The University of Minnesota Agricultural Experiment Station

A Study of the Damping-off Disease of Coniferous Seedlings

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A STUDY OF THE DAMPING-OFF DISEASE OF CONIFEROUS SEEDLINGS

By T. S. Hansen, W. H. Kenety, G. H. Wiggin, and E. C. Stakman

INTRODUCTION

The fungi which attack young coniferous seedlings and which are commonly known as the "damping-off" disease, form one of the most serious obstacles encountered in the raising of such stock in the nursery. The frequency and severity of losses from this disease among very young nursery stock have attracted the attention of pathologists, nurserymen, and foresters for many years, both in this country and abroad. Some species seem to be more susceptible than others, but practically all coniferous species handled in nurseries in this country are affected.

Spaulding and Hartley have made several studies of the organisms which cause damping-off in this country. Spaulding found more than 40 species of Fusarium which would cause the damping-off of pine seedlings. Hartley showed that both Rhizoctonia and Pythium caused very severe injury in the nurseries in Nebraska, and through experiments he worked out a system of soil sterilization for the prevention and control of the disease that was successful to a marked degree.

Unfortunately, the large number of organisms causing the disease and the great variation in climatic and edaphic factors in different parts of the country, make measures which are effective in one part of the country almost useless in another. Organisms which cause trouble in one region may be wholly lacking in another. Moreover, observations made during several years at the Cloquet Forest Experiment Station would seem to indicate that methods of nursery practice have an important influence on damping-off.

In order to determine the best fungicides for use with the native species in the Norway-jack pine type of country, which forms such a large proportion of the forest area of the Lake states, a rather elaborate project was inaugurated at the Cloquet station in co-operation with the Division of Plant Pathology and Botany. Work on the project started in 1914 and was not completed till 1919

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PART I. OUTLINE OF PROJECT

An outline of the project was drawn up to cover the following points, which were considered as having a possible bearing on the development of the damping-off fungi.

TIME OF SOWING

It was known that early and late sowing had a marked effect on the percentage of germination and the rate of development of seedlings, but no exact data had been obtained on their relation to damping-off. A series of experimental sowings was planned to cover every practicable sowing date.

PRELIMINARY TREATMENT OF SEED

It was thought that soaking the seed before sowing might, through its effect on the rate of germination, influence the percentage of injury from damping-off, and plots were planned to check this point.

Fig. 1. Sheet Iron Plate Used in Planting Square-foot Plots Plates with 100, 200 and 300 holes were used. One seed was put in each hole and pushed down the desired depth with one of the punches shown. Flanges on these punches regulated the depth. The seed were covered by rubbing soil over the plate. That which did not fall into the holes was scraped off. Uniformity in spacing and depth was thus secured. Plots were also introduced to check the effect of : depth of cover applied to seeds in the seedbeds; use of common fertilizers; use of shade of different densities; application of different amounts of water; crowning of the beds for drainage; use of different fungicides and time and method of their application.

GENERAL PLAN

The general plan was as follows: Several standard beds, 4×12 feet, were laid out in a compact block in a corner of the nursery where the conditions would be as nearly uniform as possible. Except in the case of the test for drainage the beds were built in the regular way with a crown of one inch.

In each of these beds, 18 plots one foot square were laid off in three rows, as shown in Figure 2. In the first row the seed was planted 100 to the square foot, in the second row 200, and in the third row 300.



Fig. 2. Arrangement of Beds Used in Determining Influence of Time of Sowing

Uniformity of spacing and accurate density were obtained by means of three perforated iron plates such as that illustrated in Figure 1. One plate had 100 perforations, another 200, and another 300. The plate was placed accurately on the plot. A single seed was placed in each perforation and pressed in with a shouldered peg. Uniform depth of covering was obtained by scattering dirt over the plate and rubbing off the excess which did not go into the holes.

Whenever a check was needed, at least a third, often a half and in some cases two thirds of the plots were kept as a check plot, thus obtaining an accurate, geometrically distributed check. Every seedling in all the plots was considered in arriving at results. There were no arbitrary selections.

HISTORY OF PROJECT

In 1914-15 an attempt was made to secure data from beds sown in the routine way in the regular nursery. Differences due to lack of uniformity in spacing, depth of cover, drainage, etc., that always occur in general nursery practice, completely vitiated the results. The error occasioned by arbitrarily picking out certain beds, or parts of beds, for checks, without being sure of the absolute similarity of the cultural conditions of both checks and experimental plots, made the results even more unreliable.

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In 1916 the project was carefully organized on a systematic basis. It was hoped to isolate the different factors and fix the responsibility for results. Even then results were not sufficiently conclusive on some of the points and the experiments in which results were doubtful were repeated in 1919. The 1916 experiments dealt exclusively with white, Norway and jack pine. The 1919 series included white spruce instead of jack pine.

The details of the whole series of experiments follow:

TIME OF SOWING

To determine the effect of the date of sowing on the rate and percentage of germination and the effect which these factors have on damping-off, plots were sown September 15, October 15, April 15, May 1, May 15, June 1, June 15, July 8 and July 15. Records were kept of the mean soil temperature from the time of sowing to the time of germination, and of the number of days required for germination in each case. The results are shown in Tables I and II. Figure 2 shows the arrangement of the beds.

		TABLE	I	•	
-	-		-		-

EFFECT OF DATE OF SEEDING ON RATE OF GERMINATION

···Species		Sept. 15	Oct. 15	April 15	May 1	May 15	June 1	June 15	July 8	July 15
Jack pine	Mean soil temperature from time of sowing Days for germination			35	59.6 25	67.9 16	· · · · · ·		 	77.5 11
Norway pine	Mean soil temperature* Days for germination				55.3 54	65.4 40			•	
White pine	Mean soil temperature* Days for germination				55.6 61	65.4 40				· · .*

-:. * Temperatures were taken with a soil thermometer covered the same depth as the seed. Readings were taken at the same time each day, and averaged. The longer period of sunlight in June and the more direct incidence of the sun's rays and higher temperature on certain days in the latter part of May.and June, account for the decrease in length of time for germination in these months. Fifteen days of high temperature in the last part of May averaged with 15 days of low temperature in April which might give the same or a higher average. In May and June the nights are uniformly warmer.

Table I illustrates very clearly the direct relationship between soil temperature and the rapidity of germination. 1

The results recorded in Table II show clearly that the percentage of damping-off among seedlings from seed sown in the summer is lower than among those from seed sown in the spring, especially with Norway pine. Little is known of the ecology of these fungi, but summer conditions are apparently adverse to their growth.

¹ The sowing late in the summer showed a higher percentage of germination, but the seedlings were very poorly developed at the end of the season.

