A STUDY ON A SEED CARRIED DAMPING-OFF PATHOGEN

By

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CHAPTER I

INTRODUCTION

In attempting to grow shortleaf pine seedlings for inoculations with mycorrhizal fungi, it was found that damping-off killed most of the seedlings. Since the soil had been sterilized and covered, extreme care taken in watering, and, in general, all necessary sanitation measures taken, it was very surprising that damping-off occurred. The experiment was repeated a second time, again utilizing all precautions, with the same results, damping-off destroyed the seedlings.

Upon plating out on potato dextrose agar (PDA) it was found that the seed contained a fungus. A review of the literature on damping-off of conifer seedlings revealed many reports of damping-off obtained under controlled conditions. However, the introduction of the fungus pathogen was mainly attributed to splashing water or insects. Fisher (5) indicated the possibility of the causal fungus entering the soil with the seed. He stated that there is some opportunity for organisms to enter the seed at three different times. These are: (1) before the seed completely matures in the cone, (2) as a result of injury in the extraction process, (3) or because of improper storage. However, no proof has been found to support this idea.

Since it was shown that the fungus was entering the soil with the seed, a study was undertaken to determine: (1) is the fungus in the seed or on the seed; (2) if the fungus is in the seed, does it

enter through the seed coat and how long does this take; (3) is the fungus localized in any particular tissue in the seed or does it spread throughout all the tissues of the seed; (4) to what extent is the fungus spread from infected or infested seed to clean seed during the stratification period; and (5) what is the fungus and does it cause damping-off under any particular conditions.

Answering these questions became the primary object of this study and the results are presented in this thesis.

CHAPTER II

LITERATURE REVIEW

According to Hubert (11), damping-off is the common term used to designate the rapid decay of young succulent seedlings or cuttings. Mainly it is a disease occurring in nurseries and greenhouses, but it can become epiphytotic in the field under the right conditions.

Most of this early work on damping-off was done in Germany beginning about the latter half of the 19th century as culture techniques were developed. Most of this work involved identification with little attempt to prove pathogenicity. Owens (14) notes that a species of Rhizoctonia was described in 1858 and Pythium debaryanum Hesse was reported in 1874. However, it was not until the early 20th century that these fungi were proven to cause the disease. In fact, the only fungi that were reported to be causal organisms of this disease before the 20th century were Fusarium spp. and Phytophthora fagi R. Hartig.

Damping-off was first noticed in the United States on truck and cultivated crops in the early 20th century (14). As forestry practices developed and attempts were made to grow pines in large numbers for reforestation purposes, damping-off took a large toll of the young seedlings. As this disease became an important problem, attempts to work out the causal organisms and develop control practices were undertaken.

Duggar and Stewart (4) made the first report of Rhizoctonia spp. being a causal organism of damping-off in 1901. In 1914, Spaulding (16) worked with Fusarium spp. and by 1921, Hartley (9) was working with both Rhizoctonia and Pythium debaryanum Hesse. Hartley et al. (10) worked out the parasitism of a number of strains of Corticum vagum Berk. and Curt. which is now known to be Rhizoctonia.

At the present time there is a great deal of literature on damping-off, however, most of this is descriptive and on control measures. There are more than 30 different fungi reported to cause damping-off. Some of these are as follows: Pythium spp.; Corticum vagum Berk. and Curt. (Rhizoctonia); Fusarium spp.; Phytophthora fagi R. Hartig; Phoma betae Frank; Phoma lingam (Fr.) Desm.; Phomopsis vexans (Sacc. and Syd.) Harter; Gibberella saubinetti (Mont.) Sacc.; Gleosporium spp.; Volutella spp.; Colletotrichum gossypii Southworth; Peronospora parasitica (Fr.) Tul.; Aphanomyces levis de By; Rheosporangium aphanidermatus Edson; Botrytis cinerea Pers.; Sclerotinia libertiana (Lib.) Fckl.; Sclerotium rolfsii Sacc.; Thielavia basicola (B. and Br.) Zopf.; Pestalozzia funera Desm. Trichoderma spp.; and Alternaria spp. Furthermore, under certain conditions, the bacterium, Xanthomonas malvacearum (E.F.Sm.) Dows. has been known to cause damping-off.

While many of these fungi are known parasites, many others are mainly saprophytes, which, under certain conditions, can become parasitic and cause damping-off.

The vast majority of higher plants are susceptible to damping-off in the seedling stage. According to Sprague (17), grasses which had emerged in thick stands during favorable germinating weather, damped-off during prolonged rainy periods.

The conditions which predispose the host for this disease are, in general, anything that stops the host roots and stems from becoming woody. For example, crowding a large number of seedlings in a small place. Damping-off also affects plants that are in a low state of vigor, consequently, anything that will cut down the host vigor will serve to predispose the plant to damping-off. Planting seed too deeply or sowing seed at the wrong time would tend to predispose the plant and also tend to cut down the germination (7). Whenever any right combination of conditions for causal organism growth exist, with those for lowering potential host plant vitality, damping-off can and will occur, whether the plants are in nurseries, greenhouses and gardens, or out in the fields and forests.

There are basically two types of damping-off which affect coniferous trees. These are identified as preemergence damping-off and postemergence damping-off. Preemergence damping-off is the term utilized to describe the decaying of the seed or killing of the seedlings by attacking the hypocotyl as soon as it emerges from the seed coat. Whenever seedlings fail to emerge from the soil, the losses are generally reported as due to poor germination. However, according to Hartley et al. (10), when poor emergence is followed by heavy damping-off losses, poor seed germination capacity should not be blamed. Rather, early infection and preemergence damping-off is the cause.

