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Ultrasonic Acoustic Emissions from the Sapwood of Cedar and Hemlock[1]

AN EXAMINATION OF THREE HYPOTHESES REGARDING CAVITATIONS

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ABSTRACT

Measurements are reported of ultrasonic acoustic emissions (AEs) measured from sapwood samples of *Thuja occidentalis* L. and *Tsuga canadensis* (L.) Carr. during air dehydration. The measurements were undertaken to test the following three hypotheses: (a) Each cavitation event produces one ultrasonic AE. (b) Large tracheids are more likely to cavitate than small tracheids. (c) When stem water potentials are >−0.4 MPa, a significant fraction of the water content of sapwood is held by 'capillary forces.' The last two hypotheses were recently discussed at length by M. H. Zimmermann. Experimental evidence consistent with all three hypotheses was obtained. The evidence for each hypothesis respectively is: (a) the cumulative number of AEs nearly equals the number of tracheids in small samples; (b) more water is lost per AE event at the beginning of the dehydration process than at the end, and (c) sapwood samples dehydrated from an initial water potential of 0 MPa lost significantly more water before AEs started than lost by samples dehydrated from an initial water potential of about −0.4 MPa. The extra water held by fully hydrated sapwood samples may have been capillary water as defined by Zimmerman.

We also report an improved method for the measurement of the 'intensity' of ultrasonic AEs. Intensity is defined here as the area under the positive spikes of the AE signal (plotted as voltage *versus* time). This method was applied to produce a frequency histogram of the number of AEs *versus* intensity. A large fraction of the total number of AEs were of low intensity even in small samples (4 mm diameter by 10 mm length). This suggests that the effective 'listening distance' for most AEs was less than 5 to 10 mm.

Techniques have existed for some time for detecting cavitation events in plants (1-3, 5, 6, 9) but until recently the techniques have not been widely used or reliable. Cavitation events produce acoustic emissions (AEs[2]) which can be detected and amplified to produce audible clicks using audio sound transducers and amplifiers. These AEs are very weak and easily confused with laboratory vibrations and electrical amplifier noise. Consequently it has been possible to study cavitations only when the plant is undisturbed in a soundproof chamber. Recently, Tyree and Dixon (7) have shown that water-stressed plants produce ultrasonic AEs in the frequency range of 100 kHz to 2 MHz. Using ultrasonic transducers and amplifiers, one can filter out most audible and low frequency ultrasonic laboratory vibrations (frequencies less than 50 kHz). Thus, concurrent measurements of parameters such as water potential, stomatal conductance, and stem density can be made without affecting the acoustic measurements.

The ultrasonic technique has been applied to measuring AEs from the stems of cedar under adverse environments, *e.g.* inside a pressure bomb (8) or in the field (unpublished). When a shoot is inside a pressure bomb, the xylem pressure potential and mechanical stress on cellulose fibers can be independently changed. From ultrasonic AE measurements on shoots inside a pressure bomb Tyree *et al.* (8) concluded that ultrasonic AEs were caused by cavitation events (= structural breakdown of water columns in the tracheids of cedar) and not by the breaking or deformation of cellulose fibers in the wood.

The purpose of this paper is to examine three hypotheses.

Hypothesis 1. Each cavitation event produces one ultrasonic AE, *i.e.* during the dehydration of conifer sapwood there is a one-to-one relationship between the number of detectable AEs and the number of tracheids cavitating. To test the hypothesis, we looked for a quantitative agreement between the number of AEs during the air dehydration of sapwood samples and the number of tracheids in the sample. The number of AEs could exceed the number of tracheids if the AEs are generated by structural failures partly or wholly unrelated to cavitation events (8). The number of AEs could be less than the number of tracheids in a sample for one or more of three reasons: (a) AEs are unrelated to cavitation events, (b) a significant fraction of the tracheids were already embolized (air filled) prior to the dehydration experiment, or (c) a significant fraction of the cavitations produce AEs too weak to be detected. To help reduce these problems we developed a method of mesuring AE 'intensity' so that some measure of signal intensity could be obtained. All measurements were done on small sapwood samples, thus reducing the distance that the sound waves must travel with concomitant signal loss. All samples were collected in early spring. Since the sapwood water content is highest in spring, the number of embolized tracheids is likely to be least.

