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The Effect of Some Soil Factors on the Infection and Severity of Fomes Annosus (Kr.) Karst. Root Rot on the Seedlings of Loblolly Pine (Pinus Taeda).

presented by

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has been accepted towards fulfillment
of the requirements for

M.S. degree in Forestry

Victor J. Rudolph
Major professor

Date October 16, 1978



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THE EFFECT OF SOME SOIL FACTORS ON THE INFECTION
AND SEVERITY OF FOMES ANNOSUS (Kr.) Karst.
ROOT ROT ON THE SEEDLINGS OF
LOBLOLLY PINE, PINUS TAEDA

By

Ezekiel Anwuchaepe Uche Umeokafor

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ABSTRACT

THE EFFECT OF SOME SOIL FACTORS ON THE INFECTION
AND SEVERITY OF FOMES ANNOSUS (Kr.) Karst.
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Fomes annosus is a nonhost-specific, soil-borne pathogen. The pathogen is more severe in thinned forest plantations, on some soil types and in forest plantations established on old agricultural land than in natural unthinned stands. This study investigated the effect of soil texture, soil reaction and soil micro-organisms on the infection of loblolly pine seedlings by F. annosus. The tests were made on sandy and clayey soils adjusted to pH 4.8 and 6.5. Three isolates of Trichoderma viride and four species of Trichoderma were screened for their antagonistic effect on F. annosus. Seeds of loblolly pine were aseptically planted in autoclaved soil, unautoclaved soil and soil autoclaved but later contaminated with T. viride. T. viride isolate myco 10088 was the most antagonistic of the Trichoderma species and isolates screened. There was a significantly higher ($P = 0.05$) infection rate in the sterilized soil than in the other types of soil. T. viride

isolate myco 10088 provided protection to loblolly pine seedlings against F. annosus throughout the duration of the study. In an additional study, soil samples collected in the Kellogg Forest from ten infected and uninfected spots 15 feet away were analyzed but showed no difference in characteristics.

To my mother, Mrs. Mgbugo Umeokafor,
who did not go to college but knew
the value of education and instilled in me
the need for and value of an education.

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Further thanks are due to Dr. Charles Cress for suggestions on statistical matters and to Dr. Raymond J. Kunze and Dr. Boyd G. Ellis of the Crop and Soil Science Department for their consultancy.

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CHAPTER I

INTRODUCTION

Fomes annosus (kr.) is a soil-borne pathogen that causes root and butt rot diseases in many trees. Sinclair (1964) reported that 80 species of conifers and 53 species of hardwoods are susceptible to Fomes annosus. Above ground symptoms of the disease seldom appear until the tree is virtually dead. Boyce (1961) listed some of the symptoms when apparent, as root rot, red rot, spongy rot, brown rot and butt rot. The pathogen attacks trees of all ages (Miller 1943), although contrary opinion (Boyce 1962) holds that it attacks only older trees and pole-sized trees. This disease causes considerable loss in Europe and North America.

F. annosus was reported first in the United States in 1923 by Hedgcock in North Carolina. Killing of slash pine (Pinus elliottii var elliottii) by Fomes annosus was first recorded in the Southeast in 1954 by Campbell and Hepting. Power, et al (1961), on their survey of planted and natural stands in three southeastern states for signs of F. annosus, have noted that the fungus was killing slash pines in 73% of the plantations examined. Berry and Dooling (1962) later surveyed the southern parts of

Missouri and found that this pathogen was present in shortleaf pine in many state and federal thinned pine plantations but not in natural unthinned stands.

Soil microorganisms are very complex. The fundamental knowledge of the factors contributing to this complexity is often the key to eventual development of soil borne disease control measures. Froelich, Dell and Walkinshaw (1966) considered soil pH, organic matter, and soil texture as variables most likely to influence either host resistance, root infecting fungi or soil inhabiting microorganisms that may compete with the pathogen. Other soil factors believed to play a role in F. annosus root rot development include soil moisture stress (Tower and Stambaugh 1968), and organic matter content (Froelich et al 1966; Rishbeth 1961). Similarly, Mook and Eno (1961), Peace (1936) and Powers and Boyce (1961) reported that the disease was more pronounced on former agricultural cropland than on forest land.

Knowledge of the ecology and biology of F. annosus as well as the characteristics of its host plants is necessary to develop effective control measures. It may well be that wide-spread fungistatic factors exist in the soil which inhibit the activities of F. annosus. It is likely that these fungistatic factors plus other numerous soil factors may obliterate the effect of F. annosus rather than destroy it in the soil. The removal of these inhibiting factors may allow the host species to be susceptible.

Similarly, microorganisms in the rhizosphere may exert a profound influence on the host plant by associative and antagonistic relationship, or actual parasitism.

The objectives of this study were to determine:

1. The effect of soil texture, soil microorganisms and soil pH on the incidence of F. annosus root rot on loblolly pine (Pinus taeda) seedlings.
2. The extent to which an isolated soil microorganism, Trichoderma viride, could provide protection to loblolly pine seedlings from Fomes annosus root rot.

