Ultrasonic Acoustic Emissions from the Sapwood of *Thuja occidentalis* Measured inside a Pressure Bomb[1]

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ABSTRACT

An improved method of counting acoustic emission (AE) events from water-stressed stems of cedar (*Thuja occidentalis* L.) is presented. Amplified AEs are analyzed on a real time basis by a microcomputer. The instrumentation counts AE events in a fashion nearly analogous to scintillation counting of radioactive materials.

The technique was applied to measuring ultrasonic AEs from the stems of cedar inside a pressure bomb. The shoots were originally fully hydrated. When the shoots are dehydrated in the bomb by application of an overpressure very few AEs were detected. When the bomb pressure is reduced after dehydration of the shoot, AE events could be detected. We conclude that ultrasonic AEs are caused by cavitation events (= structural breakdown of water columns in the tracheids of cedar) and not by the breaking of cellulose fibers in the wood.
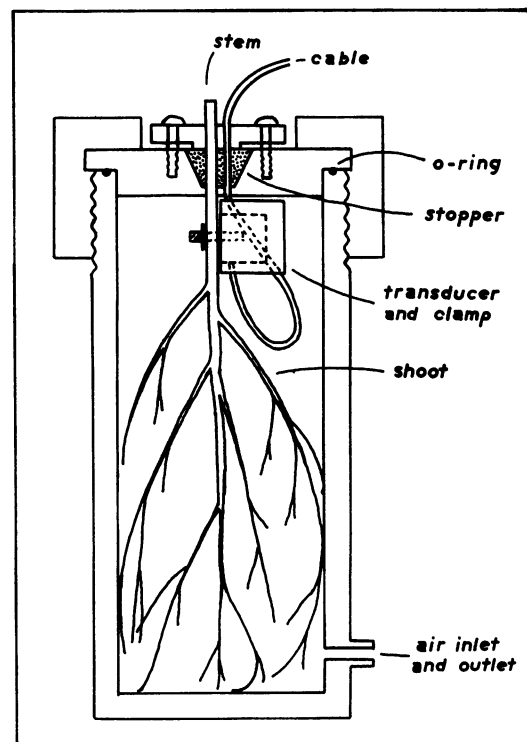
FIG. 1. A diagram (not to scale) showing an excised cedar shoot in a pressure bomb. The ultrasonic AE transducer is clamped to the sapwood inside the pressure bomb. The electric cable of the transducer and the base of the stem pass through a double-holed stopper to the outside air.

Tyree and Dixon (2) have reported that ultrasonic AEs[2] can be measured in the sapwood of eastern white cedar (*Thuja occidentalis* L.) The AEs are of an impulsive nature in the frequency range of 0.1 to 2 MHz and usually last less than 100 μs. Strong circumstantial evidence was presented to indicate that the ultrasonic AEs result from cavitation events because (a) they occurred only when the Ψ_{xp}, was more negative than a threshold level of about -1 MPa, (b) the rate of AE events increased as Ψ_{xp} decreased further, and (c) the AEs can be stopped by raising Ψ_{xp} above -1 MPa.

The AE detection technique is used extensively in the engineering sciences to detect the onset of structural failure in metals and other solids under mechanical stress (5). However, it is uncertain what kind of structural failure is being measured in cedar sapwood. As a whole shoot dehydrates in air the tension (= $-\Psi_{xp}$) on the xylem water columns increases and the xylem cell walls also experience a compressive force because the ambient air is at a higher pressure than the fluid in the xylem conduits. The structural failure events which generate AEs could originate from the water columns breaking (= cavitations) or from breaking of cellulose fibers in the xylem cell walls. It is important to know which is the case in order to test the hypothesis that ultrasonic AEs are reliable indicators of cavitation events.

