

HILGARDIA

*A Journal of Agricultural Science Published by
the California Agricultural Experiment Station*

VOLUME 13

JANUARY, 1941

NUMBER 10

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EXPERIMENTS WITH FUNGICIDES FOR USE AGAINST *SCLEROTIUM ROLFSSII* IN SOILS^{1, 2}

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INTRODUCTION

IN THE EARLY PHASES of the investigation of measures for control of southern sclerotium rot of sugar beets, caused by *Sclerotium Rolfssii* Sacc., among other lines of attack much attention was given the possibility of destroying the fungus in the soil. It was thought at that time that the fungus might be eradicated by timely application of fungicides. Now that the wide distribution of the disease and the extent of infested tracts in the Sacramento Valley have been determined, workers have realized that such an attack must have limited value.

The sclerotia of this organism are, however, discrete bodies, uniform and convenient in size, furnishing admirable material for the study of the effectiveness of fungicides. It is thought that the following work may show the relative value of several such chemicals and may aid investigators working with less easily handled organisms.

THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE DEVELOPMENT OF *SCLEROTIUM ROLFSSII*

The Influence of Hydrogen-Ion Concentration of the Medium on the Growth of Sclerotium Rolfssii on Agar Plates.—The more conspicuous characteristics of the behavior of this fungus in culture have been reported by Higgins (5),⁵ who found that measurable growth takes place

¹ Received for publication October 10, 1939.

² These investigations were made possible largely through financial contributions by the beet-sugar companies operating in California.

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⁵ Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

in standard beef-extract peptone broth with 2 per cent saccharose between the limits of pH 1.4 and pH 8.8. In media (standard broth) having a limited carbohydrate supply but differing in pH, the fungus uniformly produced an acidity of pH 4.0 except in media originally of extreme acidity—that is, greater than pH 2.0. On the other hand, where as much as 2 per cent saccharose was added to the broth the end point was changed to approximately pH 5.0, except for media originally more acid than pH 2.0. This, Higgins found to result from the ability of the fungus to produce large quantities of oxalic acid.

On solid media containing bromeresol purple we have found that acid produced by the mycelium diffuses into the agar in advance of the growing hyphae. Within the limits of pH 8.0 and pH 2.0 the fungus can grow freely, perhaps overcoming deleterious effects from the original reaction of the medium by its acid-producing ability; or, on carbohydrate-poor media of low pH, it can produce basic material, possibly ammonia as suggested by Higgins (5).

In our experiments, however, at pH 8.0 a solution of 82.4 millimols of K_2SO_4 per liter of water saturated with calcium carbonate had not killed mycelium of *Sclerotium Rolfsii* immersed in it for five days. Since no growth and no change in reaction occurred, evidently a sustained reaction of pH 8.0 in itself cannot be very toxic to even the vegetative form of the fungus. The absence of growth of the mycelium in this solution would result from lack of nutrients even if there were no inhibitory effect of the solution.

The Influence of Hydrogen-Ion Concentration in Soils on the Occurrence of Southern Sclerotium Rot in Beet Fields.—Considering the laboratory evidence just cited one can hardly expect that the hydrogen-ion concentration in soils producing profitable crops of sugar beets will itself markedly affect the development of the fungus and aid in reducing the losses in beet crops. That such is the case, has been verified by field observations. In several fields, the percentage of diseased plants was estimated in certain areas, and the pH of soil samples taken within these areas was determined. Losses of 50 per cent or more were observed in three different fields where the pH of the soil was between 7.6 and 7.7, and in one field a loss as great as 20 per cent occurred in an area where the soil reaction reached pH 8.2. Although the disease was usually more severe in areas of low pH, there was no conclusive evidence that the alkalinity of the soil—up to pH 8.0—limited the distribution of *Sclerotium Rolfsii* or strikingly reduced the severity of the disease.

The Effect of Liming Soil on Infection of Carrots.—In the past, several workers have limed the soil in the hope of reducing losses caused by root parasites. The results, however, have been very variable. Higgins

(6) showed that some control of the bed rot of sweet potatoes caused by *Sclerotium Rolfsii* was obtained by liming except where excessive organic matter was present. Wellman (15) found club root of crucifers to occur in the field irrespective of the pH of the soil. Although he markedly reduced the amount of disease in a cabbage field by using 2 tons of calcium hydroxide, he believed this to be the result of the specific toxic action of the calcium hydroxide toward *Plasmodiophora brassicae* Wor., inasmuch as calcium carbonate and calcium sulfate were not satisfactory inhibitors.

TABLE 1
EFFECT OF CALCIUM HYDROXIDE ON SOIL REACTION AND ON
INFECTION OF CARROTS IN THREE SOILS

Soil	Lime added		pH of soil	Number of carrots tested	Number of carrots infected
	Per cent of weight of soil	Pounds per acre			
Sacramento clay from Reclamation District 999.....	{ None	None	6.9	12	9
	{ 0.037	1,480	7.7	6	5
	{ 0.074	2,960	7.8	6	6
	{ 0.185	7,400	7.9	6	3
	{ 0.370	14,800	8.1	6	6
Sacramento clay from Reclamation District 1660.....	{ None	None	7.3	12	7
	{ 0.370	14,800	7.8	17	6
	{ 0.740	29,600	8.5	17	4
	{ 1.480	59,200	8.8	17	0
Sacramento clay from Reclamation District 999.....	{ ..	None	7.5	21	21
	{ ..	10,000*	8.0	18	18

* Application of lime in the field and artificial infestation with *Sclerotium Rolfsii*.

His results somewhat resembled the experience herein reported regarding the southern sclerotium rot in nontreated soils of various pH values and regarding the apparent benefit secured by liming.

Besides field observations on the relation between the pH of unaltered soils and infection of sugar beets, calcium hydroxide was used for shifting the reaction of similar soils to determine whether this change would inhibit somewhat the infection of carrot roots in these soils under greenhouse conditions. Soil from two infested fields was air-dried, and portions were mixed with amounts of calcium hydroxide. Thus a series was obtained in which the content of $\text{Ca}(\text{OH})_2$ ranged from none to 0.371 per cent of the air-dried weight of the soil, in the first case, and from none to 1.480 per cent in the second. The maximum amounts in these two trials were equivalent, respectively, to applications of 14,800 and 59,200 pounds of calcium hydroxide per acre. In addition, soil from two uninfested fields, treated with 10,000 pounds of lime per acre one and two

years previously, was seeded with sclerotia. Samples of these soils were placed in pots in the greenhouse and, after they had absorbed water from beneath for five days, were planted with carrot roots. As table 1 reveals, though the alkalinity of the soil was increased, there is no evidence that infection was inhibited by liming, except where the equivalent of about 30 tons of lime per acre was applied and a pH of 8.8 was produced.

