SOME ASPECTS OF THE DAMPING-OFF OF BLACK PINE, *PINUS NIGRA* ARN.

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SOME ASPECTS OF THE DAMPING-OFF OF
BLACK PINE, PINUS NIGRA ARN.

By
Demetrios Symeon Kailidis

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
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Demetrios Symeon Kailidis ABSTRACT

The damping-off disease in Michigan nursery soil was studied. Black pine was used as the main host. The main pathogenic fungi isolated were: Pythium irregulare, P. ultimum, P. debaryanum, Rhizoctonia solani, Fusarium solani, F. oxysporum, F. moniliforme, and Botrytis cinerea. Among Pythium the most common was P. irregulare, P. ultimum was less common, and P. debaryanum was the least common. Among Fusaria the most common was F. oxysporum, F. solani was less common, and F. moniliforme was the least common. Pythium perniciosum was isolated and identified from soil; this report on this species is the second one in this country. This species was not pathogenic to black pine seedlings.

Pythium grew in cultures better at pH 5.5, and in a temperature between 20° and 30° C. Of Rhizoctonia solani four definite strains were isolated and studied. The different strains showed different pathogenicity, and characteristic appearance in cultures. Rhizoctonia solani generally grew faster at pH 4.5 to 6.5, and its growth at pH 8.0 was also satisfactory. This fungus grew also faster between 20° and 30° C., and at the lower temperature of 12° C. tested, it grew slower than Pythium.

Fusaria proved to be "strong" species, since their growth in different environmental conditions was influenced much less than the species of the genus Pythium and Rhizoctonia. Several characteristic properties of Fusaria in cultures were also studied. Among other things it was found that several strains of Fusaria possess the ability to form zonation under all environments tested, and that at 12° C. constant temperature all pathogenic Fusaria formed zonation.

Demetrios Symeon Kailidis ABSTRACT

Several of the principal pathogenic damping-off fungi when grown in mixed cultures were found to possess antibiotic properties of which the stronger one seems to be possessed by Fusarium solani strain 3.

In greenhouse and field isolation during a continuous 12 month study Pythium was more prevalent during winter months in a closed humid well heated greenhouse, Fusarium was less prevalent, and Rhizoctonia least prevalent. During spring and autumn under a cooler environment Fusarium was more prevalent, Rhizoctonia less prevalent, and Pythium least prevalent. The same was true for summer in greenhouse and field. Pythium was not isolated at all in several cases during warm dry periods. Thus Fusarium was able to dominate the other pathogenic fungi under a variety of conditions, and it was proved to be the main cause of black pine damping-off in Michigan.

Germination studies of black pine seed proved that for good germination a medium moisture is necessary at the beginning, and after a few days good aeration proved to be the more critical factor. Thus finally better germination was obtained with the smaller quantity of moisture tested. In media (soil and plates) at pH 4.5 germination of black pine was twice that at pH 8.5. At constant temperatures two optima of germination appeared to be at 24° and 31° C. Alternating temperatures were generally favorable for seed germination. Thus under favorable moisture, pH, and temperature seeds of black pine germinated faster and gave several times higher total germination number. Thus in cases of optimum conditions germinated seeds have more possibilities to escape damping-off. Black pine seedlings became resistant to disease in 25 to 27 days after germination.

Demetrios Symeon Kailidis ABSTRACT

During warmer periods the preemergence damping-off was higher than during cooler periods.

Under a medium soil fertility in which seedlings grew in a medium way damping-off was lighter, than in sand where seedlings grew faster but were probably too tender, or in muck soil where seedlings were heavier in dry organic matter but these seedlings grew too slowly.

In cases in which seedlings of black pine were allowed to grow under favorable conditions in the field or greenhouse, and these seedlings root-rotted, isolations proved that the main pathogenic fungus was _Fusarium_. All three species of _Fusaria_ which were able to cause damping-off, were able also to cause root-rot.

A modification of the older Cholodny and the modified Krinchkova soil slide technique found by the author was used, and it proved to possess advantages over the older ones.

In nursery practice soil must be light, slightly acid, and of medium fertility. Watering after sowing must be medium during the first days, and quite light later. The time of sowing also must be tested for each locality and for each species used.

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INTRODUCTION

Damping-off is a universal disease which commonly and regularly attacks the seedlings of conifer and broad leaved species of trees as well as most herbaceous plants. This disease occurs in young tender seedlings and usually attacks the stem at the soil surface level, although it may affect the cotyledons as well as the entire root system.

In the forest tree nursery this trouble is one of the most important causes of losses in the seedling population. Severity of the disease varies from year to year even in the same nursery; in some seasons losses are negligivle while in other years heavy losses may occur.

Studies concerning the nature and control of damping-off of seedlings have been carried on for many years with various crop plants including trees.

In the work reported here it is the aim of the author to study the principal fungi which cause damping-off in conifer tree seedlings and particularly with one species, black pine, *Pinus nigra* Arnold, to obtain pertinent information concerning their habits of growth in relation to various environmental conditions of the soil, and the plants which they attack.

This species of pine was chosen because it is one of the most important species found in south and southeastern Europe where it is used extensively for reforestation purposes (56).

In Greece after the war an extensive program of reforestation was started and additional new nurseries were built. Little or no damping-off occurred in the new nurseries for several years, but, as the years passed damping-off became more and more important.

This study concerns several aspects of the damping-off of black pine seedlings and the pathogenic organisms which cause it. Naturally infested soil from the Bogue Nursery, Michigan State University, East Lansing, Michigan was used mainly in the course of this investigation. At the Bogue nursery conifer seedlings have suffered for many years from the damping-off disease (77). Soil from the Chittenden Forest Service Nursery at Wellston, Michigan was also used.

The causal organisms were identified from the two Michigan nurseries. The relative pathogenicity of each of these organisms, the percentage present, and the relationship between them throughout the year was studied. The possible influence of other soil microflora and microfauna, and particularly the influence of environmental conditions on damping-off fungi was also studied. Extensive studies were made on pure cultures of different pathogenic fungi in different environments, and on the germination of black pine seed under different disease and environment conditions.

LITERATURE REVIEW

The damping-off of forest tree seedlings and several of the causal fungi were known to European plant pathologists and foresters before the end of the last century (31). Atkinson (3) was the first to study the damping-off disease in the United States. Jones (43) and Spaulding (75) in 1908 independently published on control measures; both introduced sand as a seed bed covering; Jones used formalin, and Spaulding used sulfuric acid for the control of the disease. Hartley (33) in 1910 isolated Rhizoctonia sp. and Pythium sp. as the causal agents of coniferous damping-off. He began inoculations with these two fungi and studied their effect against conifers. He found that considerable damping-off could occur under very dry conditions.

The following year Gifford (21) reported his studies, in which he found that Fusarium alone was the cause of damping-off. He also stated that alkaline soil favored the disease. Hartley and Merril (34) in 1914 reported Pythium debaryanum and Fusarium sp. as the causal agents of damping-off of coniferous seedlings and sulfuric acid as the best control. The same year Spaulding (76) reported that damping-off of conifers is caused by several widely different fungi and occurs under a variety of conditions. Graves (24) in 1915 inoculated healthy pine seedlings with Cylindrocladium scoparium, but obtained no sign of pathogenicity. The same year Hartley and Bruner (35) found that Rhizoctonia was capable of attacking pine seedlings too mature to be killed by either Pythium debaryanum or Fusarium moniliforme.

The next important contribution on the damping-off in forest nurseries was that of Hartley (32) in 1921. He reviewed and discussed the economic significance of damping-off, its importance on conifers, the relative susceptibility of forest trees, the causal fungi, their virulence and the relation of environmental factors to the disease. In 1923 Hansen _et al_ (29) studied several nursery practices in connection with the damping-off of coniferous seedlings. They concluded that preliminary soaking of the seeds of white pine and Norway pine did not increase the rate of germination but increased injury from damping-off. Increases in depth of cover decreased germination and increased damping-off. Germination was lower and both damping-off and chemical injury were higher in clay than in either peat or sand. Half shade on the seed bed gave the best results.

Delevoy (14) in 1926 published his work with coniferous damping-off in Belgium. Damping-off there was due to _Fusarium_ sp. and _Corticium vagum_. He concluded that the best period of sowing was early spring, and the best covering was sawdust and coarse sand. He worked also on control methods with different chemicals. A number of articles to which I refer recorded damping-off counts in various sections of the United States and the results of using variously applied chemical compounds (42, 63, 91, 95).

Elianson (18) in 1928 showed that of 22 species and strains of _Pythium_ of different origin all but three caused damping-off in some conifer seedlings. However, he obtained negative results with _Alternaria_ and _Fusarium._ Rathbun-Gravatt (60) in 1931 demonstrated in cultures on agar media that strains of _Pythium ultimum_ and _Corticium vagum_ caused more apparent germination loss of certain conifers than any species of _Fusarium_ tested except _F. sporotrichioides_.

Roth (64) in 1935 in Switzerland worked with damping-off of Picea excelsa, utilizing the pathogens Fusarium bulbigenum var. blasticola, Pythium debaryanum, and Rhizoctonia solani. He found no serious losses at pH values below 5.5. His damping-off fungi were generally most destructive at intermediate temperatures. His Fusarium species caused damage at high temperatures at which Pythium and Rhizoctonia were relatively harmless. Ten Houten (78) in 1939 reported his work on damping-off of coniferous seedlings in Holland. He found less damping-off at pH 4.5. Early spring sowing gave less damping-off compared with other periods and he obtained best results when seeds were covered with coarse sand.

Roth and Riker (65, 66, 67) in 1943 studied the damping-off of red pine in Wisconsin nurseries. They succeeded in distinguishing between the field symptoms of damping-off exhibited by Pythium and Rhizoctonia. They made careful studies on the temperature and moisture relations of the two causal agents as they affected red pine. Pythium irregulare was favored by more moist soil, cool weather and pH 5.2 and above, while Rhizoctonia solani was encouraged by dry soil, warm weather and a pH 5.2 and lower. Their conclusion was that factors favoring one fungus discouraged the other, thus we are speaking of two different diseases when we refer to damping-off caused by Pythium and Rhizoctonia. They excluded Fusarium from their studies as preliminary tests showed its appearance to be negligible in Wisconsin nursery soil.

Tint (79, 80, 81) in 1945 used a great number of Fusaria of different origin in his study on damping-off of red pine. He found that the virulence of the fungi depended to a large extent upon the conditions under which they were tested, and also upon the nature of their nutrition prior to inoculation. The virulent Fusaria generally could cause relatively equal preemergence and postemergence damping-off. He found that in liquid cultures where nitrogen was deficient there was little damping-off, while on the contrary an excess of nitrogen gave severe damping-off, but with an excess of phosphorus damping-off was slight. With an excess of calcium, red pine gave higher losses from damping-off. As to the pH behavior, he found that postemergence damping-off of red pine was low in more acid samples, reached a maximum on the acid side of neutrality, fell off sharply at pH 7.5 in sterilized soil and at pH 8.1 in unsterilized soil, and then became higher in more alkaline substrate. Finally while his losses from both preemergence and postemergence damping-off were higher at higher temperatures, they were negligible at an average 10^{0} C. using in the last case as host, red pine and as pathogen, Fusarium oxysporium. Summarizing Tint found that any deficiency or excess of the nutrient, the concentration of the solution, any unbalanced ratio, the acidity etc. influenced the growth and health of the plant and pathogen, by influencing the entire metabolic process of the plant which may decrease the resistance of the seedlings to physiological and infectious diseases.

Beach (6) in 1946 in Pennsylvania reported his work on damping-off with several vegetables. He worked with spinach, peas, tomato and beets. He found that during January and February, Pythium sp. was more prevalent, Fusarium sp. was less prevalent, and Rhizoctonia sp. was least prevalent. On the contrary during May, June and July, Fusarium became more prevalent, Rhizoctonia less prevalent, and Pythium least prevalent. Beach concluded that these results were due to the influence of environmental factors.

Cox (13) in 1951 in Delaware investigated a disease causing losses in red pine and white pine seed beds. He found that the cause of the disease was a complex of Cylindrocladium scoparium, Rhizoctonia solani, and Pythium sp.. In Delaware Cylindrocladium scoparium was found to be the primary causal agent of a serious complex of diseases, including damping-off, late damping-off, root rot, and seedling blight.

Strong (77) in 1952 published a work on the control of damping-off in forest nurseries in Michigan. He found that pathogenic species of Fusarium, Rhizoctonia and Pythium were present in the nursery soils and in the soils used in the greenhouse.

Vaartaja (82, 83, 84) and Vaartaja et al (85, 86) published in recent years a number of articles on damping-off and its control in Canadian forest nurseries. They found that Rhizoctonia solani or Pythium sp. can be inhibited or parasitized by Aspergillus niger, Botrytis cinerea, Penicillium sp., Trichoderma sp. and Gliocladium sp.. In periods of heavy rains Pythium becomes high in virulence. He and his colleagues found also that differences in susceptibility to damping-off existed among tree species and among progenies of different trees. This suggested genetical difference for resistance to damping-off.

MATERIALS AND METHODS

Plantings and Soils

In the first period of this year for the occurrence and behavior of the different pathogenic to damping-off fungi, a number of flats containing naturally infected soil from the Bogue Nursery were used in the Plant Science Greenhouse. These flats of soil were regularly sowed with black pine seed throughout the year. During the summer, sowings were made out in the Bogue Nursery.

Seed were surface sterilized with 1:1000 solution of mercuric chloride for 3 minutes, then washed thoroughly with sterile distilled water (62). At the same time unsterilized seed were used, but these seed were well washed with tap water before sowing.

Seed of black pine were sowed in flats or in the nursery beds at a depth of 5 to 15 mm., usually 7 to 8 mm. (55), and were covered with the same soil. After covering, the soil surface was gently pressed down with a flat board. The number of seed sowed per row was always more than a 100 depending on the germinability of the seed. Seeds were sowed in sterilized soil in order to determine how many would germinate, then enough seed were sowed so that 100 seedlings per row would be expected to emerge. Watering was adjusted carefully to avoid over or under watering the seedbeds.