It is hoped that the results of this investigation will be useful in developing risk rating and selecting sites for growing loblolly pine seedlings without mortality due to F. annosus.

CHAPTER 2

LITERATURE REVIEW

Soil Texture

Soil-plant root relationships that lead to root and butt rot disease development and its severity are influenced among other things by the soil texture, the hydrogen ion concentration (pH), the organic matter content, temperature, aeration, moisture and microorganisms. Froelich, et al (1966) considered the soil pH, organic matter content and texture as soil variables most likely to influence host resistance. They characterized severely damaged plots as having extremely deep sand and well drained soils (high hazard sites), while they generalized that heavy clay soils with a high water table are low hazard sites.

Alexander and Skelly (1973) studied disease incidence and severity in loblolly pine planted on two soil hazard types. They found that the sand content and large pores were directly related to infection. Small pores, clay and organic matter content were inversely related to infection. Morris and Frazier (1966) defined a high hazard soil as being well-drained and having a sandy top soil 10 inches deep or more. Kuhlman et al (1976) associated

disease severity with deep well-drained soils. These findings are in agreement with earlier work by Anderson (1921), who noted that the texture of the soil appears to influence greatly the mode of Fomes attack.

However, Jorgensen, Lund, and Treschow (1939) did not establish any definite correlation between the incidence of heart rot and soil type although they stated that the disease tends to assume a more virulent character on sandy than on clayey soil.

Soil pH

The influence of pH on the incidence and growth of Fomes annosus depends on the nature of the substrate (Treshow 1941). There are therefore varying pH ranges under which Fomes annosus thrives well. Treshow also reported no variation in the growth of the fungus within a range of pH 3 to 7 on filter paper in 3% malt extract. He obtained profuse growth on a sterilized acid spruce litter (pH 4.3) and on beech mold pH 6.3, but the fungus was unable to develop on pure mineral soil. When the pH of sterile forest humus was varied by the admixture of lime in amounts ranging from 2 to 20 gm. per 50 gm. of soil, no difference in the growth of the fungus was detected. Rennerfelt (1953) in his physiological and ecological experiment with Polyporus annosus (F. annosus) found that the growth of the fungus takes place between pH 2.9 and 7.0, and between 2° C and 32° C.

Weis and Nielsen (1928) investigated the growth of the root destroying fungus Polyporus radiciperda (F. annosus) on malt extract culture of pH value from 4.0 to 4.5, and found growth rate to be modified by temperature. Tilford (1936) found that the optimum pH value for F. annosus on malt agar at 20° to 40° C were 4.91 and 5.32, respectively.

The pH of a site in Britain on which F. annosus had caused considerable damage was found to be different from that of adjacent sites by Anderson (1921). He concluded that soil acidity is the dominant predisposing cause of attack. Hopffgarten (1933) found that the disease occurs on sites in Denmark with pH ranging from 4.6 to 6.0. This compares with pH ranges of 5.7 to 6.7 in California (Wagner and Cave 1946). Etheridge (1957) studied the relationship between site and decay in subalpine spruce in Alberta and noted that a pH less than 5.95 had a positive influence on the incidence of root and butt rot disease. Grainger (1946) reported that F. annosus prefers to grow on wood that remains fairly acidic at a pH of 5.0.

On the other hand, Rishbeth (1950) and Wallis (1961) demonstrated that higher infection rates occur on alkaline soils than on acid soils. According to them, alkaline soil has limited fungi colonies and this gives F. annosus an advantage over other competing microorganisms.

Soil Microorganisms

Soil microorganisms play an important role in the infection and severity of root-borne diseases. They may promote the activity of soil-borne pathogens (Rishbeth, 1951a and 1951b). Inoculation of plants with Fomes annosus in non-sterile soil has yielded poor results (Rishbeth, 1951b, Treschow, 1941 and Wallis 1961).

Sago and Cabb (1973) studied the influence of soil biological factors on the mycelial growth of F. annosus and found that it was dramatically inhibited by antagonistic soil microorganisms. Treschow (1941) reported the failure of Trametes radiciperda (F. annosus) to grow on acid (pH 4.2) forest humus from a 20-year-old spruce natural stand, but it developed satisfactorily on the same medium subjected to sterilization. Fomes annosus made no appreciable growth on autoclaved soil inoculated with Trichoderma species, but when he introduced Bacillus subtilis or Actinomyces species into the sterilized medium, the vigorous development of F. annosus was not impeded. Rishbeth (1951a) gave an account of the interaction in culture of F. annosus with strains of Trichoderma viride, many of which were antagonistic to the former. The growth of F. annosus has been found to be antagonized by soil microorganisms other than soil fungi. Missen (1956), working on Actinomycetes antagonistic to Polyporus annosus (F. annosus), demonstrated that a number of Actinomycetes isolated from

forest soil in Denmark proved that a number of Actinomyce-
tes proved to be antagonistic in vitro. Some Streptomyce-
tes species also suppressed the mycelial growth of the
pathogen in sterile garden soil. Bjorkman (1949) investi-
gated some soil antibiotics acting against the rot fungus
Polyporus annosus, and reported that a great number of the
soil mycelia tested on malt agar plates were completely
grown over by the F. annosus mycelium. Most of the over
grown mycelia belonged to Phoma, Cladosporium, and several
Actinomycetes species. However, he observed that some
parallel seems to exist between the occurrence of the
root-rot fungus and certain soil microorganisms without
antibiotic effect.

