Acoustic emissions from transpiring plants – new results and conclusions

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Abstract

Using Ulmus glabra as an example we revealed that acoustic emissions from plants do not necessarily occur in conjunction with water stress. The waveforms in the various signals show that acoustic emissions may possibly be generated by still unknown hydraulic events, more complex than cavitation. Using a special microscopic technique we established the presence of minute gas bubbles attached firmly to the xylem vessel walls of the water-conducting conduits. The micro-bubbles accumulate especially to the poorly wettable lignin domains of the vessel pits. Our conclusion is that minute gas bubbles at the xylem vessel walls are a normal feature of actively water-conducting conduits. By means of physical model systems we ascertained that by virtue of the cohesion of water the entire system of wall adherent bubbles acts as a force-transmitting medium that creates the xylem tension. During transpiration the wall adherent gas bubbles expand, during refilling they contract. Since the bubble system always reacts intermittently this creates acoustic emissions both during transpiration and refilling. Our hypothesis is that under a gradient of the wall attraction forces of the bubble system the water column moves in a similar fashion as under a gradient of actual negative pressure. The outward appearance of xylem tension caused by the bubble system is deceptively similar to that caused by real negative pressure. Our investigations have resulted in an alternative concept of water transport in plants.

Investigations

Since the first report in 1966 about audible acoustic emissions (AAE) from plants being under water stress the acoustic signals have been considered as convincing evidence of disruptions in the water column caused by negative pressure as it is claimed in the cohesion theory (ref.1). However, when in 1983 (ref. 2) ultrasonic acoustic emissions (UAE) were introduced into the investigation it became apparent that the density of signals was then shown to be extremely high. Provided that even the UAE signals are attributable to local disruptions of the water column, an uninterrupted flow of water is only conceivable if the embolism defects are continuously being repaired. However, a generally accepted hypothesis is missing still today in order to explain the embolism repair. Therefore, the cohesion theory has been questioned since the beginning one hundred years ago and the debate still continues today (ref. 3).

Until recently, most reports on acoustic emissions were focused on plants being definitely under water stress or wilting (ref. 4). In many investigations the intensity of water stress has been assessed by the number and the amplitude of the acoustic hits versus time. In our investigation we re-examine the customary opinion that water stress is a pre-condition of acoustic emissions from plants. In order to extract more information about the mechanism of acoustic signal generation we concentrate our investigation on the frequency pattern of the emissions and the waveform of the individual signals emitted during normal transpiration without water stress. Our

acoustic data interpretation is supported by microscopic observations and newly developed physical model systems.

Acoustic emission detection

For UAE measurements we selected a 4 year old, potted Ulmus glabra which had vigorous transpiration activity. We chose this angiosperm species of tree because its ring-porous xylem facilitates microscopic examination of the vessel walls, which constitutes part of our investigation. In order to record transpiration loss whilst simultaneously monitoring acoustic data we fitted the flowerpot and the elm tree with acoustic sensors linked to a computerized electronic scale (Fig. 1). Prior to monitoring the acoustic data the elm was watered well, so that no water stress could develop in the plant during a period of 5 or 6 days. Temperature was maintained in the laboratory at 22 +/- 1.5 C° and humidity at 60 +/- 3% rel. Thus, transpiration depended primarily on the intensity of light during the rhythm of day and night. Our experiments were carried out in August 1998. Fig. 2 shows the plot "transpiration loss versus time" during a 80-hour period. The start of the measurement was at 18:00 (6:00 p.m.) To improve the assessment we calculated the derivation curve, called "transpiration intensity versus time". This curve reveals minimum transpiration intensity at night of 15 % of the maximum intensity during daylight.

Fig. 1
Experimental set-up. A young Ulmus glabra tree with acoustic sensors (above left) is placed on a computerized scale.

We detected the acoustic signals emitted by an elm leaf by means of a piezoelectric sensor SE-45 produced by Duncan Engineering Consultants USA. Normally, acoustic emissions are taken from stems, twigs or cut leaves. The advantage of detecting acoustic emissions from a living leaf is that the signal generation occurs in the immediate area of the site of transpiration. Although we applied a mild medical contact gel the mesophyll in the contact area became necrotic very soon after contact was made. This was prevented once the contact area was coated with a chemically inert silicon release agent.