The type of soil used is given a little later and was characterized with the symbol (F). The pH of the soil was 5.5.

The pH of the soil was determined with an electrode pH meter. Equal weights of soil and distilled water were stirred together in a small beaker and the pH readings made. In order to change the pH of the soil

0.1 N hydrochloric acid and 0.1 N sodium hydroxide solutions were used at first. This proved unsatisfactory, however, since the soils adjusted to the more alkaline ranges of pH developed a hard surface layer of soil after a few days. This was probably due to the formation of sodium carbonate. To avoid this condition different amounts and proportions of 0.1 M phosphoric acid and 0.1 M sodium hydroxide (5) were used to make the adjustments of the soil pH, and also as buffers for seed germination tests at different pH levels in the laboratory. When these chemicals were used in the soil the pH was checked for change after 2 or 3 days.

Isolation and Culture of Fungi

Damped-off seedlings were collected once or twice a week for isolation in the laboratory. The seedlings were washed thoroughly with tap water and surface sterilized with 70 percent alcohol or 1:1000 dilution of mercuric chloride for 2 to 3 minutes (62). Next they were thoroughly rinsed for 1 to 2 minutes in distilled water. Finally root and stem pieces from the diseased areas were planted on a nutrient medium in Petri plates. Potato dextrose agar (Difco) and malt agar were the media used (62). It was found necessary to add 2 to 3 drops of a 25 percent lactic acid to each plate in order to bring the pH of the medium below 5.0 and thus prevent bacterial growth.

For the isolation of species of Pythium from the soil a small quantity of soil was placed in a Petri dish in which enough sterile distilled water was added to cover the bottom of the plate. Sterilized half pieces of flax or hemp seed, or small pieces of boiled carrot root were then placed into the muddy water in the plate (50). When

Phycomycetes were present, hyphae would become apparent after a few days, growing out from the surfaces of the seed or carrot root. Small tips of hyphae were then transferred to the nutrient agar plate for purification. In several cases it was necessary to transfer hyphal material from these first plate cultures to a maltose-peptone agar. In this medium Pythium grew faster and could be separated from mixtures of different fungi, especially in mixture with Fusarium.

Another technique used in purification process was to examine for isolated hyphal tips under low power of the microscope (100X) and cut them out, together with an agar plug, with a small capillary tube. These plugs were then transferred to a new plate (9, 62). In other cases, using similar technique, small bits of mycelial mats were transferred aseptically to a drop of water on a microscope slide. This material was teased apart until a single sporangium could be transferred onto one needle. This was then streaked over the surface of the agar in a new plate where isolated colonies started to grow after 2 or 3 days along the streaks. From these a hyphal tips were cut and transferred to new plates, and thus pure cultures of Pythium species were obtained. The same techniques were used to obtain pure cultures of Pythium species directly from soil and from damped-off seedlings.

Several media were used for the growth and study of Pythium; potato dextrose agar, corn meal agar, oatmeal agar, lima bean agar, water agar, sterile distilled water only, and boiled halves of hemp seed and carrot.

Pythium isolates were grown in all of these media, but for each species the growth, aerial mycelia, and the time and number of sporangia and oospores formed differed considerably. Water cultures with hemp seed and carrot were used more extensively than the other media in these studies. The cultures in the carrot and water medium induced earlier and more abundant oospore formation than was obtained in the hemp seed water medium.

Single spore isolation was used for Fusarium isolations (62). For Rhizoctonia solani purification was obtained by using hyphal tip cuttings.

The fungi used to infest soil were grown on a mixture of vermiculite and cornmeal medium in Petri plates. This medium was about one half the depth of the plate and it was pressed down a little with a spatula to prevent the scattering of the particles when autoclaving. In this way contaminants could be avoided more successfully during growth of the pathogen. After suitable growth had developed in these plates measured amounts were used as inoculum to infest the soil.

The soil samples were autoclaved after placement in the pots or in the flats for one to one and one half hours at 15 pounds pressure. After removal from the autoclave they were allowed to stand for about one week in order to avoid possible undesirable influences resulting from the soil sterilization which might affect seed germination and growth, or affect the fungus inoculum (48, 70). The inoculum was then mixed with the soil in the pots or flats, and the seeds were sown.

The pots and flats were watered regularly to prevent undue drying. For a period immediately after sowing a plastic sheeting cover on a wooden frame standing one and a half feet above the soil was used.

Adjustment of the humus content of the soil was made by the method described by Piper (58) while the method for the mechanical analysis of the soil was that described by Bouyoucos (7).

The percentage content of each soil type is given below:

	Humus percent	Sand percent	Silt percent	Clay percent
Sand (SA)	0	100	0	0
Soil (D)	2.04	89.0	2.98	5.98
Soil (E)	2.61	89.43	1.99	5.97
Soil (F)	3.34	86.00	4.00	6.66
Muck (M)	46.60	--	--	--
Clay	1.25	70.75	9.00	19.00

No mechanical analysis of sand was made since it was considered to consist solely of sand without humus, silt or clay material. Only the humus content was measured in the muck soil.

The soils used were collected from the following places:

SA. Sand collected from North, Michigan.

SS. Sand like the above. In this series pots were watered with a synthetic nutrient solution for fungi.

SP. Sand like above. In this series sand was mixed with 10 percent peat used in Plant Science Greenhouse.

D. Soil collected from Woodlot Chittenden Nursery, Wellston, Michigan

E. Soil collected from seedbeds Chittenden Nursery, Wellston, Michigan

F. Bogue, University Forestry Nursery, Michigan State University, East Lansing, Michigan

M. Muck soil, used in Plant Science Greenhouse, Michigan State University, East Lansing, Michigan.

C. Clay soil, used in Plant Science Greenhouse, Michigan State University, East Lansing, Michigan.

Nematode Isolation

For the study of nematodes which were present in diseased damping-off seedlings and in soil the funnel technique was used (23). Nematodes isolated from sections of stems and roots of diseased seedlings placed in Petri plates were studied. These cultures having nematodes were put in a blender and using the funnel technique nematodes were isolated. Study of nematodes was also accomplished by the modified contact slide technique which was described by the author, and the older one of Cholodny (11) and Kzinchkova (94).

A Modification of Modified Cholodny Soil Slide Technique

For the study of soil microorganisms (microflora and microfauna) several techniques were used. One of them was the dilution plate technique. The other was the Cholodny (11) contact slide technique. It was found that this work was slow and exact identifications were not easy to do with soil fungi, since many of them grew on the slide as mycelium. Krinchkova (94) and Wright (94) used a modification of the Cholodny technique. Sterilized glass micro-slides were placed in sterile petri dishes and coated with malt agar; after hardening of the agar aseptically the slides were dried in an oven at 45° to 50°C.. These slides with the dried agar on them were put in the soil of flats in the greenhouse or in nursery beds where the damping-off study was done. A spatula was used to open a hole and make a flattened smooth surface; on that smooth surface the clean surface of the glass slide was leaned and the soil was pushed slowly onto the agar surface. The slides were left in the soil from 2 to 4 days. After that the slides were removed carefully dried, stained and examined. This technique was an improvement of the older one of Cholodny (11). Because the slides with agar were placed in the soil for only 2 to 4 days and then were dried, soil fungi etc. did not have time to establish themselves and produce the characteristic fruiting bodies necessary for identification. The slides were covered with septate and non-septate hyphae. Longer periods in the soil merely allowed the agar to dissolve in the soil moisture.

The author's improvement of the above method was as follows: After the removal of agar slides from soil, they were placed in sterilized Petri plates. Small pieces (2 X 2 mm) of the overgrown agar were transferred to 5 X 5 mm blocks of malt or potato dextrose agar on sterilized slides and this covered with a sterilized cover glass. The mount was pressed a little and put on a small piece of mesh wire in a sterilized Petri dish moist-chamber. After a few days abundant mycelia and fruiting bodies were formed and identification became easier and more accurate.

ISOLATION AND IDENTIFICATION OF SPECIES, THEIR IMPORTANCE AND PATHOGENICITY

In the course of this study several fungi and other micro-organisms were isolated, identified and studied. Isolation of a number of some of the fungi were isolated from damped-off seedlings in Bogue Nursery, and in flats of soil from the Bogue and Chittenden Nurseries.

The Genus *Pythium*

With the techniques which have already been described, the following *Pythium* species were isolated, purified, identified and studied, mainly from damped-off seedlings of black pine, and from soil. The identifications were based on the following characteristics given by several authorities.

Pythium irregulare Buisman: This species is a serious cause of damping-off of coniferous seedlings (65, 66, 67). It was isolated many times in the course of this study from damped-off seedlings and from soil. Pure cultures, which were isolated from affected seedlings and from soil, proved highly pathogenic to black pine seedlings when they were grown in artificially infested soil.

The physical characteristics of *P. irregulare* are (50, 51):

Hyphae: diameter 2-7 mu, branching freely.

Sporangia: 10-30 mu in diameter, spherical to pyriform terminal or intercalary. From sporangia zoospores formed in a vesicle, or they germinate as conidia.

Oogonia: varying in shape, smooth or irregularly echinulate. The echinulate oogonia were less than 1 percent of the total. Diameter of oogonia 24-26 mu without projections, terminal or intercalary, sessile or stalked.

Oospores: Smooth aplerotic 10-20 mu in diameter.

Antheridia: Mainly monoclinous, sometimes diclinous, usually 1 to 2 per oogonium. Wall thickness about 1.5 mu.

Pythium debaryanum Hesse: This fungus is one of the most wide-spread of the Pythium species causing damping-off in coniferous seedlings (32, 64). The fungus was isolated from damped-off seedlings and from soil. Isolates from both origins proved highly pathogenic to black pine seedlings.

Characteristics of the fungus (16, 32, 50, 51).

Hyphae: varying 3 to 7 mu in diameter.

Sporangia: 15-25 mu in diameter, spherical to oval, terminal or intercalary. Germination by zoospores, or as conidia with germ tubes.

Oogonia: Oogonia were very difficult to find. This is also reported by other workers (32). From over one hundred plates with different media and age only a few oogonia were seen in water agar cultures up to one and one half months old. In water cultures with hemp seed and carrot in which the other Pythium species studied formed abundant oogonia and oospores, this species produced no oogonia at all.
Oogonia 15-25 mu in diameter, smooth spherical terminal or intercalary.

Oospores: 12-20 mu in diamter, aplerotic, smooth, wall thinner, about 1 mu. Germination by germ tube.

Antheridia: Monoclinous and diclinous. One to as many as 6 per oogonium, when monoclinous arising some distance below the oogonium.

Pythium ultimum Trow: This species was isolated from damped-off seedlings of black pine and from soil. It is the most common of the *Pythium* species causing damping-off in the United States and is widespread all over the world; Middleton (51) gives a long list of plants and places throughout the world in which this fungus has occurred. *Pythium debaryanum* and *Pythium ultimum* are quite similar in their characteristics. Drechsler (16) considered and distinguished them as separate species.

Characteristics of the fungus (16, 50, 51).

Hyphae: 1.7-6.5 mu in diameter, long and branched.

Sporangia: 12-28 mu in diameter, spherical and terminal; or in some cases intercalary barrel-shaped 14-17 mu, to 22.9 by 27.8 mu. Sporangia act as conidia, germination only by a germ tube.

Oogonia: 19.6-22.9 mu in diameter, smooth usually terminal spherical.

Oospores: 14.7-18.3 mu in diameter, aplerotic, single spherical. Wall thickness 1.5 mu. Germination directly by germ tube.

Antheridia: usually 1 per oogonium, monoclinous, arising just below the oogonium.

P. irregulare, *P. debaryanum*, and *P. ultimum* were isolated from both damped-off seedlings and from soil isolations in nursery beds and greenhouse flats throughout the year. *Pythium irregulare* appeared to be the most common and probably the most highly pathogenic. *Pythium ultimum* was found second most prevalent, and *Pythium debaryanum* was third in prevalence. Pathogenicity of these species was proved by many isolations from black pine and scotch pine seedlings grown in sterilized soil which had been infested with these fungi.

In addition to the above, another *Pythium* species was isolated during this study. However, it was proved to be non-pathogenic to black pine seedlings.

Pythium perniciosum Serbinow: This species was first described in Russia by Serbinow in 1912 as a parasite of tobacco seedlings and several other plants (50, 51). Drechsler (17) doubted the existence of this species, and thought that Serbinow had not purified his culture. Gilman (22) did not refer to this species, but Middleton (51) described its isolation from rotted *Euphorbia pulcherima* plant.

The author isolated *P. perniciosum* from soil, and studied it in pure culture. It grows well in water cultures on hemp seed and carrot, where it produces abundant mycelium, sporangia, oogonia, and, in less numbers, catenulate spherical conidia, 3-5 in a chain. It grows and reproduced well in a number of the standard media, used for growing cultures of *Pythium*.

Hyphae: 5-9.5 mu in diameter.

Sporangia: filamentous-like hyphae or arising from sac-like structure of the hyphae, zoospores produced in a vesicle. Conidia-like catenulate bodies 3-5 in number in a series germinating directly by germ tubes.

Oogonia: 18.9-30.0 mu in diameter, smooth, spherical, terminal on short mycelia branches.

Oospores: 18.1-32.5 mu in diameter, aplerotic single, Serbinow (50, 51), and Middleton (51) state that germination is unknown. In water cultures in hemp seed after 15 months in room environment the author observed germination. This oospore germination was typically by a germ tube.

Antheridia: 1 to 2, usually one, per oogonium monoclinous or diclinous.

The Genus Fusarium.

The genus Fusarium is probably one of the most important in causing damping-off and root rot in coniferous seedlings and other crops as well, all over the world (12, 32, 64, 79, 80, 81, 85, 93). A large number of Fusarium cultures were isolated from damped-off and root rotted seedlings of black pine. No soil isolations were used, because of the large number of species and strains, the parasitic and saprophytic nature of this genus, and the difficulties of species identification. Through single spore isolation (62) pure cultures of many were obtained.

The following species were identified from the two nurseries, Bogue and Chittenden.

Fusarium solani (Mart.) Snyder and Hansen.

