

# ACETIC ACID AS A SOIL DISINFECTANT<sup>1</sup>

By WILLIAM L. DORAN

Research Professor of Botany, Massachusetts Agricultural Experiment Station

## INTRODUCTION

Acetic acid, in vinegar, is one of the most anciently used preservatives. Its toxicity to bacteria and to fungi has been recognized. Wolf and Shunk (26)<sup>2</sup> found that it was more toxic to the six species of bacteria investigated by them than was hydrochloric, sulphuric, citric, tartaric, malic, or formic acids. In the experiments of Bitting (4) acetic acid was as toxic to fungi as benzoic, boric, or salicylic acids, and it killed *Penicillium expansum*, *Alternaria solani*, and *Oidium lactis* under conditions in which citric, lactic, malic, and tartaric acids only slightly retarded their growth. According to Rideal and Rideal (20), acetic acid 0.3 per cent kills *Bacillus typhosus*. Uppal (24) found that it was toxic to the conidia of *Phytophthora colocasiae*. Wüthrich (27) reported that 0.01 N acetic acid prevented the germination of the conidia of *Phytophthora infestans* and *Plasmopara viticola* and the spores of *Ustilago carbo*. Results secured by Piemeisel (16) led him to believe that it is acetic acid which kills the spores of *Ustilago zaeae* in silage. Acetic acid, 0.1 per cent, was found by Hitchcock and Carleton (11) to prevent the germination of urediniospores of *Puccinia coronata*.

Acetic acid may have a toxic or a stimulatory effect on higher plants. In the experiments of Lövinson (14) it retarded the germination of peas. Carr and Haverkamp (6) found that the weights of plants decreased as the quantity of acetic acid, added to the soil in which they were growing, was increased. Heald (10) found that the growth of corn seedlings was prevented by a certain concentration of acetic acid, but that this concentration was greater than the concentration of hydrochloric or sulphuric acids which is toxic to these plants.

In the experiments of Small (21) a much larger percentage of cuttings of rose, privet, and veronica rooted in acetic acid, 0.01 per cent, than in water, and root systems became larger in the acid medium. Promsy (17, 18) found that the germination of seeds was hastened and the growth of seedlings increased by the application of acetic acid to the sand in which they were growing.

The toxicity of acetic acid has been considered or explained by True (23), Kahlenberg and True (13), Heald (10), Winslow and Lochridge (25), Reichel (19), Dunn (8), Cohen and Clark (7), and Uppal (24). Apparently, the toxicity of acetic acid is due partly to the hydrogen ion and largely to the undissociated molecule. Kahlenberg and True (13) found that undissociated acetic acid is toxic, and, according to Heald (10) and True (23), the  $C_2H_3O_2$  ions are not

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 279.

toxic. Wolf and Shunk (26) found that the limit for growth of *Bacterium tabacum* was  $P_H$  5.9 when the medium was adjusted with acetic acid and  $P_H$  4.6 when the medium was adjusted with malic acid. They concluded that the hydrogen ion alone is not responsible for the toxicity of acetic acid.

Soil may be changed chemically as well as biologically by the application of acetic acid. The rate of solubility of soils was found by Bouyoucos (5) to be greater after treatment with acetic acid than after treatment with nitric, hydrochloric, or sulphuric acids. According to Carr and Havercamp (6), acetic acid forms soluble and toxic salts with iron and aluminum in the soil, and they considered this to be the cause of the toxicity of acetic acid to plants.

There is, then, evidence that acetic acid is fungicidal and bactericidal, and that it may be either toxic or stimulatory to plants. In these effects it is not unlike formaldehyde.

In this paper, which is a preliminary report, results of experiments are presented which show that acetic acid applied to the soil protected plants against diseases caused by fungi in the soil and at a cost about half that of formaldehyde.

#### PREVENTION OF BLACK ROOT ROT OF TOBACCO BY ACETIC ACID

The fungus *Thielavia basicola* (Berk. and Br.) Zopf causes severe black root rot of tobacco in some of the less acid soils in the Connecticut Valley. This disease and bed rot or damping off are combated in the tobacco seed beds by treating the soil with steam or with formaldehyde.

