INTERACTION OF MICROWAVES AND GERMINATING SEEDS

DISSERTATION

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By

Floyd L. Shafer, M.S.
Denton, Texas
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This investigation was concerned with determining the interaction of microwaves with germinating seeds. This study covers two different approaches. The preliminary efforts covered the response of germinating seeds to treatment by microwaves and heat. The second phase of the investigation used microwaves as a probe to determine some of the processes of early seed germination.

The preliminary investigation measured the internal metabolic process by ATP production. Leakage of ions and organic material from germinating seeds indicated that membranes are a target of microwaves and heat. Electron photo-micrographs showed an increase in damage to membranes as heat and microwave treatments were increased.

The second phase of this investigation was concerned with determining some of the biological activity at the initiation of germination of wheat seeds, *Triticum aestivum* L., using a resonating microwave cavity oscillating at 9.3 GHz as a probe. Direct current conductivity measurements were also made on the seeds as a means of confirming the observations made with the
There was no observable difference between treatment by UHF or heat in the ultrastructure of germinating seeds. A dielectric response far above that of free water was found as live seeds of wheat began to imbibe water. This effect was assumed to be due to the release of ions, because conductivity increased as corresponding quantities of water were imbibed; and both conductivity and dielectric response decreased as imbibition progressed. Although dead seeds also imbibe water, they do not show this decrease in dielectric response. The dielectric response of live seeds was reduced after the initial imbibition, showing that water became tightly bound as imbibition progressed.

The dielectric response of actively growing shoots from the seeds was much less than that of the seeds themselves. The large quantity of water in the shoots is assumed to be immobile, being tightly bound within the membranes, enzymes, and organelles.
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CHAPTER I

INTRODUCTION

This study grew out of an interest in finding a herbicide that would leave no toxic residue. A pollution-conscious society has called for a reevaluation of the methods by which weeds are controlled. The use of microwaves is one of the innovative approaches to this problem.

Olsin found that irradiating the soil with microwaves at 2.45 GHz was lethal to certain weed seeds (1). This is the frequency used in the standard microwave oven. If the mechanism of action of microwaves on seeds could be determined, perhaps a much more effective means could be developed for using microwaves to control weeds in agriculture crops. A prototype machine has been developed to carry a microwave emitter into a field for weed control.

Electromagnetic energy is divided into several types, such as alpha-rays, X-rays, ultraviolet, visible light, infrared radiation, and radio waves (2). All are the same type of energy and differ only in the frequency and wave length. They all travel at the speed of light \(3 \times 10^8\) cm per second.

This work is limited to portions of the radio wave
spectrum. These wave frequencies are encountered in radio, television, radar, and microwave ovens. The frequencies range from $5 \times 10^6$ Hz, or cycles per second, to $1 \times 10^9$ Hz.

The present study deals with a two-phase investigation of germinating seeds. The initial work addressed the effects of potentially lethal doses of microwaves on germinating seeds and on the cells of germinating seeds and seedlings. In the second phase, microwaves were used as a probe to study germinating wheat seeds. To do so, a loaded cavity was employed which resonated at a frequency of $9.3$ GHz ($9.3 \times 10^9$ Hz) or a wave length of 3.3 cm.

Many uses have been found for microwaves besides those indicated above. Norris and Bryant (3) found that microwaves are useful for detecting biological contamination of eggs. Microwaves can be used for killing insects in grain (4, 5), but the method is more expensive than using chemicals to kill insects (6). The dielectric response of seeds is a non-destructive means of measuring moisture content in grain and other biological substances when moisture content is 10% - 25% wet weight (7, 8, 9, 10, 11). Microwave heating enhances the germination of certain seeds (12, 13, 14). This same treatment, also called dielectric heating, has been used experimentally to treat tumors in humans (15), and to increase the geological recovery of fossil fuels (16). Radio waves have been used to locate underground sources of fossil fuels (17).
Microwaves (26 to 40 GHz) are currently being used in the microwave spectroscopy laboratory as an analytical tool to determine the behavioral mode of gas molecules (18). They are also used to determine the behavior of water molecules with solutes, surfaces, and membranes (19, 20, 21, 22).

The behavior of water molecules in a microwave field is the focus of the second phase of this investigation. This phase does not address the biological changes due to microwaves but reports only the use of microwaves to indicate structural changes in water and ions as wheat seeds germinate. The water and ions of the germinating seed changes the frequency and the damping of microwaves in a resonating cavity and measurements were made only on the microwaves. This is a very sensitive method of measuring how water and ions behave during germination of wheat seeds.

In the first phase of this investigation, many experiments were carried out to determine whether there was a difference in the response of germinating seeds to heat and microwaves. At the same time efforts were made to target the interaction mechanism of each of these treatments.

The water medium in which the seeds were germinating was investigated for possible differences in heat and microwave treatments. The water medium was investigated for proteins, ions, and nitrites during germination. An
increased leakage of ions was found in the germinating medium after treatment. This indicated that the permeability of membranes was probably affected by heat and UHF exposure. Membrane permeability changes have been reported in treatments of plants with pathological organisms (23, 24, 25, 26, 27). The membrane-bound organelles of cells seem to be sensitive to foreign invasion such as bacterial toxins. Membrane permeability changes have been indicated by leakage of components from the cytoplasm of cells and by morphological changes in the ultrastructure of the organelles (23, 24, 25). Attempts have been made to correlate these physiological changes with ultrastructure changes as seen by the electron microscope. The permeability changes of the plasmalemma have not always been reflected in ultrastructure changes (25). The problem of relating membrane permeability to ultrastructure changes has been addressed in this study.

Microwaves are frequently referred to in the literature as ultra high frequency radio waves (UHF). The debate has ranged for many years as to whether there is a discernable difference between the effects of microwave and heat on biological material. No difference was found in the denaturation of a molecule of DNA when treated with heat or UHF (28). The enzyme peroxidase when exposed to UHF was not inactivated when the sample temperature was controlled constantly at 25° C. during exposure. However, the enzyme
could be inactivated by UHF exposure, allowing the temperature to increase due to the microwaves (29).

