Shear Waves in Plant Leaves at Ultrasonic Frequencies: Shear properties of Vegetal Tissues.

M.D. Fariñas and T.E.Gómez Álvarez-Arenas
UMEDIA research group.
Spanish Scientific Research Council, CSIC
Madrid, Spain
tgomez@ia.cetef.csic.es

D. Sancho-Knapik, J. J. Peguero-Pina and E. Gil-Pelegrín
Unidad de Recursos Forestales
CITA, Gobierno de Aragón
Zaragoza, Spain

***Abstract*— Shear Waves are observed in leaves of some plant species (*Epipremnum aureum* and *Vitis vinifera*) using air-coupled ultrasound, through transmission and oblique incidence. Shear waves appear as a modification of the thickness resonance pattern of the longitudinal wave measured at normal incidence. Poisson's ratio, shear wave velocity and attenuation coefficient of shear waves in the leaves are extracted from the measured resonance spectra using a bilayer acoustic model for the leaves. Influence of water content or the degree of leaf development on shear wave properties is also analyzed.**

Keywords-component; air-coupled ultrasound, shear waves, organic tissues, plant leaves.

I. INTRODUCTION.

Generation and propagation of shear waves in animal tissues and organs have already been used by different characterization, test and imaging techniques. Elastography is a well known example with a large number of medical applications. Reviews of this technique can be seen in [1] and [2].

Unlike animal cells, vegetal cells are surrounded by a cell wall. In the past, this wall was viewed as an inanimate rigid scaffold, but it is now recognized as a dynamic structure that plays an important role in controlling the development of the plant [3]. One of its functions is to withstand the osmotic pressure of the cell. So, the combination of cell pressure and cell wall strength contributes to the whole rigidity of a plant. Cell walls may differ in function and in composition. Walls surrounding growing and dividing plant cells must provide mechanical strength but must also expand to allow the cell to grow and divide. Once the cell has ceased to grow, a much thicker and stronger wall may then develops. In general, cell wall accounts for most of the carbohydrate in biomass. In addition, they may have a major impact on human life, as they are a major component of wood, are a source of nutrition for livestock and account for the bulk of renewable biomass that can be converted to fuel out of a plant.

So, as cell walls provide a relatively larger rigidity to vegetal tissues compared with animal ones, then relatively larger modulus of rigidity, lower viscosities and, consequently, faster propagation velocities and lower attenuation coefficients are expected. These features suggest that it might be possible to observe the propagation of shear waves in plant tissues at ultrasonic frequencies and that this can provide valuable information which may have significant economic implications.

Some ultrasonic techniques using longitudinal waves have been applied in the past to plant leaves [4]-[6]. More recently, air-coupled ultrasound and normal incidence have been used to excite and sense thickness resonances in plant leaves [7]-[10]. A similar technique but using oblique incidence has been used to generate and sense shear waves in other materials [11]. However, no evidence of the appearance of shear waves in plant leaves has been observed so far. [7]

II. MATERIALS AND METHODS.

A. Experimental set-up : air-coupled measurements.

A pair of air-coupled and wide band ultrasonic transducers was used to measure the phase and the magnitude spectra of the transmission coefficient at normal and oblique incidence. Transmitter transducer is driven by a Panametrics 5058 pulser. Received signal is amplified up to 40 dB, high-pass filtered (0.03 MHz) and digitized by a Tektronix 7054 digital oscilloscope. Angle of incidence is controlled by a goniometer.

First, the magnitude and the phase spectra of the transmission coefficient at normal incidence were measured. Frequency range was selected so that one or two orders of the thickness resonances were observed. Leaf properties were extracted from the analysis of these thickness resonances using a two layered (bilayer) model. Then the angle of incidence was increased from 0 to 40 degrees to find out if shear wave observation is possible. When observed, shear wave velocity and attenuation are extracted from the measured resonances using a two layered theoretical model of the leaves.

Finally, the variation of shear wave properties with leaf water content was measured. Leaves were cut, located between transmitter and receiver transducers at 30 degrees and let to dry at environmental conditions while the transmission coefficient is automatically measured every 3 minutes during a total time of three hours. Leaf weight is also monitored so that the leaf loss of water is also measured.

B. Leaf samples and method

A wide set of leaves from different species were tested. For some species no shear waves were observed (e. g. *Platanus hispanica*, *Ligustrum lucidum*, *Prunus laurocerasus* and immature -spring time- *Vitis vinifera* leaves). This can be

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attributed to a very high attenuation coefficient of shear waves. However, in some other cases, like *Epipremnum aureum* and mature (summer time) *Vitis vinifera* shear waves were clearly observed. This paper focuses on leaves of these two species.