TABLE II	
----------	--

EFFECT OF DATE OF SOWING ON DAMPING-OFF

	Early	spring		spring	Sum	mer
Species	% Germina- tion	% Damping- off	% Germina- tion	% Damping- off	% Germina- tion	% Damping- off
White pine Norway pine Jack pine	41.0 71.7 .67.0	10.4 23.3 17.0	46.1 82.6 76.0	36.7 28.6 8.0	97.0 76.0	5.0 7.0

PRELIMINARY TREATMENT OF SEED

Some coniferous seeds, especially those of white pine, do not germinate uniformly. The period of germination sometimes extends through two seasons and the rate is very irregular. It is a common practice to soak these seeds before sowing in order to hasten the rate of germination and make it more uniforn:.

To determine the result of this practice and its effect on dampingoff, a series of plots was planted with seed which had been soaked one, two and four days, and careful data were collected on the percentage of germination and the comparative loss from damping-off.

Figure 3 shows the arrangement of the beds. The results are shown in Table III.

Temp of water used 80°F

TREATMENT of SEED BEFORE SOWING.



Fig. 3. Arrangement of Beds Used in Determining the Effect of Treatment of Seed Before Sowing

EFFECT OF PRELIMINARY TREATMENT OF SEED

					Days	soaked	•			
Species -		1		2	- 4	•	Ave	rage	Ch	eck
	% Germ.	<i>%</i> D. О.	-% Germ.	% D. О.	% Germ.	D . O.	% Germ.	D. O.	% Germ.	% D. O.
White pine Norway pine	48.6 33.8	33.5 14.7	. 57.5. 34.5	30.4 28.0	70.5 40.8	30.0 35.8	58.9 36.3	31.3 26.1	70.0 .35.8	20.0 21.3

The effect of preliminary soaking on the germination of these two species seems to be very uncertain. With the exception of the four-

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day soaking, the practice did not raise the percentage of germination, and with the single exception of a one-day soaking of Norway pine, the loss from damping-off was considerably increased.

DEPTH OF COVER

The depth to which seed is covered influences the length of time between the bursting of the seedcoat and the appearance of the seedling above ground. During this period and until the root system becomes established, the seedling derives its food from the seed. Therefore the shorter this period the sooner the plant can start photosynthetic activity and the more vigorous the seedlings should be. Preliminary work in 1914-15 with Norway and white pine indicated that the lighter the cover the less the damping-off. Further experiments were carried on in 1916 with these species, and were repeated in 1919 with white spruce included.

- Figure 4 illustrates the arrangement of the beds in 1916. A similar arrangement was followed in 1919 except that white spruce was substituted for jack pine and a uniform number of seed, 200 per square foot, was sown throughout. Table IV gives the results.

	ТА	BLE I	v	
EFFECT	OF	DEPTH	OF	COVER

		White	pine			Norwa	y pine		Jack	pine	White s	spruce*
Depth of cover, Inches	191	16	. 19	19	19	16	19	19	19	16	19	19
TUCHED	Germ.	% D. O.	% Germ.	D . 0.	% Germ.	% D. 0	% Germ.	љ . О.	% Germ.	. О.	No. Germ.	D. O.
¥ ¥ ¥ ¥	46.0 42.0 31.7	9.1 18.7 16.5	11.1 13.8 13.6	5.7 8.1 14.6	79.0 82.4 	11.1 10.7 20.1	72.0 67.0 60.5	5.7 8.3 14.2	81.0 75.0 61.0	15.0 16.0 15.0	175 147 51	. 5.6 2.7 4.0

* In sowing the spruce in this experiment, it was found impossible to control the amount of seed, because of the small size and poor quality. Hence the germination is given in numbers per square foot.

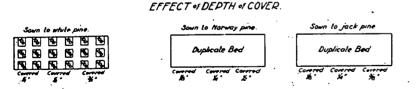


Fig. 4. Arrangement of Beds Used in Determining Effect of Depth of Cover

The figures in this table show that increase in depth of cover decreases germination and increases damping-off. White pine seems to be least affected by the depth of cover. A cover of $\frac{1}{8}$ inch seemed too thin for good practice.

FERTILIZERS

The use of manure or other fertilizers affects the development of the seedlings and makes a radical difference in the growing conditions for the fungi. Previous work with acid phosphate and sodium nitrate showed a marked tendency to increase damping-off in the beds so treated. Manure and tankage, which both contain a large variety of plant foods, were selected for the experiments in 1916. Figure 5 shows the arrangement of the beds. Table V shows the results of the experiments.

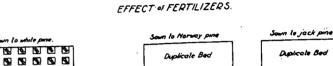


Fig. 5. Arrangement of Beds Used in Determining Effect of Fertilizers

TABLE V EFFECT OF DIFFERENT FERTILIZERS

	Wh	ite pine	Nor	way pine	Jaci	pine .
Fertilizer	~ % Germ.	% D. O '	% Germ.	% D. O.	.% Germ.	% D. O.
Manure Tankage Chcck	13.8	· 14.9 38.0 17.6	72.0 11.0 69.9	8.0 72.0 25.8	75.0 12.0 80.0	19.0 83.0 12.0

Tankage is clearly shown to reduce germination materially and to increase damping-off. The differences between the manured plots and the check plots were slight and variable. Further work must be done before any definite conclusions can be drawn.

SHADING

In order to determine the effect of different degrees of shade on the development and virulence of the organisms causing damping-off, plots were established in 1916 with three-fourths, one-half, and no shade. White, Norway and jack pine were used. Different plots were treated with different fungicides. Untreated check plots were, of course, maintained in every bed.

TABLE VI

			No shade	iade ,					14 shade	ade					3 5 1	K shade		
Treatment*		1916			1919		••	1916			1919	ŀ 1		1916			1919	
2	Germ.	D.0%	C. I.	Germ	D%	°.1.	Germ.	°.0%	ر. ۲.%	Germ.	0%0	C.1.	Germ.	D. 0.	C. I.	% Germ.	D.%	°.'.
White pine	42.1	9.8 35.6					59.5		1.7				47.1	5:3	0.3			
123 CLASO X OZ. HSO. 35 OZ. HCI. 140 CHOH. Check	36.2	: : :	0.7	6.2 4.2 0.7	24.0 10.5 21.3		50.4	18.2	- 0	9.5 9.5 13.2	2.6 18.0 8.0	5.0	45.3	14.2	: : :	13.0 13.0 10.0 14.5	11.5 20.0	16.6
Ncrway pine	77.8	10.9	42.2 14.8 100.0	58.2 68.0 60.4	2.5		80 3 980 3 85 5 85 5	1.2 16.8 16.8	15.4 0.0	66.5 62.2 67.4	1.2	9.0	64.3 50.0 82.1	8 8 10.3	1.3 10.3 100.0	71. 58.2 61.7	2.8	
White spruce- X Or. H.CO. X Or. HCI. 1:40 CHOH. Check				1 ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° °			7			+ 170 269 178 938	29.3 8.1 27.5 17.0	2.7				+ 19 45 3 258	26.3 4.4 12.4	
*HSO, = Sulphuric acid. CHOH = Formaldehyde. CuSO, = Copper sulphate. HCI = Hydrochloric acid. † Nümber of seedlings per squ	d. e. ite. per square foot.				· · · · · · · ·			11794									ļ	

DAMPING-OFF OF CONIFEROUS SEEDLINGS

These experiments were repeated in 1919, substituting white spruce for jack pine, changing some of the fungicides, and using a uniform density of 200 seeds per square foot. Figure 6 shows the arrangement of the beds in 1916. Table VI gives the results.

