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The Causes and Control of Damping-off of Tomato Seedlings

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THE CAUSES AND CONTROL OF DAMPING-OFF OF TOMATO SEEDLINGS

L. J. ALEXANDER, H. C. YOUNG, AND C. M. KIGER[1]

INTRODUCTION

Tomato plants for field transplanting in Ohio are grown in greenhouses and cold frames in the vicinity in which they are to be transplanted. Generally, these greenhouses are used for seedling production only. The seed is sown in rows in the seedbeds and the plants are grown about 21 days. They are then transplanted into flats and moved to cold frames. As soon as one crop of seedlings is removed from the seedbed, another sowing is made, and, in this way, two or three crops of seedlings are grown in the same soil each year.

With this concentration of seedling production, damping-off becomes a serious problem and necessitates the use of control measures. A few growers are equipped to steam sterilize their seedbed soil before each sowing. These have no trouble with damping-off. Others are equipped to steam sterilize their soil only once a year, and still others have no means of sterilization and must rely on changing the soil each year. Both the latter practices are unsatisfactory because steam-sterilized soil is easily recontaminated during the production of the first crop, and new soil, free of damping-off organisms, is exceedingly difficult to obtain.

In the spring and summer of 1930 an investigation of diseased seedlings, grown at the H. J. Heinz Company's greenhouse, Bowling Green, Ohio, revealed that the trouble was caused by two fungi, a species of *Pythium* and *Rhizoctonia solani*. Following this investigation, a detailed study of the diseases was initiated, the object of which was to determine the relative loss caused by each organism, to study the environmental factors affecting the development of the diseases, and to find a cheap, satisfactory control method for those growers who are not equipped to steam sterilize their seedbed soil before each seeding.

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THE DISEASES

ECONOMIC IMPORTANCE

A precise statement of the loss from damping-off of tomato seedlings is difficult to make. Damping-off is general throughout Ohio, and there are probably very few sections of the country in which it does not occur. Usually, if one seedling crop is lost, due to ravages of damping-off, there is sufficient time to replant and grow more seedlings. Where partial losses of the crop occur, many diseased seedlings do not die immediately but, when transplanted into flats, may die or live and produce undesirable plants. To the plant grower these losses are quite acute, but the true loss is often more far-reaching. Many farmers depend on producing early tomatoes for the market and, while a set-back of one or two weeks usually does not reduce the yield, it greatly reduces the net returns, because the price of late tomatoes is low. Where tomatoes are grown under contract for canning purposes, late plants mean a reduced yield and, consequently, reduced profits to the farmer and the canning company.

ETIOLOGY

A microscopical examination revealed the presence of abundant, non-septate, fungous mycelium in the discolored, cortical tissue of most diseased seedlings. Occasionally, however, a seedling was found with a septate fungus in the cortex. Numerous isolations during the fall and winter of 1930, made from diseased seedlings grown in naturally sick soil, yielded ten Pythium cultures to one Rhizoctonia culture. Occasionally, an unidentified *Fusarium* sp. occurred. In the spring of 1931, isolations were made from collections at other places in Ohio and at Princeton, Indiana. The same fungi were found in about the same ratio.

The pathogenicity of the *Pythium* sp. and *Rhizoctonia solani* has been demonstrated by numerous inoculation experiments to be described later. In the first two inoculation experiments, re-isolations were made from diseased plants, and cultures of the same fungi used for inoculating were recovered.

DESCRIPTION AND SYMPTOMS OF THE DISEASES

The absence of seedlings in the seedbed for short distances in the rows is the most common early manifestation of damping-off. These distances vary from an inch to 5 or 6 inches or more. Later manifestations are scattered spots in the seedbeds where seedlings begin to topple over. Generally, these spots enlarge from a central point or points and under favorable conditions for disease development may cover an entire seedbed.

The symptoms of the disease caused by *Pythium* sp. are of two distinct types. The one most commonly known is that in which the hypocotyl of the seedling, after emergence, becomes water-soaked and discolored at the surface of the soil. Such diseased plants fall over, quickly wither, and die. The other general symptom of damping-off is evidenced by a poor stand or stunting of the plants. In this case the hypocotyl is attacked before it emerges from the soil. The first symptoms of this attack are small, brown, water-soaked lesions which, under favorable conditions, affect the entire root system, frequently killing the seedling before it emerges. In case it does emerge, the hypocotyl and roots remain diseased, the plant is stunted, abnormally dark green, and the cotyledon leaves roll downward. This latter type of attack is frequently very destructive because such diseased plants, unnoticed by the grower, are transplanted and die later.

Fig. 1.—Malformations of tomato seedlings caused by *Rhizoctonia solani*

There are three general types of symptoms of the disease caused by *Rhizoctonia solani.* Two of these resemble very closely the symptoms caused by *Pythium* sp.; namely, the toppling over of seedlings and the general diseased condition of the hypocotyl and roots. The principal difference in detailed symptoms is a dry, shrivelled lesion instead of a water-soaked one. The third type is a deformation of the cotyledons, as shown in Figure 1. In all cases, seedlings stunted by either *R. solani* or *Pythium* sp. have an abnormally deep green color.

HISTORY OF THE CAUSES AND CONTROL MEASURES FOR DAMPING-OFF

De Candolle first described *Rhizoctonia* in 1815 as a root-destroying fungus. Since that time, it has been listed as a serious cause of damping-off of many plants. Atkinson (1), 1895, found several fungi capable of causing damping-off of plants. Among those listed as important are *Rhizoctonia* and *Artotrogus intermedius;* the latter was called *Pythium debaryanum* by Hesse in 1884. Peltier (13), 1916, listed *Rhizoctonia solani* as a parasite on 165 species of plants, including the tomato. However, he considered *Rhizoctonia solani* a weak parasite under normal conditions. Humbert (8), in Ohio in 1918, attributed most of the damping-off of tomato seedlings to *Rhizoctonia solani.* Pritchard (14), 1921, conducted inoculation experiments with *Verticillium lycopersici, Macrosporium solani,* and *Rhizoctonia solani* and found *Rhizoctonia solani* a weak parasite. Weber and Ramsey (26), in 1926, expressed the opinion that in Florida the loss from attacks of *Rhizoctonia solani* on tomato seedlings equals the loss from all other fungi combined. Small (20), 1927, found *Rhizoctonia solani* to be the cause of a disease which he called foot-rot. His experiments showed that infection only took place when plants were excessively watered. Horsfall (7), 1930, did not find *Rhizoctonia solani* a cause of damping-off in greenhouses of New York.

Pythium ultimum was described by Trow (24), in 1901, as a new species. He considered it a saprophyte. However, Drechsler (correspondence) considers it to be the most common cause of root and rootlet injury. Reference to the damping-off of tomato seedlings by *Pythium* has been made by several writers, among them Johnson (9), Humbert (8), and Weber and Ramsey (26). Horsfall (7) found damping-off of tomatoes in New York to be caused by a fungus which he thought to be a species of *Phytophthora.* Most workers refer simply to *Pythium debaryanum,* but confusion exists in the nomenclature of this group. Drechsler (4) found two water molds, *Aphanomyces euteiches* and *Plectospira myriandra,* parasitic on tomato rootlets.

Atkinson (1), 1895, first pointed out that damping-off was greatly influenced by environmental factors and suggested, as control measures, steaming the pots, maintenance of even temperatures, plentiful supply of fresh air and light, and avoidance of excessive watering. Selby (19), 1906, working on a control for damping-off in tobacco seedbeds, found a formaldehyde drench to be very effective. Johnson (9), 1914, also working on control of damping-off in tobacco seedbeds, found that steam sterilization gave the most effective control. The formaldehyde drench gave the next best control and was recommended where steam sterilization was not possible. Very little value was obtained from the use of numerous chemicals, including compounds of sulphur and copper. Thomas (22), 1927, tested a great many chemicals for the control of damping-off caused by an unidentified species of *Phytophthora* and concluded that control could be secured by the use of copper carbonate, mercuric chloride, and Uspulun. He also found that mercury compounds were effective in controlling *Rhizoctonia;* whereas copper compounds were not. May and Young (12) found that drenching the soil with 3 per cent formaldehyde gave the best control for damping-off of coniferous seedlings. Doran (3), 1928, found acetic acid to be effective as a soil drench, but it has the same disadvantages as the formaldehyde drench—it involves an undesirable wetting of the soil and causes a delay of 10 to 14 days before planting. Horsfall (7), 1930, found that by treating tomato seed with copper dusts or copper sulphate solutions, seedlings were protected from damping-off. Jozefowicz (11) found that soaking tomato seed in copper sulphate solutions did not affect the vitality but in some cases retarded germination. Tomato seed soaked in 5 per cent commercial formaldehyde solution for 10 minutes was not injured when sown wet. Also, a 10-minute treatment in a 0.05 per cent mercuric chloride solution was harmless.

