The role of leaf structure in vibration propagation

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The leaf and its structural components play a key role in the propagation of short transient signals produced by insects. In this paper, it is shown how the complex structure of an apple leaf could be modeled by a much simpler one for the analysis of vibratory signal propagation. Waves were produced by impacts of small spheres and the propagation studied using two laser vibrometers, followed by a wavelets analysis. Three components of the leaf were investigated: the midvein, minor veins, and the interspaced homogeneous regions making up the leaf lamina. The loss of signal energy over the leaf lamina and across minor veins and midvein was studied. For the midvein, the loss of energy decreased from 80% at the leaf base to 40% at the apex. For minor veins, the loss of energy decreased from 70% at the leaf base to 31% at the apex. The loss in homogeneous regions was 40%. A signal decomposition into two frequency ranges, above and below 1.7 kHz, showed that the midvein acted as a low-pass filter. As energy loss was mainly a function of vein diameter and not vein type, veins smaller or equal to 0.2 mm were considered as equivalent to homogeneous regions. Hence, a model leaf reduced to the leaf lamina and veins with a diameter >0.2 mm is retained for the study of signal propagation in a leaf. *© 2000 Acoustical Society of America.* [S0001-4966(00)02107-X]

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INTRODUCTION

Vibrations in plant leaves play an important role in many different ecological processes ranging from arthropod communication and spore liberation of fungi to gas exchange with the atmosphere (Ewing, 1989; Jones, 1992; Henry, 1994; Schuepp, 1993; Mulkey *et al.*, 1996; Stewart, 1997; Smith *et al.*, 1997). Hence, knowledge about wave propagation and vibrations in plants can not only help understand the above ecological processes, but can also have applied implications. In particular, the prediction of expected changes in boundary layers around leaves and transpiration rate due to increased air turbulence can aid in determining the expected response of vegetation to climatic change (Sellers *et al.*, 1996).

Many arthropods produce and use vibratory signals in leaves. Spiders and several insect species use leaves as a sound-radiating surface in order to increase their acoustical visibility in sexual communication (reviewed in Barth, 1998). Some spiders and predatory insects make use of the vibrations produced by their prey while walking on plants to detect and attack them. A very large group of organisms, the parasitic wasps, is able to localize and parasitize larvae of phytophagous insects living in plant materials (leaves, fruits, logs) by using the vibrations produced by the larvae when moving or eating (reviewed by Meyhöfer and Casas, 1999). We recently showed that lepidopteran larvae living inside a tunnel built within the depth of an apple leaf (called a mine) are also able to perceive and respond to vibrations produced by the hunting wasps and to behave accordingly (Meyhöfer *et al.*, 1997; Bacher *et al.*, 1997). The vibrations produced by the prey and the wasps are invariably short-term transients, almost impact-like signals lasting from 10 to 700 ms, with peaks of maximal intensity located between a few hundred Hz and 16 kHz, depending on the behavior of the insect (Meyhöfer *et al.*, 1994; Bacher *et al.*, 1996). In this case both antagonists are producing and reacting to vibrations, leading to a coupled system. We do not know which characteristics of the vibrations these animals really use. However, work on other insects and spiders has shown that most characteristics of a signal can be used, such as the amplitude, the maximal amplitude, the phase shift, the frequency content, etc. (reviewed in Barth, 1998). In all cases, the leaf acts as a transmission channel of the vibratory information, and a good knowledge of the signal propagation is therefore a requisite for further understanding systems such as those described above.

Few studies have been devoted to the analysis of vibrations in leaves, perhaps because of the difficulties associated with their delicate structures and the inherent need to use

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contact-free methods whenever possible (Martens and Michelsen, 1981; Tang *et al.*, 1986; Martens, 1990). The most complete study so far used laser doppler vibrometry of leaves in the context of noise abatement (Martens, 1990). These authors showed clearly that several theoretical models can be used as good approximations for clamped leaves. However, they also showed that none of the available models was sufficiently good for understanding naturally hanging leaves, which are too complex and require a new approach, unexplored so far.

The aim of this work was to characterize the propagation of transient vibrations in unclamped apple leaves. In particular, we aimed to identify which of the structures of the leaf must be retained in order to predict the transmission of information in the prey–predator interaction described above. Given the lack of well-founded theory of wave propagation in such structures/materials, we opted for a purely experimental approach followed by a signal analysis. Given the highly complex structure of a mine, we opted for the simpler situation of an unmined leaf.