Hypothesis 2. Large tracheids are more likely to cavitate than small tracheids. A number of people (4, 10) have suggested that there is a biological trade-off between efficiency and safety in the evolution of xylem conduits. Wide conduits (*e.g.* vessels of oak) are much more efficient water conductors than narrow conduits (*e.g.* tracheids of cedar). It is not surprising that evolution was toward wider conduits in many cases. However, there seems to be an upper limit of the useful vessel diameter of about 0.5 mm. This limit has been reached many times in diverse taxa. But it has been suggested (without much proof) that large conduits are more likely to cavitate than small conduits. This hypothesis can be tested by examining the relationship between water loss and cumulative cavitations during air dehydration of sapwood sam-

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[2] Abbreviations: AE, acoustic emission; Ψ_{xp}, xylem pressure potential.

ples. If the large earlywood tracheids cavitate before smaller latewood tracheids, then the amount of water loss per AE ought to be more at the beginning of the dehydration experiment than at the end.

Hypothesis 3. Fully hydrated sapwood contains a significant fraction of water held by 'capillary forces.' Zimmermann (10) has suggested that significant quantities of water can be held in intercellular spaces and in the tapered ends of embolized tracheid and fiber lumina by capillary forces. He estimated that most of this water would be lost as the tissue water potential falls from 0 to −0.4 MPa. Since capillary water would probably be lost without cavitation events, this hypothesis could be tested by measuring the amount of water loss prior to onset of cavitation events in sapwood samples dehydrated from initial water potentials of 0 and about −0.4 MPa.

MATERIALS AND METHODS

Shoots of eastern white cedar (*Thuja occidentalis* L.) and of eastern hemlock (*Tsuga canadensis* [L.] Carr.) were collected in the mornings on Snake Island about 80 km north of Toronto. All shoots were collected in April prior to bud break and were stripped of all leaves. The stems (still with bark) were rehydrated in water for 12 to 36 h. Sapwood subsamples of 2 to 10 mm diameter and 6 to 200 mm length were cut from the stems and stripped of bark and air dehydrated while measuring AEs. In some cases, shoots were collected when the shoot water potential was −0.35 to −0.45 MPa as measured with a pressure bomb. These samples were not rehydrated. They were immediately stripped of bark and leaves and dehydrated while measuring AEs.

The sapwood samples were clamped to a model 8312 AE transducer (Bruel and Kjaer, Naerum, Denmark) which converts an ultrasonic vibration to a voltage waveform. The point of contact between the wood and the raised contact sole of the transducer was at the midpoint of the sapwood sample on the cylindrical surface. The efficiency of conversion of vibrational displacement to voltage depends on the force with which the sapwood sample is clamped against the transducer. To standardize the force of contact, the samples were clamped against the transducer by a strip of flexed spring steel. The force of compression provided by the spring steel strip was 40 ± 4 Newtons. The AE signal was further amplified by a model 2638 wide band conditioning amplifier (Bruel and Kjaer) with a high pass filter set at 100 kHz to filter out low frequency sounds.

During air dehydration, AEs were counted by the method described by Tyree *et al.* (8). In some experiments, AE measurements were interrupted periodically to remove the sapwood sample for weight loss determination to the nearest 0.1 mg. After dehydration, some samples were examined microscopically to estimate the total number of tracheids. Samples were cross-sectioned on a sliding microtome and the average cross-sectional area of several hundred tracheids was determined microscopically. Average tracheid lengths were measured on macerated tissue using standard maceration techniques. If every intact tracheid was originally water filed then we would expect the number of AEs recorded during dehydration to equal the total number of intact tracheids in the sample. The number of intact tracheids, N, was estimated from:

$$N = \frac{A_s}{A}\frac{L - l}{l} \quad (1)$$

where A_s is the cross-sectional area of the sapwood sample, A is the average cross-sectional area of the tracheids, L is the length of the sapwood sample, and l is the average length of the tracheids.

