STUDIES ON WHITE PINE SEEDLING ROOT ROT

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Jerry William Riffle

1959

STUDIES ON WHITE PINE SEEDLING ROOT ROT

By

JERRY WILLIAM RIFFLE

An Abstract

Submitted to the College of Science and Arts
Michigan State University of Agriculture and
Applied Science in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1959

Approved Joseph C Stroy

AN ABSTRACT

The purpose of this investigation was to determine the causal organism of a root rot disease of white pine nursery seedlings at the U. S. Forest Service Chittenden Nursery in Wellston, Michigan. Efforts were made to demonstrate pathogenicity of a suspected pathogen, to extract and study the population of nematodes from soil and from white pine roots, and to study diseased root sections of white pine seedlings microscopically through the preparation of permanent slides of diseased root tissue.

Although various microorganisms were isolated on PDA medium from white pine root tissue plantings showing various degrees of root rot, Fusarium was the principal microorganism isolated, representing 30.34 percent of all tissue plantings made.

Three Fusarium species, namely F. solani, F. oxysporum, and F. moniliforme, were used in greenhouse inoculation tests. Three series of greenhouse tests were made using Fusarium species to infest the soil in which white pine seedlings and seed were planted.

Fusarium was the principal microorganism reisolated from the infected plants in the greenhouse. The percentage recovery of Fusarium due solely to the deliberate infestation with Fusarium in the first greenhouse series was 52.63 percent. The corresponding figure for the second greenhouse series was 60.26 percent. The mix-

ture of the five Fusarium isolates (FMF) was the most pathogenic inoculum used in the greenhouse tests. The percentage recovery from the infected white pine seedlings in this treatment was 89.72 percent. Fusarium oxysporum was the most pathogenic separate inoculum used, for the percentage recovery of this microorganism from infected plants was 80.80 percent.

Population studies of nematodes reveal that many nematodes were present in the soil and plant samples collected throughout the diseased areas of the nursery. More nematodes were extracted from the nursery soil than from the woodsplot soil or from the soil of a white pine plantation located near the Chittenden Nursery. Since root rot only occurs in the nursery beds, the evidence suggests that nematodes may play a part in the root rot disease, although their exact role is not known.

Nematodes were used to infest the soil in the third greenhouse series. Fusarium and Verticillium species were also used as inoculum in this series, but the latter was not pathogenic. The percentage recovery of Fusarium in this greenhouse series was 33.33 percent.

Histological studies of sections from diseased white pine roots revealed that fungus hyphae were present in the root epidermis and cortex of the diseased seedlings.

STUDIES ON WHITE PINE SEEDLING ROOT ROT

By

JERRY WILLIAM RIFFLE

A THESIS

Submitted to the College of Science and Arts
Michigan State University of Agriculture and
Applied Science in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1959

Stro
advi
Dons
acsl

Dr.
Sta
the
were

Sny
Cal
wer

of
equ

Per
and

ere
cer
".

ACKNOWLEDGEMENTS

The author is very grateful to Professor Forrest C. Strong and Professor M. C. Strong for suggestions and advice given throughout this investigation, and to Dr. Donald J. deZeeuw and Professor Putnam W. Robbins for assistance and criticism of this manuscript.

The cooperation and assistance which was given by Dr. Ralph L. Anderson of the Lake States Forest Experiment Station, and by Mr. Edward D. Clifford, Superintendent of the Chittenden Forest Nursery, during this investigation were greatly appreciated.

The author is very grateful to Dr. William C. Snyder, Professor of Plant Pathology at the University of California, who identified the five Fusarium isolates which were used in this study.

Special thanks is extended to Dr. John A. Knierim of the Entomology Department for his advice and for the equipment used in the nematode studies.

Lastly, the author wishes to thank all other persons not mentioned above who have offered suggestions and aid pertaining to this problem.

These research studies were supported in part by a grant of $2400 from the U. S. Forest Service. In addition, certain supplies and labor funds were furnished by the U. S. Forest Service.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

LIST OF PLATES

strob

this

highe

this

perc

whit

resi

plan

Fore

stri

fore

caus

tain

of o

and

blis

rest

of a

whic

thei

why

seed

are

seed

bedg

INTRODUCTION

From a commercial standpoint, white pine, Pinus strobus L., is considered the most valuable species of this type in the Lakes States Region (22). It has the highest board foot value of any conifer species grown in this region. At the Chittenden Nursery less than ten percent of the total plantings at the present time are white pine, the balance consisting of red pine, Pinus resinosa Ait. Approximately 250,000 white pine transplants are transplanted yearly on the Manistee National Forest. One of the serious limiting factors that restricts an increased number of white pine outplantings in forestation areas is the white pine blister rust disease, caused by Cronartium ribicola Fisch. The cost of maintaining Ribes free areas prohibits an increased percentage of outplantings of white pine. Ribes species, currants and gooseberries, are the alternate hosts of white pine blister rust fungus. Still another limiting factor that restricts the outplanting of white pine is the possibility of attacks by the white pine weevil, Pissodes strobi Boh., which deforms the trees and seriously interferes with their growth rate. These factors, and others, explain why less than ten percent of the total production of seedlings at the present time at the Chittenden Nursery are white pine. Since 1934, a serious loss of white pine seedlings has occurred in the seedbeds and the transplant beds of the Chittenden Nursery, Plate II. Death of the

seedlings follows what appears to be a gradual dying of the roots characteristic of the root rot disease of the white pine seedlings. This disease condition does not affect red pine seedlings grown in the same seedbeds with white pine seedlings nor does the disease continue to kill white pine after they are transplanted into a plantation. Eastern white pine is the main species affected by the disease. Although the disease has also been found on white spruce, Picea glauca (Moench) Voss, and black spruce, Picea mariana (Mill.) B.S.P., it causes very little loss to these two species.

A planting was made in a newly cleared area in the oak woods adjacent to the nursery in order to determine if a root rot pathogen capable of affecting white pine seedlings was present, Plate I. This area near the south border of the nursery was cleared in the fall of 1954. The seedbed, four feet wide and twenty-five feet long, was sown with white pine seed in the spring of 1955. In the fall of 1956, a crop of seedlings was removed and transplanted into the main nursery to see if they would be affected with the root rot disease. The woodsplot area was immediately resown with white pine seed. No case of root rot has originated from the woodsplot since its establishment. Lack of root rot in this area may be due to a different composition of microflora coupled with the fact that it is a virgin area as far as a nursery