We use impacts made by a small metal ball as a good approximation to real signals (following a preliminary study by Casas *et al.*, 1998) and a combination of two laser Doppler vibrometers. The study of nonstationary signals, where transient events appear that cannot be predicted, necessitates techniques different from Fourier analysis (Meyer, 1993). Hence, we characterize the signals in two steps. First, we use a simple and well-known criterion in signal analysis, the energy content, and measure its loss through the different structures (Cohen, 1995). Then, we refine the analysis by focusing on the frequency-dependent losses. To do so, we decompose the signals using wavelets Daubechies of order 2, because this type of wavelet is particularly well-adapted for the localization of sharp discontinuities (Misiti *et al.*, 1996). Then, we analyze the loss of energy as a function of the frequency range. We discuss the results in the light of prey–predator systems and in more general terms.

I. MATERIALS AND METHODS

The setup was composed of two OMETRON laser vibrometers VS 100, with a sensitivity of 1 μm/s and a maximal frequency range of 50 kHz, placed on an antivibration table, an unmined apple leaf, a small metal ball weighing 2.5 mg, a Siglab data recorder (Digital Signal Process Technology) working at a sampling frequency of 50 kHz per channel, and a computer. The apple leaf was maintained horizontally using the setup of Meyhöfer *et al.* (1994). The petiole was placed through a hole in the lid of a glass tube filled with water. Plasticine was used to prevent any further movement of the petiole. An unwrapped section of the petiole (at least 5 mm long) was left between the plasticine and the leaf lamina. We distinguished three structural parts of a leaf: the midvein, the minor veins radiating out of the midvein, and the homogeneous regions (Fig. 1). A homogeneous region, also called a sector, was a region of the lamina between two minor veins. In reality, these regions are made of several different tissues and are homogeneous only at a macroscopic scale.

An electrical magnet was used to drop the metal ball on specific locations on the leaf from a height of 2 cm, leading to a calculated potential energy at impact of 5×10^{-7} Nm. The impact point was always in a sector, which was the location where the prey lives. The distance between the impact point and the first measurement point was 2 mm, the smallest possible distance. The distance between the two measurement points was 4 mm. Longer distances were not possible without measuring reflections from minor veins or from the major vein. A distance of 5 mm from the border was kept to avoid undue effects from reflections of waves from the border.

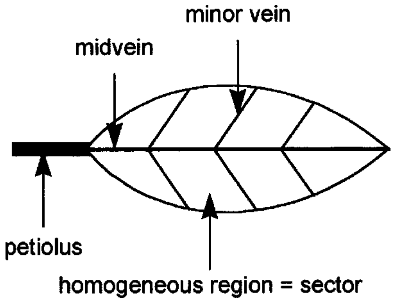

FIG. 1. Description of an apple leaf. Sectors and minor veins are numbered from base (near petiole) to apex.

Several leaves were used, as their state changed rapidly due to desiccation. Leaves were taken from young seedlings and weighed 125 mg on average (dry weight, s.d.=38.6 mg, $n=7$). The length was 5.9 cm on average (s.d.=0.63, $n=7$) and the width was 3.71 cm on average (s.d.=0.28, $n=7$). Signals were recorded for a period of 5.85 ms (300 data points). The first sampled point was always set at the same instant before the impact. The energy was calculated as

$$E=\sum_{i=1}^{300} x(t)^2,$$

with $x(t)$ being the velocity amplitude of the signal at time t. Preliminary experiments showed that at least 90% of the total energy of the signals was contained within the 5.85-ms window. This translated into a very small difference (ca. 1%) when estimating the energy losses between two measurement points, as the tails of the two signals were almost identical. Signals were analyzed using wavelets energy decomposition analysis (Misiti *et al.*, 1996). We decomposed each signal into five frequency ranges, rather than into the eight frequency levels given by the wavelet decomposition, because it gave a fine-enough frequency decomposition. We then calculated the proportion of the total energy contained in each frequency range. In order to avoid analyzing signals which were beyond the sensitivity threshold of the animals, we did a similar analysis on signals from wriggling prey pupae which were known to trigger behavioral responses in the wasps. In all cases reported here, the energy content of the signals at the second measurement point was at least one order of magnitude higher than the energy content of the weakest signals from a pupa. As done in the analysis of vibration signals (Wahl and Bolton, 1993), we presented the results of the decomposition in frequency ranges rather than in a wavelet scales.