The sensor attached to the upper side of the leaf exerted no pressure on the leaf. So, transpiration was practically unaffected at the reverse side of the leaf where the majority of the stomata is located. Close to this measuring sensor we placed another one which had no contact with the leaf. With the help of this second sensor we could eliminate disturbing noise from the acoustic data.
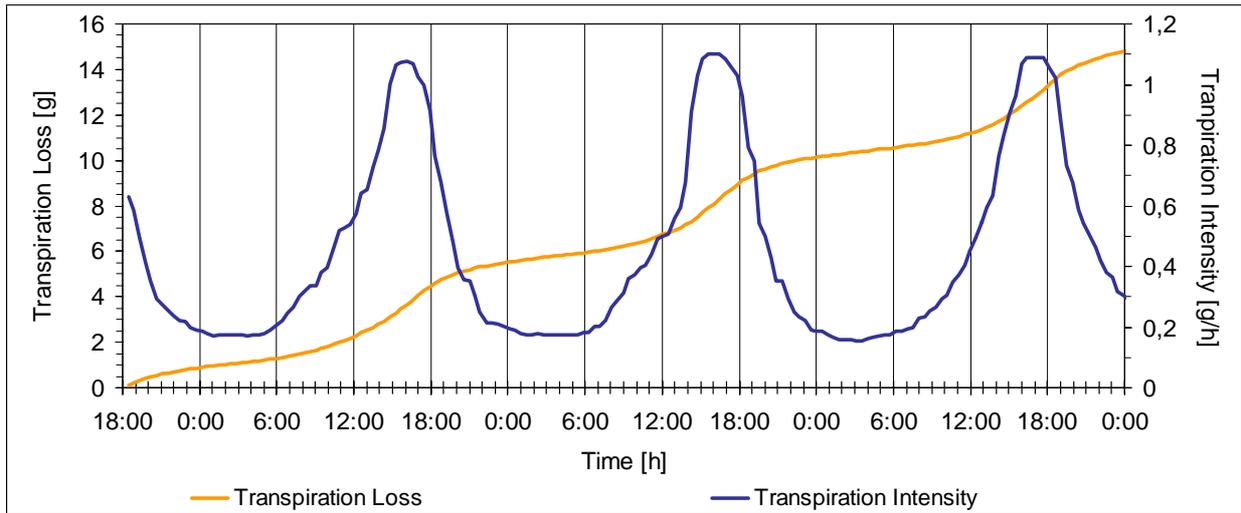
Fig. 2

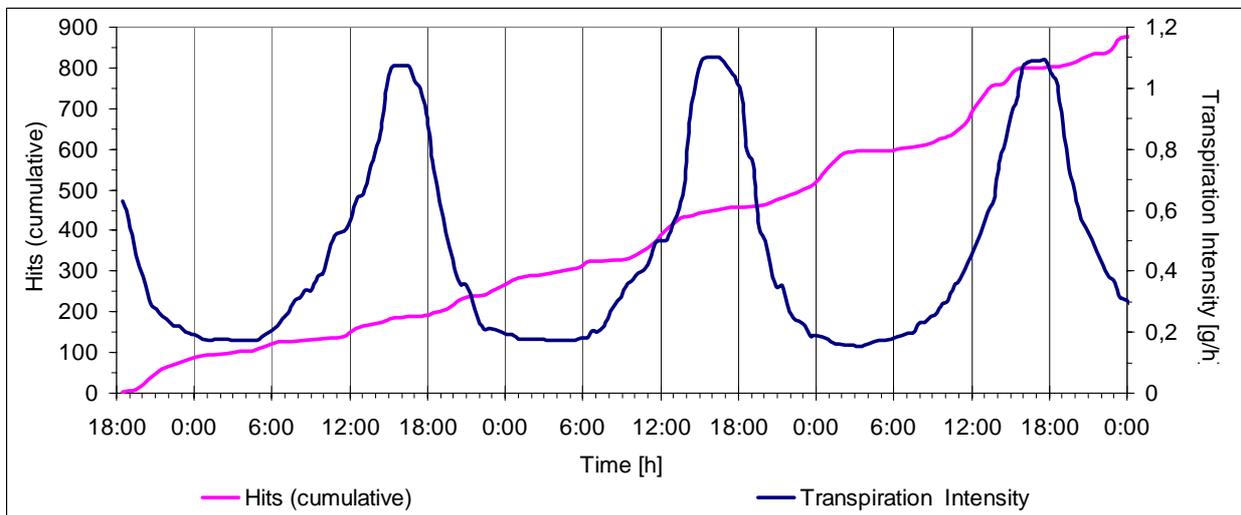Transpiration loss and transpiration intensity of the examined young Ulmus glabra tree.