Effect of Liming Soil on Infection of Sugar Beets.—Calcium hydroxide was applied in field experiments that included tests of several other materials. The application (5 tons per acre) was made to $\frac{1}{40}$ -acre plots in randomized blocks, with five replications. The stand of beets and percentage of infected beets were irregular in these plots so that the reduc-

TABLE 2
THE EFFECT UPON LOSSES IN THE SUGAR-BEET CROP OF APPLYING
CALCIUM HYDROXIDE TO INFESTED SOIL

	Range of pH of soil	Average pH in soil	Infected beets, per cent	Yield of disease- free beets, tons per acre
Lime-treated.....	7.0-8.1	7.46	45.48	10.92
Control.....	6.7-7.3	7.10	59.32	7.81
Difference.....		0.36	13.84	3.11
Difference for significance.....	19:1 odds	14.60	2.85
	99:1 odds	20.12	3.92
Calculated <i>F</i> value.....		7.03	11.95

tion in the average percentage of infected beets in treated plots is a little less than the difference required for significance with 19:1 odds as determined by the analysis of variance (13); but the increase in average yield, although significant when 19:1 odds are accepted, is not significant with 99:1 odds (table 2).

Most of the fields in northern California where *Sclerotium Rolfsii* is abundant are neutral or slightly alkaline in reaction. Under such conditions, small or moderate applications of lime produce no striking shift in reaction.

THE TOXICITY OF VARIOUS FUNGICIDES IN AQUEOUS SOLUTION

Among fungicides, formaldehyde has been popular because of its general adaptability in plant-disease control, especially where soil organisms have been involved. It may be used, therefore, as a standard for reference in placing a value on other fungicides. In the present work it has been thus treated to some extent, but with no attempt to calculate a "formaldehyde coefficient" in the sense of the "phenol coefficient." The

results given in table 3, having been obtained under uniform conditions, are comparable, although they have been collected from work extending over several years. Sclerotia from pure cultures on potato-dextrose agar were immersed in lots of 50 enclosed in cheesecloth. At the end of the test period each lot was washed in three changes of distilled water, plated on potato-dextrose agar, and incubated at 30° C for five days. The tests were conducted at room temperature.

As will be noted, the test periods are longer than those usually re-

TABLE 3
THE TOXICITY TO SCLEROTIA OF VARIOUS FUNGICIDES IN WATER SOLUTION

Materials	Fungicide, parts per million of solution	Minimum lethal exposure, in hours
Acetic acid.....	10,000	1
Ammonia (NH ₃).....	350	12
Ammonia (NH ₃).....	700	2
Ammonium thiocyanate.....	2,000	Not killed in 24 hours
Boric acid.....	10,000	Not killed in 2 hours
Calcium cyanamid (commercial).....	2,400*	120
Chlorinated lime in water.....	546†	10
CS ₂ emulsion.....	3,250‡	2
Ca (OH) ₂	1,000§	6
Dowicide B.....	500	6
Dowicide H.....	500	1
Dowicide P.....	500	6
Formaldehyde (HCHO).....	955	12
Formaldehyde (HCHO).....	3,700	2
Mercuric chloride.....	1,000	½
Phenol.....	500	2
Potassium ethyl xanthate.....	475‡	Not killed in 23 hours
Pyroligneous acid.....	10,000	Not killed in 2 hours
Sodium hydroxide.....	400	12

* Estimated available CaCN₂ solution of pH 10 determined by glass electrode.

† Available chlorine—titrated with sodium thiosulfate.

‡ Estimated available CS₂ in commercial preparation.

§ Saturated solution of pH 12 determined by glass electrode.

ported in such work. This condition is inevitable because of the size (diameter 0.5–2.0 millimeters) and the compact structure of the sclerotial bodies. With most materials in concentrations which might be used in practice, several hours are required for penetration to the point of killing all the tissue of the sclerotia. Since mercuric chloride is apparently an exception to this rule, the writers believe that it may, if absorbed in superficial tissue, prevent the emergence of mycelium from deeper living cells. The data presented are for the shortest period that resulted in the killing of all sclerotia in the test lot.

Of all the materials tested, only ammonia was definitely superior to formaldehyde in point of toxic power and cost, though carbon disulfide

emulsion proved fully as toxic as formaldehyde. The relative toxicity of ammonia and formaldehyde to *Sclerotium Rolfsii* has been previously reported (7) in some detail; and Oserkowsky (11) gives information on the toxicity of several other fungicides to *S. Rolfsii*.

Tests on Infested Soil in Greenhouse Flats.—Early in these investigations—September to November, 1932—tests were made of applications of formalin, ammonium hydroxide, carbon disulfide emulsion, mercuric chloride, and acetic acid solutions to naturally infested Sacramento clay loam. This soil had a moisture equivalent of 51 per cent. For the experiments, 2,800 grams of air-dry soil, in one series of tests, and 2,400 in the other, were made up to half the moisture-holding capacity and placed in small 8-inch flats to a depth of 2–3 inches. The solutions of chemicals were added to the surface of the soil in amounts (1,680 and 1,500 cubic centimeters) slightly exceeding the moisture-holding capacity—that is, at the rate of 1.14 and 1.02 gallons, respectively, per square foot of soil surface. Excess water was drained off through a false screen bottom, and the soil allowed to stand for 10 days. To determine the relative efficiency of the fungicides, samples of sclerotia recovered by washing the soil through a 40-mesh screen were tested for germinability, as described elsewhere (8). After being placed in cheesecloth the sclerotia were surface-sterilized in bichloride of mercury, 1:1,000, for 45 seconds, washed in sterile water, and plated on potato-dextrose agar. In one series of tests, sclerotia from the surface of the soil in the flats were plated separately.

The results (table 4) definitely indicate the relatively high efficiency of formaldehyde and the failure of the other treatments to kill sclerotia even at depths of soil as shallow as 2 or 3 inches.

In one case where a 1:400 dilution of formalin (37 per cent formaldehyde) was used, the sclerotia had so deteriorated that only 16 were recovered from one flat, whereas the soil from the other flats yielded well over 100 sclerotia each after treatment.

Where the sclerotia were recovered from the surface of the soil, none of those treated with formalin (1:100 and 1:400) or with bichloride of mercury (1:1,000) were found viable. Those surface sclerotia that were recovered after treatment with carbon disulfide emulsion (1:100 and 1:400) and with Diesel oil proved on plating to be 94.6, 97.8, and 59.3 per cent viable, respectively.