The second type of damping-off is called postemergence damping-off and it is divided into two parts, "soil-infection type" and "top damping-off" or "blighting". In the soil infection type, only the roots and lower stem are infected. The first symptoms show during the first or second month after germination. It appears at first as a water

soaked to brownish discolored area on the stems of the seedlings at a point near or just below the ground line. The reddish pigments at the base of the stem are destroyed, and, with certain causal organisms, there may be a brownish discoloration along the entire stem except for the growing tip. The most common type symptom is a constriction at the soil line and a toppling over of the plant.

Some causal organisms cause a dry and shriveled appearance of infected plant parts in early stages, whereas others do not. However, as the fungus spreads in the tissues of the host, it causes the seedlings to wilt or, in most cases, they fall over at the ground line before wilting occurs. This toppling effect occurs mainly in coniferous seedlings and is caused by the decaying of the stem tissues and not by water stoppage. In contrast to this, Wright (19) reports that broadleaf species of trees, when infected with postemergence damping-off, do not become flaccid and fall prostrate to the soil. Instead, they commonly remain in an upright position, gradually wilt, break off, and finally blow away.

In top damping-off or blighting, there is no toppling effect. In this type, the fungus will usually overgrow the young leaves of the host and smother them. This type of damping-off is usually most destructive in crowded beds following a period of cloudy or rainy weather.

Most causal organisms can live saprophytically at various depths in the soil for extended periods of time. According to Hartley et al. (10), the root of a ponderosa pine was found to be attacked at one point 11 inches below the soil surface. The majority of these weakly parasitic fungi can attack young tissues with penetration being directly

through the walls of the cells. However, entrance is easier through wounds (1).

Aerial mycelium of causal organisms varies with respect to whether they can cause damping-off by entering the hypocotyl. In a study done by Roth and Riker (15), seedling hypocotyls of 3-5 day old pine seedlings were inoculated with Pythium irregulare Buis. and Rhizoctonia solani Kuhn. R. solani surrounded the hypoctyls but could only cause damping-off by growing down through the soil and infecting the roots where it killed 93% of the seedlings. P. irregulare attacked at the inoculation point and killed 17% of the seedlings through the hypocotyl. However, after P. irregulare grew down through the soil and infected the plants, it killed 100% of the seedlings.

The fungus can grow through the root tissues of the host and back into the soil. Consequently, with roots that are too close together, the fungus spreads very easily through the soil. Other ways in which these fungi spread are through infested soil brought in from other areas by instruments, water, wind, animals, birds, and man. Chester (3) states that some Fusaria may be found in the seed of some plants, however, he does not elaborate on this point. Phoma betae has been shown to persist on sugar beet seed and cause both types of damping-off (3). Furthermore, organisms that cause other diseases but which are seed carried can also cause damping-off under certain conditions. One of these is Helminthosporium spp. which can cause either foot rots or leaf blotches on different grass hosts.

To control damping-off, many different methods are used and these can be broken down into two general categories, nonchemical and chemical. Some of the nonchemical controls are broadcast sowing, which serves to

cut down on the spread of the fungus when compared to sowing in drill rows. Avoiding excessive moisture in the air and soil by regulating the water supply, as well as early plowing, disking, and harrowing (1). The use of light, easily drained soils, sand or sphagnum mulches over the seed bed, and improved subsoil drainage are all beneficial according to Chester (3). Hansen et al. (7) found that the best results were obtained by half shading the seedlings. Transplanting healthy seedlings from infected beds into new soil helps cut down losses. Regulating the acidity of the soil may help, depending on the organism. If organic fertilizers are used, acidic forest litter or peat should be utilized. Regulation of seedling density aids in allowing the plant roots to mature and become lignified. Finally, rotating species, frequent weeding, and regulating soil temperatures if possible, or sowing seed at the time best suited for optimum growth aid in keeping down the disease. Steam or dry heat can be utilized to disinfest the soil.

Chemical controls can also be utilized to disinfest the soil. Formaldehyde treatment is used either by soaking or sprinkling the soil one week prior to sowing. Other chemicals are chloropicrin, methyl bromide, ethylenedibromide, PCNB, Ceresan M plus KI, Copozine, Manzate, New Improved Ceresan M plus KI, and Tersan which all gave good control according to Strong (18). Furthermore, according to Boyce (2), Thiram, Zineb, and Captan gave good control either as soil treatments or by pelleting the seed.

Sprinkling seed beds after sowing with different chemicals such as Semesan, Cuprocide, Tersan, and Manzate may also help. Gregory et al. (6) got some control of P. debaryanum in the laboratory

with antibiotics obtained from certain actinomycetes, bacteria, and fungi.

Seed treatment mainly to disinfest soil immediately surrounding the seed may be useful in inhibiting preemergence damping-off. To accomplish this, a number of different chemicals may be used, such as organic mercury dusts, organic sulfur compounds, carbamates, and copper carbonate.

Acidification may be achieved through the use of chemicals such as aluminum sulphate and ferrous sulfate both dry and in solution. They should be applied at the time of sowing, however, they can be sprinkled on the seed after sowing. Sulphuric acid treatment after the seed has been sown is especially good for deep acidification.