Michaelson (30) suggested that microwaves affect biological organisms only by the conversion of the absorbed energy into heat, and proposes that this is the mechanism by which cells are damaged. Rice et al. (31) reported no discernable morphological damage to plant tissue exposed to lethal dosage of UHF as observed in the transmission electron microscope. However, it was not indicated by them whether the tissue was from dormant seeds or germinating seedlings.

Wheat seed were finally chosen over millet seeds because wheat seeds had a more uniform germination percent and also germinated within 48 hours after placing in water. They were also small enough to be contained in petri dishes and be handled easily without damage. Observations of ultrastructure in electron micrographs were used as a means of identifying possible differences in treatment by UHF and heat.

To understand the second phase of this study requires a definition of some of the terms used to express the interaction of biological material with microwaves. The response of a substance to a microwave field is measured by the real and imaginary part of the complex relative permittivity or resistance. The real part is called the dielectric constant, $\varepsilon'$, or infrequently, $K'$. The
imaginary part is called the dielectric loss factor, \( \varepsilon'' \) or, \( K'' \), and is sometimes referred to as the damping term.

The dielectric constant of a material is the ratio of the capacitance of a capacitor with the material as a dielectric to that of the same capacitor with a vacuum as a dielectric. In general terms this is a measure of the ability of a material to store electrical energy. The dielectric constant indicates the ability of the dipole moments of the molecules in the material of interest to respond to an applied microwave field. The principal interaction between an applied microwave field and biological substances containing water and organic molecules is through the dipole moment of these materials. The dielectric constant is large when the dipole molecule is able to resonate with the applied microwave field (22).

The dielectric constant can be pictured as a swinging pendulum as the energy source. Adjacent to it is hanging another pendulum designated as the recipient. The recipient pendulum will start to swing to some harmonic of the first pendulum. If the natural swinging frequency of the recipient pendulum is very close to the frequency of the first pendulum, more energy is stored in the recipient pendulum and it swings in harmony with the first pendulum. This illustrates a high dielectric constant. If the natural swinging frequency of the recipient pendulum is radically different from the first pendulum, energy is
lost, and the recipient pendulum does not store much energy, and the dielectric constant would be low.

The dielectric loss factor is a measure of the microwave energy absorbed by a material from the microwave field. Dielectric loss factor is low when the relaxation time for the dipole moment is longest. Under these circumstances little energy is absorbed from the electric field. The relaxation time is the period of time for the dipoles in the electrical field to return to random orientation (22). Organic molecules in solution generally change the relaxation frequency of the water dipoles, thus reducing the dielectric loss factor of the solution and absorbing little energy. This results from stabilization of the dipoles of water which are not free to oscillate with the applied field. The stronger the binding force between water and other molecules in solution, the smaller the dielectric constant and the dielectric loss factor (32, 33).

The dielectric loss factor can be pictured as a wheat field waving in the wind, the wind being the source of energy and the waving grain as the recipient water molecules. The relaxation time of the wheat stalks is long as they return to normal orientation. In this case not much energy is absorbed. This represents a low dielectric loss factor. If the field of grain was cut, the straw, random and blowing with the wind, would receive more of the
energy from the wind. The relaxation time would be very short, thereby representing a much higher dielectric loss factor.

From the literature, it appears that the overwhelming effect of the absorption of microwave energy in biological material is thermal (17). However, despite all the vast information concerning microwaves and their influence on the water molecule of biological material, recent experiments done in West Germany indicate nonthermal effects on the growth of yeast cells exposed to 42 GHz. This would suggest an unknown system that in a yeast cell resonates at an abnormally high frequency (34).

Ionic loss is also a factor in the response of a microwave field to a substance. Roebuck and Godblith (22) have attributed this loss to the breaking of hydrogen bonds in water, thus decreasing the relaxation time of the water molecule and increasing the dielectric constant and the dielectric loss factor. This action loosens the water structure and allows the water molecule to respond to the applied microwave field.

Organic molecules in water form hydrogen-bonding sites, thus producing a type of bound water or structured water surrounding the organic molecule and reducing the mobility of the water molecule adjacent to the organic molecule (21,22). In a microwave field, the dipole moment of bound water is restricted, resulting in a decrease in
the dielectric constant and dielectric loss factor. As more free water is absorbed in biological material, the dielectric constant and the dielectric loss factor are increased because there are more polarized dipoles to respond to the microwave field.

The dielectric constant of free or bulk water is generally accepted as 80 at 400 MHz at room temperature (35), but at a thickness of 7x10^{-6} cm it was found to be 4.5 (35). This would indicate that water adjacent to surface may be different from free water. Another means of studying the property of water in close association with a surface is the disjoining pressure. This method measures the increase in pressure required to press two smooth surfaces of mica as they approach one another with water in between. There is a great increase in pressure required as the two surfaces are moved closer than 5x10^{-6} cm together. This also indicates that water in the proximity of a surface is different from what it is elsewhere (35).

One study of thin-layered water in close proximity to a surface measured the dielectric response of setting concrete (36). Although the quantity of water reduces slightly in evaporation during the early hydration period, the dielectric constant increases. Reboul attributed this to the paste becoming more conductive, with an increase in the number of ions in solution.

In a microwave study of water in starch at 1 GHz and 3
GHz frequency, the dielectric constant was shown to increase as the water mass increased. However, the dielectric loss factor increased to a maximum, then decreased as the mass of water approached 100%. Roebuck and Goddblith suggested the possibility of the influence of ionic conductivity, but made no conductivity measurements (22).

An experiment monitoring the dielectric response of freeze dried potato samples to a 3 GHz field indicated that at very low water content (less than 10%) the dielectric constant and the dielectric loss factor were very low. This indicated that the water was tightly bound to the large starch molecule. As the moisture increased to 35%, the dielectric constant and the dielectric loss factor increased rapidly due to ionization of bound salt in the free water. As the free water increased above 35% the salts were diluted and the dielectric loss factor attained a maximum at about 50% water. As the volume of water increased from 50% to 100%, the dielectric loss factor reduced to that of free water (37). This also indicates that ions in solution have an effect on the dielectric response of biological samples.