Fig. 3

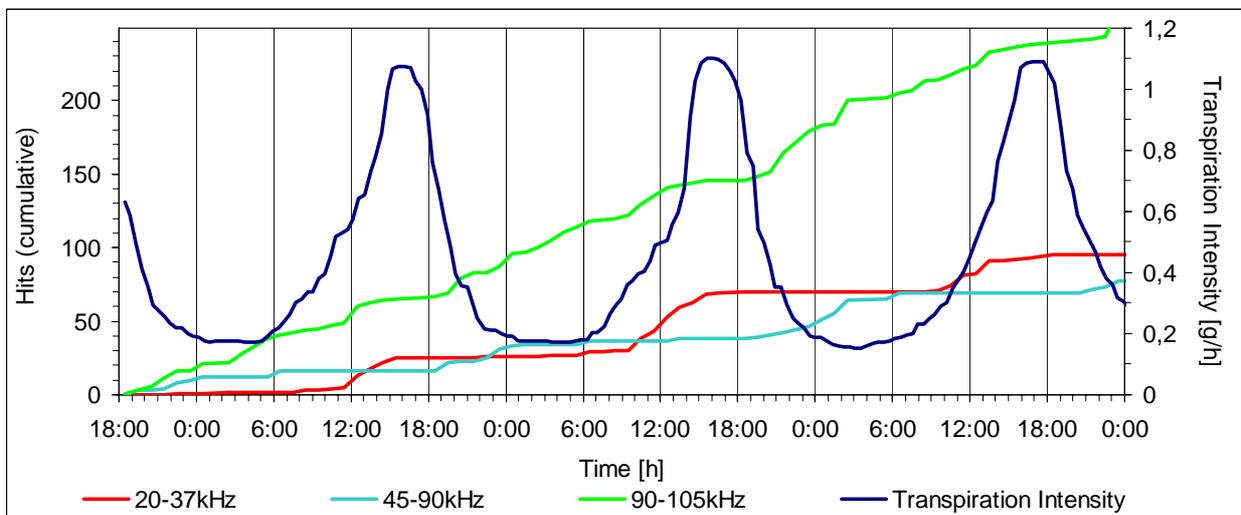Transpiration intensity and cumulated acoustic activity of the examined Ulmus glabra tree.

Fig. 4

Transpiration intensity and cumulated acoustic activity of selected dominant frequencies

In order to extract maximum information from the acoustic emissions we employed a monitoring system equipped with transient recorders. The Vallen system AMSY 4 suited our purposes very well. In parallel to normal feature extraction, the complete waveform in time/frequency can be saved in a transient recorder module. The data can be analysed by means of special Vallen software. Data compiled during a period of 77 hours is shown in Fig. 3. Intense signals occur during both maximum and minimum transpiration intensity. Interestingly, high acoustic activity developed even during the night.

The most important results of this work were obtained from the frequency pattern and the waveform of the signals. The review of stored signals revealed accumulation of signals with a maximum magnitude of the spectrum between 20 and 37 kHz (signal type A), 37 and 54 kHz (signal type B), and between 88 and 105 kHz (signal type C). Fig.4 shows the acoustic activity for these three frequency ranges. Signals of type A and type B are emitted in turn, during intense transpiration and reduced transpiration, respectively. Signals of type C are increasingly emitted between declining transpiration in the evening and increasing transpiration in the morning. The significant change of the signal-types indicates that the mechanism of sound generation during intense transpiration differs from that one during reduced transpiration and refilling, respectively. In addition to the frequency pattern we analysed the waveform of a number of signals. Fig. 5 gives the most illuminating variations of the waveform. We established very quickly that there are evanescent signals and longer, modulated signals. This indicates that similar events occur both in isolation and in a coherent sequence.

