The Heat Treatment of Soil by Microwaves to Control Pathogenic Parasitic Fungi

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THE HEAT TREATMENT OF SOIL BY MICROWAVES TO CONTROL PATHOGENIC PARASITIC FUNGI

BY

DEAN FRANKLIN RICH

A Research Report Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in Environmental Systems Management

FLORIDA TECHNOLOGICAL UNIVERSITY

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It was my privilege and good fortune to become acquainted with Drs. Gordon Grimm and John O'Bannon of the U.S. Department of Agriculture in Orlando, Florida. Without the advice and laboratory services unselfishly provided, the verification testing would have been a stunted effort.
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INTRODUCTION

The type of soil used in the production of plants and vegetables is of great importance. Of the same degree of importance are the chemical and biological constituents of the soil. The nutritional values for plant growth are chemical factors. Soil may be "rich" in food value to the plants or "poor" with little to add to plant growth.

Plant growth may also be affected by biological factors. These may include organisms which "use up," or destroy, the available food supply of the plant. Other organisms may attach themselves to the plant itself, relying on the "host" for survival.

Not all biological organisms are harmful. Some convert unavailable organic matter into useful available nutritious food stuff. These saprophytes are not only harmless but actually necessary for the natural biological and ecological processes continually taking place in the soil.

In any methods of control or elimination of pathogens in the soil, consideration must be given to the selection of those organisms which are harmful and those which are useful. This process of selective elimination of harmful organisms is the basic difference between sterilization, which voids the soil of any biological life, and pasteurization, a degree of sterilization which removes those active organisms which cause disease. Complete sterilization can sometimes cause more harm than it prevents.
Among the pathogenic species are protozoa, viruses, bacteria, nematodes, fungi, weeds and insects (28). This report concerns itself with the control of parasitic pathogens. While the bacteria and other organisms may also be pathogenic and deleterious to plant growth, the fungi are representative of the more resistant organisms (6) (28). Nematodes were tested together with the fungi to provide an indicator of the effectiveness of the test procedures and apparatus.

The method of elimination control chosen for the process was microwave energy. This form of heating has been in existence for some time under the name of diathermy. For testing purposes, standard microwave heating equipment was chosen for convenience.

The object of the report is to present a summary of the literature available and verify, through limited testing, the feasibility of microwave treatment for eliminating pathogenic parasitic fungi in the soil. The results show (1) the effectiveness of microwave energy in eliminating the fungus from the soil, (2) the effect of some of the process parameters on the fungus, and (3) some possible causes for the "heat death" in the organisms.
I. PARASITIC FUNGI IN THE SOIL

In the commercial production of plants and vegetables, the nurseryman must be concerned with the environmental factors affecting plant growth. Among these are the soil, pathogenic organisms, insects, weeds, temperature, salinity, moisture and nutrition (2). Of these, among the most difficult to control are the disease causing organisms.

Disease producing organisms, because of their size and life style, are difficult to observe. Disease is most often only detectable as a result of observable plant damage. Soil may be chosen and modified with a great deal of care and ease (2). Nutrition is man-induced and has been greatly improved and sophisticated to suit the requirements of the plant. Insects and weeds are controlled by a multitude of pesticides and herbicides available on the market today. Their control is further enhanced by the fact that they are observable. Environmental control is also sophisticated and to a large degree man induced. Large "hot houses" are available in various configurations which completely surround the plant producing area allowing humidity, ambient air temperature and salinity to be closely controlled.

There are several ways to classify harmful plant organisms. When an organism causes a plant (or another organism) to suffer, producing disease, it is a pathogen (from the Greek word Pathos meaning "to suffer"). Those disease producing organisms are of primary interest and information from the plant pathologists is useful.
The life style of the organisms is of interest in determining which of these living in the soil or in the surrounding air are pathogenic. When an organism, in the normal pursuit of life sustaining food elements, uses the supply of dead, decaying material, it is saprophytic, which literally means "rotten plant" (6). To the nurseryman, these organisms wear the "white hats" since the saprophytes help to hasten the conversion of dead animals and plants to a fertile soil and are necessary to the natural ecological cycles in the soil.

Conversely, any organism which does not wait for death to "set in" before partaking of the host is considered a "poor sport" and classed as a parasite, which literally means, "living beside the food" (6). In the scientific vernacular, these are organisms which live in (or on) another plant or animal. An obligate parasite is one which is obligated to live only on living tissue and hosts which they are suited for in particular. So the obligate saprophyte lives only on dead matter.

It is possible and practical in the "underworld" of plant life for an organism to utilize both living and dead matter for food. These are facultative (facultative parasites and facultative saprophytes) (6). Practically speaking, these are the major disease producing organisms in the plant environment and pose the greatest challenge in control and elimination.

Pathogenic parasites are primary plant enemies and consist of many types. Then why investigate the fungi? Other parasites include bacteria, protozoa, flat-worms, roundworms, insects and others of
higher order. But, fungi possess more peculiarities than most of the others and at times, hardly seem to fit either the plant or animal world.

Of the pathogenic parasites, the fungi are probably the major offenders, subject and responsive to such environmental factors as temperature, water, oxygen, acidity-alkalinity, food, minerals, vitamins, growth promoting substances and various subtle etceteras (6).

Because of the lack of green color, photosynthesis plays no part of their life functions. The fungi's environment, therefore, includes air and soil down to a foot in depth (3).

Control of parasitic fungi is important because it is a primary factor in successful plant and vegetable production and has been known to create major disasters resulting in death and migration as well as setting and upsetting the economic levels of many agriculture and foliage centers in the world (18) (19).

The fungus chosen for the study of control techniques was the Phytophthora parasitica. This is the fungus responsible for root rot and foot rot in citrus trees (28). This fungus was locally available in sample soils at the U.S. Department of Agriculture in Orlando where much cooperative work was thankfully available. Phytophthora parasitica is a facultative, parasitic fungus which can be cultivated in water and observed in 3-5 days (9). Thus, it is very suitable for experimental verification purposes.