A dielectric study was made of the absorption of microwave energy in the protein crystal in the lens of the human eye (19). This experiment demonstrated a greater absorption of microwaves than free water. The study was
developed by a theoretical model of a large protein molecule surrounded by bound water and free water containing different ionic conductivities. McClean et al. (19) proposed that at low ionic conductivity there was an increase in microwave energy absorbed. My study confirms this.

**Loss tangent** is generally expressed by the symbol $\tan \delta = \frac{\varepsilon''}{\varepsilon'}$. $\tan \delta$, like $\varepsilon''$, indicates loss of energy or dissipative character of a material for microwave energy (8, 9).

The second phase of this work reports the dielectric response of a single live wheat seed as it imbibes water. Comparisons were made with the dielectric response of a dead wheat seed as it imbibes water and of live wheat shoots.

The dielectric response of a germinating wheat seed was measured in a perturbation cavity resonating at a frequency of 9.3 GHz. For comparison, direct current conductivity was also measured on live wheat seeds as they imbibed water under the same conditions as those monitored in the perturbation cavity. The live wheat seeds were in the process of germination as each seed imbibed water. The shoots were excised from the germinating seeds and their dielectric response measured immediately. The dead seeds were allowed to imbibe water, and their dielectric response was measured at varied water levels.
CHAPTER BIBLIOGRAPHY


CHAPTER II

MATERIAL AND METHODS

Millet seeds, *Setseria italica*, and certified seeds of Coker 68-15 soft red winter wheat, *Triticum aestivum* L., grown in Texas were donated by a local seed supply house (Harpool Seed Inc.). The wheat seeds were treated with a fungicide by the supply house. The millet seeds were treated with a 5% solution of Clorox for 5 minutes to control fungi. Fifty millet seeds or 25 wheat seeds were allowed to germinate on sterile 9-cm petri dishes between two Whatman No. 1 filter papers, moistened with 10 ml of laboratory deionized water. The petri dishes with covers were placed in a dark growth chamber at 27°C. for germination and left there until time for treatment.

The source of UHF irradiation was a Model PPS 2.5AS Power Pack (Varian Industrial Microwave Operation, San Carlos, California). This machine uses a magnetron tube to deliver 1 KW of power at 2.45 Ghz to a small oven connected to the magnetron by a wave guide. The oven was equipped with a standard motor-driven rotating platform and rotating disc for exposing samples to UHF radiation. This method of exposing the samples in the oven dispersed the energy to provide a uniform distribution of the electromagnetic
field.

After germination, the seedlings were blotted dry with paper towels and placed in sterile, dry petri dishes containing filter paper. The uncovered petri dishes containing the samples were immediately exposed to a UHF chamber for a duration of 20, 40, or 70 seconds. After irradiation, 10 ml of deionized water was added to the petri dishes.

Seedlings to be heat treated were placed in small sacks of cheesecloth. These were hung in a beaker of deionized water under continuous agitation with a magnetic stirrer set at one-fourth speed and held at a constant temperature of 55° C. for 70 seconds. Immediately after the timed exposure the samples were dropped into a beaker of deionized water at room temperature.

In the direct current conductivity experiment the control and treated samples were placed in small vials containing 10 ml of deionized water. The capped vials were placed in a test tube holder for a circular fraction collector which turned on a nearly horizontal axis. This nearly vertical wheel rotated at six revolutions per minute. By this means the samples were gently "washed" overnight. Measurements were made on a conductivity instrument No. 451 Model 33, Yellow Springs Instrument Co., Yellow Springs, Ohio.

For the experiment on ATP production the seeds were
obtained untreated with fungicide so as not to affect the test. The samples containing 25 seeds were allowed to germinate from 1 to 24 hours before being exposed to UHF or heat by the method used above. After treatment, the samples were crushed in boiling Tris to extract ATP. The quantity of ATP was measured in a photometer, using a crude luciferin enzyme injected into the samples. The photometer was standardized by using known quantities of ATP.

The control and treated samples for the electron photo-micrographs were placed in 0.4 M sodium cacodylate buffer (pH 7.3), where the shoots were cut to approximately 5-mm lengths. The specimens were then fixed for 24 hours in 2% glutaraldehyde dissolved in 0.2 M cacodylate buffer (pH 7.3). This was followed by two rinses in 0.4 M cacodylate buffer for one hour each. Specimens were then postfixed for one hour with 2% osmium tetroxide dissolved in deionized water. Again the specimens were rinsed in 0.4M cacodylate buffer. All fixing and rinsing was done in small vials gently rotated in a small vertical wheel to enhance impregnation of the solution. Dehydration of the sample was done by rinsing for 5 minutes each in 10%, 20%, 40%, 60%, 80%, 95%, and then 100% ethanol. The samples were then washed twice in propylene oxide. Embedding was done by rinsing each sample for 5 minutes in 10%, 20%, 40%, 60%, and 80% epon dissolved in propylene oxide. The samples were finally placed in 100% epon for one hour.
Then the specimens were mounted in flat, open molds with epon so that shoots could be orientated to cut cross-sections with the ultramicrotome. Polymerization of the epon was done in a 60 C. oven for 48 hours. Silver-gray (90 angstroms) sections of all specimens were cut on a Sorvall Ultramicrotome, using a diamond knife. Sections were mounted on 300 mesh copper grids. They were then post-stained for 5 minutes with uranyl acetate dissolved in 50% ethanol followed by lead citrate for 5 minutes (1). The sections were then observed in an RCA transmission electron microscope at 50 KV.

For the second phase of this work wheat, seeds were obtained untreated with fungicide to prevent any interference this material might contribute to the experiment. The seeds were placed in covered petri dishes as indicated above, but germination took place at room temperature in cabinet drawers.

Dead seeds were produced from the same source as live seeds by immersing in boiling water for 5 seconds. A sample from each batch of dead seeds was placed in petri dishes with wet filter paper to test for germination. No test seeds germinated.

