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The selective effect of heat treatment on the microflora of a greenhouse soil

G. J. BOLLEN

Laboratory of Phytopathology, Wageningen

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Abstract

Detailed information about the heat tolerance of soil micro-organisms, both pathogens and their non-pathogenic antagonists, is needed for the practice of soil pasteurization.
A device for testing the thermal death point of micro-organisms in soil is described.
The selective effect of heat treatment on the total numbers of bacteria, actinomycetes and fungi of a greenhouse soil (loam) was estimated. Of these groups the fungi proved to be the most sensitive to heat treatment. The heat tolerance of the species of this group was examined in more detail. In comparing the thermal death points of fourteen pathogenic fungi with those of the saprophytic fungal flora many species of the latter proved to be more heat-tolerant.

Introduction

In the last decade increased attention has been paid to the possibilities of pasteurization instead of steam-sterilization of greenhouse soils (Baker, 1962; Baker and Olsen, 1964; Dawson et al., 1965; Olsen and Baker, 1968). Therefore detailed data about the heat tolerance[1] of the elements of the microflora are indispensable. The minimum temperature required for pasteurization is determined by the thermal death-points of the pathogens. But, in choosing the pasteurization temperature, the heat tolerance of the non-pathogenic part of the microflora has also to be taken into account, since the non-pathogens act either as competitors for nutrients or as producers of antibiotics or as hyperparasites.
One of the arguments often adduced in support of pasteurization is the slower rate of recolonization by pathogens of pasteurized soils than of sterile soils (Baker, 1962; Bollen, 1967). The higher the temperature used for treating the soil, the more organisms will be killed and the higher will be the rate of colonization by reinvading pathogens. However, exceptions can be expected if a certain pathogen has one or more antagonists, which are slightly heat-tolerant but at the same time susceptible to the antagonism of temperature-sensitive organisms in the microflora. If so, it would be possible that after killing the less heat-tolerant organisms the former antagonists would increase sharply

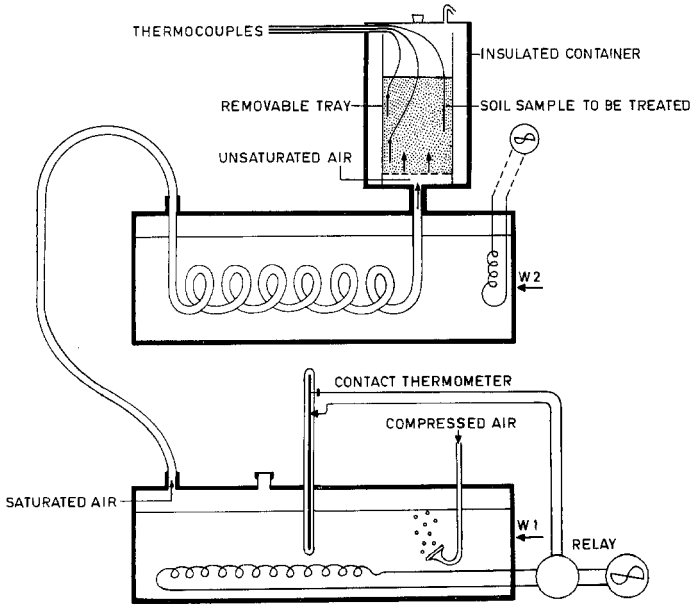
[1] The term thermotolerance has been avoided. This term is also often used for expressing the ability to survive high temperatures (e.g. Olsen and Baker, 1968). But Cooney and Emerson (1964), considering the ability of fungi to grow at various temperatures, reserved the term for "fungi which may grow at or near 50 °C, but which also grow well at temperatures below 20 °C". Our heat-tolerant isolate of *Trichocladium piriformis* is a fungus of this type.

so that particularly after soil treatment at relatively high temperatures, for instance at 70° or 80 °C, a slow recolonization of the particular pathogen could be expected.

Materials and methods

The trials were carried out with two greenhouse soils: loam, organic matter 13.8% from Bleiswijk and sand, organic matter 8.9% from Wageningen. The greenhouse soils were used for growing lettuce and tomatoes.
Samples of at most 1 kg were heated in a stream of hot moist air. The soil was kept at the temperature required for exactly 30 min, then cooled and immediately dried with cold dry air. During treatment the soil temperature was measured by thermocouples at different levels in the soil.
The samples to be pasteurized were put into a removable tray and this was placed in an insulated container (Fig. 1). The temperature and the relative humidity of the steam-air mixture used were controlled with two thermostatic waterbaths. In the first one the air was heated and water-saturated. To prevent the soil becoming too wet, the saturated air was passed through a spiral placed in the second waterbath which was kept at a higher temperature so that the air became unsaturated. The relative humidity of the air was chosen according to moistness of the soil sample and temperature at which the soil had to be treated.

