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Monitoring cavitation emissions from plants exposed to ultrasound

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Ultrasonic cavitation has been defined as the process of generation of bubbles by a sound field, the various motions of such bubbles and the physical effects brought about by the motions (1). It is the usual result of experimental studies that the degradation of polymers in solution or cells in suspension, on exposure to ultrasound, coincides with the presence of cavitation in the treated sample. The published intensity threshold values reported for the onset of cavitation in water vary widely, e.g. Iernetti included in his review (2) threshold values at 1 MHz ranging from $1 W/cm^2$ to $27,000 W/cm^2$. The exposure of humans to MHz frequency ultrasound during diagnostic and therapeutic medical applications requires that the possibility that ultrasound could cause cavitation in tissues be examined.

It has recently been recommended that the spatial peak intensity of continuous wave devices employed in monitoring pregnancies should not exceed $0.1 W/cm^2$ (3). Equipment used in physiotherapy for ultrasonic diathermy can have spatial average intensities of $3 W/cm^2$ (4) in the beam or peak intensities of the order of $10 W/cm^2$. These latter devices are in the lower part of the intensity range reported for cavitation thresholds in water at 1.0 MHz (2). Details of a number of reports of cavitation damage in mammalian tissues exposed to ultrasound are given in the Table. The threshold intensities for the observed effects varied widely. Lele et al. (6) established a cavitation threshold by monitoring subharmonic emission from cat brain while in the remaining reports (5,7,8) the presence of cavitation was deduced from the nature of the lesion or from the disappearance of the effect when the external static pressure was increased.

Table

Exposure conditions at which cavitation was believed to influence lesion production in sonicated animals

Lesion Site	Peak Intensity (W/cm^2)	Exposure Time (s)	Frequency (MHz)	Reference
Cat Brain	2,000	0.04	1.0	5
Cat Brain	400	1.0	2.7	6
Mouse Liver *in vivo*	200*	4.0*	1.0	7
Mouse	3.0-6.0	120	1.0	8

* Values deduced from author's descriptions

The blood vessels suggest themselves as sites where cavitation might occur in mammalian tissue exposed to ultrasound. Curtis (7) and Lehmann and Herrick (8) found damage, which they ascribed to cavitation, in the region of the blood vessels. Quasi-standing wave fields in the tissue, arising from partial reflections of the beam would produce peak pressure amplitude variations every half wavelength and would also introduce standing wave radiation forces on cavitation nuclei or developing bubbles in the blood stream which might trap them in the sound field so that they would grow. The histological methods employed in some of the studies in the Table to identify cavitation occurrence require that the animals be sacrificed following exposure. Any practical method of determining if cavitation is occurring in human tissue would need to be non-invasive.

Detecting emissions from non-linear bubble activity in the tissue is one such non-invasive method. Bubbles in a sound field can, under particular circumstances, emit some or all of the following, white noise, harmonics or subharmonics of the driving frequency. Prosperetti (9) and Lauterborn (10) have analysed how subharmonic and harmonic emission accompany the non-linear steady-state oscillations of driven bubbles. Prosperetti (11) also extended Neppiras' examination of subharmonic emitting sources in a cavitating field (12) by suggesting that in a transient bubble field subharmonics were generated by bubbles smaller than resonance size whose motions were perturbed by shocks from transient bubbles. Subharmonic emissions may therefore arise from stable or transient bubble fields. White noise can accompany surface wave activity or transient cavitation (13). When non-linear effects are considered there is no threshold pressure required for the emission of first harmonic from stable bubbles (9). In experimental studies with water it has been found that harmonic emission either precedes (12,14) or is coincident with (15) the onset of a subharmonic signal.

Plant tissue is a suitable material in which to investigate how cavitation emissions from a constrained environment as in a root tip would differ from emissions from bubbles in water, because material is readily available and it has been known since 1954 that cavitation will occur in plant root tips exposed to ultrasound at a frequency of 1.0 MHz (16). It was found then that if the initial temperature of the surrounding medium was high diffuse thermal damage and more localised lesions of diameter 10-100 μm occurred in onion root tips exposed to spatial average intensities of 3.3 W/cm^2 for 2 min at 0.8 MHz. The diffuse reaction was no longer observed if the temperature of the surrounding liquid was decreased but the point lesions remained. The disappearance of the point lesions when the external pressure was increased indicated that they were cavitational in origin. The presence of point lesions when the roots were exposed to sound while in an agar gel showed that the cavitation was occurring within the root tip rather than in the surrounding medium. More recently it has been shown that exposure of root tips to megahertz frequency ultrasound could result in growth retardation or death of the tip, depression of the mitotic index and induction of chromosomal anomalies (see ref. 17). The extent of growth retardation is a function of intensity and time of irradiation. The threshold intensity at frequencies of 0.75 and 1.5 MHz for an inhibitory effect on the growth of the root tip of the bean Vicia faba was in the range 0.4 - 0.7 W/cm^2 where the intensity was averaged over the meristen and elongation region of the root tip and treatments lasted from 90 to 180 min (18). Root tip death at 2.0 MHz follows exposure to 12 W/cm^2 for 10 min.(23)Thin sections of bean root tip have been sonicated in a special chamber which enabled cinephotomicrographs to be obtained during treatment (19). Intra-cellular movements in areas of the cells proximate to intracellular gas spaces were observed at very low intensities, several orders of magnitude below those at which a subharmonic signal was detected.

