SOIL STERILIZATION FOR SEED-BEDS AND GREENHOUSES

By Walter G. Sackett
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*On leave, 1926-1927.
SOIL STERILIZATION FOR SEEDBEDS AND GREENHOUSES

By Walter G. Sackett

Our estimate of values and the significance we attach to objects around us are determined largely by our personal contact and acquaintance with them.

A fishmonger from the Atlantic Coast would be interested in visiting the fish markets of Chinatown, San Francisco, but he would care very little for the exhibits of old china in the museum at Golden Gate Park. However, a connoisseur of fine porcelain could spend days at the museum without tiring, but he would not even take the trouble to inquire the location of the fish markets.

So it is with the city business man or woman who has had little opportunity to become acquainted with agriculture and its related branches. To them, the thousands of acres of summer fallow so common in the dryland sections of the West present nothing but dust, dirt and dreary desolation. Yet, the farmer who has cultivated this land, who has harvested the crop, and who knows its potential possibilities, gives it a very different rating. He thinks of these apparently barren wastes in terms of waving grain and golden corn and refers with pride to the wonderful productivity of his soil. If he is cultivating new land, he is apt to be quite optimistic regarding its fertility. This is perfectly natural, since to him, the soil is an inexhaustible storehouse of plant food composed of a mixture of minerals and organic matter capable of producing crops if properly tilled and watered.

But is this conception entirely correct? Is the supply of plant food inexhaustible? Is the soil merely an accumulation of inert, lifeless ground rock and humus? Because of his limited technical knowledge, the average farmer cannot answer these questions since they involve principles not gained thru practical experience, and he must turn to the specialist for further light.

The investigations of the microbiologist have shown that the soil is populated with countless millions of living, moving, breathing micro-organisms, and that its continued and increasing fertility is due, in a large measure, to the ceaseless activity of this invisible multitude. It is indeed difficult to visualize our fields and garden plots, our seedbeds, flats and greenhouses as being literally alive with bacteria, fungi (molds) and protozoa, so small as to be seen only with the aid of a powerful microscope, but this is true nevertheless, and what they lack in size is compensated for in numbers. The bacteria and fungi belong to the plant world, while the protozoa are classified in the animal kingdom. Many of these are indispensable to the maintenance of soil fertility, and therefore the farmer and truck grower should follow such practices as will favor the growth and development of the beneficial species.

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But here the story changes. Just as there are undesirable members in the society of men who are unwilling to work and who resort to the foul practices of the outlaw and the bandit to make a living, so too, there are bacteria and fungi in the soil population which thrive at the expense and even extermination, not only of the beneficial bacteria, but also of cultivated plants. Thousands of dollars worth of field and garden crops are lost annually thru the ravages of these plant diseases commonly described as blights, wilts, rots, galls, leaf spots and rusts. Is there no relief from this condition of affairs? Must man forever submit to this insult to his intelligence and meekly admit that he has been outwitted by an army of microbes, or is there some hope that thru investigation and education he will find means, eventually, for the annihilation of his arch-enemy, the disease germ?

Decided progress has been made in the control of animal diseases by observing recognized sanitary and hygienic measures, and there is no reason why comparable results should not be obtained with plant diseases if similar fundamental principles are applied here. The chief obstacle to advancement along these lines is the fact that the average gardener will take no preventive steps as long as he can produce a fifty percent crop, and it is not until he is face to face with total failure that he will give the recommendations of the specialist even a trial. This usually comes too late to be of any value, and the next season, instead of following the professional advice given him, he hopes against fate that somehow conditions may be different this year, and the precautionary preventive measures of the preceding year are either ignored or forgotten with the same inevitable results.

Until the grower learns that it is just as important for him to follow the detailed instructions of the plant-disease specialist to the letter, as it is for him to give medicine according to the doctor's orders, we can hope for but little progress in the control of plant diseases. Prevention, not cure, is the recognized method of controlling disease whether we have in mind man, animal or plant.

It is a well-established fact that many plants become diseased while still in the seedbed or flat and are so weakened by this attack that they either do not develop into normal plants when set out in the field, or they succumb more readily to later infection.

In the pages which follow, methods are described for sterilizing the soil of seedbeds and greenhouses. If this is done carefully and thoroly, disease-producing micro-organisms will be destroyed, and hardy, vigorous plants, better able to withstand adverse conditions, will result.

**METHODS OF STERILIZATION**

Altho different chemical agents, particularly formaldehyde, have been advocated for sterilizing soil, their use has been rather limited and con-
fined largely to experimental work. For large acreages the expense is prohibitive, not to mention the questionable efficiency and possible detrimental residual effect upon the soil.