Fig. 1. Apparatus for pasteurizing small amounts of soil



W1. AND W2.–THERMOSTATIC WATERBATHS

Fig. 1. Apparaat voor het pasteuriseren van kleine hoeveelheden grond

Because of the low specific heat of air in relation to water, the temperature in the first waterbath mainly determined the temperature required in the soil, whereas the temperature of the second waterbath controlled the moisture of the soil during treatment. The soil moisture was adjusted to about 50 % of field capacity.
With this device it was possible to heat the soil quickly to the temperature required, for instance within 3 min to 50 °C and within 7 min to 80 °C. At the beginning of the treatment the flow rate of the air was adjusted to 35–40 litres per minute. As soon as the temperature required had been attained, the flow rate was decreased to 10–15 l/min.
The microflora after heat treatment was analysed by the soil-dilution plate method and the soil-washing technique. The media applied in the first method were: (a) soil agar (bacteria and actinomycetes; Lochhead, 1940, modified by Johnson et al., 1959); (b) soil agar with 50 ppm Terramycin (fungi); (c) potato dextrose agar with 25 ppm Terramycin and 50 ppm pimaricin and cherry agar with 50 ppm pimaricin (pythiaceous fungi), and (d) cellulose agar (cellulose decomposing fungi; Witkamp, 1960). In the soil-washing method 2 g soil were thoroughly washed with sterile water thirty times by a modification of Parkinson's soil-washing apparatus. The soil particles were plated out on soil agar with 50 ppm Terramycin. Since all the plates were incubated at 23 °C, the thermophilic microflora was not included in our trials.

Results

Heat tolerance of the various groups of the soil microflora
Differences in sensitivity to heat of the various soil organisms were indicated by observing the pasteurized soils in sterilized glass tubes 2–3 weeks after incubation. If moist soil was kept in daylight, algae, mostly unicellular Chlorophyceae developed abundantly in samples treated at temperatures up to 65 °C. On soil treated at 70 °C algae rarely grew; if treated at higher temperatures no algae could be detected.
At the same time clear white or greyish colonies of Streptomycetes developed on soil samples treated at temperatures from 60° or 70 °C and kept in sterilized glass tubes at room temperature for about 2 weeks, particularly if the soil was aerated with sterile air. In practice greenhouse soils often become red, brown or green from various fungi after steaming. This is more likely to be due to reinvaders than to survivors.
The surviving bacterial, actinomycetal and fungal flora of the greenhouse loam soil (Bleiswijk) was estimated by the soil dilution plate method. Fig. 2 summarizes the results of seven trials with 2–5 replicates for each medium and each dilution. The fungi proved to be the most sensitive; heat treatment at 60 °C reduced the number of colonies to 5 % of those from untreated soil.
Heating the soil for 30 min at 80 °C decreased the number of colonies of bacteria to 1.8 %, of actinomycetes to 1.0 % and of fungi to 0.1 %.

Heat tolerance within the group of fungi
The composition of the fungal flora surviving heat treatments was estimated by the soil dilution plate method and the soil-washing technique. The heat tolerance of the various species was very different. In seven trials using both greenhouse soils data obtained in any one species proved to be fairly consistent. Thus the heat tolerance seems to be species-specific.
The Oömycetes proved to be very sensitive. Although about 300 colonies per gramme

Fig. 2. Survival of micro-organisms after heating the soil for 30 min

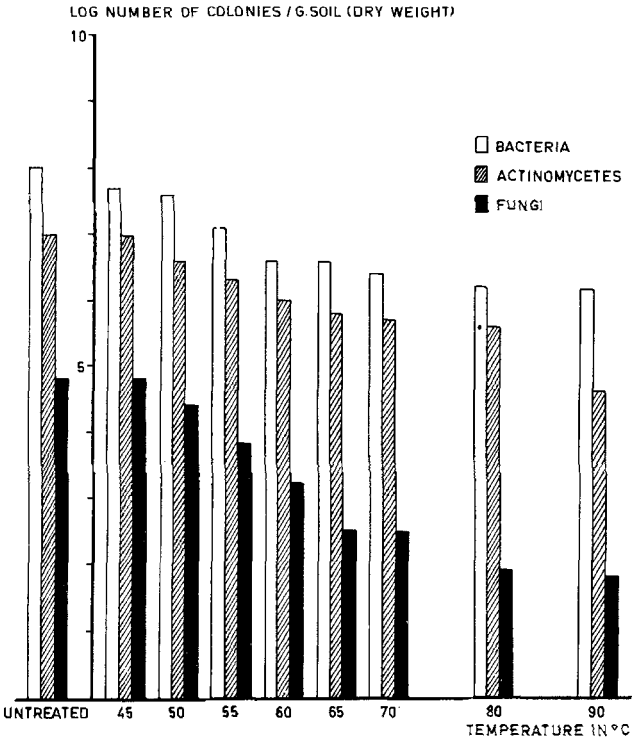


Fig. 2. Overlevende microörganismen na een warmtebehandeling van de grond gedurende 30 min

