A PRELIMINARY STUDY OF SOIL PASTEURIZATION

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There are many micro-organisms living in the soil, some of which are of parasitic nature and capable of producing serious plant diseases. Various methods of treating the soil to destroy these disease-producing organisms are recognized as important means of plant disease control. Some of the methods, at least, are commonly referred to as soil *sterilization.* This term is misleading, however, since it implies the destruction of all micro-organisms in the soil, some of which may be distinctly beneficial ones. Either of two terms might be used instead of the term sterilization. The term *disinfect* may be used to describe methods that free the soil of the disease-producing organisms, since these organisms do "infest" the soil but do not "infect" it. The term *pasteurization* may also be used to describe methods that destroy or inactivate or attenuate certain organisms, thus destroying their disease-producing capacity, without similarly affecting other components of the soil microflora.

In this paper we shall use the term *pasteurization* to imply destruction or inactivation of the disease-producing organisms. Pasteurization has proved quite satisfactory in greenhouses, seed beds, etc., where various methods are used extensively.

The soil may be pasteurized in various ways. The application of heat, either dry or in the form of steam, is advocated by some as being efficient and inexpensive, in spite of the fact that considerable equipment must be installed and maintained. A newer method of applying heat to the soil is by electrical means, using either a heating cable or copper electrodes placed in shallow soil 2 to 4 inches apart.

The use of various chemicals, such as the mercury salts, formaldehyde, acetic acid, caustic lime, copper sulphate, zinc chloride, sulphuric acid, calcium cyanamide, and others, has been recommended as very efficient in pasteurizing the soil, the fungicides varying considerably in concentration and method of application, depending on the type of soil, moisture content, and the organisms in the soil.

All of these methods have been used experimentally and some are being used commercially in the larger greenhouses and forest nurseries, where it is less expensive to pasteurize the soil than to furnish a new supply. There has been considerable thought that the temperatures used in pasteurization have been too high and that many of the beneficial organisms have been destroyed unnecessarily. Little work has been done on the determination of the thermal death point of the pathogenic soil fungi in order to know more definitely the temperature to which the soil should be subjected either to destroy these organisms or else inactivate them so that they will be unable to harm crop plants. It may be that the most objectionable organisms may be inactivated at such a low temperature as not to disturb other soil relationships. This study was undertaken to determine the thermal death point of some of the more important fungi causing "damping-off" of seedlings. It is concerned only with the thermal death point determinations *in vitro* in pure culture.

The following fungi commonly found in the soil were studied: *Sclerotium rolfsii, Fusarium lycopersici, Fusarium eumartii, Sclerotium*

delphinii, *Pythium debaryanum*, *Macrosporium solani*, and *Botrytis cinerea*.

Review of Literature

The results obtained by investigators vary considerably, due to difference in methods used and also to variation in organisms studied. A summary of results, however, seems to indicate that a temperature of 100° C. is beyond the temperature necessary to inactivate the pathogenic fungi studied. Bewly and Budden (**1**), in their study of the pathogenic organisms found in the water used for greenhouse purposes, claim that a temperature of from 50° to 70° for 1 minute is sufficient to kill the mycelium and summer spores of the fungi found in the water supply. In the study of greenhouse sterilization by Brown, Baldwin and Conner (**2**) the following soil fungi among others can be controlled by a temperature of 60° C.: Rhizoctonia on lettuce, and Pythium. The method is not given. Pathogenic organisms in the soil can be destroyed in 4-inch pots according to Byars and Gilbert (**3**) by submerging them for 5 minutes in water at 98° C. and in 8-inch pots by the application of 3,000 cc. of water heated to 98° C. This method varies considerably from some of the others in that these organisms are growing naturally in the soil.

In relation to *Sclerotium rolfsii*, Higgins (**4**) claims that its maximum temperature for growth is 40° C., but that 37° seems to be the maximum for continued normal growth. Jorgensen (**5**), in testing soil disinfectants, says that heating soil containing *Pythium debaryanum* 2-3 hours, 90°-95° C., definitely controls this fungus. Using electricity as a source of heat in sterilizing soil, Newhall (**6**) claims that *Rhizoctonia solani*, *Pythium ultimi* and *Fusarium* sp. can be killed in varying lengths of time by raising the temperature to 71° C. or less. According to Newton (**8**), however, the lethal temperature for *Rhizoctonia solani* is one hour at 50° C. for both mycelium and sclerotia. In order to get partial sterilization of soil for greenhouse work, Russell and Hutchinson (**9**) hold that the soil should be heated from 82°-94° C. in order to get the most satisfactory results, although Scheffer (**10**) claims that, in order to disinfect coniferous seed beds efficiently, they should be subjected to a temperature of 70° C. for a few minutes insuring this temperature to a minimum depth of 4 inches. Scott (**11**), using electricity to pasteurize soil in greenhouse flats and benches, holds with Russell and Hutchinson when he recommends a temperature of 82° C. as sufficient for sterilization and claims that electricity is a less expensive source of energy. *Macrosporium solani*, according to Wallace and Tanner (**12**), shows considerable variation in resistance to heat, depending on the solution in which it is placed while being heated. In salt solution it resisted 50° C. for 30 minutes, but was killed at 55° in 10 minutes. In a sugar solution the suspension was killed within 25 minutes at 50° C. and in 5-10 minutes at 55° C. In the case of *Sclerotium rolfsii (Corticium vagum)*, Watanabe (**13**) found that the sclerotia immersed in water were destroyed in 50 minutes at 50° C., and the mycelium in 30 minutes at 45° C., while in the air, a period of 60 minutes was necessary to kill sclerotia and 20 minutes for the mycelium.