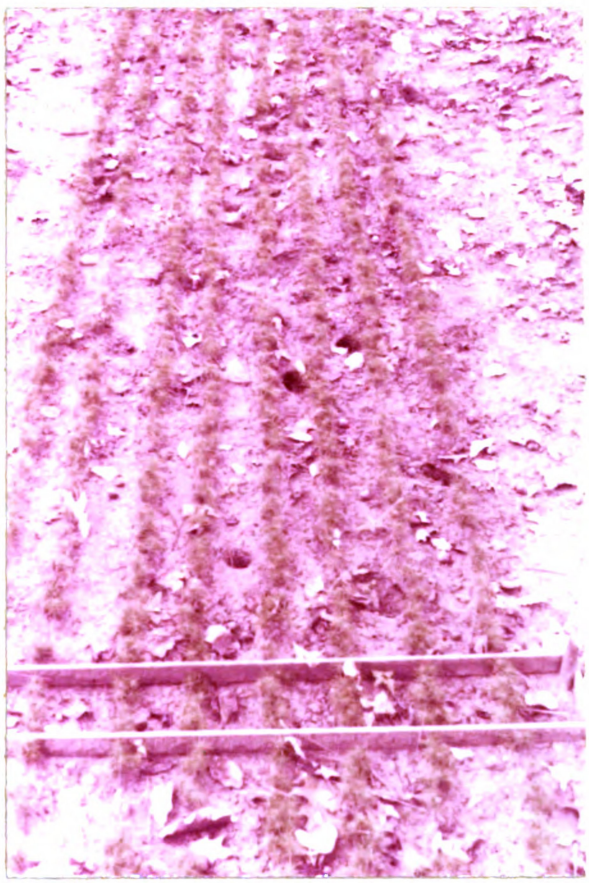
seedb
remov
nurse
bili
imme
tive
on t
the
has
othe
pine
rot
the

den
the
At
In
oth
dis
tra
ag
was
in
The
of
los

seedbed is concerned. The second crop of seedlings was removed in the fall of 1958 and transplanted into the nursery as before in order to determine their susceptibility to the root rot pathogen. The area was to be immediately resown with white pine seed. It is tentatively planned to continue growing white pine seedlings on the area to determine how long it will remain free of the root rot disease. The fact that the root rot disease has not developed in the woodsplot area supports the other observations made on surveys of outplanted white pine plantations. Very few white pine succumb from root rot after they are outplanted on planting sites within the forest.

A review of the production records of the Chittenden Nursery since its establishment in 1934 shows that there have been consistent losses in the white pine beds. At first these losses were attributed to white grubs (2). In 1943, it was found that white pine were dying from some other cause than white grubs. In 1948, the root rot disease was recognized in the 2-1 and 2-2 white pine transplants, and a large number of trees were lost in both ages of stock (2). The possibility of white grub injury was investigated, but there was no evidence of such injury on the majority of the transplants that had died. The root rot disease was extremely serious in 1949. Most of the 1-0 white pine seedlings were killed and heavy losses occurred in the 2-0, 2-1, and 2-2 beds (2). In

PLATE I



Portion of the woodsplot area where no root rot occurs.

1950, 250,000 white pine seed were sown and from this thirty-six percent were available for transplanting and twenty percent for shipping.

It was learned that the W.W. Ashe Nursery in Mississippi had suffered heavy losses of their southern pines from a root rot disease. It was found that if the nematodes present at the nursery were controlled, the root rot disease failed to develop. Samples of 2-1 and 2-2 white pine from the Chittenden Nursery were sent to Dr. G. Steiner, principal nematologist at the Plant Industry Station at Beltsville, Maryland, in 1949. He found several nematodes, most of which were different from those at the Ashe Nursery. In a letter to Mr. E.D. Clifford, Superintendent of the Chittenden Nursery, dated August 3, 1949, Dr. Steiner said that in general the nematode complex was quite similar to the one seen at the Ashe Nursery. Additional samples of stock were sent to Dr. G. Steiner in 1950, and he found the same nematodes in these samples as in the past and a few additional ones. (Table XIII). The genera found were Alphelenchus, Alphelenchoides, Acrobeloides, Ditylenchus, Dorylaimus, Metaphelenchus, Metarhabditis, Tylenchus, Plectus, Panagrolaimus, Acrobeles, Diplogaster, and Seinura. The Aphelenchus and Panagrolaimus genera were the most numerous and both are considered to be decay nematodes (2).

Dr. Gerald Thorne, a noted nematologist, visited

the Chittenden Nursery in 1950 with Dr. Curtis E. Dieter, of the Dow Chemical Company, and collected soil and plant samples. They found several nematodes in the samples, of which the following are able to cause injury to plants: Leptonchus granulosus, Tylenchorhynchus dubius, Aphelenchoides sp., Ditylenchus sp., and Tylencholaimus proximus (Table XIV) (2).

Dr. Berch Henry, from the Ashe Nursery, visited the Chittenden Nursery in the spring of 1950. He and others established experiments employing the use of soil fumigants and insecticides in an attempt to control the root rot disease. The disease was not generally serious in 1950, and results of the experiments showed that the fungicide and insecticide plots failed to give any indication of control of the disease (2). During 1951, Dr. Henry grew red pine and white pine in infected areas at the Ashe Nursery. Both species proved to be susceptible to the Ashe root rot, while only the white pine seemed to be susceptible to the root rot found at the Chittenden Nursery.

Since 1952 various experiments have been carried out in an effort to control the root rot disease. Soil fumigants have been tested on a large scale, and some tests have been made with fungicides, insecticides, and fertilizers. Results of tests as of 1956 by the Lakes States Forest Experiment Station involving over 500,000 white pine seedlings indicate the following (1):

1. Methyl bromide[a] applied at the rate of two pounds per 100 square feet, supplemented with a Parzate (zinc ethylene bisdithiocarbamate) foliage spray, has increased production of seedlings by as much as 150 percent over that of untreated beds. Methyl bromide alone and methyl bromide plus Fermate (ferric dimethyl dithiocarbamate) sprays also show large increases in production, Plate IV.

2. Formaldehyde (forty percent commercial) applied at 1.5 ounces per square foot, or about three times the dosage generally recommended for damping-off control, has given excellent control of the root rot disease, equal to or better than methyl bromide, Plate III.

3. The use of methyl bromide and formaldehyde has resulted in heavier seedlings even though there was a denser stand in these beds.

Thus the white pine root rot problem at the Chittenden Nursery has received some attention for several years. With the cooperative help of the nursery staff, the Dow Chemical Company, the pathologists and nematologists of the Plant Industry Station, and the Lakes States Forest Experiment Station disease research personnel,

[a] Methyl bromide (ninety-eight percent methyl bromide plus two percent chloropicrin) contributed by the Dow Chemical Company, Midland, Michigan.

several experiments have been conducted in an effort to find satisfactory control measures. At the present time, methyl bromide applied at the rate of 1.5 pounds per 100 square feet, and formaldehyde, applied at the rate of 1.5 ounces per square foot, are the most effective of the various chemicals used to control the disease.

Before fumigation of the soil with methyl bromide was adopted, at least ninety percent of the white pine stock going out of the nursery was affected with root rot ranging in severity of infection from light to heavy. White pine seedlings exhibiting extreme infection were culled. In the last two years, through the use of methyl bromide, this percentage has been greatly reduced. It was estimated by Mr. Clifford that less than five percent of the white pine stock which left the nursery in the fall of 1958 was affected with root rot.

In 1954 a survey of three white pine plantations which were planted in 1952 revealed that the average loss of white pine was ten percent. Two percent was entirely accountable to root rot, and the other eight percent was due to transplanting. It was found some time later that death of white pine due entirely to root rot does not occur after two years in the field, although death may result from a combination of root rot and transplanting injury. After the third year in the field, death due to the combination of root rot and transplanting does not occur.