Any method of sterilizing soil which is to become a general practice among gardeners and greenhouse men must be cheap, practicable and efficient.

Sterilization by means of live steam satisfies all of these requirements and can be carried out according to either one of two procedures:

1. The perforated-pipe system, suited to either indoor or outdoor conditions.
2. The inverted-pan method, better adapted to outdoor use.

The Perforated-Pipe Method

Briefly, this method consists in heating the soil to a depth of 10 or 12 inches by means of live steam under pressure introduced thru perforated pipes laid under ground.

Preparation of Pipes.—The description of the equipment given here is that of the installation used by the Toothaker Greenhouse Company of Denver, Colorado. The method has proved eminently satisfactory, and while the general arrangement of the pipes might be modified to suit the shape and size of the area to be sterilized, it is recommended that the plan as a whole with respect to size of pipes, perforations, hose connections, steam pressure, depth of pipes and time of exposure be adhered to quite closely.

Material Required for Sterilizing 400 Square Feet at One Setting.—

11 pieces 2-inch steam pipe, 20 feet long, threaded at both ends.
11 2-inch iron caps.
10 reducing elbows—2-inch to 1-inch.
10 pieces 1-inch pipe, 20 inches long, threaded both ends.
10 pieces 1-inch pipe, 6 inches long, not threaded.
10 steam valves for 1-inch pipe.
10 2-inch brass nipples.
10 pieces 1-inch steam hose.

Fig. 1.—Diagram showing position of holes in perforated pipes with reference to circumference.
20 hose clamps for 1-inch steam hose.
10 1-inch hose couplings.
1 2-inch union.
1 2-inch steam valve.
2 2-inch nipples—3 inches long.
1 2-inch elbow.
1 2-inch T.
2 pieces 2-inch pipe, 1 foot long, threaded 1 end.

The 2-inch pipe, thru which the steam is released in the soil (10 pieces 20 feet long) is to be drilled with 3 lines or rows of 3/16-inch holes, the latter 6 inches apart. In drilling these, it is important that the lines be properly placed and spaced with relation to each other and to the circumference of the pipe. First drill one row in a straight line from end to end; next, rotate the pipe a quarter turn or thru an angle of 90 degrees, then turn it back a quarter of an inch so that a hole drilled with the pipe in this position will come just a little below the diameter drawn at right angles to the one passing thru the first line. Alternate these holes with those of the first row so the holes in the two lines will be 3 inches apart in a lengthwise direction. The third row is drilled on the opposite side of the first and bears the same relation to it as the second row. The holes should be opposite those of the second and alternate with those of the first. The arrangement of the holes is shown diagrammatically in Fig. 1.

When the pipe is in position for use, the middle line of holes is on the bottom. It has been found that if the lateral rows are drilled a little below the center, the steam penetrates the soil to a greater depth before escaping than when they are made exactly in line with the diameter. This seems to be due
to the slightly downward rather than lateral direction of the steam when it first enters the soil.

After the pipes have been drilled, each is fitted with a cap on one end and a reducing elbow on the other; one end of a one-inch pipe, 20 inches long, is screwed into the latter, and a steam valve which carries a short brass nipple is attached to the other end. This assembly is shown in Fig. 2.

The remaining 2-inch 20-foot pipe is for the header which supplies the 10 perforated pipes with steam. Ten 1-inch holes are drilled in this, with 24-inch centers, lengthwise in a straight line; placing the first hole 6 inches from the end. Into each of these a piece of 1-inch pipe, 10 inches long, is welded (Fig. 3). A piece of 1-inch steam hose is next slipped over each of the 1-inch pipes and clamped in place. The free end of the hose is equipped with a 1-inch hose coupling which connects with the brass nipple of the steam valve on the perforated pipe assembly (Fig. 2). This completes the pipe connections except for the 2-inch union which is attached to one end of the header and the 2-inch cap which closes the other end (Fig. 4).

The blowout assembly is placed between the union and the steam supply by means of a T and is constructed as shown at the left in Fig. 5. The union connects the sterilizing device with the steam boiler.

Sterilizing Procedure.—The description which follows applies to surface beds, but is equally applicable to benches.

Begin the operation either at one end or at the side of the house by
digging a narrow trench 10 inches deep adjacent to the outside wall and then an additional trench parallel to the first for each of the perforated pipes, spacing them approximately 2 feet apart. (Fig. 6.) Lay the pipes
Fig. 6.—Trenches ready for perforated pipes.

Fig. 7.—Perforated pipes in position.
Fig. 8.—Covering pipes and spading soil between trenches.