Taxonomically, the fungus is of the phylum Eumycophyta (true fungi), the class of Phycomycetes, the order of Peronosporales which
has three families, Peronosporaceae, Albuginaceae and Pythiaceae (9). The Pythiaceae are distinguished from the other two families in that they produce sporangia on unspecialized hyphae and are mostly soil inhabitants. It contains two genera; Pythium and Phytophthora. The Phytophthora parasitica causes foot rot, a disease which becomes apparent above soil level indicating more serious root disease problems. It is characterized by sporangiophores with ovoid and papillate sporangia. Normally the sporangia function as such and produce zoospores within an extruded vesicle. The species produces zoospores and thickened resting bodies as chlamydospores which retain their germinating capacity for a long period (28).

Fig. I-1.—Sporangia of the Phytophthora Parasitica Fungus (Courtesy of the U.S. Department of Agriculture, Orlando, Florida).
Control of Parasitic Fungi in Soil

As competition becomes greater for the nurseryman and farmer, the operating margins become smaller. Crop losses from diseases (particularly fungi and nematodes) average between 1-10 percent, with losses of 50 percent or more not too infrequent (3). In the small nursery, the cost of installing larger operating equipment (such as steam generators, hot water heaters, hot air generators or other soil conditioners) is prohibitive, feasible only for larger producers. Economical, effective methods of control are imperative.

Competition is not restricted to the market place for the farmers and the nurserymen. The environmental constraints existing and pending also present a new set of problems. Chemical pesticides are coming under fire for their polluting effects which are due to both accumulative effects and increased usage (32). In 1967, the total U.S. production of pesticides increased 18 percent from 1965 production (1). More than one billion pounds were produced of which 17 percent were fungicides. Even more significant are the problems of application which requires special equipment. After initial application, a new formed plant immunity may cause that particular fungicide to become ineffective requiring changes in either application dosage or methods.

Fungicides present an additional problem of residues on the plant. The Food and Drug Administration provides strict control and enforcement of allowable tolerances in residual toxic chemicals on plants and vegetables.
External chemical additives are subject to weather and other natural elements. Rain may cause loss of 40-50 percent of the applied chemical in a relatively short period of time (29).

Economic reasons demand control of diseases for successful plant production. Environmental factors support that conclusion. Agricultural space in the world is decreasing while the demand for food and foliage is increasing. This requires new and better plant and vegetable producing techniques; techniques which will not leave permanent negative ecological effects on the environment as we utilize it.

Selectivity

In the control of any pathogens, careful consideration must be given to the preservation of the non-pathogens to prevent harmful imbalance in nature's processes. Without this important consideration, one cure could produce an equally bad or worse condition. Many cases of this are a part of our history; for example, the discovery of dichloro-diphenyl-trichloroethane (DDT) in 1874 by the Austrian scientist Othmar Zeidler (32). DDT was not used extensively until World War II as an insecticide. Since then, DDT has been found in Antarctic penguins and may well be a hazard in areas of the world where it was never used. DDT is not a selective insecticide and is a long term degradent, requiring long period of time to be reduced.

All fungi, however, are not pathogenic or otherwise harmful. On the contrary, mass elimination of fungi might produce more harmful effects than helpful. Inman and Ingersoll (17) of Stanford Research
Institute have shown that significantly increased production of carbon monoxide in the United States in the past 15 years has not resulted in corresponding increased residual levels in the atmosphere in part due to the discovery that at least 16 strains of fungi absorb CO in the soil (nature's carbon monoxide sink).

Emphasis on selectivity in control of fungi would seem to be prudent. This leads one to contemplate the concepts of sterilization and pasteurization. While sterilization is complete and total elimination of all organisms, pasteurization eliminates the more harmful ones (at least in vegetative form) and allows the others to survive. These terms are associated with temperature control methods but the idea is fundamental and meaningful for other methods.

Methods of Control

There are four methods of controlling pathogens in the nursery;
(29) exclusion of the pathogen, eradication of the pathogen, protection of the host, and immunity (breeding for resistance to disease) of the host.

Protection consists of inserting a shield of protection between the pathogen and the host. Primarily, protection is the application of chemicals to the plant itself. Protection is not permanent, causes pollution, may be toxic to humans and its effectiveness is subject to weather conditions.

Immunization is the technique of breeding the plant to resist the pathogen or conversely, offer no advantage to the parasitic pathogen.
Exclusion involves the prevention of the spread of pathogens to an area in which they do not exist. The procedure involves the detection of diseased sister plants or seedlings by inspection and legislation to prohibit import.

Eradication is necessary when exclusion is not successful. It involves either removing the host plant or treating the host environment to kill the pathogen "in vivo." This last method is going to be emphasized and discussed here and does not include filtration by airborne pathogens.

There are several well-known methods for eliminating pathogens from the soil: 1) hot water immersion (4), 2) soil steam heat treatments (5), and 3) application of chemical solutions (28).

The hot water immersion process is costly, leaves the soil wet requiring a long drying period and provides no method for selectivity. In addition, soaking, leaching and percolation affect the soil structure and chemical composition adversely.

Steam sterilization is an improvement over the hot water method but has the disadvantage of requiring a steam generator (boiler) and while temperatures of 80-100 degrees C can be reached in 30 minutes or less throughout the soil mass, sufficient to kill most pathogens in the soil, it leaves the soil void of any organisms making recolonization of the pathogenic organisms easy if recontaminated.

Use of chemicals such as methyl bromide and choropicrin or methylmercury dicyandiamide (PANOGEN) is effective but the chemicals are toxic, and cause pollution. The method itself is costly and results
below the surface are unpredictable without considerable knowledge of the individual soils and the inhabitants.

**An Electronic Method**

Research indicates work has been done recently in heat treating several types of pathogenic soil electronically. O'Bannon and Good (22) demonstrated that heating with microwave energy was effective in reducing and eliminating nematodes in soil. Other electromagnetic and sonic sources have been utilized (21)(30)(31) for control of insects while Davis, Wayland and Merkle (8) applied a microwave source to selectively control weeds.