Depths to Which Formaldehyde, Ammonia, and Sodium Hydroxide Solutions Are Effective When Applied to the Surface of the Soil.—Field applications of solutions of formaldehyde, ammonia, and sodium hydroxide were made in the summer of 1933 (July–September) in three representative districts in the Sacramento Valley. In the test with Yolo loam at Davis, samples of sclerotia were plated at 6-inch and 12-inch depths

before treatment, whereas the other two experiments were on naturally infested soils from which diseased beets had just been removed. The rates of application were 1½ and 3 gallons per square foot on plots 4 square feet in area. The formalin solutions used ranged in strength from 1 volume of commercial formalin (37 per cent formaldehyde) in 50 volumes of water to 1 volume in 1,000. Ammonia water (28 per cent anhydrous ammonia) was diluted to 100 volumes with water. Sodium

TABLE 4

THE EFFECTIVENESS OF AQUEOUS SOLUTIONS OF SEVERAL FUNGICIDES AS WELL AS CARBON DISULFIDE EMULSION AND DIESEL OIL IN KILLING SCLEROTIA IN SOIL

Material	Dilution ratio	Application of the pure chemicals, pounds per acre	Number of trials	Number of sclerotia plated	Viable sclerotia, per cent
Formalin (37 per cent HCHO).....	1:100	1,530	3	335	1.2
	1:200	765	2	252	2.0
	1:400	382	3	276	2.8
	1:1,000	153	2	238	22.7
Ammonium hydroxide (28 per cent NH ₃).....	1:60	960	2	100	10.0
	1:180	320	1	50	74.0
	1:360	160	1	50	76.0
Acetic acid (glacial).....	1:100	1,875	1	50	28.0
Mercuric chloride (dry salt).....	1:1,000	167	1	50	48.0
CS ₂ emulsion (65 per cent CS ₂).....	1:100	1,088	1	50	82.0
	1:400	272	1	50	80.0
Diesel oil (sp. gr. 0.87).....	Without dilution	416,000	1	50	66.0
Control H ₂ O.....	3	297	88.9

hydroxide solution was made up by dissolving 1 part by weight in 100 parts of water.

The soil was ridged up around the plots to provide a basin. Careful excavating after an application of 3 gallons of 1:100 formalin revealed a limited lateral spread (6 inches) of the solution beyond the measured boundary of the plot, and a corresponding reduction of the depth of penetration in the marginal zone of the treated areas to 16 inches, as compared with 21 inches in the middle of the plot. The plots in the sugar-beet fields were sampled with a soil tube 8 to 10 days after treatment in horizons of 0-6 inches and 6-15 inches. As previous trials (8) had shown, a very small proportion of the sclerotia in an infested field are to be found below 15 inches, most of them being located in the surface 6 to 8 inches. These soil samples were washed through a 40-mesh screen in the laboratory,

TABLE 5
THE EFFECTIVENESS OF FIELD APPLICATIONS OF AQUEOUS SOLUTIONS OF FUNGICIDES IN KILLING SCLEROTIA

Chemical	Soil type and locality	Gallons applied per sq. ft.	Sampling horizon, inches	Per cent* of sclerotia viable after treatment of soil with solutions of the given strengths						Controls H ₂ O
				1:50	1:100	1:200	1:400	1:1,000	Controls H ₂ O	
				..	0	74	72	94		
Formalin (38 per cent HCHO).....	{ Yolo loam, Davis.....	3	{ 0-6 6-15	..	0	74	72	94	32 62†	
	{ Sacramento clay, Clarksburg.....	3	{ 0-6 6-15	..	0	32	64	91	..	
	{ Sacramento clay, Clarksburg.....	1½	{ 0-6 6-15	..	6	8	56	94	..	
	{ Sacramento clay, Woodland.....	3	{ 0-6 6-15	..	0	24	38	96	..	
Aqua ammonia (28 per cent NH ₃)....	{ Sacramento clay, Woodland.....	3	{ 0-6 6-15	..	34	46	80	90	98†	
	{ Yolo loam, Davis.....	3	{ 0-6 6-15	6	6	2	14	80	..	
Sodium hydroxide (1 per cent NaOH).	{ Sacramento clay, Woodland.....	3	{ 0-6 6-15	64	37	50	71	93	87†	
	{ Sacramento clay, Woodland.....	3	{ 0-6 6-15	..	76	32 62	
	{ Sacramento clay, Woodland.....	3	{ 0-6 6-15	..	94	
	{ Sacramento clay, Woodland.....	3	{ 0-6 6-15	..	92	87†	
	{ Sacramento clay, Woodland.....	3	{ 0-6 6-15	..	70	
	{ Sacramento clay, Woodland.....	3	{ 0-6 6-15	..	78	87†	

* Percentages based on samples of 50 sclerotia recovered from the soil 10 days after treatment. (In eight cases less than 50 sclerotia were recovered.)
† Horizon 0 - 12 inches rather than 6 - 15 inches.

and samples of 40 to 50 sclerotia were plated on potato-dextrose agar after surface sterilization for 45 seconds with 1:1,000 HgCl₂. In 8 tests out of the 59, fewer than 50 sclerotia were recovered; and in these cases all the sclerotia were plated.

Sclerotia placed in soil before treatment were made up in lots of 50 and tied in cheesecloth before being planted at depths of 6 inches and 12 inches. The soil was thoroughly packed around the samples after placement.

Table 5 gives the percentage of viable sclerotia in the various test samples. As will be noticed, a 1-100 dilution of commercial formalin was highly effective to a depth of 6 inches, but not lower. More dilute solutions had little effect on the viability of sclerotia. Aqua ammonia and sodium hydroxide diluted 1-100 were not lethal to sclerotia in soil.

To complete the information given in table 5, we must mention the development upon plated sclerotia of other organisms apparently growing from within the mercuric-chloride-treated surface. These organisms were characteristically of two sorts: first, a white bacterium appearing consistently upon sclerotia recovered from control plots wet only with water; second, a *Trichoderma* species appearing upon considerable numbers of the sclerotia treated with formalin. The presence of the bacteria or fungi may have considerably affected the percentage germination given above, for the controls in the experiments with Yolo loam soil showed a lower percentage of viable sclerotia than did the treatments with 1:1,000 dilution of formalin. In this instance, out of the 50 nontreated sclerotia recovered from the 6-inch level, 34 did not germinate; and from all these 34 there grew the white bacterium. That a higher percentage of germinated sclerotia might have been secured is suggested by the more recent experience of Leach and Mead (9), who indicate a preference for plating on peat soil without sterilization.

The relation of *Trichoderma* to sclerotia from soil treated with formalin is even more striking. The percentage of sclerotia from which *Trichoderma* grew decreased along with the strength of the solution used for treating the soil. In other words, *Trichoderma* apparently involved the interiors of the sclerotia as they became weakened or killed, the invader finally becoming deeply seated enough to be below the zone affected by the surface application of mercuric chloride. The data, showing the effect of applying 1½ gallons per square foot, are given in table 6.

Judging from these results *Trichoderma*, instead of being an active parasite of *Sclerotium Rolfsii*, invades only the sclerotia killed or weakened by chemical treatment. This opinion is supported by the fact that *Trichoderma* is only occasionally recovered from either viable or nonviable sclerotia washed from nontreated soils.