Fig. 9.—After spading the bed, it is covered with heavy canvas.
in the trenches with the valves upright and all in the same direction (Fig. 7). Replace the soil in the trenches and spade deeply all of the intervening space. (Fig. 8). Wherever a path crosses a bed or extends thru the house, see that a pipe is laid on each side of the walk so that the passageway is heated as well as the planting surface. Cover the area to be heated with a heavy canvas (Fig. 9) and connect the valves with the steam hose of the header (Figs. 10 and 11). Close all the perforated pipe valves and open the blowout valve. Turn steam into the line from the boiler under about thirty pounds pressure, and as soon as all the water of condensation has escaped from the line, close the blowout and open the farthest perforated pipe valve. When vapor escapes from the soil over this pipe, open the one next to it and so on until all are in operation. It is better to open them one at a time as directed rather than all at once, since the greater pressure maintained by this procedure tends to clear the holes more effectively.

After about one-half hour, a dense cloud of vapor should be visible above the canvas (Fig. 12) but this does not mean that the soil thruout the bed has reached the proper temperature. We have found by repeated tests that after three to three-and-a-half hours' heating a thermometer placed anywhere in the bed shows a temperature around 203 degrees F. When this has been accomplished, the steam may be turned off, and the bed kept covered for twenty-four hours to confine the heat.

After a plat has been treated, the greatest care must be exercised not
Fig. 11.—Steam hose connection made between header and perforated pipes.

to contaminate it by walking over it or allowing any tool, flat or other article that has not been sterilized, to come into contact with it.

In a large house it is usually more convenient to make the second setting in line with the first than at the side of it. This is done most

Fig. 12.—Steaming process in operation.
easily by continuing the original trenches in the same direction and by withdrawing the perforated pipes lengthwise into the new trenches. (Figs. 6 and 7.) This obviates the necessity of digging up the pipes or lifting them out of the soil.

All flats, tools and other equipment to be used in connection with heated soil, should be sterilized by putting them under the canvas while the soil is being heated. Large pans of three percent lysol should be placed at frequent intervals in the paths and at outer entrances of the houses. All persons should be required to immerse the soles of their shoes in this disinfectant before entering the houses or walking on the heated beds (Fig. 13). These are precautions which may cause some to smile, who are not acquainted with the habits of micro-organisms, but it would be the height of folly to go to the expense of sterilizing the soil, and then contaminate it immediately by the thoughtless introduction of infectious material on the shoes. As an added precaution, chlorinated lime or bleaching powder may be sprinkled in the passageways. There is much to be said in favor of changing both the shoes and overalls when going from outdoor to inside work where the soil has been sterilized; in fact, this practice is in force in some of the largest greenhouse establishments in the country.

With a steam pressure of 30 pounds it is possible during the summer months, to raise the temperature of the entire bed, approximately 400 square feet, to 200 degrees F. to a depth of 10 inches in 3 to 3½ hours. Considering the length of time required to accomplish this, the probability is that most of the harmful animal parasites and plant-disease micro-organisms will have been killed. Along with these, many of the beneficial bacteria (nitrogen fixing and nitrifying) may have perished also, but these will be restored to the soil in a relatively short time thru watering, cultivation and the dust in the air.
Brown\(^2\) gives the following table relating to the temperatures necessary for the control of common greenhouse soil pests:

**Table No. 1—Temperatures Necessary for the Control of Common Greenhouse Pests.**

<table>
<thead>
<tr>
<th>Pest</th>
<th>Temperature Controlling Pest—Few Minutes Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nematodes(^2)</td>
<td>140° F.</td>
</tr>
<tr>
<td>Pythium(^2)</td>
<td>140° F.</td>
</tr>
<tr>
<td>Rhizoctonia (on lettuce)(^2)</td>
<td>176° F.</td>
</tr>
<tr>
<td>Sclerotinia (on lettuce)(^3)</td>
<td>176° F.</td>
</tr>
<tr>
<td>Septoria lycopersici-spores (on tomato)(^4)</td>
<td>127° F.</td>
</tr>
<tr>
<td>Colletotrichum lindemuthianum</td>
<td></td>
</tr>
<tr>
<td>(Anthracnose on beans)</td>
<td>119° F.</td>
</tr>
<tr>
<td>Spores(^5)</td>
<td></td>
</tr>
<tr>
<td>Mycelium(^5)</td>
<td>149° F.</td>
</tr>
</tbody>
</table>

From the standpoint of effective heating, we feel that the perforated-pipe method is a success. It was checked in one instance where the soil

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*Fig. 14.—Cucumbers growing in soil heated for 3½ hours at 200 degrees F.*
was known to contain both Fusarium and Nematodes, by heating it for three and one-half hours and then planting it to cucumbers and tomatoes (Figs. 14 and 15). At no time during the growth of the vines was there any evidence of Fusarium. After the crop was harvested, the roots were dug up, and out of 206 cucumber and 1977 tomato plants, not a single root showed the slightest trace of nematode injury where the soil was heated to 200 degrees F. However, beside one path, where the soil did not reach the temperature mentioned, there were about 80 tomato plants with nematode root galls.

