

# SOME CHEMICAL TREATMENTS AND THEIR INFLUENCE ON DAMPING-OFF, WEED CONTROL, AND WINTER INJURY OF RED PINE SEEDLINGS<sup>1</sup>

By A. J. RIKER, professor, R. H. GRUENHAGEN, L. F. ROTH, formerly research assistants, Department of Plant Pathology, Wisconsin Agricultural Experiment Station, and W. H. BRENER, Manager, Griffith State Nursery.<sup>2</sup>

## INTRODUCTION

The control studies described herein were made because damping-off continues to influence production in Wisconsin forest nurseries. The causal fungi seem to be distributed widely in cultivated soil, but to be relatively scarce in unbroken forest soil (7).<sup>3</sup> While serious outbreaks are sporadic, small losses are expected each year. The unpredictable occurrence of epidemic attacks in the various nurseries is a handicap to the sustained production demanded by an extensive planting program.

In Wisconsin damping-off is caused principally by two fungi, *Pythium irregulare* Buisman and *Rhizoctonia solani* Kühn, although seedlings are occasionally damped-off by other micro-organisms. These two fungi induce damping-off with somewhat different symptoms and are active under different circumstances, so that control measures against one may have little effect on the other.

For example, *Pythium* operates in Wisconsin at relatively cool temperature, high humidity, and in neutral to slightly acid soil. In contrast, *Rhizoctonia* is active particularly in relatively warm, dry soil with an acid reaction (8, 9). Nevertheless, control measures need to be effective against both, and they are not especially differentiated in this paper.

Reasonable commercial control has often been secured in Wisconsin by planting on newly cleared forest land, by fall seeding, by covering late spring seeding with sand, and by soil treatments, especially with dilute sulfuric acid. These methods have been used alone or in various combinations. Nurserymen used new land as long as the nurseries were expanding, but, eventually, they reached a limit when they had to replant land that had already grown tree seedlings. Fall seeding has been the more common practice, but sometimes circumstances have required spring planting. Late spring planting

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<sup>3</sup> Italic numbers in parentheses refer to Literature Cited, p. 95.

with the seed covered by sand is a nursery application of the greenhouse practice common with many kinds of susceptible plants.<sup>4</sup>

Both the sterilizing effect of sulfuric acid and the increased soil acidity prevent damping-off and kill many weed seeds. However, acid treatment is relatively expensive to apply and is harmful to the nurseryman's clothes and skin if improperly handled. There is some possibility of chemical injury to the seedlings, but worst of all (10) is the fact that the acid undesirably disturbs the base exchange capacity of the soil.

This paper presents studies aimed at a control of damping-off as efficient as sulfuric acid but at one that will not change conspicuously the nutritional balance in the soil. An abstract covering some of the work has already appeared (6).

#### MATERIALS AND METHODS

In the early stages of the work, many different trials were made in 4- and 6-inch pots in the greenhouse where a number of unpromising materials were eliminated.

The soil employed was Plainfield sand secured from near the location of nursery trials at Wisconsin Rapids in order that the greenhouse studies might be more directly applicable to nursery conditions in central Wisconsin. An analysis of this soil is available (8).

Seed from local red pine (*Pinus resinosa* Ait.) was used for the most part, but some observations were also made with seed from Scotch pine (*P. sylvestris* L.) and from Austrian pine (*P. nigra* Arn.).

The fungi used for soil inoculation were *Pythium* and *Rhizoctonia*, as described by Roth and Riker (8). Greenhouse pots were planted with a counted number of seeds, usually 25, 50, or 100 seeds per pot, depending primarily upon its size. The different treatments were made with suitable numbers of replications. The soil in some of the trials was steamed for 20 minutes at 15 pounds pressure in 6-inch pots. However, most of the trials had a natural woods soil practically free (8) from damping-off fungi.