The above observations offer an interesting comparison when related to outplantings with diseased stock at the Ashe Nursery by Dr. Perch Henry. Data in a letter from Dr. Henry to Dr. Curtis May, principal nematologist of the Bureau of Plant Industry, dated September 12, 1949 indicated that of the outplantings made from 1947 experimental plots at the Ashe Nursery, the first year survival of "plantable" slash pine seedlings, Pinus elliottii Engelm, was not adversely affected by the root rot. The survival of longleaf pine, Pinus palustris Mill., after one year in the field, ranged from fourty-four to ninety-two percent. Dr. Henry indicated that Dowfume W-40, applied at the rate of thirty-two gallons per acre, 2.5 to 5 weeks before spring sowing, has given satisfactory root rot control of slash and longleaf pine seedlings in the Ashe Nursery for two consecutive years.

It has become apparent that the cause of the root rot disease at the Chittenden Nursery must be determined so that better control methods can be applied. Studies were initiated in June 1957 to determine the actual cause of the root rot disease, and they were continued through August 1958. These studies were divided into three phases, which are the isolation and reisolation of micro-organisms from diseased white pine seedlings, the extraction of nematodes from plant and soil samples for population studies, and the histological studies of sections of diseased white pine seedlings.

Description of the disease

Root rot occurs on white pine nursery stock of all ages, from 1-0 seedlings to 2-2 transplants, throughout the nursery. It is common to find healthy and affected trees in all stages of decline intermixed in the white pine beds of the nursery. The aboveground symptoms of the root rot are chlorosis and stunting of the foliage, and a stunting of the tree itself. The stunting and chlorosis of the foliage appears to progress from the top of the seedling downward. The color of the foliage changes from a dark bluish green to a color ranging from a pale green to a light yellow. The color change starts on the extreme tips of the needles and advances toward their base. Later the needles droop, dry up, and turn brownish red, from the tip downward. The seedlings die in an upright position after the bark and cambium are killed by the pathogen, Plate VI.

The small rootlets rot first, followed by the lower portion of the primary root, Plate V. Some of the lateral roots on the healthy appearing trees have root rot symptoms. The cortex is sloughed off from the affected lateral roots as they become well decomposed with the advance of the disease. Before the cortex is sloughed off, the epidermis turns from a light tan to a black color. This color change is probably due to the breaking down of the root tissue by the pathogen. Free hand cross sections

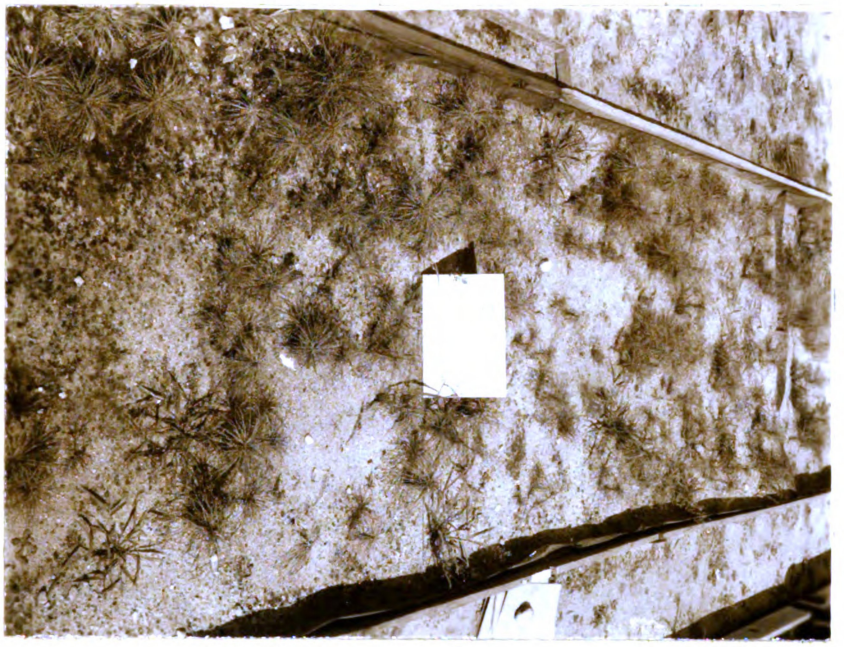
made at points where the epidermis is blackened reveal a light brownish discoloration of the woody tissue. The root rot progresses from the young tissues of the root toward the older tissues, and proliferation occurs above the rotted portions of the lateral roots. Later, portions of the lateral roots and primary roots completely slough off leaving only a small part of the root system. Trees at this stage of decline are sustained by a few lateral roots restricted to the upper few inches of the soil.

PLATE II

Nursery control plots showing 2-0 white pine seedlings.[a]

[a] Black and white photograph courtesy of Dr. Ralph L. Anderson of the Lakes States Forest Experiment Station.

PLATE II



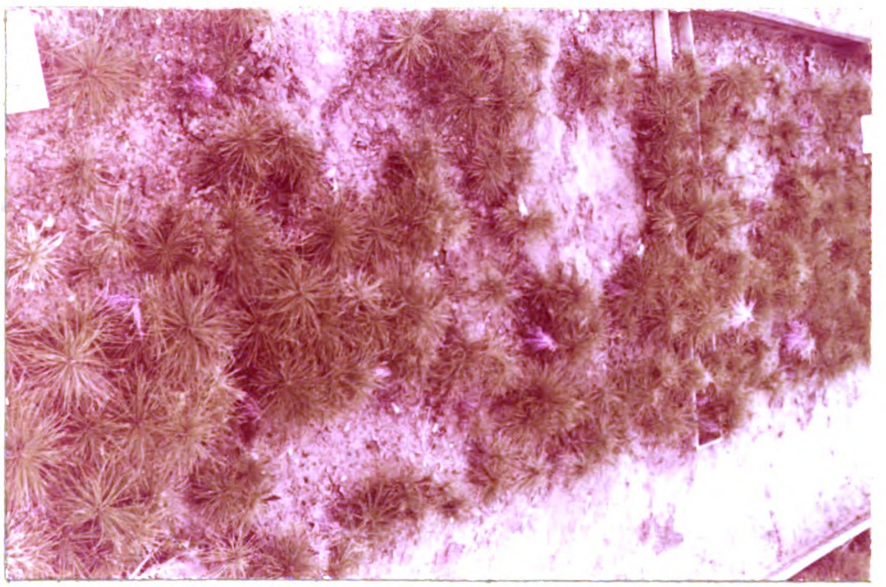


PLATE III

Nursery plot containing 2-0 white pine seedlings in which the soil was treated with formaldehyde (forty percent commercial) at the rate 1.5 ounces per square foot.

PLATE IV

Nursery plot containing 2-0 white pine seedlings in which the soil was fumigated with methyl bromide at the rate of two pounds per 100 square feet.[a,b]

[a] The darkened areas in the foreground of the colored photo are shadows.

[b] Black and white photograph courtesy of Dr. Ralph L. Anderson of the Lakes States Forest Experiment Station.