In a pressure bomb it is possible to manipulate independently

the compressive force on the xylem walls and the tension in the water columns. If an excised shoot is placed inside a pressure bomb with the cut end passing through an airtight seal to the outside air (Fig. 1), the shoot can be dehydrated by increasing the air pressure in the bomb. During dehydration the water columns are at or above zero Ψ_{xp}, *i.e.* there is no tension, but the xylem walls are being radially compressed by the air inside the bomb in a manner causing distortion almost identical to that experienced by the intact plant at negative water potential, Ψ. After a period of dehydration in the bomb the P_B can be determined; the Ψ_{xp} is zero at P_B. If the bomb pressure is subsequently decreased to a value equal to P, then Ψ_{xp} grows negative by an amount equal to $P-P_B$. But the distortion causing forces on the xylem cell wall do not change since the pressure difference between the xylem water and the bomb pressure, P, does not change. In short, during compressive dehydration the compressive forces on the walls increase with no tension on the xylem water; during decompression of the bomb the compressive forces

[2] Abbreviations: AE, acoustic emission; Ψ_{xp}, xylem pressure potential; Ψ, water potential; P_B, balance pressure; P, bomb pressure; EPM, events per minute.
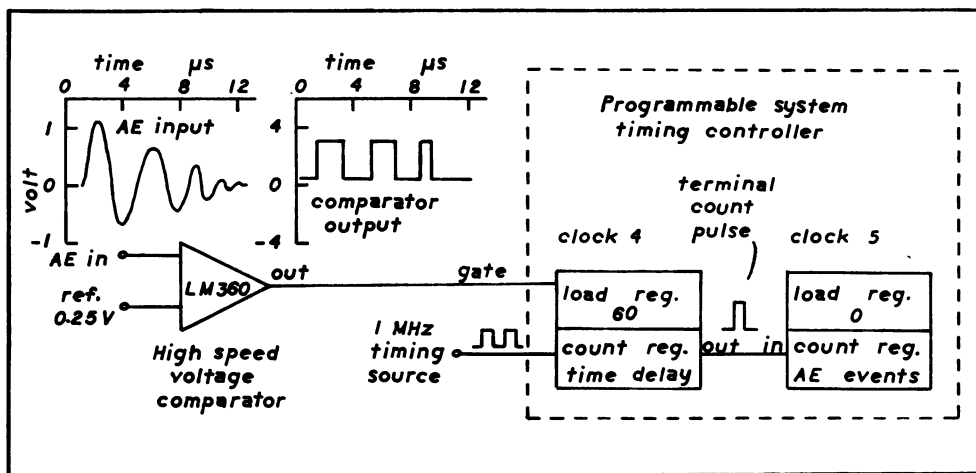
FIG. 2. A block diagram illustrating the principle of operation of the event counter for counting ultrasonic AEs. The amplified AE signal is passed through a voltage comparator which compares the AE waveform to a reference voltage of 0.25 v. The comparator converts all positive peaks exceeding 0.25 v to square wave pulses of 3-v amplitude. The square wave pulses go to clock No. 4 on a programmable system timing controller (an Am9513 manufactured by Advanced Micro Devices). Clock No. 4 effectively filters out all but the last pulse which is sent to clock No. 5. Clock No. 5 uses the pulse to increment the count in a count up register. Thus, this register contains a count of the number of AEs detected by the transducer. See the text for more details.

on the wall are unchanged since the xylem water tension (= $-\Psi_{xp}$) increases by an amount equal to the decrease in bomb pressure.

In this paper we present experiments in which the AE transducer is mounted inside a pressure bomb clamped against a stem of a cedar shoot also inside the bomb. By measuring AEs during compressive dehydration in the bomb and during subsequent decompression we can determine the origin of AEs.

We also present a much improved computer assisted method of measuring AE events. This instrumentation counts AE events in a fashion analogous to scintillation counting of radioactive materials.

MATERIALS AND METHODS

Experiments were done on excised terminal shoots of *Thuja occidentalis* collected from the forest understory on Snake Island about 80 km north of Toronto. The shoots were about 60 cm long and had stems about 10 mm diameter at the base. A model 8312 AE transducer (Bruel and Kjaer, Naerum, Denmark) was clamped to the stem about 12 cm from the cut end. The bark was removed from half of the circumference of the stem over a length of 7 cm to provide direct contact between the transducer and the sapwood. The transducer was held in place by a spring-loaded clamp and the exposed surface of the wood was covered with grease to prevent surface drying. The AE signals were amplified by a model 2638 wideband conditioning amplifier (also from Bruel and Kjaer) with a high pass filter set at 100 kHz to filter out low frequency sounds. The overall amplification of the system was set at 72 to 74 decibels. The shoot and transducer were placed inside a large pressure bomb with only the cut end of the stem passing through the airtight seal (Fig. 1). A small hole had been drilled into the case of the AE transducer to allow pressure equilibration with air in the pressure bomb.