For those treatments employing seed dusted with a disinfectant, the seed and dust were placed in a drum about 4 inches in diameter and 7 inches long which was provided with 1-inch baffles. The drum was closed and slowly rolled for 10 feet. The seed was removed from the drum, separated from excess dust over a fine mesh screen, and placed in packets prior to planting.

For nursery plantings one could easily treat the seed in grain dusters, such as those in common use (4).

The nursery trials were made in three Wisconsin locations. Nurseries 1 and 2 were located on Plainfield sand in central Wisconsin, while No. 3 was on Vilas light sandy loam in northern Wisconsin. Both of these soils had a pH near 5.7, and for some years previous had been employed in growing nursery pine trees. The seed and fungi were similar to those in the greenhouse studies. Where the soil was inoculated, damping-off fungi were introduced from sand-corn-meal cultures grown in mason jars. The fungus and its corn-meal substrate were passed through a quarter-inch wire mesh and worked

<sup>4</sup> The Evergreen Nursery, Sturgeon Bay, Wis., has eliminated its damping-off problem by covering the seed with Lake Michigan sand.

into the surface soil of the seedbed at the rate of 1 pint of culture for 16 square feet.

Dry soil treatments that were strong enough to injure the seed were applied evenly over the surface of the soil, were worked in to a depth of 1.5 inches, and were allowed to stand 3 days before seeding. Seeding was made without disturbing more than this top 1.5 inches of soil, and the calomel treatment was made at this time. The chemical was dusted into the seed trenches at the rate of 0.4 gm. per square foot of plot area.

All of the liquid treatments were applied just after seeding, at the rate of one-half pint per square foot. The liquid-treated beds were then leached with an equal volume of clear water.

All of the seedbeds were covered with wire screen and half shade to prevent injury by rodents, birds, and excessive sunshine. In both the smaller and larger field plantings the various treatments in each replication were distributed by chance.

Records of emergence and damping-off of the small-scale plantings were made at frequent intervals. Records on germination included the entire area planted, but those for damping-off excluded 3 inches marked off around the edge to eliminate marginal effects.

Seedlings which were damped-off and those which were chemically injured were differentiated according to the descriptions of Hartley (2, 3) and Roth and Riker (7). All standing seedlings were given a slight brushing pull upward. If they came up, they were counted according to the character of the lesion. If they resisted the pull, they were considered healthy. This gave the benefit of the doubt to seedlings with minor chemical injury.

## EXPERIMENTAL RESULTS

### GREENHOUSE STUDIES

The following chemicals were employed in the greenhouse studies in liquid soil treatments: Sulfuric acid, sodium dinitro-ortho-cresolate, mercuric chloride, ferrous sulfate, copper oxide, copper chloride, and aluminum sulfate. In dry soil or seed treatments the following were used: Mercuric phenyl cyanamide and cadmium oxide, mild mercurous chloride, ethyl mercury iodide, paraformaldehyde, cuprous oxide, cuprous chloride, and cuprous carbonate, ethyl mercury phosphate, finely divided metallic mercury in talc, ammonium phosphate, tetramethyl thiuramdisulfide, hydroxymercurichlorophenol, tetrachloroparabenzquinone, sodium salt of 2-, 4-, 5-trichlorophenol, and pentachlorophenol.

Various plant materials were used also to determine whether they might either contain antibiotics against the damping-off fungi or stimulate the growth of micro-organisms antagonistic to these pathogens. These materials included ground oak leaves, pine needles, alfalfa, grass, autoclaved oats, sawdust, and corn meal. Since none were effective, further mention of them is omitted.

Nine different sets of greenhouse trials were made between 1937 and 1945 in order to determine which materials were sufficiently promising for nursery testing. Because of their volume, they are not described in detail.

From these greenhouse trials several materials appeared encourag-

ing as used, and worthy of nursery trials. The most promising were a seed dust of mercuric phenyl cyanamide and cadmium oxide and more particularly a soil application of calomel. The combination of these treatments indicated a control of damping-off approximately as good as that from sulfuric acid. However, this acid apparently killed many weeds which were not affected by these mercurials.