Shoots were excised from germinating seeds after they were from 1 to 3 cm long. All samples were held in the end of an open nylon sample tube by friction and suspended in a perturbation cavity resonating at 9.3 GHz (Fig.1).
Figure 1. Diagram of resonant cavity and micrometer drive mechanism.
The water vial was made from the same size nylon tubing used above; however, the bottom end was closed with a .5-mm plug of wax.

Measurements were made with the microwave instrument (Fig.2) containing the perturbation cavity. The shift in the frequency of the resonating cavity with the seed in place was obtained on the oscilloscope (Fig 3). The energy absorbed by the imbibed seed in the cavity was measured by the change in resonant frequency and width at half-power of the wave absorption in the cavity. The half-power condition was determined by the change in the width [designated by the symbol Δ (1/Q)] of the "butterfly" on the oscilloscope trace (Fig. 3). The "butterfly" is the first derivative of the fundamental frequency wave of the cavity absorption.

The experimental set-up is designed to measure both the real and imaginary terms of the material's dielectric response ε(ƒ) as the sample of material perturbs the resonant cavity. Once the dielectric constant of the material is determined, its susceptibility χ(ƒ), which is a measure of its electrical properties, can easily be deduced by using the standard equation

$$ε(ƒ) = 1 + 4πχ(ƒ).$$  \hspace{1cm} (1)

Both the real and imaginary parts of χ(ƒ) can be
Figure 3. Dual trace oscilloscope scan of the first derivative of the cavity absorption profile with frequency markers on second trace.
obtained by utilizing suitable equations for perturbation of resonant cavity. A number of approaches to this technique of perturbed cavities can be found in the literature, but the comprehensive perturbation equation shown below was used in this experiment (2):

\[
\frac{\Delta(1/Q) - 2j(\Delta f/f)}{\int_0^\infty \vec{E} \cdot \vec{E}_0 \, dv} = j[(\varepsilon' - 1) - j\varepsilon''] \frac{\int_0^\infty \vec{E} \cdot \vec{E}_0 \, dv}{\int_0^\infty \vec{E}_0 \cdot \vec{E}_0 \, dv} \tag{2}
\]

The symbols \(\Delta(1/Q)\) is Q-change and \(\Delta f\) is frequency shift from the resonant frequency \(f\) of a cavity of volume \(V\) when it is perturbed by a sample material of dielectric constant \(\varepsilon\), and volume \(v\). The real and imaginary parts of susceptibility are \(\varepsilon'\) and \(\varepsilon''\). The induced electric field within the material sample is \(\varepsilon \varepsilon \hat{E}\), and \(E_0\) is the unperturbed field for the resonant cavity. The denominator of equation (2) gives the energy stored in the cavity volume \(V\) per cycle.

Wheat seeds and seedlings are non-magnetic or weakly magnetic; so the perturbation of the magnetic field \(H\) within the cavity is not considered. It is assumed that the sample volume \(v\) is very small compared to the cavity volume \(V\), and the perturbation of the fields due to the sample materials is small, so that \(\hat{E}\) is very nearly equal to \(E_0\).

Equating real and imaginary parts of equation (2)
gives the cavity perturbation equations in a form suitable to our investigations, as follows:

\[ \Delta f = \frac{-\varepsilon' + 1}{2} \frac{\int V \hat{E} \cdot \hat{E}_0 \, dv}{\int V \hat{E}_0 \cdot \hat{E}_0 \, dV} \]  
(3)

\[ \Delta (1/Q) = \varepsilon'' \frac{\int V \hat{E} \cdot \hat{E}_0 \, dv}{\int V \hat{E}_0 \cdot \hat{E}_0 \, dV} \]  
(4)

The two parameters \( \Delta f \) and \( \Delta (1/Q) \) can be measured as the resonant cavity is perturbed by a controlled volume of the sample.

The simplified form of the equation holds for non-magnetically active samples. It is clear from the equations that a given sample of known volume can be used to calibrate the instruments for \( f \) and \( 1/Q \), with relative changes measured from this point.

Equations (3) and (4) may be simplified further to a more usable form as follows:

\[ \frac{\Delta f}{\Delta m} = B = f_0 \frac{(\varepsilon' - 1)}{2 \int \frac{F(E)}{F(E)} \, dV} \]  
(5)

and

\[ \frac{\Delta (1/Q)}{\Delta m} = D = f_0 \varepsilon'' F(E) \]  
(6)
where $F(E)$ is a functional form of how the field interacts with the sample in changing frequency of resonance and Q-factor for the cavity.

If $F(E)$ is of the same form for all the samples, there arises a characteristic $B_x$ and $D_x$ for each sample $X$, and if $B_s$ and $D_s$ are corresponding values for a chosen standard, we can express relative values for a dielectric material as follows:

$$\frac{B_s}{B_x} = \frac{(\varepsilon_x' - 1)}{(\varepsilon_s' - 1)} \quad (7)$$

and

$$\frac{D_s}{D_x} = \frac{\varepsilon_s''}{\varepsilon_x''} \quad (8)$$

Thus, once $B_x$, $B_s$, $D_x$, and $D_s$ are experimentally determined, the dielectric response in terms of $\varepsilon_x'$ and $\varepsilon_x''$ can be deduced using standard values of the $\varepsilon_s'$ and the $\varepsilon_s''$.