The number of tracheids in a given sample (N) was compared to the cumulative number of AEs which is known to a high degree of accuracy (±0.0005 times the count). The uncertainty of the AE count arises primarily from the correction for background counts. The uncertainty in the estimation of N is considerably more; N is calculated from equation 1 and is subject to the combined uncertainties of A_s, L, A, and l. The values of A_s and L are subject to errors in distance measurements which we take to be ±0.03 and ±0.01 times their respective values. The mean value of A was computed from cross-sectional area measurements of several contiguous groups of 50 to 100 tracheids, and l was obtained from measurements on a few hundred tracheids. The standard deviations of A and l (S and S', respectively) were computed and expressed as a fraction of the means S/A and S'/l, respectively. The combined uncertainty of N was then estimated from:

$$\text{uncertainty in } N = \pm N\,[(0.03)^2 + (0.01)^2 + (S/A)^2 + (S'/l)^2]^{1/2}$$

The method of AE signal intensity measurement used is very similar to that used in the Bruel and Kjaer model 4429 pulse analyser described elsewhere (7). The system described below has improved measurement resolution and costs less than one-tenth the price of the model 4429 analyser.

Acoustic emission intensity was measured on a real time basis on a CompuPro 8-16 microcomputer (Godbout Electronics, Oakland, CA). The microcomputer was programmed in FORTRAN and assembler languages to utilize 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 are used as signal timers. All 5 clocks were programmed in mode E (as defined in the application sheet for the Am9513 chip). In mode E, each timer counts clock cycles from a 6-MHz square wave source (Fig. 1A) whenever its gate pin is held at a high voltage (logic 1 state). The gates are controlled by the AE signal and five high speed comparators (LM360's). One input of each comparator is connected to a reference voltage source. The reference voltages are 0.25, 0.5, 1.0, 1.5, and 2.0 v. The other input of each comparator is connected to the AE signal. The output of each comparator is connected to a different clock gate. Whenever the AE signal exceeds the reference voltage of a comparator, the output of the comparator goes high and triggers the corresponding timer to count clock cycles until the signal drops below the comparator's reference voltage. The area under the curve was approximated by up to 5 rectangular areas as indicated in Figure 1B. The output of the 0.25-v comparator was connected in parallel to another Am9513 chip programmed as an event counter as described elsewhere (8).

The intensity of each AE is defined here as the area under the positive half of the curve of voltage *versus* time (Fig. 1B). After each AE event, the computer read the value of each timer and computed the area under the curve using an equation like that in Figure 1B. The area resolution of the intensity measurement was 0.25 v times the smallest time interval that could be counted (⅙ μs); so areas were measured in multiples of 4.17×10^{-8} vs. During the dehydration experiments the computer constructed a frequency histogram which was a plot of the number of AEs *versus* intensity.

It is worth noting that all AE counting and intensity determination can be done on the Bruel and Kjaer 4429 pulse analyser by making full use of the IEC/IEEE computer interface provided on the 4429. Programs were developed to use this interface and all our preliminary measurements were done on the model 4429 analyser. Although the results obtained from the two techniques are similar, the Bruel and Kjaer instrumentation was eventually discarded since the performance of it was inferior to the instrumentation described above.