C. Two-layered (bilayer) acoustic model of the leaves.

To theoretically analyze the presence of shear waves, the analysis of the transmission coefficient spectra cannot be limited to the vicinity of the first thickness resonance but must include a larger frequency window. Therefore, as in [7] and [12], the layered structure of the leaf must be considered. To further illustrate the limitations of the one layer model Figure 1 is shown. Theoretical predictions using the one layer model and the data extracted from the analysis of the first thickness resonance are extrapolated up to the second resonance, the discrepancy between model predictions and experimental data is quite clear. However, a two layers model provides a very good fitting into the experimental data.

In order to minimize the number of layers in the model, we considered a two layered model, see Fig. 2. The first layer comprises the upper epidermis and the palisade parenchyma (PP) while the second one comprises the spongy mesophyll (SM) and the lower epidermis. Effective density of the first layer is close to the density of its main constituents: water (1000 kg/m^3), cellulose (1500 kg/m^3), ligning (1300 kg/m^3) and wax (950 kg/m^3). The second layer can be considered a cellular material [13] and its properties are determined by its very high open porosity which is linked to its physiological role: gaseous interchange with the surrounding air. Thicknesses of these two layers are considered equal as in [14] and [15], and also the attenuation coefficient and the Poisson ratio.

D. Extraction of lead data from transmission coefficient spectra measurements.

First, the one-layer model is employed to extract effective leaf properties from measurements at normal incidence (Figs. 1 and 3.a.) in the vicinity of the first resonance as in [10].

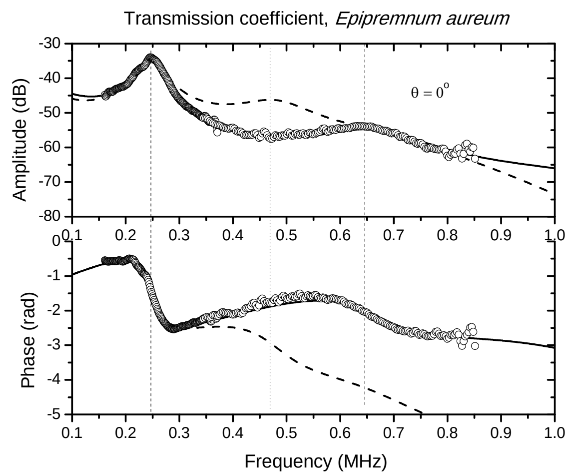

Figure 1. Magnitude and phase of the transmission coefficient of *Epipremnum aureum* leaves versus frequency at normal incidence. Dashed line: 1 layer, Solid line: 2 layers. See data in Table I. This is species that exhibit the largest anisotropy between layers, so the discrepancy between both models is, in this case, maximum.

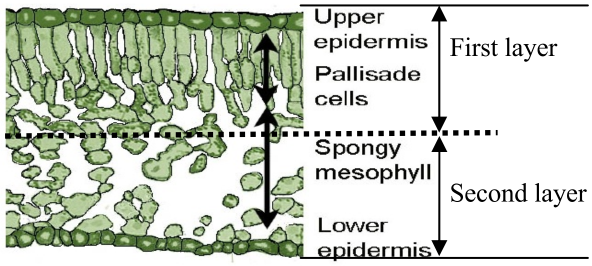

Figure 2. Schemmatic and microscopic wiev of the cross-section of a dicotiledonean leaf and the proposed bilayer acoustic model.

The effective properties (density and ultrasound velocity) so obtained are used as initial values for the bilayer model. Then these parameters are changed (become stepwise more different, i.e. anisotropy between layers is increased) until the fitting of the theoretically calculated transmission coefficient into the experimental data reaches an optimum value.

Once these data are determined, the measurements at oblique incidence are analyzed. Magnitude and the phase spectra of the transmission coefficient are theoretically calculated using the set of parameters obtained at normal incidence only allowing to change the Poisson ratio. It is changed from -1 to 0.5 in steps of 0.01. Negative values of the Poisson's ratio are considered because such values have been suggested before for some vegetable tissues [16].

III. RESULTS.

A. Normal incidence measurements.

Averaged leaf data extracted from measurements at normal incidence and from the two models considered (one layer and bilayer) are summarized in Table I. Data obtained by the bilayer model are similar to those obtained for other plant species following a similar procedure [12]. It is worthwhile noting that attenuation figures are smaller than those found in other species where shear waves are not observed [7], this could explain why in this cases it is possible to observe shear waves. In addition, anisotropy is larger for *Epipremnum* leaves.