The microwave instrument (Fig. 2) consisted of a tuneable X-band wave guide with a klystron at one end to supply microwave energy at 9.3 GHz. Attached to the wave guide was a tuneable cavity with a small iris on top to admit a nylon sample holder containing the sample of interest (Fig. 1). The wave guide contained an attenuator to control the power from the klystron. The frequency of
the klystron was caused to sweep across the central
frequency of 9.3 GHz by a few MHz with the sweep voltage
from the oscilloscope. The sweep voltage is also called a
ramp voltage because it gradually increases then suddenly
drops as the oscilloscope makes another sweep. This sweep
voltage was "chopped" by a 31-KHz square-wave signal which
gave the first derivative of the fundamental absorption
peak of the cavity. The ensuing "butterfly" display
shifted in frequency and changed in width as the cavity was
perturbed (Fig. 3). In order to measure accurately the
frequency shift of the perturbed cavity, a marker mixer
circuit was employed to produce a harmonic-wave of the
fundamental frequency of the klystron. This was monitored
with a radio receiver. The output of the radio receiver
produced two sharp vertical lines on the second trace of
the dual trace oscilloscope as the klystron was swept by a
few MHz in frequency (Fig. 1). A variable marker was
produced from another signal generator connected to the
marker mixer circuit. This was tuned to a harmonic of the
center frequency and was variable over a narrow range of
frequencies. The variable marker was superimposed as a
sharp vertical line on the second trace of the
oscilloscope. The variable marker was moved manually to
the center of the "butterfly" to measure the change in
frequency of resonance of the cavity and then moved to the
turning points of the "butterfly" to measure the cavity
resonance width at half-power points. These measurements were designated $\Delta F$ and $\Delta(1/Q)$ respectively. The amount of frequency shift was determined on the empty and loaded cavity and read on the vernier scale of the signal generator.

The water contained in the seeds and shoots was determined by weighing each sample as it was removed from the microwave cavity. The samples were then dried at 105° C. for 48 hours and reweighed. Perturbation determinations were made on measured quantities of deionized water using a nylon vial of the same size as the seed sample holder. In the Introduction the dielectric constant of free water was reported to be 80 at 400 Khz; however, at 9.3 GHz and room temperature, the dielectric constant is 64.5 and the dielectric loss factor is 27 (3). The relative perturbation of measured quantities of deionized water and the equivalent quantity of water in the samples was used to calculate the dielectric constant and the dielectric loss factor of seeds and shoots.

The conductivity apparatus (Fig. 4) consisted of a small test tube 2 cm x 15 cm with silver plates separated by 90 wheat seeds. These seeds were continually "washed" with deionized water at 120 ml per minute. A continuous flow of water from the bottom of the test tube to the overflow at the top was employed to measure only the conductivity of the seeds and not the ions extracted from
Figure 4. Diagram of the d.c. conductivity measuring apparatus.
the seeds. This enabled the conductivity measurements to be made of the seeds in approximately the same condition as those taken from the petri dishes for cavity perturbation measurements. The silver plates were connected to a direct current conductivity instrument, described earlier.

The conductivity was measured at timed intervals as the live seeds imbibed water from the continuous flow. Measurements were continued 48 hours. Moisture content of the seeds was determined by weighing 50 seeds that had imbibed water for the same period of time that had elapsed as the conductivity measurements were made. The seeds were then dried for 48 hours at 105° C.

In an effort to obtain the optimum size sample vial for use in the resonating cavity, some rather interesting observations were made for which there seemed to be no explanation. The loss tangent (\(\tan\delta\)) indicates the ability of the sample to dissipate energy and should be constant for water regardless of small changes in mass, cross sectional area of the sample, or position of the sample above the bottom of the cavity. The loss tangent \((\tan\delta)\) may be expressed by

\[
\tan \delta = \frac{3 \Delta (1/Q)}{2 \Delta F}
\]

(4). This, of course, eliminates any relative volume of sample to cavity, cross sectional area of the sample, or height of the sample to height of cavity. Repeated
readings were made of water in different sized vials. Equation (9) gave a value that varied directly with the cross sectional area of the sample vial (Fig. 5). From these data, the small vial with cross-sectional area of 1.724 mm$^2$ was used to take all the readings for water because the calculated loss tangent was (.60), which was nearest to the loss tangent found in the literature of (.42) (Fig. 5). This size vial gave a nearly uniform loss tangent as the volume of water increased in the vial. However, using a larger vial of the same material, Teflon, 8 mm$^2$ in cross sectional area, the calculated loss tangent varied directly as the volume of water increased in the vial (Fig. 5). For the same volume of water in large and small sample vials, the level was much higher in the small vial than in the large vial. This raised the question that perhaps the position of a small volume of water above the floor of the cavity would give a more nearly reproducible value for the loss tangent. The calculated loss tangent value was measured as 5 microliters of water was raised in fixed increments above the bottom of the cavity. The measured loss tangent varied inversely with the distance above the bottom of the cavity (Fig. 6). Although increases in the level of water as the quantity increased in the small sample vial caused practically no change in the calculated loss tangent (Fig. 5) a small mass of water in the sample vial at measured intervals above the center
Figure 5. Loss tangent of water vs water volume for two different radii sample holders.
Figure 6. Loss tangent of water vs position of vial in perturbation cavity.
of the cavity caused the measured loss tangent to decrease substantially (Fig. 6).

In order to compensate for variations of field geometry in the cavity, all samples were placed in the same position using a micrometer drive and sample holder. The resonant cavity and drive mechanism are shown in (Fig. 3)

The data collected on all samples were fitted to 3rd degree polynomials. The following equations were used for each set of data. The symbol X was used to represent microliters of water.

Live seeds $\Delta F = 0.0527 + 0.0860X - 0.00324X^2 + 0.00078X^3$

Live seeds $\Delta 1/Q = 0.0437 + 0.0237X + 0.00148X^2 - 0.00008X^3$

Shoots $\Delta F = 0.0312 - 0.0077X + 0.00275X^2 - 0.000024X^3$

Shoots $\Delta 1/Q = 0.0255 - 0.0160X + 0.00232X^2 - 0.000024X^3$
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CHAPTER III
RESULTS AND DISCUSSION

In the first experiments, air-dried millet seeds, *Seteria italic*a, were exposed to microwave irradiation at 2.45 GHz and tested for germination. Microwave irradiation of dry seeds had very little effect on the number of millet seeds germinated even with 50 minutes of exposure at 1 Kw of power. Millet seeds that had imbibed water for a period of several hours prior to exposure were much more susceptible to the effects of microwave irradiation than were dry seeds (Fig. 7).