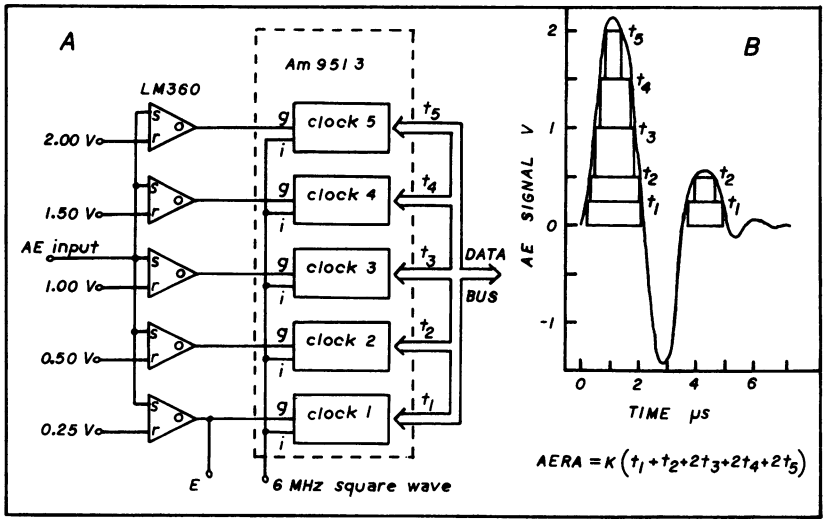

FIG. 1. A, Diagram showing the major components of the computer interface used to measure AE intensity. The triangles are high speed voltage comparators (LM360); r, reference voltage input; s, AE signal input; o, output. The Am9513 is a programmable timer/counter chip containing 5 independent 16-bit clocks; g, a gate which enables the clocks to count a timing signal from a 6-MHz square wave source when programmed in mode E (as defined in the applications sheet of the Am9513 chip); i, the input for the 6-MHz square wave source. The double shafted arrows represent the computer's data bus for transfer the times t_1 to t_5. B, Illustrates how the area under the curve of an AE signal is estimated by the computer. Clock times t_1 through t_5 are represented by the width of the rectangles. Times t_1 and t_2, in this example, equal the combined width of the rectangles in the two positive peaks. The computer uses an equation like that below B to compute the area under the curve.

RESULTS

The average intensity of AEs detected by the transducer depended on the size of the sample clamped to the transducer. The average intensity measured from large samples was always less than from small samples. The average intensity of AEs changed during the time course of the dehydration of sapwood samples; the intensity generally increased initially, remained high for most of the experiment, and eventually fell to lower values near the end of the dehydration experiment (Fig. 2A).

Several frequency histograms of the number of AEs *versus* intensity were measured during the dehydration of cedar and hemlock samples from full hydration to air-dryness. Two typical results for hemlock are shown in Figure 2B, the plots are marked S and L for small and large samples, respectively, and the arrows point to the mean AE intensity. Frequency histograms for cedar were qualitatively similar but the mean intensities were less, *i.e.* 0.5 to 0.8 times the mean intensity of AEs in hemlock samples of comparable size.

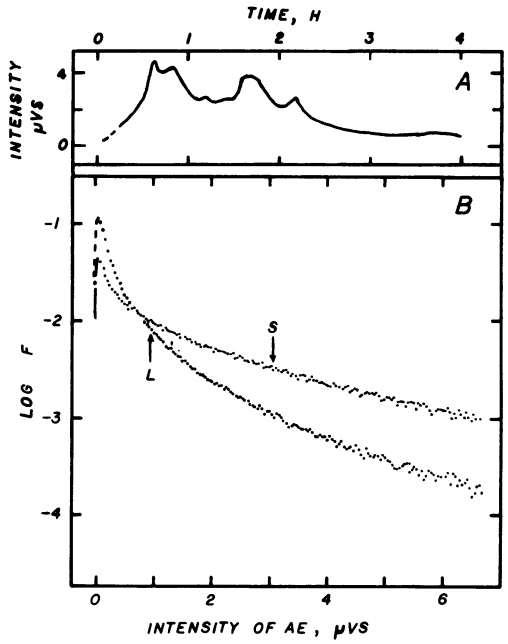

FIG. 2. A, The time course of the average intensity of AEs during the dehydration of sample *S* below. The intensities plotted are the average of 20 min counting period or of 5,000 events, whichever occurs first. B, A frequency histogram of the relative number of AEs *versus* their intensity. The ordinate is the log base 10 transformation of F = (the number of AEs of the plotted intensity)/(the total number of AEs in the histogram) and the abscissa is the intensity in μvs. The arrows point to the mean intensity of two histograms obtained during the dehydration of two hemlock stem segments from full hydration. *S*, a small stem segment 4.3 mm diameter and 11.5 mm long and *L*, a large segment 8.2 mm diameter and 87.8 mm long.