Experiments with Formalin in Preventing Plant-to-Plant Spread of the Rot in Field Rows of Sugar Beets.—Frequently, in fields where southern sclerotium rot occurs, infected plants are found adjacent in the rows, suggesting that the disease has been transmitted by growth of the causal fungus through the soil. If such be the case, removal of single affected plants as soon as possible after symptoms appear might be expected to effect a material saving in the crop, especially if the operation were accompanied by treatment of the soil from which the plants were removed. The logic of the procedure seems well founded, since the extensive development of mycelium in the soil depends upon the presence of abundant nutritive material such as is furnished by the sugar beet.

TABLE 6
THE RELATION OF STRENGTH OF FORMALIN SOLUTION TO
GERMINATION OF SCLEROTIA AND TO INVASION
OF SCLEROTIA BY TRICHODERMA

Strengths of formalin solutions used in treating soil	Per cent sclerotia recovered from soil	
	Germinating	Showing growth of <i>Trichoderma</i> on plating
1:100.....	8.5	57.0
1:200.....	30.8	21.4
1:400.....	64.8	15.8
1:1,000.....	86.5	3.0
Control.....	98.0	0.0

The method was tested in June, 1934, by a field experiment in the Holland land tract. Because the crop was subirrigated, there was no carrying of sclerotia such as might occur with surface water moving along the rows. In this field, at the start of the experiment, approximately 100 plants per acre or 0.4 per cent of the stand exhibited symptoms of the disease.

The experimental area was divided by the irrigation ditches into three plots of about the same size (1.5 acres). In one plot the diseased plants were located and marked with stakes but not removed, and no formalin was applied to the soil. In a second plot some 150 diseased plants were located and removed, together with the soil immediately surrounding each diseased beet root. After removal of each root, 3 quarts of a solution consisting of 1 part of commercial formalin to 50 of water were poured into the space previously occupied by the beet. In a third plot 20 infected plants were found and removed, and the adjacent soil treated with 2 gallons of 1:50 formalin, the disinfectant being retained by 12-inch

TABLE 7

THE EFFECT OF REMOVING DISEASED BEETS FROM THE ROW, WITH SUBSEQUENT LOCAL SOIL STERILIZATION, UPON THE INFECTION OF ADJACENT BEETS

Procedure	Number of diseased plants	Plants becoming infected within 20 days after removal or marking of adjacent diseased plants	
		In same row	In next row
Plot 1: Plants not removed; soil untreated.....	57	23	12
Plot 2: Plants removed; soil treated with 3 quarts formalin (1:50) per plant.....	83	17	13
Plot 3: Plants removed; soil treated with 2 gallons formalin (1:50) per plant.....	20	3	13

metal cylinders open at both ends, the lower edge being thrust into the soil. On June 25, twenty days after treatment, counts were made of the numbers of diseased plants adjacent in the same row, and in the rows next on each side, to spots from which plants had been removed, as compared with the numbers of diseased plants found adjacent to those pre-

TABLE 8

THE EFFECTIVENESS OF FORMALIN IN KILLING SCLEROTIA IN INFESTED SOIL* FROM WHICH DISEASED PLANTS HAVE BEEN REMOVED

Treatment and plant number	Sclerotia recovered per 200 grams of soil	Per cent germination	
Plants not removed; untreated.....	1.....	333	100
	2.....	165	98
	3.....	915	98
	4.....	572	100
	5.....	585	98
	<i>Average</i>	<i>514</i>	<i>98</i>
Plants removed; space treated with 3 quarts formalin (1:50).....	1.....	63	0
	2.....	21	0
	3.....	43	2
	4.....	20	0
	5.....	55	48
	<i>Average</i>	<i>40.4</i>	<i>10</i>
Plants removed; space cylinder-treated with 2 gallons formalin per diseased plant.....	1.....	249	0
	2.....	2	0
	3.....	46	0
	4.....	67	0
	5.....	252	0
	<i>Average</i>	<i>123.2</i>	<i>0</i>

* Soil sampled to a depth of about 1 foot.

viously marked as diseased, but not removed. The numbers of diseased plants subsequently occurring in the same row as the removed or marked plants show (table 7) that the amount of disease was reduced as the result of treatment or removal. The spread to plants in adjacent rows appeared, however, not to be affected by the methods used.

In the field where these trials were conducted the percentage of diseased plants was, as will be noted, very small. Where the disease is more abundant the methods used in this experiment could be of little practical value.

At the time of the final counts, soil samples were taken from several treated spots and also from untreated areas around diseased plants that had not been removed. Sclerotia recovered by washing the soil samples through a screen were plated on potato-dextrose agar after surface sterilization for 45 seconds with 1:1,000 HgCl₂. Table 8 shows the effectiveness of the treatments in reducing the number of viable sclerotia left in the treated areas. Evidently, where the disease is localized in a very small area, such timely treatment of diseased plants may reduce the amount of infective material left in the soil. Probably, where as much as 90 per cent of the sclerotia in the treated spots are killed, as in the present case, the incidence of the disease in subsequent crops would be considerably reduced.

THE TOXICITY OF VOLATILE FUNGICIDES TO SCLEROTIA

The Toxicity of Ammonia, Formaldehyde, Naphthalene, Xylene, Tetrachlorethane, Pentachlorethane, Chloropicrin, and Carbon Disulfide to Wet and Dry Sclerotia.—Since Oserkowsky (11) found chloropicrin, tetrachlorethane, and pentachlorethane ineffective against *dry* sclerotia and since Godfrey (4) has pointed out that *wet* sclerotia are readily killed by chloropicrin, all these materials were tested against air-dry sclerotia and against sclerotia soaked in water for at least 15 minutes before treatment.

Sclerotia were subjected to the vapors of ammonia and formaldehyde over aqueous solutions; the solutions of ammonia contained 0.14, 0.07, and 0.028 per cent of NH₃, and the formaldehyde solutions 0.4, 0.2, 0.1, and 0.04 per cent of formaldehyde. Tests of naphthalene, xylene, tetrachlorethane, pentachlorethane, chloropicrin, and carbon disulfide were conducted with saturated atmospheres over the surface of the chemical.

The sclerotia used in testing the ammonia and formaldehyde solutions were from plate cultures on potato-dextrose agar. For the other materials the sclerotia were obtained from field-infected sugar beets. Those produced on beets are characteristically larger than those from plate cultures.

After treatment with gaseous ammonia and formaldehyde, the sclerotia were plated directly on potato-dextrose agar. In the other tests they were plated on unsterilized peat soil as described by Leach and Mead (9).