Since UHF irradiation caused no damage to dry seeds and lethal damage to seeds that had imbibed water, it followed that perhaps the major effect of microwaves was the heating of water. This led me to try to differentiate between heat and microwave treatments. An attempt was made to determine if hot water had a different effect on germinating millet seeds than exposure to microwaves in an oven. The first step was to establish a germination LD 50 treatment for microwaves and for heat, and to see if there were differences between the two treatments.

Millet seeds that had imbibed water for 16 hours and were irradiated for 25 seconds in the microwave oven had
Figure 7. Foxtail millet seeds exposed to UHF 1 KW power.
the same LD 50 (measured by percent germination) as imbibed seeds subjected to 20 seconds submersion in water at 45°C.

One of the classical effects of heat is to disrupt the integrity of the cell membrane. The conductivity of water was measured in the germinating medium to see if this would indicate any difference between treatment at the same LD 50 by heat or microwaves. Conductivity measurements indicated that there was more leakage of ions from the seeds treated with UHF and heat than from untreated seedlings (Tab. 1), but it failed to distinguish between the two.

The production of ATP was used as a means of determining damage to cell membranes by UHF and heat. In the first experiment the results were somewhat erratic, but consistent enough to suggest that heat at 55°C. is more destructive to ATP production than irradiation with microwaves (Fig. 8). In subsequent experiments consistency in measurements of ATP production, became even more erratic and further efforts in this direction were abandoned.

Damage to the plasmalemma and organelle membranes of wheat seedling cells treated by UHF irradiation has been documented with electron photo-micrographs (1). Membrane damage was expected to be the most likely cause of increased leakage of ions into the germinating medium of UHF or heat-treated wheat seedlings. The data in Tab. 1 indicate that as the exposure to heat or UHF increases, more ions are discharged into the growth medium as measured
### TABLE 1

CONDUCTIVITY MEASUREMENTS OF THE GERMINATING MEDIUM OF UHF IRRADIATED AND HOT WATER TREATED WHEAT SEEDLINGS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conductivity *</th>
<th>Range **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>42</td>
<td>a</td>
</tr>
<tr>
<td>UHF 20 seconds</td>
<td>118</td>
<td>b</td>
</tr>
<tr>
<td>60 C. water 5 seconds</td>
<td>132</td>
<td>b</td>
</tr>
<tr>
<td>UHF 40 seconds</td>
<td>153</td>
<td>b</td>
</tr>
<tr>
<td>60 C. water 10 seconds</td>
<td>154</td>
<td>b</td>
</tr>
<tr>
<td>60 C. water 20 seconds</td>
<td>211</td>
<td>c</td>
</tr>
<tr>
<td>60 C. water 40 seconds</td>
<td>246</td>
<td>c</td>
</tr>
</tbody>
</table>

* average of 5 replications of 10 seedlings.
** Duncan's multiple range test.
Figure 8. ATP in germinating wheat seeds vs. hours imbibed.
by conductivity.

There are factors other than heat that can cause cellular membrane damage. Experimenters working with pathogenic bacteria on plants have found permeability changes in the cellular membrane (2, 3). Ultrastructural changes in plant cells similar to my observations of wheat seedlings have been attributed to pathogenic bacterial infection of plant root and leaf tissue (4, 5, 6). Cook and Staal (2) proposed the S-S bond cleaved in membrane protein tertiary structure as the cause of cellular membrane alteration and permeability changes by pathogenic bacteria.

This study of membrane alteration was started by looking at two electron photo-micrographs of a cross-section of a normal root cell from a two-day-old wheat seedling (Fig. 9, 10). The various organelles are intact. The plasmalemma is appressed to the cell wall and is continuous. The membrane is very thin. The limiting membranes of the nucleus and the mitochondria stand out as double membranes. The endoplasmic reticulum with ribosomes is easily observed. The Golgi apparatus (dictyosomes) with secretory pouches can easily be seen. The electron-dense nucleolus and electron-dense areas of nuclear material stand out clearly. A few single-membrane microbodies may be observed in the cytoplasm. The tonoplast (between the cytoplasm and the vacuole) may be seen as a very thin
Figure 9. Electron photomicrograph of untreated wheat seedlings.
Figure 10. Electron photo-micrograph of untreated wheat seedlings.
membrane. Under high magnification infrequent locations may show a double membrane strand.

Observation of tissue from germinating seedlings exposed to UHF radiation for 40 seconds shows clear evidence of electron lucid breaks in the plasmalemma (Arrow No. 1, Fig. 11). There is also some evidence that this membrane is pulling away from the cell wall (Arrow No. 2). In some cells the plasmalemma has completely disappeared with the appearance of a number of small membrane-bound vesicles (Arrow No. 3). The mitochondrial envelope is partly disorganized but the interior portions are more electron lucid (Arrow No. 4, Figs. 11, 12). Parts of the endoplasmic reticulum are disorganized (Arrow No. 5, Fig. 12), but the ribosomes are still very much intact (Arrow No. 6). The nuclear envelope is disorganized but the electron dense nuclear material within remains unchanged (Arrow No. 7). Golgi apparatus are reduced in number. In one case an intact Golgi was found with the secretory vesicles reduced in number (Arrow No. 8, Fig. 13). It should be noted that there is no evidence of change in the cell wall, nor was any expected.