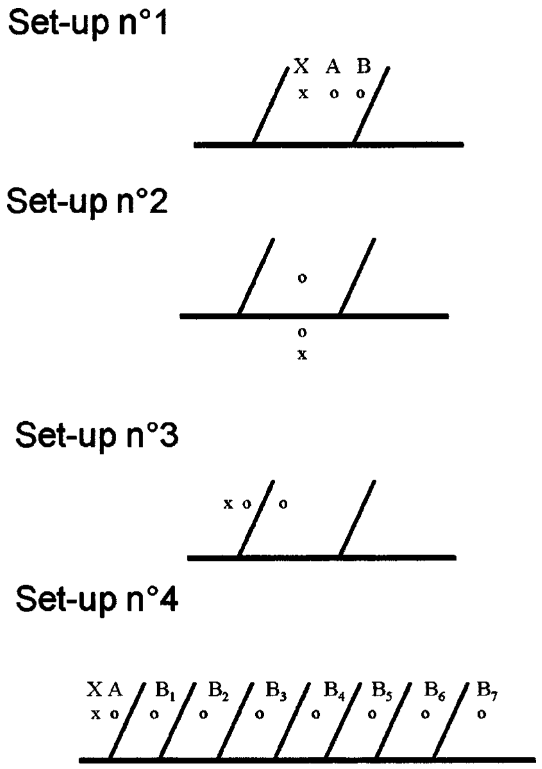

FIG. 2. Description of the measurement setups. *X* is the impact point, *A* is the first measurement point, always located near the impact and used to estimate the input to the system, and *B* is the second measurement point. In the last setup, B_i refers to the different locations of the second measurement point.

II. RESULTS

We first analyzed the role of structural elements and their geometry in determining the loss of total energy between the first and second measurement points. We then considered frequency-dependent energy losses through the same structural elements.

A. The role of structural elements on the loss of total energy

For the ease of presentation, we equated the source location (the impact point) and the first measurement. The second measurement point was then referred to as ''the receiver.''

The loss of energy within a sector was estimated by locating the two measurement points and the impact point within one sector (Fig. 2, setup no. 1). When the source of the signal and the receiver were located on the same sector, the average percentage of energy loss was 44% (s.d.=12%, 42 impacts in 11 homogeneous regions on three leaves). There was no significant difference between sectors near the stem and at the tip of the leaf.

The loss of energy through the midvein was estimated by locating the two measuring points on each side of it (Fig. 2, setup no. 2). Experiments were conducted on two leaves (six sectors in the first leaf and ten in the second, 39 measurements total). When the midvein was located between the source and the receiver, the percentage of energy lost through the midvein varied along the midvein (Fig. 3). The loss was 80% near the stem and decreased linearly to 40% at the apex.

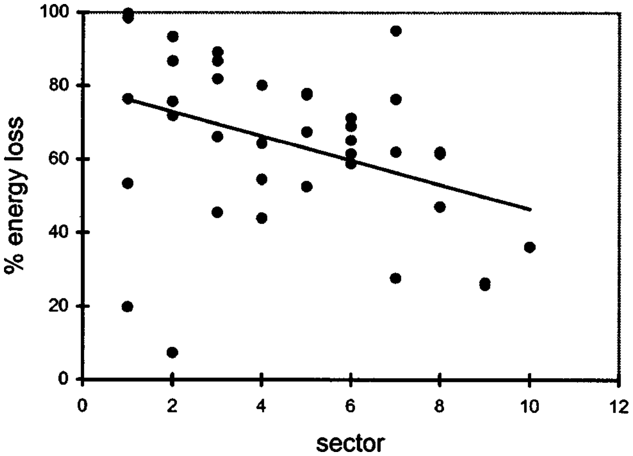

FIG. 3. Percentage of energy loss through the midvein as function of the position of the sector. Sector number 1 is near the petiole and sector number 10 is at the apex of the leaf. Black points are measurements and the straight line was obtained by linear regression ($y=79-3.3x$, $t=-2.47$, $P=0.0195$). Leaving the two outliers near the origin gives $y=90.28-4.9x$, $t=-4.97$, $P=0.0000$.