Tests were run for 24 and 72 hours at room temperature in 22-mm test tubes, fitted with rubber stoppers except that with chloropicrin and carbon disulfide, glue-coated corks were used. Sclerotia were in cheese-cloth sacks suspended above the chemicals.

The results of the experiment, presented in table 9, show that sclerotia

TABLE 9
THE TOXICITY OF VAPORS OF VOLATILE FUNGICIDES TO WET
AND DRY SCLEROTIA

Material	24 hours' exposure				72 hours' exposure			
	Wet sclerotia		Dry sclerotia		Wet sclerotia		Dry sclerotia	
	Plated	Viable	Plated	Viable	Plated	Viable	Plated	Viable
Ammonia (NH ₃)								
0.14 per cent solution.....	20	0	20	0
0.07 per cent solution.....	20	2	20	13	20	0	20	2
0.028 per cent solution.....	20	20	20	20	20	20	20	19
Formaldehyde (HCHO)								
0.4 per cent solution.....	20	0	20	0
0.2 per cent solution.....	20	5	20	1	20	0	20	0
0.1 per cent solution.....	20	19	20	18	20	0	20	0
0.04 per cent solution.....	20	20	20	19	20	19	20	20
Naphthalene.....	40	24	40	22
Xylene.....	19	16	19	18
Tetrachlorethane.....	19	0	19	19
Pentachlorethane.....	21	0	20	19
Chloropicrin.....	50	0	50	50
Carbon disulfide.....	50	0	50	3

were killed by vapors of the more concentrated solutions of both ammonia and formaldehyde. There is some indication that wet sclerotia were more readily killed by ammonia vapors than dry sclerotia. Evidently, however, moisture-saturated atmospheres at room temperature will supply sufficient moisture to make ammonia an effective killing agent. Further information on this point is furnished by another experiment in which air-dry sclerotia were placed in suction flasks, which were evacuated to give a pressure of 38 centimeters of mercury (about $\frac{1}{2}$ atmosphere). Anhydrous ammonia was then run into the flask until atmospheric pressure was restored. In the first of two trials all the sclerotia were killed after three hours' exposure, but in the second, 10 sclerotia out of a sample of 50 remained viable.

Again table 9 clearly shows the difference in the susceptibility of the

wet and dry sclerotia to chloropicrin, tetrachlorethane, and pentachlorethane.

A striking feature of the data is found in the fact that sclerotia (both wet and dry) were extremely resistant to vapors of xylene and naphthalene; indeed those of the latter have been found according to data not included in table 9 as merely inhibiting growth without further apparent injury to the fungus even after 5 days' exposure. This was surprising because of the effectiveness of xylene reported by Ezekiel and Taubenhau (2) in field tests with *Phymatotrichum omnivorum* and because of the results of Weiss and Evinger (14), who found that naphthalene vapor kills sclerotia in 3 to 4 days' exposure at 30° C. In a later experiment, however, we have found that air-dry sclerotia soaked for only 15

TABLE 10
EFFECTIVENESS OF XYLENE AGAINST SCLEROTIA SOAKED IN
WATER FOR DIFFERENT PERIODS PREVIOUS
TO TREATMENT

Time sclerotia soaked in water	Per cent germinated after:		
	24 hours' over xylene	48 hours' over xylene	1 week over xylene
Dry sclerotia.....	100	100	76
15 minutes.....	38	14	0
45 minutes.....	28	4	0
3 hours.....	0	0	0

minutes in water are still relatively resistant to exposures of 48 hours to saturated vapors of xylene, as are those soaked for as long as 45 minutes; whereas sclerotia soaked for 3 hours were all killed by an exposure as short as 24 hours (table 10).

Just why the dry sclerotia must be soaked so long before the beginning of the test is a point of interest. We assumed that initiation of growth processes might be necessary. The recent experience of Pinckard and his associates (12), however, concerning the increased toxicity of benzene vapors to tobacco leaves after wetting opens the question of the extent to which water had penetrated the sclerotial "tissue" within the shorter periods of soaking. Thus if the toxicity of the fungicide were to depend upon its accumulating in the water in contact with the cells, one might judge that only the outer sclerotial cells were wet, leaving the interior dry and not exposed to the fungicide in the condition in which it could be effective. These points we have not had the opportunity to investigate.

The Effectiveness of Anhydrous Ammonia Against Sclerotia in Soils of Different Moisture Content.—With a view to injecting anhydrous

ammonia as a volatile fungicide into infested soils, we investigated, as follows, the physical condition of the soil necessary to the effective use of that chemical. Seemingly, if the gas could be made to permeate dry soil and kill sclerotia, there would be reasonable hope for its practical application.

To establish the lower limit of soil moisture necessary to the effective use of anhydrous ammonia against sclerotia, two soil types were investigated: a Yolo fine sandy loam with moisture equivalent of about 17 per cent, and Columbia silty clay loam with a moisture equivalent of 28 per cent. Test samples of sclerotia were placed in a median position in columns of 30 grams of oven-dry soil. The columns were made up by placing

TABLE 11
THE RELATION OF THE MOISTURE CONTENT OF SOIL TO THE EFFECTIVENESS
OF ANHYDROUS AMMONIA IN KILLING SCLEROTIA

Moisture content of soil	Per cent of sclerotia surviving treatment						
	After 2 hours' exposure in:		After 6 hours' exposure in:		After 12 hours' exposure in:		After 24 hours' ex- posure in Columbia silty clay loam
	Yolo fine sandy loam	Columbia silty clay loam	Yolo fine sandy loam	Columbia silty clay loam	Yolo fine sandy loam	Columbia silty clay loam	
Oven dry.....	..	64	..	48	36	18	14
5 per cent.....	100	96	0	0	0	0	0
12.5 per cent.....	91	98	0	0	0	0	0
27.3 per cent.....	..	94	..	100	..	96	60

the soil in a short length of 22-mm glass tubing, the ends of which were closed with cheesecloth to retain the soil. The moisture contents of other samples of the same soil were made up to 5 per cent, 12.5 per cent, and 27.3 per cent, the last being close to the field capacity of the Columbia silty clay loam. The tubes were placed in 2-liter suction flasks (one to a flask); and, as in the previous experiment, ammonia to give a partial pressure of $\frac{1}{4}$ atmosphere was introduced by replacement. The results on plating the sclerotia after different periods of treatment are given in table 11, which shows the percentage survival in samples of 50 sclerotia. Evidently, air-dry sclerotia in contact with soil containing 5 per cent soil moisture are rendered susceptible to the relatively heavy dosage employed. The crumb structure of the soil containing 27.3 per cent moisture was broken down in the process of packing the tube, and the plug thus made prevented killing by the gas within the time of the exposure.