Fusarium oxysporum (Schl.) Snyder and Hansen.

Fusarium moniliforme (Sheld.) Snyder and Hansen.

The Genus Rhizoctonia.

Rhizoctonia solani Kuhn is also a fungus which causes severe losses to coniferous seedlings and other crops, causing both damping-off and root rot (57, 64, 65, 66, 85). Many isolations of Rhizoctonia solani were made. After careful observation and study four distinct strains of the fungus were established on their pathogenicity, cultural appearance, growth and behavior (15, 46, 49).

Rhizoctonia solani strain N=1, isolated from damped-off seedlings.

Rhizoctonia solani strain N=2, isolated from damped-off seedlings.

Rhizoctonia solani strain N=4, isolated from damped-off seedlings.

Rhizoctonia solani strain N=87, isolated from root-rot seedlings.

Other Genera

Botrytis cinerea Persoon, was isolated from damped-off seedlings and from soil. It is of much less importance in causing damping-off of pine than the above three genera.

A number of other fungi and microorganisms were also isolated throughout the course of this study. These isolations were obtained from soil, through the modified contact soil slide technique or by dilution plates, and from plates in which diseased tissues of damped-off seedlings were planted.

Saprophytes contaminants or secondary parasites isolated from diseased seedlings and identified as to species or to genera were as follows:

Mucor sp., from seedlings and soil.

Mucor hiemalis, from seedlings and soil.

Rhizopus nigricans, from seedlings and soil.

Alternaria sp., from seedlings and soil.

Aspergillus sp., from seedlings and soil.

Penicillium sp., from seedlings and soil.

Zygorhynchus sp., from soil.

Geotrichum sp., from soil.

Bremia sp., from soil.

Nigrospora sp., from soil.

Botryotrichum sp., from soil.

Torula sp., from seedlings and soil.

Actinomyces sp., from soil.

Streptomyces sp., from soil.

Bacteria, different species, from seedlings and soil.

Nematodes, different species, from seedlings and soil.

Protozoa, different species, from soil.

These fungi which were isolated from both soil and from diseased plant tissues are generally considered to be saprophytes. In several cases various workers (82, 85, 98) have considered them as possible true parasites, however, although a number of organisms other than the accepted damping-off parasites were found growing on the tissue planting plates it is probable that they were secondary. In some cases the pathogens may have been killed, when decayed tissues absorbed too much surface sterilizing chemicals, and thus allowed the appearance of the saprophytic fungi or bacteria more resistant to the surface sterilization. In the favorable environment of the Petri plates bacteria or saprophytes multiplied abundantly and often covered the surviving pathogens.

The author tested one of the so-called saprophytes, *Rhizopus nigricans*, by infesting sterile soil in three pots. The pots kept well watered and covered with cellophane bags. Surface sterilized black pine seed were sowed in these pots. In a few days the pots were covered with dense mycelium of this fungus. Complete killing of the seedlings occurred, mainly as postemergence damping-off. In natural environments, however, these fungi must be considered saprophytes and only in conditions very favorable for their development as weak parasites.

OBSERVATIONS ON CULTURAL APPEARANCE, GROWTH

The fungi isolated during the course of this work were studied as to their growth at different pH levels and temperatures. The behavior of these fungi in different artifical environmental conditions is important because it explains, in part, their differences in frequency and severity in natural environments.

Pythium.

It can be seen from inspection of the data in Tables I and II that the optimum temperatures for growth of the genus *Pythium* at a pH of 5.5 in potato dextrose agar are between 20 and 30° C. Roth and Riker (66) found that the optimum for *Pythium irregulare*, a Wisconsin strain, was 28° C. Middleton (51) found that in corn meal agar at pH 6 the best average growth in length over a 24-hour period for *P. debaryanum* was at 28° C., for *P. perniciosum* at 31° C., and for *P. ultimum* at 28° C.. The different *Pythium* species generally produced their best growth between 20° and 30° C.. After further studies of their growth habits at lower temperature of 12° C. a great difference was noted; *P. debaryanum* started to grow by the second day, *P. irregulare* by the third day but *P. perniciosum* made no growth at all.

According to several investigators (36, 64, 66) working with pH relationships in the genus *Pythium* the minimum growth occurred between pH 3.1 and 4.6 and pH 7.2 to 9.6 while maximum optimum growth took place between pH 3.5 to 8.3. In the present work species of *Pythium* from Michigan were isolated and tested on potato dextrose agar at various pH levels adjusted with phosphate buffers. The cultures were grown at an optimum constant temperature of 24° C.

Table I. Growth of various fungi at different pH levels, in an incubator in the dark at 24° C.

Species	pH	Average growth of colonies in radius in mm., on potato dextrose agar.						
		1 day	2 days	3 days	4 days	5 days	6 days	7 days
<u>Pythium debaryanum</u>	4.5	17	26	39				
	5.5	20	45	45				
	6.5	12	26	42				
	8.0	0	0	0				
<u>Pythium irregulare</u>	4.5	19	44					
	5.5	17	45					
	6.5	15	39					
	8.0	11	26	44				
<u>Pythium ultimum</u>	4.5	23	45					
	5.5	18	45					
	6.5	13	26	40				
	8.0	2	8	12	18			
<u>Pythium perniciosum</u>	4.5	20	45					
	5.5	23	45					
	6.5	18	37					
	8.0	11	32	45				
<u>Rhizoctonia solani</u> No. 1	4.5	15	35	45+				
	5.5	13	34	45+				
	6.5	15	33	45				
	8.0	8	18	40				
<u>Rhizoctonia solani</u> No. 4	4.5	8	18	25	31	36	43	
	5.5	11	30	44				
	6.5	1	5	18	25	34	45	
	8.0	0	1	8	15	21	30	
<u>Rhizoctonia solani</u> No. 87	4.5	23	45					
	5.5	14	45					
	6.5	0	12	26	43			
	8.0	0	0	0	0			
<u>Fusarium solani</u> No. 3	4.5	6	10	15	22	28	35	41
	5.5	5	10	16	22	28	36	42
	6.5	6	10	17	23	30	37	43
	8.0	4	10	16	21	27	33	39
<u>Fusarium solani</u> No. 25	4.5	8	14	20	28	34	44	
	5.5	6	13	20	28	36	44	
	6.5	8	14	23	30	40	45	
	8.0	6	12	20	27	34	42	
<u>Fusarium moniliforme</u> No. 24	4.5	7	14	20	27	34	42	
	5.5	4	10	18	26	34	42	45
	6.5	8	15	23	31	41	45	
	8.0	4	12	18	26	33	41	
<u>Fusarium oxysporum</u> No. 15	4.5	5	10	17	26	32	41	
	5.5	6	13	20	29	36	44	
	6.5	8	14	22	30	39	45+	
	8.0	6	12	18	25	32	40	43

TABLE II. Growth of various fungi in different temperatures in dark on potato dextrose agar at pH 5.5

Species	Incubated °C.	Radius growth in mm in Potato Dextrose agar with pH 5.5													
		Days													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Pythium debaryanum	12	0	3	9	15	21	27	35	42						
	16	5	16	33	45										
	20	14	32	45+											
	24	20	45												
	30	20	44												
	37	0	0	0	0	0	0								
Pythium irregulare	12	0	0	5	14	21	27	36	45						
	16	0	0	17	31	45									
	20	8	16	41											
	24	17	45												
	30	15	30	45											
	37	0	0	0	0	0	0								
Pythium perniciosum	12	0	0	0	0	0	0	0	0						
	16	3	7	18	27	37	45								
	20	15	31	45											
	24	23	45												
	30	20	45												
	37	0	0	0	0	0	0								
Rhizoctonia solani No. 1	12	0	0	0	1	1	2	3	5	6	7	10	12	13	15
	16	6	12	18	25	31	37	45							
	20	13	28	45											
	24	13	34	45+											
	30	15	37	45+											
	37	15	33	43											
Rhizoctonia solani No. 4	12	0	0	0	0	0	0	1	2	3	4	5	7	9	11
	16	4	17	24	29	34	41								
	20	13	28	45											
	24	11	30	44											
	30	11	24	35	45										
	37	0	1	2	2	3	3	4	4	5	6	7	8	9	

Rhizoctonia	12	0	1	10	20	31	43								
solani No. 87	16	3	21	44											
	20	13	37	45											
	24	14	45												
	30	23	45+												
	37	0	0	0	0	0	0	0	0	0	0				
Fusarium	12	0	0.5	0.5	1	2	2	3	4	5	6	7	9	11	13
solani No. 3	16	1	2	7	8	11	13	15	18	20	22	24	26		
	20	3	6	13	20	25	30	37	44						
	24	5	10	16	22	28	36	42							
	30	5	11	19	27	33	41	45							
	37	0	2	4	8	10	11	12	13	15	17				
Fusarium	12	0	0	1	2	3	4	6	7	10	11	12	13	17	20
solani No. 25	16	1	3	10	13	16	20	24	28	30	33	36	40		
	20	4	11	17	25	32	40	45+							
	24	6	13	20	28	36	44								
	30	3	9	14	23	28	35	43							
	37	0	0	0	1	1	1	1	1						
Fusarium	12	0	1	2	3	4	6	7	9	11	13	15	18	21	
moniliforme No.24	16	2	4	8	12	15	18	22	26						
	20	4	11	17	25	32	40	45+							
	24	4	11	18	26	34	42	45+							
	30	10	14	20	26	34	39	45							
	37	0	1	2	3	4	4	5	5	6	6	7			
Fusarium	12	0	0	1	2	4	5	7	9	10	11	13	16	18	
oxysporum No. 15	16	1	4	9	12	15	18	21	25	27	30	33	36		
	20	4	11	17	26	31	39	45							
	24	6	13	20	29	36	44								
	30	5	11	18	24	30	37	40							
	37	0	1	1	2	2	2	2							
Fusarium	12	0	1	2	3	4	5	7	10	11	14	16	20	23	26
roseum	16	4	8	13	17	24	28	35	41						
	20	5	12	22	32	41	45+								
	24	4	12	16	21	28	35	42							
	30	4	12	18	25	32	40	45							
	37	0	0	0	0	0	0	0	0						

Optimum growth of the *Pythium* species took place between pH 4.5 and 6.5 with the best growth at pH 5.5. At pH 8.0 *Pythium debaryanum* failed to grow, but the other three species grew satisfactorily (Table I).

Rhizoctonia.

Rhizoctonia solani Kühn is a species found all over the world, which can cause damping-off to coniferous seedlings (14, 20, 32, 39, 64, 65), and is pathogenic on many other hosts (15, 45, 57). Strains of this fungus are known to have a wide range of pathogenicity on the many different hosts (13, 15, 32, 45, 57, 65). There are many strains, and these strains have different cultural appearances and growth rates in different media and environmental conditions (15, 46, 57).

Growth studies at different pH and temperatures were made of *R. solani* cultures No. 1, 2, 4, and 87 (Tables I, II). Potato dextrose agar was used as culture medium. Small pieces, one-half to one centimeter in diameter, of inoculum were placed in the middle of each Petri dish. Growth measurements recorded were the average of the largest and smallest diameter of each colony.

The general appearance of the strains isolated remained constant on the same medium at different pH levels and at room temperature; on same medium, same pH and at different temperatures; and at same pH, same temperature but different media.

The characteristics in appearance of the strains isolated are shown in Table III.

From an examination of Table II it is seen that the four different strains of *Rhizoctonia solani* differ in growth rates when grown on the same medium at the same pH of 5.5 but at different temperatures. These

TABLE III. Cultural characteristics of strains of Rhizoctonia solani

Strains	One day old culture			Seven day old culture		
	Mycelium appearance in agar plate	Concentric growth rings	Sclerotia	Mycelium appearance in agar plate	Concentric growth rings	Sclerotia
No. 1	white thin	none	none	white thin	none	present
No. 2	white thick	none	none	white heavy thick	present	none
No. 4	white to brown thick	none	none	white to brown thick	present	none
No. 87	white quite thin	none	none	white quite thin	none	none

results corroborate the findings of other owrkers (15, 46, 57). Rhizoctonia solani strain 87 grew rapidly at 16° C while strains 1 and 4 grew less rapidly than the Pythium species. At 12° C Rhizoctonia strain 87 grew rapidly but strains 1 and 4 produced quite small colonies and the total growth was less than the two pathogenic Pythium species, P. debaryanum and P. irregulare. (This shows that the growth of strains 1 and 4 of Rhizoctonia is negligible at temperatures lower than 12° C as compared with the pathogenic species of Pythium.)

Rhizoctonia solani, a species which has a large number of races, is expected to have a wide response to its environmental conditions, It has been shown that the lower limits of growth are between pH 2.5 to 3.8, optimum growth between pH 4.5 to 7.0, and its upper limits of growth from pH 6.7 to 9.1 (46, 64, 66). In our studies of the strains of Rhizoctonia solani (Table I) strain No. 1 grew well from pH 4.5 to pH 8.0. The strain No. 4 showed a wider variation, best growth at pH

5.5, less at pH 4.5 and 6.5, and still less at pH 8.0. The strain No. 87 grew fastest at pH 4.5 and 5.5, less at pH 6.5, with no growth at all at pH 8.0.

The pathogenic ability of the different Rhizoctonia solani strains is shown in Table XIV.

Fusarium.

Fusarium sp. are reported frommany countries as causing damping-off and root-rot of coniferous seedlings (14, 32, 64, 79, 80, 81, 82, 85), and other hosts (12, 74, 93).

For the growth rate study of Fusaria, different species and strains, were grown on potato dextrose agar at different pH levels at 24° C and in the dark (Table I). The differences found between the species and strains of this genus were small and not significant under the conditions of the experiment. This indicates that these species of Fusarium studied, at least, should be considered stable culturally as to pH effects.