Soil known to be infested with *Thielavia basicola* was placed in 2-gallon crocks, and to it was applied 7, 14, and 21 c. c. normal acetic acid per 100 gm. dry weight of soil, with treatments in duplicate. With the acid, enough water was added to bring the soil to 60 per cent of its water-holding capacity, and at that degree of saturation it was maintained.

Tobacco seeds planted in this soil four weeks after the application of the acid germinated well. While the heaviest application of acetic acid interfered somewhat with growth, the beneficial effect of appropriate quantities was apparent. When the average area of leaves of control plants was 0.37 square inch, the average area of leaves of plants in soil treated with acetic acid (7 c. c. normal acid per 100 gm. soil) was 1.86 square inches (planimeter measurements), or five times as large as that of the controls.

When these plants were 7 weeks old, their roots were examined for infection by *Thielavia*. The results are recorded under the first series in Table 1. Black root rot was severe on control plants, and infection was prevented by acetic acid.

TABLE 1.—Effect of acetic acid on black root rot of tobacco

Number of cubic centimeters of N/1 acetic acid applied per 100 gm. of soil	Infection of roots of tobacco by <i>Thielavia</i>	
	First series	Second series
0 (Control).....	Severe on all.....	Severe on all.*
7.....	None on 83 per cent, trace on 17 per cent.....	None.
14.....	None.....	Do.
21.....	.....do.....	Do.

\* An average of 30 lesions per root.

Tobacco plants, from steamed soil, and therefore free from black root rot, were set in this same soil 12 weeks after the application of the acid. As before, the plants grown in the treated soil grew better than the controls with the exception of those receiving the heaviest application of acetic acid, which was so large as to be somewhat toxic to the plants. Black root rot on control plants seriously interfered with their growth.

Eight weeks after these plants were set, their roots were examined for black root rot; the results are recorded under the second series in Table 1. Infection was severe on all control plants, but there was no infection whatever on plants in soil to which acetic acid had been applied.

Equivalent quantities of citric, tartaric, lactic, and malic acids of the same normality were applied to this soil infested with *Thielavia basicola*, but only acetic acid prevented infection.

It has been found by the writer that orthophosphoric acid applied to soil infested with *Thielavia basicola* is very favorable to infection of tobacco by this fungus. Acetic acid together with orthophosphoric acid was therefore applied to such a soil. The soil was kept watered to 60 per cent of its water-holding capacity, and 18 days after the application of the acids tobacco plants from steamed soil were set in it. Six weeks later their roots were examined for black root rot. Infection was severe on control plants and was very mild on plants in soil to which had been applied 9 c. c. normal acetic acid and 8 c. c. normal orthophosphoric acid per 100 gm. of soil. Infection was much reduced, although not entirely prevented, by acetic acid with orthophosphoric acid, and orthophosphoric acid used alone resulted in infection considerably more severe than on control plants.

In order to secure more information about the relative safety or toxicity of acetic acid to tobacco, acetic acid, 56 per cent, was applied to soil in the field at the rate of 3,000 pounds per acre. Two weeks after the application, tobacco plants were set in this soil. No toxicity to plants resulted, and in the absence of black root rot, plants in soil treated with acetic acid had the same average weight at the end of the season as control plants.

In one series of pot experiments, acetic acid was applied to a well-buffered soil (a water-deposited silt or fine sandy loam) and to a poorly buffered soil (an ice-deposited stony loam). Acetic acid was not toxic to tobacco plants (set one week after the application of the acid) in the well-buffered soil, but was toxic to them in the poorly buffered soil.

#### EFFECT OF ACETIC ACID ON SOIL REACTION

It has been pointed out by Stephenson (22) that such organic acids as acetic acid are too rapidly oxidized in the soil to cause an increase in soil acidity. But since it has been found that acetic acid can prevent black root rot of tobacco, and since Anderson, Osmun, and Doran (2) and Morgan and Anderson (15) have shown the relation which exists between the  $P_H$  value of soil and black root rot of tobacco, it is interesting to see whether or not this effect of acetic acid can be correlated with soil reaction.