In our investigation we provide proof that acoustic emissions from plants do not necessarily occur in conjunction with water stress. Apparently, it is not possible, with our current level of knowledge, to describe properly the complex acoustic activity of plants. Therefore, we attempt to search for other sources of acoustic emissions from the water conducting system of vascular plants.
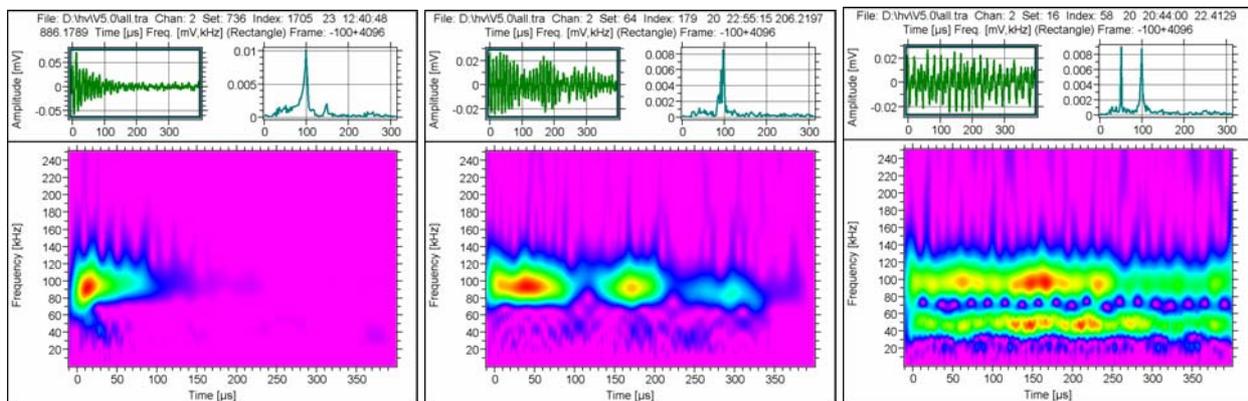

Fig. 5
Typical variations of the waveform of the signals.
Top left: time signal. Top right: result of the Fast-Fourier-Transformations.
Bottom: Wavelet Diagram. The magnitude of the spectrum is encoded in rainbow colours.
Violet shows the lowest magnitude and red the highest.

Microscopic examinations

In textbooks the wetting ability of the xylem vessel walls is considered to be perfect. However, in a previous investigation we proved that the wetting behaviour of the vessel walls is a more complex one caused by inhomogeneous distribution of perfectly wettable cellulose and poorly wettable lignin (ref.5).

It is known that lignin can be detected by its yellow-green auto-fluorescence when exited by blue light (ref. 6). But in a normal epi-fluorescence microscope the intensity of the lignin fluorescence is low and in general the source of the fluorescence cannot be localised sharply due to the stray light and the depth of focus is too low. This disadvantage is avoided by confocal laser scanning microscopy (CLSM).
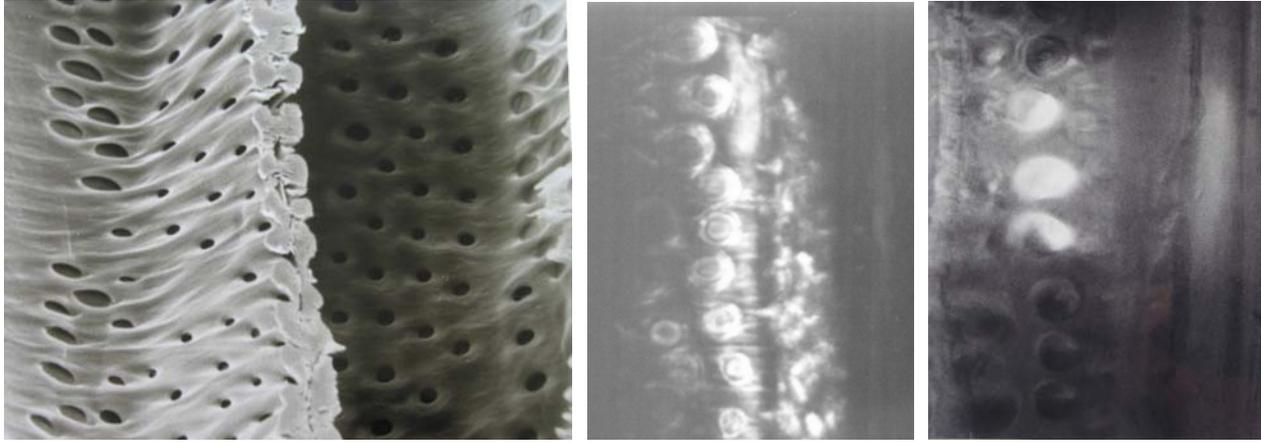