THE PATHOGENS

TAXONOMY

Two cultures of the non-septate fungus were submitted for identification to Dr. Charles Drechsler of the Bureau of Plant Industry, Washington, D. C. He identified the organism as *Pythium ultimum* Trow. The characters of the septate fungus agreed in all essential details with those given for the fungus *Rhizoctonia solani* Kuhn (*Corticium vagum* B. & C., var. *solani* Burt). Earlier workers have ably described both fungi and no attempt is made to repeat the descriptions.

RELATION OF TEMPERATURE TO GROWTH IN PURE CULTURE

Pythium ultimum and *Rhizoctonia solani* have been grown on Petri plates of potato-dextrose agar over a range of controlled temperatures. Five plates were used at each temperature. The results for each fungus are given in Table 1.

TABLE 1.—Summary of Results of Growth-Temperature Experiments *Pythium ultimum* and *Rhizoctonia solani* Grown on Potato-Dextrose Agar

Temperature °C.	3.5–6.5	10.0–10.5	15.5–16.5	19.5–20.5	24.0–26.5	30.0–31.5	33.0–34.5	37.5–38.5
Pythium ultimum 34-hour cultures...	0.0	0.0	25.6	52.4	85.0	85.0	74.8	....
Rhizoctonia solani 44-hour cultures...	0.0	0.0	6.0	35.3	79.3	87.4	8.5	0.0

Figures indicate radial growth in millimeters.

After 34 hours *P. ultimum* had made no growth at the two lowest temperatures. These cultures were kept several days longer, with the result that the cultures at 10 degrees C. finally grew across the Petri plates. Under these conditions the greatest linear growth occurred between 25 and 30 degrees C.

Rhizoctonia solani has a narrower temperature range than *P. ultimum.* After 44 hours no growth occurred at either of the two lowest temperatures. The optimum temperature for linear growth under these conditions occurred at 30 degrees C. Above this temperature the rate of linear growth decreased very rapidly.

PATHOGENICITY

In addition to causing damping-off of tomato (*Lycopersicum esculentum*), *Pythium ultimum* readily produced the disease on tobacco (*Nicotiana tobacum*), leaf lettuce (*Lactuca sativa*), celery (*Apium graveolens*), and sugar beets (*Beta vulgaris*). No further attempt has been made by the authors to determine the host range, but it is assumed to be very broad. No effort was made to test the pathogenicity of the strain of *R. solani* on hosts other than the tomato, but the work of Peltier (13) and others showed that the number of hosts for *Rhizoctonia solani* is large.

METHODS AND MATERIALS

The same method was adopted for isolating both *R. solani* and *P. ultimum.* The procedure consisted of removing a segment of discolored tissue from each of a number of diseased seedlings, washing the pieces of tissue several times in sterile distilled water, and planting directly on water agar. Transfers to potato-dextrose agar slants were made as soon as fungous growth occurred from the plantings. The water or clear agar was prepared by dissolving 1.7 grams of agar agar in 1000 c. c. of distilled water and autoclaving at 15 pounds pressure for 30 minutes. The potato-dextrose agar used throughout this work contained 2 per cent dextrose and 1.7 per cent agar.

In all experiments pure culture inoculations were used. Inoculum was grown in Petri dishes on potato-dextrose agar for 7 to 10 days. An effort was made to use equal amounts of inoculum in all experiments. In the soil temperature-moisture experiments, inoculum was mixed with the top 3 inches of soil, but where pots or flats were used the inoculum was mixed with the entire mass of soil.

Soil used throughout this work was secured from the compost pile of the H. J. Heinz Co., at Bowling Green, Ohio. Before composting, the soil is classed as a Newton sandy loam. The water-holding capacity of the compost was about 46 per cent of its oven-dry weight. For pure culture inoculation experiments, this soil was sterilized in a soil autoclave at 15 to 18 pounds pressure for 2 to 4 hours, the time varying with the size of the container. In most cases, after mixing inoculum with sterilized soil, it was allowed to stand several days before using.

Soil temperature and soil moisture studies were conducted in especially constructed tanks. In each tank there were eight metal cans supported at the rim and surrounded by water, the temperature of which was thermostatically controlled. The cans were all ballasted to weigh alike and were then filled with the same amount of soil by weight. Different soil water contents were used and these are expressed in percentages of the water-holding capacity of the soil. These water contents were maintained by weighing the cans of soil daily and adding distilled water to restore to original weight.

Where flower pots were used in any experiment, they were ballasted, filled, and watered in the same manner as the tank cans. This procedure reduced the effect of variations in the soil moisture content to a minimum.

INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE UPON DAMPING-OFF

Since tomato seedlings are grown in greenhouses where soil temperature and soil moisture can be easily controlled by the growers, it was deemed highly desirable to make a critical study of the effect of these factors on the development of the diseases. The study was divided into two parts: (1) the effect of temperature and moisture on the emergence and development of tomato seedlings in sterilized soil; (2) the effect of temperature and moisture on seedling emergence and subsequent development of the diseases—in naturally infested soil, in sterilized soil to which pure cultures of *P. ultimum* were added, and in sterilized soil to which pure cultures of *R. solani* were added.

EXPERIMENTAL

Two soil temperature and moisture experiments were conducted, one essentially a duplication of the other. The results of both experiments agreed in all essential details and only one will be described here.

At each of six soil temperatures (15, 18, 21, 24, 27, and 30 degrees C.) and four soil moisture contents (35, 45, 55, and 65 per cent of the water-holding capacity of the soil), four cans were planted with 150 Cobourg tomato seeds each. One can contained naturally infested soil. The other three contained the same soil sterilized, to one can of which Pythium inoculum was added, to another Rhizoctonia inoculum, and the third was a sterilized-soil check.

The relation of soil temperature to rate of emergence of tomato seedlings, grown in sterilized soil maintained at 45 per cent of its water-holding capacity, is shown in Figure 2. The most rapid germination and highest percentage of emergence occurred at 27 degrees C. There was very little difference in rapidity of emergence between 24 and 30 degrees C. Apparently, a temperature of 30 degrees C. is slightly above whereas 24 degrees C. is slightly below the optimum for germination and emergence.

A summary of the results of growing tomato seedlings in sterilized soil for 19 days is shown in Table 2A. The highest percentage of germination and emergence occurred at the highest temperature and in the driest soil. However, when the plants were examined at the conclusion of the experiment, the roots of plants grown in the driest soil (35 per cent) were in poor condition;

whereas those of plants grown in soil with more moisture were very white and in excellent condition. Near the end of the experiment some damping-off occurred, which, undoubtedly, resulted from contamination by *P. ultimum* from adjoining cans.

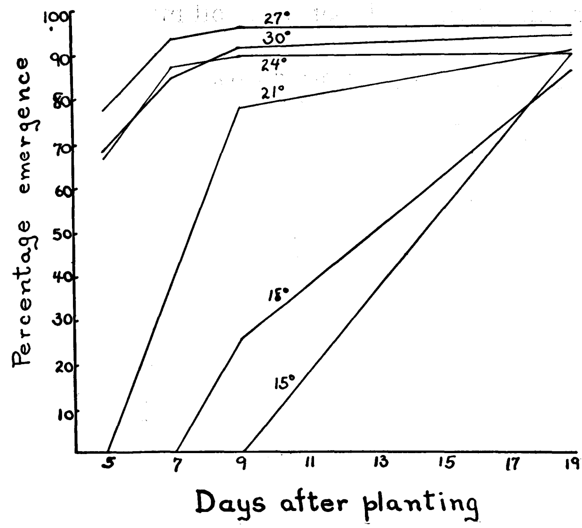

Fig. 2.—Relation of soil temperature to rate of emergence of tomato seedlings in sterilized soil. Moisture content 45 per cent of the water-holding capacity of the soil.

A summary of the results of growing seedlings in sterilized soil, naturally infested with *R. solani* and *P. ultimum,* is given in Table 2B. The highest percentage emergence occurred at 30 degrees C. and in the driest soil. At lower soil temperatures and higher percentages of soil moisture, the percentage of emergence fell off markedly. With respect to damping-off after emergence, there was no significant variation with changes in either temperature or moisture.

A summary of the results of growing tomato seedlings in sterilized soil to which Pythium inoculum had been added is given in Table 2C.

Pythium ultimum greatly reduced the percentage of emergence of seedlings and caused damping-off at all soil temperatures and moistures. The greatest percentage of seedlings emerged in the driest soil, 35 per cent moisture, at the highest temperature, 30 degrees C. The fewest seedlings emerged between 18 and 24 degrees C., which would indicate that the optimum for the development of the disease lies between these temperatures. At 27 degrees C. a few more seedlings were able to emerge, and at 30 degrees C. still more emerged. Damping-off of seedlings after emergence occurred most severely between 15 and 24 degrees C. and was least severe at 30 degrees C. The disease development reached its maximum at 45 per cent soil moisture.