In some experiments pressure volume curves were measured by the Hammel method (3) except that the P was returned to zero after each stage of dehydration, *i.e.* after each overpressure. Ultrasonic AEs were monitored during the entire experiments.

In other experiments the shoots were dehydrated in the pressure bomb by applying compressed air at a pressure of 2.0 to 2.5 MPa for 3 to 6 h. During dehydration recordings were made of AEs. At the end of the dehydration period the P_B was measured. The Ψ_{xp} was decreased by known amounts in a stepwise fashion by lowering the P below P_B. Ultrasonic AEs were monitored continually during the stepwise decompression.

All P adjustments up or down were done at a standard rate of

150 to 200 kPa min^{-1} and Ψ_{xp} was computed from the difference between the current P and the P_B, $P-P_B$. The xylem solute potential was occasionally measured on expressed sap and found to be negligible.

The amplified AEs were analyzed on a real time basis on a microcomputer programmed to count AEs in a mode analogous to that used in scintillation counting of radioactive material. AE events were counted only if the signal exceeded a preset lower level; this allowed elimination of unwanted background electrical noise. The AE counter had a programmable time delay analogous to the dead time in a geiger tube. The dead time was set at 60 μs. This permitted counting of AE events at rates up to 10^4 EPM with loss of less than 1% of the total events. The computer program plotted the results graphically on a dot matrix printer as rates with a running mean of 1 to 10 min (set by software) and as cumulative events. The AE counts were also saved in memory every 30 s and eventually recorded on a floppy disk for later analysis.

The principle of operation of the AE counter is presented below for interested readers, but the experimental results presented later can be understood without reading the following section.

Principle of Operation of the Acoustic Emission Counter. AE event counting was done on a real time basis on a CompuPro 8-16 microcomputer (Godbout Electronics, Oakland, CA). The microcomputer was programmed in FORTRAN and assembly language to make use of an Am9513 programmable system timing controller which was supplied as part of an S-100 analog to digital board (model AD212, TecMar Inc., Cleveland, OH). The Am9513 contains 5 clocks which can be used as timers or event counters. Only 2 clocks are needed for AE counting. Clock No. 4 was programmed in 'mode R' permitting us to convert a complex AE event consisting of one to many electrical wave forms into a single pulse which was sent to clock No. 5. Clock No. 5 was programmed in 'mode A' to count the number of pulses coming from clock No. 4. (The modes referred to above correspond to programmable modes discussed in detail in the technical application sheet for the Am9513 chip.)