The possible advantages of these or similar chemicals were as follows: (1) They had no accident hazard such as that provided by pouring water into concentrated sulfuric acid. (2) They were less injurious to the skin or clothing on contact than dilute sulfuric acid. (At the same time it is clear that these chemicals are poisonous if eaten, that the dust should not be inhaled in any quantity, and that continual skin contact might be harmful.) (3) They were easier to handle. (4) They had no obviously ill effect on the fertility of the soil. Since traces of mercury had disappeared within a few months (5), there was no fear that the minute amount employed per square foot might accumulate.

The more promising of these substances along with certain other commonly used chemicals were employed in experimental tree nursery trials.

#### SMALL SCALE NURSERY TRIALS

Of the chemicals tried in the greenhouse, all were employed in small nursery beds except cuprous oxide, cuprous chloride, cuprous carbonate, and metallic mercury.

Small-bed nursery trials were made during the period 1937 to 1943, inclusive. There were two trials in nursery No. 1, seven in nursery No. 2, and four in nursery No. 3. The individually treated areas were either 2 by 2 or 3 by 4 feet in size. Each trial consisted of a number of treatments (3 to 22) and replications (4 to 6) as appeared necessary. Although most of the details of these studies have been omitted because of their volume, some of the results of a trial on inoculated soil are given in table 1, as representative of the group.

The percentages of damping-off for each of the six replicates in the three critical treatments shown in table 1 were as follows: No treatment—18, 7, 45, 11, 10, and 42; sulfuric acid—2, 8, 3, 12, 7, and 30; combination—4, 1, 3, 3, 2, and 1. No statistical analysis seemed necessary to conclude that in this trial the combination was significantly superior to no treatment and as effective as the sulfuric acid

TABLE 1.—Averages from 6 replications of small-scale nursery trials on the control of damping-off of red pine seedlings, made in 1939, on inoculated soil at nursery No. 1<sup>1</sup>

Treatment	Seed planted that emerged	Emerged seedlings that were—		
		Damped-off	Chemically injured	Healthy
	Percent	Percent	Percent	Percent
None.....	70	22	0	78
Sulfuric acid <sup>2</sup> .....	66	10	2	89
Combination <sup>3</sup> .....	83	2	0	98
Seed dust alone.....	80	15	0	85
Soil dust alone.....	84	7	0	93

<sup>1</sup> 11 of the less promising among 16 treatments have been omitted.

<sup>2</sup> 2 percent sulfuric acid, 3 gallons per 4 by 12 foot bed (235 ml. per square foot).

<sup>3</sup> Seed dust of mercuric phenyl cyanamide, 8 percent, and cadmium oxide, 2.5 percent (commercial Barbak C); soil dust treatment of calomel, 0.4 gm. per square foot.

against damping-off. Particularly in the sulfuric acid plots, some of the seedlings classed as damped-off may have been chemically injured.

Isolations were made from damped-off seedlings in various treatments. *Pythium* was rather less prevalent than *Rhizoctonia* (ratio 15 to 21) in the untreated plots and was a poor second to it (7 to 22) in the sulfuric acid plots. However, *Pythium* appeared exclusively (16 to 0) in the plots treated with calomel.

The combination treatment appeared sufficiently promising to justify trials on a commercial scale.

#### COMMERCIAL SCALE TRIALS

Commercial nursery trials were made with the best of the treatments from the 9 greenhouse and 13 small-bed nursery trials, viz, (1) sulfuric acid, (2) mercuric phenyl cyanamide with cadmium oxide seed dust, (3) calomel on the soil, and (4) a combination of (2) and (3).