Cumulative AEs were measured as a function of weight loss from cedar and hemlock sapwood samples. These data are plotted in Figure 3 as cumulative per cent AE events *versus* per cent total water loss. In Figure 3, A and B, initial water potential was nearly zero. Some samples were taken directly from the tree when the shoot water potential was −0.35 to −0.45 MPa (Figure 3, C and D). Note that in these cases the amount of water that evaporated before the commencement of AEs was reduced by 10 to 15%. The percentage of water contained in the severed tracheids of the two cut ends of the stem segments was estimated from 100% l/L, where l is the average tracheid length and L is

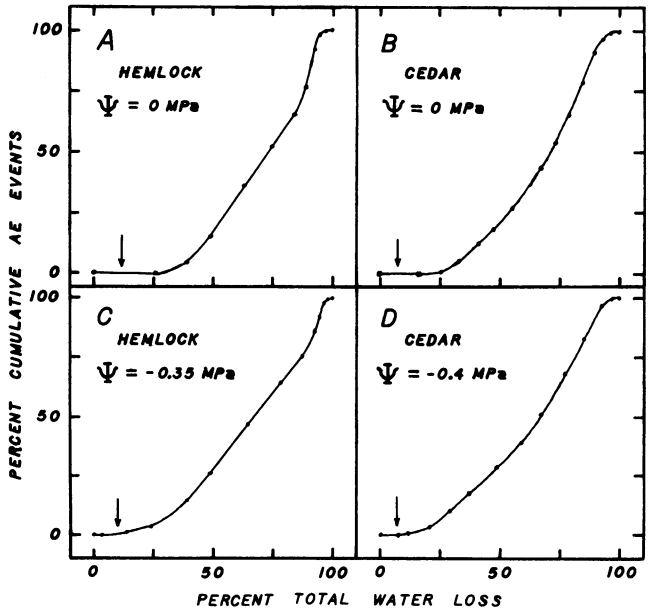

FIG. 3. Plots of the per cent cumulative AEs *versus* the per cent total water loss from hemlock and cedar stem segments dehydrated from various initial water potentials as indicated on the figure. The arrows indicate the estimated per cent of the total water contained in the severed tracheids at both ends of the stem segment.

Table I. *Acoustic Emissions and Tracheid Numbers*

Small sapwood samples, originally fully hydrated, were air-dehydrated while counting the cumulative acoustic emissions. Subsequently, tracheid dimensions were measured to obtain an estimate of the total number of tracheids in the sample from equation 1.

Sample	Cumulative AEs	Number of Tracheids
		$\times 10^6$
Cedar 1	3.23	3.91 ± 0.65
Cedar 2	1.98	2.15 ± 0.70
Cedar 3	2.50	2.72 ± 0.73
Cedar 4	2.19	1.97 ± 0.22
Hemlock 1	1.00	1.14 ± 0.25
Hemlock 2	1.22	0.93 ± 0.24
Hemlock 3	0.82	1.25 ± 0.38

the length of the stem segment; these values are indicated by the arrows on Figure 3.

In some parallel experiments, the lengths and diameters of hemlock and cedar sapwood segments were measured during air dehydration. Measurements were made with a vernier caliper to the nearest 0.02 mm. For samples of 4 to 8 mm diameter and 20 to 40 mm length no change in diameter or length was detectable during air dehydration. We estimate the percentage volume change of the sapwood to be less than 1% during air dehydration.

The relationship between AEs and the total number of intact tracheids in small samples of cedar and hemlock sapwood is shown in Table I. In all cases, the number of AEs detected falls within the estimated uncertainty in the measurement of the number of tracheids (see "Materials and Methods").

DISCUSSION

The intensity of an AE detected at the point of attachment of the AE transducer will depend on the intensity of the AE vibration at the point of origin and on the amount of signal loss while the emission propagates to the detector. The amount of signal loss will depend on a number of poorly understood factors including: (a) attenuation in the propagation medium, (b) reflections of the waves from internal surfaces, (c) inverse square law signal dispersion, and (d) conversion in the mode of wave propagation at reflective surfaces. Although it is difficult to quantify all these factors, one would expect that the AE signal would grow progressively weaker the farther it has to travel to reach the transducer. In large samples the average distance that an AE has to travel to reach the transducer is greater than in a small sample; this explains why the average intensity of AEs is less in large samples.