CHAPTER III

MATERIAL AND METHODS

Pinus echinata Mill. (shortleaf pine) seed was obtained from the State of Oklahoma Division of Forestry. Two seed lots that had been stratified on 7 January 1965 and 23 May 1965, were obtained in June 1965 and labeled Group 1 and Group 2 respectively. A third lot of unstratified seed was also obtained in June 1965 and a portion of this lot was stratified on 1 August 1965 and labeled Group 3, while the unstratified portion was labeled Group 4. Three more lots of unstratified seed were obtained in May 1966. This seed had been stored in a cold room at a temperature of -19 to -23 C, and was labeled as Groups 5, 6, and 7.

The stratification of pine seed is intended to soften the seed coat, after-ripen the seed, allow the seed to absorb water and germinate. The seed in this experiment was stratified by soaking in water, then all excess water was shaken away and the seed placed in a sterilized jar in a refrigerator at 0-5 C. After the first 3 days, the seed was wetted again, drained and replaced in the refrigerator. Following a 30-day stratification period, the seed was kept dry in the refrigerator until used.

Potato dextrose agar (PDA) was prepared by combining 5 g of French's instant potatoes, 5 g of dextrose, and 7 g of agar with 500 ml of water. This was autoclaved for 15-20 minutes at 14 pounds of

pressure per square inch at 120 C. The medium was then poured into sterilized petri dishes, allowed to solidify, then the plates were placed in sterilized paper bags and kept in the transfer room in the Plant Pathology Laboratory where the temperature is kept between 24.5-30.0 C.

The surface sterilizing solution was prepared by adding 1 ml of commercial Clorox Bleach, which contains 5% sodium hypochlorite, to 10 ml of distilled water and a surfactant, Tween 20, (two drops to 1000 ml), was added to this solution.

In order to determine whether the fungus was in the seed or on the seed, methods were utilized that would show both directly and indirectly where the fungus was located. To show the fungus location indirectly, random samples of 100 seed were taken from each seed group, then each sample was divided into two equal portions. The first half was rinsed with sterile distilled water, then placed on the PDA plates, 2 seeds per plate, and kept in the culture room at 24.5-30.0 C for 3 days, when any fungus growth was recorded. The remaining half of the seed was placed in a .5% sodium hypochlorite solution for surface sterilization, for 15-20 minutes, then placed in two successive rinses of sterile distilled water and plated out on the PDA. There were 2 seeds to a plate with 5 replications. These were kept in the culture room for 3 days, and the results were recorded.

To determine where the fungus was located, seed was surface sterilized and rinsed as described, then dissected aseptically and the seed coat, testa or aril, endosperm, and embryo were separated out. Each of these parts was placed on agar in a petri dish and after 3 days results were recorded. This method showed directly where the fungus

was located in the seed.

To determine to what extent the fungus was spread in the stratification process, and how much time was required by the fungus to grow into the seed, unstratified pine seed was surface sterilized and plated out on PDA. After a 3-day incubation period, the seed that showed no fungal or bacterial growth was harvested in sterile jars and kept in the refrigerator until a sufficient quantity of seed was obtained. At the same time, previously stratified seed that was known to be infested, was marked with yellow enamel by dipping a very fine camel's hair brush in yellow paint and lightly touching it to the side of each seed. Preliminary tests had shown this paint to have no deleterious effect on either the fungus or the seed. When enough clean seed was accumulated, the paint-marked infested seed was mixed with them in a ratio of 3 healthy seeds to 1 infested seed. All the seed was then stratified as described previously and after the 30-day stratification period, the unmarked seed was divided into 2 portions. The first half was rinsed with sterile distilled water, plated out on PDA, and incubated for 3 days. The second half was surface sterilized then plated out, and the seeds from the second half that showed infection were dissected as previously described. This was to determine if the fungus could grow through the seed coat in the 30-day stratification period.

The last 3 groups of seed was utilized to find out to what extent the fungus is prevalent on shortleaf pine seed in Oklahoma. The seed from each lot was divided into two parts. The first half was rinsed with sterile distilled water and plated out; the second half was surface sterilized and plated out. For comparison, one group of loblolly pine

seed, Pinus taeda L. was treated in the same manner as the shortleaf pine seed.

To prove pathogenicity of the fungus an experiment was devised using 6 replications. There were 4 pots per replication, 2 control and 2 inoculated pots. These pots were kept in the greenhouse from April through July 1966. From May through the first half of July, the temperature in the greenhouse reached 35 C or above nearly every day and many of the young seedlings fell over from the heat. When damping-off occurred, the seedlings were plated out, the fungus reisolated, obtained in pure culture, and used as a source of inoculum for further replications.

CHAPTER IV

RESULTS

Fungi Found Associated with the Seeds

There were many fungi observed to be associated with the seeds. With the exception of the pathogen of this study, Mucor racemosus Fries., the most commonly observed fungus was Penicillium spp. Other fungi found to some extent in the seeds were Aspergillus spp., Rhizopus spp., and Fusarium spp. Bacteria did not cause much of a problem although a few seeds were found to contain them. One year after this study began however, when Groups 1 and 2 were taken out of the refrigerator where they had been kept, and were plated out, the majority of the seeds were found to contain bacteria.