Since there is a precedence for an electronic radio frequency (RF) radiation technique for controlling or eliminating pathogens from the soil, further research and experimental verification is in order to:
1) Determine the feasibility of eliminating parasitic fungi from plant soil, 2) Study the parameters affecting the process, and 3) Analyze its usefulness for commercial applications.

**Fungi Physiology**

Successful control of harmful fungi (in the soil) can only be achieved when its composition and structure, life functions and environmental activities are known. For control optimization, the choice of elimination, migration or modification of the pathogen (or the victim) necessitate knowledge and understanding of the ecological factors. Presentation of the treatment of the physiology of the fungi will be limited to those facts necessary to evaluate and conclude the
feasibility and usefulness of applying microwave energy to control fungi in the soil. It is not intended that a basic and complete treatment of cell, plant and animal physiology be presented.

The *Phytophthora parasitica* fungus is a member of the soil microflora which consists of the following (12) taxonomic divisions of micro-organisms: Fungi, Bacteria, Actinomycetes and Algae. Of these, only the algae are green colored and photosynthesize carbohydrates from carbon dioxide and water through the process of chlorophyll pigments contained in their cells. This form of nutrition is called autotrophic. In contrast, fungal cells are colorless and as such do not make use of photosynthesis. It must, as all colorless plant and animal organisms do, depend on the products of green celled organisms for energy and nutrition and is therefore called heterotrophic. This fact is important in the determination of a fungus population at various depths below the soil surface. Being facultative parasites, capable of receiving nutrition from both live hosts or dead organic material, the *P. parasitica* can exist at depths greater than most organisms (3). This is one of the reasons why the *P. parasitica* is more difficult to control than those pathogens living closer to the surface.

Fungi as a group share the following three characteristics: (6)

1. Lack of chlorophyll--This means they cannot manufacture their own organic food such as starches, fats, sugars, proteins and celluloses from carbon dioxide and water as green photosynthetic plants do, a fact which makes them dependent on the green plants for life.
2. Morphologically they lack plant body (i.e. they have no stem, root or leaves). Instead they have long, hollow, branching cells which in the aggregate are called Mycelium.

3. Reproduction is by means of spores which can remain dormant for long periods of time or become active and grow when the environment is favorable. Reproduction may be either sexual or asexual which results in the large population of fungi in the world.

Cell Physiology

All cells have two basic elements, a cell wall and inner material, protoplasm. From this point, the only other predictable characteristic between various types of cells is that reproduction is always performed by duplicating itself (12).

Some cells contain the necessary constituents which allow the manufacture of food by photosynthesis, a process utilizing solar energy. For those cells not possessing chlorophyll, as the fungi, the energy must be provided from organic material which somewhere in the chain originated from a photosynthesizing cell (6).

The fungal cell produces enzymes and acids which diffuse through the cell walls and into the material on which the fungus is growing. There they break down the cellulososes, starches, sugars, proteins, fats and other constituents into simple compounds which diffuse or are transported back into the cell and supply the food and energy for
growth. The diffusion phenomenon will be discussed later with regard to the possible effects on cell death from irradiation.

The fungal nucleus is responsible for initiating the basic actions of the cell such as reproduction and manufacturing of enzymes and other proteins. The nuclei contain nucleoproteins and DNA (Deoxyribonucleic acid). Through an ingenious coding scheme and molecular mechanism, these elements are responsible for the selective production of precisely the right enzymes and proteins. These, in turn, provide the characteristics of the species during growth. In addition, the DNA are present during reproduction in the chromosomes which divide and miraculously form another cell.

**Fungus Growth**

Growth of the *P. parasitica* fungus begins with the germination of spores. As previously mentioned, the spores may be produced sexually or asexually. In the soil, germination takes place only when proper stimulation exists. It does not occur in either dry soil or in elevated temperatures (above 35° C) (13). Without germination activity, the propagule remain in a dormant stage, a fact which must be examined before selection of a successful fungi control technique.

As germination proceeds, hollow tubes, 3-10 microns in diameter, are secreted by the protoplasm in the cell causing it to grow. This tube is called the hypha and collectively they form the mycelium (13). Growth is longitudinal by extension of the hyphal cells which consist of a cell wall which contains the protoplasm and may contain more than...
one nuclei. The rate of growth is of the order of $1/8000$ inch per minute and depends on the nutrients available in the substrate. In 24 hours, a fungal colony can produce a total length of over one-half mile mycelium.

The *P. parasitica* produce zoosporangia which transport zoospore (eggs) by migration of water in the soil or by insects to which the zoosporangia become attached. After coming to rest, the motile, flagellate zoospores are released where they locate and attach themselves to a host.
II. ENVIRONMENTAL FACTORS AFFECTING FUNGI CONTROL

The ecology of the world in which the fungi exist is important. This is especially true whenever permanent or long-term alterations to nature are considered. Not only is the influence of the environment and its inhabitants on the fungi important, but conversely, the effects of the fungi on the community. The factors involved are numerous and presented in great detail in several writings (7) (13). Only those factors pertinent to the study of the control of parasitic fungi by microwave radiation will be presented in this paper.

Of special interest are those factors considered as possible contributors to the death of the fungus. The specific cause of death in fungi, or any death for that matter, has long been a matter for speculation (16). Therefore, any theories referenced are hypothetical and proof will be left for future study and research.

The environment of the P. parasitica is the soil except when present in vivo on the host plant. The physical factors present in the soil and directly affecting all microorganisms include temperature, moisture content, oxygen, nitrogen, organic food, light, other radiation and other chemical constituents. These are discussed in the following paragraphs.

Temperature

Most microorganisms exist over a limited temperature range of about 10 - 45° C (75 - 139° F) called the "Biokinetic Zone" (16).
All life is not limited to this range, however, and microorganisms are generally classed as being psychrophilic (heat sensitive), 0°-20°C; mesophilic, 25°-40°C; and thermophilic (heat loving), over 40°C (16) (45°-60°C) (11).