The trials consisting of five treatments and six replications were made in 1940, 1941, and 1942. The field manipulations were similar to those described for the small-bed trials. The results with controls, sulfuric acid, and the seed and soil treatments alone and combined (table 2) show some of the variability encountered. The combina-

TABLE 2.—Averages from 6 replications of large-scale nursery trials on the control of damping-off of pine seedlings

Location, year, and pine seed	Treatment	Seed planted that emerged	Emerged seedlings that were—		
			Damped-off	Chemically injured	Healthy
Nursery No. 1:		Percent	Percent	Percent	Percent
1940 Austrian	None	27	16	0	84
	Sulfuric acid <sup>1</sup>	32	2	4	94
	Combination <sup>2</sup>	28	6	0	94
	Seed dust alone	28	7	0	93
	Soil dust alone	28	7	0	93
1941 Red	None	79	12	0	88
	Sulfuric acid	76	5	0	95
	Combination	74	1	0	99
	Seed dust alone	71	10	0	90
	Soil dust alone	77	2	0	98
1942 Scotch	None	18	30	0	70
	Sulfuric acid	16	11	2	87
	Combination	20	9	0	91
	Seed dust alone	19	25	2	73
	Soil dust alone	20	8	1	91
Nursery No. 2:					
1940 Red <sup>3</sup>	None	84	2	0	98
	Sulfuric acid	75	3	15	82
	Combination	80	1	0	99
1941 Red <sup>3</sup>	None	81	8	0	92
	Sulfuric acid	78	2	7	91
	Combination	81	2	0	98
1942 Red <sup>3</sup>	None	87	2	0	98
	Sulfuric acid	87	1	1	98
	Combination	86	1	0	99
Nursery No. 3:					
1940 Red	None	83	4	0	96
	Sulfuric acid	88	1	1	98
	Combination	85	1	0	99
	Seed dust alone	81	3	0	97
	Soil dust alone	85	1	0	99
1941 Red	None	76	25	0	75
	Sulfuric acid	74	13	2	85
	Combination	70	19	0	81
	Seed dust alone	71	41	1	58
	Soil dust alone	73	30	1	69

<sup>1</sup> 2 percent sulfuric acid, 3 gallons per 4 by 12 foot bed (235 ml. per square foot).

<sup>2</sup> Seed dust of mercuric phenyl cyanamide, 8 percent, and cadmium oxide, 2.5 percent (commercial Barbak C); soil dust treatment of calomel, 0.4 gm. per square foot.

<sup>3</sup> Results from seed dust alone and soil dust alone are omitted, because there was too little damping-off to indicate effectiveness.

tion treatment, and frequently the calomel soil dust alone, caused less chemical injury to seedlings than sulfuric acid and was as effective in controlling damping-off. On the other hand, sulfuric acid eliminated many weeds that were not affected by the other treatment.

#### REDUCTION IN NUMBER OF WEEDS

Because weeds often present a critical problem in the seedbed, an effort was made to find a chemical that would destroy many weed seeds, and control damping-off, without causing either injury to the pines or undesirable changes in the soil.

The following materials were tested: In liquid soil treatments, sulfuric acid and sodium dinitro-ortho-cresolate; in dry soil treatments, tetramethyl thiuramdisulfide, ethyl mercury iodide, ethyl mercury phosphate, tetra-chloro-parabenzoquinone, sodium salt of 2-, 4-, 5-trichlorophenol, mercurous chloride, and hydroxymercurichlorophenol.

These materials were employed in four trials of from three to six replications each in the greenhouse in 1941, 1942, and 1943, and in four trials each with four small-bed replications in two forest nurseries. Although several of the materials gave fair weed control, all but tetramethyl thiuramdisulfide, as used, failed to meet other requirements. A summary of portions of the field trials is given in table 3. For brevity the treatments with little promise have been omitted.