soil on dry basis were obtained, both in untreated soils and in soils treated at 45 °C, not a single colony could be isolated after treatment at 50 °C. As will be seen later two pathogenic *Pythium* spp. could just survive this treatment.

Of the Zygomycetes, many *Mucor* and *Mortierella* spp. were killed after treatment at 50 °C. A few *Mucor* spp. and *Zygorhynchus moelleri* survived treatment at 50 °C but not at 55 °C.

A wide range in the thermal death points was found in the Ascomycetes. *Chaetomium* spp. were killed between 50° and 55 °C. For many other species the thermal death-point was between 55° and 60 °C, for instance, of *Preussia fleischhakii* and some *Sordaria* spp.

Sporormia aemulans and *Sordaria carbonaria*, were killed between 60° and 65 °C.

The spores of species of Eurotiaceae occurring in these soils proved to be highly resistant. They were killed by soil treatments at 65° to 80 °C. For instance, *Talaromyces helicum*, *T. wortmanni* and *T. vermiculatum* survived 65 °C, *Carpenteles brefeldianum* and *C. baarnense* 70 °C and *Sartorya fumigata* 80 °C. *Byssochlamys nivea* (Gymnoascaceae) was still present after treatment at 70 °C.

Few data can be given on the Basidiomycetes. They were not often isolated by the soil

dilution plate method nor with the soil-washing technique. Sometimes *Coprinus fimetarius* was found, but never in soils heated to at least 50 °C. But there were too few isolates to draw definite conclusions about the exact thermal death-point.
The most common soil-inhabiting fungi, the Deuteromycetes, were the most variable in sensitivity to heat. Data were obtained for sixty species. Many *Fusarium* spp., some *Paecilomyces* spp. and most of the *Trichoderma* isolates were rather temperature-sensitive, for they were not detected after soil treatment at 50 °C; however, *Trichoderma lignorum* sometimes appeared on the soil dilution plates with up to 55 °C. The following species were moderately heat-tolerant (thermal death-point between 50 ° and 60 °C): *Cladosporium herbarum*, *Hormiscium* sp., some *Phialophora* spp., *Rhinocladiella mansonii*, *Stachybotrys atra*, *Doratomyces* spp., *Myrothecium verrucaria*, *Verticillium* spp., a few *Fusaria* such as *F. oxysporum* and *F. redolens*, some *Aspergilli* and nine out of the fourteen *Penicillium* spp., which had been isolated.
For the following heat-tolerant species, the maximum temperatures at which they survived are recorded between brackets: *Stemphylium botryosum*, *Phialophora mustea* and *Penicillium corylophilum* (60 °C), *Aspergillus fumigatus* (65 °C), *Penicillium funiculosum* and *P. lapidosum* (70 °C), *Phoma herbarum* (75 °C) and *Trichocladium piriformis* (80 °C). *Gilmaniella humicola* was the most heat tolerant fungus found (90 °C).

Heat tolerance of some pathogenic fungi
To compare the temperature sensitivity of pathogens with saprophytic fungi, fourteen pathogens were chosen from different systematic groups. Pure cultures in soil amended with 5% oatmeal or not amended were treated at various temperatures with intervals of 2.5 °C. Table 1 shows that none of the pathogens tested had been able to survive at 60 °C.

Table 1. Tolerance of some pathogens to heat treatment for 30 min

Pathogen	*Host plant*	*45.0*	*47.5*	*50.0*	*52.5*	*55.0*	*57.5*	*60.0*	*± 0.2 °C*
Cylindrocarpon destructans*	cyclamen	+	+	−	−	−	−		
Didymella lycopersici*	tomato	+	+	−	−				
Fusarium oxysporum	freesia			+	+	+	+	−	
F. redolens	carnation			+	+	+	+	−	
Phialophora cinerescens	carnation		+	+	+	−	−		
Phytophthora cryptogea*	gerbera	+	+	−	−	−			
Pythium irregulare	pea	+	+	+	−				
P. ultimum	pea	+	+	−	−				
P. sp.*	spinach	+	+	+	−				
Rhizoctonia sp.*	cyclamen		+	+	−	−	−		
R. solani*	tomato		+	+	−	−	−		
Thielaviopsis basicola	cyclamen	+	−	−	−				
Verticillium albo-atrum	potato		+	+	−	−	−		
V. dahliae	tomato			+	+	+	−	−	

The heat-tolerance of the pathogens marked with * was estimated both for pure cultures in soil and for diseased plant material buried in soil before treatment. (+) and (−) mean survival and death, respectively.