The important aspects of the AE signal conversions are shown in Figure 2. The amplified AE signal arrives at the input of a high speed voltage comparator. The AE signal level is compared to a constant reference voltage set at 0.25 v. The comparator ignores any signal less than 0.25 v, thus filtering out all electrical background noise. Whenever the AE signal exceeds the reference voltage, the output of the comparator goes from a low voltage
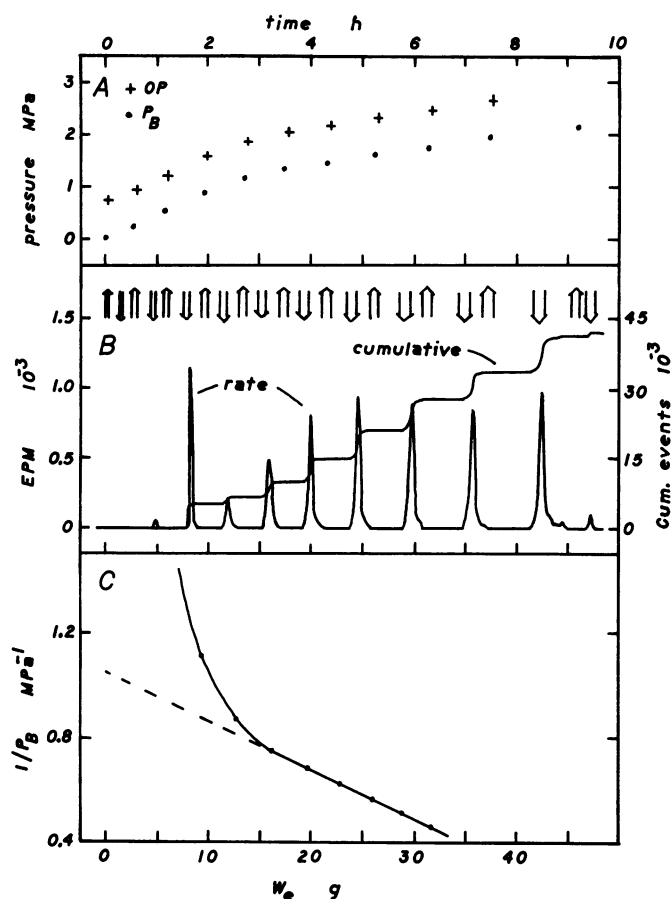
FIG. 3. Time course of AEs during the measurement of a pressure-volume curve. A, A plot of P_B and the overpressure (OP) *versus* time during the dehydration of a shoot. The double-shafted arrows indicate when the P was increased (arrow up) or decreased (arrow down). The left shaft of each arrow indicates the start of the pressure change and the right shaft indicates when the pressure change stopped. All pressure increases were from 0 MPa to the indicated OP (pluses) with a short pause to confirm the P_B (dots). All pressure decreases were from the OP to 0 MPa. B, Time course of AEs during the pressure changes in A. The rates in EPM are based on a running mean of the previous 4 min of events. Cumulative events are read from the right ordinate. (The peak rates of AEs immediately following a decrease in P did not follow a consistent pattern between replicates of this experiment. At this time no significance can be attached to the large peak rate at 1.8 h in this experiment.) C, The pressure-volume curve resulting from this experiment. The inverse P_B is plotted *versus* the weight of water expressed (W_e) from the shoot.

state (about 0.5 v) to a high voltage state (about 3 v) within 16 ns. In Figure 2 the timing sequence of the comparator is illustrated by an AE signal consisting of 3 waves which are translated by the comparator into 3 square wave pulses. The square wave pulses from the comparator are connected to a gate on clock No. 4. When programmed in mode R, the rising edge of the square wave causes the contents of the load register of clock No. 4 to be read into the count register. (The load register has been previously programmed to contain the number 60.) The count register counts down from 60 to 0 in 1-μs intervals using a 1-MHz square wave generator as its timing source. When the count register reaches 0 (= the terminal count) a terminal count pulse is sent to clock No. 5 which records a single event. However, if the gate receives another pulse before the terminal count is reached, then 60 is loaded back into the count register of clock No. 4, and the countdown is restarted at 60. (In the example shown in Figure

2, clock No. 4 is started at 60 three times, once for each pulse.) In short, clock No. 4 filters out all but the last peak of the AE signal and sends a terminal count pulse (which records the AE event) only if at least 60 μs has elapsed before a new pulse is sensed by the comparator. The time delay of 60 μs was chosen because it is equal to 6 times the period between peaks of a 100-kHz wave. This is the longest wavelength we can measure because lower frequency sounds are filtered out by the AE amplifier. If no peaks are seen within 6 wave periods it is safe to assume the AE event has ended. If another AE event occurs within 60 μs of the end of the previous event it is not counted; it is treated as part of the first AE event. So the time delay of 60 μs is like a dead time on a geiger counter.

Our program reads the contents of the count register of clock No. 5 once every 2 s and resets the counter to 0. The data read are used for further processing. The same AE counting technique can be used for lower frequency AE counting; it is only necessary to increase the time delay used in clock No. 4, *e.g.* to work with frequencies down to 100 Hz the time delay would have to be 60,000. A longer time delay would decrease the counting efficiency of the system; if the time delay is 60,000 then lost events would exceed 1% of the total counts if the events occurred at rates in excess of 10 events per min. Readers wishing more details and/or a program listing should contact the authors. The program is not reproduced here because it is very long and is hardware specific.