TABLE 2.—Relation of Soil Temperature and Soil Moisture to Emergence and Damping-off

Soil temperature °C.	Soil-moisture-holding capacity							
	35%		45%		55%		65%	
	Emergence Pct.	Damped-off Pct.	Emergence Pct.	Damped-off Pct.	Emergence Pct.	Damped-off Pct.	Emergence Pct.	Damped-off Pct.
A. Sterilized soil								
15	86.6	0.0	92.0	0.0	82.0	0.0	35.3	0.0
18	98.7	0.0	85.4	0.0	92.6	0.0	30.0	0.0
21	96.7	1.4	91.3	2.2	92.0	2.2	71.3	1.9
24	95.4	0.0	90.0	0.0	90.6	2.2	80.0	8.2
27	97.3	3.8	95.3	4.2	90.0	11.1	67.3	4.9
30	96.7	0.0	94.0	2.1	86.0	0.8	52.3	10.0
B. Unsterilized soil								
15	58.0	5.7	64.7	21.1	70.0	27.6	23.3	17.1
18	67.3	20.8	81.4	21.3	78.6	0.8	28.7	16.3
21	83.3	24.8	49.3	18.9	82.0	4.9	74.0	5.4
24	95.3	26.6	80.0	14.6	66.7	13.0	82.0	8.9
27	94.7	14.8	93.3	15.0	100.0	7.3	95.3	17.2
30	98.0	4.8	96.0	3.5	80.7	23.0	80.0	25.8
C. Sterilized soil plus Pythium inoculum								
15	12.0	22.2	0.0	0.0	1.3	0.0	0.0	0.0
18	6.6	40.0	0.6	100.0	0.0	0.0	0.0	0.0
21	7.3	90.9	0.0	0.0	0.0	0.0	0.0	0.0
24	18.6	85.7	2.6	100.0	1.3	50.0	2.0	33.3
27	24.7	78.4	4.0	83.3	7.3	81.8	2.0	66.6
30	62.7	51.1	17.3	61.6	10.0	93.4	52.5	50.3
D. Sterilized soil plus Rhizoctonia inoculum								
15	64.6	2.1	66.0	2.0	68.6	0.0	7.5	0.0
18	68.6	0.0	81.3	0.8	81.3	0.0	1.3	0.0
21	60.0	6.7	38.0	10.5	76.0	0.9	52.6	2.5
24	58.6	6.8	45.3	17.9	32.6	38.8	34.6	7.7
27	69.3	35.6	91.3	46.0	76.0	61.3	36.7	60.0
30	43.3	29.3	68.7	50.2	74.0	60.0	45.3	61.8

Rhizoctonia solani was also destructive over a wide range of soil temperature and soil moisture, Table 2D. However, the seriousness of damping-off caused by *R. solani* is considerably less than that caused by *P. ultimum.* The fewest seedlings emerged at 24 degrees C., which indicates an optimum for development of the disease at that temperature. After emergence, damping-off was most serious at the two highest temperatures, 27 and 30 degrees C.

However, the air temperature was considerably lower than these two soil temperatures. Consequently, the temperature at the soil surface, where the fungus was active, was lower than the recorded soil temperatures would indicate. The percentage of emergence did not differ consistently between 35, 45, and 55 per cent soil moisture but was considerably reduced at 65 per cent. After emergence, the least damping-off occurred at 35 per cent soil moisture.

DISCUSSION OF EXPERIMENTAL RESULTS

The optimum temperature for germination and emergence of tomato seedlings was found to be 27 degrees C. This is in close agreement with Clayton (2), who concluded that the optimum temperature for the development of tomato plants was between 24 and 31 degrees C. The fact that the highest percentage of germination and emergence occurred in soil too dry to support good growth is a little difficult to explain. However, the soil was watered from the surface, and probably the surface soil had more than the calculated amount of water.

It was not surprising to find that both *P. ultimum* and *R. solani* caused damping-off over a wide range of temperatures. Hemmi (6) states that *Pythium debaryanum* was most active on garden cress seedlings between 20 and 30 degrees C. The present findings showed *P. ultimum* to be capable of causing severe damping-off of tomato seedlings from 15 to 30 degrees C. but was most active between 18 and 24 degrees C. Jones and Drechsler (10), working with root-rot of peas, found *Aphanomyces euteiches* to be active over a wide range of temperature, 15 to 30 degrees C.

The optimum temperature for damping-off of tomato seedlings by *R. solani* was found to be about 24 degrees C. This is above the optimum temperature given by Richards (15) for the Rhizoctonia disease of potatoes. It is, however, in fairly close agreement with the work of Gratz (5) on the wire-stem disease of cabbage and with that of Walker (25) on the sore-shin disease of cotton. The relation of temperature to damping-off of tomato seedlings, caused by *P. ultimum* and *R. solani,* is shown in Figure 3.

The relation of soil moisture to development of the disease was more striking than the relation of temperature, as shown by the photographs in Figure 4. The disease, caused by *P. ultimum,* in soil sufficiently moist for good growing conditions, (45 per cent), was practically as severe as in soils of still higher moisture contents. Only in the very dry soil (35 per cent) was it checked. *R. solani* was less active over the entire range of soil moistures but was very destructive in the wettest soil.

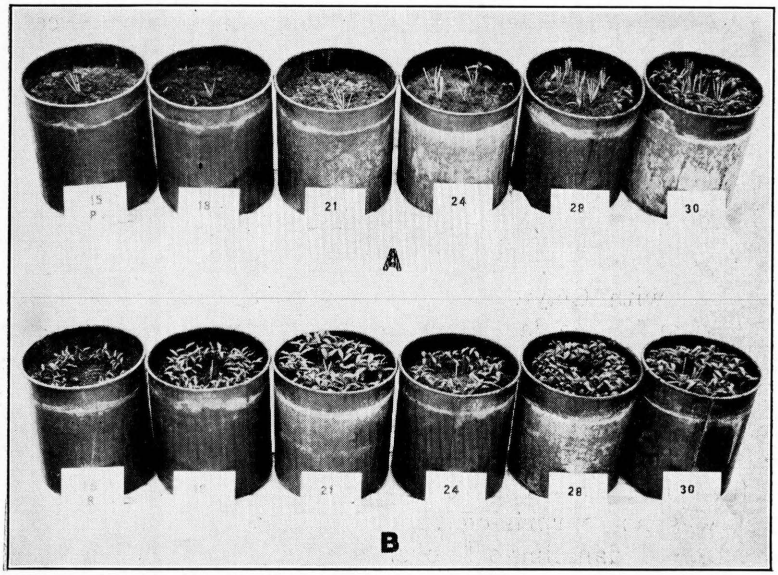

Fig. 3.—Effect of temperature on seedling emergence and subsequent damping-off. A. Sterilized Pythium-infested soil. B. Sterilized Rhizoctonia-infested soil. Toothpicks indicate damped-off plants. Soil at 45 per cent of its water-holding capacity. Temperatures given in degrees Centigrade.

The results of these experiments show clearly that damping-off can not be controlled by regulation of soil moisture and temperature. However, where growers do not sterilize their soil, certainly the ravages of the disease can be modified by germinating the seed in soil which is maintained at high temperatures and with just sufficient moisture to support growth.

CONTROL

Seed treatments for the control of soil-borne seedling diseases have generally met with failure. The only hope of controlling such diseases by seed treatment is that a sufficient quantity of fungicide be carried on the seed into the soil to protect the seedling until it becomes well established. Certain types of seed, such as the tomato, will carry large quantities of material, but, when highly toxic compounds are used, there is almost always considerable damage done to the seed. Nevertheless, certain dust and wet seed treatments were tried and are herein reported.

The most common chemical means of soil disinfection is by the use of a formaldehyde drench. This method is very effective but necessitates a thorough wetting of the soil and a delay of 10 to 14 days before planting to allow the gas to escape from the soil. Further, it cannot be used in greenhouses with growing plants. The ideal fungicide for soil disinfection purposes should be a dust that can be mixed with the soil and that causes no injury when seed is planted immediately.