Conceivably, since such a relatively low moisture content of the soil would render the use of ammonia effective, one might utilize anhydrous

ammonia in the field by injecting it into the soil. Accordingly a galvanized iron cylinder with a perforated bottom, a diameter of 12 inches, and a depth of 24 inches was obtained. A large sample of Columbia silty clay loam, dried to a moisture content of 7 per cent, was screened through 0.25-inch mesh. A piece of glass tubing was bent into a 10-inch circle and pierced at eight points, spaced equally along its length, by holes about 1 mm in diameter. One end was sealed, the other attached to a rubber tubing that led from a cylinder of anhydrous ammonia. The flow of ammonia from the cylinder was controlled by a needle valve calibrated (by collecting the gas over saturated Diesel oil) to deliver an average of 364 cubic centimeters in 20 seconds. In setting up the experiment, four test lots consisting of 35 to 60 sclerotia in cheesecloth were distributed in each

TABLE 12
KILLING RANGE OF ANHYDROUS AMMONIA IN AIR-DRY SOIL

Distance from level at which NH ₃ was introduced, centimeters	Per cent of viable sclerotia found 20 hours after introducing NH ₃							
	Above level of introduction				Below level of introduction			
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 3	Sample 4
Same level.....	0	0	0	0
7.6.....	0	0	0	0	0	0	0	0
15.2.....	83	76	82	77	10	0	86	0
22.8.....	63	85	82	77	32	34	35	39

of seven horizontal planes separated by 3.45 kilograms of soil at vertical distances of about three inches. The delivery tube was set in the median horizontal plane. When the experiment was thus set up, the needle valve was opened to deliver ammonia for 91 minutes at the rate indicated. Thus there was introduced some 80 liters of gas or an estimated 60 grams of ammonia, at standard conditions of temperature and pressure. There was no convenient way of checking the actual delivery; but because of the constant pressure in the tank it is supposed not to differ greatly from expectation.

Twenty hours after starting the delivery of ammonia, the test samples of sclerotia were recovered from the soil, surface-sterilized for 45 seconds in 1:1,000 HgCl₂, washed, and plated on potato-dextrose agar. Table 12 shows the percentage of viable sclerotia at the various distances from the level at which the ammonia was introduced.

Thus, as will be seen, a volume of ammonia (80 liters) several times the approximate pore space (24 liters) of the volume of soil (37 liters) used was insufficient to penetrate the mass of soil even with as little as 7 per cent moisture. The rate of injection was slow, somewhat less than a

liter per minute, because of the need for controlling the amount used. It is unknown what might be the result of using the much greater rates of delivery possible, and thus perhaps forcing the gas through the soil mass more rapidly than the soil could absorb it.

Laboratory Tests of the Toxicity of Vapors of Xylene, Tetrachlorethane, Pentachlorethane, Chloropicrin, and Carbon Disulfide to Sclerotia in Soil.—The literature regarding the use of volatile disinfectants in soils has been reviewed by Bliss (1).

A simple technique was adopted for these tests: a weighed amount of soil, adjusted to the desired moisture content by the method of Nichols (10), was placed in quart Mason jars; and the material to be tested was introduced from a graduated pipette through a glass tube which was placed vertically in the jar at the time of filling the latter with soil and which reached to the bottom of the jar.

Immediately after introduction of the fungicide, the tubes were sealed at the upper end with cork stoppers freshly coated with animal glue. The jars were sealed with glass tops and rubber rings after vapor clinging about the top of the jar had been blown away and after sclerotia soaked for at least 15 minutes had been placed on small squares of cheesecloth on top of the soil. This procedure resembles that used by Ezekiel and Taubenhau (2) in tests with *Phymatotrichum*.

The soil-moisture content was adjusted to three levels: 10 to 11 per cent, 15 to 17 per cent, and 23 to 25 per cent. The last approached the moisture equivalent of the sample of Columbia silty clay loam used. The jar was completely filled by 970 grams of oven-dry soil made up to between 23 and 24 per cent soil moisture. With less soil moisture, the jars were not quite filled. In some tests with chloropicrin, samples of screenings from two beet-receiving stations were used. These were passed through a 4-mesh screen to remove large parts of sugar beets and leafy material. Although these samples possibly included several soil types and certainly contained undetermined amounts of organic matter, the results did not differ markedly from those obtained with the Columbia silty clay loam. Most of the tests were made at room temperature; others in a thermostat at 25° C. The time between introduction of the fungicide and recovery of sclerotia from the jars was 24 or 48 hours. Sclerotia used for these tests were obtained from diseased sugar beets and were produced either in the laboratory or in the field. After exposure to the test materials, the sclerotia were plated on peat soil without surface sterilization and were incubated at 30° C. The tests were repeated at least twice, usually three and four times, with close agreement. The figures presented in table 13 are for a single representative trial with soil at 10 per cent and 25 per cent moisture, and a test period of 48 hours.

Chloropicrin is conspicuously more effective than any of the other materials. Carbon disulfide, which approaches it most closely, is only about one fifth as toxic. Xylene, tetrachlorethane, and pentachlorethane were definitely ineffective under the experimental conditions. Here we must recall that the sclerotia were soaked for 15 minutes before being placed in the jars. As previously stated, it has since been learned that the duration of soaking the sclerotia within the range used in the experiment is of vital importance to the results. Possibly, therefore, these three fumigants would have been much more effective if the sclerotia had been

TABLE 13
THE COMPARATIVE EFFECTIVENESS OF FIVE VOLATILE FUNGICIDES AGAINST SCLEROTIA EXPOSED FOR 48 HOURS IN SOIL IN CLOSED GLASS CONTAINERS

Fungicides	Per cent moisture in soil	P.p.m. of fungicide in dry soil	Sclerotia in test sample	Viable sclerotia
Xylene.....	{ 11	10,000	20	20
	{ 25	10,000	20	19
Tetrachlorethane.....	{ 11	5,000	20	20
	{ 25	5,000	15	15
Pentachlorethane.....	{ 11	5,000	20	20
	{ 25	5,000	20	15
Chloropicrin.....	{ 11	100	20	0
	{ 11	50	20	20
	{ 25	100	40	0
	{ 25	50	40	40
Carbon disulfide.....	{ 10	450	40	19
	{ 23	500	40	12
	{ 23	600	40	0

soaked for at least 3 hours. Nevertheless the dosage of fumigant used sufficed to saturate the space within the jar in each case; and sclerotia similarly treated except for the presence of soil succumbed to both tetrachlorethane and pentachlorethane, though not to xylene. The soil has therefore undoubtedly reduced their effectiveness by restricting diffusion, and probably by absorption. Variations in moisture content of the soil did not change the results. It should also be pointed out that the soil moisture was sufficient in all cases to saturate the atmosphere within the jars with water vapor.

Depth to Which Sclerotia Are Killed by Chloropicrin in Soil When the Gas Is Confined at the Surface.—Though the tests in Mason jars offer a convenient means of comparing different fungicides and soils, the values obtained for such a small, confined space can scarcely serve as a basis for dosage in the field, where continuous diffusion and downward movement

by convection take place in the soil. The following experiment, accordingly, was set up in the laboratory with chloropicrin as the fungicide.