The 1916 series showed a larger amount of damping-off in the unshaded than in the shaded plots. This was true for both treated and untreated beds. The 1919 plots confirmed this conclusion, but not to such a marked degree. The spruce seed used in 1919 was of such poor quality that it was impossible to draw any definite conclusions in regard to that species.

Shading seemed to have little effect on the amount of chemical injury.

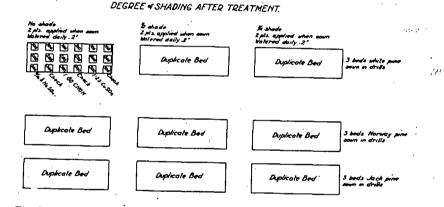


Fig. 6. Arrangement of Beds Used in Determining the Effect of Shading After Treatment

WATERING

In order to determine the effect of watering on the efficacy of the different fungicides, a series of plots was arranged as shown in Figure 7. The effect of watering on damping-off was studied in both treated and untreated plots. Watering was classified as light—onehalf inch per week; medium—one inch per week; and heavy—two inches per week. Due allowance was, of course, made for the rain that fell. Table VII gives the results.

The amount of water did not seem to have any direct bearing on the effect of fungicides. During the first month after germination, the rainfall was quite uniform and heavy. In general, the heavy watering seemed to increase the amount of damping-off, but this did not hold true in every case, nor was the increase marked enough to be certain that it was the effect of the heavy watering.

This work was not continued in 1919 because it was strongly indicated in 1916 that enough water would be applied under general nursery practice to prevent concentration.

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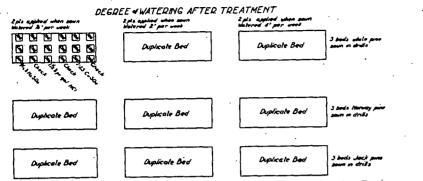


Fig. 7. Arrangement of Beds Used in Determining the Effect of Watering After Treatment TABLE VII

				Er	FECT O	F WATE	RING				·	
		Light		. :	Mediun	3	· ·	Heavy			None	
Treatment*	% Germ.	D . O.	с. 1.	% Germ.	% D. O.	% C. I.	% Germ.	% D. O.	% С. I.	% Germ.	% D. O.	с. I.
White pine					[[{	[ļ	
% Oz. H₂SO4	36.0	18.9	1.3	45.3	7.0	1.4	40.3	13.6	0			
⅔ Oz. HCl	46.0	14.4.	1.1	47.6	11.9	o	49.3	19.2	0.3			
1:25 CuSO4	45.3	9.2	24.6	48.8	9.5	17.7	46.0	9.0	38.0		26.2	0.2
Check												
Norway pine							(l		(1	
% Oz. H\$SO4	82.0	3.2	0.4	93.1	7.6	0.2	74.6	20.7	0.9			
⅔ Oz. HC1	93.3	7,1	0.3	86.1	11.0	0.0	•83.1	5.8	0.6		•	
1:25 Cu SO4	79.0	3.1	88.4	73.8	2.5	92.1	90.6	7.5	.82.7			
Check	73.0	26.7	0.4	74.2	27.0	· · · · · ·	77.7	29.7	5.0			
Jack pine											1	
⅓ Oz. HsSO4	70.3	4.0	.0	76.0	1.9	3.7	59.6	10.8	2.8			
⅔ Oz. ' HC1	66.5	4.0	0	85.0	4.5	- 0	83.3	4.2	1.0			
1:25 Cu SO4	32.8	2.0	8.3	60.1	0.3	92.	56.6	0.3	94.			
Check	46.5	19.0	0	45.1	.17.	0.4	65.8	18.8	1.8	····	[¹	
						·			<u> </u>	·	<u></u>	

* H₁SO₄ = Sulphuric acid.

14

HCl = Hydrochloric acid.

Cu SO4 = Copper sulphate.

MULCHING

To determine the effect of different mulches, applied immediately. after sowing, on the development of damping-off disease a series of plots was arranged as shown in Figure 8. Table VIII shows the results. A burlap mulch was beneficial on white pine, seemed to have very little effect on Norway pine, and was decidedly detrimental on jack pine. Sphagnum produced the highest germination in every case, and reduced the amount of damping-off except in the jack pine plots.

·,	Mulch										
Species	Exp	osed	Sphar	gnum	Burlap						
	% Germ.	% D. O.	% Germ.	% D. O.	% Germ.	% D. O					
White pine	53.7	21.8	59.9	16.6	55.4	15.3					
Norway pine	75.0	17.0	81.4	9:5	72.0	17.2					
Jack pine	78.0	10.0	79.0	15.0	73.0	22.0					

EFFECT & DIFFERENT MULCHES.

Sown to while pine.

· . .

Sown to Harway pine Sown to jack pine
Duplicate Bed
Duplicate Bed
Temuch Schamm Burdas
Nemuch Schamm Burdas

Fig. 8. Arrangement of Beds Used in Determining the Effect of Different Mulches

DENSITY OF SOWING

To determine the effect of the density of seedlings on damping-off, the results from all the 1916 beds, which were sown 100, 200 and 300 seeds to the square foot, were tabulated. The results for the jack pine beds are shown in Tables IX, X and XI. The results for the other species were similar.

	•				
INFLUENCE OF DENSIT	ч ог Јаск	PINE SEEDLINGS ON	DAMPING-OFF	IN TREATED	BEDS

Bed No.	. 10	ю	20	00	· 300		
	Germ	D. O.	Germ.	D. 0.	Germ.	D. 0.	
31	467	31	673	41	1253	43	
32	343	23	758 ·	· 33	1386	58	
33	331	15	626	. 37	· 1122	61	
34	313	47	. 714	104 .	1227 [·]	108	
35	313	8.	775	47	1314	85	
36	188	18	388	54	498	41	
Total	1955	142 .	39.34	316.	6800	.396	
Per cent	54.3 ·	. 7.2	54.6	8.0	62.9	5.8	

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·. ·			No. of seeds p	per square foot			
Bed No.	10	ю	20	00	300		
	Germ.	D. O.	Germ.	D. O.	Germ.	D. O	
1	151	15	284	39	370	85	
2	140 ·	18	302	18	472	41	
3	150	6	311	22	455	43	
9	483	72	930	159	1354	212	
12	341	39	903	133	1370 [°]	218	
13	. 170	7	346	12	462	. 13	
14	159	20	327	16	512	37	
15	121	10	320	29	435	65	
.8	300	39	636 ⁻ ·	80	922	175	
Total	2015	226	4359	508	6352	887	
Per cent	71.6	11.2	77.8	11.6	75.6	13.9	