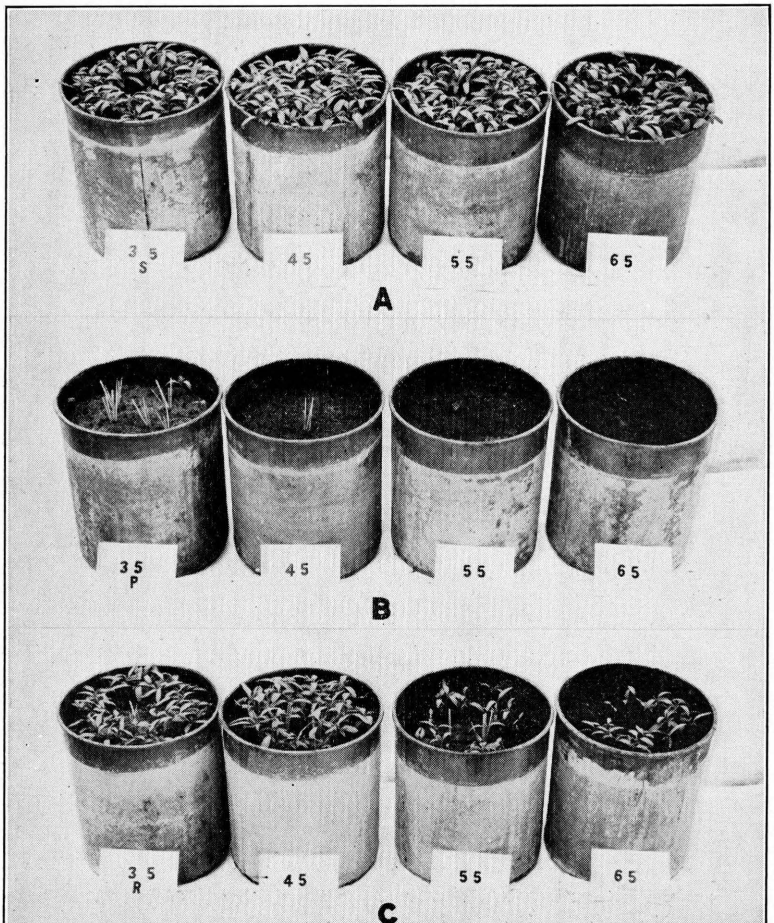

Fig. 4.—Effect of soil moisture on seedling emergence and subsequent damping-off. A. Sterilized soil. B. Sterilized soil plus Pythium inoculum. C. Sterilized soil plus Rhizoctonia inoculum. Toothpicks indicate damped-off plants. Soil temperature 24° C. Soil moisture in per cent of its water-holding capacity.

Sayre (16) and Sayre and Thomas (17, 18) were the first to employ formaldehyde as a fungicide in dust form. They made the dust by adsorbing sufficient 40 per cent commercial formaldehyde (formalin) on diatomaceous earth to make a 4 per cent dust. This dust is now used commercially for the control of oat smut. Almost simultaneously, Stirrup and Cranfield (21) published an account of controlling bunt of wheat by a formaldehyde dust. They used gypsum as a carrier.

Following this same line of thought, 40 per cent commercial formaldehyde, acetic acid, monohydrated copper sulphate, Ceresan, and leaded zinc oxide have been made into dusts and mixed with the soil for the control of damping-off.

SEED TREATMENTS

Dust seed treatments.—Five fungicides, to be applied to the seed as dusts, were selected. The seed was treated by placing an excess of the dust in a jar with the seed and shaking it vigorously 100 times. The excess dust was removed by later shaking the seed in a cheesecloth bag. One hundred seeds were sown in naturally infested soil in 4-inch flower pots. All treatments were run in duplicate. As described before, equal amounts of soil were placed in the pots, and all watering was done by weight. The results obtained were so variable that no definite conclusion could be arrived at. After repeating the experiment, it was concluded that dust seed treatments could not be depended upon to control damping-off.

Wet seed treatments. *Experiment I.*—The purpose of the first of two experiments with wet seed treatments was to determine if damping-off of tomato seedlings in naturally infested soil could be controlled by soaking the seed in solutions of copper sulfate, formaldehyde, and mercuric chloride. One, two, five, and ten per cent solutions of copper sulphate and of formaldehyde were used. Two strengths of mercuric chloride were used; namely, 0.05 per cent and 0.1 per cent. Different lots of seed were soaked in the copper sulphate solutions for 5, 10, 15, 20, and 30 minutes. For the mercuric chloride and formaldehyde solutions the 20- and 30-minute treatments were omitted. Immediately after treating, the seed was washed 5 minutes in distilled water and then spread out on blotting paper to dry one-half to one hour before sowing. Seed was soaked in water for each of the time periods used and planted as checks. One hundred seeds were sown in each pot, and all treatments were in duplicate.

The pots were filled and watered as described in the previous experiment. The final counts were made 30 days after seeding. A summary of the results is given in Table 3.

Soaking the seed in the different solutions of formaldehyde and of mercuric chloride gave no control of damping-off. In fact, the formaldehyde treatments reduced the percentage of emergence, and in all cases a higher percentage of damping-off occurred than with the water-treated checks. Seed treated with the copper sulphate solutions gave a higher average percentage of emergence than the checks, and in only one instance did the percentage of damping-off equal or exceed the checks.

Experiment II.—After a consideration of the results secured (with copper sulphate seed treatment) in the preceding experiment and those secured by Horsfall (7), it seemed advisable to conduct an experiment using stronger copper sulphate solutions and longer periods of treatment; also to plant the treated seed in (1) unsterilized soil, (2) sterilized soil to which pure cultures of *P. ultimum* were added, and (3) sterilized soil to which pure cultures of *R. solani* were added.

The soil to be inoculated with *P. ultimum* and *R. solani* was steam sterilized in flats, then mixed with the inoculum and allowed to stand 24 hours. It was then shovelled over thoroughly and weighed into clean, sterilized, 4-inch flower pots, as in the preceding experiment. Lots of 100 seeds each were sown in duplicate for each treatment. The soil was maintained at approximately 55 per cent of its water-holding capacity during the first 14 days but much wetter than this during the last 7 days.

A summary of the results is given in Table 4.

TABLE 3.—Results on Control of Damping-off by Wet Seed Treatments

One hundred seeds sown in duplicate for each treatment.
Soil naturally infested

Treatment	Concentration	Length of treatment in minutes									
		5		10		15		20		30	
		Emergence Pct.	Damped-off Pct.	Emergence Pct.	Damped-off Pct.	Emergence Pct.	Damped-off Pct.	Emergence Pct.	Damped-off Pct.	Emergence Pct.	Damped-off Pct.
Water	......	89	32.6	77	33.3	96	33.4	90	22.2	83	20.5
Copper sulphate	1%	93	10.7	89	16.8	88	22.8	95	10.5	87	15.0
	2%	89	3.4	80	36.2	92	9.8	94	3.1	92	14.1
	5%	99	10.0	96	3.1	97	12.4	98	7.1	95	13.7
	10%	94	15.0	96	13.5	100	3.0	92	8.7	80	11.2
Formaldehyde	1%	58	83.0	69	55.1	68	67.6	......	......	......	......
	2%	70	70.0	41	80.6	70	71.4	......	......	......	......
	5%	70	53.0	75	34.8	54	61.2	......	......	......	......
	10%	75	61.0	62	54.8	44	60.0	......	......	......	......
Mercuric chloride	0.1%	94	21.1	93	31.1	91	6.6	......	......	......	......
	0.05%	85	52.6	92	9.8	89	36.0	......	......	......	......

TABLE 4.—Results on Control of Damping-off by Soaking Seed in Copper Sulphate Solutions

Concentration of copper sulphate solution	Length of treatment											
	15 minutes		30 minutes		1 hour		2 hours		12 hours		24 hours	
	Emergence	Damped-off	Emergence	Damped-off	Emergence	Damped-off	Emergence	Damped-off	Emergence	Damped-off	Emergence	Damped-off
	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
Unsterilized naturally infested soil												
2%	94	4.3	99	9.1	93	11.8	98	3.1	99	1.1	96	15.6
5%	86	8.1	91	6.6	99	3.1	96	5.2	89	5.6	92	10.9
10%	94	2.1	93	5.4	92	2.2	96	6.3	94	2.1	93	3.2
20%	94	2.1	89	3.4	99	1.1	95	1.5	97	2.1	93	0.0
Saturated	91	2.2	94	5.3	97	3.1	94	1.1	96	5.2	94	4.3
Water-treated	89	9.0	90	13.3	93	10.7	95	9.5	89	14.6	93	5.4
Sterilized soil plus Pythium inoculum												
2%	79	30.4	54	42.6	91	30.8	89	37.1	76	31.6	83	31.3
5%	79	30.4	92	25.0	86	26.8	87	25.3	84	34.5	92	19.6
10%	90	27.8	94	14.9	88	15.9	86	24.4	93	29.0	89	19.1
20%	91	15.4	87	26.5	92	23.9	89	19.1	90	24.4	90	28.9
Saturated	90	8.9	93	20.4	90	13.3	96	17.7	91	22.0	90	15.6
Water-treated	68	36.8	35	68.6	68	58.8	55	51.0	42	42.9	72	33.3
Sterilized soil plus Rhizoctonia inoculum												
2%	88	18.2	89	21.3	86	14.0	71	28.2	81	18.5	87	20.7
5%	84	36.9	90	23.3	84	32.1	94	12.8	89	18.0	90	10.1
10%	89	16.9	89	29.2	91	17.6	93	9.7	90	16.7	86	10.0
20%	73	8.2	92	20.6	80	15.0	86	9.3	85	10.1	90	4.4
Saturated	90	5.6	91	12.1	92	25.0	97	3.1	89	7.9	89	11.2
Water-treated	75	37.3	70	11.4	70	15.7	70	14.3	41	17.1	77	7.8

In all cases, the copper-sulphate-treated seed gave a higher percentage of emergence than the water-treated checks. This was especially true where the seed was planted in the sterilized soil to which Pythium inoculum had been added. In this case increasing the duration of the treatment did not increase the percentage of emergence but did decrease the percentage of damping-off. In the case of the Rhizoctonia-infested soil, increasing the duration of treatment did not increase the emergence and did not consistently decrease the percentage of damping-off. Generally a 10 per cent solution gave better results than a 2 or 5 per cent solution.