The loss of energy through the minor veins was estimated by locating the two measurement points on each side of a minor vein (Fig. 2, setup no. 3). The loss of total energy between the source and the receiver was on average 49% ($n=108$ impacts on four leaves, s.d.=19%). The loss of energy through a minor vein was 70% near the petiole and 31% near the apex (Fig. 4). There are no gradient of energy loss along a minor vein.

The dependency of energy loss on the position of the minor veins may have an important effect on the energy propagation through the whole leaf. In order to study the cumulative effect of several minor veins, we predicted the loss of energy through the whole leaf under two different assumptions, the first using the calculated relationship between energy loss and vein position (Fig. 4), and the second

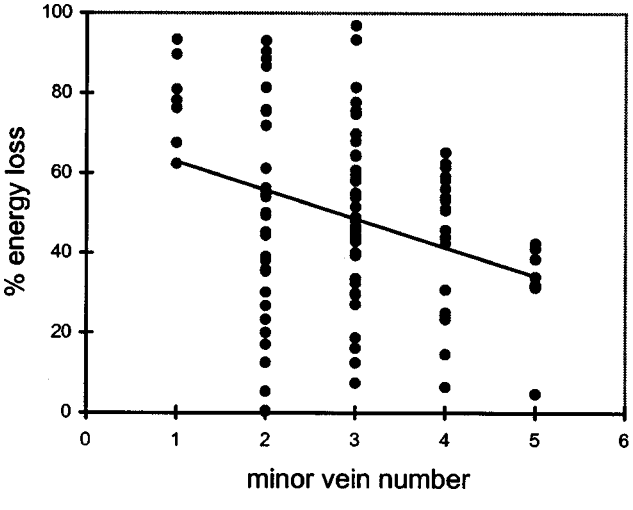

FIG. 4. Percentage of energy loss through a minor vein as function of its position. Minor vein number 1 is near the petiole, irrespective of its location on the side of the midvein, and minor vein number 5 is at the apex of the leaf. Black points are measurements and the straight line was obtained by linear regression ($y=70-7.2x$, $t=-3.51$, $P=0.0007$).

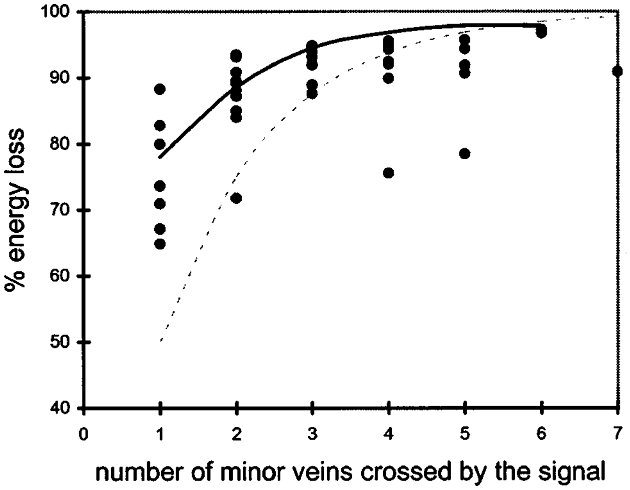

FIG. 5. Percentage of energy loss as function of number of minor veins crossed by the signal. Black points are measurements. The dotted line represents predicted values under the assumption of a constant energy loss of 49%. The black line represents predicted values using observed values of the position-dependent energy loss given in Fig. 4.

using a position-independent mean value of energy loss (49%). We then measured experimentally the loss on two leaves using a new setup (Fig. 2, setup no. 4, 12 measurements on one leaf and 29 measurements on a second leaf). The impact point and the measurement point *A* were, as before, in the same homogeneous region. The measurement point *B* was located on the other side of a minor vein. In each successive experiment, the number of crossed minor veins was incremented. There was a maximum of seven minor veins between points *A* and *B*. The increasing distance between the two measurement points implied that the signal measured in *B* incorporated the effects of multiple reflections from borders and veins. Hence, our setup cannot identify the sole distance effect.

The energy loss increased with the number of minor veins crossed by the signal, and approached 100% at the apex (Fig. 5). The loss of energy through several minor veins was quite well predicted when the energy loss was a function of the position of the vein. The assumption of constant loss through minor veins, however, strongly underestimated the loss of energy.