We are currently examining the effects of 1.0 MHz ultrasound on the root tip of green long pod beans in a study which includes the measurement of growth and root tip death subsequent to treatment, the detection of emissions from the root during treatment and the microscopic examination of sections of the treated roots. The methods of root growth and treatment have already been described (20). The root tips are exposed to 1.0 MHz sound at the last axial pressure maximum in the near field in a tank of autoclaved degassed water. Emissions from the root tip are monitored with a ring hydrophone placed 5 mm from the root tip. The experimental arrangement is shown in Fig. 1. The first order subharmonic, second harmonic and 600 kHz white noise signals are selectively amplified and examined. Growth of the root tips is measured daily for a number of days after treatment and is compared with the growth of non-irradiated control roots.

The dependence of G_1 (the growth on the first day following treatment expressed as a fraction of the growth of control roots) and root tip death on the intensity and time of irradiation are shown in Fig. 2. The intensity quoted is the peak intensity in the beam at the point of treatment. In order to examine any correlation between root tip growth and cavitation emissions roots were treated individually rather than in batches as in some other studies. It was therefore not practical to attempt to establish a statistically significant clearly defined threshold for long duration low intensity exposures. However we were able to demonstrate a threshold for an effect on G_1 in the region 2 - 4.5 W/cm^2 for treatments of 40 min duration, which was broadly comparable with previous results (16). Our treatment of 16 W/cm^2 for 5 min necessary to kill the root tip was also in broad agreement with previous reports (23).

A continuous low level subharmonic and harmonic signal which was detected even in the absence of a root was found to vary in amplitude even during the exposure of the autoclaved degassed water alone. The detection of a modulation was a much more clear cut end point and attention was directed to the detection of modulated emissions. The onset of strong subharmonic, first harmonic and white noise signals was coincident and occurred at an intensity of 25 W/cm^2 (Fig. 2) which was in excess of those required to produce growth inhibition and even root tip death. Sporadic low amplitude bursts of subharmonic and harmonic were detected below the intensity level for the strong signals but these were of such low amplitude that it has not proved possible to integrate them to investigate whether their occurrence was related to biological damage (20).

Several indirect approaches indicate that the emissions we detect originate in the root rather than elsewhere in the system. An ongoing histological study of treated root tips is designed to investigate this point further. Preliminary results with thermocouples inserted in the roots suggest that our treatments at 50 W/cm^2 could produce temperature rises which may be sufficiently high to damage the root but the temperatures associated with treatments at 4.5 W/cm^2 which produce growth inhibition do not result in thermal damage. This latter result is in agreement with previous reports (21).

At this stage of our work it appears that at high intensities, where white noise, harmonics and subharmonics suggest transient cavitation, growth inhibition and root tip death occur. The temperatures during some treatments may be high enough to produce thermal damage. At lower intensities, around 4.5 W/cm^2, growth inhibition is observed in the absence of strong emissions under conditions where thermal effects can be disregarded. These effects may arise from the intracellular streaming observed near gas spaces in root tip sections at intensities below those required for subharmonic emission (19). If such is the case then emissions from nonlinear motions of such gas spaces are not readily detectable. The consequences,for the induction of intercellular motion, of having free gas available in plant tissue prior to treatment, compared with a situation which might exist in mammalian tissue where bubbles would need to grow from nuclei, have recently been discussed by Miller (22).

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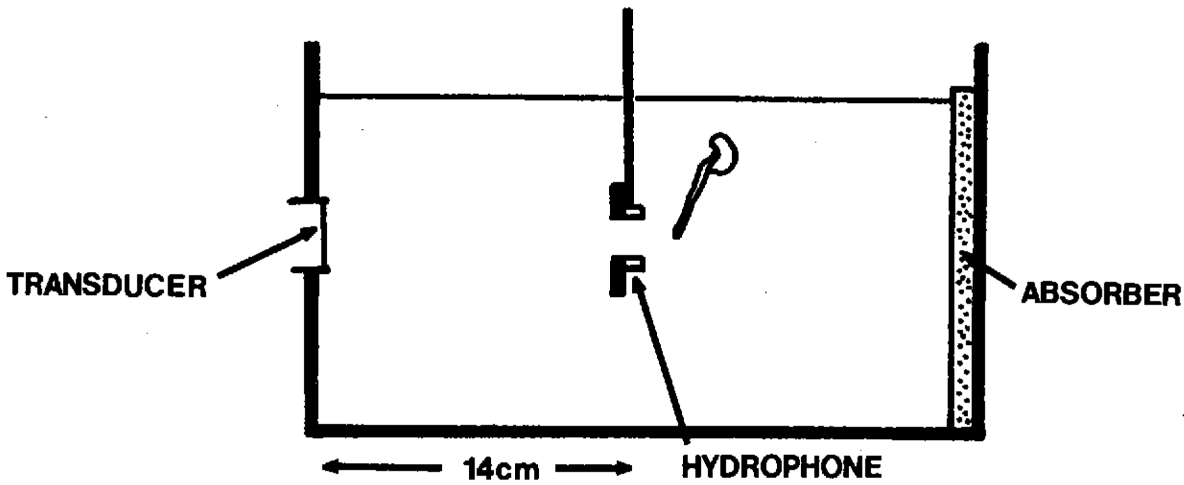