Acetic acid was applied to soil having an initial  $P_H$  value of 6.1, and the  $P_H$  values of this soil were determined<sup>3</sup> thereafter at frequent intervals. The results of these determinations are shown in Table 2.

TABLE 2.—Effect of acetic acid on the  $P_H$  value of soil

Number of cubic centimeters of N/1 acetic acid applied per 100 gm. of soil	$P_H$ value of soil after—					
	6 days	12 days	15 days	31 days	41 days	63 days
0 (control).....	6.1	6.1	6.1	6.1	6.1	6.1
7.....	4.9	6.1	6.1	6.1	6.1	6.1
14.....	4.6	5.4	5.9	6.1	6.1	6.1
21.....	4.4	4.6	4.9	5.0	6.1	6.1

The application of acetic acid temporarily increased the acidity of the soil, but within a few days or a few weeks, depending on the quantity used, the  $P_H$  value of the soil to which it was applied reverted to its original value. When normal acetic acid, 7 c. c. per 100 gm. of soil, was applied, the  $P_H$  value of the soil was changed from 6.1 to 4.9, but 12 days after the application of the acid the  $P_H$  value of this soil was back to 6.1. In the case of the application of normal acetic acid, 14 c. c. per 100 gm. of soil, the  $P_H$  value of the soil was changed from 6.1 to 4.6, but 31 days after the application of the acid the  $P_H$  value had reverted to 6.1.

When acetic acid, 56 per cent, was applied to soil in the field at the rate of 3,000 pounds per acre, and determinations made 4, 8, and 10 weeks after the application, the  $P_H$  value of the soil was found unchanged.

When acetic acid was applied to a well buffered soil and to a poorly buffered soil, the  $P_H$  value of neither was changed, as determined 3, 6, and 12 weeks after the application of the acid.

Acetic acid was found to surpass either nitric or sulphuric acid in preventing infection of tobacco by *Thielavia basicola*. But the  $P_H$  value of the soil treated with acetic acid was 5.9, which, in the absence of acetic acid, is favorable to this fungus (2), while soil to which the inorganic acids were applied became considerably more acid ( $P_H$  5.6 to 5.4). This is in agreement with the results of Cohen and Clark (7), who found that the growth of certain organisms was inhibited at  $P_H$  5.45 when acetic acid was used to adjust the reaction of the media, but that these same organisms grew at  $P_H$  5.0 when adjustment was made with hydrochloric acid.

Acetic acid has no lasting effect in increasing soil acidity, and its effect on fungi in the soil is not exerted through the factor of soil reaction.

#### EFFECT OF ACETIC ACID ON GERMINATION AND DAMPING OFF OF LETTUCE, CUCUMBER, TOMATO, AND TOBACCO

Acetic acid, in the concentrations named in Table 3, or formaldehyde, 1 : 50, was applied to a soil known to be infested with species of *Pythium* and *Rhizoctonia*. The soil was 4 inches deep, in flats, and the diluted acetic acid or formaldehyde was applied at the rate of 2 quarts per square foot. The soil was stirred 4 days after appli-

<sup>3</sup> The  $P_H$  values of soil were determined colorimetrically with the Stirlen double wedge comparator, using the method described by Anderson and Morgan (7).

cation of the chemicals and 10 days after their application seeds were planted. There were planted per flat and for each treatment 70 cucumber seeds, 100 tomato seeds, and 200 lettuce seeds. An equal weight of tobacco seeds was planted in each flat and for each treatment. The soil in all flats was kept at 70 per cent of its water-holding capacity and the flats were in air of high relative humidity.

When it was evident that all seeds had germinated which were going to germinate, the percentage of seeds which failed to germinate in each treatment was determined. The results are recorded in Table 3.