Fig. 6   10μm   10μm   10μm

Xylem vessel walls of Ulmus glabra. Left: Scanning electron micrograph. Center: Confocal laser scanning micrograph in the fluorescence mode. The concentrations of lignin appear brightly. Right: accumulation of gas bubbles at the vessel pits.
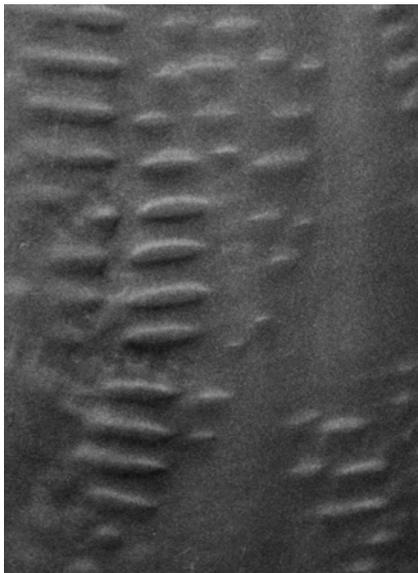

Fig. 7   10 μm   10 μm   Fig. 8   10 μm
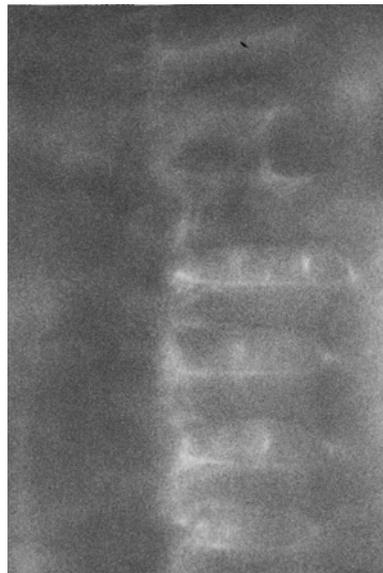Gas accumulations at the scalariform   Gas pads at the xylem
vessel pits of Vitis vinfera.   vessel walls of Ulmus glabra.

Fig. 6 shows CLSM fluorescence images of the vessel walls of Ulmus glabra. In order to reveal the implication of the lignification pattern and the wetting ability of the vessel walls we have carried out special microscopic examinations. From our potted elm a branch was bent down into a container of water and a sample of about 3 cm in length was cut off. On this sample a longitudinal cut was made and then the sample, still immersed, transferred under the microscope equipped with a lens for water immersion (high resolution objective lens "PLABO"; 160x/1.25W, Leitz Wetzlar, Germany). The whole procedure took only a few minutes. As it is visible in Fig. 6, the vessel wall shows bubble-shaped gas accumulations at the pits. Under certain conditions of illumination some of the bubbles show intense light reflection, which is

convincing proof of the existence of gas/water interfaces. We are aware that the gas bubbles, which we have first described in 1989 (ref. 5), are not maintained in the original shape as in the intact vessel tubes in vivo. By opening up of the vessel tubes there resulted a drop in tension in the xylem resulting in a contraction of the bubbles. However, new bubbles may scarcely develop during sample preparation. We ascertained the genuine nature of the wall adherent bubbles not only for Ulmus glabra but also for Quercus robur, Acer pseudoplatanus, Betula alba, Populus nigra, Salix alba and Vitis vinifera. We observed repeatedly that the accumulation of expelled gas takes place at the lignin domains of the vessel pits**.**