The germination tests showed that UHF exposure for 70 seconds is lethal to most wheat seedlings. Only 2% to 3% survive. Examination of the ultrastructure indicates serious disruption of the plasmalemma (Figs. 14, 15). There are many more single membrane-bound vesicles (Arrow
Figure 11. Electron photo-micrograph of wheat seedlings irradiated 40 seconds with UHF. Arrow 1 shows lucid breaks in plasmalemma, arrow 2 plasmalemma away from cell wall, arrow 3 membrane-bound vesicles, arrow 4 mitochondrial envelope partly disorganized.
Figure 12. Electron photo-micrograph of wheat seedlings irradiated 40 seconds with UHF. Arrows 4 shows mitochondrial envelope partly disorganized, arrow 5 endoplasmic reticulum disorganized, arrow 6 ribosome intact, arrow 7 nuclear envelop disorganized.
Figure 13. Electron photo-micrograph of wheat seedlings irradiated 40 seconds with UHF. Arrow B shows intact Golgi with secretory vesicle.
Figure 14. Electron photo-micrograph of wheat seedlings irradiated 70 seconds with UHF. Arrow 9 shows single membrane-bound vesicles, arrow 11 partly organized interior of nucleus, arrow 12 expanded nuclear membrane, arrow 13 probably endoplasmid reticulum or Golgi.
Figure 15. Electron photo-micrograph of wheat seedlings irradiated 70 seconds with UHF. Arrow 9 shows membrane-bound vesicles, arrow 10 shows interior of mitochondria mostly organized, arrow 11 interior of nucleus is partly organized, arrow 12 nuclear membrane breaks and expanded, arrow 13 no recognizable endoplasmic reticulum or Golgi.
No. 9, Fig. 14) than are present in cells exposed for 40 seconds (Figs. 11, 12, 13); evidently these form from pieces of the plasmalemma as it breaks up. The interior of the mitochondria is mostly organized; cristae can still be observed (Arrow No. 10, Fig. 15). The interior of the nucleus is partly organized with electron dense material slightly more scattered than in untreated cells (Arrow No. 11, Figs. 14, 15). The nuclear membrane shows breaks and disruptions. Several cells show greatly expanded, though still mostly unbroken, nuclear membranes (Arrow No. 12). The nuclear contents do not appear to expand to fill the space enclosed. There is no recognizable endoplasmic reticulum or Golgi apparatus (Arrow No 13).

Wheat seedlings from germinated seeds immersed in 55°C. water for 70 seconds show ultrastructural damage similar to that caused by UHF exposure at 40 seconds (compare Figs. 11, 12, 13 with Fig. 16). The plasmalemma is pulled away from the cell wall. It is disrupted and evidently forms a number of small membrane-bound vesicles (Arrow No. 15). The mitochondria have almost completely disappeared. Some disorganized structures observed may be partially destroyed mitochondria (Arrow No. 16). Golgi apparatus and endoplasmic reticulum are not observable. The interior of the nucleus remains mostly organized, with electron-dense areas (Arrow No. 17). The nuclear membrane is no longer in evidence (Arrow No. 18).
Figure 16. Electron photo-micrograph of wheat seedlings heated in 55°C water 70 seconds. Arrow 14 shows plasmalemma away from cell wall, arrow 15 small membrane-bound vesicles, arrow 16 partially destroyed mitochondria, arrow 17 nucleus mostly organized, arrow 18 no nuclear membrane.
Electron photo-micrographs of rapidly growing wheat cells treated with UHF do not appear different from those treated with heat. The most striking effect of these two treatments is disruption of the various membranes of the cell. This correlates well with my conductivity experiments, indicating increased leakage of ions into the germinating medium after UHF and heat treatments of wheat seedlings and the data in Tab. 1 also shows no difference between heat and microwave treatment. This agrees with Michaelson's work that the cellular membrane is deranged by the heat produced from UHF irradiation (1).

Since lethal effects could only be inflicted by heat and microwaves on seeds containing water after the germination process started, my interest shifted to the germination process itself. In the microwave spectroscopy laboratory I found a very sensitive and non-intrusive method to monitor this process. The study was then shifted to this laboratory.

Germinating seeds perturb a resonant microwave cavity, and it was hoped this would provide insights into germination not available by more classical methods. The remainder of this work concentrates on the use of low-wattage microwaves as a measuring device to probe germinating wheat seeds.

The use of microwaves is a noninvasive system, but it carries with it some unfortunate problems. One of them is
the determination of the specific nature of the molecules that perturb the field. My earlier work indicates that microwaves interact mostly with water molecules, but the literature indicates that ions and some organic molecules may in some circumstances also be involved. It is not possible to separate the effect of organic molecules, water, and ions in a microwaves field.

It is generally accepted that the dipole of molecules of the sample that can resonate with the microwave field is the target of these waves. With the use of wheat seedlings the question was whether the total weight of all the organic material or only one organic molecule was the cause of microwave interaction.

In the microwave spectroscopy laboratory hyaluronic, acid had been shown to interact with microwaves (7). Hyaluronic acid in a water solution was shown to be the primary cause of the perturbation of the microwave field. Various concentrations and pH of hyaluronic acid have shown the dielectric response of microwave frequencies to be dependent upon the relaxation mechanism of the hyaluronic acid molecule, which is affected by its water environment. Hyaluronic acid, though much larger than the water molecule, is still very small when compared with the 3.3-cm wave length of UHF at the 9.3 GHz frequency used in this study. Also, this molecule is polar in water and would be expected to respond to the microwave field.
An attempt was made to determine whether there were organic molecules in the shoots that would perturb microwaves as does hyaluronic acid. This was done by determining the dielectric constant and the dielectric loss factor of dry shoots and deducting the dielectric constant and the dielectric loss factor of free water. This result is shown in Fig. 17. Decrease in dielectric constant and dielectric loss factor as the dry mass of biological material increases gives a reverse relation that indicated organic material was not the cause of the microwave interaction. If the organic mass caused the dielectric response, one would expect the dielectric constant and the dielectric loss factor to increase as the mass of organic material increased, but the opposite was true.

When the change in the dielectric constant and the dielectric loss factor was compared to the water contained in the specimen I concluded that water was the major factor in the microwave field response as germination proceeds. This agreed with tests conducted on dormant field-dried seeds containing about four microliters of water, which showed very little perturbation of the resonant cavity. This perturbation was generally within the lower limits of the sensitivity of the equipment. Therefore, I concluded that direct microwave interaction with organic molecules was minimal on wheat shoots (Fig 17).

The dielectric constant and dielectric loss factor of
Figure 17. Dielectric constant and dielectric loss factor vs dry mass of shoots.
wheat seeds that was found differs greatly from results of other investigators (Figs. 18, 19). This is attributable to several differences in the way the experiments were done. My measurements were made on only one seed at a time in a highly sensitive perturbation cavity, while others used a quantity of field-dried seeds in a wave guide. In addition, my data were collected at the initiation of germination instead of on dormant seeds as others have done. My seeds, therefore, had much higher moisture content.