TABLE 3.—*Effect of acetic acid and of formaldehyde on determination and damping off of cucumber, tomato, and lettuce*

Treatment *		Per cent lost								
Pounds of 56 per cent acetic acid in 50 gallons of water	Per cent acetic acid equivalent	By failure to germinate			By damping-off			By failure to germinate and by damping-off.		
		Cucumber	Tomato	Lettuce	Cucumber	Tomato	Lettuce	Cucumber	Tomato	Lettuce
0.00	0.0	50	30	36	25	0	17	75	30	53
2.98	.4	26	13	7	6	0	4	32	13	11
5.95	.8	11	5	7	3	0	1	14	5	8
8.93	1.2	2	3	0	0	0	0	2	3	0
Formaldehyde 1 : 50		2	0	0	0	0	0	2	0	0

\* Applied to soil at rate of 2 quarts of the solution per square foot.

In control flats, 50 per cent of the cucumber seed, 30 per cent of the tomato seed, and 36 per cent of the lettuce seed failed to germinate; and the seeds which failed to germinate were found to be attacked by soil fungi. In soil to which 1.2 per cent acetic acid (8.93 pounds of 56 per cent acetic acid in 50 gallons of water) was applied, none of the lettuce seed, only 2 per cent of the cucumber seed, and 3 per cent of the tomato seed failed to germinate.

There was considerable loss of seedlings of cucumber, lettuce, and tobacco (but not tomato) by damping off in untreated soil. Species of *Pythium* and *Rhizoctonia* were present in the damped off seedlings. In the untreated soil 25 per cent of the cucumber seedlings and 17 per cent of the lettuce seedlings damped off. There was no damping off of cucumber and lettuce seedlings in soil to which acetic acid, 1.2 per cent, was applied. The application of this strength of acetic acid was as efficient as formaldehyde in preventing damping off. In the untreated soil 75 per cent of the cucumbers, 30 per cent of the tomatoes, and 53 per cent of the lettuce plants were lost by failure to germinate and by damping off combined. In soil to which 1.2 per cent acetic acid was applied, the loss from these causes was only 2 per cent of the cucumbers, 3 per cent of the tomatoes, and none of the lettuce. Acetic acid 0.8 per cent was nearly as effective in preventing damping off.

In Figure 1 the effect of acetic acid in preventing damping off of tobacco seedlings is evident. Acetic acid 0.4 per cent was insufficient for protection but acetic acid 0.8 or 1.2 per cent prevented damping off.