Huppel (1968) worked on the protection of coniferous
seedlings from attack by F. annosus and showed that 40% of
the isolates of soil fungi were characterized as mycorrhi-
zal fungi which provided protection to the roots of pine
seedlings. Dobbs and Hinson (1953) reported the presence
of widespread fungistatic factors in the soil. It is
likely that these fungistatic factors may be responsible
for the distribution of Fomes annosus on a particular site.

Adam, Witcher and Lane (1964) studied microorganisms
in soils from Fomes annosus infected pine stand and reported
that there were significantly fewer colonies of fungi from
the soil around infected trees than from soil around non-
infected trees. Manka (1968) attempted to classify myco-
flora of the soil environment (root, rhizosphere and litter)

into communities arranged into so-called "biotic series" to show the function of these communities in relation to F. annosus. Of the 15 species of fungus he isolated from the soil environment, Trichoderma glaucum showed the highest degree of biotic influence. Manka (1968) also found that there were more biotic influences in a healthy stand than in an infected stand and that communities of fungi were more abundant in the root and rhizosphere of the healthy stand.

Other soil factors

Other soil factors such as aeration, temperature, humus and moisture are also interrelated with the factors already discussed. In a similar way these factors are linked to the incidence of Fomes annosus. Tower and Stambaugh (1968) found that soil temperature was a significant factor in the longevity of F. annosus inoculum in the soil. They also found that more seedlings of loblolly pine die from Fomes infection when subjected to temporary wilting than in soil maintained at field moisture capacity. Hopffgarten (1933) observed that permanently moist sites are not invaded by the fungus in the absence of aeration, but infection is prevalent on temporarily damp areas.

Mode of Entry

The mode of infection and spread of F. annosus has been reviewed by Hodges (1969), who stressed that the

spread of the disease is accomplished by spores and by growth of mycelium. Evidence abounds that roots of freshly cut stumps and even living trees around them become infected by spores. On the other hand little experimental evidence is available on infection through above ground wounds. Besides, tree species differ in their ability to resist the attack of F. annosus through trunk wounds. Rishbeth (1951a) reported one case where F. annosus may have infected a suppressed tree through pruning wounds and eventually reached the root system. However, he did not succeed in inoculating cut branches of Scotch pine (Pinus sylvestris) with F. annosus. Witcher and Beach (1962) on the other hand reported infection initiated from pruning wounds.

F. annosus spreads from diseased to adjacent trees by root contact. Rishbeth (1951a) observed that the mycelium of the fungus passes directly from the bark of the infected roots to that of living roots. The work of Dimitri (1962) and Bassette, et al (1967) tends to implicate the presence of a toxin in the infection process. This toxin was named fomanosin, although it has not been possible to isolate it from infected wood.

CHAPTER 3

MATERIALS AND METHODS

Collection of Soil Samples

Two soil samples for green house studies were collected from two different sites to reflect differences in soil texture. The first site was located along College Road on the University Farms which has clayey soil. The other site was located next to the University Married Housing apartments along Mount Hope Avenue and has a sandy soil. The soil samples were collected with an auger to a depth of 10 inches.

Mechanical Analysis

The sand, silt and clay content of the soil samples were determined by the hydrometer method. Fifty grams of each sample were mixed with a dispersing agent consisting of 33.5 grams of sodium oxalate and 2.0 grams of sodium hydroxide in one liter of water. The soil sample was placed in a one-liter cylinder and the dispersing agent added until the whole volume was brought to 1000 ml. The cylinder was shaken vigorously, a hydrometer inserted, and readings taken after 40 seconds and one-hour intervals; the former gave the sand content in percent while the

latter gave the clay content in percent. The percent silt was obtained by adding the two readings and subtracting the total from 100. The clay loam soil has 32.4% sand, 35.2% clay and 32.4% silt, whereas the sandy loam soil has 72.5% sand, 15.0% clay and 12.5% silt.

Soil pH

The pH of the soil samples was obtained by mixing 20 grams of each soil sample with 25 ml. of 0.01 normal calcium chloride solution and after 10 minutes the pH was determined with a 9600 Zerometic pH meter.