Most microorganisms (including *P. parasitica* fungus) are mesophilic and are characterized in the following Table.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>below 10°C</td>
<td>dormancy—seldom death unless extreme cold</td>
</tr>
<tr>
<td>21°-32°C (70°-90°F)</td>
<td>most favorable activity</td>
</tr>
<tr>
<td>35°-43°C</td>
<td>slowness, restricted activity, some dormancy</td>
</tr>
<tr>
<td>55°-65°C</td>
<td>reversible suspension of life functions for short periods, irreversible cessation of life functions over a period of hours</td>
</tr>
<tr>
<td>above 65°C</td>
<td>instant death, irreversible.</td>
</tr>
</tbody>
</table>

Dormancy can occur when the normal life functions of the fungi are threatened due to abnormal thermal conditions. Since fungi lack the temperature control mechanisms of higher organisms, they simply minimize their activities and "hold their breath" until more favorable conditions occur. This mechanism is characterized by dehydration and, at extremely elevated temperatures, by production of
sporangia. Lack of water prevents harm to the cell through freezing. Return to normal generally occurs upon contact with water.

Killing a dormant organism is basically different than killing a non-dormant organism. Elimination of the vegetative stages is a process called pasteurization (16) occurring around 60°C for one-half hour. Sterilization, on the other hand, involves killing the dormant cells and spores as well. This is accomplished at temperatures of 150°C-170°C for a half-hour or so and is dependent on the moisture present. Boiling (a common bacterial sterilization method) at 100°C in water may kill the vegetating cells and some spores but usually requires repeated applications, a process known as Tyndallization.

Pasteurization is used to kill the harmful organisms in a substance, while leaving those chemical and biological components whose presence is either necessary or desirable intact. Because of the harmful effects of heat on the ecology of the soil, the term pasteurization will be used to describe a process of selective control of pathogens in the soil instead of complete sterilization.

The greater thermal resistance of dormant organisms is not completely understood. It is known that the temperature required to denature proteins varies inversely with water content as shown in Figure II-1 for a sample of egg albumin.

It is theorized (23), that some thermophilic microorganisms withstand heat better because they synthesize enzymes faster than they are destroyed but only when sufficient nutrients are available. Without nutrients, they are reduced to mesophiles. Another theory
Fig. II-1.--Relation between relative humidity and temperature for denaturation of egg albumin. The data are for denaturation of half of the egg albumin in 60 minutes. (From Barker, 1933: J. Gen. Physiol. 17:24).

states that thermophiles have a stronger protein hydrogen bonding than their lower temperature comrades (16). Ribonuclease (RNA) can withstand boiling temperature for relatively long periods. DNA (Deoxynucleic Acid) is denatured when heated to 70°C for a short period of time (14).

Heat Death

The four factors affecting the heat death of fungi are: (24)

1. The previous temperature range to which the fungus has been adapted.

2. The activity level of the mycelium or spores—decreased activity generally increases heat resistance.

3. The heat exposure or total irradiation energy—increased exposure time increases the lethal probabilities.
4. The form of the applied energy, i.e., thermochemical, electromagnetic, molecular friction, etc.

Heat death may be due to any or all of the following causes:

(16) (23)

1. irreversible inactivation of the enzymes
2. dissociating of the enzymes from the aggregate of which they are a part
3. Derangement of the lipids (fats, etc.) which may rupture or destroy a portion of the cell wall
4. liberation of a coagulating enzyme causing gel to form around the cell wall prohibiting the passage of nutrients literally starving the cell (this is caused by releasing calcium from the cell walls).
5. disruption of cell reproduction causing death—this may be done by either mutation or malfunction of the coding functions of the DNA or denaturing the DNA helix structure blocking the production of nucleic acids and enzymes.

Heat curves for the killing of bacteria indicate that the lethal point is reached as the result of a single event, probably a reproductive malfunction (16). There is reason to believe that fungi react in the same manner (7).

Moisture Content

As previously mentioned, fungi lacking water, or moisture, become dormant or sluggish. However, the water necessary for their
growth (not survival) also makes them vulnerable to lethal heat rays. Whenever the humidity exceeds 70 percent, the fungi will flourish (7). P. parasitica were germinated and colonized in water samples for evaluation (see Figure II-2). Time for germination in water was 3-5 days. Fungi deprived of water will not grow.

**TABLE II-2**

**LEthal Temperatures for Pathogenic Plant Organisms with 30 Minutes Exposure Using Steam or Dry Heat (4)**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>46°C</td>
<td>water molds</td>
</tr>
<tr>
<td>49°C</td>
<td>Nematodes</td>
</tr>
<tr>
<td>52°C</td>
<td>Fungus, <em>Rhizoctania solani</em></td>
</tr>
<tr>
<td>60°C</td>
<td>Most Plant Pathogenic Fungi and Bacteria, Worms, Slugs, Centipedes</td>
</tr>
<tr>
<td>60°-71°C</td>
<td>Most Soil Insects</td>
</tr>
<tr>
<td>71°C</td>
<td>All Plant Pathogenic Bacteria, Most Plant Viruses</td>
</tr>
<tr>
<td>70°-80°C</td>
<td>Most Weed Seeds</td>
</tr>
<tr>
<td>93°-100°C</td>
<td>Resistant Weed Seeds and Resistant Plant Viruses</td>
</tr>
</tbody>
</table>

Groundwater and rainwater also aid in the transport of fungi to suitable environments.
Fig. II-2.--Phytophthora Parasitica Germinating in Watered Soil Samples (Courtesy of the U.S. Department of Agriculture).

Oxygen (Respiration)

The fungi require free oxygen for growth. In this sense they are aerobic. There are no obligate anaerobic fungi as there are among the bacteria—and important factor. Fungi not only require oxygen but are poisoned by carbon dioxide. Concentrations greater than 50 percent, regardless of the quantity of oxygen, will inhibit or kill the fungi.

Nutrition

Basically, fungi will utilize almost any type of element around except metal. However, certain species of fungi, such as P. parasitica, are extremely selective, resulting in a large number
of families, each peculiar to its own host (for parasites) and environment. Some fungi attack other fungi, a control factor itself.