RESULTS

Several control experiments were done to check the performance of the AE detection equipment. When AE activity is measured with the transducer clamped to dry wood or in contact only with air, the background count rate measured over 24 h averaged 0.5 EPM or less depending on the gain setting used. In other control experiments the AE transducer was sealed inside a pressure bomb either alone or clamped to a short piece of dry wood or fresh sapwood. The P could be raised or lowered at rates of 200 kPa min⁻¹ or more without detecting AE counts above the background rate.

Five pressure-volume curves were measured while monitoring AE activity. A typical result is shown in Figure 3. The P_B and the overpressures at each stage of dehydration are shown *versus* time in Figure 3; the periods of time during which the pressures were increasing or decreasing are indicated by arrows. It can be seen that AE activity occurred only during compression phases and never while the bomb pressure was increasing. Typical pressure-volume curves were obtained in all five experiments.

Five other experiments were done in which a shoot initially near full hydration was dehydrated by a single large overpressure. The P was subsequently decreased in a stepwise fashion over the next few hours. A typical result is shown in Figure 4. Note that very little AE activity was detected during the dehydration phase when compressive forces were increasing on the xylem cell walls. Most AE activity occurred while the P decreased, *i.e.* while the tension on the water columns increased but the compressive forces on the walls remained unchanged (because the sum of xylem tension and P remains unchanged). Note also that AE activity stopped at 7.7 h when the P was increased to redetermine the P_B. In all experiments we found that the P_B had decreased 0.08 to 0.21 MPa during the decompression. Also, the cumulative number of AEs measured in this series of experiments was always less than the number of AEs detected during a pressure-volume curve experiment (Fig. 3).

DISCUSSION

It is common practice in many laboratories to measure Ψ isotherms of leaves and shoots by progressive air dehydration of
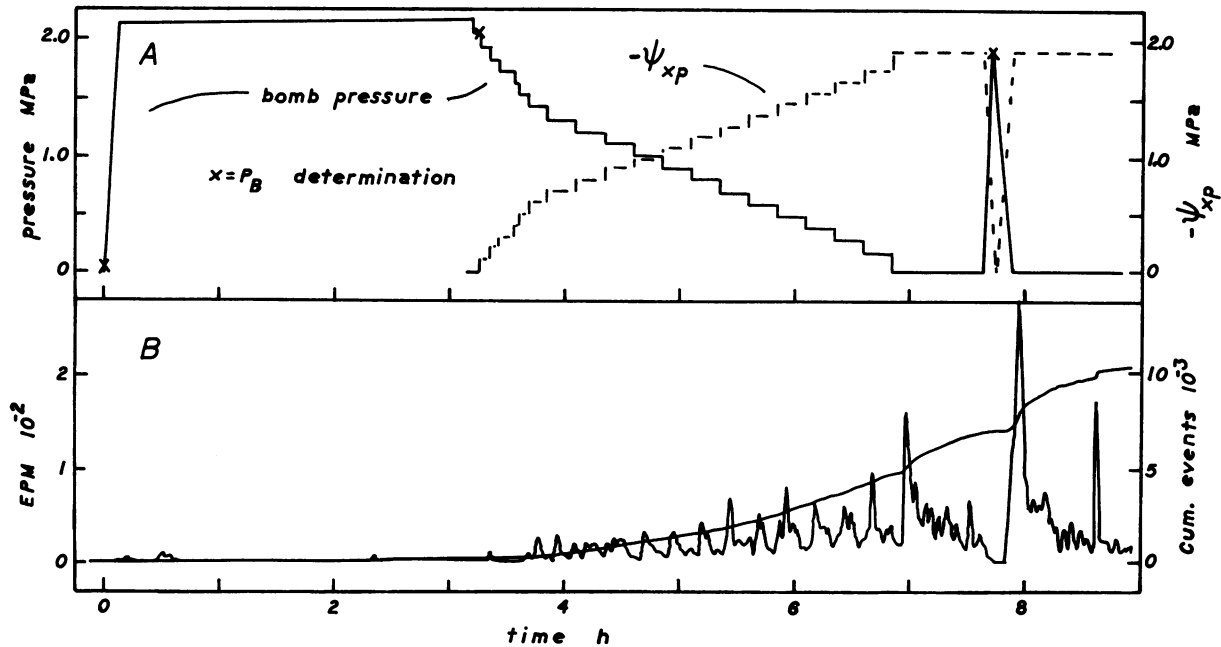
FIG. 4. The time course of AEs during a one-step dehydration of a shoot followed by a stepwise release of the bomb pressure. A, The time course of the P changes (———). The X's on the curve indicate the P_B as determined at the beginning (0 h), after the dehydration (3.25 h), and after pressure release (7.7 h). (- - -), the time course of the computed xylem water tension (= $P_B - P = -\Psi_{xp}$). Since P_B decreased from 3.25 to 7.7 h, the tension was computed assuming P_B decreased linearly with time. B, The time course of AE events during this experiment. Rates of AE events in EPM are based on a running mean of the previous 2 min of events. Cumulative events are read from the right ordinate.