Tabel 1. Tolerantie van enkele pathogenen ten opzichte van een warmtebehandeling gedurende 30 min

The morphological form in which the pathogens in the soil occurred was not exactly known. Although the trials had been triplicated it is possible that the most resistant structure was not present in the soil treated.

Discussion

Fig. 2 shows that with increasing temperatures from 60° to 80 °C, the survival of fungi decreases much faster than of bacteria. This phenomenon is probably due to spore formation of the heat-resistant bacteria. Baker et al. (1967) and Olsen and Baker (1968), showed that the antibiotic activity of some strains of the ubiquitous and heat-tolerant *Bacillus subtilis* is very high, so that these bacteria are suitable for biological control, for instance of the common pathogen *Rhizoctonia solani.*

Of the fungi it was obvious that those *Penicillia* and *Aspergilli* possessing a perfect stage (*Carpenteles, Talaromyces, Sartorya* and *Eurotium*) were less sensitive to heat treatment than those species lacking cleistothecia. Exceptions were *Penicillium funiculosum* and *Aspergillus fumigatus.* Some of the heat-tolerant ones are known for their antagonism to pathogens, e.g. *Talaromyces vermiculatum* (Husein and McKeen, 1962).

The widest range in heat tolerance was found within the Deuteromycetes: *Thielaviopsis basicola* being very sensitive and *Gilmaniella humicola* even surviving 90 °C. This diversity is correlated with the great differences within this systematic group.

Trichoderma spp. are often considered to be survivors after soil steaming in practice (Welvaert, 1962). But we found little evidence that these fungi could survive even a moderate heat treatment. Their appearance in the surface soil within a few days of steaming must be due rather to rapid colonization by rapid mycelial growth and abundant sporulation, than to survival.

Some Ascomycetes (*Byssochlamys nivea, Carpenteles brefeldianum* and *Sartorya fumigata*) and few Hyphomycetes (*Gilmaniella humicola* and *Trichocladium piriformis*) were only found after treatment of the soil at 55 °C or more. Warcup and Baker (1963), using ethanol as a selective agent for Ascomycetes, described the increase in colonies of Ascomycetes and some Hyphomycetes on soil-dilution plates after heating the soil. This was caused by breaking the dormancy of spores. In our trials selective agents have not been used, so that it cannot be here concluded that the phenomenon was caused by breaking the dormancy of these fungi. Perhaps the growth of these fungi was suppressed by the less heat-tolerant species in the dilution plates of soils, which were either left untreated or treated at lower temperatures.

Comparing the heat-tolerant fungi from our experiment with the fungi tolerant to the fumigants most frequently applied (Domsch, 1959, 1963; Wensley, 1953), it can be concluded that heat-tolerance is by no means correlated with chemotolerance.

The heat-tolerance of the pathogenic fungi tested (Table 1) was usually low so that control of these fungi by pasteurization has good prospects.

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Samenvatting

Het selectieve effect van warmtebehandeling op de microflora van kasgrond

In verband met de toenemende belangstelling voor pasteurisatie van kasgrond is een grondige kennis omtrent de afstervingstemperaturen van de bodemorganismen – zowel pathogenen als hun niet-pathogene antagonisten – noodzakelijk. In een voor dit doel geconstrueerd apparaat werd vochtige lucht van verschillende temperaturen door kleine hoeveelheden kasgrond geblazen. Daarna werd de grond geanalyseerd op de overlevende microflora. De schimmels bleken als groep verhitting minder goed te kunnen doorstaan dan de bacteriën en de actinomyceten. Vooral de Oömyceten waren zeer gevoelig. Zeer tolerant waren enkele donkersporige Deuteromyceten en die *Penicillium*- en *Aspergillus*-soorten, welke een perfecte vorm hebben. De als antagonisten van pathogenen beroemde *Trichoderma*-soorten bleken weinig tolerant.
De afstervingstemperaturen van veertien pathogene schimmels bleken lager te liggen dan die van veel saprofyten.

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