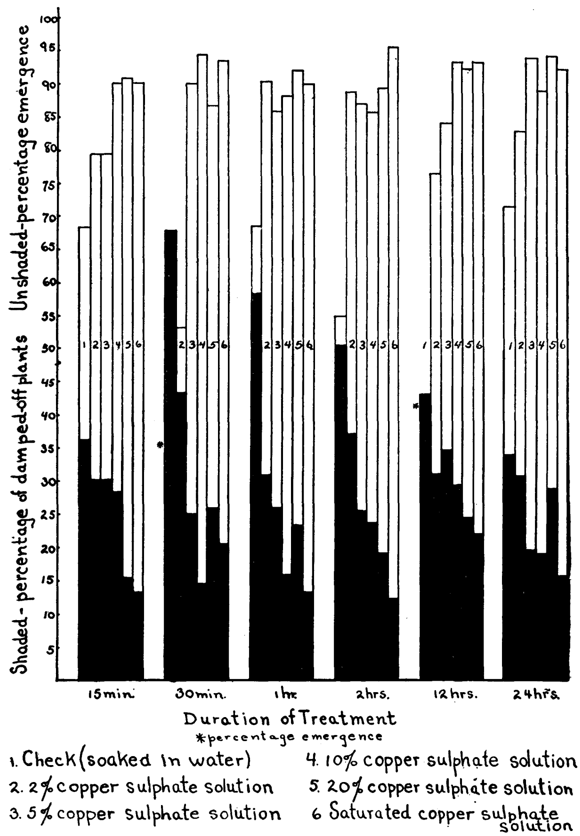

Fig. 5.—Control of damping-off by soaking seed in copper sulphate solutions. Sterilized soil plus Pythium inoculum

(Figures 5, 6, and 7). Attention should be called to the fact that the copper-sulphate-treated seed gave a high percentage of emergence and very few seedlings damped-off until the soil moisture content was later increased; whereas the water-treated seed gave a poor emergence, and the seedlings began to damp-off immediately. It is also interesting to note that the percentage of damping-off in the water-soaked checks decreased with increased duration of treatment, when the seed was planted in sterilized soil plus Pythium inoculum. (Figure 5).

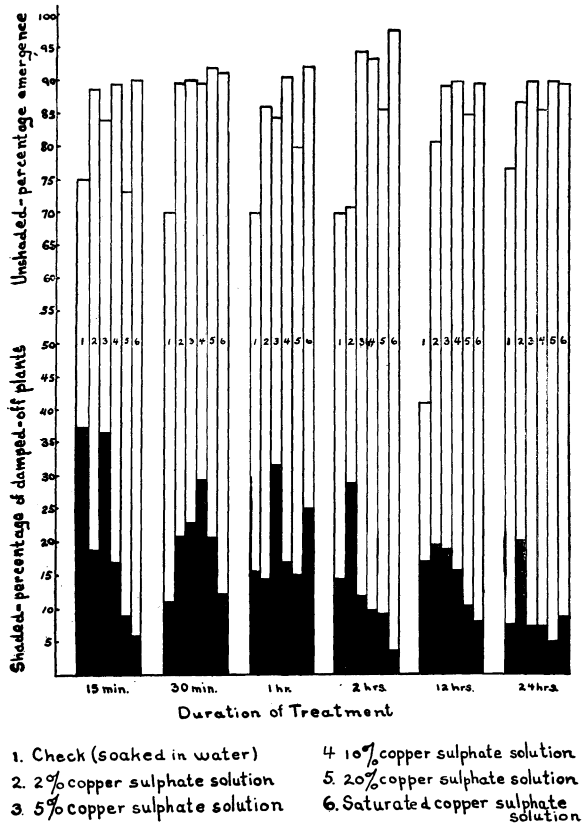

Fig. 6.—Control of damping-off by soaking seed in copper sulphate solutions. Sterilized soil plus Rhizoctonia inoculum

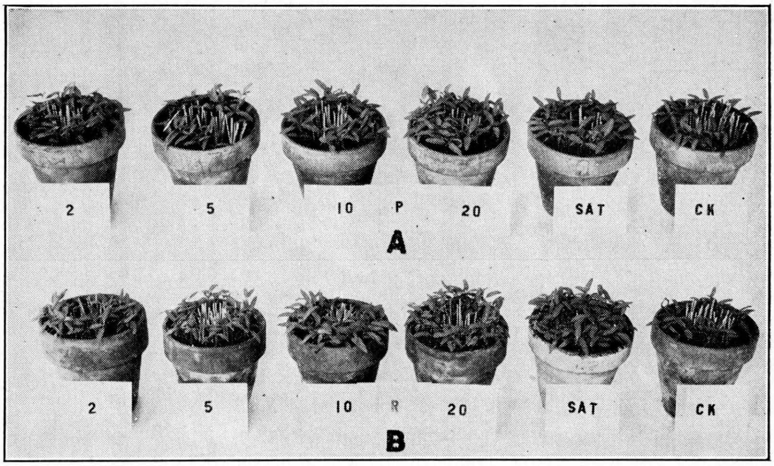

Fig. 7.—Control of damping-off by soaking seed in copper sulphate solution. Figures on pots indicate concentration of solution in per cent. Toothpicks represent damped-off seedlings
A. Sterilized soil—Pythium infested
B. Sterilized soil—Rhizoctonia infested

SOIL DISINFECTION

Experiment I.—In this experiment three dusts (namely, formaldehyde, monohydrated copper sulphate, and a Ceresan-kaolin mixture) were tried as soil disinfectants for the control of damping-off.

An 8 per cent formaldehyde dust was made by slowly pouring and stirring 20 c. c. of 40 per cent commercial formaldehyde into 80 grams of diatomaceous earth. Since lumps were formed, screening was necessary. The dust was then stored in air-tight containers. The 50 per cent monohydrated copper sulphate dust was made by mixing equal parts by weight of this material with kaolin. Ceresan, a compound recommended only for grain seed treatment, was prepared by mixing it with equal parts of kaolin by weight. Kaolin is a finely ground clay.

The flats used were 2½ inches deep and had a surface area of 1 square foot. The soil was treated by thoroughly mixing the dusts with it. It was then placed in the flats, after which the seed was sown and the soil thoroughly watered, using a Ross nozzle. Two lots of soil were used, one the naturally infested soil and the other sterilized soil to which Pythium inoculum had been added. Fifty untreated seeds were sown in each of three rows for each treatment.

The quantities of dust used and the results obtained are given in Table 5.

TABLE 5.—Control of Damping-off by Soil Disinfection with Chemicals

Treatment	Rate of treatment in grams of dust per square foot of soil									
	Check		6		12		18		24	
	Emergence Pct.	Damped-off Pct.	Emergence Pct.	Damped-off Pct.	Emergence Pct.	Damped-off Pct.	Emergence Pct.	Damped-off Pct.	Emergence Pct.	Damped-off Pct.
Unsterilized naturally infested soil										
Formaldehyde dust	86.6	8.5	100.0	1.3	100.0	0.7	95.3	1.4	100.0	0.0
Monohydrated $CuSO_4$ dust	86.6	8.5	98.6	19.6	88.6	1.5	99.3	5.4	94.0	7.1
Ceresan-kaolin dust	86.6	8.5	95.3	3.5	100.0*	0.0	100.0*	0.0	96.6*	0.0
Sterilized soil plus Pythium inoculum										
Formaldehyde dust	79.4	36.1	90.6	4.4	88.0	6.7	100.0	5.3	95.3	7.0
Monohydrated $CuSO_4$ dust	79.4	36.1	60.0	37.8	50.6	30.9	64.6	29.9	64.6	16.5
Ceresan-kaolin dust	79.4	36.1	78.0	12.0	96.0*	9.0	100.0*	6.0	90.6*	2.2

*Plants stunted.

In both lots of soil the formaldehyde dust gave very excellent control of damping-off. The strongest treatment, 24 grams of dust per square foot, was considered the best because the rootlets were free of lesions. The Ceresan-kaolin mixture gave excellent control at the rate of 12, 18, and 24 grams of dust per square foot of soil but was toxic to the plants. Emergence was slightly delayed, and the seedlings had an abnormally dark green color. Little or no control was secured from the use of the monohydrated copper sulphate dust.