The pH of the sandy loam soil was 7.2 and that of the clay loam 6.7. Two pH levels, 4.8 and 6.5, were selected for this study. To bring the pH of the soil samples to those levels, varying concentrations of hydrochloric acid (0,1,2,4,10,16,22,32,Me^{H+}) were mixed with 50 gms of the sample in seven 100 ml. beakers for each sample. These were left for one week, after which 20 mls. of distilled water were added to each beaker, stirred and the pH determined with a 9600 Zeromatic pH meter. The pH values against the concentrations of hydrochloric acid were plotted for the two samples (Figure 1). These curves were then used to adjust the two soils to pH 4.8 and 6.5.

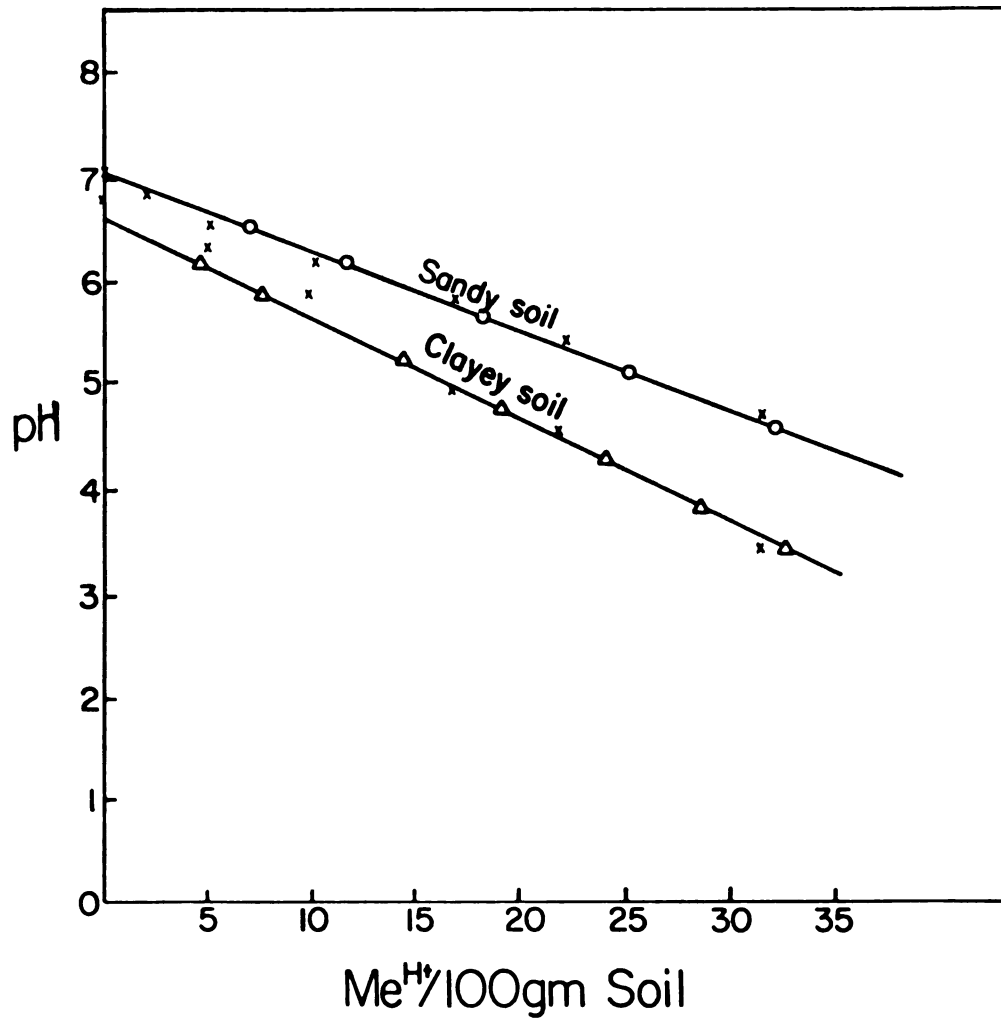


Figure 1. Amount of HCl acid required to adjust 50gms of soil to varying pH levels.

Isolation of *Fomes annosus* from Infected Roots

Cultural medium for the isolation of *Fomes annosus* from infected roots was prepared using the modification of Russell (1956), with O-phenylphenol medium as applied by Miller and Arthur (1966). Components of the cultural medium include 17 gm. agar, 20 gm. malt extract, 60 mg. O-phenylphenol, 100 mg. streptomycin sulphate, and 1.5 ml. of 50% lactic acid in 1000 ml. of distilled water. These components were added aseptically after the malt agar was autoclaved. *Fomes annosus* inoculum was obtained from the roots of Scotch pine within an infected pocket in the Carl G. Fenner Arboretum along Mount Hope Avenue in Lansing (Figure 2). The *Fomes annosus* isolate was obtained after two weeks (Figure 3) and was used in the inoculation of 2.0% of malt extract agar (MEA 2% malt extract, 2% agar, 2% dextrose, and 0.1% peptone in distilled water). This was used subsequently in the inoculation of test seedlings (Figure 4).