PLATE IV

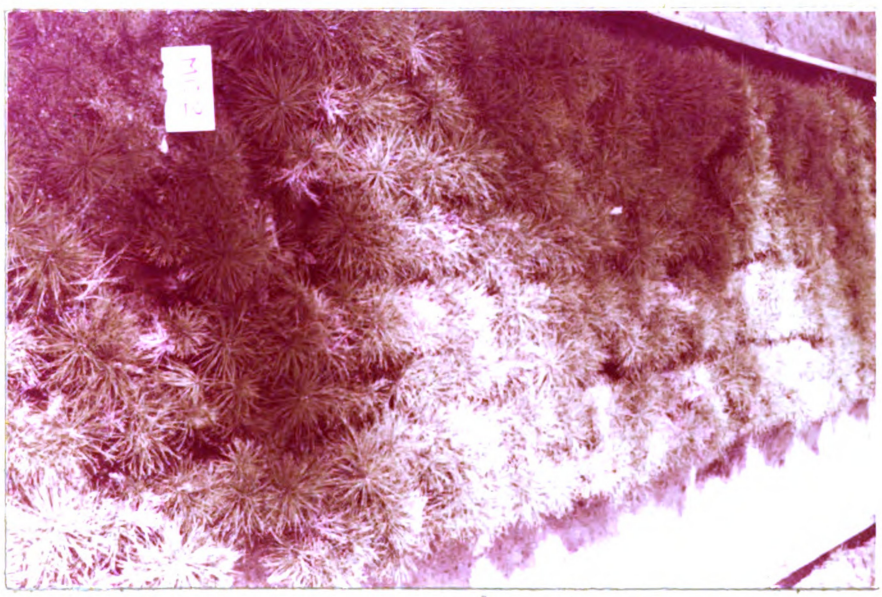
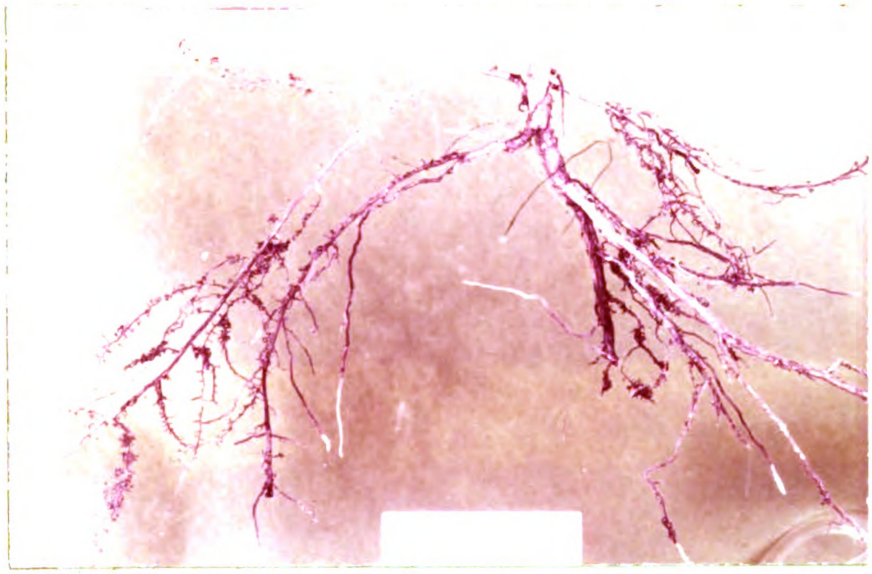


PLATE V



Root rot symptoms on a 2-2 white pine transplant.

PLATE VI

Stages of root rot symptoms on 2-2 white pine transplants. Healthy transplant on left, diseased transplant in middle, and dead transplant on right.[a]

[a] Courtesy of Dr. Ralph L. Anderson of the Lakes States Forest Experiment Station.

REVIEW OF LITERATURE

Many early workers have found that Fusarium species were pathogenic on pine seedlings. Inoculation experiments by Spaulding (17) established that some strains of Fusarium were parasitic and caused damping-off of pine seedlings. Fusarium moniliforme Sheldon was one of the more virulent species. A few years later Gifford (5) repeatedly isolated a species of Fusarium capable of causing damping-off and root rot from the roots of two-year-old white pine seedlings. The findings of Hartley et al (8) indicated that Fusarium solani (Mart.) Appel & Wr. was a weak parasite on jack pine, Pinus banksiana Lamb., while the most destructive of the species of Fusarium tested was F. moniliforme. Variable results were obtained by Rathbun (15) using strains of Fusarium species involved in a root rot of jack pine and red pine, root rot being more severe in the Fusarium inoculations than in the controls. Eliason (3) found that white pine and white spruce were intermediate in susceptibility to a Fusarium root rot. In this study there was a definite relation shown of buckwheat residue to root rot. The root rot was more severe in the nursery areas which were successively sown for three or more years with buckwheat as a cover crop. Tint (18) demonstrated that resistance to invasion of Fusarium oxysporum Schlect. in seedlings of red pine increased directly with increasing age of the seedlings. Various other workers have reported

damping-off of pine seedlings caused by Fusarium (10, 19, 21).

A Fusarium has been found in what appears to be a nematode-fungus complex in the Ashe Forest Nursery in Mississippi. Longleaf pine, slash pine, and loblolly pine, Pinus taeda L., were susceptible to root rot (14). In plots most severely affected, fifty percent of the loblolly pine, ninety percent of the spring sown longleaf pine, and ninety-five percent of the slash pine died. It appeared, from the survival in the nursery and the survival in the field, that the root rot affected spring sown longleaf pine most severely, loblolly pine next, and slash pine the least. Fusarium, two other species of fungi, and representatives of at least ten genera of nematodes were found. Strong evidence indicated that the cause of the disease was a nematode-fungus complex, since application of the nematocidal fumigant ethylene dibromide to the seedbeds at the rate of twenty-four gallons per acre of a twenty percent by volume solution two to three weeks prior to spring seeding gave excellent root rot control. No phytotoxic effects resulted from this rate of application (9).

Nematologists have established the fact that parasitic nematodes are associated with the roots of all the major southern pine species (7). A parasitic nematode identified as Criconemoides rusticum (Micoletzky) Taylor

was associated with the deterioration of the fine roots and mycorrhiza of one-year-old potted shortleaf pine seedlings, Pinus echinata Mill. (12). Hopper (11) made a survey of the plant parasitic nematodes in the soils of thirty-five southern forest nurseries and found that Meloidodera and Tylenchorhynchus were the only nematode genera found to be directly associated with seedling injury. Tylenchus was frequently associated with root rots but was not considered responsible for this injury.

Root-knot nematodes have been found in connection with Fusarium wilt of mimosa, Albizzia julibrissin Durazz (6). More wilting of mimosa occurred when the soil was infested with Fusarium oxysporum f. perniciosum (Hepting) Toole and Meloidogyne incognita (Kofoid & White) Chitwood or M. javanica (Treub) Chitwood in combination than in soil infested with Fusarium alone. The nematodes alone reduced the size of the mimosa seedlings.

MATERIALS AND METHODS

Isolation and identification of microorganisms

The isolation and identification of microorganisms from white pine seedlings showing symptoms of the root rot disease was initiated in June 1957 at the U.S. Forest Service Chittenden Nursery at Wellston, Michigan. Working on the premise that a soil inhabiting fungus might be the cause of the root rot, tissue plantings were made from the roots of white pine seedlings in various stages of decline. The nursery stock selected for tissue planting was brought into the laboratory located on the nursery grounds. The roots were thoroughly washed in flowing tap water in order to remove excess soil and other foreign matter. Roots showing early stages of browning were selected and cut into sections one-quarter to one-half inch in length.