TABLE X

INFLUENCE OF DENSITY OF JACK PINE SEEDLINGS ON DAMPING-OFF IN CULTURAL BEDS

TABLE XI

INFLUENCE OF DENSITY OF JACK PINE SEEDLINGS ON DAMPING-OFF IN CHECK PLOTS OF TREATED BEDS

-			No. of seeds p				
Bed No.	10	00	2	00	30	00	
· .[Germ.	D. O.	Germ.	D. O.	Germ.	D. 0.	-
31	233	· 10	421	26	668	* 30	
32	152	15	306	• 16	648	. 38 .	
33	160	39	244	26	476	48	
34	145	16	336 2	× 13	607	48	•
35	135	3	s 357	. 30	624	56	
36	92	16	<u>\$4</u> 7	-42	255	36	
Total	917	89	1811	158	3278	256	· · ·
Per cent	50.9 ⁸	9.7	50.3	8.7	60 .7 بخ	7.8 9 37*	

The germination is from 4 to 8 per cent higher in the more densely sown beds. Possibly this is because the larger number of seeds break the crust and let the weaker seedlings through. There is also, on the whole, less damping-off in the more thickly sown beds.

DRAINAGE

In order to determine the effects of drainage, a series of beds was constructed which varied from each other only in the shape of the surface. In some of the beds the surface was crowned up one inch in the center, in others the surface was flat, and in still others the surface was hollow or sunken in the center, like the crowned bed inverted.

The arrangement of the beds is shown in Figure 9. The results are given in Table XII.

EFFECT & DRAINAGE & BEDS.



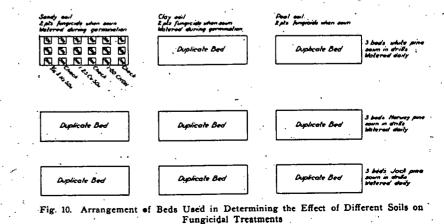
Fig. 9. Arrangement of Beds Used in Determining the Effect of Drainage

		TABLE XII		
EFFECT OF	BED	CONSTRUCTION	ON	DAMPING-C

Species	Crown		Leve	l bed	Sunken bed		
	% Germ.	% D. O.	% Germ.	% D. O.	% Germ.	% D. O.	
White pine	47.1	12.2	51.0	14.7	34.2	30.0	
Norway pine Jack pine	87.5 81.0	17.1 3.0	83.9 83.0	10.1 7.0	70.3 73.0	31.0 12.0	

The crowned bed, which gives the best drainage, shows the least damping-off, except in Norway pine, and even there the difference is not so marked that the increase can be definitely attributed to the method of bed construction.

. EFFECT & SOIL . FUNGICIDAL TREATMENTS



•

CHARACTER OF SOIL

To determine the effect of different kinds of soil a series of beds was arranged as shown in Figure 10 using sand, clay and peat soils. Unfortunately an accident destroyed the jack pine and white pine beds. The results for the Norway pine bed are given in Table XIII.

TABLE XIII

EFFECT OF KIND OF SOIL ON DAMPING-OFF OF NORWAY PINE SEEDLINGS

	Clay			Peat			Sand		
Treatment*	% Germ.	<mark>%</mark> D. О.	c. 1.	Germ	р %о.	% C. I.	% Germ	•% D. O.	% С. I.
% 01. H ₂ SO, H ₂ O daily 1:25 CuSO4, H ₂ O daily 1:80 CHOH, No H ₂ O No treatment, No H ₂ O	36.3 56.0 25.5 36.0	10.1 33.6 .43.1 41.9	73.0 32.4 22.8 4.0	65.0 56.3 77.5 82.1	5.1 13.0 6.4 12.0	0 10.0 1.3 0.6	74.6 90.6 92.3 85.5	20.7 7.5 ·4.5 16.7	0:9 82.7 0 0

* H₂SO₄ = Sulphuric acid. CuSO₄ = Copper sulphate CHOH = Formaldehyde.

HrO = Water.

Germination was lower in clay than in muck or sand, while damping-off and chemical injury are relatively higher in clay, except where copper sulphate was used. Copper sulphate caused a great deal more injury in sand, because there is a possibility of a greater concentration in sand than interther clay or peat.

FUNGICIDES

In order to determine the efficiency of different fungicides in a -preliminary treatment of the soil before sowing, a series of plots was arranged as shown in Figure 11.

So little was known of the fungi that the fungicides were selected at random. All solutions were based on the application of a certain amount of fungicide per square foot. Water was considered only a medium for securing even distribution of the fungicide. Three different strengths were tried in 1916 of sulphuric acid, formaldehyde, copper sulphate, zinc chloride, hydrochloric acid, and lime-sulphur solution. The results are given in Table XIV.

Sulphuric acid, hydrochloric acid, and formaldehyde were the most effective. Copper sulphate, zinc chloride, and lime-sulphur were not nearly so effective as fungicides and caused more loss from chemical injury than there was from damping-off in the check plots.