In this set of experiments, microwaves were used as a probe to investigate the relative abundance of free water in the germination process, although I did not overlook the influence of ions at particular points. The dynamic biological activity early in germination makes a difference in the dielectric response.

As live wheat seeds begin to imbibe water, the dielectric constant is very much higher than bulk or free water (Fig. 18). Free water would be expected to be tightly bound and not respond to the applied field. However, measurements of the direct current conductivity of the seeds were also very high (Fig. 20). This is attributable to the release of free ions by the germinating seed (8). These free ions within the seed perturb the applied electric field and produce a dielectric constant far above that of free water. It is interesting to note
Figure 18. Dielectric constant for wheat seed vs microliters of water.
Figure 19. Dielectric loss factor for wheat seed vs microliters of water.
Figure 20. Direct current conductivity of germinating wheat seed vs volume of water per seed.
that this phenomenon also occurs in the early hydration of setting concrete, when the dielectric constant increases rapidly due to the release of ions (9).

Dead seeds do not produce the high dielectric response of live seeds (Fig. 18), although they imbibe water at about the same rate. This is attributed to the absence of ions in the initiation of water absorption. This difference reflects the rapid chemical reactions that take place in living seeds at the onset of imbibition. In dead seeds the enzymes that would initiate the germination process had been destroyed by heat, which eliminated the production of ions as in live seeds. Therefore, the data show only a gradual increase in the dielectric constant as water is imbibed.

As more water is imbibed in living seeds the dielectric constant is much lower, and finally becomes less than that of free water. Again, this correlates with the conductivity measurements and indicates that the ions are becoming confined (Fig. 20). The decrease of dielectric constant below that of free water occurs because water molecules are less mobile as they become more tightly bound to organic molecules and membranes.

It is interesting to note that the water in living shoots does not perturb the microwave field as much as the water in germinating seeds. Shoots contain approximately 90% water, while living seeds contain less than 50% water
at 30 microliters of water (Fig. 18). The data indicate that water in living shoots is more tightly bound than in living seeds. This water in shoots is apparently confined within and adjacent to membranes and enzymes as the quantity of water increases to 25 microliters. Evidently there is less free water space than in live seeds. In the profile of increasing mass of water in living seeds and shoots, the microwave field responds approximately the same at the maximum of 30 microliters. This may be due to the large volume of the shoots, which is about 5 times the volume of the living seed, and this large volume in the small cavity may perturb other parts of the microwave field. It can readily be observed that the dead seeds and shoots cause nearly the same response in the microwave field until there are more than 20 microliters of water involved. Then the dead seeds held the remaining 10 microliters of water more tightly than either live shoots or live seeds.

The dielectric loss factor of living seeds approaches that of free water at the start of imbibition (Fig. 19), despite the fact that much of the water is probably held as bound water. This is due to the large amount of ions released in the early hours of germination (Fig. 20). As the imbibition of water continues the dielectric loss factor becomes less than that of free water. The ions are no longer free as indicated in the conductivity profile in
(Fig. 20), and the water molecules are also bound and not free to respond to the field. At the maximum imbibition of 30 microliters of water the dielectric loss factor is again equal to that of free water. In this case ions are no longer involved (Figs. 19, 20). As seeds continued to imbibe water above 30 microliters of water they became seedlings with roots and shoot which could not be fitted into the same resonating field as the seed, and the ability to monitor a continual expanding organic material came to an end. With the data collected to this point it is not understood why the dielectric loss factor should increase to become equal to that of free water just before the seed sprouts roots and shoot (Fig. 19), but it is possible that 30 microliters of water was near the limits my equipment could measure.

As dead seeds imbibe water the dielectric loss factor increases. This would be expected, because more water is free to respond to the microwave field and is not all in a bound or structured condition.

The dielectric loss factor of shoots is less than that of either dead or live seeds (Fig. 19). This is another indication that there is apparently less free water in shoots than in the seeds. The water is not free to respond to the microwave field. Of course this is in spite of the fact that shoots contain approximately 90% water, indicating much less organic mass than seeds which at
maximum imbibition contain less than 50% water.

The loss tangent (Tan δ) expresses the relative ability of the material to dissipate electrical energy (Fig. 21). It is readily noted that shoots dissipate less energy than either dead seeds or live seeds. Shoots are larger and less dense than seeds with the same water content. Water is the main component that absorbs and dissipates the electrical energy. The conclusion that can be drawn from this is that water is much different in the shoots than in the dead and live seeds. Water in shoots is more restricted.

The data in this study correlate with conventional wisdom concerning bound and free water. Noggle and Fritz describe the nature of cellular water (10). They indicate that a small fraction of water is held tenaciously by plants, and they point out that water is dipolar and forms hydrogen bonds. The cell parts mentioned that are enveloped by bound water are endoplasmic reticulum, plasma membrane, tonoplast, and water held in interstices within proteins. Comparing growing shoots and germinating and dead seeds, I was able to show that a larger portion of the water was restricted in growing shoots, thus differing somewhat from Noggle and Fritz, who indicated that only a small fraction of the water was bound and the balance was free of bulk water. Salisbury and Ross refer to bound water or water of hydration (11). They indicated there is
Figure 21. Loss tangent of germinating wheat seed vs microliters of water.
a higher level of bound water in frosthardy plants and high temperature hardy plants. The concept of bound water indicated in the introduction of this report (page 8) is consistent with my data. Organic molecules reduce the mobility of the water molecules. The reference to "vicinal water" on the same page, measured by disjoining pressure, also agrees with my data.

The concept of bound water in cells has been accepted by plant biologists for many decades, but precise measurements of its extent in botanical tissue is very complex. Nuclear magnetic resonance studies confirm that notable amounts of water in cells interact differently from free water (12), but quantitative measurements are impractical. The lowered vapor pressure measurements of water in capillaries was also examined for constrained water adjacent surfaces. The distribution of ions inside and outside of the cellular membrane has led to the conclusion that water is more ordered intracellular than extracellular (13).
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