Inoculation

Fomes annosus isolate was grown in MEA at 25° C in the dark for 21 days. Then, one ml. of conidial mycelia suspension was harvested by flooding the inoculated 2% MEA plate with distilled water, stroking the surface, separating the suspension and diluting it with 100 ml. of distilled water. Ten weeks after germination, the seedlings were



Figure 2. Fomes annosus infected spot in the Carl G. Fenner Arboretum, Lansing, Michigan, from where the inoculum was obtained.



Figure 3. Isolation of F. annosus from infected wood.

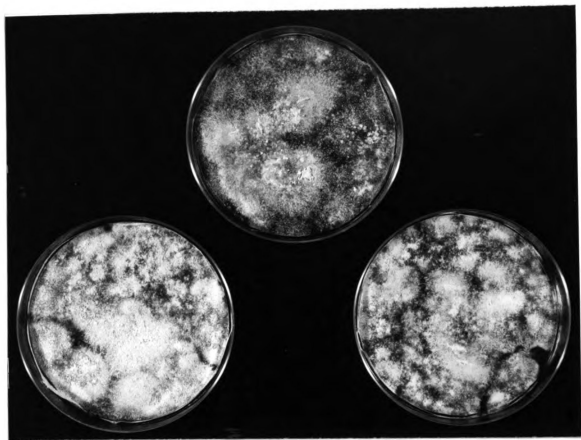


Figure 4. Oedocephalum lineatum a conidial stage of F. annosus on 2.0% M.E.A.

inoculated with 10 ml. of the above suspension.

Identification of the Fungus in Culture

The conidial state of F. annosus (Figure 4) has been called Oedocephalum lineatum (Bakshi, 1952). The fungus rarely forms basidiocaps in culture. For this reason, identification is based on the features of the conidial stage. The identification of the fungus was based on Nobels' (1965) method, and all tests performed on malt agar prepared according to the following formula: malt extract 12.5, and agar 20.0 gm. in 1000 ml. of distilled water. The fungus for identification was grown on the above medium in a petri dish and incubated for 4 weeks at 25° C in the dark, being brought out to the light for observations only. The test of the presence or absence of extra cellular oxidase was determined by dropping an alcoholic solution of guaiacum (0.5 gm. gum guaiac in 30 ml. of 95% ethyl alcohol) on the fungal mycelia mat. Blue color which appeared indicated the presence of extra cellular oxidase. The fungus has an oval conidia attached by a short sterigmata to a club-shaped conidiospore. The mycelial mat has a white color initially which changes to pale color with time. The fungus has thin-walled hyphae with simple septa. The rate of growth was slow and the petri dish was covered within 14 days.

Screening *Trichoderma* Species for Antagonistic Effects on *F. annosus*

Four species of *Trichoderma* and three isolates of *T. viride* from Mycological Service Amersta, Massachusetts (*T. viride* myco. 10043, *T. viride* myco. 10087, *T. viride* myco. 10088, *T. harzianum* myco. 10090, *T. koningii* myco. Ga) were each inoculated at one edge of four petri dishes. The opposite edge was inoculated with pieces of *F. annosus*. The medium was 2.5% MEA with a pH of 6.5. The control included only inoculates of one species or isolates arranged as above. The radius of each fungal growth was determined for 4 days (Table 1) and further observed for 21 days (Figure 5). Another medium was prepared but acidified with cactic acid and buffered with potassium sulphate (KH_2PO_4) to lower the pH to 4.8. The inoculation of this second medium was as above. On this basis isolate myco. 10088 was the most antagonistic and therefore was selected for the study.

Preliminary Studies

A pilot experiment was set up to determine soil moisture levels for the study. A given volume of water was used to irrigate equal amounts of soil samples. The water retained after a period of time was highly varied and it was observed that it is difficult to maintain a given moisture level and to have the water uniformly

Table 1. Growth Rate of Fomes annosus and Trichoderma Species and Isolates in mm of Radius for 4 Days in 2.5% MEA of pH 6.5.

Day	<u>F. annosus</u>	<u>T. viride</u> myco 10088	Day	<u>F. annosus</u>	<u>T. harzianum</u>
1	5	12	1	7	13
2	11	22	2	13	23
3	16	43	3	19	42
4	23	47	4	22	44

Day	<u>F. annosus</u>	<u>T. viride</u> myco 10087	Day	<u>F. annosus</u>	<u>T. Longibrachiatum</u> myco 9930
1	5	11	1	6	13
2	12	20	2	10	23
3	17	39	3	15	42
4	24	41	4	22	45

Day	<u>F. annosus</u>	<u>T. viride</u> myco 10043	Day	<u>F. annosus</u>	<u>T. koningii</u>
1	6	9	1	5	10
2	14	18	2	13	19
3	18	30	3	18	34
4	26	39	4	25	40