**Light**

Fungi require little or no light to grow, existing well in darkness. *P. parasitica* grow in either light or darkness.

**Radiation**

Since fungi are not photosynthetic, the mechanism for converting solar energy into chemical energy is not an integral part of the cell makeup.

Several factors are worthy of mention here. First, solar energy in the visible and near-visible wavelengths, effect the colored cells. Most fungi including the *P. parasitica*, have some color and will heat due to absorption of the light energy. In the fungus tested, the heating due to solar light absorption is insignificant.

Secondly, experiments have shown (16) that initial doses of ultraviolet radiation inhibit DNA synthesis and halt all cell division. Larger doses of ultraviolet light inhibit RNA and finally the protein synthesis process in that order. Some genes of fungi have been "inactivated" by ultraviolet light and later "reactivated" by visible light indicating "healing" was possible--the process of deactivation was not irreversible under certain conditions. Photo-reactivation appears to involve a photchemical reaction (absorption of visible and long ultra-violet light in the chromophore) followed by a thermochemical reaction. This process may also be duplicated...
using a salt solution for recovery (16). This is called "dark recovery" since it may be accomplished in the absence of light.

Soil

Two soil factors may influence the control of pathogenic fungi using a RF energy source: 1) the physical and dielectric characteristics, and 2) the effects of diurnal heating on the microorganism population density at various depths below the surface.

The physical characteristics affecting the microorganisms in the soil are acidity, salinity (2), size and shape of the granules, organic content, moisture and thermal diffusivity (5) or conductivity factors.

The factors governing the effectiveness of microwave irradiation are density, humidity, color, thermal diffusivity, absorptivity and dielectric constants. The heat capacity (number of calories required to raise one gram through one degree centigrade) is given by the following equation (24):

\[ C_v = \frac{C_w}{D} = 0.58 \]

where: 
- \( C_v \) = thermal capacity per unit volume
- \( C_w \) = thermal capacity per unit weight
- \( D \) = density

With a natural soil, containing air spaces and water, the porosity, \( P \) (as a fraction of air volume) and the water content,
W (in grams water per grams soil) can be allowed for in the equation:

\[ C_v = \frac{0.58(100-P) + W}{100} \]

Typical residual temperatures in soil with various types of soil covers are given for daytime periods in Table II-3 below:

**TABLE II-3**

DAILY HEAT STORAGE IN SOIL UNDER VARIOUS TYPES OF VEGETATION
(From Sutton, 1953)

<table>
<thead>
<tr>
<th>Type of Soil Cover</th>
<th>Heat Content During Daytime Hours (calories/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woods on sandy soil</td>
<td>15-34</td>
</tr>
<tr>
<td>Moorland (marshes)</td>
<td>33-43</td>
</tr>
<tr>
<td>Bare Sandy Soil</td>
<td>95-105</td>
</tr>
<tr>
<td>Grass on Sandy Soil</td>
<td>56-67</td>
</tr>
<tr>
<td>Bare Granite</td>
<td>128</td>
</tr>
</tbody>
</table>

The diffusivity and time lag equations are: (24)

Amplitude Ratio: \( \frac{\theta_1}{\theta_2} = (d_2-d_1) \left( \frac{\pi}{kT} \right)^{\frac{1}{2}} \) \hspace{1cm} (2-1)

Diffusivity constant: \( k = \frac{\pi}{T} \frac{d_2^2-d_1^2}{\log_\theta_1^{\theta_2}} \) \hspace{1cm} (2-2)

Time lag: \( T = \frac{1}{2}(d_2-d_1) \left( T/\pi k \right)^{\frac{1}{2}} \) \hspace{1cm} (2-3)

In general, the microorganism population at any particular depth in the soil may be estimated by computing the temperature at
the desired depth and applying one of the growth rate equations, i.e., Chick's Law, Arrhenius molecular activity law, etc. (24). To illustrate, Figure II-3 provides a quick evaluation of the constant $k$ for the Arrhenius equation:

$$k = \frac{2.303 (\log x_2 - \log x_1)}{t_2 - t_1}$$

where: $x =$ densities at times $t_1, t_2$

![Arrhenius Plots of the Specific Growth Rates of a Psychrophilic pseudo-monad, a Mesophilic Strain of E. Coli and a Thermophilic Strain of Bacillus Circulars (24).](Figure II-3)

**Fig. II-3.** -- Arrhenius Plots of the Specific Growth Rates of a Psychrophilic pseudo-monad, a Mesophilic Strain of E. Coli and a Thermophilic Strain of Bacillus Circulars (24).
III. MICROWAVE ENERGY – A METHOD OF FUNGI CONTROL

What is microwave energy? Why should it be considered in a control application for fungi? How effective is it? How is it generated? How does it affect the environment? The answers to these questions constitute the purpose for this study and will be pursued through the survey of available literature and limited experimental testing to determine or verify the feasibility.

Microwave Energy

The effect of RF (radio frequency) microwave energy have been made evident in detailed reports from the users of radar during the World War II period. As a result of the "sterilizing" and lethal powers of the radiated energy, much research was performed culminating in a strict set of safety and precautionary rules regarding its use.

Medical science picked up the phenomenon of RF energy and made use of its therapeutic qualities in diathermy (25). Diathermy machines are now commonplace in hospitals and doctors offices using wavelengths from one millimeter to one meter. These radiations are close to the infrared region in the electromagnetic spectrum.

It is the heating phenomenon of RF waves which have attracted the attention of agriculturists and nurserymen toward use in the control of pests and pathogenic organisms in the environment. The sterilizing effects of ultraviolet light on bacteria was evident many years
ago (25). Since then the use of RF energy for control of insects, (21) elimination of nematodes in soil, (22) elimination and control of weeds, (8) pasteurization of soil infected with fungi using RF energy in the 27.12 MHz region (10) have been reported.