A cylinder was made of a special chloropicrin-proof kraft paper supplied by Dr. G. H. Godfrey, who has investigated the efficiency of such materials (3). This paper consists of two thicknesses glued together and has one side treated with paraffin. In making up the cylinder a strip 24 inches wide was wrapped around a section of 3-inch pipe, and the free edge secured by Scotch masking tape. The top and bottom of the tube were made of the same paper, the bottom being folded over the paper at the end of the pipe and secured by more tape.

Upon removal of the paper cylinder from the pipe, the paper tube was filled with fractional amounts of Columbia silty clay loam made up to a moisture content of 15 per cent. As each fraction of 1,165 grams of soil was placed in the tubes and tamped firmly, a test sample of 40 sclerotia was placed on the leveled soil surface. Thus the sclerotia were spaced at intervals very close to 15 centimeters. When the tube had been filled with four such fractions, the last sample of sclerotia was covered with a centimeter depth of soil; and the top end of the tube was closed by a cap secured by masking tape.

The dosage of chloropicrin was calculated on the basis of 2 cubic centimeters per square foot of soil surface. This requires 0.136 cubic centimeters of fungicide for the 63.6 square centimeters of cross-sectional area of the tube. The chemical was applied by means of a 2-cc glass hypodermic syringe with a 2.6-cm needle. A puncture was made in the tube 16 centimeters from the upper end; and after the injection of the chemical on the insertion of the full length of the needle, this puncture was closed with a small piece of Scotch tape. In actually making the injection, a slight excess or 0.14 cubic centimeter of chloropicrin was introduced. The delivery of the hypodermic according to the graduation of the cylinder was found to have an extreme error of ± 3 per cent when checked by weighing, on a chemical balance, the amount delivered. The tube was stood upright after introduction of the chloropicrin.

The sclerotia were recovered from the tube after 48 hours by sectioning the tube and soil column with a large knife at the points where the test samples had been placed, starting with the lowermost samples. As each cut was made, the soil surface exposed was carefully examined for chloropicrin odor. The sclerotia recovered were immediately plated on peat. Table 14 gives results of the examination and plating.

The chloropicrin is of course very easily detectable in extremely low concentration by smell. Evidently little of the vapor, if any, penetrated as far as 30 centimeters. On the other hand, sclerotia were killed at distances of 15 centimeters above and below the points of injection. An ap-

plication of 2½ cc per square foot of soil surface injected to a depth of 6 inches should then be adequate to kill sclerotia to a depth of 12 inches with proper confinement of the gas at the surface. Apparently, furthermore, downward movement of the vapors by displacement of air is very slight with such small doses and within the limited depths of soil under treatment.

The Effectiveness of Chloropicrin in Killing Sclerotia When Applied to an Infested Area in a Beet Field.—In August, 1934, 400 square feet of sugar-beet field on Grand Island, Sacramento County, was treated with chloropicrin. Nearly all the beets in this area had been killed by *Sclerotium Rolfsii*, and the rotted roots were removed before treatment. The soil, belonging to the Egbert series, appeared dry and friable. The

TABLE 14
THE KILLING RANGE OF CHLOROPICRIN IN SOIL CONFINED
48 HOURS IN A GLUED PAPER CYLINDER

Distance of sclerotia from point of injection, centimeters	Per cent of sclerotia found viable	Chloropicrin detectable
15 above.....	0	Strong odor
0.....	0	Strong odor
15 below.....	0	Strong odor
30 below.....	67	No odor
45 below.....	98	No odor

surface soil was worked into loose condition by hand-digging, and half the area was moistened by applying a quart of water per square foot before and again after working of the soil. By means of a Vermorel applicator 1-cc amounts of chloropicrin were injected at intervals of 1 foot each way to a depth of 6 inches over the entire area. The total amount delivered was checked by measuring the amount of residue in the applicator. As the area was treated, the surface was covered with kraft wrapping paper sized on both sides with 10 per cent animal glue as suggested by Godfrey (3). As successive strips of paper were laid down over the treated areas, their edges were secured by Scotch masking tape. The edges of the paper at the periphery of the area were carefully buried under several inches of soil.

Two days after the application of the chloropicrin the cover was found to have been badly whipped by the wind. There was at least one considerable tear, and the Scotch tape had pulled loose in several places. The confinement of the gas was therefore not complete.

Samples of sclerotia were recovered from the treated area to a depth of 8 inches by screening them out of a number of soil-tube cores taken

both before and after the chloropicrin was applied. The sclerotia were surface-sterilized with 1:1,000 HgCl₂ for 45 seconds before being plated on potato-dextrose agar. The results of viability tests of these sclerotia appear in table 15. Evidently, the percentage of viable sclerotia was reduced from 90 to between 20 and 30. The sclerotia from the wetted area showed a slightly lower percentage of germination than those from the dry areas.

The dosage of 1 cubic centimeter per square foot of soil, if assumed to penetrate uniformly to a depth of 1 foot, would be at the rate of about 60 parts per million of dry soil. This is less than the effective rate (100 parts per million of dry soil) with nearly perfect confinement of the gas under laboratory conditions and is only half the dose found necessary

TABLE 15

THE VIABILITY OF SCLEROTIA FROM A FIELD PLOT TREATED WITH CHLOROPICRIN AT THE RATE OF ONE CUBIC CENTIMETER PER SQUARE FOOT OF SOIL SURFACE

Samples	Number of sclerotia plated	Number of sclerotia viable	Per cent of sclerotia viable
Sclerotia from soil samples taken before treatment.....	200	181	90.5
Sclerotia from soil samples taken two days after treatment, soil left dry.....	200	55	27.5
Sclerotia from soil samples taken two days after treatment, soil wet.....	197	45	22.8

to kill to a depth of 1 foot when allowed uninterrupted downward movement. A higher degree of effectiveness can probably not be expected under the circumstances. The rate of application used was equivalent to applications of 200 pounds per acre; greater dosages would probably be prohibitive in cost. Beside the high cost, the greatest handicap to practical application of chloropicrin soil treatment in open fields is the difficulty of providing complete confinement of the gas.

The Effectiveness of Chloropicrin Against Sclerotia in the Surface Layers of Piles of Screenings at a Beet-Receiving Station.—To eliminate the most important method of spreading *Sclerotium Rolfsii* and other parasites from one beet field to another, the screenings at the beet-receiving stations in California are piled on nonagricultural land instead of being returned to each grower's truck as was formerly done. As an additional precaution, destruction of sclerotia in such piles seemed desirable.