Seventy percent ethyl alcohol was used as a surface sterilizing agent. Each root section was immersed in this solution for thirty to sixty seconds and then aseptically transferred into a bath of sterile distilled water for one minute before planting aseptically with forceps on sterile medium in a Petri plate. Five root sections were planted in each plate. The principal medium used was potato dextrose agar (PDA) acidified with twenty-five percent lactic acid. The tissue planted plates were then labeled, wrapped in wax paper to prevent aerial contamination, and incubated at room temperature. These plates were observed

macroscopically three to six days after plating.

The colonies growing from each tissue planting were identified to genus as far as possible by examining them on microscope slides under a compound microscope. The fungi that did not sporulate were transferred to PDA slants and attempts were made to identify them approximately one to two weeks later. All transfers were made from the outer edge of the young, actively growing colonies. After identification, the cultures were transferred to PDA slants and maintained in the refrigerator. The frequency of microorganisms isolated in this study is shown in Table I.

Greenhouse inoculation technique

Since Fusarium species appeared consistently and predominantly throughout the isolation studies conducted during the summer of 1957 (Table I), it seemed desirable to test the pathogenicity of these organisms. Five Fusarium isolates, numbers 33, 42, 46, 49, and 62 were selected at random from the numerous collection of pure culture isolates of 1957. The source of these isolates is shown in Table II. The five isolates were grown on two different media. One medium consisted of cornmeal plus vermiculite and the other was a synthetic liquid medium.

The cornmeal plus vermiculite medium was prepared by mixing 130 ml. (by volume) of yellow cornmeal in a one

liter Erlenmeyer flask with 130 ml. each of water and vermiculite. These materials were stirred with a glass rod until thoroughly mixed. Six of the flasks thus prepared were then autoclaved for twenty minutes at fifteen pounds pressure, allowed to cool, and each flask was planted with one of the selected Fusarium pure culture isolates. One flask was left unplanted for use as control inoculum.

The synthetic liquid medium was prepared by adding the following material to one liter of distilled water in a sterile Erlenmeyer flask: 7.2 grams sucrose, 3.6 grams glucose, 1.23 grams $MgSO_4$, 2.72 grams KH_2PO_4, and 2.02 grams KNO_3 (16).

Ten liter flasks, each containing 200 ml. of medium, were prepared. The flasks were autoclaved for twenty minutes at fifteen pounds pressure and allowed to cool. Five flasks were then planted each with one of the selected Fusarium pure culture isolates. Five flasks were left unplanted, three of them for control inoculum and two for use in suspending the mycelial mats when they were macerated in the Waring blendor. The inoculum used for the first greenhouse series was allowed to grow on the medium for eighteen days.

The pure sand which was to be infested with the inoculum was obtained from a sand pit located approximately five miles east of the Chittenden Nursery. The sand was collected in metal containers at a depth of thirty-five

feet below the ground level, and it was considered free of organic matter and reasonably sterile. Danger of introducing the root rot pathogen from this source was considered to be negligible because of the very low organic content of the sand. The sand was brought to East Lansing and placed in newly constructed flats in the Plant Science Greenhouse. The inoculum, consisting of the contents of one Erlenmeyer liter flask, was dumped on the sand surface, broken into small pieces, and mixed into the sand by hand. After thoroughly mixing the inoculum into the sand, the sand surface was leveled and a new metal divider was placed in the flat crosswise to separate it into two equal parts. One-half of the flat was planted with one hundred white pine seeds from the 1956 crop, fifty seeds per row. The other half of the flat was planted with fifty woodsplot seedlings collected at the Chittenden Nursery and transported to East Lansing in sphagnum moss in plastic bags. These seedlings had been planted in the fall of 1956 in a woodsplot adjacent to the south side of the nursery proper. No root rot has occurred in this plot, but some of the seedlings from it that have been planted in the nursery developed root rot.

These woodsplot seedlings were transplanted into greenhouse flats as quickly as possible. Fifty seedlings, five rows of ten each, were planted in each flat. The flats were put in four inch deep metal pans and watered from below. The soil in the rest of the flats receiving

inoculum grown on cornmeal vermiculite was infested in the same manner. The check flats were prepared first in order to avoid contamination from the other flats.

The synthetic liquid medium was decanted from the mycelial mats of the five Fusarium isolates into a liter flask and was used for one treatment. Unplanted synthetic liquid medium served as a control treatment. The mycelial mats of the five Fusarium isolates, which were grown on the synthetic liquid medium for eighteen days, were suspended in 400 ml. of unplanted synthetic medium in a Waring blendor. The mycelial mats were macerated for one minute.

After the greenhouse flats were planted with one hundred white pine seed and fifty woodsplot seedlings, the mycelial fragments and spores suspended in the liquid medium were sprayed on the surface of the sand in the flat with a rubber bulb sprayer. The same inoculation procedure was used for the 600 ml. of unplanted liquid medium control and for the decanted liquid from the mycelial mats.

The first greenhouse series consisted of ten flats as follows:

1. Unplanted liquid medium check (PLM)
2. Unplanted cornmeal vermiculite medium (CCV)
3. Control - no medium or inoculum added (CNM)
4. Decanted liquid from the five Fusarium mycelial mats (LFM)
5. Five Fusarium mycelial mats macerated in sterile liquid medium (FMM)

6. Fusarium isolate 33 - Cornmeal vermiculite medium
7. Fusarium isolate 42 - Cornmeal vermiculite medium
8. Fusarium isolate 46 - Cornmeal vermiculite medium
9. Fusarium isolate 49 - Cornmeal vermiculite medium
10. Fusarium isolate 62 - Cornmeal vermiculite medium

Periodic counts were made on the number of seed germinating and on the number of seedlings dying. When the seedlings looked as though they were starting to decline or become discolored, they were removed and used in attempts to reisolate the original Fusarium infested into the soil. Some of the seedlings were collected for histological studies.

The second greenhouse series was begun in December 1957. The same procedures and inocula previously employed were used with the following exceptions:

1. The inoculum was allowed to grow on the two types of media for forty-three days.
2. The liquid from the five Fusarium mycelial mats was discarded.
3. The same type of sand was used except that it was steam sterilized for two hours.

4. The white pine seeds were surface sterilized in 1:3000 $HgCl_2$ for five minutes and rinsed in flowing tap water for twenty minutes.
5. The flats were watered from above.
6. Even though the white pine seed were subjected to the standard cold treatment, they germinated very poorly in the flats of this second greenhouse series. Therefore, an additional one hundred seeds of the 1957 crop were surface sterilized as above and planted in these flats.

The second greenhouse series consisted of ten flats as follows:

1. Unplanted liquid medium check (PLM)
2. Unplanted cornmeal vermiculite medium (CCV)
3. Control - no medium or inoculum added (CNM)
4. Control - no medium or inoculum added (CNM)
5. Five Fusarium mycelial mats macerated in sterile liquid medium (FMM)
6. Fusarium isolate 33 - Cornmeal vermiculite medium
7. Fusarium isolate 42 - Cornmeal vermiculite medium
8. Fusarium isolate 46 - Cornmeal vermiculite medium

9. Fusarium isolate 49 - Cornmeal vermiculite medium

10. Fusarium isolate 62 - Cornmeal vermiculite medium

The flats of the first and second greenhouse series were fertilized once with Folium, a completely soluble fertilizer. Two gallons made at the recommended rate of one ounce per gallon were used. Also one application of the insecticide dieldrin was applied.

