure in the vacuole membrane produced by the loss of water.

V. CONCLUSIONS.

The technique presented permitted us to determine, simultaneously, thickness of plant leaves and velocity and attenuation coefficient of ultrasounds. The low values of the velocity can be linked to the microstructure of the leaf: air, fibres and water filled vacuoles. Measurements obtained from different leaves suggest a close relationship between ultrasound velocity and leaf development stage. Two models, 1-layer and 4-layers, have been proposed. The 4-layers model gives a more accurate description of the experimental data, though a more complete knowledge of the leaf is required. The 1-layer model is simpler and provides a reasonably good description of the first thickness resonances, at least in the vicinity of the resonant frequency. In addition, ultrasound velocity shows a great sensitivity to any variation of the water content and water status in the leaf. The possibility of monitoring water content changes in a transpiring leaf will provide is of great importance for plant physiologists.

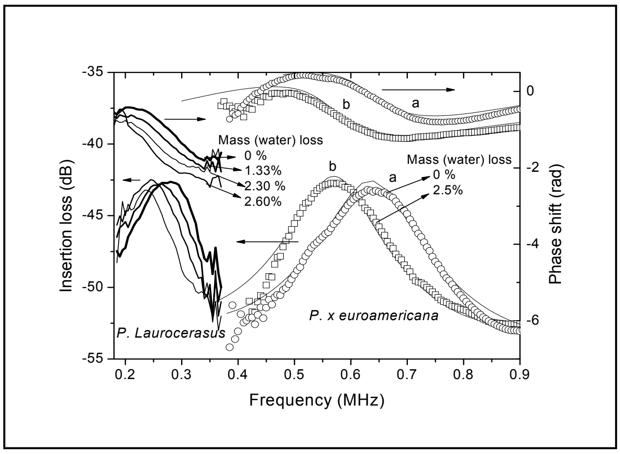

Figure 5. Variation of the first thickness resonance vs. frequency (magnitude and phase) as leaves loss water due to evaporation.

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