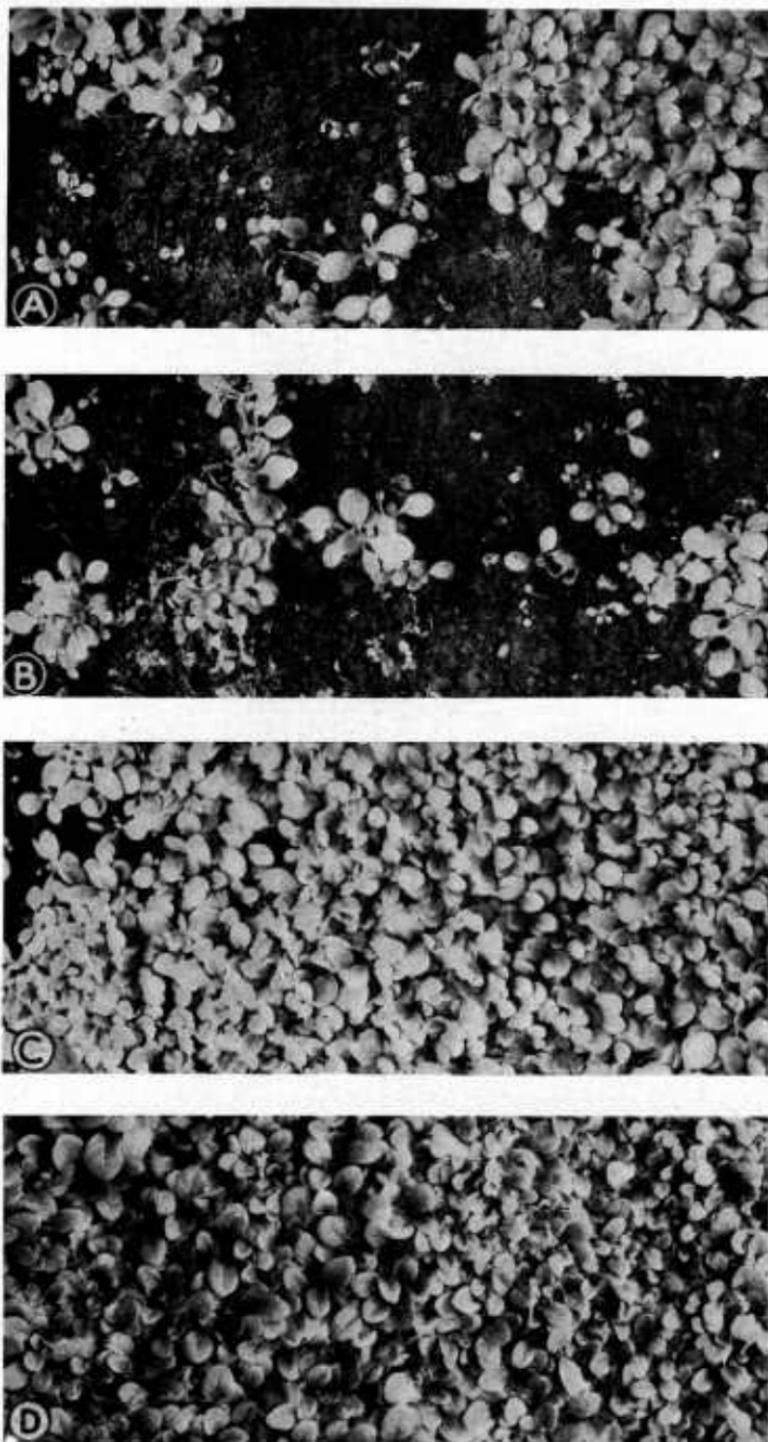


FIG. 1.—Control of damping off of tobacco by the use of acetic acid. A, No acetic acid; B, acetic acid, 0.4 per cent; C, acetic acid, 0.8 per cent; D, acetic acid, 1.2 per cent

Tobacco plants which did not damp off in each of the several treatments were counted. For each 100 plants living in the untreated soil, there were 367 plants living in the soil treated with acetic acid 0.8 per cent and slightly more than this in the soil treated with acetic acid 1.2 per cent, which treatment gave results equal to those secured with formaldehyde.

EFFECT OF ACETIC ACID ON BED ROT AND ON BLACK ROOT ROT OF TOBACCO IN SEED BEDS

Acetic acid, at the rate of 2 quarts per square foot of a solution of 7.5 pounds of 56 per cent acetic acid in 50 gallons of water (equivalent to acetic acid 1.008 per cent), was applied to tobacco seed beds known to be infested with *Thielavia basicola* and with bed-rot fungi, *Rhizoctonia solani* and *Pythium* sp.<sup>4</sup>

TABLE 4.—Effect of acetic acid on black root rot and on bed rot of tobacco in seed beds

Seed bed No.	Per cent of plants showing black root rot		Number of centers of infection of bed rot	
	In control	In acetic-acid treated area	In control	In acetic-acid treated area
1-----	100	19	0	0
2-----	26	0	15	0
3-----	18	0	0	0

In seed beds No. 1 and No. 2 (see Table 4) tobacco seeds were sowed one week after the application of the acid, and in these plots germination was not as good as in the control plots. In bed No. 3, seed was sowed 20 days after the application of the acid, and germination was better in this plot than in its control plot. On the basis of this and other experiments it seems unsafe to sow tobacco seeds as soon as one week after the application of acetic acid to soil. Many weeds came up in the control plots in these seed beds, but very few weeds came up in the plots to which acetic acid was applied.