Temperature, on the contrary, influences the growth rate of Fusaria considerably (Table II). The same medium, potato dextrose agar at pH 5.5 and same technique of measuring and recording was used as with Pythium and Rhizoctonia. The optimum growth temperatures for the Fusaria studied were between 20° to 30° C., with smaller differences among the species and strains. At 12° C. Fusarium solani strain No. 3 showed a small total growth, about equivalent to Rhizoctonia solani strains No. 1, and No. 4. The difference being, however, that the Fusarium had a small and constant rate of growth from the first day of planting of the inoculum on the medium while Rhizoctonia did not begin to grow for several days. The growth of the other Fusaria at 12° C. was faster than that of Fusarium solani strain No. 3, but much less than that of the Pythiums at the same temperature. Fusarium roseum[1], did not grow at 37° C. and other species of Fusarium grew very slowly at this high temperature although there was generally some growth at 37° C. with the Fusaria studied it was about the same as or less than that of Rhizoctonia solani strains No. 1 and No. 4. This was in contrast to Pythium which did not grow at all.

Numerous observations on cultural characteristics, of a number of Fusaria isolations were made when grown on different media at pH levels and under other environmental conditions. Differences in virulence are shown in Table XIV. All of the species were isolated from both damped-off and root-rotted specimens of black pine seedlings. It was found that all the species isolated from diseased seedlings can cause both damping-off and root-rot on black pine. Fusarium oxysporum was the most common of a large number of isolations made during this study. The other two species, F. solani, and F. moniliforme, were much less abundant and of about equal frequency.

All of the fungus cultures used in this experiment all originated from single spore isolations.

Pigmentation of Fusaria.

A large number of cultures were studied in replicate. They were grown on potato dextrose agar and malt agar at pH 5.5. The plates, after planting, were held in three different environments, namely: at room temperature about 21° C. in the dark (covered with Kraft paper); at room temperature under diurnal light conditions (Not covered with Kraft

[1] F. roseum was not isolated from diseased seedlings in any study, but it was given to me by Mr. E. H. Pepper, and it is really F. roseum f. cerealis (Cke) Snyder and Hansen (73).

paper); and in an incubator at 25° C. in the dark. Pigmentation was generally more abundant in the absence of light or in reduced light than when the cultures were grown under diurnal light conditions. This is in agreement with the findings of other investigators (72, 96). Pigmentation varied with the medium used as has been reported by Brown and Horne (10).

Zonation of Fusaria.

Previous workers (72, 96) have reported that light, especially fluctuations in light, as well as temperature influence variations in growth to produce zonation in the *Fusaria*. In the course of this study several species and strains of *Fusarium* were grown on potato dextrose agar at different pH levels and in incubatorsset at different temperatures and without light. The results are shown in Tables IV and V.

TABLE IV. Zonation of *Fusarium* cultures in potato dextrose agar at different pH, in incubator, in dark, at 25° C.

pH	*F. solani* No. 3	*F. solani* No. 19	*F. moniliforme*	*F. oxysporium*
4.5	+[a]	+	-[b]	-
5.5	+	+	-	-
6.5	+	+	-	-
8.0	+	+	-	-

[a]+ zonation formed, [b]- no zonation.

F. solani (strains 3 and 19) formed zonation in potato dextrose agar in the dark at 25° C. at all pH levels tested. The *F. moniliforme* and *F. oxysporium*, under these conditions did not form zones. Thus diurnal light or fluctuating temperature (72, 96) are not the only factors which cause zonation in Fusarium. From the results it seems that several species and strains possess the ability to form zones when grown without light at certain temperatures as well as when grown in diurnal light or at fluctuating temperatures. Different levels of pH had no effect on zonation of F. solani.

TABLE V. Zonation of Fusarium cultures in potato dextrose agar pH 5.5, at different temperatures in dark.

Temp. °C.	F. solani No. 3	F. solani No. 25	F. moniliforme	F. oxysporium	F. roseum
12	+[a]	+	+	+	-[b]
16	+	-	-	-	-
20	+	-	-	-	-
24	+	-	-	-	-
30	+	-	-	-	-
37	+	-	-	-	-

[a]+ means zonation formed, [b]- means no zonation.

F. solani strain No. 3 was the only one (Table V) forming zones on potato dextrose agar at pH 5.5 in the dark at all temperatures tested (12°-37° C.). This strain obviously represents a group possessing the ability to form zonation under widely differing temperatures. From the data in Tables IV and V it seems that temperature is a more important factor in zonation than pH, since with different temperatures we have more variations than with different pH levels within the same species. F. moniliforme and F. oxysporium formed zonation at 12° C. constant temperature, but no zonation at any other temperatures. F. roseum gave negative results in all temperatures tested.

Sporodochia.

Sporodochium formation was also studied. According to the literature (30, 72, 96) sporodochia are generally formed more abundantly in light than in dark. At room temperature in potato dextrose agar pH 5.5 *Fusarium* species produced sporodochia more abundantly under the influence of diurnal light, than those placed in the dark at room temperature. At the same temperature on malt agar at pH 5.5 sporodochia were produced in larger numbers under the influence of diurnal light than in darkness. Some were also formed in the dark on malt agar. These results, thus, generally confirm the reports of others on the conditions influencing sporodochial production (10).

Many other studies on factors affecting variations in *Fusarium* have been reported (10, 30, 52, 53, 54, 71, 72, 96). These variations were not included in this study but a few other observations were made. *F. oxysporum* strain No. 15, for instance, in several cases under different environmental conditions showed a turning of hyphal tips at about 45 degrees from left to right.

Nematodes.

Nematodes were isolated from damped-off and root-rotted seedlings in the Petri plate plantings of diseased stem and root tissues several times in the course of this study. Nematodes have often been referred to as causing diseases in plants. In coniferous seedlings they have been reported to cause disease, reduction in yield, poor quality plants, and that they are in a complex association with damping-off and root-rot disease in several nurseries (19, 28, 37, 38, 41, 92).

During a year's study of damping-off of black pine seedlings, and from several thousand isolations the presence of nematodes in the damped-off seedlings did not appear to be significant. Table VI shows the number of seedlings from which nematodes were isolated, and the period of the year when it occurred. Nematodes isolated from pieces of diseased tissues in plates, from soil, and by the contact slide technique, revealed that the most abundant nematodes were species of the Family Rhabditidae which are saprozooans. Another nematode which is parasitic was isolated from diseased seedlings but in less numbers. The presence of the characteristic stylet was observed in this nematode indicating that it was a parasitic. However, it did not possess a vascular medium esophageal bulb. No identification was made of any of these nematodes.

In Table VI the time of year and the number of nematodes recovered is shown. Isolations from diseased seedlings were made from May, to October. The number of nematodes recovered from diseased seedlings was quite small. Thus in this case at Bogue Nursery nematodes seemed not to play any important role in the appearance and severity of damping-off.

SEASONAL DAMPING-OFF OF BLACK PINE

At the Bogue Nursery at Michigan State University, East Lansing, Michigan, damping-off disease has been common since its establishment. Strong (77) in 1952 published a report on control studies in this nursery.

Isolation studies.

Isolation from damped-off seedlings of black pine were made periodically by the technique described previously. Table VI shows the results of the isolations throughout the period of one year. In this Table the main pathogenic fungi isolated are listed individually. Miscellaneous fungi include many different fungi which normally are considered to be saprophytes or molds: *Mucor*, *Aspergillus*, *Penicillium*, *Alternaria*, *Geotrichum*, *Rhizopus* and others.

At the same time studies of the soil microflora and to some extent soil microfauna were made. The dilution plate method was used. The contact slide technique of Cholodny (11) as modified by Krinchova (94) and the author was also used extensively. This study was repeated several times throughout the year. At different periods the following fungi and microorganisms were isolated: different species of bacteria, in colonies or as single bacteria; *Streptomyces* sp., *Pythium* sp., *Botrytis* sp., *Rhizoctonia solani*, *Bremia* sp., *Fusarium* sp., *Nigrospora* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus nigricans*, *Actinomyces* sp., *Mucor hiemalis*, *Zygosporium* sp., *Aspergillus* sp., *Geotrichum* sp., *Botryotrichum* sp., many non-sporulating colonies both with septate and non-septate, hyaline and colored hyphae which could not be identified. Nematodes, adults, larvae and eggs and different protozoa were also found on the agar surface on the glass slides.

TABLE VI. Damping-off of Black and Scotch pine and occurrence of pathogens throughout a period of a year under greenhouse and field conditions.

Months	Greenhouse Field	Plant Species	Preemer. D. O. %	Postemerg. D. O. %	Healthy %	Organisms isolated from diseased seedlings: Pythium %	Fusarium %	Rhizoctonia solani %	Botrytis cinerea %	Bacteria %	Misc. Fungi %	Nematode isolated cases %
Jan.-Feb.	Greenhouse	Black pine	58	28	14	45	18	14	4	12	7	-
Jan.-Feb.	Greenhouse	Scotch pine	55	30	15	52	20	11	-	6	8	-
Jan.-Feb.	Greenhouse	Nor. Spruce	34	24	42	32	19	15	-	22	12	-
March	Greenhouse	Black pine	24	64	12	65	14	8	-	6	7	-
March	Greenhouse	Scotch pine	26	61	13	60	16	12	-	6	6	-
April	Greenhouse	Black pine	30	62	8	7	68	18	-	7	-	-
May	Greenhouse	Black pine	28	76	6	8	60	24	-	-	8	12
June	Greenhouse	Black pine	54	38	8	-	80	8	-	7	5	11
June	Field	Black pine	28	30	42	-	91	3	2	4	-	13
July-Aug.	Greenhouse	Black pine	60	32	8	8	60	32	-	-	-	4
July-Aug.	Field	Black pine	39	33	28	-	57	33	6	-	-	6
September	Greenhouse	Black pine	62	27	11	-	62	21	-	12	5	-
October	Greenhouse	Black pine	28	58	14	39	34	-	8	4	8	
November	Greenhouse	Black pine	59	32	9	15	38	22	2	11	12	-
December	Greenhouse	Black pine	63	19	18	39	19	13	3	16	10	-

Table VI shows the frequency of appearance of the principal pathogenic genera causing damping off of black pine throughout the year.

Pythium was more prevalent as the cause of damping-off, and dominated the other pathogenic species during warm and moist conditions of the heated greenhouse during the winter months. During this period the greenhouse was heated and close, thus moisture was high, no evaporation of water from flats and pots where seedlings were grown took place. Thus this period must be considered the period when in flats and pots more water was present, and at the same time enough temperature was present too.

Fusarium was more prevalent and dominated all other species under a wider range of environmental conditions. Thus under cooler environment of spring and autumn, and under warmer and drier conditions of summer *Fusaria* were the more prevalent species causing damping-off, with second the *Rhizoctonia solani*, and third *Pythium*. This was probably due to the fact that, *Fusaria* as we mentioned previously are "strong" species which means that they can live and grow satisfactorily under a wider range of conditions of environment as compared with *Pythium* and *Rhizoctonia*, which are influenced more by their environment.

These results agree in a general way with those of Beach (6) in Pennsylvania with several vegetable crops.

In Table VI is shown that the *Fusarium*, and especially *F. solani* (Schl.) Sny. and Han., *F. oxysporum* (Schl.) Sny. and Han., and *F. moniliforme* (Sheld.) Sny. and Han., are the most important year-round cause of damping-off of black pine in Michigan, and possibly other conifers as well. *Rhizoctonia solani* (Kuhn), *Pythium irregulare* Buisman, *Pythium ultimum* Trow, and *Pythium debaryanum* Hesse, are only secondary in importance. This is in contrast to the reports of Röth and Riker (65, 66, 67) who found that in Wisconsin nurseries Pythium irregulare and Rhizoctonia solani are the main cause of damping-off on red pine.

With Fusarium, the most common year-round species was F. oxysporum, followed by F. solani, and the least common F. moniliforme. With Pythium generally throughout the year P. irregulare was the most common, P. ultimum less common, and P. debaryanum least common.

A number of questions have arisen during the process of this study. One of these was; do the isolated fungi studied show any ability and preference to attack seedlings from the time of emergence until they become resistant to damping-off some 3 to 4 weeks later? The above question is concerned with the postemergence damping-off of black pine. From several thousands of isolations throughout the year, no species of the three main pathogenic genera showed any special ability to attack earlier than any other species. The ratio of isolated species from the early time of damping-off to late time of damping-off was the same as the ratio of the most common species to less common and least common, which occurred at each period.

Another question was; do pathogenic fungi which cause damping-off work together and cause damping-off in the same individual seedling, or do they avoid each other by competition or by some antibiotic means? From the discussion of isolated organisms from damped-off seedlings throughout the year and from Table VI we found that in many cases bacteria and various other fungi (Aspergillus sp., Penicillium sp., Mucor sp., etc.) were present. However, bacteria, Aspergillus sp., Penicillium sp., Mucor sp., etc. are saprophytes or weak parasites. Thus the presence of all of them in so many cases must be considered as an excellent example of suppression and antibiosis of the main pathogenic fungi. It is probably easy for someone to say that the main pathogen was already dead or was killed by the sterilizing agent, why then were not the saprophytes or bacteria also dead or killed. From the work of others it has been found that Pythium can be supressed by bacteria (83); or that with the presence of eleven different saprophytes in sterilized soil Pythium caused less damping-off than Pythium alone (32).

During this study it was found that bacteria and saprophytes in plate cultures could suppress the main parasites. There is a considerable amount of literature on the subject of suppression, antibiosis, and parasitism of several pathogenic fungi by saprophytic ones (25, 26, 47, 59, 68, 69, 87, 88, 89, 90, 94).