Midveins as well as minor veins showed a strong decrease of diameter from petiole to apex. This varying diameter had an important role in the transmission of energy, as the average loss of energy was constant and equal to 45% (s.d.$=$18%, $n=95$) for a diameter inferior or equal to 0, 2 mm (Fig. 6) and increased up to 90% for larger diameters. The loss was nearly complete when the signal crossed the midvein near the petiole.

B. Frequency-dependent losses through structural elements of a leaf

After the decomposition of each signal into five frequency levels, we pooled values to obtain two frequency ranges: lower frequencies, below 1.7 kHz, and higher frequencies, between 1.7 and 25 kHz. The reason for doing so was twofold. First, the energy content in the five levels differed substantially, while the two ranges each contain roughly half the total energy. Calculating losses from frequency levels with very low energy content was imprecise due to the small energy involved (for example, 0.6% of the total energy was contained in the level corresponding to the highest frequencies) and the high signal-to-signal variability at these levels. Second, the splitting frequency of 1.7 kHz corresponded roughly to the borderline frequency (2.5 kHz) of Meyhöfer *et al.* (1994) between ‘‘low-frequency’’ signals produced by prey moving or feeding and ‘‘high-frequency’’ signals of prey wriggling in order to escape stings of the wasps.

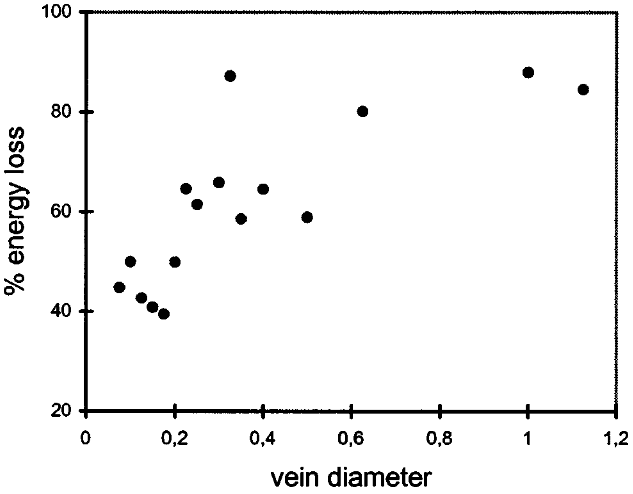

FIG. 6. Average percentage of energy loss through midveins and minor veins as function of vein diameter.

In a given sector, the average loss of energy was 45% (s.d.$=$22%, $n=42$) for high frequencies and 18% (s.d. $=$35%) for low frequencies. Minor veins reduced energy by 44% (s.d.$=$29%, $n=108$) on average for high frequencies, and by 32% (s.d.$=$24%) on average for low frequencies. The midvein reduced higher frequencies by 72% (s.d.$=$18%), and lower frequencies by 43% (s.d.$=$29%, $n=39$). Hence, the loss of total energy was mainly due to a loss of high frequencies. Signals changed in composition, sometimes strongly. The proportion of frequencies higher than 1.7 kHz was reduced by 18% when the signal crossed the midvein (Fig. 7). Hence, the midvein acted as a low-pass filter. The decrease was much less important for minor veins (4%) and homogeneous regions (6%, Fig. 8).

III. DISCUSSION

A. The leaf as channel in vibratory communication in prey–predator systems

The use of small dropping balls was chosen to produce short, transient vibrations similar to those observed in the leafminer system. A wavelet analysis of several signals from wriggling pupae confirmed the qualitative similarities with the signals produced by impacts, but showed quantitative differences. In particular, the energy contained in the biological signal was at least one order of magnitude less than in the signals produced by the impacts, and around two-thirds of the energy contained in biological signals was located