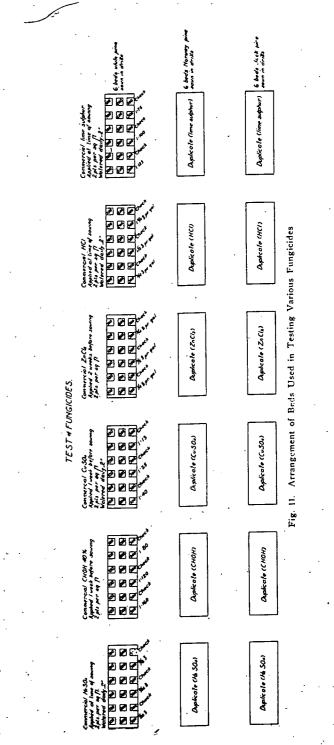


TABLE XIV

EFFECT OF VARIOUS FUNGICIDES, 1916

•	w	hite pi	ne	No	rway p	ine	Jack pine		
Treatment [*] in 2 pints H ₂ O per square foot	% Germ	% D. O.	% С. I.	% Germ.	D. 0.	с. I.	% Germ.	D . 0.	с. 1.
HsSO: 🔏 02	51.8	·10.6	0.6	81.6	2.0	0.2	60.8	5.8	0.8
HsO: 36 uz		10.3	1.7	80.3	1.2	0.2	54.8	3.0	0.6
H ₃ SO ₄ 5/2 oz		12.6	0.0	62.1	3.0	0.8	63.1	4.8	0.1
Check		19.4	0.6	85.5	16.7	0.0	73.0	5.0	0.5
СНОН 1:160	48.1	10.4	0.0	87.6	13.8	0.0	79.5	3.5	0.0
СНОН 1:120	59.6	8.1	0.0	90.0	6.5	0.0	74.8	3.3	.0.2
СНОН 1:80		8.5	0.0	92.3	4.5	0.0	74.1	2.4	-0.0
Check	50.4	18.2	0.0	88.7	10.9	0.0	62.0	5.6	0.0
Cu SO4 1:40	48.3	20.7	1.0	89.6	17.1	18.4	74.8	4.6	26.5
Cu SO4 1:25	51.0	11.7	1.0	98.0	16.8	15.4	67.3	6.1	62.
Cu SO4 1:15		7.0	3.4	91.0	16.4	34.4	51.0	2.0	72.5
Check		11.8	0.1	82.0	29.0	4.7	48.0	9.6	12.0
ZnCh 🔏 oz	33.8	15.2	2.4	89.1	11.2	2.4	62.8	8.5	14.0
ZnCl: 3/2 02	35.6	20.1	18.2	88.3	8.1	44.3	63.8	21.1	39.4
ZnCh 💃 oz	47.0	17.3	16.3	86.6	11.7	1.5	68.1	16.3	·81.9
Check	41.4	20.5	1.8	·83.7	37.0	1.9	57.0	7.6	9.5
HCl ¾ oz	58.1	18.3	1.1	93.0	12.7	0.4	73.8	4.2	0.2
HCl 34.0z	54.8	8.3	0.6	94.0	~7.0	0.5	70.3	4.7	0.0
HCl ½ oz	55.5	8.7	2.1	90.0	3.7	0.4	73.6	3.8	0.2
Check	49.6	18.5	0.5	87.0	23 : 7	0.1	61.0	7.7	0.0
Ca S 1:125	56.1	11.6	0.6	82.5	29.0	3.2	22.0	1.5	11.3
Ca S 1:100	57.3	11.3	2.4	88.1	16.6	7.5	24.3	2.7	18.0
Ca S 1:75	54.1	8.9	3.1	72.1	11.7	12.9	40.6	8.5	9.8

* H₃SO₄ = Sulphuric acid.

HCl = Hydrochloric acid.

CHOH = Formaldehyde.

Cu SO₁=Copper sulphate. Zn Ch=Zinc chloride.

HrO = Water.

Ca S=Lime sulphur solution.

As the largest amounts of sulphuric acid, hydrochloric acid, and formaldehyde used in 1916 caused very little chemical injury, further experiments were tried with them in 1919, to determine the maximum strength usable and to provide a more complete sterilization of the soil.

Table XV gives the results of the 1919 experiments.

In this series the 'wo acids proved more satisfactory. The stronger solutions, however, not only caused heavy chemical injury, but greatly reduced the percentage of germination. In White spruce the reduction of germination greatly exceeded the loss from dampingoff in the check plots.

TABLE	xv
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EFFECT OF VARIOUS FUNGICIDES, 1919

	w	hite pi	ne	Norway pine			White spruce		
Treatment [*] in 2 pints H ₂ O per square foot	% Germ.	% D. O.	% С. I.	% Germ.	D. O.	% С. 1.	No. Germ.	D. O.	% C.I.
⅓ oz. H₂SO₄ ¼ oz. H₂SO₄ 1 oz. H₂SO₄ Check	9.7 9.5 9.2 13.2	5.0 2.6 8.0	 18.9	71.0 66.5 13.0 67.4	1.4 1.5 5.7 11.2	1.0 1.1 46.1	267 170 58 938	31.8 29.3 3.4 17.0	 1.5
¼ oz. HCl. ½ oz. HCl. 1 oz. HCl. Check.		10.0 18.0 	· · · · · · · · · · · · · · · · · · ·	60.7 62.2 26.5 62.0	1.6 1.2 6.6 6.0	4.7	391 269 187 914	37.3 8.1 19.2 21.2	1.4 10.1
1:80 CHOH 1:40 CHOH 1:20 CHOH Check	3.0 0.5 0.0 12.8		8.0 100.0	10.7 2.7 0.0 64.4	4.6 5.5	9.0	277 178 34 1107	7.2 27.5 8.0	1.4 2.7 20.5

* HrO = Water.

CAPT.

H₃SO₄ = Sulphuric acid. HCl = Hydrochloric acid. CHOH = Formaldehyde.

CHOH Brontaldenyde.

TIME OF APPLICATION OF FUNGICIDES

To determine the best time for applying the fungicides, a series of experiments was planned in 1916 using sulphuric acid and zinc chloride and formaldehyde.

Figure 12 shows the arrangement of the beds and Table XVI the results of the experiments.

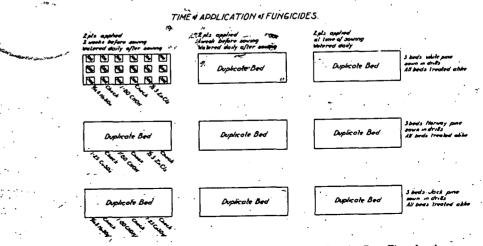


Fig. 12. Arrangement of Beds Used in Determining the Best Time for the Application of Fungicides

			TA	BLE	XVI			
Effect	OF	TIME	OF	APPL	CATION	OF	FUNCICIDES	

	2 week	s before	sowing	1 week	before	sowing	At ti	me of s	owing
Treatment* in 2 pints HrO per square foot	% Germ.	D. 0.	с. 1.	% Germ.	% D. О.	% С. І.	% Germ.	% D. О.	с". с. 1
White pine—									
5 oz. H3SO4	33.8	6.4		32.5	9.7	0.0	29.8	16.2	0.0
1:80 CHOH	29.1	7.4	5.1	35.5	16.9	0.0	38.3	31.7	0.5
⅓ oz. ZnCl₂	32.5	9.2	1.0	41.0	22.7	1.2	37.0	28.0	7.7
Check			• • • • • •	•••••			29.1	23.6	0.4
Norway pine									
Cu SO4	91.2	0.0	98.0	61:8	0.0	100.0	17.0	0.0	100.0
1:80 CHOH	84.0	7.5	0.0	81.6	16.7	0.0	34.6	49.0	0.0
' ⅓ oz. ZnCl₂	58.5	8.5	1.4	80.0	12.4	1.8	61.5	23.0	0.0
Check				· · · · · ·			65.3	25.4	· 0.3
Jack pine									
⅓ oz. H₂SO₁	51.0	9.0	0.0	57.0	16.0	0.0	35.0	3.5	0.0
1:80 CHOH		10.6	6.0	46.0	13.0	1.0		100.0	0.0
1/2 oz. ZnCl2.		1.0	93.0	+	0.0	100.0	+		100.0
Check							49.0	14.0	0.0

* HrO = Water.

H₂SO₄ = Sulphuric acid.

CHOH = Formaldehyde.

ZnCl2 = Zinc chloride.

 $CuSO_4 = Copper sulphate.$

† Normal.

The plots treated in advance of sowing show a higher rate of germination, and, except in the case of jack pine, considerably less damping-off. The difference in chemical injury was negligible.