Our original question was whether the main parasitic fungi among species of the same genus or among species of different genera were working together in an individual diseased seedling or avoiding each other by any means of competition or antibiosis etc., In order to give an answer to the above question many combinations of the main pathogenic fungi were planted in potato dextrose agar at pH 5.5 at room temperature in the dark. Three plates were used in each case. The results found are as follows:

Fusarium solani strain 3 and *F. oxysporum*	+
Fusarium solani strain 3 and *F. moniliforme* strain 24	+
Fusarium solani strain 3 and *F. roseum*	+
Fusarium roseum and *F. moniliforme*	-
Fusarium moniliforme and *F. oxysporum*	-
Fusarium roseum and *P. debaryanum*	-
Fusarium solani strain 3 and *P. irregulare*	-

Fusarium solani strain 3 and *P. ultimum*	-
Fusarium solani strain 3 and *R. solani* strain 1	+
Fusarium moniliforme and *R. solani* strain 1	-
Fusarium moniliforme strain 24 and *R. solani* strain 87	+
Fusarium moniliforme and *P. debaryanum*	-
Fusarium moniliforme and *P. irregulare*	-
Fusarium oxysporum strain 1 and *R. solani* strain 1	-
Fusarium oxysporum strain 15 and *P. ultimum*	±
Rhizoctonia solani strain 1 and *P. ultimum*	+
Rhizoctonia solani strain 1 and *P. debaryanum*	-
Rhizoctonia solani strain 1 and *P. irregulare*	±
Pythium irregulare and *P. ultimum*	-
Pythium irregulare and *P. debaryanum*	-
Pythium ultimum and *P. debaryanum*	-

In the above test - means no antibiosis apparent (fungi mixed), + means antibiosis apparent (a clean zone was formed between the fungi tested) and ± means a slight antibiosis probably present, (a situation among the first two.) In this third case the hyphae tips of the two opposite different fungi were approached by each other and probably mixed a little between them, but a lighter zone was apparent.

From the above it was apparent that certain antibiosis was present. For instance *F. solani* strain 3 showed antibiosis with the three *Fusaria* tested, *F. oxysporum*, *F. roseum* and *F. moniliforme*. The same fungus showed also an antibiosis with *Rhizoctonia solani* strain 1, but it does not show any antibiosis with *Pythium*. (Gregory *et al* (25) found also that a *Streptomyces* isolate produced an antibiotic which was active against some fungi but had low activity towards other fungi and bacteria).

The same was also true of several other fungi and combinations used in that test (Fig. 1). From the above it is clear that in pure cultures several of the main pathogenic fungi avoid each other, several do not. But the author, from the above results and his isolations from damped-off seedlings believes that in nature as a general rule only one species attacks and invades each individual damping-off seedling. In exceptionally few cases the author isolated from the same seedling more than one species of different genera; the same was found also by Brandt (8) who in soil infested with pure culture mixtures, only rarely reisolated Rhizoctonia and Fusarium together in the same diseased stem.

Another question was; when did the peak loss of seedlings take place after emergence, and when was resistance attained? Roth and Riker (67) found in Wisconsin on red pine and with Pythium irregulare and Rhizoctonia solani that the highest percentage of damping-off occurred in seedlings 11 to 13 days old, and relative resistance occurred after about 1 month. Tint (80) by planting red pine seedlings of different ages found that seedlings from 1 to 7 days old had the highest percentage of postemergence damping-off from Fusarium oxysporum and the reduction in damping-off corresponded with the increasing age of the host.

The data below (Table VII) illustrate a similar effect.

Seedling emergence started at eight days during the warmer summer months, and under greenhouse heated conditions during winter months. During spring and autumn cool conditions emergence started after 10 days. It appears from this that, since the necessary moisture is available, temperature is the next most important factor for seed germination. At medium high temperatures germination is faster than at lower temperatures. In nature seed germination is influenced by fluctuating temperature, but

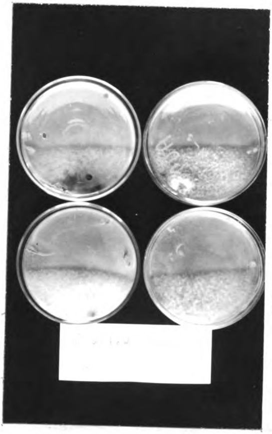

Fig. 1. Upper half <u>Rhizoctonia solani</u> strain 87, lower <u>Fusarium moniliforme</u>, the two species did not mix, a clear line is apparent between them.

under experimental conditions, medium high temperatures are more favorable for seed germination than lower ones (2, 27, 66, 81).

TABLE VII. Time of emergence and highest damping-off percentage on black pine seedlings, as compared with the age of seedlings.

Time period	Days to emergence	Highest percentage postemergence damping-off	Seedlings developed resistance
Winter months Greenhouse heated	8 days	9 days after emergence	25 days after emergence
Spring months Greenhouse cool	10 days	11 days after emergence	27 days after emergence
Summer months Greenhouse-Field	8 days	8 days after emergence	25 days after emergence
Autumn months Greenhouse cool	10 days	11-12 days after emergence	27 days after emergence

Black pine seedlings become resistant to damping-off nearly one month after emergence (Table VII), the same as red pine in Wisconsin (67). In Wisconsin the highest percentage of post-emergence damping-off occurred at 11 to 13 days during summer months (67) but in Michigan with black pine seedlings the highest damping-off during summer was found to be 8 days after seedling emergence. This variance probably was due to the different host, different pathogenic fungus, varying soil type and pH, and finally the climatic conditions, since all of them influence not only the good growth of the seedlings, but the pathogenic fungi also.

With lower temperatures in Spring and Autumn black pine showed the highest postemergence damping-off with seeds starting to emerge at 11 to 12 days respectively. From the data it is apparent that at higher temperatures in summer or in a heated greenhouse, seedlings emerged earlier, more damping-off occurred earlier, and finally the resistance of seedlings was attained earlier, than at the lower temperatures of Spring and Autumn. This is due probably to the better conditions of plant growth which allowed the seedlings to pass all the susceptible stages in a shorter time.

The total damping-off (preemergence plus postemergence) was found to be quite high all year around under greenhouse conditions, this being in agreement with others (77, 79). From Table VI one can see that preemergence damping-off is higher at the higher temperatures. Thus, in the heated greenhouse, and during summer months in the nursery, the preemergence damping-off was higher than the postemergence damping-off, and preemergence damping-off was also higher at warm periods of winter and summer than in cooler spring and autumn.

The preemergence and postemergence damping-off is the same disease expressed on the time and size of seedling attacked. We can consider that preemergence damping-off is a more severe type than postemergence damping-off, since the same microorganisms cause both of them, but in the first case it happens very early just as the seed starts to germinate; in the postemergence damping-off it happens later, and after hypocotyles and roots of seedlings are exposed to the fungi attack for a longer period of time.

From Table VI we found that the higher the temperature the higher the preemergence damping-off throughout the year, and this should naturally happen with postemergence damping-off. Tint (81) found also that the higher the temperature within limits the higher the preemergence damping-off.

Emergence of black pine seedlings was higher at warmer periods than in cooler ones (Table VII) and the pathogenic fungi isolated grew better at temperatures between 20° and 30° C. than at lower temperatures they could not escape because the pathogens grow faster also.

Leach (44) has pointed out that the severity of damping-off in all combinations of host and pathogens was determined to a considerable degree and was most severe at temperatures that were relatively less favorable to the host than the pathogens as measured by the ratio of their growth rates.

MODIFIED CHOLODNY SOIL SLIDE TECHNIQUE

This method was discussed under the **Materials** and Methods section. The fungi found were reported in the chapter "Isolation and Identification of Species, their Importance and Pathogenicity."

These main pathogenic fungi were always present in the soil. *Pythium* sp. were found to be abundant in the soil, by using the above technique, even in periods in which they were not isolated from damped-off seedlings. The other soil fungi and microorganisms were referred to in an earlier chapter.

Data obtained in this study of soil microflora and microfauna by the slide technique were poorly correlated with the damping-off of black pine. The action of the main pathogenic fungi in this study under greenhouse conditions, seems to be correlated with the health and growth of host and pathogens as they are influenced by the inorganic environment rather than the organic environment. In the greenhouse the pathogenicity of damping-off fungi became greater than the competitive ability of secondary microorganisms of the soil flora and fauna. It has been pointed out in the literature, nevertheless, that the organic environment under certain or special conditions can reduce or control damping-off (25, 26, 32, 47, 87, 89).

BLACK PINE SEED GERMINATION

The damping-off disease occurs only from the time seed starts to germinate until about 5 weeks after sowing. Since seedlings become resistant in about 3 to 4 weeks, after emergence and since timing is a very important factor for damping-off, it is easy to understand that seed with a high germinative capacity and germination speed should have more possibility of escaping the disease. The different germination ability of seed depends upon its inherited capacity, the maturity of seed, and the treatment and care of seed from the time of collection until the time of sowing.

Many species of seeds are treated before they are sown in various ways in order to produce better germination (1). Even although quick seed germination is desirable, very little knowledge exists on the effect of such presowing treatments on the health of germinated seed in relation to damping-off disease. One of these seed treatments, the presoaking of seeds after Roth and Riker (66), had no apparent ill effects on the seedlings. This is in contrast to one report (97) in the literature where dormant seeds of conifers had lower losses than pre-germinated ones in *Fusarium* infested soil. The same was reported by Hansen *et al* (29), that the soaking of the seed of white pine and Norway pine did not increase the rate of germination but increased injury from damping-off.

Black Pine Seed Germination and Moisture.

The first and most important factor in the germination of seed is probably free moisture. Since the presence of free moisture is necessary for seed germination, the next consideration must be the amount of water. The amount of water is important because it is inversely related to seed aeration and that is also an important factor in seed germination. An experiment was designed to study these relationships. A series of Petri plates were used. One filter paper was put on the bottom of each plate, and one for seed covering. A series of plates contained various quantities of water ranging from minimum to excess of the required for normal germination. The results for the different moisture levels are given in Table VIII.

All plates were held at room temperature (about 22° C.) and covered with Kraft paper in order to have dark environment. Part of the plates were sealed with wide rubber bands in order to prevent evaporation, and retain constant moisture. The treatments were replicated four times and the experiment performed twice. Germination was considered complete when the radicle was at least 2 mm in length.

Table VIII shows that seed required enough water quickly at the beginning of germination. Thus there was better germination up to the 8th day in series B, C, D, E, in which seed could absorb more water than in series A. With more water (seed 3/4 or completely covered) there was less germination, since aeration was the critical factor (series F and G).

Although enough free moisture is necessary to initiate germination, aeration seems to be the critical factor after 8 days, in this experiment. Series A, with the best aeration, had the best germination of all. Table VIII shows that with less water and better aeration the germination was better while with more water and poor aeration the germination was reduced.

The sudden gain in germination of seeds in Series D after 11 days

TABLE VIII. Black pine seed germination in different quantities of water at room conditions in darkness.

Water per plate	Percent germination in days 7	8	11	13	15	21	Remarks
A. 5 cc. not sealed[a]	16	28	76	96[b]	100	100	Seed and paper enough moisture. In 13 days 2 cc. H_2O was added.
B. 5 cc. sealed	20	40	72	84	92	96	Seed and paper enough moistened, more than in A.
C. 10 cc. not sealed	20	40	52	84	92	96	Seed covered at least 1/2 with water.
D. 10 cc. not sealed	20	40	52[c]	92	96	96	Same as C. but in 11 day excess water was poured off.
E. 10 cc. sealed	20	36	40	61	84	92	Seed covered at least 1/2 with water.
F. 15 cc. sealed	16	32	36	60	84	92	Seed covered at least 3/4 with water.
G. 25 cc. sealed	8	20	22	40	60	92	Seed completely covered with water.

[a]Sealed with wide rubber band seal for dishes.

[b]Filter paper started to dry, 2 cc. more water was added per plate.

[c]On that day after counting the excess water was poured off.

can be attributed to the improved aeration from draining the excess water. Series C, where the water was not drained, showed less increase in germination. This experiment indicates that free moisture is required for seed germination, but the necessary moisture is really quite low. Although we had a better germination at the beginning with medium moisture, after a few days it was evident that germination increased at the lower moisture content (better aeration) more than at medium moisture content, and lowest germination occurred in the series with most water (less aeration).

The above results apply to germination of black pine until the thirteenth and fifteenth day. At the end of the twenty-first day, it was found that coniferous seed like black pine could give satisfactory germination (92 percent) even if the seed were completely covered with water as in series G. This germination would be considered satisfactory except for the fact that it required approximately half again as long as with less water (series A). This is an unfavorable condition since these poorly aerated germinating seeds would be exposed for a longer time to fungus attack.

Germination of the same seed lot in greenhouse tests varied from 78 to 84 percent but in Petri dishes (Table VIII, A) it was 100 percent.

Black Pine Seed Germination and Temperature.

An experiment was designed to find the relationship of temperature and germination of black pine seed. Four plates each containing 50 seeds were used per treatment. Watering was optimum and the plates were held in incubators in dark at the designated temperatures. Part of the treatments were constant temperatures (Table IX, No's. 1-8) and the remainder (No's. 9-17) were 14 hours of a higher temperature and

TABLE IX. Black pine seed germination at different temperatures in the dark.

No.	Temperature	Days					
		7	9	11	14	16	19
1.	6°C (Constant)	0	4	12	16	28	32
2.	12°C (Constant)	0	4	12	16	28	32
3.	16°C (Constant)	0	12	24	40	72	82
4.	20°C (Constant)	4	20	32	56	80	84
5.	24°C (Constant)	16	36	56	80	92	92
6.	28°C (Constant)	12	28	44	60	72	84
7.	31°C (Constant)	12	36	56	72	88	92
8.	37°C (Constant)	0	16	20	20	40	44
9.	24°C(14 hrs), 16°C(10 hrs)	12	28	52	72	94	96
10.	24°C(14 hrs), 12°C(10 hrs)	16	32	52	72	92	92
11.	24°C(14 hrs), 6°C(10 hrs)	12	20	36	60	84	92
12.	37°C(14 hrs), 16°C(10 hrs)	40	52	68	92	92	92
13.	37°C(14 hrs), 6°C(10 hrs)	34	46	60	80	84	84
14.	31°C(14 hrs), 16°C(10 hrs)	14	20	40	80	96	96
15.	31°C(14 hrs), 6°C(10 hrs)	16	24	48	80	88	92
16.	28°C(14 hrs), 16°C(10 hrs)	8	16	24	60	84	88
17.	28°C(14 hrs), 6°C(10 hrs)	10	18	36	64	84	88

10 hours of a lower temperature. The results (Table IX) show that, in incubators with constant temperatures, there was better germination between 16° and 31° C.. At the two lowest temperatures (6° C. and 12° C.), the germination was identical and only reached 32 percent throughout the period. Only when the temperature reached 16° C. was the germination significantly increased to 82 percent. Two maxima, 24° C. and 31° C., were obtained for constant temperature conditions. Other workers (4, 66) have reported optimum germination around 24-26° C. for red pine and Haasis (27) found two optima at 24° and 36° C. for red pine and for lodgepole pine two optima at 24° and 40° C. as well. At 37° C. constant temperature there was another drop although still above that at 6° C. and 12° C..