Experiment II.—This experiment was a continuation of the preceding one, except that larger amounts of the formaldehyde and monohydrated copper sulphate dusts were used. Since the Ceresan-kaolin mixture gave both disease control and seedling injury, a further test was made using a narrower range of amounts, to determine, if possible, a point of safety and control. In addition to the two lots of soil used in the preceding experiment, sterilized soil plus Rhizoctonia inoculum was added. The results are summarized in Tables 6 and 7.

TABLE 6.—Control of Damping-off by Soil Disinfection with Ceresan-kaolin Mixture

Soil used	Rate of treatment in grams of dust per square foot of soil							
	Check		12		15		18	
	Emergence Pct.	Damped-off Pct.	Emergence Pct.	Damped-off Pct.	Emergence Pct.	Damped-off Pct.	Emergence Pct.	Damped-off Pct.
Naturally infested soil	87.3	7.6	94.6	0.0	91.4	0.0	96.0	0.0
Sterilized soil plus Pythium inoculum	22.6	38.3	94.0	0.7	94.0	2.1	88.0	0.8
Sterilized soil plus Rhizoctonia inoculum..	57.3	4.7	92.0	0.0	98.0	0.0	94.0	0.0

In all cases, the Ceresan-kaolin dust gave good control of damping-off. However, the plants were delayed in emergence, abnormally dark green, and slightly stunted.

The formaldehyde dust gave excellent control in all amounts used for the naturally infested soil and sterilized soil with Pythium inoculum. Good control was also secured in the sterilized soil with Rhizoctonia inoculum but slightly less than with the other two soils. With increased amounts of formaldehyde dust there was increased control up to 36 grams per square foot of soil. The root systems on all seedlings were white, free of lesions, and well developed.

TABLE 7.—Control of Damping-off by Soil Sterilization with Chemicals

Treatments	Rate of treatment in grams of dust per square foot of soil													
	Check		18		24		30		36		42		48	
	Emergence	Damped-off	Emergence	Damped-off	Emergence	Damped-off	Emergence	Damped-off	Emergence	Damped-off	Emergence	Damped-off	Emergence	Damped-off
	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
Naturally infested soil														
Monohydrated $CuSO_4$ dust	87.3	7.6	88.0	5.3	84.0	2.4	99.4	0.7	91.3	1.5	94.6	0.0	92.6	1.4
Formaldehyde dust	87.3	7.6	96.0	0.0	94.6	0.0	96.0	0.7	92.0	0.7	96.6	0.0	97.3	0.7
Sterilized soil plus Pythium inoculum														
Monohydrated $CuSO_4$ dust	22.6	38.3	20.0	53.3	34.0	27.4	45.3	19.1	31.4	34.0	46.0	17.4	58.6	30.4
Formaldehyde dust	22.6	38.3	85.3	3.9	89.3	6.7	84.0	7.9	97.3	0.7	97.3	0.0	99.4	0.0
Sterilized soil plus Rhizoctonia inoculum														
Monohydrated $CuSO_4$ dust	57.3	4.7	69.3	4.8	71.4	0.0	90.6	2.2	95.3	0.0	89.3	0.0	94.0	2.8
Formaldehyde dust	57.3	4.7	78.6	0.0	93.3	0.7	88.0	1.5	84.7	0.0	90.0	0.7	94.6	0.7

The control of damping-off from the use of the monohydrated copper sulphate dust was very poor. With increasing amounts of dust, slightly better control was secured, but in the Pythium-infested soil the highest percentage of emergence was 58.6 per cent, which is too low to be of value. In the Rhizoctonia-infested soil better results were secured; the 48-gram treatment gave a 94 per cent emergence against 52.6 per cent in the check. However, the addition of such large quantities of monohydrated copper sulphate to the soil was injurious to seedling development. The plants grew slowly, the cotyledon leaves were abnormally dark green, and the true leaves decidedly yellow. Figure 8 shows the results secured when Pythium-infested soil was treated with each of the three dusts.

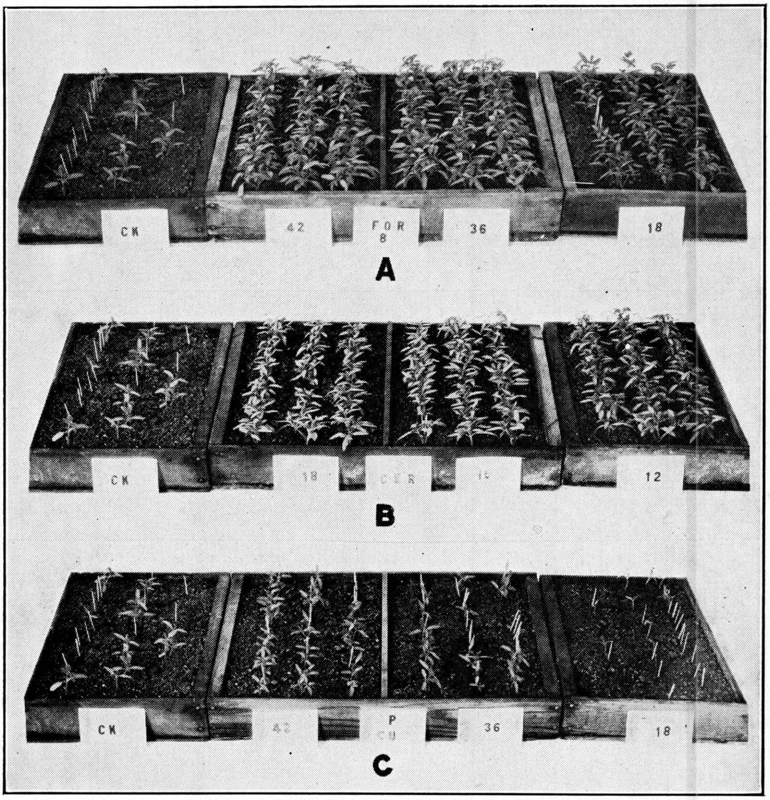

Fig. 8.—Control of damping-off by chemicals. Sterilized soil—Pythium infested. Figures indicate rate of treatment in grams per square foot of soil. A. Formaldehyde dust. B. Ceresan-kaolin dust. C. Monohydrated copper sulphate dust.

Experiment III.—Three additional dusts—a 20 per cent glacial acetic acid, a 20-80 copper-lime, and a 10 per cent leaded zinc oxide (3 per cent)—were tested for control of damping-off. In addition, a 6 per cent formaldehyde dust and steam-sterilized soil were used for comparison. In this experiment, only steam-sterilized soil plus Pythium inoculum was used.

The 20 per cent glacial acetic acid dust was made by adsorbing 20 c. c. of the acid on 80 grams of diatomaceous earth. The dust was screened and stored in an air-tight container. The 20-80 copper-lime dust was made by thoroughly mixing 20 parts by weight of monohydrated copper sulphate with 80 parts of hydrated lime. The leaded zinc oxide was made by mixing 10 parts, by weight, of a 3 per cent leaded zinc oxide dust with 90 parts of kaolin. The 6 per cent formaldehyde dust was made by adsorbing 15 c. c. of 40 per cent commercial formaldehyde (formalin) on 85 grams of a mixture of diatomaceous earth (2 parts) and kaolin (1 part). The addition of kaolin makes a dust that is less bulky and flows more freely.

In Table 8 the quantities of dusts used and the results obtained are recorded.

TABLE 8.—Per Cent Stand When Sterilized Soil, Pythium-Infested, was Disinfected with Chemical Dusts

Treatment	Rate of application in grams of dust per square foot of soil						
	Untreated	20	30	40	50	60	70
20% glacial acetic acid dust	18.2	13.2	13.2	7.1	8.8	2.2	1.3
20–80 copper-lime dust	18.2	59.2	84.4	87.5	85.3	87.0	87.5
10% of a 3% leaded zinc oxide dust	18.2	20.0	25.6	25.6	43.5	....	58.2
6% formaldehyde dust	18.2	....	....	93.3	....	95.0	....
Sterilized uninfested soil	87.5	....	....	....	....	....	....

The glacial acetic acid and zinc oxide dusts, when added to the soil, proved to be quite toxic to developing seedlings. The 20-80 copper-lime dust, when used at the rate of 40 grams or more per square foot of soil, gave considerable control of damping-off, as shown by the high percentage of emergence. However, it was quite toxic to the seedlings when used at this rate. The symptoms were the same as those exhibited by the seedlings grown in soil to which the 50 per cent monohydrated copper sulphate dust was added. The 6 per cent formaldehyde dust gave excellent control and even a higher percentage of emergence than the steam-sterilized, uninfested soil, and the rootlets were well developed and free of lesions.

Experiment IV.—Since the formaldehyde dust, when mixed with the soil at the rate of 42 grams per square foot, gave control, and treating the seed with a 10 per cent copper sulphate solution for 15 minutes gave partial control when used in the small flats, it was decided to try these two treatments on a much larger scale. The seed was planted in a bench duplicating commercial practices as far as possible, except that sterilized soil plus Pythium inoculum was used. The bench, which was 30 inches wide, 12 feet long, and 2½ inches deep, was divided into four sections and an equal amount of the Pythium-infested soil was placed in each. Sixteen rows, 2 inches apart, were planted in each section, a row containing approximately 350 seeds, estimated by weighing.