Radio frequency waves are electromagnetic in nature. This distinguishes them from the subsonic, sonic and ultrasonic energy which is propagated through the air as vibratory mechanical waves causing molecular reactions due to small pressure differences. All electromagnetic waves are similar in nature and can be described with the basic equations:

\[ \lambda = \frac{c}{v} \quad \text{and} \quad E = hv \]

where, \( c \) = the speed of light, \( 3 \times 10^{10} \) cm/sec

\( h = \) Planck's constant, \( 6.62 \times 10^{-27} \) ergs sec.

\( \lambda = \) the wavelength in meters

\( v = \) frequency in Hertz (cycles per second)

\( E = \) energy of quantum or photon

Basically, (1) as the frequency of wave occurrence becomes greater, the wavelength becomes smaller and (2) as the frequency increases (wavelength shortens), the quantum energy of electromagnetic energy increases.

These two fundamental facts cause the radiation to have different effects on materials at various frequencies. Generalizing, the longer wavelengths produce heating while the shorter wavelengths produce an electro-chemical effect, creating ionization in the molecular structure.
The shorter, higher energy wavelengths may have a disruptive effect on the molecular bonding forces. When these bonds are broken or "ionized", the action is usually permanent and irreversible as is the case with the higher radiations, i.e. X-rays and gamma rays. Because of the irreversibility, exposure to these higher frequency radiations can cause an accumulative effect with each new exposure adding to the effect of the last.

There are advantageous characteristics of the shorter wavelengths. These include the ability of the waves to be deflected, diffracted and refracted utilizing equipment of a practical size. Unfortunately, they are also easily absorbed and lose energy rapidly when encountering high absorptive materials.

Below the infrared frequency range are the "work horse" radio frequencies. Exposure to these frequencies induces a less permanent effect characterized by heating instead of ionizing, a phenomenon which is usually reversible, i.e. disappears with healing. To oversimplify, the reason for the milder reaction is because at longer wavelengths, the electromagnetic energy has less effect on the molecular binding forces, producing a "stretching" instead of "breaking" action. At much lower frequencies, the wavelengths are not as easily directed (although the energy loss is not prevalent).

UHF

There is a frequency band below the infrared and visible spectrum which retains many of the desirable characteristics of the shorter wavelengths but produces the heating effect of the lower
frequencies. Microwaves are short RF waves in the 300-3,000 MHz, UHF (ultra high frequency) range with wavelengths between 0.1 - 1.0 meters (10 - 100 centimeters). The relationship between UHF waves and the other regions of the electromagnetic spectrum are given in Figure II-1.

The highly effective heating ability of the upper UHF wavelengths has resulted in industrial and commercial applications including microwave ovens used in the preparation of food (27). The feasibility of using microwaves for control or elimination of pathogenic fungi was investigated using such a device.
Microwave Heating

Commercial microwave heating is done at or near two ranges, 915 MHz and 2450 MHz. These frequencies were chosen because of their physical dimensional relationship for resonance in a multimode cavity (20).

As previously mentioned, UHF waves interact in three ways with material with which they come in contact. They can be reflected, transmitted or absorbed. Of the three, absorption is the interaction which causes heating.

Most metals reflect microwaves as a mirror reflects light. In general, all good electrical conductors will cause reflection.

Materials such as paper, glass, ceramics and some plastics will not react to microwave radiation in any appreciable manner. These materials transmit the energy waves without extracting any and are "transparent" to microwaves.

When a material "reacts" with the radiated wave and extracts energy, it is said to be "lossy". As the energy is reluctantly given up by the microwave radiation, heating results. This phenomenon is technically called "dielectric heating" and as the term implies, it is primarily due to the dielectric properties of the material.

The two characteristics of an absorbing substance are; 1) the dielectric constant and 2) the dissipation factor (loss factor). The dielectric constant is a measure of the effects of a material on a capacitive system compared to the same system in a vacuum (standard minimum loss condition). It is a function of the polarization of the
molecules in a material when the presence of an electric field. Polarization is a term used to describe the extent these "centers of charge" can be separated by the electric field. A measure of this characteristic is called the "dipole moment" of the molecule. Water, for instance, has a dipole moment without an externally applied field. This provides a measure of predictability regarding the separation characteristics after the field is applied.

The second factor has to do with the ability of the molecules to "align" themselves physically in the direction of the field at the rate of change in the field of polarization, i.e. 2450 MHz. This is the dissipation factor and losses are due to "friction" in the molecular structure. The total loss resulting in heat is the product of these two factors.

Water has a dielectric constant of 83.6 (20) at 17°C for a 38 cm. wavelength. When frozen it changes to approximately 3.2 indicating the relative difference between a liquid and a solid. The heating effect will be, to a large extent, dependent on the quantity of liquids and principally water present in the material. The dielectric constant is also affected by temperature, increasing with an increase in temperature.

The depth of penetration of a microwave is dependent on the strength of the field and the amount of energy absorbed from it by substances coming in contact with it for a given frequency. The depth at which power is reduced by one-half value in cold water is approximately four cm. at 1,000 MHz and 0.5 cm. at 3,000 MHz (20).
While the practical factors involving making microwave ovens are not pertinent, a word on safety is important to anyone considering investigating this method. Federal standards governing the manufacture and use of microwave devices are strict and designed for health and welfare of the users. Injury due to microwave radiation is not easily detected at the time of contact, therefore, caution should be exercised at all times.
IV. TESTING THE MICROWAVE METHOD

To determine whether microwave energy can be utilized to control or eliminate parasitic fungi in soil, a microwave oven similar to that used in the preparation of food was employed. The oven was a Litton Model 500 with an 8 3/4 inch x 15 inch x 12 inch cavity. The microwave oven output was rated at 575 watts with an input of 120 VAC, 14 Amps (1680 VA). The actual measured microwave power absorbed in the center of the cavity was approximately 240 watts or 42 percent efficient. This was measured by heating a 50 ml sample of distilled water and converting the temperature to energy.

Experimental Procedure

The tests were performed by using a special mixture of three parts peat, one part vermiculite and one part Lakeland fine sand infected with Phytophthora parasitica which was supplied by the local U.S. Department of Agriculture.