SPRAYING

In 1919 a series of plots was established to determine the ef_{τ} ficiency of spraying with sulphuric acid, hydrochloric acid, and formaldehyde in preventing infection from the aerial spores of Fusarium.

The beds all received a soil treatment based on the results of previous work. White pine, $\frac{1}{4}$ oz. sulphuric acid per square foot; Norway pine, $\frac{1}{2}$ oz. hydrochloric acid per square foot; white spruce, $\frac{1}{4}$ oz. hydrochloric acid per square foot.

In addition to the treatment of the soil, a spray solution was applied after the seed had begun to germinate. The spray used varied in strength as indicated in Table XVII. No definite amount of the spray was applied, but the plots were sprinkled lightly with the solution of different strengths.

Table XVII shows the effect of spraying with fungicides.

		TABLE	XVII	
EFFECT	OF	SPRAYING	WITH	FUNGICIDE

	W	/hite pi	1e	No	rway pi	ine	WI	nite spr	uce
Spray treatment*	% Germ.	љ D. О.	% C. I.	% Germ.	<mark>%</mark> D. О.	с. I.	No. Germ.	D. O.	с. 1
4 oz. H.SO4 per gallon 1 oz. H.SO1 per gallon 4 oz. H.SO4 per gallon Check	3.2 1.5 4.7 5.3	13.0 5.2 14.0	66.6 73.0	58.2 63.7 50.5 71.8	0.3 0.4 5.5	11.4 72.9 99.0	316 220 223 859		15. 78. 100.
½ oz. HCl per gallon 2 oz. HCl per gallon 8 oz. HCl per gallon Check	11.0 12.0 3.0 9.8	4.1	4.5 14.5 92.0	68.2 53.2 51.7 81.8	0.9	8.0 84.9 100.0	210 68 173 700	4.0 16.2	22. 75. 99.
oz. CHOH per gallon	10.7 9.0 8.5 7.2	2.3 5.8 	4.6 27.7 41.2 1.1	66.0 58.7 47.2 80.3	6.9	97.7 99.1 98.3 14.1	268 31 189 860	6.2	88. 77. 94.

* H₂SO₄ = Sulphuric acid.

HCl = Hydrochloric acid.

CHOH = Formaldehyde.

The weaker solutions had very little effect on damping-off. The stronger solutions caused disastrous chemical injury. In almost every case the combined loss from chemical injury and damping-off exceeded the loss from damping-off in the untreated check plots.

AGE AT WHICH SEEDLINGS ARE MOST SUSCEPTIBLE TO DAMPING-OFF

In order to keep an accurate record of the life history of the seedlings, each week's germination was marked with a colored toothpick stuck in the ground beside it. A different color was used for each count. By this means it was possible to tell the exact age of the seedling when it was affected with damping-off.

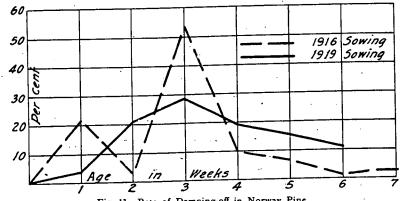


Fig. 13. Rate of Damping off in Norway Pine Average of all plots, both treated and untreated

Figures 13 to 20 show the behavior of the seedlings in general, and under separate treatments.

These curves show clearly that the critical period in the life of the seedling is the first four weeks. After this they are practically safe from damping off. In most cases the use of a fungicide seems to reduce the development of the disease, but to extend the period of its virulence.

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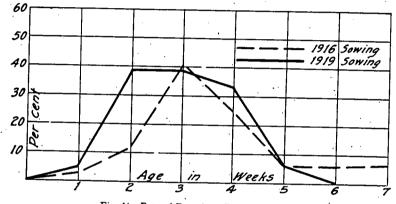


Fig. 14. Rate of Damping-off in White Pine Average of all plots, both treated and untreated

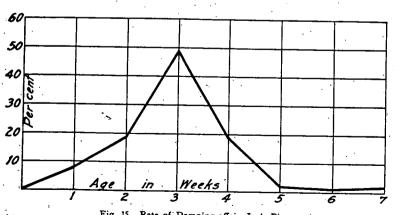
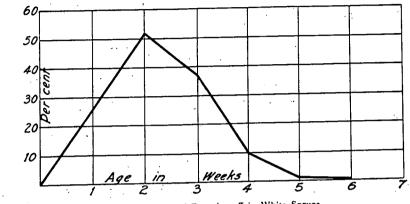
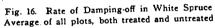


Fig. 15. Rate of Damping-off in Jack Pine Average of all plots, both treated and untreated





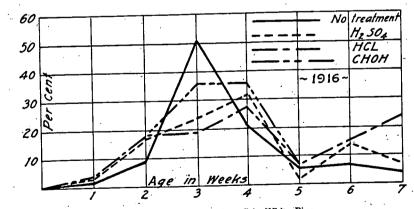


Fig. 17. Rate of Damping-off in White Pine Each treatment given separately

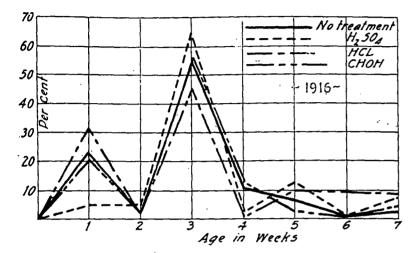


Fig. 18. Rate of Damping-off in Norway Pine Each treatment given separately

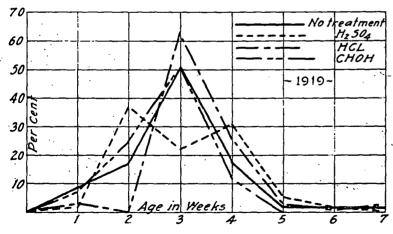
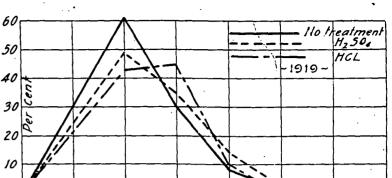


Fig. 19. Rate of Damping-off in Jack Pine Each treatment given separately



Age in Weeks 2 3 4 5 6 Fig. 20. Rate of Damping-off in White Spruce Each treatment given separately

GENERAL SUMMARY OF CONCLUSIONS

1. To obtain rapid germination, seed should not be sown until the soil termperatures are above 60 degrees F. Too late planting, on the other hand, produces poorly developed seedlings at the end of the first season.

2. Seed sown in the early summer is much less subject to damping-off than that sown in the early spring. Late spring would seem to be the most satisfactory time, all things considered.

3. Preliminary soaking of the seed of white and Norway pine does not markedly hasten or increase the rate of germination. It does increase the injury from damping-off. It should not be practiced.

4. Increase in depth of cover decreases germination and increases damping-off. The seed should be covered as lightly as is consistent with good nursery practice.

5. Manure can be used as a fertilizer without increasing damping-off. Tankage decreases germination and increases damping-off. It should not be used.