Bed rot, caused by *Rhizoctonia solani* and *Pythium* sp., developed in only one seed bed (No. 2). In the control plot of this seed bed, 15 centers of infection appeared and from these bed rot spread rapidly within the plot. There was no bed rot in the plot to which acetic acid was applied. In this and the other seed beds, control plots were of the same area as treated plots and adjacent to them.

The percentages of plants found to be infected with black root rot are recorded in Table 4. In the control plots of seed beds No. 2 and No. 3, 26 and 18 per cent of the plants, respectively, were infected by *Thielavia basicola*. There was no infection in the plots to which acetic acid was applied in these beds. In seed bed No. 1, infection was unusually severe in the control plot, for in it 100 per cent of the plants had black root rot. When acetic acid was applied in seed bed No. 1, there was already so much water in the soil that much—probably one-third—of the solution of acetic acid ran off the bed. Under these conditions black root rot was not entirely prevented by

<sup>4</sup> Acknowledgment is made of the cooperation of Walter W. Sanderson and T. L. Warner, tobacco growers, on whose farm these experiments were conducted.

acetic acid but was reduced to 19 per cent as compared with 100 per cent in the control plot.

In order to learn whether or not dilute acetic acid can be applied to living tobacco plants in the seed bed to control bed rot, acetic acid in several concentrations was sprayed on growing tobacco seedlings among which bed rot had appeared. The spread of the disease was checked, but living plants were injured by the application of a 0.5 per cent solution of the acid. Concentrations so low as not to injure the plants did not prevent the spread of bed rot. The usefulness of acetic acid, as of formaldehyde, for the control of bed rot of tobacco in seed beds is confined to its application to the soil before plants are growing in it.

#### EFFECT OF ACETIC ACID ON DAMPING OFF OF SEEDLINGS OF WHITE SPRUCE

Acetic acid of two concentrations, 8.33 pounds and 12.5 pounds, of 56 per cent acetic acid in 50 gallons of water (equivalent respectively to 1.12 and 1.68 per cent acetic acid) was applied at the rate of 1.64 quarts per square foot to seed beds in a forest nursery.<sup>5</sup> A control seed bed, not treated, was adjacent to them. Seed beds were 44 square feet in area. One-half pound of seeds of white spruce was planted in each bed seven days after treatment.

Although the interval of time between the application of acetic acid and the planting of seeds was relatively short, germination was not injured. Germination of white spruce seeds was much better and there were fewer weeds in the beds to which acetic acid was applied than in the control bed. Loss by damping off was severe in the control bed, as may be seen by reference to Figure 2, A. There was very little damping off in the beds to which acetic acid was applied. Four months after seeds were planted, the number of seedlings in 5 representative square feet in each bed were counted. The average number of seedlings per square foot in each bed and the number of seedlings in each seed bed figured from this are recorded in Table 5.

TABLE 5.—Effect of acetic acid on damping off of white spruce seedlings

Treatment <sup>a</sup>	Equivalent per cent of acetic acid	Average number of seedlings		Seedlings per unit area, expressed in relative numbers
		Per square foot	Per seed bed	
Pounds of 56 per cent acetic acid in 50 gallons of water				
8.33.....	1.12	746	32,824	315
12.50.....	1.68	665	29,260	281
0 (control).....	0	237	10,428	100

<sup>a</sup> 1.64 quarts of diluted acetic acid per square foot.

For each 100 seedlings in the untreated soil there were 315 seedlings in soil to which 1.12 per cent acetic acid was applied. This concentration of acetic acid gave better results than the higher concentration (1.68 per cent acetic acid) for in soil to which the higher con-

<sup>5</sup> The cooperation of John Palmer, superintendent of the State forest nursery in Amherst, Mass., is acknowledged.

centration was applied there were 281 seedlings for each 100 seedlings in untreated soil.