In the first test 50 cc sample volumes of soil, loosely packed at a depth of 3.0 cm., were placed in paper containers with lids, marked and placed in the microwave oven. The moisture content was 15 percent on an oven dry basis. The soil samples were set on a glass shelf 2.5 cm. above the floor of the oven. Exposure times of 0 (control), 5, 10, 15, 20, 25, 30, 35, 45, 60 and 300 seconds were used individually. Temperatures were observed and recorded at the end of each period using thermocouples placed in the center of the soil samples immediately after
exposure. The time constant for the thermocouple to reach 95 percent of full scale was three seconds. A calibrated Simpson Model 389 meter was used to observe the temperature readings. Ambient (initial) temperature of the soil was constant for all samples tested.

In the second test (actually run simultaneously) 50 cc volumes of the same original soil containing 15 percent moisture and P. parasitica were mixed with 30 cc of distilled water and the test repeated. This was done to observe the effects of the moisture content on the environment of the fungus. Both of the above tests were repeated several times weeks apart to provide collaboration. Repeatability was good.

In the third test, 50 cc and 400 cc samples of the 15 percent moist soil were exposed in the same manner to provide a temperature relationship with increased volume. Unfortunately, tests were not performed on the high moist samples - a point later to be recognized as significant.

Determination of the effectiveness in killing the fungus was accomplished by immersing the samples in water, adding small chopped sections of leaf and allowing to germinate for five days or until the colonization was observed in the control samples (9). No further germination was possible after the incubation period. Much of this work was performed at the U.S. Department of Agriculture laboratories at Orlando, Florida.

A fourth type of test was performed using a special treated Astatula fine sand with seven percent peat moss by volume. The moisture
content was 12 percent (dry oven basis) and the soil was infected with *Tylenchulus semipenetrans*, the citrus nematode. The same energy source and soil conditions were used as in the fungi tests.

Kill evaluations of the irradiated samples were done by the Nematode Investigations Section, U.S. Department of Agriculture in Orlando under Dr. John O'Bannon (22). Living nematodes were collected by sieving them through a 44 micron screen, washing them into a Petri dish and observing them with a dissecting microscope at x30 magnification.

Results obtained from nematode tests agreed with the published information by O'Bannon and Good (22) which indicated that the techniques used were valid.

**Results**

The results of the tests are given in tabular and graph form to provide a better prospective. (See Figures IV-1, Tables IV-1).

The results show that there was killing of fungus in less than 30 seconds in both dry and wet soil (15 percent and 45 percent moisture respectively). The nematodes were killed in less than 15 seconds using dry soil only (15 percent moisture). Expressed in equation form:

**Phytophthora parasitica** lethal points

- **Time:** \( 25 \leq t \leq 30 \) seconds
- **Temperature:** \( 78^\circ C \leq T \leq 85^\circ C \)
- **Energy:** \( 14,375 \leq E \leq 17,250 \) watt-seconds

(without the efficiency factor)
### TABLE IV-1a

<table>
<thead>
<tr>
<th>Time (seconds)</th>
<th>Population</th>
<th>Nematodes 50 cc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fungi (P. Parasitica)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dry 50 cc</td>
<td>Wet 50 cc</td>
</tr>
<tr>
<td>0*</td>
<td>Inf.</td>
<td>Inf.</td>
</tr>
<tr>
<td>5</td>
<td>Inf.</td>
<td>Inf.</td>
</tr>
<tr>
<td>10</td>
<td>Inf.</td>
<td>Inf.</td>
</tr>
<tr>
<td>15</td>
<td>Inf.</td>
<td>Inf.</td>
</tr>
<tr>
<td>20</td>
<td>Inf.</td>
<td>Inf.</td>
</tr>
<tr>
<td>25</td>
<td>Inf.</td>
<td>Inf.</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>45</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>120</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>300</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Control Sample

Note: Sample of 50 cc containing P. parasitica weighed 121.8 gm or 4.29 oz (.268 lbs.).

**Numbers represent area counts at specified intervals.
<table>
<thead>
<tr>
<th>Time (Seconds)</th>
<th>Temperatures (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 cc (Dry samples)</td>
</tr>
<tr>
<td>0</td>
<td>25.2</td>
</tr>
<tr>
<td>5</td>
<td>31.8</td>
</tr>
<tr>
<td>10</td>
<td>43.0</td>
</tr>
<tr>
<td>15</td>
<td>54.4</td>
</tr>
<tr>
<td>20</td>
<td>66.5</td>
</tr>
<tr>
<td>25</td>
<td>77.8</td>
</tr>
<tr>
<td>30</td>
<td>85.0</td>
</tr>
<tr>
<td>35</td>
<td>86.2</td>
</tr>
<tr>
<td>40</td>
<td>--</td>
</tr>
<tr>
<td>45</td>
<td>--</td>
</tr>
<tr>
<td>50</td>
<td>--</td>
</tr>
<tr>
<td>55</td>
<td>--</td>
</tr>
<tr>
<td>60</td>
<td>--</td>
</tr>
<tr>
<td>300</td>
<td>93.2</td>
</tr>
</tbody>
</table>

Note: 1) Sample of 50 cc containing *P. parasitica* weighed 121.8 gms.
2) Temperatures readings represent mean average of 4 separate tests.
Tylenchulus semipenetrans (Nematodes) lethal points

Time: \(10 \leq t \leq 15\) seconds

Temperature: \(43.0^\circ C \leq T \leq 54.4^\circ C\)

Energy: \(5750 \leq E \leq 8625\) watt-seconds

(excluding power efficiency factor)
RF Energy Source = 575 Watts
2450 MH
Dry off
point

50 cc Samples (122 gm.)
400 cc Samples

Fig. IV-1.--Time vs temperature for microwave irradiated soil samples containing Phytophthora parasitica (Fungus) and Tylenchulus semipenetrans (Nematodes).
V. DISCUSSION AND CONCLUSION

To satisfy the objectives stated at the onset, three determinations are in order:

1. How effective was the technique of microwave irradiation in controlling the fungus in the soil?
2. What effect did the process parameters have on the result?
3. What factors are involved in killing of organisms by microwave radiation exposure?