Description of the Pathogen

The fungus most prevalent was found to be, Mucor racemosus. It was isolated from seeds and diseased plants continuously and plated out in pure cultures for identification. These cultures were eventually used as a source of inoculum for proof of pathogenicity. The pathogen's description follows rather closely the description given by Jensen (12), Naumov (13), and Zychia (20). In culture the fungus mycelium was at first white to yellowish white, but as the colony ages it turned brownish to yellowish brown. The colonies were of indefinite height. The mycelium was coenocytic, however in older hyphae there were some

crosswalls. Sporangiophores were rigid, erect, 5-40 mm. long, 8-20 u thick, and richly branched. According to Jensen (12), the side branches are sometimes branched, however, this was not observed. Furthermore, Zychia (20) states that the long sporangiophores on the edge of the culture may fall and become highly branched and this was not seen either. The sporangia were small and spherical, 43.7-75.4 u in diameter and varied in color from a very dark yellowish brown to black. In young cultures, the smooth, hyaline sporangial wall was deliquescent, whereas in older cultures the walls were broken.

The columella was hyaline with a somewhat regular form. However, according to Jensen (12), they may be pyriform to elliptical orbicular, or campanulate. According to Naumov (13), the sizes of the columella range between 14-60 u long by 7-45 u broad. The spores were hyaline to slightly yellowish and varied in shape from ellipsoidal, which seemed most prevalent, to globose or somewhat angled. The average spore size was 8.47 u long by 6.78 u wide.

The fungus is heterothallic according to Jensen (12). The zygospores, which were not observed, are globose, 70-85 u in diameter with a brown warty exospore. The suspensors are smaller than the spores (12). Chlamydospores were very numerous, being globose to oblong and measuring 13.0 u by 28.2 u. They were hyaline to yellowish with a smooth membrane.

Indirect Determination of Where the Pathogen is Located

By rinsing the seed with sterile distilled water and plating them out, it was ascertained that the fungus, Mucor racemosus, was present. However, since no attempt was made to sterilize the surface of the seed

at this time, it was impossible to determine if the fungus was located within the seed or simply on the seed. After showing the fungus was present by surface sterilizing the seed, rinsing in sterile distilled water, and plating them out, one could determine if the fungus was on the seed coat or within the seed coat. This was done with a group of seed from each seed lot with a .5% sodium hypochlorite solution containing Tween 20. Figure 1 shows the results from this set of experiments using stratified seed. From this graph it can be seen that the fungus was prevalent in the stratified seed. Seed Groups 1 and 2 were obtained after they had been stratified, so that the presence of the fungus in any unstratified seed of this seed lot is unknown.

In the stratified condition, Groups 1 and 2, which had been stratified on 7 January 1965 and 23 May 1965 respectively, showed 100% contamination when unsterilized. After surface sterilization, the seed showed 56% and 54% infestation respectively. This drop of 44% and 46% would indicate that over 50% of the seed in these groups were invaded internally. However, as previously mentioned, Groups 1 and 2 were from the same seed lot and, since the initial contamination or infestation of the unstratified seed was unknown, the spread of the pathogen during stratification of this seed lot cannot be ascertained.

Group 3 and 4 were from the same seed lot and Group 3 had been stratified while the seed in Group 4 was unstratified. By comparing Group 3 in Figure 1 with Group 4 in Figure 2, it can be seen that there was a small increase of 4% contamination between the surface sterilized, stratified Group 3 and the surface sterilized, unstratified Group 4. However, in the seed from both groups that was not surface sterilized, the stratified seed had 28% more infested seed than the unstratified seed.