In October and November 1934, several attempts were made with chloropicrin to kill sclerotia planted in the surface material of piles of screenings accumulated at a beet-receiving station. In earlier experiments^o the heat of fermentation in such piles had proved sufficient to kill

^o Unpublished data.

sclerotia throughout the mass except for a comparatively thin zone at the surface, usually not more than a foot thick. The work described here was intended to kill the sclerotia that might be present in these surface zones.

The screenings are composed of variable amounts of soil from different fields and of leaves and the slender, broken taproots of the beets. On drying at 110° C for 48 hours, samples of such material, taken before the fall rains, show a moisture loss of 12 to 17 per cent. It was in newly made piles (not more than one to two days' accumulation) of such material that the experiments were conducted. The piles had been made with a dump truck, each load being deposited against the previous one, constituting a heap about 12 feet wide, 3 feet in extreme depth, and of indefinite length. For purposes of experiment about 15 feet of a pile was used. Even such loosely built, shallow piles heated readily, reaching 60° C in a day or two at a depth of 1 foot.

For convenience, test samples of 50 sclerotia were mixed with several grams of soil, and the mass tied in cheesecloth parcels to which tags on strings were attached. Such samples were distributed with uniform spacing at distances of several feet over the whole surface of several piles and were placed at depths of 12 inches, 9 inches, 6 inches, and 3 inches. Others were placed on the surface and just barely covered with screenings. At the time of placing the sclerotia, the temperature of the pile was recorded for the position of each test sample.

Chloropicrin was then injected into the pile with spacing of the points of application 1 foot each way. The dosage was 2½ cubic centimeters of chemical for each square foot of surface. Immediately after injection of the chloropicrin, the pile was covered with the glue-and-paraffin-treated kraft paper "chloropicrin proof" obtained from Dr. G. H. Godfrey. As before, the edges of the strips of paper were jointed by a strip of Scotch tape. At the periphery, the paper was secured by loading it with a continuous heap of soil. Again difficulty was experienced when the Scotch tape loosened in spots, partly as a result of moisture condensing on the lower side of the paper. Thus, too, a rather rapid escape of the gas took place, for there was no appreciable odor of chloropicrin about the surface of the pile when the test samples were removed 48 hours after the injection.

The sclerotia were removed from the cheesecloth, washed free of soil on a screen in the laboratory, and surface-sterilized for 45 seconds with 1:1,000 HgCl₂ before being plated on potato-dextrose agar. The results of culturing the sclerotia are presented in table 16, where the numbers of sclerotia tested in the various trials have all been combined for economy of space.

As will be noted, some few sclerotia escaped at a depth of 12 inches where the temperature might itself have been lethal (50° C killing sclerotia in water within 2 hours). On the whole, however, the figures show that killing, whether by high temperature, by the action of chloropicrin, or by both, was much more complete at the greater depth, up to 15 per cent of the test sclerotia surviving on the surface. It seems obvious that some means of making a tight cover is essential to such work.

The danger of disseminating sclerotia from piles of screenings to un-

TABLE 16
EFFECTIVENESS OF CHLOROPICRIN IN KILLING SCLEROTIA IN THE SURFACE LAYER
OF PILES OF SCREENINGS AT A BEET-RECEIVING STATION

Depth of sclerotia, inches	Number of trials	Number of test lots	Sclerotia plated	Viable sclerotia	Per cent of sclerotia germinated	Temperature of screenings at position of test lots, degrees centigrade
Surface.....	2	26	1,300	193	14.80	35°
3.....	4	42	2,100	55	2.60	30°-55°
6.....	3	37	1,850	3	0.16	38°-55°
9.....	3	21	1,050	0	0.00	Unknown
12.....	3	34	1,700	7	0.41	55°-67°
Control.....	2	2	100	89	89.00

infested fields has been removed by moving the screenings to locations that present no menace to cropped land. Thus the necessity for using fungicides is now avoided.

SUMMARY

Sclerotium Rolfsii, which causes southern sclerotium rot of sugar beets, has been found to grow on media having a wide range of hydrogen-ion concentration, rapid growth taking place up to pH 7.9. In the field the occurrence of rot on sugar beets is apparently not greatly affected by soils having values up to pH 8. Liming of soils, except with excessive amounts, has failed to reduce significantly infection of carrots in the laboratory; and in a single trial, moderate applications of lime to soils in the field reduced only slightly the amount of infection in a sugar-beet crop.

Seventeen water-soluble chemicals and fungicidal agents considered to have possibilities for use against soil fungi have been subjected to laboratory tests in vitro, and most of them also by application to soils. These are namely: acetic acid, ammonia, ammonium thiocyanate, boric acid, calcium cyanamid, chlorinated lime, carbon disulfide (water emulsion), calcium hydroxide, Dovicides B, H, and P, formaldehyde, mercuric chloride, phenol, potassium ethyl xanthate, pyroligneous acid, and sodium

hydroxide. None of these agents has proved more effective than formaldehyde under all conditions, though several, including ammonia, are more potent in vitro.

Solutions of formaldehyde, ammonia, and sodium hydroxide have been tested further in infested fields. With one exception, formalin (37 per cent formaldehyde) diluted 1:100 and applied at 3 gallons per square foot of soil surface killed sclerotia completely to a depth of 6 inches. Ammonia (28 per cent NH_3) and sodium hydroxide in the same strengths and at the same rates failed to reduce noticeably the numbers of viable sclerotia in the surface 6 inches.

Early removal of diseased beets from infested fields and chemical disinfestation of the surrounding soil reduced the occurrence of the disease on adjacent plants in the same row, but the effect of the treatment did not extend to the other rows. The number of viable sclerotia that remained to affect subsequent plantings was materially reduced.

The use of anhydrous ammonia by injection has been considered, but, even with almost air-dry soil, the absorptive capacity of the soil used in the experiment seems to preclude the possibility of thoroughly permeating soil in the field with ammonia.

Five volatile fungicides, insoluble in water, have been submitted to laboratory test. Xylene, tetrachlorethane, and pentachlorethane failed to kill sclerotia in doses as great as 5,000 parts per million of soil when the sclerotia were at least partially wet and the atmosphere saturated with water vapor; chloropicrin and carbon disulfide killed in doses as low as 100 and 600 parts per million of soil respectively. When properly confined, 2 cubic centimeters of chloropicrin per square foot of soil surface, injected at a depth of 6 inches, suffices to kill to a depth of 1 foot in the soil types considered.

Though the use of chloropicrin in the field has been attempted, the results secured in the single trial were unsatisfactory, no doubt both because of too small an application of the chemical and because of failure to confine the gas adequately.

When chloropicrin was used to kill sclerotia in the surface zone of piles of screenings at sugar-beet receiving stations, about 97 per cent of the sclerotia were killed; but it was difficult to confine the gas. Disposal of screenings in isolated spots has rendered treatment with fungicides unnecessary.

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