The stands in the several sced beds three months after seeds were sowed may be compared by reference to Figure 2. The control of damping off secured by both concentrations of acetic acid is evident, and it is likewise evident that the optimum concentration of acetic

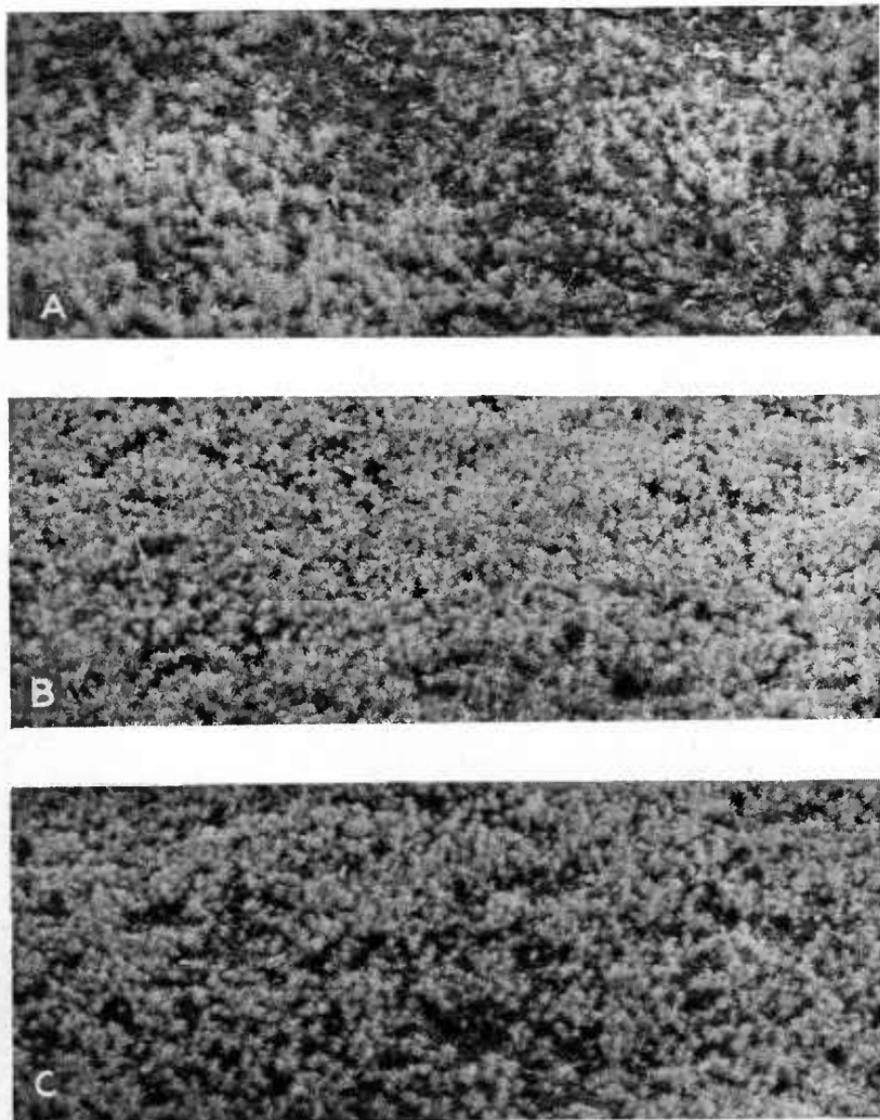


FIG. 2.—Control of damping off of white spruce seedlings by the use of acetic acid. A, No acetic acid; B, acetic acid, 1.12 per cent; C, acetic acid, 1.68 per cent

acid is nearer 1.12 per cent than 1.68 per cent. The fact that acetic acid 1.12 per cent used in this way protected seedlings of white spruce against damping off without injuring the seeds or seedlings is of interest because of the results secured by Hansen, Kenety, Wiggin, and Stakman (9). In their experiments, the application of sulphuric acid, hydrochloric acid, or formaldehyde greatly reduced the ger-

mination of seeds of white spruce, and they considered as questionable the use of any of the common fungicides for the prevention of damping off of seedlings of this species.