When the expense of treating the soil in this way is considered, there are two objections to the method. One is the time required to dig trenches and fill them up again; the other is the length of time necessary to raise the soil temperature to 200 degrees F. throughout the bed. If the heating period could be cut in half, it would mean a very material saving in fuel cost. A suggested modification of the present system which might remove these difficulties follows:

In place of the perforated pipe, there might be substituted a battery of 2-inch pipes with 1-inch nipples 10 inches long, welded into the pipe every 6 inches. The lower end of the nipple would be capped and just above the cap 4 to 6 3/16-inch holes would be drilled. It is believed

Fig. 15.—Tomatoes growing in soil heated for 3½ hours at 200 degrees F.
that if the beds were spaded deeply, this assembly of nipples could be forced into the soil the desired 10 inches, and thus the necessity of digging a trench would be obviated. In order to shorten the heating period, the number of units could be doubled so that there would be a line of nipples every foot instead of every 2 feet as with the perforated-pipe scheme. The plan is essentially a modified steam harrow.

The Inverted-Pan Method

The inverted-pan method for sterilizing soil utilizes the heat of live steam for accomplishing the desired end just as described above, except that the steam is applied in a different manner. The equipment required is more simple and the expense is correspondingly less. Briefly, it consists of a shallow wooden box, steam tight, turned upside down over the soil, beneath which steam under pressure is liberated. (Fig. 16). However, it cannot be depended upon for deep sterilization, does not give as satisfactory distribution and penetration of the steam, and is somewhat more unwieldy to handle. For outdoor seedbeds, where the surface 4 to 6 inches is all that need be considered, it serves the purpose very satisfactorily.

Construction of Steaming Pan.—The size and shape of the pan will be determined largely by the beds where it is to be used. If the soil to be sterilized is that of hotbeds or coldframes, the pan should be wide enough to fit into the frame snugly and of such a length that it can be moved lengthwise of the bed conveniently.

Fig. 16.—Inverted steaming pan. (After Reinbort Farmers' Bul. 996, U. S. Dept. of Agriculture.)
A pan 6 feet wide, 12 feet long and 4 inches deep is well adapted to general use. This gives an area of 72 square feet and covers as much surface as can be sterilized properly with a 20 to 25 horse-power boiler maintaining 80 pounds pressure.

A rectangular frame 6x12 feet is made of 2x4-inch material. This is covered with 4-inch matched siding, ⅞ of an inch thick, the boards being laid crosswise of the frame the 6-foot way. In order to prevent the escape of steam, it is advisable to fill the joints between the boards with white lead as each is nailed in place. To keep the cover from pulling apart and warping, it is necessary to secure it further by two 10-inch boards laid lengthwise of the frame. The matched boards are nailed firmly to these from the inside, the nails being set at an angle and driven thru the tongue just as in laying flooring. After this is done, the frame may be turned right side up and the boards nailed well at the ends.

A ⅜-inch hole is next bored at one end in the middle of the 2x4 frame. Into this is inserted a ¾-inch threaded pipe nipple, 6 inches long. The latter is held in position by lock nuts inside and outside the 2x4 with leather washers next to the wood to make the joint tight. By means of this, the pan is connected with the steam hose from the boiler.

If the pan, as described thus far, were to be inverted over the soil and the steam turned on, it would be found that there would be very little penetration of the heat due to the escape of steam between the edge of the frame and the soil. To overcome this difficulty, a narrow strip of felt packing, ¼-inch thick and 2 inches wide, is tacked around the entire frame, on the outside, flush with the lower edge. On top of this and encircling the frame, there is nailed, at frequent intervals, a strip of hoop iron or angle iron, one-half the width of which projects below the bottom edge of the 2x4. This arrangement gives a knife-edge contact with the soil and the felt packing reduces the leakage between the frame and hoop iron.

To facilitate moving the pan a large screw eye should be inserted at each corner thru which a pick handle or bar can be thrust to serve as a handle.

**Preparation of the Seedbed for Steaming.**—As steam penetrates frozen ground with difficulty, beds in this condition should be covered with glass sash 2 to 3 weeks in advance of steaming so the soil may thaw. The beds are prepared by spading to a depth of 8 to 10 inches, the desired fertilizer is applied, mixed well with the soil and the surface is left in a finely pulverized condition so that, after steaming, a light raking is all that will be required before seeding. Any dilution material which is mixed with the seed to facilitate sowing should be sterilized first.