Day	<u>F. annosus</u>	<u>T. reesie</u>
1	4	10
2	12	19
3	18	30
4	25	34

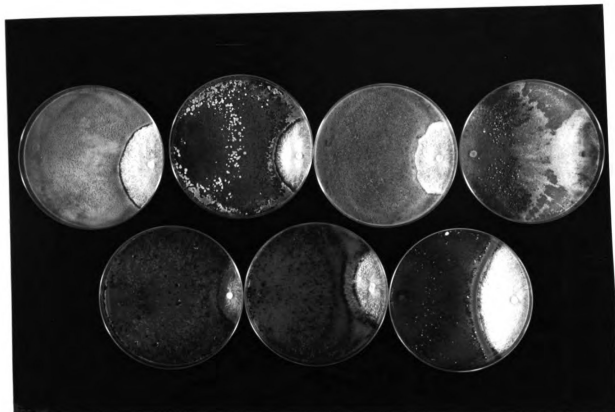


Figure 5. Growth of F. annosus and Trichoderma species after 21 days. Trichoderma on the left of petridish and Fomes on the right.

Top row: T. harzianum and F. annosus; T. longibrachaeatum and F. annosus; T. Koningii and F. annosus; and T. reesei and F. annosus.

Bottom row: T. viride myco 10078 and F. annosus, T. viride myco 10043 and F. annosus, and T. viride myco 10088 and F. annosus.

distributed. For this reason differing soil moisture levels were not used in the study.

In December 1977, a study was initiated to determine the length of period the soil sample would remain sterile under experimental conditions. For this purpose, six 125 ml. Erlmeyer flasks and six 38 mm. culture tubes, each containing 60 gms. and 30 gms. of soil samples, respectively, were autoclaved for two periods of 60 minutes each 24 hours apart. The irrigation of the soil was with sterile water. Small samples of soil from each container were tested for sterility every 14 days. After three months, the samples in the culture tubes still maintained their sterility.

Experimental Procedure

Loblolly pine seeds from an improved seed source in Georgia were surface sterilized by immersion and occasional shaking in 30% hydrogen peroxide for one hour. They were rinsed with sterile distilled water and stratified by soaking in wet, filter paper in petri dishes and kept at 30° F. in a cold room for 35 days.

The soil samples were thoroughly mixed and irrigated with water to slightly below field capacity. Samples were weighed into 38 mm. culture tubes. These were autoclaved for two periods each lasting 60 minutes 24 hours apart. The tubes were plugged with loose cotton

and covered with steel caps in the last autoclaving. Then 5 ml. of sterile malt extract in distilled water were added to each tube. The pH of the malt extract corresponds with that of the soil into which it was poured. The surface sterilized seeds were planted aseptically in the tubes which were covered as described above and left at 30° C for the duration of the study.

The medium to be treated with T. viride myco. 10088 was first sterilized and after two days was inoculated with T. viride. Two weeks later sterilized loblolly pine seeds were shown in the medium previously inoculated with T. viride and other media used in this experiment. Ten weeks after germination the seedlings were inoculated with Fomes annosus.

Field Study

Ten Fomes annosus infected spots were located in the Kellogg Forest near Battle Creek, Michigan, in red pine, white pine and Scotch pine plantations. A soil sample was taken within each infected spot and also in an uninfected spot adjacent to it. A soil core of approximately four inches in diameter was taken to a depth of ten inches on each spot. These samples were taken into the laboratory and analyzed mechanically and the pH determined with a 9600 Zeromatic pH meter.

Statistical Methods

The greenhouse experiment was a 3x2x2 factorial arranged in a completely randomized design with five seedlings per treatment and three replications. In all, 180 seedlings were used. Symptoms of root rot disease began to show ten days after inoculation. The number of days it took an infected seedling to die or show wilting symptoms was recorded and ranked for virulence as follows:

<u>Number of days after inoculation that death or wilting occurred</u>	<u>Ranking of the virulence of <i>F. annosus</i></u>
12	4
14	3
16	2
18	1
20	0

The ranked mortality data were subjected to an analysis of variance and multiple comparisons among means were made with the least significant difference test set at the 5% level.

CHAPTER 4

RESULTS AND DISCUSSION

Screening *Trichoderma* Species for Antagonistic Effects on *F. annosus*.

Trichoderma viride myco. 10088 was the most antagonistic of the three isolates of *T. viride* and four of *Trichoderma* species screened. Antagonism is based on the width of the bare zone between *F. annosus* and each of the other species or isolates of *Trichoderma* grown on 2.5% MEA in a petri dish for three weeks (Figure 2).

At pH 6.5, *Trichoderma* species and isolates showed slightly faster rate of growth on malt extract agar than they did on the same medium acidified to pH 4.8. On the other hand, *F. annosus*' rate of growth was higher on MEA of pH 4.8 than in one with pH 6.5. These differences may be partially attributed to the high temperature under which the fungi grew. This is similar to the observations of Rennerfelt and Paris (1953) on the physiology and ecology of *Polyporus annosus* (*F. annosus*), when they noted that the mycelium of *T. viride* overgrew that of *F. annosus* at 22° C but at 12° C *F. annosus* overgrew *T. viride*. Bjorkman (1949) worked on soil antibiotics acting against

the root rot fungus Polyporus annosus and he interpreted the presence of the bare zone between F. annosus and some soil microorganisms as an indication of antibiotic activity, even if only temporary.