Conclusion Number 1

The microwave energy source was 100 percent effective in eliminating the Phytophthora parasitica in the soil after exposure time of 30 seconds.

Discussion

The fungus was killed in less than 30 seconds using a 575 watt, 42 percent efficient microwave source in a resonant cavity oven at 2450 MHz. There was some uncertainty of time and temperature relationships due to the 5 second intervals used in the test procedure. The energy requirements to accomplish this were:

(theoretical) 100 percent efficiency 14,375-17,250 watt-sec. (joules)

or (575 watts x 25-30 seconds)

(measured) 42 percent efficiency 6,040-7,250 watt sec. (joules)

or (14,375 x .42%) and (17.250 x 42%)
On a per gram basis, this was 49.4-59.3 joules/g absorbed energy using the 42 percent efficiency factor.

Calculation: \[ \frac{6,040 \text{ joules}}{122 \text{ gm.}} = 49.4; \quad \frac{7,250 \text{ joules}}{122 \text{ gm.}} = 59.3 \quad \text{(joules/g.)} \]

The specific heat is 0.22-0.24, a reasonable figure for the soil. Water is slightly greater than 1.0.

Specific Heat = \[ \frac{59.3 \text{ joules/g.}}{85^\circ \text{C}-25^\circ \text{C}} \times 0.239 \text{ calories/joule/g.} \approx 0.24 \text{ calories/}^\circ \text{C.} \]

The nematodes, too, were completely eradicated but at lower time and temperature values. The energy required to kill the nematodes was about one-half that of the fungus (19.8-29.7 joules/g). The specific heat of the soil containing nematodes was calculated to be approximately 0.24, which agrees fairly well with the fungus soil sample. To verify these figures with other reported data, Davis, Wayland and Merkle (8) report damage to plants was noticeable for energy levels of 45 joules/g with the most noticeable effect occurring in the first 60 minutes. In a more directly comparable study, O'Bannon and Good (22), using a 1250 watt microwave oven (efficiency unknown) reported complete killing of nematodes after 15 seconds at temperatures over the 49°-88°C range using two soil volumes.

Conclusion Number 2

The parameters affecting the microwave irradiation technique are: 1) water content, 2) soil type, 3) energy source, 4) organism type 5) energy penetration factors.
Discussion

The water content is an obvious factor since the soil is moist initially and dries off with time and temperature. This was observed and in fact was the basis for measuring the moisture content (dry weight basis). The slope of the time-temperature (t-T) curve is indicative of the rate of moisture dissipation (this also varies with energy). As the water becomes scarce (see Figure IV-1) at the upper end of the curve (saturation) the slope of the soil absorption rate dominates.

The t-T curve has three distinct phases. At the lower end, the slope increases from the initial temperature conditions with a "lag" period. This period is probably from the magnetron tube warm up time. The second phase is the water dissipation phase. During this period the water (moisture) is dissipating which in itself could be fatal to the aerobic fungus. After the moisture burn-off, the curve enters phase three which is, for soils, an area where the heat capacity, mentioned in section III, ranges from 0.2 to 0.8 (24) or one-fifth to four-fifths that of water. This is clearly observable on the curve in Figure IV-1. Davis, et. al. (8), report a definite increase in radiation susceptibility with higher water content.

The soil type is another observable factor and somewhat predictable from the difference in dielectric constants. (See section III) The soil characteristics dominate when the moisture has been removed and is responsible for the decrease in the curve slope in phase three. An index of soil heat capacity and dielectric constants was
not available but would be invaluable in further study efforts in this area.

The energy source and coupling efficiency are certainly factors in the system effectiveness. The abrupt changes are observable when the source is turned off or on. The exact relationship between the slopes in phases 2 and 3 of the curve and the applied energy would be necessary to optimize the equipment in any practical application.

The type of organism is a variable, observed by comparing the nematode "heat death" point (or uncertainty zone) with that of the fungus. The energy relationships are distinguishably different, indicating the possibility of a characteristic killing energy level, unique for each type of organism. To go further than this would be speculation. Much further work is needed to provide a further basis for action.

The depth of penetration was not a major factor in this test since the depth was not sufficient (approximately 3.0 cm) for limiting and analysis. However, there is adequate information regarding the characteristics of microwaves (section III) and the diurnal effects in soil (24) to conclude that there is an energy loss gradient. Further experience in this subject should provide the relationship between the energy density and the absorption levels required for extermination of the pathogens.

Conclusion Number 3

The thermal "kill mechanism" in organisms is a complex reaction and not definable from the experimental results. Other reports (8) (30)
note the same fact, namely, that much work is needed to provide the reaction parameters involved in "heat death" by microwave irradiation.

Discussion

Because of the uniqueness of the energy levels between the types of organisms and the fact that no appreciable difference was noted when irradiated with 45 percent moisture than with 15 percent moisture plus the soil volumes (50 cc, 400 cc) did not affect the kill times would provide a good basis for further study to determine the extent that the organism is directly influenced, regardless of its environmental test conditions.

Selectivity, or pasteurization, has been stressed throughout the report. The purpose of selectivity is to allow for a minimum disruption of the ecosystem while still providing a positive control of the undesired pathogens. If, as has been reported (8) (30) (31) and observed in these tests, the energy levels are unique for each organism, elimination by energy level (heat x time) selection is possible. Again, more information is needed to provide the relationships of other factors such as frequency, soil mass, organism mass, etc.

The feasibility of using microwave energy to control pathogenic, parasitic fungi in the soil has been demonstrated. The degree that it can be put to practical use is a matter for the design engineer. Meanwhile, commercially available microwave ovens could be used to control heat sensitive organisms in small soil samples such as the ones sent from engineering laboratories for analysis. Because it is
clean, non-polluting, portable and relatively inexpensive, this technique of heat treating can become a powerful tool to the small nurseryman and the larger "grower" as well.
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