Since steam penetrates moist soil rather slowly, it is important to have it as dry as possible before starting the operation. Similarly, the heavier clay soils do not heat as readily as the lighter sandy loams, and for this reason additional time must be allowed for steaming them.
Operation of the Steaming Pan.—To accomplish the work satisfactorily, it is necessary to have an adequate and dependable source of steam. According to Beinhart\(^1\) whose bulletin I have taken as the basis for this part of the subject, a 20 horse-power boiler will fulfill the requirements. If a portable boiler or steam tractor is used, it should be located as near the beds as possible in order to reduce the amount of condensation in the supply line as well as the expense of a long steam hose. A steam pressure of 80 to 100 pounds should be maintained in the boiler all the time the pan is in operation, for effective heating. Pressure is necessary for penetration.

The pan is placed over the bed with the iron edge in contact with the soil, and the latter banked up all around the outside to prevent the escape of steam from beneath. The feed line from the boiler is next connected with the pan by means of the nipple in the end of the frame as described previously, and the steam is turned on. The bed is heated in this manner for 30 minutes at the end of which time the steam is turned off and the pan moved to an adjoining area, the precaution being taken to allow an overlapping between settings of about 6 inches. The plot just heated should be covered at once with heavy canvas to retain the surface heat and induce further penetration.

Under average conditions, this treatment will give the following soil temperatures after 30 minutes:

- Surface 2 inches: \(208^\circ-212^\circ\) F.
- 3 to 4 inches: \(170^\circ-180^\circ\) F.
- 6 inches: \(120^\circ\) F.

If the bed is covered with canvas, the temperature at 6 inches will be found to have raised to 160 degrees F. at the end of 2 hours. A simple way of telling whether the job has been well done is to bury a medium-sized potato 4 inches deep in the soil; when the pan is removed this should be well done and mealy like a baked potato.

Cost of Steaming.—The expense of carrying on this work will be determined largely by two factors—whether the grower has a boiler available or must rent one, and whether he has unemployed labor at home or must depend upon additional outside help. Ordinarily, this steaming can be done early in the spring before general farming operations are in progress, and when the regular laborers are not particularly busy.

Approximately one-half a ton of coal is consumed in steaming 1,000 square feet which is the average area covered in a 10-hour day. The time of one man is required throughout the day to fire the boiler and three helpers must be available for short periods to assist in moving the pan.

The item of weeding should not be overlooked in considering the expense of heating seedbeds. It is estimated by those who are following this practice that two good weedings usually costs as much as the steaming which eliminates the weeds entirely, and therefore, the soil treatment has
cost nothing, not to mention the benefits which have resulted. Due to the fact that the soil is warm, the seed that is planted germinates more quickly, more uniformly and in larger numbers; the plants are more vigorous and are ready for transplanting 10 to 14 days earlier than those grown under ordinary conditions. Because of this improved condition for germination and the absence of damping-off diseases, the amount of seed sown can be materially decreased since no allowance need be made for those unfavorable factors which so often deplete the stand of seedlings.

TECHNICAL CONSIDERATION

There is a popular notion among some growers, apparently without experimental or scientific foundation, that plants cannot be grown successfully to maturity in heated soil. Granted that this is the case, which is contrary to fact, the alleged unfavorable condition would probably have resulted from one or more of three factors, namely: A change in the physical condition of the soil, which would affect the tilth and water-holding power; second, an increase in the water-soluble salts and organic matter, which might bring the concentration of the soil solution to a point where it would be harmful to growth; third, the destruction of the beneficial bacteria and molds, which might retard temporarily the fixation of atmospheric nitrogen and the ammonification and nitrification of organic fertilizers and ammonium salts, as well as the solution of certain insoluble mineral soil constituents. We have endeavored to gain some first-hand information on these points by a laboratory investigation of the particular soil under observation. This is a sandy loam from a virgin prairie, buffalo sod. The samples examined were taken from greenhouse beds where the soil was heated for 3½ hours at 200 degrees F.

Effect of Heating on Physical Condition

The organic colloidal aggregates appear to have been broken down by this heating which resulted in setting free an increased amount of colloidal material. The greater surface exposed by these colloids for the absorption of soil moisture would doubtless reduce somewhat the available water, and slightly more water might be required to grow the crop. The texture of the soil might become a little more compact with a tendency to be sticky; water would penetrate it more slowly and it would be a little more difficult to cultivate. None of these is sufficiently serious to affect the growth of the crop materially.