Soil Sterility and Microorganisms

The number of seedlings infected and not infected by F. annosus are shown in Table 2, while Table 3 lists the severity of the disease based on the mortality ranking of each treatment.

Table 2. Number of Seedlings Infected and Not Infected by Fomes annosus

Soil pH		Sterilized		Unsterilized		Sterilized soil but treated with <u>T. viride</u>	
		Sandy loam	Clay loam	Sandy loam	Clay loam	Sandy loam	Clay loam
4.8	Infected	14	12	1	2	2	0
	Non Infected	1	3	14	13	13	15
6.5	Infected	13	13	1	1	1	1
	Non Infected	2	2	14	14	14	14

Table 3. The Severity of the Infection Based on the Sum of All Rankings for Each Treatment.

Soil pH	Sterilized Soil		Unsterilized Soil		Sterilized but treated with <u>T. viride</u>	
	Sandy loam	Clay loam	Sandy loam	Clay loam	Sandy loam	Clay loam
4.8	39	28	3	2	3	0
6.5	33	26	3	2	1	1

The number of seedlings in the sterilized medium killed by F. annosus was significantly greater ($P=0.05$) than those killed in the unsterilized medium and sterilized but later contaminated with T. viride (Table 4). There was no significant difference between the infection rate of seedlings in unsterilized soil and in sterilized soil later contaminated with T. viride. The high infection rate of F. annosus on loblolly pine seedlings under sterile conditions is probably due to the absence of antagonistic soil microorganisms. This absence of microorganisms gave F. annosus the ability to express its potential as a pathogen. The sterile condition may have eliminated fungistatic factors which are widespread in the natural soil.

The severity rating was also highest in the sterilized soil than in the unsterilized soil later contaminated with T. viride (Table 2). Seedlings on the unsterilized soil did not show any significant difference in either

Table 4. Mean Severity Rating of Infection for Media, Soil Texture and Soil Reaction.*

Main Effect	Mean Severity Rating
<u>Media</u>	
Unsterilized soil	9.67 ^a
Sterilized soil	0.83 ^b
Sterilized inoculated soil	0.42 ^b
<u>Soil Texture</u>	
Sandy loam	4.56
Clay loam	2.72
<u>Soil Reaction</u>	
Sandy loam	4.11
Clay loam	3.17

*"a" significantly different from "b" (P = 0.05). Statistical analyses performed on the CDC 6500 computer.

degree of severity or number of seedlings infected from those in Trichoderma contaminated soil (Tables 2 and 3). This is indicative of T. viride's ability to provide protection to loblolly pine seedlings from the attack of F. annosus. The ability to provide protection may be due to its toxic properties which according to Weindling (1932) are composed of two distinctly different substances, gliotoxin, which is active both as a bactericide and a fungicide, and viridin, exclusively a fungicide.

The low infection rate and low degree of severity of F. annosus root rot on unsterilized soil is due to a combination of factors. Perhaps the most important of these is the presence of soil microorganisms which are antagonistic to F. annosus. This is in conformity with Bjorkman (1949) who noted that some parallel seem to exist between the occurrence of the root-rot fungus and certain soil microorganisms.

Although temperature was not monitored in this study, there is every likelihood that temperature favored the competitive ability of T. viride particularly if one takes into consideration the high average daily temperature during the months of June to August.

Soil pH

The effects of soil pH together with other factors are shown in Tables 2 and 3. There is a slight difference between the severity of infection on pH 4.8 and pH 6.5. The severity of the disease tends to be greater on soils with pH 4.8 than on soils of pH 6.5. However, when the means were compared using the least significant difference, there was no significant difference between the means at both ($P = 0.05$) and ($P = 0.01$). It is likely that the range of pH chosen was so small that it did not show any effect on disease severity and infection rate. In nature, soil reaction may range from pH 3.4 in acid soil to 8.5

or more in alkaline soil. Some workers have shown that Fomes annosus grows favorably under a wide range of pH. Rennerfelt, et al (1953) stated that the growth rate of F. annosus takes place between pH 2.9 and 7.0. On 3% malt extract there was no variation in the growth of F. annosus within a range of pH 3.0 to 7.0 (Treschow 1946). He also did not detect any difference in the growth of the fungus when he varied the pH of sterile forest humus by adding lime ranging from 2 to 20 gm per 50 gm of humus soil. In all cases the growth rate of the fungus is indicative of its potential to cause root rot. Etheridge (1955) observed that all the isolates of F. annosus he investigated had a similar pH optimum for growth, 4.6 to 5.5. This optimum range falls within the range of pH used in this study. It is not surprising, therefore, that no significant difference in the effect of pH was detected.