A third greenhouse series, using artificially infested soil in metal cans, was begun in May 1958 for the purpose of demonstrating a possible nematode-fungus complex. The soil used in this series was obtained from the root rot infested white pine beds at the Chittenden Nursery. The soil was put in no. 10 metal cans and steam sterilized for one and one-half hours.

Three Fusarium isolates, namely numbers 33, 42, and 62, were grown on cornmeal vermiculite for forty-four days. The series consisted of the following treatments:

1. Unplanted cornmeal vermiculite medium (CCV)
2. Control - no medium or inoculum added (CNM)
3. Nematodes only
4. The three Fusarium isolates only
5. Nematodes plus the three Fusarium isolates
6. The Verticillium isolate only

Each treatment was replicated four times. Fusarium

was mixed into the soil by pouring enough soil to fill eight no. 10 metal cans into a fiberglass container and mixing uniformly into this soil the three Fusarium isolates. The Verticillium isolate was mixed into the soil in the same manner. After the fungus inoculum-soil mixture was placed in the no. 10 cans, ten woodsplot seedlings were planted in the soil of each can.

In the tests involving nematodes[a], five times the normal load of nematodes present in the soil was added to each can. Care was taken to see that the soil in these cans remained moist so that the nematodes would not die from dessication.

Nematology

Samples of affected white pine stock and soil samples were collected from infested areas for the purpose of extracting nematodes. The root systems of the affected plants were carefully lifted from the soil with a spade. Most of the soil samples were collected with a soil stab auger from areas representing different age classes of the white pine nursery stock. Soil samples were collected from the upper layer of the soil in which the root systems of the white pine seedlings were growing. Each sample consisted of approximately one quart of soil. The soil stab auger was wiped with a clean cloth after taking each

[a] See pages 29-33 for extraction of nematodes.

sample in order to reduce contamination among the samples. The soil auger was sunk into the soil four times per plot, and a total of three plots represented one sample. The plant and soil samples were collected in polyethylene bags, and care was taken to see that they were kept in a moist condition and were not allowed to become overheated.

Extracting nematodes from pine root tissue

The affected plants were taken to Dr. Knierim's nematology laboratory where the root systems were carefully washed in flowing tap water to remove the excess soil adhering to the roots. The roots were then cut into pieces approximately one-half inch long. The sections were put into a Waring blendor with a small amount of water and macerated for two minutes. The sample was washed from the blendor with a small stream of water into the inner plastic basket of the two cloth bottomed plastic baskets supported in the inverted bottle which was half full of water, Plate VII. This arrangement allows the nematodes to settle out into the culture tube which is attached to the neck of the inverted bottle by a short piece of rubber hose. The cloth bottoms of the plastic baskets stop the residue from entering the culture tube. The sample remained in the inverted bottle for three days, during which time the culture tube containing the nematodes was changed daily.

After the nematodes were collected in the culture tube, water was drawn off until one-half ml. remained, and

the t

for s

tion

nega

up a

(36.

tive

Extr

fest

sam

eno

sam

all

sus

mor

th

an

s

co

th

p

a

t

n

t

the tube was then immersed in a hot water bath at 145°F. for sixty seconds to kill the nematodes. A fixing solution was then added to the culture tubes to preserve the nematodes for later study. The fixing solution was made up as follows: eight parts water, one part formaldehyde (36.8%), and enough glacial acetic acid so that the fixative solution would contain two percent acetic acid.

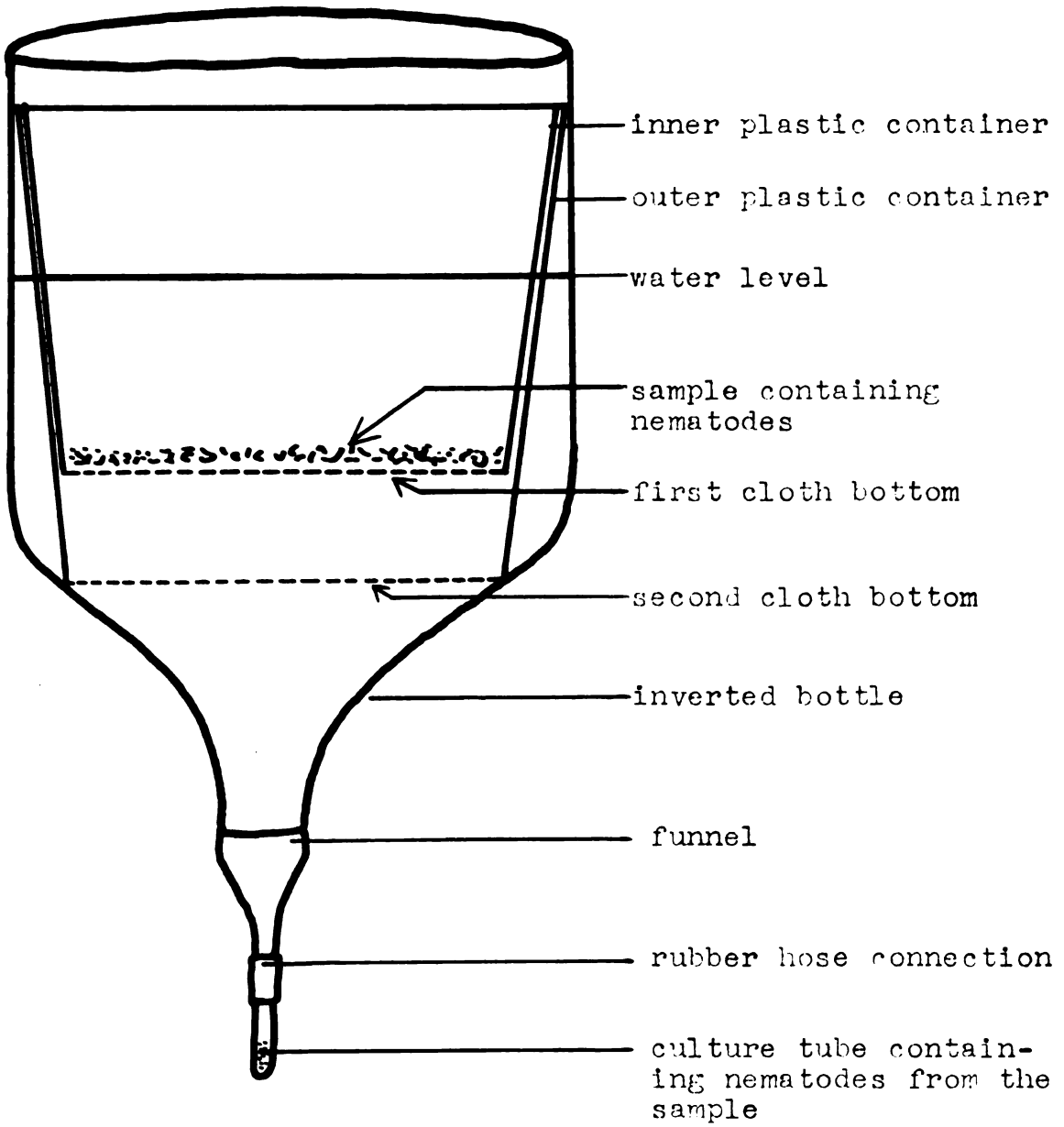
Extracting nematodes from soil samples

The soil samples which were collected from infested areas were taken to the nematology laboratory. A sample was placed into a plastic container containing enough water to thoroughly cover the soil sample. The sample was then stirred and worked with the fingers until all the soil lumps were pulverized and the nematodes were suspended in the water. The soil was allowed to settle momentarily. The water was then decanted from the soil through a screen having openings of 140 microns into another plastic container. More water was added to the soil, and the sample was swirled again. Then the water containing the nematodes from the soil sample was poured through the screen into the other plastic container. This procedure of adding water to the soil, stirring the soil, and pouring the water containing the suspended nematodes through a screen into the other container was repeated nine times for each sample. The water in the other plastic container which contained the nematodes in suspension