Effect of Heating on Water-Soluble Salts and Organic Matter

One hundred grams of soil, before and after heating, were extracted with 250 c.c. distilled water by shaking for four hours. The suspensions were allowed to stand over night and the supernatant fluid removed by decantation. The extracts were then partially clarified by centrifuging and subsequently filtered thru a Mandler filter. Fifty c.c. of this filtrate were evaporated to dryness on a waterbath for the determination of dissolved solids.
Table II—Dissolved Solids in Raw Soil

<table>
<thead>
<tr>
<th>Material</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-soluble Material</td>
<td>326</td>
</tr>
<tr>
<td>Loss on Ignition (OM)</td>
<td>138</td>
</tr>
<tr>
<td>Salts</td>
<td>188</td>
</tr>
<tr>
<td>Chlorides (Cl)</td>
<td>.656</td>
</tr>
<tr>
<td>Carbonates (CO₃)</td>
<td>44.560</td>
</tr>
</tbody>
</table>

Table III—Dissolved Solids in Heated Soil

<table>
<thead>
<tr>
<th>Material</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-soluble Material</td>
<td>273</td>
</tr>
<tr>
<td>Loss on Ignition (OM)</td>
<td>145</td>
</tr>
<tr>
<td>Salts</td>
<td>123</td>
</tr>
<tr>
<td>Chlorides (Cl)</td>
<td>.972</td>
</tr>
<tr>
<td>Carbonates (CO₃)</td>
<td>65.360</td>
</tr>
</tbody>
</table>

The analyses show that there was actually less water-soluble material in the heated soil than in the raw. The salt content was less in the former also, but the soluble organic matter was slightly more. While the percentage of carbonate appears to be greater in the heated sample, the actual amount is practically the same owing to the higher salt content of the raw soil. It is apparent from these results, that so far as this particular soil is concerned, no harm would result to plant growth from an increase in the water-soluble material due to heating.

Other investigators working in this line have reported an increase in the soluble mineral as well as organic matter in the heated soil, but in nearly every case they have used temperatures higher than ours and have not employed a process of heating which is comparable. This may account for the slight differences observed.

The Effect of Heating on Beneficial Bacteria

The effect of heating the soil upon 4 groups of bacteria has been studied, namely: Nitrogen-fixing, ammonifying, nitrifying-nitroso and nitrifying nitro.

1. Nitrogen-Fixing Bacteria—Azotobacter—

   Culture Solution—
   
   Mannite ........................................ 16 grams
   K₂HPO₄ ........................................... 0.5 grams
   MgSO₄ ........................................... 2 grams
   CaCl₂ ........................................... .02 grams
   10 percent Solution FeCl₃ ........................ 1 drop
   Distilled Water ................................ 1000.00 c.c.

   Reaction adjusted to —2 Fuller's Scale with N/1 NaOH (pH 8.0)

This solution was distributed in 500 c.c. Erlenmeyer flasks in 50 c.c portions and sterilized in the autoclave for 5 minutes at 120 degrees C. Each flask was then inoculated with 1 gram of soil from the respective samples. The cultures were incubated at 28 degrees C. for five days and then examined for Azotobacter films and microscopically for typical cells. The results follow:

Table IV—Effect of Heating Soil Upon Nitrogen-Fixing Bacteria

<table>
<thead>
<tr>
<th>Depth</th>
<th>Raw</th>
<th>Heated</th>
<th>3 Months After Heating</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4 inches</td>
<td>Azotobacter present</td>
<td>Azotobacter absent</td>
<td>Azotobacter present</td>
</tr>
<tr>
<td>4-8 inches</td>
<td>Azotobacter present</td>
<td>Azotobacter absent</td>
<td>Azotobacter present</td>
</tr>
<tr>
<td>8-10 inches</td>
<td>Azotobacter present</td>
<td>Azotobacter absent</td>
<td>Azotobacter present</td>
</tr>
</tbody>
</table>

The results show that the nitrogen-fixing bacteria as represented by Azotobacter were destroyed by the heating, but that the soil was reinoculated naturally after three months.
2. Ammonifying Bacteria.—

Culture Solution: Dunham’s peptone solution—

Peptone ........................................... 10 grams
NaCl .............................................. 5 grams
Distilled Water .................................. 1000 c.c.

Fifty c.c. portions of this solution in 500 c.c. Erlenmeyer flasks, sterilized for five minutes at 120 degrees C. were inoculated in duplicate with 1-gram quantities of the respective samples. One set of the duplicates was sterilized at once for 5 minutes at 120 degrees C. as a check on the ammonia already present in the soil, and was then incubated with the other set for 16 days at 28 degrees C. At the end of this time, two drops were removed from each flask to a milk-glass plate and tested for ammonia with 1 drop of Nessler’s solution. In the presence of ammonia an orange colored precipitate is formed. The results are given in Table 5.