TABLE 3.—Averages from 4 replications of soil treatments against weed seeds and damping-off in red pine seedbeds

Soil treatment	Measure used	Amount per square foot	Weeds per 12 square feet	Seed planted that emerged	Emerged seedlings that were—		
					Damped-off	Injured by chemicals	Healthy
<b>1942 Nursery No. 2:</b>							
None			Number	Percent	Percent	Percent	Percent
Calomel <sup>1</sup>	Grams	0.4	102	85	6	0	94
TMTD, pure <sup>2</sup>	do	.3	55	83	3	2	95
Do	do	.6	57	86	1	1	98
Do	do	1.2	24	85	2	1	97
Sulfuric acid, 2 percent	Milliliters	236	9	86	1	2	97
			17	77	2	5	93
<b>1942 Nursery No. 3:</b>							
None			105	72	2	0	98
Calomel <sup>1</sup>	Grams	.4	85	73	2	0	98
TMTD, pure <sup>2</sup>	do	.3	86	64	1	1	98
Do	do	.6	24	71	1	0	99
Do	do	1.2	10	64	1	1	98
Sulfuric acid, 2 percent	Milliliters	236	5	80	0	1	99
<b>1943 Nursery No. 2:</b>							
None			448	89	8	0	92
Calomel <sup>1</sup>	Grams	.4	264	90	5	0	95
TMTD, 50 percent <sup>3</sup>	do	1.2	107	86	12	0	88
Do	do	2.4	58	86	7	0	93
Do	do	4.8	17	95	4	0	96
Sulfuric acid, 2 percent	Milliliters	236	63	86	25	0	75
<b>1944 Nursery No. 4:</b>							
None			70	76	13	0	87
TMTD, 50 percent <sup>3</sup>	Grams	4.8	10	61	0	0	100
Sulfuric acid, 2 percent	Milliliters	236	28	81	2	0	98

<sup>1</sup> Seed also treated with mercuric phenyl cyanamide and cadmium oxide (commercial Barbak C).

<sup>2</sup> Tetramethyl thiuramdisulfide (pure was commercial Tuads, 50 percent was commercial Thiosan).

<sup>3</sup> This increase in the amount of damping-off following acid treatment has never been encountered in any other trials. The readings for the 4 replications, respectively, were as follows: 23, 27, 25, and 25 percent. Similar increases appeared with 2 other chemical treatments. *Rhizoctonia* was particularly severe in 1943.

The amount of damping-off in these trials, especially in 1942, was not large enough to give much information about damping-off control in the nursery. However, in these trials tetramethyl thiuramdisulfide appeared equal to sulfuric acid in respect to (1) weed control, (2) control of damping-off, (3) chemical injury to seedlings, and (4) seedling emergence. The significance of the weed control was apparent from inspection.

One of these experiments (1943, nursery No. 2) was in progress during a season when winter injury was severe.

#### WINTER INJURY

The winter of 1943-44 was particularly severe on pine seedlings in several Wisconsin forest nurseries. There was no rain, sleet, or snow during December. The lack of adequate snow cover caused a large amount of frost heaving and winter drought. However, in the spring of 1944, some plots that had been treated and seeded in 1943 survived the winter better than others.

The treatments and seedings were made in April 1943 with three replications. The results (table 4) were significant by inspection, so no statistical analyses were made. The data indicate that the use of increasingly larger amounts of the chemical gave concomitantly improved protection against winterkilling. The results presented are on only 1 year's observations in one locality. Since such winters are unusual, the opportunities are limited for repeating such observations. The writers hope they will be tried in regions where such winter injury is common. At the same time the influence of hydrophilic colloids in the soil (1) deserves consideration.

TABLE 4.—*Summary of survival of red pine after various soil treatments in relation both to damping-off and to winter injury*

Soil treatment	Year	Measure used	Amount per square foot	Seedlings from seed planted
None:				<i>Percent</i>
Emergence, June and July .....	1943			88
Seedlings living, August .....	1943			81
Seedlings living, July .....	1944			33
Sulfuric acid (2 percent):				
Emergence, June and July .....	1943	milliliters	235	86
Seedlings living, August .....	1943	do	235	64
Seedlings living, July .....	1944	do	235	20
Calomel †:				
Emergence, June and July .....	1943	grams	4	90
Seedlings living, August .....	1943	do	4	85
Seedlings living, July .....	1944	do	4	33
Tetramethyl thiuramdisulfide:				
Emergence, June and July .....	1943	grams	1.2	86
Seedlings living, August .....	1943	do	1.2	76
Seedlings living, July .....	1944	do	1.2	63
Tetramethyl thiuram disulfide:				
Emergence, June and July .....	1943	grams	2.4	86
Seedlings living, August .....	1943	do	2.4	80
Seedlings living, July .....	1944	do	2.4	71
Tetramethyl thiuramdisulfide:				
Emergence, June and July .....	1943	grams	4.8	95
Seedlings living, August .....	1943	do	4.8	92
Seedlings living, July .....	1944	do	4.8	83