Soil Texture

The degree of virulence was higher on sandy soil than on clay soil particularly within the sterilized soil lot. There was a significant difference (5% level) between the rate at which the seedlings planted on sandy soil died and the rate at which those on clay soil died. More seedlings died on the sandy soil than on clay soil. The sandy soil not only favored an easy penetration of the roots into the soil, but it also offered better growth

conditions to the fungus. Treschow (49) noted that the growth of F. annosus depends not only on the pH of the substrate but also on its nature. The puddling tendency of clay soil after long periods of autoclaving may have reduced the porosity of the clay soil to the point that growth of the fungus may have been slightly affected.

Field Study

The results of soil mechanical analyses and soil reaction of the infected and apparently healthy spots in the field are summarized in Table 5. There was no significant difference (5%) between % sand, silt and clay from diseased spots and healthy spots adjacent to them using the "t" test. Similarly there was no significant difference between their pH although there are few spots where the pH of the infected site was less acidic than those of the healthy spots. The infected spots may have initiated from inoculum left over on stumps of thinned trees or the stumps of cutover vegetation prior to plantation establishment. It is noteworthy to mention that 80% of the infected spots throughout the plantation occurred at a lower elevation or at the base of the hills where the soil was deeper than farther up the hills.

Table 5. Soil Data from Diseased and Adjacent Healthy Spots in the Kellogg Forest*

Spot	Diseased				Healthy			
	Sand %	Clay %	Silt %	Reaction pH	Sand %	Clay %	Silt %	Reaction pH
a.	65	10	25	5.1	59	14	27	5.0
b.	60	15	25	4.9	60	15	25	5.1
c.	64	12	24	4.8	64	13	23	4.8
d.	63	14	23	4.9	62	15	23	4.7
e.	64	13	23	4.7	64	14	22	4.7
f.	68	12	20	4.7	69	12	19	4.5
g.	65	15	20	5.2	65	15	20	4.9
h.	61	12	27	5.3	61	12	27	5.1
i.	68	13	19	4.7	69	13	18	4.4
j.	59	20	21	4.6	59	20	21	4.6

*Approximate distance between the points of sampling at each site was 15 feet. No significant difference between % sand, silt and clay (5%).

CHAPTER 5

SUMMARY AND CONCLUSIONS

The objective of this study was to determine the effect of some soil factors, namely texture, pH, and isolated microorganism Trichoderma viride on the incidence and severity of F. annosus root rot on loblolly pine seedlings, and to determine the extent to which the isolated case of soil microorganism could provide protection to loblolly pine seedlings from F. annosus infection.

Three isolates of T. viride and four species of Trichoderma were tested with F. annosus conidial mycelium on a petri dish containing 2.5% MEA. The fungi were placed opposite each other on the plate. The bare zone between them was the basis for the selection of the most antagonistic of the Trichoderma isolates and species. Trichoderma viride myco 10088 was selected as the most antagonistic because it showed the broadest bare zone. Two soil samples typical of clay loam soil and sandy loam soil were collected and their mechanical properties confirmed by mechanical analysis. The pH of these soils was determined with a 9600 Zeromatic pH meter and thereafter adjusted to pH levels 4.8 and 6.5.

The soil samples were divided into three parts, one autoclaved, the second unautoclaved and the third autoclaved but later contaminated with T. viride. Surface sterilized and stratified seeds of loblolly pine were aseptically sown in these soils. Ten weeks after germination the seedlings were inoculated with conidial mycelia suspension of F. annosus. The numbers of seedlings that died or wilted were recorded.

Soil samples were collected from ten infected spots and apparently uninfected spots fifteen feet away from them. The soil samples were analyzed in the laboratory for mechanical properties and soil reaction.

The soil texture and soil pH levels tested here do not tend to have much influence on the incidence of the disease, probably because a very narrow range of pH was used. More important to the direct effect of soil texture and pH on the fungus is the effect of these factors on the seedlings. Any site which stresses the seedlings or trees eventually leads to susceptibility to F. annosus infection.

It is noteworthy to observe that T. viride was able to provide protection to loblolly pine seedlings under the experimental conditions. The high mortality rate of seedlings grown on sterilized soil may partially be due to the absence of mycorrhiza forming fungi as a result of prolonged autoclaving. Huppel (1968) has demonstrated that certain mycorrhiza forming fungi provided protection to

pine seedlings from F. annosus attack.

The interaction of T. viride with other soil microorganisms and their antagonistic effects are not well known. Therefore the possibility of using the aggressive and antagonistic features of T. viride as a method of control or selecting suitable soil media for raising seedlings of loblolly pine is subject to further investigation. A very important point is that changes in soil temperature may render T. viride inactive and thus put F. annosus to an advantage. From the ecological standpoint, the temperature of the soil may be a selective factor determining the microorganisms present on a particular site at any given time.

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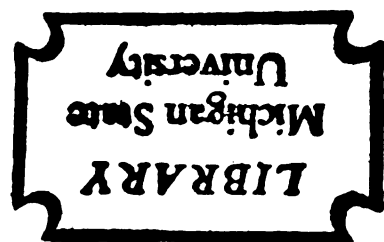
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