Table V—Effect of Heating Soil Upon Ammonifying Bacteria
Prepared October 27, 1926

<table>
<thead>
<tr>
<th>Treatment . . .</th>
<th>Raw Sterilized</th>
<th>Raw Check Sterilized</th>
<th>Heated Sterilized</th>
<th>Heated Check Sterilized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth, 0-4&quot;....</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Depth, 4-8&quot;....</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Depth, 8-10&quot;...</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>+Ammonia absent.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results shown in Table 5 indicate that heating had practically no effect upon the ammonifying bacteria due to the fact that many of this group form heat-resistant spores.

Baldwin7 has noted an increase in the activity of ammonifying bacteria following partial sterilization (140° F. for 12 hours).

Russell and Hutchinson8 heated soil to 98° C. and observed an increase in ammonia production following the treatment, indicating that the ammonifying organisms had survived.

3. Nitrifying Bacteria.—

A. Nitroso

- Nitroso Solution
  - (NH₄)₂ SO₄ .......................... 2.0 grams
  - NaCl ................................ 2.0 grams
  - K₂H PO₄ .............................. 1.0 grams
  - MgSO₄ ................................. 0.5 grams
  - FeSO₄ ................................ 0.4 grams
  - Distilled Water ..................... 1000.00 c.c.

B. Nitro

- Culture Solutions
  - NaNO₂ ................................ 1.0 grams
  - Na₂ CO₃ ................................ 1.0 grams
  - K₂HPO₄ ................................ 0.5 grams
  - NaCl .................................. 0.5 grams
  - FeSO₄ .................................. 0.4 grams
  - MgSO₄ .................................. 0.5 grams
  - Distilled Water ..................... 1000.00 c.c.

These two solutions were distributed in 50 c.c. portions to 500 c.c. Erlenmeyer flasks, but before sterilization each flask received 0.5 grams magnesium carbonate (MgCO₃). One set of flasks prepared from each solution was then inoculated with 1 gram of the several samples and sterilized for 5 minutes at 120 degrees C. as checks on the nitrite and nitrate contained in the soil itself. At the same time a second set of flasks, containing the culture solutions only, was sterilized. These were then inoculated with 1 gram of soil and all the flasks were incubated at 28 degrees C. for 41 days, tests being made for nitrates and nitrates after 10, 24, and 41 days. The results are given in Tables 6 and 7.
Table VI—Effect of Heating Soil in Nitroso (Nitrifying) Bacteria. Production of Nitrites from Ammonium Sulfate. Prepared October 19, 1926.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oct. 29</th>
<th>Nov. 12</th>
<th>Nov. 29</th>
<th>Oct. 29</th>
<th>Nov. 12</th>
<th>Nov. 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date Examined</td>
<td>NO₂</td>
<td>NO₃</td>
<td>NO₂</td>
<td>NO₃</td>
<td>NO₂</td>
<td>NO₃</td>
</tr>
<tr>
<td>Depth, 0-4&quot;</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth, 4-8&quot;</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth, 8-10&quot;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oct. 29</th>
<th>Nov. 12</th>
<th>Nov. 29</th>
<th>Oct. 29</th>
<th>Nov. 12</th>
<th>Nov. 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date Examined</td>
<td>NO₂</td>
<td>NO₃</td>
<td>NO₂</td>
<td>NO₃</td>
<td>NO₂</td>
<td>NO₃</td>
</tr>
<tr>
<td>Depth, 0-4&quot;</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth, 4-8&quot;</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth, 8-10&quot;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ Nitrites present. — Nitrites absent.

The results indicate that the nitroso bacteria were present in the raw soil, but were destroyed by heating.


<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oct. 29</th>
<th>Nov. 12</th>
<th>Nov. 28</th>
<th>Oct. 29</th>
<th>Nov. 12</th>
<th>Nov. 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date Examined</td>
<td>NO₂</td>
<td>NO₃</td>
<td>NO₂</td>
<td>NO₃</td>
<td>NO₂</td>
<td>NO₃</td>
</tr>
<tr>
<td>Depth, 0-4 inches</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth, 4-8 inches</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth, 8-10 inches</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oct. 29</th>
<th>Nov. 12</th>
<th>Nov. 28</th>
<th>Oct. 29</th>
<th>Nov. 12</th>
<th>Nov. 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date Examined</td>
<td>NO₂</td>
<td>NO₃</td>
<td>NO₂</td>
<td>NO₃</td>
<td>NO₂</td>
<td>NO₃</td>
</tr>
<tr>
<td>Depth, 0-4 inches</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth, 4-8 inches</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth, 8-10 inches</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results show that the nitro bacteria were present in the raw soil, but were destroyed by the heating.