was then poured through another screen having an opening of forty microns. This screen allowed water to pass through but retained the nematodes. The nematodes were washed from the screen into a container and then into a container in the inverted bottle, Plate VII. This washing containing the nematodes remained in the inverted bottle for 24-72 hours, at which time the culture tube at the base of the inverted bottle was removed. The nematodes in the culture tube were taken to the greenhouse, and the soil containing white pine stock was now infested with nematodes by making two openings in the soil with a knife and pouring the nematodes into these openings.

For the purpose of nematode population studies, soil and plant samples were collected from the nursery. A total of seventy-four samples were collected, eighteen of which were plant samples and fifty-six of which were soil samples. The soil samples were processed by placing each sample into a plastic container and mixing it thoroughly by hand. A 250 ml. soil sample from each collection was placed into the inverted bottle without processing it through the screens, Plate VII. It was not considered necessary to process the soil samples through the series of screens for the population studies alone. The culture tube at the base of the inverted bottle was changed every twenty-four hours during a three day period. The samples were replicated three times. The container used to scoop up the soil was washed in flowing tap water

PLATE VII



Diagrammatic sketch of inverted bottle used to collect nematodes from soil and plant samples.

after handling each sample in order to reduce factors which would give inaccurate population counts. The plant samples were processed in the same manner as described previously.

Microtechnique studies

In order to determine early progress of the pathogen in the host, 106 outwardly healthy plants were collected from the inoculated and control flats in the greenhouse. Newly germinated seedlings as well as woods-plot seedlings were collected. Diseased and healthy white pine seedlings were also gathered from the Chittenden Nursery.

One hundred and thirty-seven sections one-half inch long were cut from these specimens. The sections were then killed and fixed in FAA (13). The sections remained in this solution for a minimum time of fifty hours, and then they were dehydrated with ethyl alcohol and tertiary butyl alcohol by passing them through a series of changes over a period of two days, the last change being pure tertiary butyl alcohol. The root sections were then transferred to a mixture of equal parts of paraffin oil and butyl alcohol for three hours. Following this, a vial three-fourths full of paraffin, 56°-58°C. melting point, was cooled to the solidification point. The contents of the vial containing root sections in paraffin oil and tertiary butyl alcohol was poured on top

of the solidified paraffin. This open vial was then placed in the embedding oven for three hours. The solution was then replaced with three changes of fresh paraffin over a period of twenty-four hours, the final change being embedding tissuemat. The solution was left in the oven for three hours, after which the sections were cast into blocks.

The embedded sections were then mounted on wooden blocks, and serial sections were cut at twelve microns with a rotary microtome. The sections were then mounted with Haupt's adhesive (13). Two stains, Stoughton's Thionin and Orange G, and Pianese III B stain were used for staining (13). Permanent mounts were made with the synthetic resin "clarite".

EXPERIMENTAL DATA AND RESULTS

Isolation studies

The isolation of microorganisms from white pine nursery stock was carried out during the summer months of 1957 and 1958. Five tissue plantings from the root systems of white pine seedlings exhibiting various degrees of root rot were planted in each Petri plate. The percentage occurrence of each microorganism was calculated and listed in Table I and Figure I. Table I lists the microorganisms isolated from the white pine nursery stock, the number of times each microorganism was isolated, and its percentage occurrence in the total tissue plantings. The non-sporulators represent all the microorganisms which failed to sporulate on the medium used for the isolation studies.

The results of the isolation studies made throughout this investigation were arranged according to age of white pine nursery stock, and they appear in Tables III-VI and Figures 2-7. These tables show the microorganisms isolated from the total tissue plantings made in this study as to 1-0, 2-0, 2-1, and 2-2 nursery stock. The first number of these values above refers to the number of years a seedling was grown in the seedbed, while the second number refers to the number of years it was grown in the transplant bed.

White pine seedlings grown in the root rot free woodsplot near the south border of the nursery also were collected for isolation studies. The results of these

isolations are shown in Table VII and Figure 8. Table VIII and Figure 9 give the isolation results from nursery transplant bed seedlings that had previously had two years growth in the woodsplot.

Tissue plantings were made on unacidified PDA medium to determine the presence of secondary organisms and contaminants. Many colonies of bacteria and other microorganisms appeared around several of these tissue plantings, and in some instances the bacteria covered a considerable portion of the Petri plate. In another series of isolations made, tissue sections were planted directly onto the medium without surface disinfection. It was found that many of the lower fungi appeared in greater numbers on these sections as compared to the root sections with surface disinfection.

Inoculation studies

Table II shows the source of the isolates which were used in the greenhouse studies. The soil used in greenhouse flats to grow white pine seed and seedlings from the woodsplot was infested with the different *Fusarium* isolates. These isolates were grown on cornmeal vermiculite medium and also on a synthetic liquid medium. The microorganisms which were reisolated from the seedlings grown in the infested soil are shown in Tables IX-XII and Figures 10-13. The reisolation results from seedlings grown in the first greenhouse series are shown in

Table X, and the results from the second greenhouse series appear in Table XI. Plate VIII shows the growth on PDA medium of Fusarium reisolated from white pine seedlings of the second greenhouse series. The reisolation results of the first and second greenhouse series were combined into one table, and they are shown in Table IX. In Table XII are listed the microorganisms reisolated from the seedlings which were grown in soil in the third greenhouse series.

Nematode studies

Tables XIII and XIV list the genera of nematodes found by nematologists from soil and white pine seedlings grown in the nursery. Five species of nematodes considered parasitic on white pine are also shown in Table XIV. The number of nematodes extracted from soil samples collected from different areas in the nursery during the 1957-1958 studies are shown in Table XV. In Table XVI is shown the number of nematodes extracted from the roots of diseased white pine seedlings collected during this study.