6. Half shade gives the best results and there is no advantage in removing the shades after each rain.

7. The amount of watering does not materially affect the amount of injury from damping-off or from chemical treatment. The water called for by the best nursery practice may be applied without danger.

8. The use of sphagnum moss as a mulch increases germination and decreases damping-off with the possible exception of Jack pine.

9. The more densely seeds are sown—up to 300 per square foot, the higher the germination and the less the injury from damping-off.

10. A crown of one inch in the surface of the bed improves drainage and decreases the amount of damping-off.

11. Germination is lower, and both damping-off and chemical injury are higher in clay than in either peat or sand.

12. The following are the best treatments for the sterilization of seedbeds and should be applied at the time of sowing: For white pine, $\frac{1}{4}$ ounce sulphuric acid per square foot; for Norway pine and white spruce, $\frac{1}{2}$ ounce hydrochloric acid per square foot; for jack pine, 7/16 ounce hydrochloric acid.

13. The application of any fungicide greatly reduces the germination of white spruce. The use of fungicides with this species is questionable.

14. The expense of applying fungicides in advance of sowing is too great and the results are not worth while.

15. No satisfactory spray for the control of Fusarium has yet been found.

16. There is no great danger of loss from damping-off after the seedlings are four weeks old.

17. Before any satisfactory methods can be worked out for the control of damping-off, the life history and ecology of the fungi causing the disease must be fully worked out.

DAMPING-OFF OF CONIFEROUS SEEDLINGS

PART II. FUNGI CAUSING DAMPING-OFF OF CONIF-EROUS SEEDLINGS IN MINNESOTA

By E. C. Stakman¹

INTRODUCTION

It is well known that under proper environmental conditions many different species of fungi may cause damping-off of coniferous seedlings. Those species, however, which are the most important in one locality may not always be the most important in other localities. It is known that different fungi react differently to the physical and chemical environment. It is very desirable, therefore, in conducting experiments on the control of damping-off to know what particular fungi are the most important in causing the disease, and which are the most resistant to the control measures applied. It should also be known whether the different species of fungi attack all species of conifers equally, or if certain fungi attack certain conifers more vigorously than they attack others.

The objects of the work reported below were as follows:

1. To ascertain what fungi are responsible for damping-off of coniferous seedlings in Minnesota.

2. To determine whether the same fungi attack all species of conifers equally.

3. To determine whether there was any correlation between the symptoms produced and the kind of fungi causing them.

4. To determine the resistance of the various fungi to soil treatment.

5. To determine which species of fungi were principally responsible for reinfestation of treated soil.

r. Lord

FUNGI FOUND ASSOCIATED WITH THE DISEASE

During August and September, 1916, isolations were made from 205 diseased seedlings. The fungi obtained from these plants are listed in Table I.

In 1919 another lot of seedlings was examined and the same fungi were found in approximately the same proportion. It will be observed that species of Fusarium, Pythium, Rhizoctonia, Alternaria, and Botrytis were the fungi most commonly present in the diseased seedlings. Species of Fusarium were especially common, having been isolated from 48.8 per cent of the plants examined in 1916. Pythium was second in frequency of occurrence, having been isolated from 35 per cent of the plants. Rhizoctonia was third and was found in 15.7

¹ L. L. De Flon, Miss Elsa Horn and J. L. Seal worked at various times on this phase of the project.

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per cent of the plants. Alternaria was fourth and Botrytis fifth in order of prevalence. Often several of the above mentioned fungi were isolated from the same diseased seedling; 29 per cent of the specimens examined were infected with more than one species. The most frequent combination was that of Fusarium and Alternaria. These fungi were found associated in 13 per cent of the specimens examined.

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FUNGI FOUND ASSOCIATED WITH DISEASED SEEDLINGS AT THE CLOQUET FOREST EXPERIMENT STATION. 1916

Fungus	No. of . seedlings attacked	Percentage of seedlings attacked
Fusarium	54	26
Pythium	29	14
Rhizoctonia	26	13
Alternaria	7	3
Fusarium and Pythium	24	12
Rhizoctonia and Alternaria	6	3
Rhizoctonia and Pythium	4	2
Pythium and Alternaria	2	1 1
Fusarium and Rhizoctonia	3	1
Fusarium and Alternaria	12	6
Fusarium, Pythium, and Alternaria	1	1/2
Fusarium, Pythium, and Rhizoctonia	2	1 '
Fusarium, Rhizoctonia, and Alternaria.	3	1
None	30	15
•Doubtful	2	1
		991/2

HOST RANGE OF THE FUNGI CAUSING DAMPING-OFF

An attempt has been made to find out whether any of the fungi were particularly virulent on certain kinds of seedlings. Altho fairly extensive observations were made, the indications are that the fungi causing damping-off are not restricted to any particular species of conifer. Fusarium, Pythium, and Rhizoctonia were commonly found on Norway, white and jack pine in the nurseries. In the greenhouse Fusarium and Botrytis were commonly found on Scotch and jack pine and also on blue and white spruce. A summary of the observations made in 1916 and 1919 is given in Table II.

		Hosts	TABLE II Hosts Attacked by Various Fungi	TABLE II KED BY VARI	OUS FUN	15			•			
		Norwa	Norway pine			Jack	Jack pine			White	White pine	
Fungus	1916	9	19	. 6161	-10	1916	61	1919	19	1916	19	1919
	No. Pl.	Pct.	No. Pl.	Pct.	Np. PI.	Pct.	No. Pl.	Pct.	No. Pl.	Pct.	No. PI.	Pct.
Fusarium	16	24	7	31		12	1	œ	52	34	14	35
Pythium	2 7	ۍ :		s ç	° .	25	•	34	15	٥ <u>:</u>	4:	0 8
Alternaria	: 9	15	с m	2 E	-	4	1		2 0	<u>י</u> ה	11	07
Fusarium and Pythium			1	Ś					14	6	S	13
Rhizoctonia and Pythium									\$	4	-	3
Rhizoctonia and Alternaria.	•	v.	1	s	-	4				2	-	£
Fusarium and Alternaria.	4	s ,	E	13		4	-	80	4	£		••
Rhizoctonia, Alternaria, Fusarium								ø	-		-	£
r usarıum, ryumum, Anternaria Rhizoctonia, Pythium, Fusarium				· · · · · · · · · · · · · · · · · · ·					2	1		
Doubtful			_			_	•		2	-		
Nothing	17	26	3	80	. 12	20		34	31	20	2	s
Total	66		23		24		12		154		39	

:30

in PENSIA FRANCIS

Percentage

36 10

28

3/ 13

> 3 3 5

101

53

7

13

13

:7

7

100

15

TECHNICAL BULLETIN 15

RELATION OF FUNGI PRESENT TO SYMPTOMS PRODUCED

The appearance of seedlings infected with damping-off is not al-. ways the same. Often the seedlings may be completely destroyed before they come through the ground. Again the plants may be attacked after they have emerged, but the entire plant will be completely destroyed. One of the most common types, however, is that in which the stem is attacked only at the ground line, the roots and lower stem being destroyed while the upper part of the stem is not attacked until after the plant has fallen. In some cases there is a general wilting of the entire plant and it soon dries up but remains standing. Often when older plants are attacked the roots are destroyed while the rest remains free of fungous infection until after the plant is dead. Frequently when the plants are not killed at once the stems become more or less swollen. Many minor variations of these symptoms have been observed. It is only reasonable to suppose that different fungi might consistently bring about more or less characteristic symptoms. However, a summary of the observations made in 1916 does not substantiate this view. There appears to be no appreciable correlation between the effect of the disease on the plant and the particular fungus causing the disease. A summary of these observations is given in Table III.