Fig. 7 shows the formation of gas bubbles at the scalariform vessel walls of Vitis vinifera. The formation of gas pads due to the coalescence of single bubbles is shown in the example of Ulmus glabra in Fig. 8. In this it becomes visible that each flat gas pad integrates numerous single bubbles.
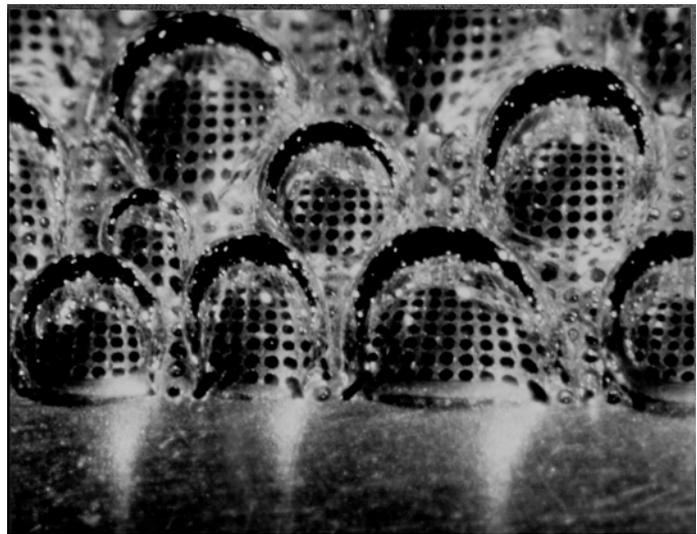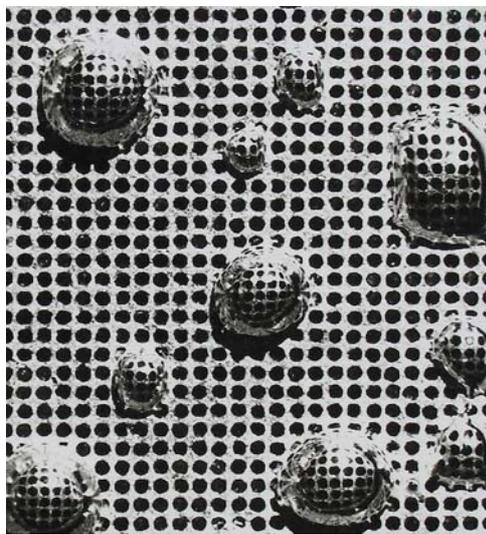

Fig. 9   1 mm

Physical model system. Gas pads adherent at the holes of an aluminium plate. The depth of the holes is 0,05 mm. Left: Birds eye view. Right: Angle of viewing 45°.

Physical model systems

We studied the properties of wall adherent gas pads by means of several physical model systems. Compared with the geometrical dimensions of the bubble systems in the xylem vessel tubes our model systems are larger by two orders of magnitude. However, the advantage of these macro models is that they reveal clearly the peculiar surface curvature of the wall adherent gas pads.

Fig. 9 shows wall adherent gas pads in several phases of coalescence. Affected by the buoyancy, each bubble tries to detach from the solid structure. However, since the energy needed to detach cannot be obtained, the bubble aggregates cannot achieve their spherical equilibrium shape so, they remain in non-spherical metastable configurations. It is obvious that at the undulated rims of the gas pads tension develops. The rims act like elastic bonds. Some of the hump-shaped gas pads integrate up to 35 single bubbles. Of course, at the xylem vessel walls due to the dense pattern of the very minute pits many more bubbles are able to coalesce, so that sizeable gas pads develop (Fig. 8). We observed under the microscope that the bubbles in our model system intermittently coalesce quite violently. It is our hypothesis that in plants it is the transpiration induced intermitting alteration of the wall adherent bubble system that creates acoustic emissions.

Fig. 10 shows a glass capillary perforated by two rows of minute lateral holes. The holes were made by means of a laser. A limited length of the water column in the capillary is balanced since the lateral concave menisci create a resultant force against gravity. Our conclusion is that in plants the countless number of wall adherent gas bubbles and gas pads restrain the water column against gravity.
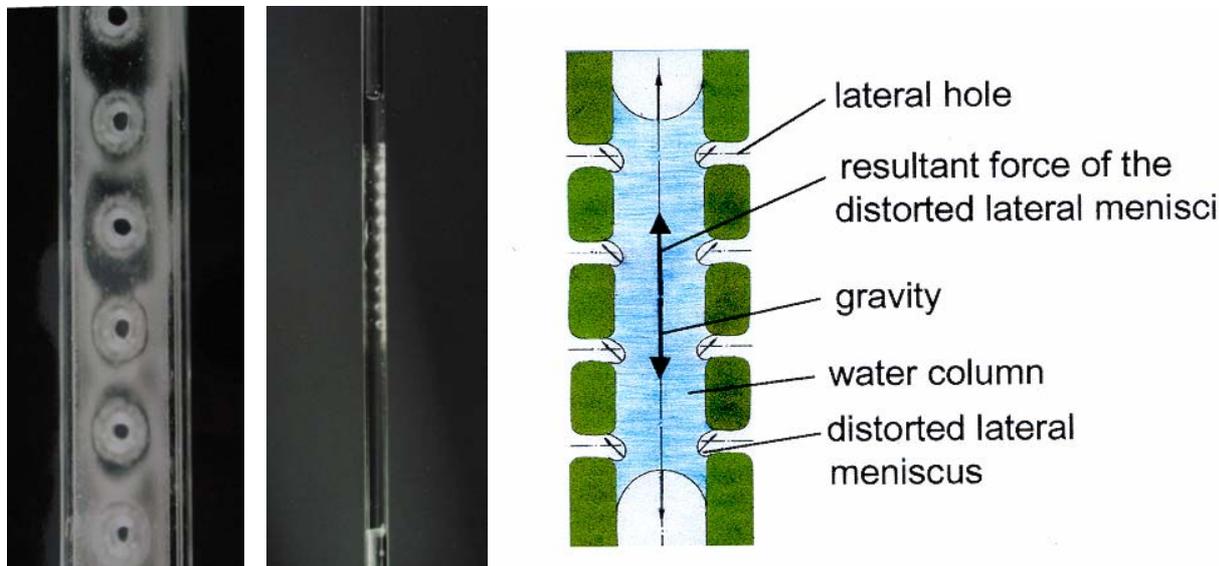