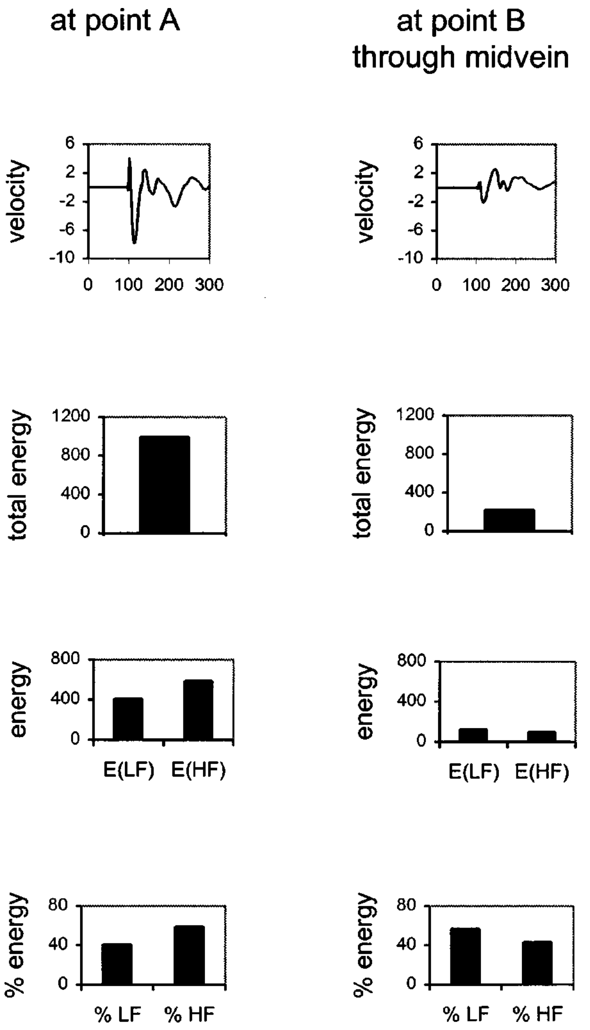

FIG. 7. Recorded signals at point *A* and point *B* and their energy analysis. The signal crosses a midvein. We represent the velocity of the signals (mm/s), the total energy, the energy contained in lower frequencies below or equal to 1.7 kHz, and in higher frequencies above 1.7 kHz, and its relative values (energy expressed in mm^2/s^2).

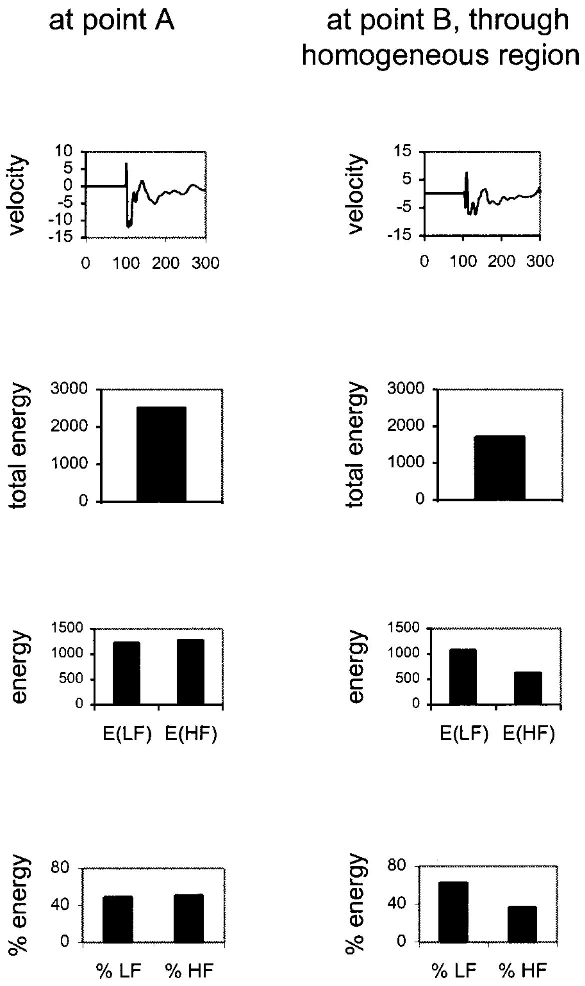

FIG. 8. Recorded signals at point *A* and point *B* and their energy analysis. The signal goes through a homogeneous region. See the legend of Fig. 7 for further information.

above 1.7 kHz. We do not understand how insects are able to create signals having a much larger proportion of high frequencies than low frequencies, a point also raised by Michelsen *et al.* (1982). A thorough study of the coupling of a vibrating insect to the plant structure may provide part of the explanation.

