tained under field conditions.[2,3] Tetrachloro-para-benzoquinone serves in the dual capacity of seed protectant and growth stimulant when applied to pea seed. Like any other good seed protectant it can increase the yield under severe disease conditions by preventing seed destruction. In addition, it can stimulate plant growth even in the absence of disease. From a practical view-point this is tremendously important. Heretofore, seed treatment has been recommended as a form of crop insurance against disease because it is needed in one of every three or four fields. As a growth stimulant, the material should pay dividends in practically every field, irrespective of disease conditions. It is of interest to note that the need for such a dual treatment has been previously recognized, and attempts have been made[4] to secure it by using a mixture of a fungicide and various plant stimulants.

It is not known how many species of plants will give the same response to tetrachloro-para-benzoquinone as peas. There is good circumstantial evidence from field observations that lima beans[5] and sweet potatoes[6] may be stimulated, but positive proof has not yet been provided. These field tests and the experiments described herein were made with the commercial product sold under the trade-name of Spergon, which contains one per cent. of a buffering agent. It has been shown that the growth stimulation of peas is dependent on the fungicide and not the buffering compound.

These observations on growth stimulation are of considerable fundamental significance, since they reveal a promising new field of study into fungicides. Further study is needed on the various halogenated members of the quinone series to see if they have properties similar to tetrachloro-para-benzoquinone and whether the growth stimulation and fungicidal properties are dependent upon one and the same chemical grouping within the compound. There is an outside possibility that the growth stimulation comes from decomposition products of tetrachloro-para-benzoquinone, since it is relatively unstable in alkaline media such as some of the soils used in these tests. Regardless of the exact relationship of chemical structure to biological responses, these observations point to a field of study in which both plant pathologists and plant physiologists are vitally concerned.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE BIOLOGICAL APPLICATION OF FOCUSED ULTRASONIC WAVES

HIGH intensity ultrasound above a frequency of 500 kilocycles can best be produced through driving a quartz crystal to expand and contract along its x-axis by an alternating current which is timed to the same frequency of the crystal. The lethal effects of such supersonic waves were first carefully studied by Harvey and Loomis[1] 14 years ago. They used a 50-kilowatt power source and produced rapid death of bacteria and red blood cells in suspensions and of frogs. Since that time many biological and industrial applications of supersonic waves have been found, such as the sterilization of solutions, the homogenizing of oil-aqueous mixtures and of molten metals which would otherwise be immiscible.[2] In all these applications the sound waves have been allowed to proceed in parallel paths from the flat surface of the quartz crystal generator and no effort has been made to increase their intensity and local effect by bringing them to a focus. The authors' studies have utilized the curved quartz crystal method of Greutzmacher,[3] developed in his physical laboratory, and applied it with various improvements and adaptations to the production of focal changes in non-living as well as in living biological material.

The apparatus is essentially an 835 k.c. radio transmitter which provides the radio frequency current for driving the focusing crystal in the ultrasound generator. Ordinary 110 v. 60-cycle current is transformed, rectified and fed into the plate circuit of the first or oscillating stage of the radio frequency generator. This is kept stable, and all tuning is eliminated by introducing into the circuit a master control crystal which has been ground to exactly the same natural frequency as the ultrasound crystal, namely, 835 k.c.[4] After passing through a final amplifying

[1] E. N. Harvey and A. L. Loomis, *Biol. Bull. Marine Biol. Lab.*, 55: 459, 1928.
[2] Ludwig Bergmann, "Ultrasonics and Their Scientific and Technical Applications," p. 264. Translated by H. Stafford Hatfield. New York: Wiley. 1939.
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[4] This circuit was designed by A. E. Miller, Piezo-electric Laboratories, North Bergen, N. J.

stage, the radio frequency current is fed through condensers into the ultrasound generator.

The generator consists of the crystal and its holder housed in a transparent bakelite container which is filled with transformer oil kept cool by water circulating through copper coils. The entire generator is attached to a universal joint on the end of a flexible extension arm which permits its safe and ready application to living biological material. The design of the crystal holder permits maximum freedom from dampening and torsion effects, the maintenance of an oil-tight air chamber behind the crystal with doubling of ultrasound output, and finally, it permits the operator to vary the position of both crystal and holder with respect to the container and so vary the depth of application of the focused ultrasound. The focused beam of sound passes out of the generator through a Cellophane diaphragm and into any living or dead specimen which has been applied to its outer surface.

RESULTS

Propulsion of oil: The activity of the focused ultrasonic waves was made visible by floating a film of oil on the surface of the diaphragm. When the current was turned on, the oil was driven upward in a conical column, its height being roughly proportional to the radio frequency current applied to the crystal. A large number of experiments of this nature were performed with single curved crystals and mosaics, mounted so as to focus on a common point.

Paraffin wax blocks: Preservation of the conical effect in solid form was obtained by placing cubes of paraffin wax or beeswax of known melting point on the diaphragm over the point of focus. Perfect cones were melted out of the wax blocks if low intensity current was used over a long period of time. The authors were naturally interested in obtaining a maximum melting zone at the point of focus with a minimum effect at the base, since the focal effect would be much more useful biologically. This was obtained by utilizing an ultrasonic beam of maximum intensity applied for a few seconds only.

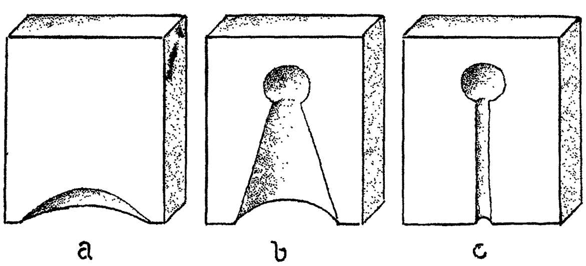

FIG. 1. Melting defects in paraffin blocks produced by focused ultrasound. (a) Low intensity for 30 seconds; (b) medium intensity for 30 seconds; (c) high intensity for 10 seconds.

Animal tissues: Blocks of fresh animal tissue were also treated, and liver was found to give the best results because the heated portion "cooked" whitish-brown, leaving the rest of the liver a deep maroon color. On sectioning, it was easy to see with the naked eye not only the area of basal absorption where the liver was in contact with the diaphragm, but also the more deeply situated focal region of high ultrasonic concentration. Examination of sections under a microscope confirmed the effect of focal destruction of cells.

Living animals: The production of lesions in living animals is made more difficult by the fact that circulating blood absorbs and disperses ultrasonic waves to some extent. The brain of the dog was chosen for the experiment because any destructive effects would be apparent by disturbed motor activity as soon as the animal was allowed to come out from under the anesthetic. In spite of the probability that the focus was not sharp because of refraction from the solid bone and tissue phases of the head, positive results were obtained. By the transcranial application of focused ultrasound to the appropriate areas of the cerebral cortex, disturbances in muscular coordination, some paralysis and in one case blindness were obtained in association with corresponding gross and microscopic brain lesions. Unfortunately, there was always a necrosis of the scalp where the apparatus was applied.

CONCLUSIONS

An apparatus for the production of focused ultrasound has been constructed and applied to biological material. Focal lesions were produced in fresh tissue and living animals.

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