† Seed also treated before planting with mercuric phenyl cyanamide and cadmium oxide.

## DISCUSSION

The variability inherent in the damping-off problem has indicated that any control measure may give fluctuating results from place to place in the same year and from year to year in the same place. In Wisconsin, the activity of the two principal fungi changed with different circumstances. For example, *Pythium* ordinarily was active in cool, wet, and slightly acid to neutral soil, while *Rhizoctonia* appeared in warm, moderately dry, and relatively acid soil (7, 8, 9). The Plainfield sand is light enough to absorb rain easily and to blow when not protected. In another locality and with a different kind of soil, one might expect these or different pathogenic fungi to operate in various ways—and correspondingly that other control measures might be necessary.

An increase in damping-off has appeared from time to time when the chemical treatment was quite mild. Examples appear in table 2 (nursery No. 3, 1941 trials), in the 1943 trials of table 3, and have been encountered frequently among chemical treatments not recorded because they were not effective against damping-off. Of the factors that might influence this increase, two deserve special mention.

Chemical injury and damping-off are difficult enough to distinguish when they are operating separately. As Roth and Riker (7) have pointed out, seedlings that have been weakened may be more susceptible to damping-off than vigorous seedlings. As a result, under certain conditions a chemical injury too mild to be classed as such may open the way for damping-off fungi and thus provide more damage than no treatment at all.

A more complicated consideration involves antagonisms between different biological agents in the soil. Damping-off fungi have to compete against other kinds of soil fauna and flora, including *Trichoderma*. Perhaps some of the mild chemicals injure or destroy the inhibitors of the damping-off fungi and, thus, indirectly favor the development of damping-off.

The effectiveness of tetramethyl thiuramdisulfide is difficult to explain. Possibly its well-known antioxidational properties would particularly affect the *Rhizoctonia*. Further study is suggested on its repellent or destructive effect on soil fauna. For example, if it inhibited worms that attacked roots, and flagellates that killed nitrifying bacteria, the seedlings would have improved water-absorbing systems and a better nutritive balance to withstand winter injury. If further trials were made with this chemical, the related but less expensive commercial "Thiuram M" might be included.

While the authors have achieved their purpose in finding chemicals, in addition to sulfuric acid, that control damping-off and that do not obviously upset the nutrient balance in the soil, the question of relative costs is quite pertinent. These chemicals cost more than sulfuric acid. However, they need less labor to apply, and they require no supplementary application of fertilizer to restore the desirable base exchange capacity disturbed by the acid. Counting everything, the cost of using these chemicals seems close enough to that of sulfuric acid to deserve consideration.

In any case, it seems better, when possible, to avoid damping-off by such cultural means as may best suit the circumstances; e. g., (1) the use of newly cleared land, (2) fall seeding, or (3) late spring seeding with the seed covered by sand rather than soil.

#### SUMMARY

A search has been made for a means of controlling damping-off in Wisconsin forest nursery seedlings which would be as effective as dilute sulfuric acid, but which would not obviously upset the nutritional balance in the soil.

A seed treatment of mercuric phenyl cyanamide with cadmium oxide, supplemented by a soil treatment with calomel, was relatively effective against damping-off from *Pythium* and *Rhizoctonia* in Wisconsin's sandy soil.

A soil treatment with tetramethyl thiuramdisulfide in limited trials was relatively effective not only against this damping-off but also against many weed seeds and winter injury.

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