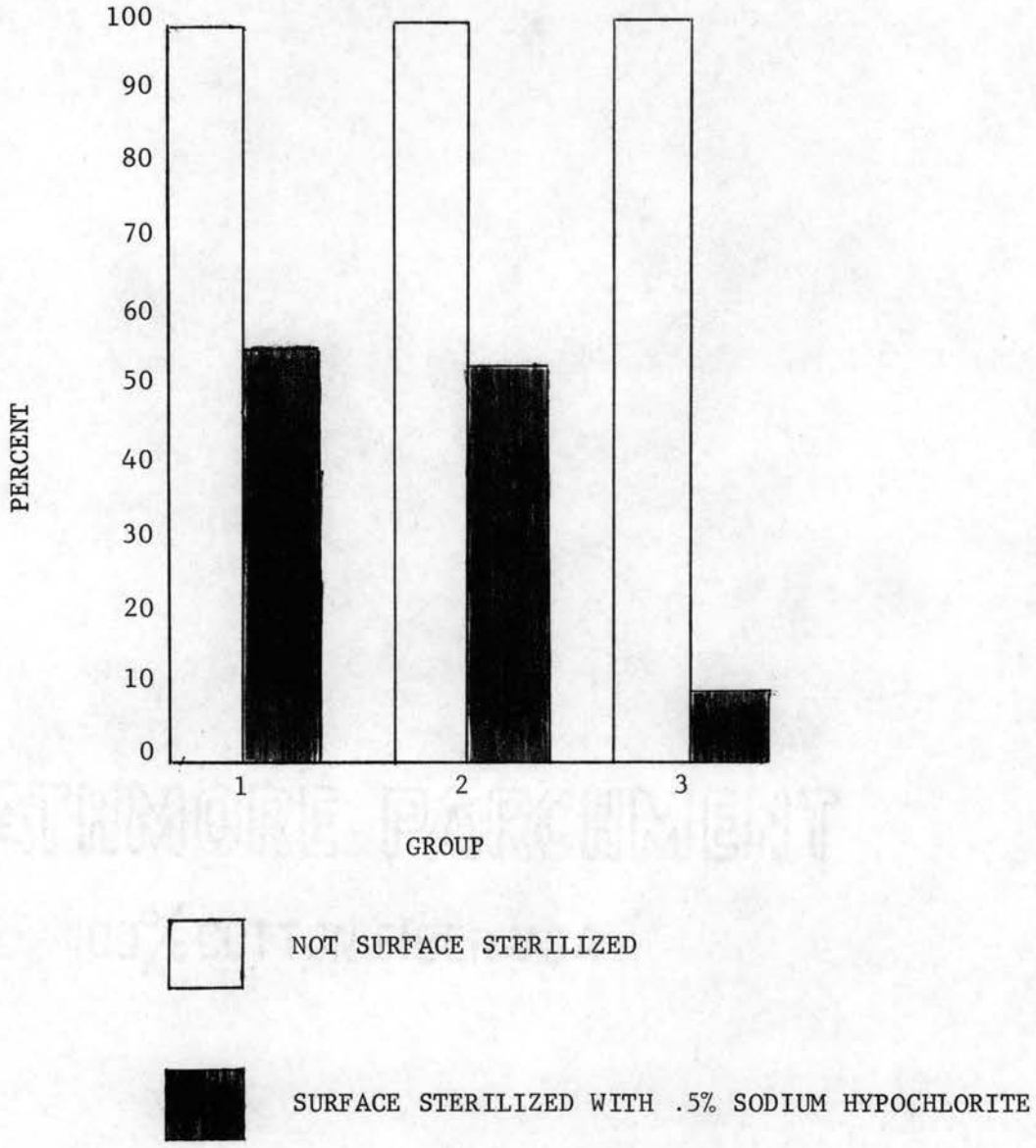


Figure 1. The Percentage of Stratified Shortleaf Pine Seed, Both Unsterilized and Surface Sterilized, Infested with Mucor Racemosus

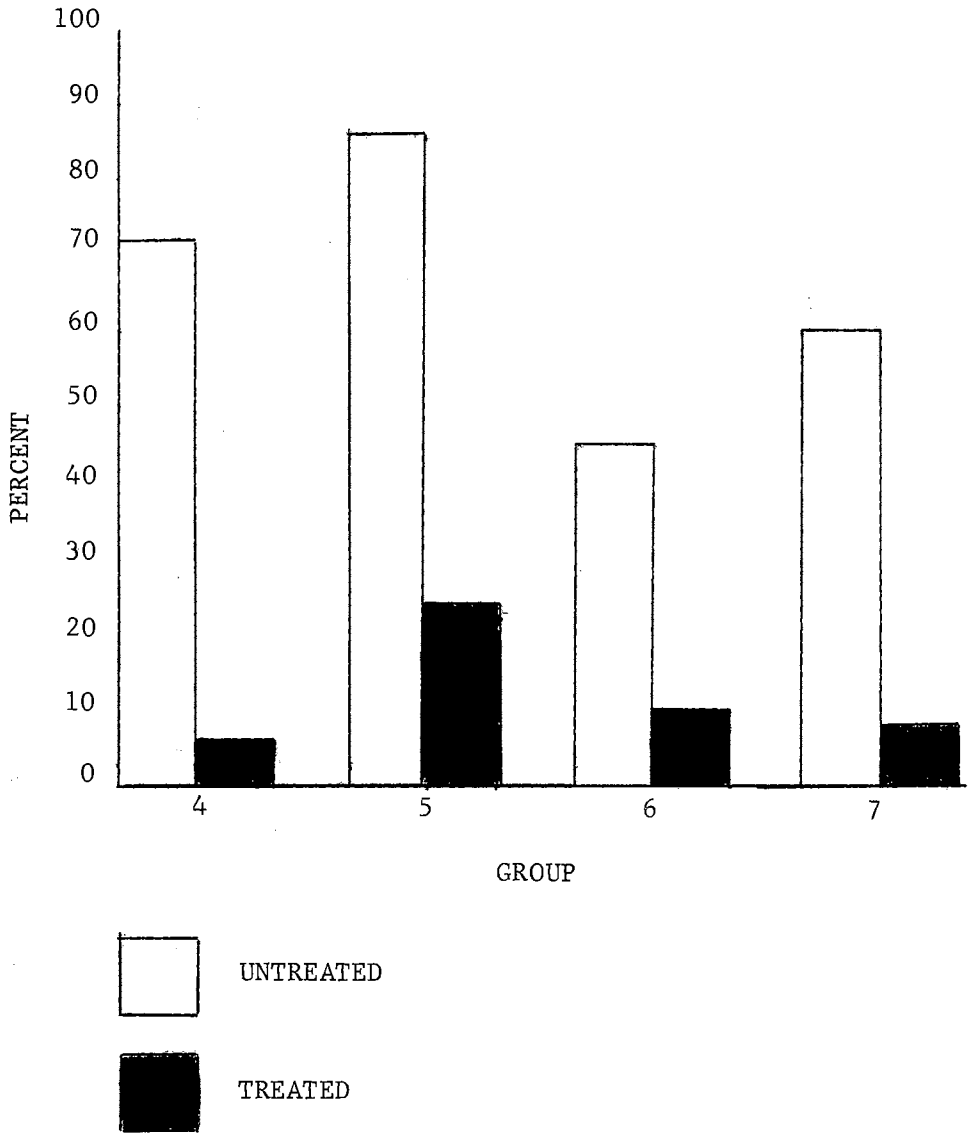


Figure 2. The Percentage of Unstratified Shortleaf Pine Seeds, Both Unsterilized and Surface Sterilized, Infested with Mucor Racemosus.

Figure 2 shows the infested and infected seed in four groups of unstratified seed. Group 4 shows that about 66% of the infested seed had the fungus only on the surface of the seed, whereas 6% carried the pathogen inside the seed coat. A total of 72% of the unstratified seed in this Group carried the fungus.