TABLE I. EFFECTIVE LEAF PARAMETERS EXTRACTED FROM THE EXPERIMENTAL DATA WITH THE ONE LAYER (1L) AND THE BILAYER (2L) MODELS. NORMAL INCIDENCE.

	Thickness (µm)	***Density (kg/m^3)***	***Long. wave Velocity (m/s)***	***Long. wave Attenuation (Np/m)***
Epipremnum a. (1L)	340	1000	170	640
Epipremnum a. (2L)				
I. PP	170	1200	500	590
II. SM	170	400	155	590
Vitis v. (1L)	295	890	315	800
Vitis v. (2L)				
I. PP	147.5	950	500	750
II. SM	147.5	678	274	750

TABLE II. LEAF SHEAR PARAMETERS OBTAINED FROM THE ANALYSIS OF THE TRANSMISSION COEFFICIENT AT OBLIQUE INCIDENCE.

	Poisson's ratio	*Shear wave Velocity (m/s)*	*Shear Wave Attenuation (Np/m)*
Epipremnum a.			
I. PP	0.35	240	1032
II. SM	0.35	75	
Vitis v.			
I. PP	0.35	245	1600
II. SM	0.35	135	

B. Oblique incident measurements: shear properties.

Fig. 3 shows measured and calculated amplitude and phase spectra of the transmission coefficient at different incidence angles for a *Vitis vinifera* leaf (similar results were obtained for the *Epipremnum a.* leaves). The appearance of the shear wave is clear beyond 20 degrees. Table II show shear wave data extracted from these measurements. Obtained Poisson's ratio values agree with available estimations for different tissues: 0.28 for lignin, and 0.18-0.4 for onion epidermis [17]-[21].

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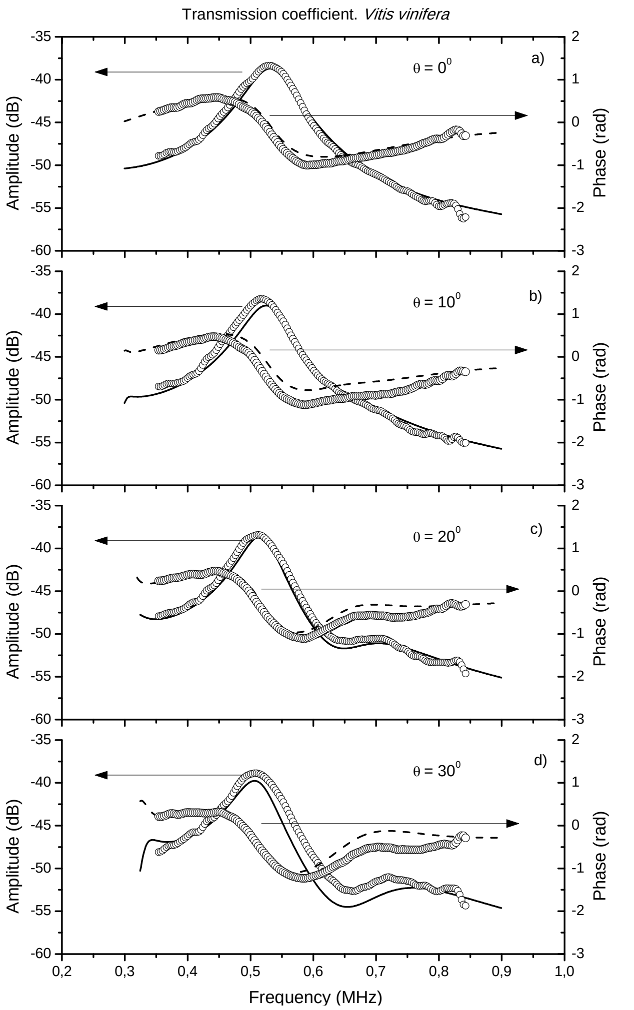

Figure 3. Magnitude and phase spectra of the transmission coefficient of *Vitis vinifera* leaves versus frequency at several incidence angles (0, 10, 20, 30). Solid and dashed line: Calculated values according to the bilayer model.

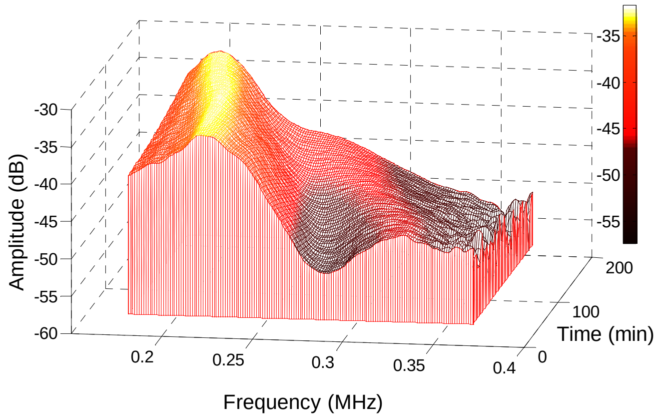

Figure 4. Measured magnitude spectrum of the transmission coefficient in one Epipremnun aureum leaf versus frequency at incidence angle of 30 degrees, during dehydratation.