Newhall and Nixon present evidence to show that a temperature

above 70° C. in the presence of adequate soil moisture is not necessary to kill several soil pathogenes.

Method

Several preliminary trials were made with a number of these soil fungi using variations in method until the following technique was standardized. One-half cubic centimeter of distilled water was put into each of a number of small thin glass test tubes (½x4 in.). The tubes were plugged and autoclaved at 15 lbs. for 20 minutes, then removed and allowed to cool. A circular piece of agar on which the fungus had been growing was placed in each tube aseptically. A pure culture of the fungus was made on potato dextrose agar, and pieces were cut out of it with a small sharpened cork-borer. The tubes containing the fungus were then placed through holes bored in a large cork and floated on the surface of a water-bath with a constant temparture regulator. The tubes projected down into the water to a depth of 4-5 in. A thermometer was inserted through a hole in the center of the cork so that the lower end was at the same level as the bottom of the tubes. Another thermometer was suspended in the water-bath, giving readings at a lower level. For higher temperatures paraffin oil was poured on the surface of the water forming a thin film, which reduced evaporation considerably.

The tubes and the cork were then allowed to float on the surface of the water, and the tubes were removed at five minute intervals up to 30 minutes. As each tube was removed it was taken to the inoculation booth and the contents poured on a petri plate containing solidified potato dextrose agar, and each plate was labeled. A series of six plates were so prepared for each temperature and then incubated at a temperature of 25° C. for 24-48 hours. Readings were then made to determine the highest temperature at which the various fungi were able to continue to grow. Identical sections of inoculated agar were plated in each case as controls at the same time as the test tubes were prepared. Only six tubes were prepared at one time, the maximum for a certain tempera-

TABLE I.

Pathogen	Period of Heating Minutes	Highest Survival Temperature Recorded Degrees C.	Period of Heating Minutes	Lowest Lethal Temperature Recorded Degrees C.
Sclerotium rolfsii	5	50	10	50
Fusarium lycopersici	5	65	10	65
Fusarium eumartii	10	55	15	55
Sclerotium delphinii	5	50	10	50
Pythium debaryanum	5	40	10	40
Macrosporium solani	30	45	5	50
Botrytis cinerea	10	55	15	55

ture. The maximum time for each temperature was 30 minutes, the tubes being removed at 5 minute intervals. The results are given in Table I.

It might be well at this time to call attention to the work of Kendrick and Gardner (13) in which they describe an apparatus to maintain a constant temperature in a water bath, by the addition of ice water or hot water as the need may be, this apparatus being for the purpose of determining the thermal death point. The paper was presented to the Academy meeting in 1922.

Discussion

There are several factors involved in thermal death point determinations which may under certain conditions alter the results. Some of the soil fungi form spores very readily, and the spores, because of their structure, would be able to withstand higher temperatures than the mycelium alone. The majority of the fungi used in this study form spores readily with the exception of *Pythium debaryanum*, in which case only the mycelium was used. In the case of *Sclerotium rolfsii* and *S. delphinii*, only the sclerotia were used, as they are quite large and easily handled.

All of the fungi studied were grown in pure culture on potato dextrose agar. It is quite possible that some of them might have developed into more resistant forms if grown on some other culture medium.

The technique could also be improved, I think, by the use of a liquid culture medium such as Richard's or Czapek's in the test tubes instead of the sterile distilled water, as the entire contents of the tube were poured on the agar plate. There is a possibility that the water on the agar plug may have had a tendency in some cases to inhibit the growth of both spores and mycelium.

The recommendation is generally made that greenhouse soil to be sterilized should be subjected to a temperature of 180° F. or 82° C. From the brief results obtained it might seem that this temperature could be somewhat reduced. However, it must be remembered that these determinations were made *in vitro* in pure cultures. The next step is to make thermal death-point determinations of these fungi in the soil.

The variation in the results for *Fusarium lycopersici* may be due to the fact that in the first test uniform sections of agar plus mycelium, 6 mm. in diameter were used, while in the second test the sections of agar and mycelium were not of uniform size. This also holds true for *Fusarium eumartii*, which gave a result of 45° C. for 20 min., using the uniform size, but was contaminated and therefore was not included in the results. Uniform sections were used, however, with Pythium, Macrosporium, and Botrytis, the diameter in the first two being 9 mm. and 6 mm. in the latter.

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