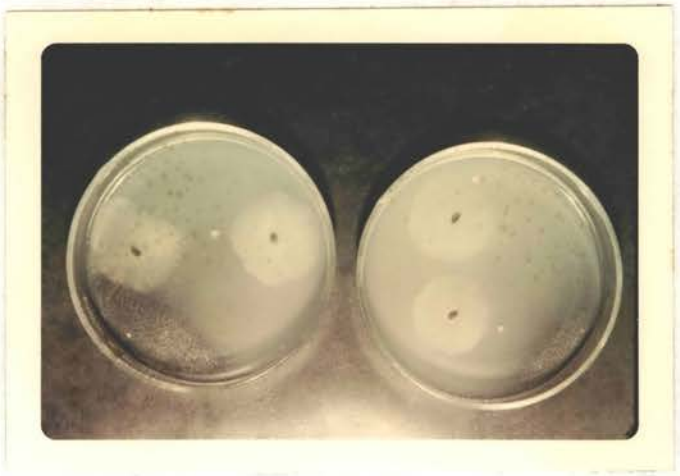
Group 5, which was the unstratified seed lot obtained in June 1966, showed the highest percentage of infestation and infection of all the unstratified groups tested. Of the unsterilized seed, 86% carried the pathogen, M. racemosus. However, since 24% of the sterilized seed carried the pathogen inside the seed coat, it can be deduced that 62% of the unsterilized seed carried the fungus on the surface.

Group 6, which was another unstratified seed lot obtained in June 1966, had the lowest percentage of infestation of the unsterilized seed examined. Only 35% carried the fungus on the surface, since 10% of the seed was found to contain the fungus inside the seed coat when the seeds were surface sterilized. Group 7, which was the third unstratified seed lot obtained in June 1966, showed 52% of the seed infested only on the surface of the seed coat, since 8% of the surface sterilized seed contained the fungus.

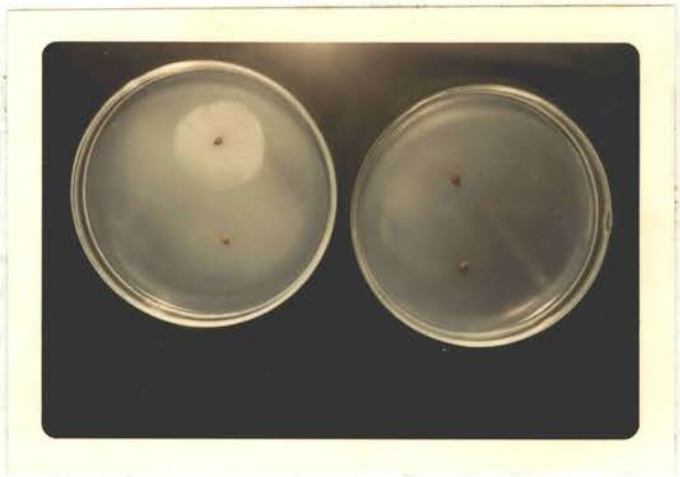
Figure 3 shows unstratified and stratified seed that was plated out on PDA showing the typical growth of the fungus.

Direct Determination of the Location of the Fungus in the Seed

Since the first set of experiments established by indirect methods that the fungus was located in the seed, it became necessary to locate the fungus by direct methods. This was accomplished by surface sterilizing seed and dissecting them aseptically. The dissected parts



SEEDS NOT SURFACE STERILIZED



SEEDS SURFACE STERILIZED

Figure 3. Seed Plated Out on PDA Showing the Typical Growth of the Fungus

were then plated out on PDA and the results are recorded in Table 1. The table shows the tissues or combination of tissues in the seed in which the pathogen was most prevalent.

TABLE I

TISSUES INFECTED WITH M. RACEMOSUS IN SURFACE STERILIZED SHORTLEAF PINE SEED

	Infected Seed			
Tissues infected	Group 1	Group 2	Group 3	Group 4
	%	%	%	%
Seed coat	24	18	2	2
Seed coat and aril	8	12	0	2
Seed coat, aril, and endosperm	8	10	2	0
Completely infected	10	4	2	0
Total % infected seed	50	44	6	4

It can be seen that the majority of the seed was infected solely in the seed coat. Group 2 was perhaps the most characteristic of all the groups tested because it characteristically showed the general trend of the fungus being less prevelant deeper in the seed. The total percentage of infected seeds that had been dissected agreed fairly closely with the percent infection in seed that had not been dissected but indicated internal infection when plated out after surface sterilization.

Figure 4 shows seed that had been dissected and the parts plated out on PDA showing the growth of the fungus.