Fig. 10
Left: Glass capillary with lateral holes. Hole diameter 0,15 mm.
Center: A water column of 23,5 mm length is balanced in the capillary by the forces of
          the lateral menisci.
Right: Forces acting on the water column in the capillary.

Discussion

In our opinion the acoustic emissions from plants do not necessarily occur in conjunction with water stress. The frequency pattern and the waveform in the various signals show that acoustic emissions may possibly be generated by still unknown hydraulic events, more complex than cavitation. We provided evidence of accumulation of gas bubbles at the vessel walls of the water conducting conduits of Ulmus glabra and some other angiosperm tree species. Encouraged by these findings we conclude that free gas in the active conduits does not represent a disturbing factor in long-distance water transport. Clearly, the persistent presence of gas spaces in the active conduits can never be reconciled with negative pressure in terms of the cohesion theory. It belongs to the basic concept of this theory to consider the transpiration stream to be forced merely by the surface tension of the transpiring menisci in the leaves. We take a distinctive view. In our opinion the water column is forced over the whole length of the xylem conduits. Our experiments with physical model systems give rise to the assumption that in plants the whole water column is balanced by a wall adherent micro-bubble system extending in the xylem conduits from the roots to the leaves. According to the principle of buoyancy the system water/gas moves its centre of gravity into the lowest possible position. Since the wall adherent bubbles are coupled both with the liquid and the wall, tension develops when the bubbles get distorted due to the weight of the clinging water column. Thus, we re-define the xylem tension as being a distortion-tension of a wall adherent bubble system rather than a gradient of negative pressure in terms of physics. We consider the outward appearance of xylem tension induced by the wall adherent bubble system to be deceptively similar to negative pressure. In these premises we also explain the famous pressure chamber test applied to the determination of the water potential of plants. Seen from our point of view the chamber-pressure exerted to compensate the

xylem tension represents mainly the pressure in order to compress the wall adherent bubble system. The stronger the tension of the bubble system the higher the chamber pressure needed to alter the reluctant bubble system. So the readings of the pressure chamber test are in correlation with the state of the bubble system. We consider the surface energy of the bubble system to be part of the water potential.

Our transport model might be described as an energy-storage-model. Without influence of external forces the system "bubble layer/water column" shifts due to an exchange of internal energy into the minimum of free energy. Transpiration induced loss of water deflects the system from the minimum of free energy. Since the entire bubble system works as force-transmitting medium, the deflection propagates slowly in basal direction to the roots. (In the cohesion theory, it is assumed that a change of negative pressure propagates with the velocity of sound through the whole plant). (ref. 8). The propagation of the energy-deflection is accompanied by a rearrangement of the wall adherent bubble system. This rearrangement undoubtedly creates acoustic emissions. When transpiration declines (at night) then the deflected system returns to the energy minimum by uptake of water from the roots. According to the laws of thermodynamics, the return to zero is not exactly the reversal of the initial energy deflection. This might be the reason why the frequency pattern of acoustic emissions is different during the transpiration phase than in the refilling phase. We assume the long-distance water transport in plants to be a non-metabolic physico-chemical mechanism, however, we do not doubt that the installation and the maintenance of the wall adherent bubble systems in the xylem is the work of metabolic forces.

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