Fig. 1. Treatment of a root at the last near field intensity maximum. The ring hypophone was 5 mm from the tip.

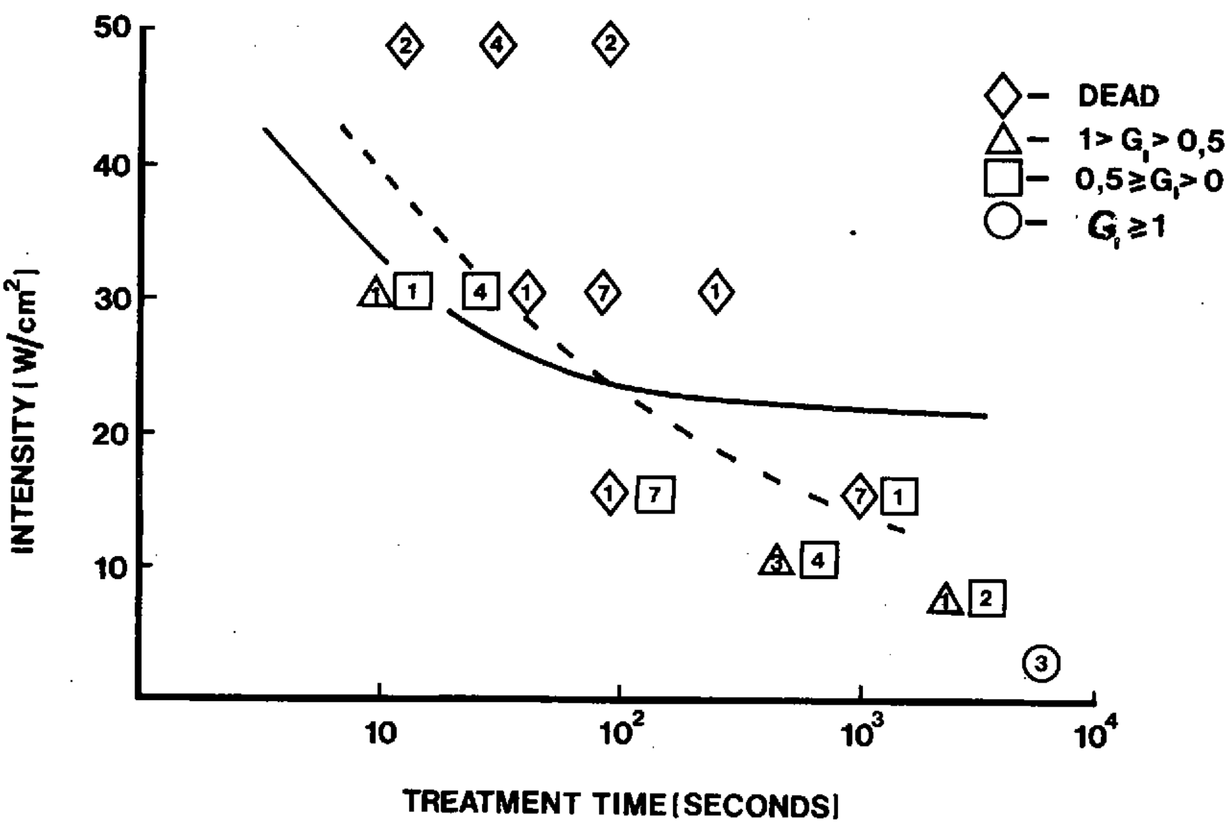

Fig. 2. Effect of sonication on G_1 (first day growth as a fraction of the average control value). The dashed curve separates lethal and non-lethal regimes. The continuous curve separates the regions of strong subharmonic from its absence. The number within the symbol is the number of results obtained.

DISCUSSION

Professor Nyborg said that he had carried out experiments using Elora leaves. In these leaves, free gas can be detected between the cells. If these leaves were irradiated at intensities as high as 25 watts per cm^2 (the threshold for subharmonic observed by the Authors) the gas would come out, but no subharmonic was detected. Dr.Coakley said he was unsure whether the subharmonic that they detected was coming from the bean-roots themselves or from the water surrounding them.