C. Variation of shear properties with the leaf water content.

Measured amplitude spectra for one *Epipremnum aureum* leaf are shown in Fig. 4. Initially, the longitudinal thickness resonance appears at 0.22 MHz, while the interference due to the shear wave appears at about 0.32 MHz. As the leaf dries, thickness resonance shifts towards lower frequencies (as in Refs [7]-[10]). The shear wave interference shifts towards lower frequencies (the modulus of rigidity decreases) and the amplitude of the interference is reduced, which is due to an increase of the attenuation of shear waves.

The variation with the relative water content (RWC) of the measured thickness resonant frequency and of the other leaf parameters extracted from the measurements is shown in Figure 5.

Resonant frequency (corresponding to the longitudinal thickness resonance) follows a sigmoid behavior as in [7]-[10] and the point of turgor loss can be determined from the point of inflection. In Fig. 4 it is located at x_0 = RWC$_0$ = 0.935. This corresponds to 132 minutes. This point of inflection is calculated by fitting the logistic curve (Eq. 1) into the experimental data.

$$f_R = f_R^0 + \left(f_R^{dry} - f_0\right)/\left(1 + \left((x/x_0)\right)^p\right) \qquad (1)$$

where f_R is the thickness resonant frequency (longitudinal wave), superscripts *0* and *dry* denote the full turgor (water saturation) and the dry cases, respectively, x is the RWC and x_0 is the value of RWC at the point of inflection.

In Fig. 5, it can be observed, that beyond the point of turgor loss the variation rate of the shear wave attenuation coefficient (increase) and of the shear wave velocity (decrease) change notably. In addition, Poisson ratio also exhibits a relatively higher variation rate beyond the point of turgor loss. All these features can be explained by the loss of rigidity in the vegetal tissues produced by the decay of the pressure of the cells against the cell wall.

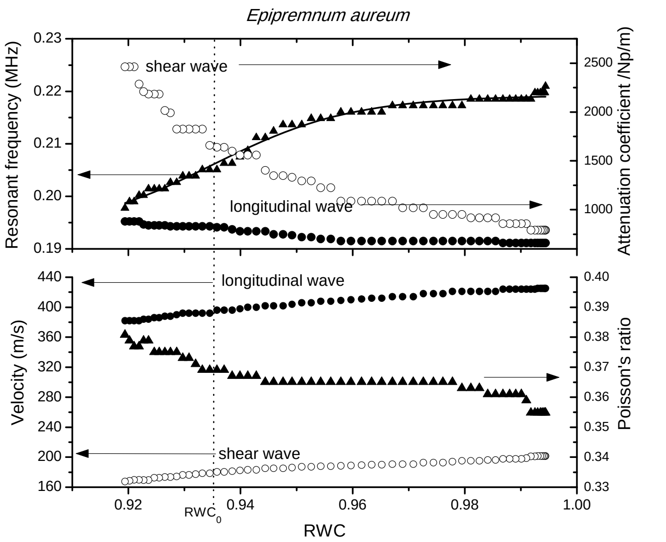

Figure 5. Variation of *Epipremnum a.* leaf properties during dehydratation.

IV. CONCLUSIONS.

The present paper shows that it is possible to generate and detect shear waves in some plant leaves using a trough transmission technique and wideband air-coupled ultrasounds to measure magnitude and phase of the transmission coefficient. Shear wave is detected at oblique incidence as a modification of the resonance pattern of the longitudinal wave measured at normal incidence.

In *Vitis vinifera*, appearance of shear waves strongly depends on the degree of development of the leaf. For leaves collected in spring time, the longitudinal attenuation coefficient is very high (from 2600-3000 Np/m at 700 kHz) and there is no evidence of the shear waves. On the contrary, for leaves collected in summer and fall, the attenuation is smaller (1200 Np/m) and the shear wave is clearly detected. This can be produced by the further evolution of the cell wall produced when the leaf (and cell) growth finishes.

Propagation of shear waves also depends on turgor pressure. When the leaves dehydrate, the observed shear wave decreases until it completely disappears. This can be explained considering that the rigidity of the plant is the result of the cell wall strength and the cell pressure, so a decrease of the RWC leads to a decrease of the leaf rigidity.

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