We always found that the most frequently occurring AE intensity (= the peak in histogram, Fig. 2B) had the smallest detectable area (4.17×10^{-8} vs). This was true even for the smallest samples we could use in our apparatus (2 mm diameter by 6 mm long). Since the peak in the histogram did not shift substantially to the right of the origin in the smallest samples, we cannot say with confidence that we are able to detect all AEs. This could mean that either a large fraction of AEs are originally of low intensity or that the predominant frequency of a large fraction of AE events is beyond the range of our present apparatus. Emissions of frequency less than 100 kHz are removed from the signal by a passive filter and frequencies above 2 MHz are too fast for the amplifier.

Although a substantial fraction of the AEs are of barely detectable intensity, a reasonably good agreement exists between the number of AEs and the estimated number of tracheids (N) in the samples. In every case the agreement is within the estimated error of the measurement of N (Table I). It seems likely that hypothesis 1 is correct, *i.e.* an approximately one-to-one relationship exists between AEs and cavitating tracheids. Agreements as good as those in Table I are obtained only on small samples (typically less than 5 mm diameter and 16 mm long). On larger samples the number of tracheids exceeds the number of AEs detected. This suggests that the maximum distance from which the weaker AEs can be detected is of the order of 0.5 cm. Table I provides further confirmation that ultrasonic AEs are indeed reliable indicators of cavitation events.

The suitability of our instrumentation for measuring cavitation events in a variety of other species (including maple, beech, corn, and palm) was assessed. The intensity of emissions in these species generally averaged 2 to 10 times that in cedar. It may be that conifers are among the weakest of acoustic emitters (unpublished results).

A surprisingly large percentage of water evaporated from fully hydrated samples before AEs started (Fig. 3, A and B). The xylem pressure potential, Ψ_{xp}, must fall to about −1 MPa before cavitations can begin (7). Before sufficiently negative Ψ_{xp}'s can develop, all the water from cut tracheids on both ends of the stem segment must evaporate away. This will account for about 8 to 12% of the total water content (see arrows in Fig. 3, A and B). Water loss associated with volume changes during dehydration is less than 1%. So 10 to 15% of the water loss observed before AEs start is still unaccountable. It is possible that the first cavitation events do not produce AEs in the measured frequency range of 100 kHz to 2 MHz. Another possibility is that the water that must evaporate before cavitations start is capillary water as recently discussed at length by Zimmermann (10). Sapwood could contain a substantial amount of water held by capillary forces in intercellular spaces and in embolized tracheids and wood fibers. Air-seeded fiber and tracheid lumina will never be completely air filled; they will contain some water at the tapered tips. Zimmermann calculated that most capillary water is held at water potentials between 0.0 to −0.5 MPa. If this is correct, then sapwood samples starting at a water potential of about −0.4 MPa ought to start cavitating after much less water loss than fully hydrated samples. Our results fully support Zimmermann's hypothesis and these data may be the first experimental results confirming the existence of capillary water in sapwood (compare Fig. 3, C and D to Fig. 3, A and B).

When AEs do start the amount of water lost per emission is more at first than toward the end of the dehydration period, *i.e.* note the gradual increase in slope (Fig. 3). It has often been suggested that large xylem conduits are less resistant to cavitations than small conduits (4, 10). Cavitations probably initiate from very small loci, *e.g.* perhaps a very small hole in the conduit wall which allows air to enter (10), or a small air bubble embedded in the inner wall surface of the lumen (4), or perhaps adhesion between the water and the wall breaks down at a small hydrophobic imperfection in the wall. The probability of finding such a locus in a large conduit is greater than in a small conduit because there is more wall surface area in a large conduit. Our data may provide the first experimental confirmation of the proposition that large tracheids are more likely to cavitate than small ones. The shapes of the curves in Figure 3 fit with this hypothesis; in Figure 3, B and D, there is a gradual change in slope. This corresponded to an observed gradual transition in tracheid size between earlywood and latewood in cedar samples. In contrast, the hemlock samples had more distinct earlywood and latewood regions; this correlated with a sharper change in slope in the hemlock dehydration curves (Fig. 3, A and C).

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