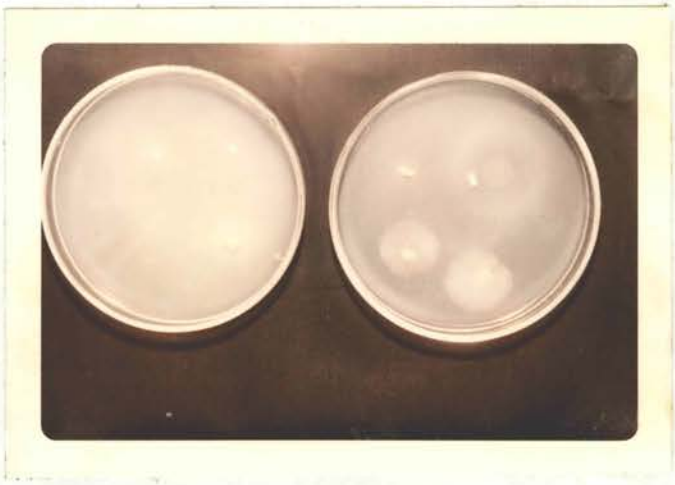


Figure 4. Dissected Seed Parts Plated Out on PDA Showing the Growth of the Fungus

Results of Dissected Known Infected Seed

Seed that had been previously surface sterilized and then plated out to prove that the seed was infected, were resterilized and then dissected. This was done to see if the tissues infected in seed not previously known to be contaminated, correlated with those tissues that were infected in seed that was previously known to be contaminated. Since the seed had been surface sterilized and still showed the fungus, by resterilizing, a check would be made on the sterilization techniques. All of the seed (Table II) that showed the pathogen to be present after the first sterilization also showed the fungus still present after the seeds were resterilized and then dissected. None of the seeds that did not show the fungus after the first sterilization showed the fungus after the second sterilization and dissection.

Tables I and II show that the unknown infected seed correlated with the known infected seed. For the three groups tested in Table II, an average of 76% of the seed was infected only in the seed coat. Fifteen percent were infected in the seed coat and aril, 7% in the seed coat, aril, and endosperm, and only 2% infected throughout all the seed tissues. In Group 5 none of the seed tested was infected throughout. In observing the three groups tested (Table II) and the average, it can be seen that the presence of the fungus decreases toward the inner tissues of the seed and the seed coat contains the pathogen more than any of the other tissues.

Pathogen Spread During Stratification

Seed from Group 4 was surface sterilized, plated out, and only the seed that showed no contamination or infection of any sort was

TABLE II

LOCATION OF M. RACEMOSUS IN THE TISSUES OF SHORTLEAF PINE SEED

	Infected Seed			
Tissues infected	Group 4	Group 5	Group 6	Average
	%	%	%	%
Seed coat	73	75	80	76
Seed coat and aril	14	17	12	15
Seed coat, aril, and endosperm	9	8	6	7
Completely infected	4	0	2	2
Total % infected seed	100	100	100	100

used in this series of experiments. These were stratified with seed from Group 3, that was known to carry the pathogen and had been marked with yellow paint. The seed was thoroughly mixed in a ratio of 3 uninfected seeds to 1 infected seed, then stratified for 30 days. Figure 5 shows the results of this experiment and Table III shows the results from the infected seed that was dissected.

It can be seen from Figure 5 that all of the unsterilized seed became contaminated following the stratification process. After surface sterilization, only 10% of the contaminated seed showed the fungus indicating that it was carried, for the most part, on the outer portions of the seed coat.

Surface sterilized seed that showed contamination was surface sterilized a second time, rinsed twice with sterile distilled water, dissected, and plated out on PDA. Table III shows these results. Almost 50% of the infected seed had the fungus solely in the seed coat

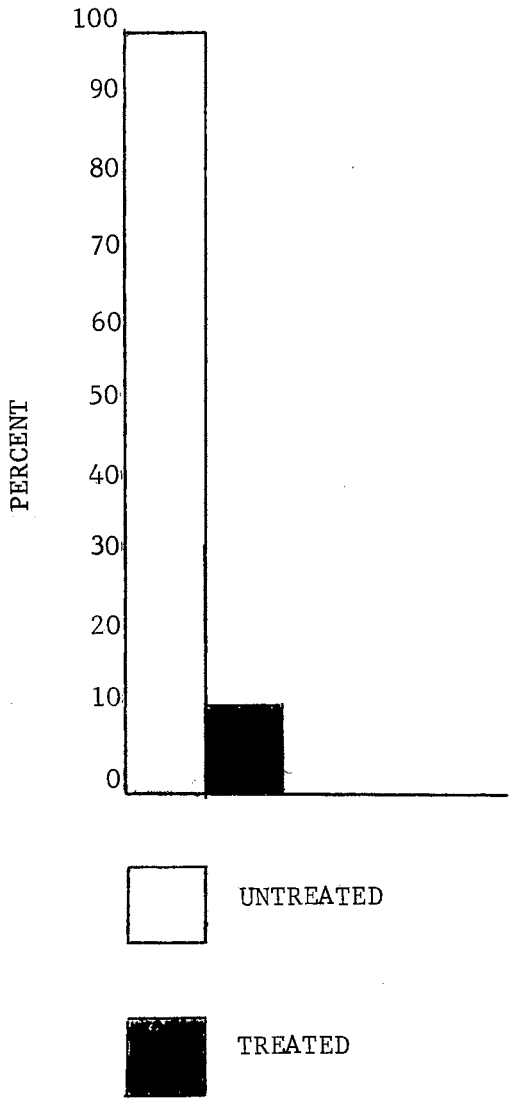


Figure 5. The Percentage of Shortleaf Pine seeds Contaminated Upon Stratification with Seeds Known to be Contaminated with Mucor Racemosus

and only 9% were completely infected throughout the seed. Again there was a decrease of the fungus toward the inner tissues of the seed.