In a check section, neither the seed nor the soil was treated. In another section, the soil was treated with an 8 per cent formaldehyde dust at the rate of 42 grams (approximately 1½ ounces) per square foot. A third section was planted with seed treated in a 10 per cent copper sulphate solution for 15 minutes, and in the fourth section the soil was treated with formaldehyde dust and the seed was treated with the copper sulphate solution.

For the first few days of the experiment, the soil was kept fairly dry, but during the latter part a much wetter condition was maintained to favor damping-off. The results are tabulated in Table 9.

Seedlings emerged in the untreated and formaldehyde-treated plots one or two days in advance of the two plots planted with copper-sulphate-treated seed. Damping-off began to occur in the check plot as soon as the seedlings emerged and continued throughout the experiment. At the end of the experiment only 52.1 per cent of the seedlings remained alive, and most of these were unfit for transplanting. The plot planted with copper-sulphate-treated seed had 74.4 per cent alive at the end of the experiment, but the root systems of many of these were badly diseased. Damping-off did not occur until the seedlings were several days old. The plot in which the soil was treated with the 8 per cent formaldehyde dust and planted with copper-sulphate-treated seed had 83.6 per cent alive at the end of the experiment, and almost no damping-off occurred.

At the end of the experiment there was 97.2 per cent of seedlings alive in the plot treated with 8 per cent formaldehyde dust. Damping-off occurred in three places on the side of the plot adjoining the check. It is likely that contamination occurred from the adjoining untreated plot, or else sufficient care was not taken in mixing the dust with the soil. (Figure 9).

TABLE 9.—Control of Damping-off in Pythium-infested Soil by Soil Disinfection and Seed Treatment

Untreated			8 per cent formaldehyde dust			Seed soaked in 10 per cent copper sulphate solution 15 minutes			Combination of first two treatments		
Emer-gence	Damped-off	Alive after 21 days	Emer-gence	Damped-off	Alive after 21 days	Emer-gence	Damped-off	Alive after 21 days	Emer gence	Damped-off	Alive after 21 days
Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
64.4	15.5	52.1	98.6	1.8	97.2	78.6	5.6	74.4	84.3	0.8	83.6

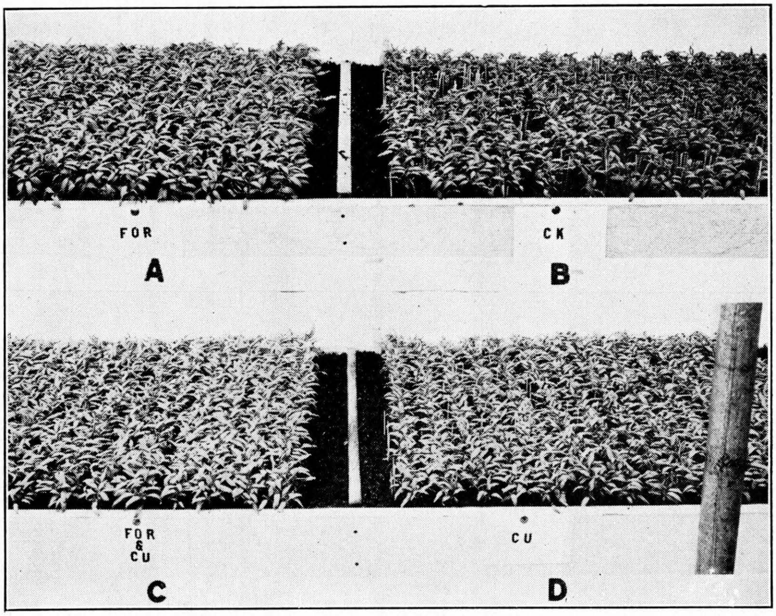

Fig. 9.—Control of damping-off in Pythium-infested soil. Toothpicks represent damped-off plants

A. Soil treated with 8% formaldehyde dust, 1½ oz. per square foot.

B. Check—soil and seed untreated.

C. Soil treated with formaldehyde dust and seed treated 15 minutes in 10% copper sulphate solution.

D. Seed treated 15 minutes in a 10% copper sulphate solution.

DISCUSSION OF EXPERIMENTAL RESULTS

In this investigation it was impossible to include all the available seed-treating compounds; hence, an effort was made to select a limited number that seemed most likely to control damping-off. These compounds failed to control damping-off in naturally infested soil, and it was concluded that they would be worthless for trial in sterilized soil artificially infested with either *P. ultimum* or *R. solani.*

Again, the possibilities in the line of wet seed treatments were far from exhausted. The copper sulphate seed treatments in all cases gave an increased percentage of emergence over the check, even in the sterilized soils to which Pythium and Rhizoctonia inoculum had been added. The seedlings were protected until they were several days old, after which they damped-off rather severely. Soaking the seed in formaldehyde and bichloride of mercury solutions gave no beneficial results.

The most effective method for the control of damping-off was by soil disinfection, either with chemicals or steam. Fortunately, the first experiment included formaldehyde dust, and it was with the use of this dust throughout the investigation that the best results were obtained. In the first experiments an 8 per cent dust was used, ranging in applications from 6 to 60 grams per square foot of soil 2½ to 3 inches deep. Six grams gave some control, but better results were secured by additional amounts up to 36 grams per square foot. Above that, very little difference was noted. In later experiments and when the dust was used commercially, a 6 per cent dust was found to be as effective as an 8 per cent dust. Forty-two grams are equivalent to approximately 1½ ounces, and, since a general recommendation must be made in ounces, it was concluded that the ideal treatment consisted of 1½ ounces of the 6 per cent dust.

The use of the Ceresan-kaolin dust gave considerable promise when mixed with the soil. However, the difference between the amount necessary to control, when used in sterilized soil plus Pythium inoculum, and the amount that injures was very slight. Twelve grams of the diluted dust per square foot of soil were the least that could be used to give good control; yet this amount caused injury to the seedlings. The cost of such treatment would also exceed that of formaldehyde dust. Hence, the product was not considered a safe remedy for use by commercial growers.

A 50 per cent monohydrated copper sulphate dust and a 20-80 copper-lime dust, when used in sufficient quantity to give a good percentage of emergence, caused severe stunting and yellowing. The least injury was secured with the 20-80 copper-lime dust. Hence, it was concluded that these two dusts were of little value for the control of damping-off.

Glacial acetic acid dust proved very toxic and gave a low percentage of emergence and only a slight degree of control. The zinc oxide dust gave some control of damping-off but was also too toxic to the plants.

The results secured from the large scale experiment were quite interesting. Regardless of the thickness of seeding and the large amounts of water used, the 8 per cent formaldehyde dust, at the rate of 42 grams (1½ ounce) per square foot, gave almost perfect control. The results secured by treating the seed in copper sulphate solutions were disappointing since, when the seed was planted thickly as in commercial practice, a delayed germination resulted, and the protective action did not extend over a sufficiently long period. There did not seem to be much in favor of using both the copper sulphate and the formaldehyde dust, because the formaldehyde dust alone gave excellent control.

CONTROL OF DAMPING-OFF OF OTHER THAN TOMATO SEEDLINGS

In March of 1931 some celery growers in the Cleveland area inquired about a means of controlling damping-off in greenhouse celery seedbeds. They were supplied with a 6 per cent formaldehyde dust to be mixed with the soil at the rate of 1½ ounces per square foot of seedbed 2½ inches deep. These growers secured an excellent stand of seedlings and had no trouble from damping-off. Somewhat later, in another greenhouse, two plots were seeded to celery. In one plot the soil was steam sterilized just previous to seeding, and in the other the soil was treated with a 6 per cent formaldehyde dust at the rate of 2¼ ounces per square foot. The seedbed was 6 inches deep. In this case, the formaldehyde dust was worked into the top 2 or 3 inches of soil. At the time of transplanting, the seedlings in the formaldehyde plot were as good as those in the steam-sterilized plot.

In late April, 1931, some tobacco seedbed plots at Germantown, Ohio, were treated and planted. A 6 per cent formaldehyde dust was applied at the rate of 1, 2, and 3 ounces per square foot of seedbed. The dust was worked into the top 2 or 3 inches of soil, the seed sown, and the soil then watered heavily. At the time of transplanting, the plants in the check plot were light green, 3 inches high, and none were considered worth using. In the 1-ounce plot, the plants were 5 inches high, medium dark green, and 50 per cent were good plants. In the 2-ounce plot, the plants were very dark green, 6 inches high, and 85 per cent were good plants. In the 3-ounce plot, the plants were very dark green, 7 inches high, and all were good plants. These plants compared favorably with those grown in the steam-sterilized plots.