The strong, frequency-dependent attenuation implies that vibratory signals change over a short distance on a leaf. This applies in particular when signals cross veins, but even the leaf lamina dissipates a lot of energy. These results have two implications for understanding the leafminer system. First, they imply that the low energy signals produced when a larva moves in the mine or feeds will most likely not propagate much further than the mined area, as a mine is located between two minor veins. Hence, only specialized natural enemies may be able to perceive and react to such signals from any distance on a leaf using specifically tuned receptors. Second, a wasp on a leaf will obtain signals that vary greatly depending on its location on the leaf relative to the source. This gradient of information may be used by the wasp for locating the source. For example, the frequent and rapid swaps between several mines on the same leaf while foraging for prey may be explained by the fact that wasps receive signals coming from mines other than the one currently being searched (Casas *et al.*, 1993). In more general terms, the biological message is that the leaf architecture generates a set of options for vibratory communication, a level of complexity that would be absent in a veinless, plate-like leaf. For the sender it means stay within the same sector as the receiver if you want the signal to be well-detected, but keep veins between you and the receiver if you want to hide. For the receiver, it suggests moving to the same sector as the sender to increase signal reception.

We have shown in the previous paragraph how predators and prey can improve their situation relative to vibration reception by moving around on a leaf. Arthropods are even able to use vibration without moving. A difference of intensity of a signal between two of the legs is likely to be used by spiders living in orb webs or hunting in the vegetation to turn and locate a vibrating source (Hergenröder and Barth, 1983; Landolfa and Barth, 1996). The species *Cupiennius salei*, which hunts animals moving on the vegetation, has a leg span of 10 cm. A loss of 10 dB between two legs triggers correct turning behavior. Potentially, big arthropods can also use the frequency composition in different sensors to locate a

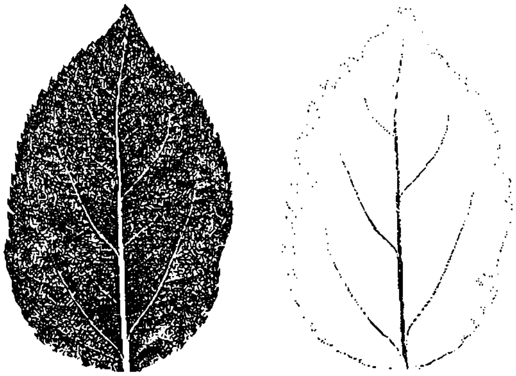

FIG. 9. An apple leaf (left) and the outline of the structures retained for modeling the propagation of waves due to impacts (right).

vibrating source, but results obtained so far are inconclusive. For *C. salei*, differences in time of arrival between legs at the peak frequency of the courtship signal are between 2 and 10 ms, which is a range known to be effective for orientation. However, prey signals contain mostly higher frequencies, leading to times of arrival between legs that are too small to trigger any behavioral response (Barth, 1998). The use of time-of-arrival and phase cues is uncertain in web spiders due to the high propagation speed of vibrations (Barth, 1998). Given the inconclusive results obtained with relatively large spiders, arthropods with a leg span of a few millimeters, such as the leafminer parasitoid, are most likely unable to use the frequency composition of vibratory signals for mate and prey recognition. Our findings suggest, however, that these arthropods could be better served using the loss of total energy, or any related statistics, which is important even over a very short distance within homogeneous regions.

B. Relative importance of structural elements in the propagation of vibrations

A real leaf is made of a few basic structural components that vary substantially at the microscopic scale. The results obtained in this study enable us to focus on the relevant level of detail for modeling the propagation of mechanical waves produced by impact-like behaviors of insects. We showed the important role of veins in determining the position-dependent energy loss through a whole leaf. We intend next to study experimentally the channeling of energy through the veins, either into other regions of the leaf or into the air. This step will enable us to characterize the radiation properties of the energy and the mechanisms by which veins act as low-pass filters. A study combining laser doppler anemometry and laser doppler vibrometry showed indeed that air movement triggered by leaf vibrations caused by such impacts could be measured up to several cm away from a leaf (Casas *et al.*, 1998).

The loss of energy in crossing a vein was also shown to be strongly dependent on the vein diameter. There is a gradient of vein diameter over a leaf, the midvein and minor veins being thicker near the petiole and becoming thinner towards the apex. Hence, we regard the diameter as being the relevant variable in determining energy loss through structures, rather than the position *per se*, and also discard the former distinction between midvein and minor veins. Veins with diameter smaller or equal to 0.2 mm dissipate as much energy as the leaf lamina. Therefore, we end up with a much simpler leaf (Fig. 9) and consider only veins of diameter >0.2 mm and the leaf lamina as being the necessary structural elements to retain in a future model of vibration propagation in apple leaves.

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