At fluctuating temperatures we obtained the following results: The germination was more or less the same for 24°-16° C., 24°-12° C., 24°-6° C. as at a constant 24° C.. This indicates that if one temperature was favorable for enough hours per day, the alternate temperature did not change the amount of germination. When a higher temperature of 37° C. (known to be unfavorable when constant) was combined with alternating lower temperatures of 6° and 16° C. a favorable germination condition was produced. Of the two, 16° C. was superior to 6° C. germination being 92 and 84 percent respectively (Table IX, No. 12 and 13). At constant temperature of 37° C., 16° C. and 6° C. the germination was 44, 82 and 32 percent respectively. At 31° C. higher temperature much the same results were obtained with 24° C. (Table IX, No's. 14, 15, and 9, 10, 11). At 28° C., as higher alternating temperature, there also was better germination than at the constant temperatures 28° C., 16° C., or 6° C..

Summarizing the influence of alternative temperatures on black pine seed germination, if one temperature of the alternative combination is favorable or optimum in the case of constant temperatures, the change of temperature had little influence and gave more less the same germination number as the favorable constant temperature. On the contrary, if alternative temperatures are neither one favorable, the alternative temperature stimulated germinatinn above that at any constant temperature used.

The Effect of pH on Black Pine Seed Germination.

Among other factors which may influence the germination of black pine seed is pH. A series of experiments were conducted in order to find any relationship of pH solutions and the germination of black pine seed.

Seeds of black pine were put in Petri dishes with two layers of filter paper at the bottom in order to retain more uniform moisture; then seeds were covered with one filter paper. Four plates were used in each treatment and the experiment repeated twice. Buffers were made by using different combinations of 0.1 M phosphoric acid and 0.1 M sodium hydroxide (5). Watering was normal to give best aeration and germination. The plates were stored at room temperature and covered with Kraft paper in order to provide a dark environment. The results of these germination tests are shown in Table X.

A soil test was also used to determine the influence of pH on the germination of black pine seeds. This experiment was conducted in the Plant Science Greenhouse. The soil used contained 3.34 percent humus, sand 86.00 percent, silt 4.00 percent, and clay 6.66 percent (Type F, Table XVII). The soils were also buffered with 0.1 M phosphoric acid and 0.1 M soidum hydroxide used in different proportions to give the desired pH. Soil was sterilized etc. as described in Materials and Methods. Four replicates were used for each pH value; 50 seeds were sown in each pot. The watering was normal. The germination of black pine seed in pot soil at different pH levels is shown in Table XI.

During the course of the study germination of black pine seed was observed and also the influence of pH on the length of root and stem was measured. The results of these observations are shown in Table XII.

By examination of Tables X and XI in which the germination of black pine seed is shown in Petri plates and pot soil at different pH; it can be seen that the pH of the solution strongly influenced total germinative capacity and the germination curve of black pine seed. The influence of pH on earliness of germination is shown well in Table XI. This test in soil at different pH levels showed that seed started to germinate two days later at pH 7.0 than at pH 4.5, and at pH 8.5 seed started to germinate two days later than at pH 7.0.

The total germinative capacity of the same seed in Petri plates as compared with that in soil differs for each pH value. This is probably due to poorer aeration in pot soil. The germination capacity at each pH value was also generally different. Thus the germination capacity at pH 4.5 in both Petri plates and soil was twice as large as that at pH 8.5; the germination at pH 7.0 was found to be intermediate. These findings are the opposite of those of Tint (80) who found a reduction in emergence of Pinus nigra var. austriaca in acid media.

By comparing the two Tables X and XI it is found that the rate of germination differs considerably. In Petri plates seed was considered to have germinated when the radicle appeared. In pot soil, germination

TABLE X. Black pine seed germination at different pH values in Petri plates in dark at room temperature.

pH	Total number of seed germinated. percent by days						
	6	7	9	11	12	13	16
4.5	60	68	86	88	88	88	88
7.0	38	42	54	59	59	59	59
8.5	26	31	44	48	48	48	48

TABLE XI. Black pine seed germination at different pH values in pot soil in greenhouse.

pH	Total number of seed germinated, percent in days											
	12	14	16	19[a]	21	23	26	28	31	33[a]	35	37
4.5	11	15	31	50	56	68	72	74	74	78	78	78
7.0	--	5	17	32	42	48	50	56	56	56	56	56
8.5	--	--	4	8	23	29	32	32	32	32	32	32

[a]Time seedling root and stem measurements were taken (data in Table XII).

TABLE XII. Root and stem length of black pine seedlings at different pH values in pot soil in greenhouse.

pH	19 days after sown			33 days after sown		
	Root cm.	Stem cm.	Total length cm.	Root cm.	Stem cm.	Total length cm.
4.5	5.45	3.25	8.70	6.50	3.45	9.95
7.0	3.90	2.60	6.50	5.80	3.20	9.00
8.5	3.30	2.50	5.80	5.00	3.20	8.20

whole germination process after seed started to germinate lasted only five days. In soil after germination of seed started. it continued for more than 15 days.

The results of root and stem measurements of seedlings grown at the different pH levels is given in Table XII. Ten seedlings were collected at random 19 and 33 days after sowing from each of four pots. The nin-teenth day was the time of highest damping-off, and the thirtyieth was the time when the seedlings had passed their susceptible stage.

In 19 days the length of roots at varying pH levels were different and were longest at pH 4.5, shorter at pH 7.0, and shortest at pH 8.5. Thus, at the time of highest damping-off and fungus activity the differ-ences in root length were most striking for the various pH levels. The same trend was apparent at 33 days but there were smaller differences. Stem lengths differed also but in less degree, at different pH levels on the 19th day. They were the same at 33 days at pH 7.0 as at 8.5 and only slightly longer at pH 4.5. Jackson (40) in liquid cultures at different pH found total length of Douglas fir was greater at pH 3.5 and 5.5, and in quartz sand and with liquids the total length of Douglas fir and Ponderosa pine were greater at pH 4.5. Roth and Riker (66) in pot soil with different pH found that the optimum range for root growth of red pine extended from about pH 4.7 to 6.0. From the above results one can see that the influence of acidity on root and total seedling growth is better at the acid side than at the alkaline side of the pH scale. Different species show a varying ability to grow faster at the different levels on the acid side of the pH scale (40, 66).

Relative Pathogenicity of Different Strains and Species.

In an earlier section we have discussed the fungus species isolated from black pine damped-off seedlings. In order to determine if these isolated fungi were the real cause of the disease, experiments were conducted with pure cultures in soil.

The soil used in this experiment was at pH 5.5, and contained humus 3.34 percent. Four or more pots were used for each treatment and the experiment repeated one or more times. The soil was sterilized and treated as described earlier. Cornmeal-vermiculite medium in Petri plates was used for growing inoculum. One fourth of a plate of inoculum was mixed into each 4 inch pot of soil in the first trial. Since this amount of inoculum was found to be too heavy, one eighth of a plate per pot was used thereafter. Appropriate controls were used in each set of experimnts in order to compare the percentage of healthy germination, and from this the percentage losses by damping-off. Watering of the pots was as usual.

In order to have an accurate record of the temperature and relative humidity a hygrothermograph was placed among the pots. Table XIII presents the record of temperature and relative humidity during this test.

It can be seen that the minimum temperature was more or less constant throughout the period at about 22° C.; the higher temperatures were unfavorable for seed germination, but the alternation of temperature has been shown (Table IX) to reduce the unfavorable effects of high temperatures.

The relative humidity plays a considerable role to the maintenance of soil moisture, and has less influence on damping-off. When a 100

TABLE XIII. Record of temperature and relative humidity in the greenhouse during testing of relative pathogenicity of species and strains isolated.

	Days												
	1[a]	2	3	4	5	6	7	8	9	10	11	12	13
Temperature °C.													
Maximum	37.8	38.9	32.2	34.4	34.5	35.0	34.4	35.6	35.8	34.4	31.7	37.2	28.9
Minimum	22.8	22.4	22.2	22.2	22.2	21.6	21.7	21.7	21.8	22.2	22.8	22.4	22.2
Rel. Humidity													
Maximum	86	82	83	75	74	97	99	84	74	72	64	71	75
Minimum	28	26	30	29	26	53	47	62	30	30	32	40	64

	Days												
	14	15	16	17	18	19	20	21	22	23	24	25	26
Temperature °C.													
Maximum	23.3	34.4	37.8	34.4	38.3	35.6	38.9	37.8	39.4	35.0	32.2	30.6	28.9
Minimum	22.2	22.2	22.8	22.2	22.2	22.2	22.2	20.6	22.2	22.2	21.1	21.1	21.1
Rel. Humidity													
Maximum	77	72	76	74	73	64	60	61	88	90	70	68	82
Minimum	32	30	54	30	26	33	31	26	34	48	60	36	40

[a]First day considered the day seed was sown. This was done from the 25 of March to the 30 of April 1958. The second repetition was started a week later.

percent relative humidity was obtained, however, in watered pots covered with cellophane bags, Rhizoctonia solani grew abundant aerial mycelial and could attack the above ground part of seedlings. This confirms the observations by Roth and Riker (67). In this type of covered pots the author got also complete postemergence damping-off of black pine seedlings using the common black mold Rhizopus nigricans.

Table XIV represents the relative pathogenicity of different species and strains of pathogenic fungi isolated from black pine seedlings. Of the Pythium species the isolate of P. irregulare was the most pathogenic. This species was the commonest isolated from diseased seedlings and from soil from nursery and flats in the greenhouse. P. perniciosum, as was expected, did not cause any damping-off of black pine seedlings.

The different strains of Rhizoctonia solani whose cultural and growth characteristics were described in Tables I, II, and III showed that they possess different pathogenic ability also. Even though all of them showed a high total damping-off, there were noticeable differences in the preemergence and postemergence damping-off. Others have also reported that strains of R. solani have different pathogenic ability (13, 15, 32, 57, 65).

In the Fusaria the different species and strains showed also certain differences in their total damping-off. Preemergence damping-off was generally more severe than postemergence but strain differences in this respect could be seen as in F. solani strain 3 and 19 or 25. In this study only in Fusarium solani were distinct strains found. For the two other species F. oxysporum and F. moniliforme the many different isolations studied proved to be culturally and pathogenically identical.

TABLE XIV. Relative pathogenicity in the greenhouse of strains and species of isolated fungi on black pine seedlings.

Species	Number of Pots	Damping-off percent			Healthy seed-lings
		Preem.	Postem.	Total	
Pythium irregulare	4	83	11	94	6
Pythium ultimum	4	59	26	85	15
Pythium debaryanum	4	49	33	82	18
Pythium perniciosum	4	0	0	0	100
Rhizoctonia solani strain 1	4	55	40	95	5
Rhizoctonia solani strain 4	4	85	13	98	2
Rhizoctonia solani strain 87	4	70	18	88	12
Fusarium solani strain 3	4	49	39	88	12
Fusarium solani strain 25	4	85	10	95	5
Fusarium solani strain 19	4	68	6	74	26
Fusarium oxysporum strain 15	16	74	11	85	15
Fusarium moniliforme strain 24	12	76	16	92	8

Damping-off of Black Pine Seedlings and pH.

It has been known for many years that acid soils reduced damping-off losses, and in alkaline soil damping-off losses were heavy (32, 40, 64, 66, 78, 80). This seems inconsistent with the present work since it was found that the pathogenic damping-off fungi really thrive in acid environment, and in alkaline environment they grow less vigorously. It is further assumed that in natural acid soil the antagonistic bacteria should compete with fungi better than in alkaline soil. As was discussed earlier, when media with pH 5.5 were used for isolation of pathogenic fungi from damped-off seedlings, bacteria almost always covered the entire tissue sections. It was necessary to put 2-3 drops per plate of a 25 percent lactic acid solution in order to have a medium with pH 4 to 4.5, and in that medium only were the undesirable bacteria avoided.

Although the pathogenic fungi thrive in acid media, and the competitive bacteria in alkaline media, coniferous seedlings avoid damping-off better in acid media (soils). The reason for this is easy to find, in that the seedlings themselves are favored by acid soils and grow in a healthier condition. In the slightly acid soils minerals are easier available to plants, and thus plants live in a healthier condition, grow faster, and mature earlier (Table XV).

Black pine seed were sowed again in the same soil which was used earlier. The pH of the soil was adjusted with buffers, and the soil was sterilized as usual, and seeds were sterilized with 1:1000 mercuric chloride. Fifty seeds were sowed per each 4 inch pot. Two fungi, Fusarium moniliforme and Rhizoctonia solani, were grown in Petri plates in cornmeal and vermiculite. One eighth of plate inoculum was used per

TABLE XV. Damping-off of black pine seedlings in soil at different pH levels

pH	Organism	Germ-ina-tion Days	Percentage postemergence damping-off in days								Damping-off			
			17	20	21	23	25	28	30	33	Preem.	Postem.	Total	Alive
4.5	*F. moniliforme*	11		3	5	8	18	20	21	21	32	21	53	47
7.0	*F. moniliforme*	13			13	13	14	14	14	14	71	14	85	15
8.5	*F. moniliforme*	15	4	4	4	4	4	4	4		96	4	100	0
4.5	*R. solani* No. 4	11	4	7	11	13	15	15	15	15	65	15	80	20
7.0	*R. solani* No. 4	--	--	--	--	--	--	--	--	--	100	--	100	0
8.5	*R. solani* No. 4	--	--	--	--	--	--	--	--	--	100	--	100	0

TABLE XVa. Record of temperature and relative humidity

	Days															
	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Temp.																
Maximum	22.2	31.7	32.2	33.3	30.0	33.3	37.8	35.6	33.3	34.4	33.3	33.3	28.3	22.8	30.6	35.6
Minimum	21.1	22.0	21.6	21.7	21.5	21.0	22.0	27.2	21.7	21.2	22.2	21.7	21.7	21.7	22.2	21.7
Rel. Hum.																
Maximum	99	80	96	96	62	64	99	99	88	68	56	58	50	84	46	48
Minimum	30	42	43	46	39	36	40	58	50	38	30	32	36	38	26	29

[a]Seed sown.