#### EFFECT OF ACETIC ACID ON BROWN ROOT ROT OF TOBACCO

¶ When acetic acid was applied to "brown root-rot soil," that is, soil known to have in it the cause of brown root rot, tobacco plants subsequently set in the treated soil grew without showing symptoms of brown root rot. Johnson, Slagg, and Murwin (12) found that when formaldehyde was similarly applied to brown root-rot soil, the ability of this soil to produce symptoms of brown root rot of tobacco was destroyed.

The cause of brown root rot is at present unknown, and the results with acetic acid are presented here not because of any light they may shed on the nature of the disease, but because there is in them additional evidence of the effectiveness of acetic acid as a substitute for formaldehyde for soil treatment.

Tobacco seeds were sowed in pots of brown root-rot soil three weeks after the application to it of 1 per cent acetic acid. Seeds were also sowed in pots of this soil without acetic acid. Brown root rot was found on seedlings in untreated soil when they were 4 weeks old. Roots of all plants were examined when they were 9 weeks old. Brown root rot was found to be severe on all plants in untreated soil, but there was not even a trace of the disease on plants in soil to which acetic acid had been applied.

The severity of brown root rot on plants in untreated soil seriously retarded their growth. When the average dry weight of plants in untreated soil was 0.90 gm., the average dry weight of plants in soil treated with acetic acid was 4.76 gm., or more than five times as great as that of the controls.

#### COST OF USING ACETIC ACID COMPARED WITH COST OF USING FORMALDEHYDE

A cheaper chemical than formaldehyde for the partial sterilization of soil is desirable. Beach (3) applied formaldehyde at the usual rate and reported that it cost (in 1926) \$0.21 for the 1.4 pounds used to treat 18 square feet of soil, or \$508 per acre.

One-half gallon of dilute acetic acid per square foot of soil has been found to be sufficient, and this is the rate at which formaldehyde 1 : 50 is usually applied. The relative cost of these chemicals may therefore be compared by comparing the cost of 50 gallons of each.

Formaldehyde 1 : 50 contains 1 gallon (9.1 pounds) of the commercial 40 per cent solution of formaldehyde in 50 gallons of water. This is now purchasable (in lots of 100 pounds) for about \$0.12 per pound. The cost of formaldehyde for 50 gallons (enough for 100 square feet) is therefore \$1.09 and the cost of formaldehyde for 1 acre is \$475.

The concentrations of acetic acid which were found to control the plant diseases named in this paper were 1.2 to 1 per cent. These are equivalent to 8.93 to 7.44 pounds of 56 per cent acetic acid in 50 gallons of water. Acetic acid of several different concentrations (28 to 99 per cent) is sold, but the cost per unit of actual acetic acid is not greatly different. Acetic acid 56 per cent is now sold (in lots

of 100 pounds) for \$0.066 per pound. When 8 pounds of this chemical is used in 50 gallons of water (equivalent to 1.07 per cent acetic acid) the cost of acetic acid for 50 gallons (enough for 100 square feet) is \$0.53 and the cost of acetic acid for 1 acre is \$231.

The cost of acetic acid is therefore about 49 per cent of the cost of formaldehyde for soil treatment.

#### SUMMARY

The application to soil of 1 to 1.2 per cent acetic acid (equivalent to 7.44 to 8.93 pounds of 56 per cent acetic acid in 50 gallons of water) at the rate of about one-half gallon per square foot was found to protect tobacco against black root rot, brown root rot, and bed rot or damping off; and to protect cucumber, tomato, lettuce, and white spruce against injury by damping off during and after seed germination.

For the treatment of a unit area of soil, the cost of acetic acid is about 49 per cent of the cost of formaldehyde.

Acetic acid is toxic to seeds and plants with which it comes in contact. The exact time interval which must elapse between the application of the chemical to soil and the planting of seed will depend upon the species of plant and upon the soil; 14, 10, or, in some cases, 7 days have been long enough.

Acetic acid has no lasting effect on the reaction, i. e., the  $P_H$  value of soil to which it is applied.

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