The nitrite tests were made by removing two drops of the culture to a milk-glass plate and adding 1 drop of Griess's reagent. In the presence of nitrites a pink color is produced.

The nitrate tests were made by adding 1 drop of the culture to 2 drops of diphenylamin reagent on a milk-glass plate. In the presence of nitrates a blue color is produced. If nitrites were present, they were first removed by boiling 8-10 drops of the culture with 1 c.c of saturated ammonium chloride and the test was then carried out as above.

Baldwin states that in soil heated for 12 hours at 140 degrees F. the nitrifying bacteria, being non-spore formers, were killed by the sterilizing process.

Deherain and Demoussy prevented nitrification in two samples of soil by heating them for 1 hour at 120 degrees C.

Russel and Hutchinson found that the growth of nitrifying bacteria was inhibited by heating soil to 98 degrees C.

Three months after the soil was heated, samples of the same were collected from the greenhouse and examined for nitrifying bacteria. The results of the test are given in Table 8.

<table>
<thead>
<tr>
<th>Organism ..........</th>
<th>Nitroso Bacteria</th>
<th></th>
<th>Nitro Bacteria</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Date Examined ......</td>
<td>Jan. 28</td>
<td>Feb. 10</td>
<td>Jan. 28</td>
<td>Feb. 10</td>
</tr>
<tr>
<td>Depth, 0-4 inches....</td>
<td>NO₂</td>
<td>NO₃</td>
<td>NO₂</td>
<td>NO₃</td>
</tr>
<tr>
<td>Trace</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Depth, 4-8 inches....</td>
<td>Trace</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Depth, 8-10 inches....</td>
<td>--</td>
<td>--</td>
<td>Trace</td>
<td>--</td>
</tr>
</tbody>
</table>

The results show that after three months both the nitroso and the nitro bacteria, which constitute the nitrifying organisms, were again present.

Summarizing the results of the bacteriological tests, we find that both the nitrogen-fixing and the nitrifying bacteria were destroyed by heating the soil, but the ammonifying were not, and that after three months both of the former groups were again present. As related to crop production, this means no permanent injury to the soil, but rather a change in fertilizer practice if fertilizers are to be applied during the first three months after heating. This point is discussed under "Fertilizers." If it is not the custom to use fertilizers, then we can say that the temporary loss of the bacteria would not be felt economically.

In the light of our studies, there seems to be little scientific basis for the claim that heating soil renders it unfit for plant growth either from the standpoint of soil physics, soil chemistry or soil biology. In fact there is ample evidence1,2 that partial sterilization, such as we are advocating, improves the productiveness of the soil and increases plant growth.

**Fertilizers**

Because of the absence of the nitrifying organisms from heated soil, no nitrogenous fertilizer in the form of an ammonium compound, cottonseed meal, dried blood or tankage, should be used for two or three months after heating or until the soil becomes reinoculated. Not only are such fertilizers of no immediate value to the crop, but they may be actually harmful. This is due to the fact that the ammonifying bacteria which are not destroyed convert the nitrogen of the cottonseed meal and other nitrogenous organic fertilizers into ammonia, and because there are no nitrifying bacteria present at the same time to change the ammonia to nitrates, the former may accumulate and burn the plants. Before ammonia compounds can be used by the crop, they must be changed to nitrates which transformation is accomplished by the nitrifying bacteria.

Therefore, if any commercial fertilizer is to be used on recently heated soil, it should be applied in a readily available, water soluble form: The nitrogen as sodium nitrate, the phosphate as acid or superphosphate, and the potash as potassium sulphate. After two or three months have elapsed, the usual organic materials may be employed.

**SUMMARY**

1. The soil of greenhouses and seedbeds can be effectively sterilized for the control of plant diseases and animal pests by the perforated-pipe method.
2. With the equipment described and 30 pounds steam pressure, it is pos-
sible to raise the temperature of the soil 10 inches deep to 200 degrees F. in 3 to 3½ hours.

3. Altho the nitrogen-fixing and nitrifying bacteria are destroyed by the heating, the soil becomes reinoculated after three months by natural agencies. The ammonifying bacteria are not affected.

4. All fertilizer used for three months after the soil is heated should be in a readily available form and water soluble.

5. The steam-pan method is satisfactory for seedbeds where shallow sterilization only is required.

6. Heating soil as described herein does not render it unfit for plant growth, but rather improves it.

ACKNOWLEDGMENT

During the pursuit of this work, I have received many valuable suggestions from Mr. Carl Toothaker and Mr. Bert Toothaker of the Toothaker Greenhouse Company, Denver, Colorado, whose courtesy and hospitality I take pleasure in acknowledging at this time.

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9. Smith, Ora

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