	Days														
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Temp.															
Maximum	33.9	33.3	32.2	32.2	34.4	31.7	30.0	24.4	29.4	33.3	28.3	36.1	36.7	32.8	37.8
Minimum	21.1	21.7	20.0	21.7	22.8	21.1	18.9	16.1	21.1	21.7	21.1	25.6	21.7	21.1	21.7
Rel. Hum.															
Maximum	40	60	40	50	70	99	99	99	62	60	68	65	70	81	75
Minimum	28	35	27	32	41	55	50	32	26	30	47	42	35	44	20

Fig. 2. Black pine damping-off at different pH levels of the soil. Left pots with pH 4.5 less damping-off occurred, than at pH 7.0 center, or pH 8.5 right.

4 inch pot. Four pots were used, repeated twice, for each pH value; watering was as usual. Temperatures and humidity during the course of this study were measured and are shown in Table XVa. Table XV gives the results of this experiment. At pH 4.5 the preemergence damping-off and especially the total damping-off was smaller than at pH 7.0 and pH 8.5. This is the case especially for Fusarium moniliforme (Fig. 2). In *Rhizoctonia solani* strain No. 4 we had a total number of damping-off of 80, alive 20, at pH 4.5 and 100 percent damping-off at pH 7.0 and pH 8.5. *Fusarium moniliforme* was less pathogenic in general than *R. solani* strain No. 4 at the two lower pH levels but equally severe at pH 8.5.

In general with both fungi we had less damping-off in acid soil than in alkaline soil, this being in agreement with others (32, 40, 64, 66, 78, 80).

Measurements of length of roots and stems at different pH of soil were shown in Table XII. Thus it was found that the root lengths generally were greater at pH 4.5, less at pH 7.0, and least at pH 8.5; this being in agreement with the work of others (40, 66). Stem and total length were less clearly affected.

Table X shows the influence of pH on seed germination. Thus at pH 4.5 seeds started to germinate two days earlier than at pH 7.0, or 4 days earlier than at pH 8.5. But in acid soils not only germination started earlier but it was approximately twice that in alkaline soils (Table XI). Thus it is clear from all the above that in acid soils less damping-off occurs not only because seedlings are grown better, but seeds also germinate earlier, faster, and in a total germination twice that of alkaline soils. Thus seedlings in this condition have more possibilities to escape damping-off, and reach the maturity stage earlier.

Black Pine Damping-off and Soil Quality.

It was referred earlier that temperature, moisture, aeration, pH are some of the environmental factors which influence the germination, the emergence, and the good and fast growth of seedlings, but at the same time the growth condition and health of the pathogens. It was also accepted that the main factor generally which determines the quantity of damping-off is the growth condition and vigor of the seedlings, rather than that of the pathogens.

Under good growth conditions vigor of the seedlings is influenced not only by the quantitative and qualitative expression of environmental factors such as temperature, moisture, aeration, pH, but also by the quality (fertility etc.) of the medium (soil) in which seedlings are grown. In nature the medium used is the soil and its type influences the health and balanced growth of the seedlings, the pathogens, or both of them, to affect the amount and kind of damping-off.

In order to find what relationships exist between soil quality and damping-off, soil of different types was used. The origin and composition of soils was discussed under Materials and Methods. Composition of the soils is given further in Table XVII.

All soils, before sterilization, were equalized at pH 6.0, by using different proportions of phosphoric acid and sodium hydroxide buffers. After sterilization the pH was checked and corrected if necessary. Inoculum was supplied from Petri dish cultures of vermiculite and cornmeal. Two types of inoculations were made: "heavy" (Table XVI) in which one third of a Petri dish culture was used per 4 inch pot, and "light"

TABLE XVI. Comparative damping-off of black pine by several fungi in different soil types at pH 6.0 in the greenhouse. Heavy inoculum.

Soil Type	*P. irregulare*			*R. solani* No. 4			*F. oxysporum* No. 23			*F. moniliforme* No. 24			*F. solani* No. 25		
	Damping-off			Damping-off			Damping-off			Damping-off			Damping-off		
	Pre-emer.	Post-emer.	Healthy	Pre-emer.	Post-emer.	Healthy	Pre-emer.	Post-emer.	Healthy	Pre-emer.	Post-emer.	Healthy	Pre-emer.	Post-emer.	Healthy
SA. Sand	88	6	6	100	--	--	82	12	6	79	24	7	66	22	22
SS. Sand + Nutrient solution	96	4	--	100	--	--	100	--	--	88	12	--	82	12	6
SP. Sand + peat	100	--	--	100	--	--	100	--	--	94	6	--	73	17	16
D. Soil	84	12	4	100	--	--	47	35	18	68	16	16	45	41	14
E. Soil	85	8	7	96	4	--	56	24	20	70	12	18	66	12	22
F. Soil	84	8	8	100	--	--	45	25	30	44	20	36	56	16	28
M. Muck	94	6	--	100	--	--	60	40	--	88	12	--	100	--	--

TABLE XVII. Black pine seedling damping-off caused by _Fusarium_ _solani_ strain 25 in different types of soil at pH 6.0, in the greenhouse. Light inoculum.

Soil Type	Soil content				Damping-off			Seed-lings heal-thy	Seedling[a] length Av. 33 plants			Seedling Weight Av. 33 plants		
	Humus %	Sand %	Silt %	Clay %	Pre-emer.	Post-emer.	Total		Root cm	Stem cm	Total cm	Green root + stem gr.	After drying gr.	Dry wt. per cm. length gr.
S Sand (without nutrient)	0	100	0	0	35	37	72	28	7.80	4.96	12.76	2.28	0.225	0.0177
D Soil	2.04	89.00	2.98	5.98	46	12	58	42	7.69	4.35	12.04	2.19	0.205	0.0171
F Soil	3.34	86.00	4.00	6.66	20	22	42	58	7.40	4.32	11.72	2.34	0.205	0.0175
M Muck	46.60	--	--	--	28	40	68	32	5.55	5.03	10.58	2.11	0.201	0.0189
C Clay	1.25	70.75	9.00	19.00	78	19	97	3	--	--	--	--	--	--

[a] These data referred to 16 day old seedlings.

TABLE XVIIa. Records of temperature and relative humidity during greenhouse damping-off tests of various soils at pH 6.0.

	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Temp.																		
Maximum	28.3	28.9	28.3	32.8	29.4	28.3	28.3	36.1	35.0	31.1	29.4	32.8	36.1	40.6	37.8	28.9	32.2	32.2
Minimum	21.1	23.3	23.9	22.8	22.8	22.8	22.2	23.3	25.6	23.3	25.6	25.6	25.0	25.0	25.2	25.6	22.8	24.4
Rel. Hum.																		
Maximum	45	64	58	50	45	59	58	52	49	52	52	50	62	58	52	44	58	61
Minimum	34	38	39	34	35	38	39	37	37	38	36	43	42	42	40	38	42	46

	Days 19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Temp.																	
Maximum	35.6	41.1	37.2	30.0	28.9	32.2	31.7	29.4	28.9	38.3	30.0	32.2	31.7	30.0	30.0	42.2	42.2
Minimum	22.8	25.6	24.4	25.6	25.0	22.8	23.3	25.6	25.0	25.0	25.0	26.1	23.9	23.3	23.3	25.0	25.6
Rel. Hum.																	
Maximum	54	52	62	52	70	58	57	55	60	63	61	67	58	52	54	62	70
Minimum	40	36	40	39	40	42	41	36	46	44	41	48	44	42	37	38	42

inoculum (Table XVII) in which one eighth of a Petri dish culture was used per 4 inch pot. Eight pots were used for each soil type and pathogen. Water was as usual. Temperature and relative humidity noted during the progress of this test is given in Table XVIIa.

On comparing the damping-off for the various pathogens and soils under two rates of inoculum it can be seen that the heavy type of inoculation (Table XVI) was more severe than the light inoculation used later (Table XVII).

In pure sand (series SA) there was less damping-off than in SS or SP sands containing a fungus nutrient solution or peat (Table XVI).

In soils D, E, F with various percentages of humus, sand, silt, and clay (c.f. Table XVII) we had less damping-off. It was lowest in series F containing humus (3.34 percent) which probably represented a better balanced fertilizer condition. In muck soil which contained 46.60 percent humus, we had most damping-off.

Rhizoctonia solani strain 4 caused heavy losses in all types of soils (Table XVI). Pythium irregulare gave heavier losses with muck soil and in sand with fungus nutrient solution or with peat, and less with D, E, F kind of soils. Even in the latter soils, however, the total losses must be considered heavy. Pythium sp. are predominant in soils with high humus content, and Rhizoctonia solani in soils with low humus content (32, 65). In the case of Pythium the present work gave more or less the same results. With Rhizoctonia solani, however, in pure culture inoculations there were heavy losses in all kinds of soils. These losses were probably due in part to heavier amounts of inoculum and the favorable greenhouse environment. With Fusaria, again, there were heavy losses on muck soil and sand plus nutrient fungus solution, sand plus peat, and less damping-off with soil type F.

In Table XVII it can be seen that a light inoculum with Fusarium solani produced most losses in clay soil, next most in the muck soil and sand, less with soil D and least in soil F.

From the above it is clear that the type of soil is an important factor in the occurrence of damping-off. The quality of soil influences, along with the environmental conditions, both fungus growth, and the balanced growth and vigor of the host. Thus it is apparent that the physical, chemical and fertility properties of the soil are important factors in damping-off. The experimental work confirms, in part the results of Tint (80).

In Table XVII one can see that the root length and total length of black pine seedlings 16 days old were greater in sand (no humus at all) than in muck soil (humus 46.60 percent). Stems were shorter than roots in all the soils tested, but in the case of muck soil the root-stem ratio was smallest. The longer length of roots in sand is probably due to better aeration which also influences seed germination. The increased root lengths found in lower moisture, better aerated soils confirms a report by Roth and Riker (61) on red pine.

The length and weight of green and dry seedlings are also shown in Table XVII. Thirty three seedlings 16 days old were collected at random from pots of each soil type. The average dry weight per centimeter of seedling length was calculated also, since the length of seedlings varied with the soil type. There was no correlation between dry weight per centimeter and severity of damping-off.

The less damping-off, or the greater number of healthy seedlings occurred in soil of series F and D. This probably was due to a balanced condition of soil fertility in which seedlings were grown neither so fast, nor so slow. In case of sand seedlings grew too fast, and tissues were too tender and attacked easier by pathogens. In muck soil seedlings grew too slow, thus they were exposed to fungus attack for a prolonged period. Probably seedlings in soil of series F and D possessed a medium condition of growth between sand and muck, and thus less damping-off occurred.

Relationship of Damping-off and Root-rot of Black Pine Seedlings.

In many cases seedlings in flats in natural infected soil, or in pots of pure cultures, or in the nursery, escaped damping-off and were allowed to remain for more than a year. In a number of these cases, especially in the favorable environment of the greenhouse, root-rot sooner or later developed. Isolations were made from representative root-rotted seedlings taken from natural infected soil in greenhouse and nursery. In all cases *Fusaria* were found to be the primary cause of root-rot. In a few cases *Rhizoctonia solani* was isolated also, but it was doubtful that it was the root-rot agent since *Fusarium* was also isolated from the same root section.

In several cases the phenomenon of weak parasitism, as pointed out by Tint (79), was also observed. Black pine seedlings that escaped damping-off in natural infected soil, started to show dryness of their lower needles after several months. Root isolations proved that *Fusaria* were present. If these seedlings were retained for one half to two years they continued to be alive, even though their lower needles were dried and even though *Fusaria* were living in their roots.

F. solani, *F. oxysporum* and *F. moniliforme* strains from the Chittenden nursery, and strains of the same species from the Bogue nursery caused both damping-off and root-rot on black pine seedlings in pure cultures in pot soil. The same Chittenden strains can cause a root-rot complex with nematodes on white pine at the same nursery (61).

No *Pythium* or *Rhizoctonia* alone were isolated from root-rot seedlings of black pine in the greenhouse or in the nursery. These results were similar to those of Riffle (61) with white pine.

DISCUSSION

Black pine damping-off was examined in natural infections in Michigan nurseries and research was done mainly in the greenhouse. During the summer field experiments were also made in the nursery.

Under greenhouse conditions damping-off was heavier than in the field, this being in accordance with work of others (77, 79, 82). It was found also that if the same infested soil, either natural or inoculated, was used repeatedly the damping-off became more severe. This means that under greenhouse conditions the main pathogens predominate against their competitive microflora and microfauna.

In Michigan soil the following damping-off fungi pathogenic to black pine were isolated: *Pythium irregulare* Buismann, *P. ultimum* Trow, *P. debaryanum* Hesse, *Fusarium solani* (Mart.) Snyder and Hansen, *F. oxysporum* (Schl.) Sny. and Han., *F. moniliforme* (Sheld.) Sny. and Han., *Rhizoctonia solani* Kühn, and *Botrytis cinerea* Persoon. Of the above several *Fusarium* sp. were the most important pathogens causing damping-off of black pine under a wide range of conditions, and secondary in importance were *Rhizoctonia* and *Pythium*. In the genus *Fusarium* the most common species isolated under a wide range of conditions was *F. oxysporum*, and in *Pythium* it was *P. irregulare*.

Pythium perniciosum was isolated from soil, and was of interest because this is the second time in which this Pythium was reported in the United States (50, 51). In this fungus oogonium germination was unknown (50, 51), but the author observed germination in two cases in water cultures. These occurred on hemp seed held in this culture for 15 months in room environment. Germination was by a germ tube which had a branch about 40 mu from its base. This species showed no pathogenicity to black pine seedlings.