Following the experimental part of this work with formaldehyde dust, Mr. P. E. Tilford (23) conducted a series of experiments with 22 species of flower seedlings for the control of damping-off. Many species of flower seedlings are far more susceptible to damping-off than tomato seedlings. In all cases, he secured excellent control with the 6 per cent formaldehyde dust when mixed with the soil at the rate of 1½ ounces per square foot. He also found that air-dried muck would serve as an excellent carrier for the formaldehyde.

GENERAL DISCUSSION

Throughout this investigation the organism which caused the greatest amount of damping-off was *Pythium ultimum. Rhizoctonia solani* was found to cause an appreciable loss, but this was slight when compared with that caused by *P. ultimum.* Isolations from diseased plants yielded cultures of *P. ultimum* and *R. solani* in the ratio of ten to one, respectively.

In many of the experimental flats in which Rhizoctonia inoculum had been mixed with the soil and no attempt at control made, the percentage of emergence was not greatly reduced and relatively few seedlings damped-off. However, many of the seedlings showed symptoms of damping-off, in that the cotyledons were deformed. Many of these deformed plants later recovered sufficiently so that they could be used for transplanting.

In the flats in which Pythium inoculum was mixed with the soil and no attempt at control made, very few plants emerged and many of those that did emerge soon damped-off. In fact, usually there was not a single healthy plant in these flats.

When two environmental factors (soil temperature and soil moisture) were varied, it was found that these two fungi have a wide range of parasitism. At a high soil-temperature and a low soil-moisture content the attack of each fungus was somewhat arrested. Under these conditions more seedlings emerged and damping-off was less severe. However, root injury was quite severe in the cans containing the Pythium inoculum.

The severity of the disease caused by *P. ultimum* increased as the soil temperature was lowered to 18 degrees C., even when the soil moisture was maintained at 35 per cent. As the soil-moisture content was increased at these low temperatures, practically no emergence occurred. *R. solani* was most severe around 24 degrees C. at all soil-moisture contents. It must be concluded that the most favorable environmental conditions for arresting the disease can not be depended on to give a satisfactory control of damping-off.

The general method now in use for the control of damping-off diseases is steam sterilization of the soil; this has proven to be entirely effective when properly done. In many cases the use of this method is impracticable or impossible, and, frequently, after steaming, recontamination takes place and failure to control results. In many places where seedlings are grown, the soil is sterilized in one place and then transferred to seedbeds or flats; with such a procedure it is virtually impossible to prevent recontamination. The only successful steam-sterilization methods are those in which the soil of the seedbed is sterilized by the use of the inverted-pan or the buried-pipe method. By either method the soil can be sterilized between each seedling crop. Since many growers of seedlings are not equipped with suitable apparatus, they either do not attempt to control damping-off or resort to other methods.

One of the first substitutes for the control of damping-off has been the use of various seed treatments. Since damping-off organisms are primarily in the soil and not on the seed, there seems to be little hope for control by this procedure. However, Horsfall (7) found that a copper sulphate seed treatment gave control of damping-off of tomato seedlings. These results were confirmed only in part. It was found that the copper-sulphate-treated seed germinated and a high percentage of seedlings emerged. However, after emergence, damping-off became severe. This was particularly the case when seed was sown in sterilized soil to which Pythium inoculum had been added. Just why this protective action occurred for even a short time is difficult to explain. Possibly, however, enough copper sulphate was carried on the seed and absorbed by the soil to produce a partial disinfection around the developing seedling. Other seed treating compounds, such as formaldehyde and mercuric chloride, gave no such protection.

Many attempts to control damping-off have been made by applying chemical disinfectants to the soil. Formaldehyde has probably been the one most generally employed. The common method of applying it has been to saturate the soil with a 2 to 3 per cent solution. The saturated soil is covered with burlap or heavy paper for 24 hours to retain the gas and then aerated for a few days to allow it to escape. If carefully done, this method insures excellent control. However, there are several objections to the use of a formaldehyde drench. In the first place, it can not be used in greenhouses where there are growing plants because of gas injury. In the second place, in outside seedbeds there must be considerable delay between treatment and seeding during which time recontamination is likely to take place. Also, if the weather happens to be rainy, seeding is further delayed.

A method that would be most practical and have the least objections would be one in which a chemical disinfectant could be added to the soil and the seed sown immediately. Formaldehyde dust seems to meet these requirements. The formaldehyde being adsorbed, at least in part, on a carrier seems to be released rapidly enough to kill damping-off fungi but not in great enough quantities to cause injury to germinating seed. The release of formaldehyde is also in too small quantities to cause any injury to plants growing in the same greenhouse in which the treatment is made.

The formaldehyde dust has been used successfully in greenhouses where several million seedlings were grown. The practice there was to remove the seedlings from a part of a seedbed, mix formaldehyde dust with the soil, reseed, and water thoroughly. When first used on this large scale, it was found that in part of the seedbeds, where the soil had not been properly wet, some injury to the rootlets occurred. One of the most important steps in this procedure was to water thoroughly following planting. The treatment caused a slight delay in emergence, but by the time of transplanting, the seedlings were larger than those grown in steam-sterilized soil.

The use of a formaldehyde dust seems thoroughly practical and insures disease-free seedlings. Under most conditions, the cost of its application does not exceed that of steam sterilization.

SUMMARY

Two fungi, *Pythium ultimum* and *Rhizoctonia solani,* were found to cause serious damping-off of tomato seedlings in seedbeds.

Isolations made from diseased plants yielded cultures of the two fungi in the ratio of ten *P. ultimum* to one *R. solani.*

Inoculation experiments proved both fungi to be pathogenic on tomato seedlings and demonstrated that *P. ultimum* was the major aggressor.

Seedlings were subject to attack by both fungi from the time the hypocotyl emerged from the seed coat until several weeks old.

The symptoms caused by *P. ultimum* were brown, water-soaked lesions; whereas those caused by *R. solani* were brown, more or less shrivelled, and not water-soaked. In addition, *R. solani* causes the cotyledons to be seriously malformed.

Pythium ultimum and *Rhizoctonia solani* were grown on potato-dextrose agar at several controlled temperatures. The former made its maximum linear growth between 25 and 30 degrees C., and the latter at 30 degrees C.

The influence of soil temperature and soil moisture upon growth of the tomato seedling and upon development of damping-off, caused by *P. ultimum* and *R. solani,* has been studied in tanks with controlled soil temperature.

Tomato seedlings grow well from 20 to 30 degrees C., but the most rapid emergence and growth took place at 27 degrees C. Excessive soil moisture reduces the percentage of emergence. A medium dry soil was found to be optimum for quick emergence and rapid growth.

Pythium ultimum caused damping-off at all soil temperatures from 15 to 30 degrees C. and at soil moistures varying between 35 and 65 per cent of the water-holding capacity of the soil. The highest percentage of emergence and the least damping-off occurred at 30 degrees C. in soil at 35 per cent of its water-holding capacity.

Rhizoctonia solani reduced the percentage of emergence of tomato seedlings at all temperatures but was most destructive at 24 degrees C. After emergence the greatest percentage of damping-off occurred at the two highest temperatures, 27 and 30 degrees C., but the temperatures at the surface of the soil where the plants were attacked were lower than the recorded tempera-

tures because of a cooler air temperature. In wet soil the percentage of emergence was lower than in drier soil and the percentage of damping-off was greater.

No control of damping-off in naturally infested soil was secured by dusting the seed with each of five different fungicides.

Soaking the seed in formaldehyde and in mercuric chloride solutions gave no control of damping-off.

Soaking the seed in copper sulphate solutions gave fair control of damping-off in naturally infested soil. In sterilized soil plus Pythium inoculum and sterilized soil plus Rhizoctonia inoculum, the copper sulphate treatment protected the seedlings until after emergence when damping-off occurred seriously. Increasing the strength of the copper sulphate solution up to 10 per cent generally gave better control, but increasing the duration of treatment made little or no difference.

Soil disinfection by the use of copper sulphate, either as a monohydrated copper sulphate dust or a 20-80 copper-lime dust, was unsatisfactory. When sufficient fungicide was mixed with the soil to control damping-off, the seedlings were injured.

Soil disinfection by the use of a Ceresan-kaolin dust gave some promise, but it was found that the least amount that would control damping-off was slightly toxic to tomato seedlings.

A 20 per cent glacial acetic acid dust and a 10 per cent leaded zinc oxide (3 per cent) dust were also toxic to plants.

Excellent control of damping-off, in naturally infested soil, in sterilized soil to which Pythium inoculum had been added, and sterilized soil to which Rhizoctonia inoculum had been added, was secured by disinfecting the soil with a 6 per cent formaldehyde dust.

The recommended method of control is to mix thoroughly the 6 per cent formaldehyde dust with the soil at the rate of 1½ ounces per square foot of seedbed 2½ or 3 inches deep, sow the seed immediately, and water the soil thoroughly.

The dust was used commercially on a large scale for the control of damping-off of tomato seedlings this past spring (1931) by the H. J. Heinz Company.

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