TABLE III

SPREAD OF M. RACEMOSUS THROUGH TISSUES OF CLEAN SEED OF SHORTLEAF PINE, WHEN STRATIFIED WITH CONTAMINATED SEED

	Infected Seed
Tissues infected	Group 4
	%
Seed coat	45
Seed coat and aril	28
Seed coat, aril and endosperm	18
Completely infected	9
Total % infected seed	100

Proof of Pathogenicity

No previous reports of any of the Mucorales being the cause of damping-off have been found. The only reference found concerning a member of the Mucorales was by Hartley (9), in which he stated that Rhizopus nigricans Ehr. was not a causal organism of damping-off. The Mucors are collectively known to be mainly saprophytes with some being very weak parasites. This is the case with Mucor racemosus. In these experiments, the plants were kept in the greenhouse during the months of May, June, and July, of 1966. The temperatures during these days were extremely hot, ranging from 35-41 C during the day, to 23 C at night. Many of the young pine seedlings wilted during the day, but

revived at night under cooler temperatures. In this experiment two pots were inoculated and two were kept as controls and this treatment was repeated six times. Plants were periodically dug from both the control and the inoculated pots. Within 6-10 days, those plants that had been inoculated with the fungus showed typical damping-off symptoms. In the inoculated pots only the plants that damped-off produced a brownish colored, constricted lesion while a number of plants in the uninoculated control pots, produced lesions but did not damped-off. The lesion was attributed to the excessive heat and not to the damping-off. Upon plating out the damped-off seedlings, M. racemosus was obtained.

Figure 6 shows some of the healthy and damped-off pine seedlings. Figure 7 shows the damped-off seedlings plated out.

CONTROL

INFECTED

Figure 6. Healthy and Damped-off Pine Seedlings as They Occurred in These Experiemnts

Figure 7. Damped-off Pine Seedlings Plated Out on PDA Showing The Growth of the Fungus

CHAPTER V

DISCUSSION

It has been known for some time that it is possible for a damping-off pathogen to enter the soil as a seed contaminant. However, the time at which the pathogen comes in contact with the pine seed and the source of the fungus is unknown. Fisher (5) states that there is some opportunity for organisms to enter pine seed at one of at least three different times. These are (1) before they completely mature in the cone; (2) as a result of injury in the extraction process; and (3) because of improper storage after seed extraction. In this study, seed Groups 5, 6, and 7 were taken directly from the cold room storage where they were kept, plated out, and shown to contain the fungus. As the cold room was kept at -19 to -23 C, the probability of the fungus growing and spreading at these temperatures was remote, although the fungus did remain alive within the seed. It is assumed that sometimes between the maturing of the seed in the cone and storage, the fungus came in contact with the seed, so it was present by the time the seed was stored.

The pathogen was identified as Mucor racemosus Fres. In identifying this fungus, the characteristics followed rather closely the description given by Jensen (12), Naumov (13), and Zychia (20). The size of the spores, sporangia, columella, and chlamydospores matches their description, as well as did the color of the colony and growth habit.

The fungus was shown to spread during a 30-day stratification period from contaminated seed to previously tested clean seed. The latter was then surface sterilized and plated out and the seed that contained the fungus inside the seed was dissected and plated out.

The fungus predominantly was found to be on the surface of the seed, however, it was shown to have penetrated inside the seed coat of many seeds. In the seed, the fungus was found mainly in the layers of the seed coat, although it was found throughout some of the seeds. The occurrence of the fungus decreased toward the center of the seed indicating that it had entered through the seed coat and worked its way into the inner tissues.

In a study done by Hartley (8) on "Whitespot" injury, he found that, by subjecting plants to strong light and high summer temperatures, a lesion developed on the base of the stems and after eight days the plants toppled over and died. He further states that the lesion may extend up the stem. He put these plants in a moist chamber, and isolated Alternaria and Fusarium from the lesions. In the present studies, plants were set in the greenhouse during the high hot summer temperatures and inoculated with M. racemosus. In the control pots, all plants recovered from midday wetting whereas those inoculated with M. racemosus died. Plants that did not wilt from the heat either were not affected by the fungus or damped-off after 15-21 days. The fungus seemed to be a relatively weak pathogen, affecting mainly those plants that had been predisposed by the high temperatures. To what extent this fungus causes damping-off in nature is not known. However, based on the results of this study, unless seeds germinate at a time when they would be subjected to high temperatures, it should not be much of a problem.

In working on this study, two interesting side lights were noted. Some of the seeds tested were what Fisher (5) regards as "pops". This was seed that, either did not fill properly, or whose internal tissues were destroyed by some unknown agent. This seed could be distinguished by the fact that, after being stratified, if the seed was placed in water, the "pops" floated whereas most of the "good" seed sank. The fungus could not be isolated consistently from this seed, so no relationship could be determined.

In plating out to find uninfested seed, the darker colored seeds were found to have more fungus present than the lighter colored seeds. Whether the fungus had something to do with the darkness of the seed, or whether it was "attracted" to the darker seed is unknown.

CHAPTER VI

SUMMARY

1. Seed of Pinus echinata Mill., shortleaf pine, were plated out to find a seed-borne damping-off organism. Mucor racemosus Fres. was found to be the causal organism that induced the disease usually when the seedling plants had been weakened by high temperatures.

2. The fungus was found to be present on almost all the seed tested that had been stratified. Surface sterilizing with 0.5% sodium hypochlorite eliminated 60% of the infection indicating that only 40% of the seed was infected internally. Only 58% of the unstratified seed contained the fungus and only 12% was internally invaded.

3. Plating out dissected parts of infected seed showed the fungus predominately in the inner layers of the seed coat and evidence of the fungus diminished toward the center of the seed.

4. Stratifying clean seeds with known infected seed produced 100% infection. In the 30 day stratification period, the fungus penetrated the internal tissues of 11% of the seeds.

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