The principal pathogenic species and strains isolated showed differences in culture appearance, growth, and pathogenicity. Growth studies of these fungi were done in different environments. Pythium grew satisfactorily from pH 4.5 to 8.0, with pH 5.5 being the most favorable, this agrees with the work of others (36, 40, 64, 66). Pythium also grew better in potato dextrose agar at pH 5.5 between 20° C. and 30° C., this also being in a general agreement with the work of others (51, 66).

From Rhizoctonia solani four different strains were isolated; these were found to have stable cultural appearance in different environments, different growth, and pathogenicity. Rhizoctonia solani grew faster between 20° C. and 30° C. generally for all strains. At 12° C. the different strains grew in different ways, and two strains of it slower than Pythium at the same temperature. The growth of Rhizoctonia in different pH varied. Best growth generally occurred in the slight acid media, and its growth in alkaline media was less, this being in agreement with others (46, 49, 64, 66).

In the case of the Fusaria growth rates were smaller than Pythium and Rhizoctonia but as a whole the different species and strains tested showed less differences under the varying environments used. This means that Fusaria are less influenced by the changes of their environments and that they are really "strong" fungi. The above probably explains why Fusaria predominate under a variety of conditions throughout the year. Others have found that Fusaria are widely adapted, and generally can cause severe losses in higher temperatures (64, 81, 93). Pigmentation in *Fusaria* was found to be more abundant under dark, and it is influenced also by the media used, this being in agreement with others (10, 72, 96). The results of this study showed that pH of the medium is not an important factor for zonation. And further it was found that all the damping-off *Fusaria* formed zonation in the lowest constant temperature of 12° C.. This is not in agreement with the work of others (72, 96), that zonation in *Fusaria* is influenced only by fluctuating temperature or light. It was also found that several strains of different species can form zonation under a wide range of environmental conditions. Sporodochium formation was proved to occur more abundantly in light than in dark, this being in agreement with others (30, 72, 96). Media influenced also sporodochia formation.

Isolations from damped-off seedlings of different fungi throughout the year proved that species of the main pathogenic genera *Fusarium*, *Pythium*, *Rhizoctonia*, and their importance in different seasons of the year are the same in Michigan with black pine as in Pennsylvania with several vegetable plants (6).

Pythium was more prevalent under the warm and moderately humid conditions of closed heated greenhouses during winter months. *Pythium* is responsive to moisture and caused more severe damping-off of seedlings and predominated more over the other main pathogens the more the soil was saturated. *Pythium* causes negligible losses in dry soils (6, 61). During the warmer and drier summer months, *Pythium* losses were negligible in open, well aerated greenhouses or in the field. Other pathogenic genera predominated over *Pythium* at that time. The above means that, in a natural infested soil at medium high temperatures in which *Pythium* lives together with other pathogenic damping-off fungi and with a great number of other competitive microorganisms, *Pythium* predomination depends mostly on moisture. The higher the soil moisture content for a prolonged period of time the higher the losses from damping-off of *Pythium* on black pine seedlings. In drier conditions the other pathogenic fungi were predominant.

Fusaria were predominant under a variety of conditions throughout the year. As was mentioned earlier, *Fusaria* are less influenced by environment than *Pythium* or *Rhizoctonia* which are influenced by more limited conditions. *Fusaria* are "strong" species while *Rhizoctonia* become secondary in importance in many periods of the year. The present work also confirms that of Roth and Riker (66) who pointed out that *Rhizoctonia* was influenced less by environment than *Pythium*.

From several thousands of isolations throughout the year in natural infected soil the main damping-off causing fungi did not show any special ability to attack black pine seedlings earlier than did other species. The rate of isolation of different species of fungi was according to the final percentage ratio per each pathogenic genus for each set of seedlings.

When the main pathogenic fungi were allowed to interfere with each other in culture media it was found that several of them possessed a strong antibiotic property. Gregory *et al* (25) found an antibiotic produced by a *Streptomyces* which was active against some fungi, but had low activity towards other fungi and bacteria. The earlier results of the author's work and from his isolation studies, showed that in natural infected soil in which damping-off occurs, individual damping-off seedlings can be attacked and invaded by one only and in very few cases by more than one pathogenic fungus.

Black pine seeds germinated two days earlier in warm than in cooler periods, and black pine seedlings developed resistance to damping-off after 25 and 27 days after emergence respectively. During warmer periods both the preemergence damping-off and postemergence damping-off of black pine was higher than during cooler periods. This confirms the results of others (32, 43, 44, 66), who found that conifer damping-off was generally more severe in the warmer soils and that seedlings were better able to escape the disease under cool conditions.

The author's modification of the older contact-slide technique for the study of soil microflora (11, 94) was used. The advantages of the new modified technique was that it was faster, and more accurate for the identification of soil fungi.

Seed germination is an important factor in damping-off disease, since it is evident that in case of slow germination, pathogenic fungi have more opportunities to attack and cause severe types of preemergence damping-off. Black pine seed germinated under different free moisture conditions showed that a medium amount of moisture is necessary at the beginning for a good start, but within a few days about the time the new radicle started to appear, aeration became the most important factor for black pine seed germination. In other words germination was favored by medium moisture initially and a reduction in moisture after the process had begun. The above is of importance in nurseries practice, because it is clear that a few days moderate watering is necessary after sowing and then watering must be much lighter thereafter. In the lower moisture content test and in good aeration 100 percent germination resulted with the same seed lot which in the practice gave 78 to 84 percent germination. The reason for this difference must be probably due to the poorer aeration of seed in soil, as compared with the better aeration in low moisture content in Petri plates in the above experiment.

At constant temperature with normal moisture black pine germination showed a double peak curve with its optima at 24° C. and 31° C.. The same was found for other species by Haasis (27). Alternating medium temperatures showed good germination as well (Table IX). Alternating a low temperature with an unfavorably high temperature produced favorable germination conditions.

At different pH levels of 4.5, 7.0 and 8.5, in Petri plates and soil there was better germination at the lower pH 4.5 and successively poorer at the more alkaline. Lower pH also induced earlier germination. Here again there was better germination in Petri plates than in soil at each pH level, probably due also to the poorer aeration in soil. Aeration also is probably the reason that germination was completed in 5 to 6 days, in plates after it had started, while in soil the same process required about 20 days.

From all the above it is apparent that germination is a very important factor in connection with damping-off disease, since under special environments germination takes place quickly, and a high total germination number occurs, thus seedlings more readily escape the disease. Under other environments on the contrary, germination starts later, continues for a longer time, and gives finally a lower germination number. All these conditions are favorable for the development of severe damping-off.

Black pine damping-off was lighter in soil with pH 4.5 than in soils with pH 7.0 or pH 8.5; this being in agreement with the work of others (32, 40, 64, 66, 78, 80). Black pine was found to grow better at pH

4.5 than at pH 7.0 and pH 8.5; this was also in agreement with the work of others (40, 66) on red pine. This is probably one explanation for the lower damping-off in medium acid soil; plants grew faster and were in a nutritional equilibrium of minerals made possible by the soil acidity. But this is not the only explanation of the less occurrence of damping-off in acid soils, because it was found by the author that in acid soils germination of black pine seeds started earlier, the speed was greater, and the total germination number twice as much as that in alkaline soils, and hence the seedlings reach the maturity stage earlier, when they become resistant to damping-off.

Soil type and fertility influence plant growth, the pathogens, and the soil microorganisms. Less damping-off of black pine occurred in soils of medium fertility and in conditions in which black pine grew well on the average than in sand, muck, or in more clayey soil. Black pine grew larger seedlings in sand soils, and plants with heavier unit of dry weight per length in muck soil. It is postulated that, under a medium type of soil condition and fertility, seedlings did not grow so fast as to become excessively tender and susceptible to pathogenic fungi as they did on sandy soil. Neither did they grow so slowly as to be exposed to fungus attack for long periods as was the case on muck soil.

In several reports (75, 78, 79), less damping-off occurred with coniferous seedlings in coarse sand cultures or in cases in which coarse sand was used as seed cover. In our case a fine sand less than 1 mm. in diameter particle was used in the greenhouse. In Table XVII it was found that neither the longer seedling length in sand soil nor the heavier seedlings in muck soil were correlated with a lower incidence of damping-off.

Seedlings of black pine escaping damping-off in greenhouse flats with natural infected soil; in pots in pure cultures; or in nursery beds were allowed to continue growth. The root-rot was heavier in greenhouse (77, 79, 82) probably due to more favorable environment for the pathogenic organisms and the tenderness of seedlings than in the nursery. In seedlings of one or more years, weak parasitism was found to be associated with Fusarium sp.. These seedlings grew satisfactorily and only their lower needles started to dry, these seedlings in the opinion of the author were good stock for planting. From the results of this study weak parasitism seems to depend on the age in which the host was attacked and also to the environmental conditions. Tint (79) wrote that weak parasitism and virulence of Fusaria was dependent to the species and the nature of their nutrition prior to inoculation and the conditions under which the experiments took place.

SUMMARY

The causal agents of damping-off of black pine in Michigan forest nursery soils proved to be *Pythium irregulare*, *P. ultimum*, *P. debaryanum*, *Rhizoctonia solani*, *Fusarium solani*, *F. oxysporum*, *F. moniliforme*, and *Botrytis cinerea*. Culture and soil isolations proved that prevalence of *Pythium* was; *Pythium irregulare* first, *P. ultimum* second, *P. debaryanum* third. Among *Fusaria*, *F. oxysporum* isolations were more common, *F. solani* was second, and *F. moniliforme* was third.

From soil isolations *Pythium perniciosum* was identified, this report being the second in this country, but when tested proved to be non-pathogenic to black pine seedlings.

In culture studies *Pythium* generally grew better at pH 5.5, and in a temperature between 20° and 30° C. Four definite strains of *Rhizoctonia solani* were isolated with characteristic cultural appearances and different pathogenicity. The strains showed also differences in growth in different temperatures and pH of medium. *Rhizoctonia* in cultures generally grew faster between 20° and 30° C., and at the lowest temper ature tested 12° C. was slower than *Pythium*. *Rhizoctonia* generally grew well at pH 4.5, 5.5 and 6.5, and its growth at Ph 8.0 was satisfactory.

Fusaria grew slower than *Pythium* and *Rhizoctonia*, but the differences among species and strains used in different environments were less than those of *Pythium* and *Rhizoctonia*. Pigmentation in *Fusaria* was more abundant under dark, and was influenced by the medium used. Zonation in *Fusaria* was not influenced by the pH of the medium; it was influenced by diurnal light; and at the lowest temperature tested 12° C. all pathogenic *Fusaria* formed zonation. It

was also found that several strains of *Fusaria* possess the ability to form zonation despite their enviornmental conditions.

Antibiotic properties were found when the main pathogenic fungi were grown together to interfere with each other. Thus *Fusarium solani* strain 3 proved to possess considerable antibiotic ability when it was allowed to grow with several other of the pathogenic fungi; several of the other fungi proved also to possess antibiotic ability in less degree than *F. solani*, these fungi are *Rhizoctonia solani* strain 1, *Fusarium moniliforme*, and *F. oxysporum*.

From isolations of damping-off seedlings and the above antibiosis of several fungi, it was concluded that in natural infected soil, generally only one of the pathogenic genera attack and invade each seedling; more than one genera were isolated from the same seedling only in a few cases.

Nematodes saprophytic and parasitic species were isolated from black pine damped-off seedlings, but due to the small number of cases, they cannot be considered important factors in the occurrence of damping-off.

Isolations from greenhouse and field around a year showed that *Pythium* was more prevalent during winter months in a closed heated greenhouse, with *Fusarium* second in importance and *Rhizoctonia* third. In spring in a greenhouse which was open and cooler *Fusarium* became the more prevalent species with *Rhizoctonia* second, and *Pythium* third in importance. During summer months in greenhouse and field *Fusarium* was the more prevalent, *Rhizoctonia* was second in importance, and *Pythium* was third. In several cases during summer *Pythium* did not appear at all in isolations. In fall again in greenhouse which was cooler, Fusarium was most prevalent, Rhizoctonia was less prevalent and Pythium was least prevalent. From the above it is clear that Fusarium is the main cause of damping-off in Michigan soils, and that Fusarium is really a "strong" species since in a natural infected soil and under a wide variety of conditions it dominated in many cases the other pathogenic fungi.

During warmer periods the preemergence damping-off was higher than during cooler periods. The same was true for postemergence damping-off and consequently for total damping off.

Studies on black pine seed germination showed that at the beginning a medium quantity of moisture, and not too much moisture, is necessary for initiation of seed germination. After a few days aeration became the critical factor, and total germination proved best at the lower moisture content tested. At constant temperature black pine seed germination showed two optima at 24^{0} C. and 31^{0} C.. Alternating temperatures resulted in the same or better germination than constant temperatures. As to the relation of black pine and germination and pH of the medium, it was proved that at pH 4.5 total germination number was approximately twice that at pH 8.5, and it occurred in much less time. From the above it is clear that pH, moisture, and temperature, are very important factors for the rapid and better germination of black pine seeds, and this of course is corellated to the occurrence of damping-off disease since rapidly germinating seed and fast growing seedlings reach their resistant stage sooner. Black pine seedlings reached maturity 25 to 27 days after emergence, and seeds germinated under favorable environments reach this stage earlier, and can escape damping-off.

The soil type and fertility influence also the growth of plants, of pathogens, and the soil microorganisms generally. Thus less damping-off occurred in black pine seedlings in a soil of medium fertility where seedlings did not grow so fast as in the case of sand, or so slow as in the case of muck soil. It was found that the larger seedlings grew in sand but were probably tender, while the seedlings in muck soil were heavier in dry organic matter, but they grew too slowly and were proved to be attacked more severely by damping-off fungi.

For the study of soil microflora and microfauna a modification of the older Cholodny and Krinchkova soil slide technique was used. This modification proved useful since less time and more accurate identification of soil fungi were obtained.

The practical applications of this study are that in forest nurseries in order to avoid as much as possible damping-off, the soil must be light, slightly acid, and of medium fertility. After sowing the watering, when no progerminated seeds are used, must be medium for several days and quite light after. And since the temperature is also an important factor, time of sow in g must be tested in each locality and for each plant species used.

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