the sample. Water loss is determined from weight loss of the sample and the air dehydration process is interrupted periodically to measure the P_B of the sample in the pressure bomb. It is clear from this work and other studies of cavitation (1 and 4) that samples dehydrated in air will experience extensive cavitation. The water released from cavitated xylem conduits will rehydrate the leaf cells and consequently distort the shape of the water-potential isotherm. In many of our experiments we noted a decrease in P_B after cavitations had occurred (see that the second 'X' in Fig. 4 is higher than the third X), but we are not sure all the decrease in P_B was caused by water released from cavitated tracheids. There may have been a small change in osmotic solute concentration in the cell over the time course of the experiment, or the initial P_B may not have been a proper equilibrium value. How serious this problem is cannot yet be determined because we do not have enough information about the physics of sound propagation in our samples to estimate what fraction of the total number of xylem conduits have cavitated. Also, we do not know how many of the cavitated conduits are refilled while measuring the P_B. The potential exists to answer these questions with our ultrasound techniques.

We suspect that the tracheids of cedar are more likely to cavitate once they have been exposed to compressed gases in the pressure bomb than when dehydrating in air. In this study and (2), cavitations started in shoots in the pressure bomb when the Ψ_{xp} reached about -0.6 MPa, whereas in whole shoots dehydrated in air cavitation started at -1.0 MPa (2). Cavitations at higher Ψ_{xp} inside the pressure bomb might be caused by gas bubbles coming out of solution. In a pressurized pressure bomb the concentration of the gases in the water of the shoot will increase while approaching equilibrium with the elevated gas pressure in the bomb. Upon decompression, the gases will diffuse back out or come out of solution as a bubble inside a tracheid. The formation of an air bubble inside a tracheid would cause a cavitation, i.e. the shoot gets 'the bends' in the same way as a scuba diver would if he comes to the surface (decompresses) too

quickly. Carrying this analogy further, we would expect fewer pressure-bomb-induced cavitations if the shoot is decompressed slowly, thus allowing time for the dissolved gases to escape by diffusion rather than by bubble formation. This is exactly what we have found. Rapid decompression induced more cavitations than slow decompression in several unpublished experiments; this can also be seen by comparing the total number of AE events in Figure 3 where the shoot was rapidly compressed and decompressed several times and the lesser number of AE events in Figure 4 where a similar shoot was decompressed slowly from 3.2 to 6.5 h. Also (Fig. 4) it can be seen that the final rapid decompression produced a larger number of AEs than any of the previous smaller decompression steps. Very few cavitations will occur if pressure-volume curves are obtained by the Hammel method (3) because the P is not allowed to fall far below the P_B in this technique. Anyone wishing to avoid cavitation problems would be advised to use the Hammel method.

The most important observation in these experiments is that ultrasonic AEs from the sapwood of cedar arise only when the pressure is decreasing in the pressure bomb and not when the pressure is increasing. This strongly suggests that the ultrasonic AE are associated with true cavitation events and not just structural failure of the xylem cell walls under compressive stress. We know of no publications which provide stronger evidence that the sound emissions from water-stressed plants arise from cavitation events rather than other sources.

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