TABLE III

Fungi Found Associated with Diseased Seedlings Manifesting Different Types of Injury, 1916

WHITE PINE

Dried, standing, swollen type. I C 2.

Fungus Fusarium	No. seedlin; attacked 14
Pythium	4
Rhizoctonia	11
Rhizoctonia and Pythium	1.
Fusarium and Pythium	5
Rhizoctonia and Alternaria	1
Fusarium, Rhizoctonia, and Alternaria	1
Nothing observed	2
	39
Bent seedling, D. O. below the leaves. II C 3a.	
Fusarium	8
Pythium	1.
Rhizoctonia	0
Fusarium and Pythium	2
Fusarium and Alternaria	2
Doubtful	1
Nothing observed	1

Disease	No. seedling attacked	Percentage
Bent seedling, D. O. all the way. II C 3b.		50
Fusarium	6	50
Pythium	1	8
Fusarium and Pythium	3	25
Rhizoctonia and Pythium	1	8
Rhizoctonia, Alternaria, and Fusarium	1	8
		·
	12	99
Limp, swollen type. II C 2.		
Fusarium	0	
Rhizoctonia and Pythium	1	25
Fusarium and Pythium	.1	25
Fusarium, Pythium, and Alternaria	1	25
Nothing observed	1	25
Nothing observed		
	4 ·	100
Apparently dried, swollen type except that upper stem is green and softer. I C 2^1 .	ı	
Pythium	1	50
Fusarium and Pythium.	1	50
rusanum and rythum		
	2	100
Standing but stem soft and flabby. I C 2 ² .		
Rhizoctonia, Pythium, and Fusarium	2	100
	-	
Straight stem with D. O. below leaves. II C 4.	2	100
Fusarium	2	100
Apparently dried, swollen type but fallen. I C 3 ¹ .		
Fusarium and Alternaria	1	100
D. O. at groundpoint, stem above is normal.		
I C 3.		
Alternaria	1	100
Leaves only apparently D. O., stem dried. I. C. 2 ³ .	1 -	· 100
Pythium and Alternaria	1	100
• • • •		
NORWAY PINE		
Dried, standing type, not swollen. I A 1.	•	
Fusarium	7	30 -
Pythium	1	4
Rhizoctonia	5	22
Alternaria	3	13
Fusarium and Pythium	1 .	4
Fusarium and Alternaria	3	13
Rhizoctonia and Alternaria	1	4
Nothing observed	2	9
ATOMINA ODDUTTUL TITTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT		

Fungus	No. seedlings attacked	Percentage	
Dried, standing type, swollen. I A 2.		20	
Rhizoctonia	2	29 14	
Alternaria	1.	14	
Fusarium and Pythium	••	14	
Fusarium and Alternaria	1	43	
Rhizoctonia and Alternaria	3	43	
	7	100	
Apparently dried type but fallen. I A 1 ¹ .		F 0	
Fucarium	1	50	
Fusarium and Alternaria	· 1 ·	50	
•	2	100	
Apparently dried with green stem, slightly soft.	•		
1a. 1b.	_		
Doubtful	1	100	
More or less limp but standing. I A 1b ¹ .		100	
Fusarium and Alternaria	1	100	
Fallen, limp, and soft. II A 2.		100	
Fusarium	1	100	-
D. O. at groundpoint, stem above ground. I A la.		100	
Fusarium	1	36	
Pythium	4	30	
JACK PINE			
Dried, standing type. I B 1.	1	9	
Alternaria	1	9	
Fusarium and Alternaria		9	
Rrizoctonia, Alternaria, and Fusarium	-	36	
Nothing observed			
	11	99	
Apparently dried type but fallen. II B 2.		100	
Fusarium	1	100	•

In 1919 isolations were made from a large number of seedlings manifesting many distinct types of injury, but no correlation could be made between the types of injury and the fungi causing them.

Altho excellent control was obtained by chemical treatment, a small amount of damping-off occurred in the treated beds. From the results of the isolations which are summarized in Table IV it can be seen that Fusarium, Pythium, Rhizoctonia, and Alternaria occurred in soil after treatment, but in about the same relative proportion in which they occurred prior to treatment. This does not justify the assumption that any of the fungi causing damping-off are to any great extent more resistant to the treatment than others. It is recognized here that it is entirely possible that many of the fungi found in the treated beds may have been due to reinfestation from outside sources, since no precautions were taken to prevent this.

DAMPING-OFF OF CONIFEROUS SEEDLINGS

FUNGI WHICH SURVIVE TREATMENT

TABLE IV

FUNGI ISOLATED FROM DISEASED SEEDLINGS GROWN ON CHEMICALLY TREATED SOIL IN 1916

•	Upper stem	Lower stem	Total	Per cen
	35	40	75	30
Fusarium	13	9	22 ·	9
Pythium	17	16	33	13
Rhizoctonia	7	8	16	6
Alternaria	10	4	14	5
Fusarium and Pythium	2	4	6	2
Rhizoctonia and Pythium	4	3	7	. 3
Rhizoctonia and Alternaria	6	2	. 8	3
Fusarium and Alternaria.	1	0	1	1
Rhizoctonia, Alternaria, and Fusarium	1	0	1	1
Fusarium, Pythium, and Alternaria	· ·	- 1	2	1
Rhizoctonia, Pythium, and Fusarium	2	2	4	2
Doubtful	28	35	63	21
Nothing	<u>-</u>			99

SUMMARY

1. The damping-off of coniferous seedlings in Minnesota is due to facaltative parasites found more or less universally in the soil, such as Fusarium, Pythium, Rhizoctonia, Botrytis, and possibly Alternaria. They are here given in the order of their prevalence.

2. The fungi mentioned are often associated on the same host plant.

3. Nothing conclusive has been found to show that one host species is more susceptible to an organism than another host species to the same organism.

4. The different types of injury could not be correlated with the presence of the different kinds of fungi found in the lesions. Each kind of fungus acting alone or in combination with other forms apparently can cause any or all of the different symptoms.

5. All the organisms except Botrytis were found in beds that had been previously treated. They were much less abundant than before treatment but in about the same relative proportions.

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