Table I. Frequency of microorganisms isolated from white pine nursery seedlings showing various degrees of root rot.[a]

Microorganisms isolated	Number of times isolated	Percentage[b]
Fusarium	565	30.84
Penicillium	154	8.41
Trichoderma	122	6.66
Rhizopus	121	6.60
Bacteria	75	4.09
None (sterile)	54	2.95
Hormiscium	25	1.36
Alternaria	20	1.09
Zygorhynchus	15	0.82
Rhizoctonia	9	0.49
Verticillium	5	0.27
Aspergillus	4	0.22
Chaetomium	3	0.16
Botrytis	2	0.11
Cephalothecium	2	0.11
Curvularia	1	0.05
Phoma	1	0.05
Non-sporulators	654	35.70

[a] Isolations from 1832 diseased white pine seedlings from June to September of 1957 and June to September of 1958

[b] Expressed as percentage of total isolations

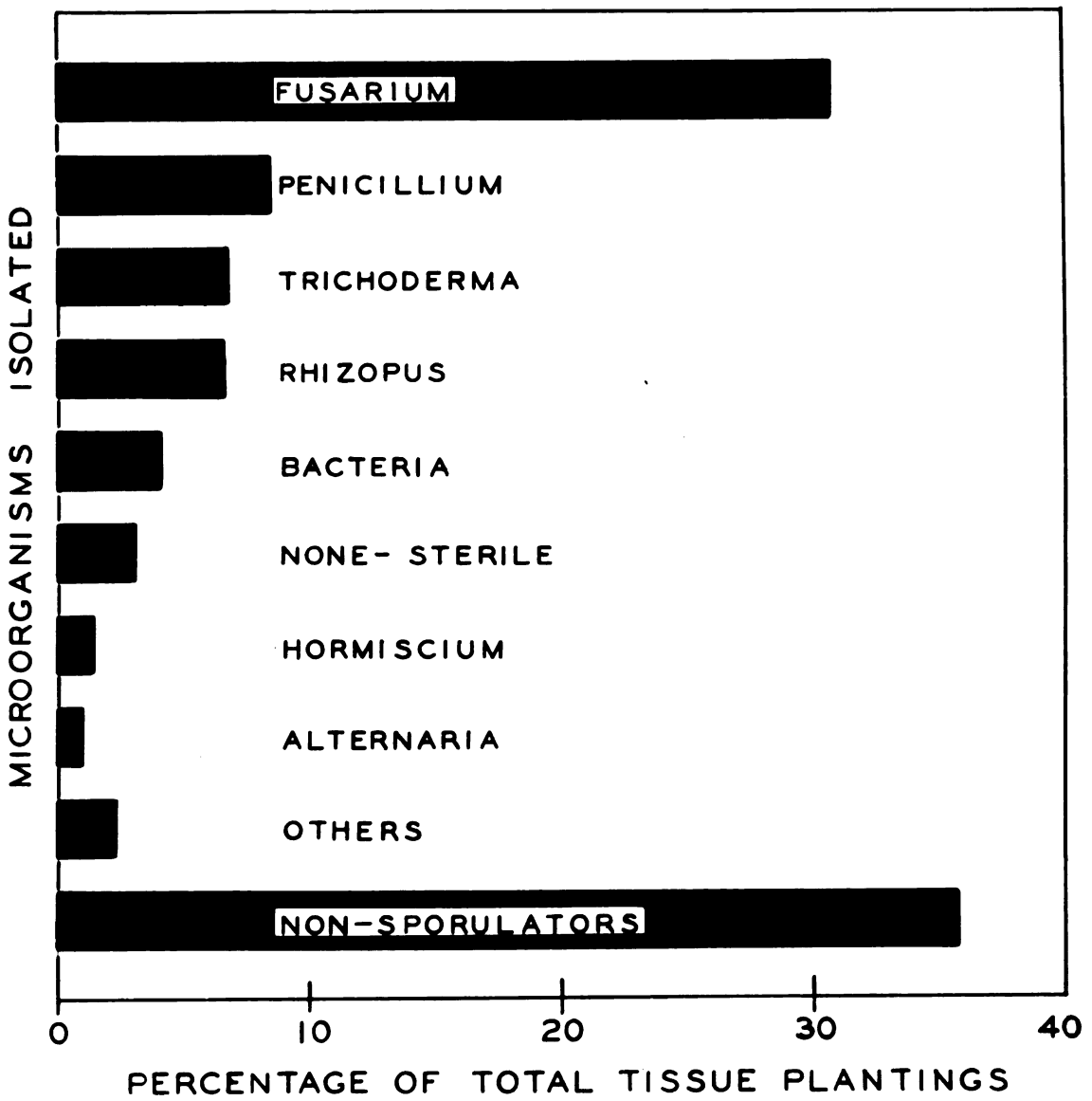


Figure 1. Frequency of microorganisms isolated from white pine nursery seedlings showing various degrees of root rot.

Table II. Source of Fusarium isolates used in inoculation tests.

Isolate number	Location in the nursery: Block number	Section[a] number	Bed number	Plot number	Identification of isolates[b]
33	1	2	2	3	Fusarium solani
42	1	2	4	12	F. moniliforme
46	1	2	3	12	F. solani
49	1	2	4	12	F. moniliforme
62	1	2	2	6	F. oxysporum

[a] Sown with white pine seed in the fall of 1955

[b] By Dr. William C. Snyder, Professor of Plant Pathology, University of California

Table III. Frequency of microorganisms isolated from 1-0 white pine nursery seedlings showing various degrees of root rot.[a]

Microorganisms isolated	Number of times isolated	Percentage[b]
Fusarium	78	30.00
Rhizopus	27	10.38
None (sterile)	16	6.15
Trichoderma	11	4.23
Rhizoctonia	6	2.31
Penicillium	5	1.92
Alternaria	4	1.54
Bacteria	1	0.38
Non-sporulators	112	43.07

[a] Isolations from 260 1-0 white pine seedlings

[b] Expressed as percentage of total isolations from 1-0 white pine seedlings

Table IV. Frequency of microorganisms isolated from 2-0 white pine nursery seedlings showing various degrees of root rot.[a]

Microorganisms isolated	Number of times isolated	Percentage[b]
Fusarium	365	33.70
Trichoderma	80	7.39
Penicillium	78	7.20
Rhizopus	51	4.71
Bacteria	30	2.77
None (sterile)	24	2.22
Alternaria	8	0.74
Zygorhynchus	8	0.74
Verticillium	5	0.46
Chaetomium	3	0.28
Botrytis	2	0.18
Rhizoctonia	1	0.09
Phoma	1	0.09
Curvularia	1	0.09
Aspergillus	1	0.09
Non-sporulators	425	39.24

[a] Isolations from 1083 2-0 white pine seedlings

[b] Expressed as percentage of total isolations from 2-0 white pine seedlings

Table V. Frequency of microorganisms isolated from 2-1 white pine nursery seedlings showing various degrees of root rot.[a]

Microorganisms isolated	Number of times isolated	Percentage[b]
Fusarium	73	36.14
Rhizopus	21	10.40
Hormiscium	19	9.41
Bacteria	16	7.92
Penicillium	12	5.94
Trichoderma	6	2.97
Alternaria	5	2.48
None (sterile)	1	0.49
Zygorhynchus	1	0.49
Non-sporulators	48	23.76

[a] Isolations from 202 2-1 white pine seedlings

[b] Expressed as percentage of total isolations from 2-1 white pine seedlings

Table VI. Frequency of microorganisms isolated from 2-2 white pine nursery seedlings showing various degrees of root rot.[a]

Microorganisms isolated	Number of times isolated	Percentage[b]
Penicillium	26	17.69
Bacteria	23	15.65
Trichoderma	21	14.28
Rhizopus	17	11.56
Fusarium	8	5.44
Hormiscium	6	4.08
None (sterile)	4	2.72
Alternaria	2	1.36
Cephalothecium	2	1.36
Zygorhynchus	2	1.36
Rhizoctonia	1	0.68
Aspergillus	1	0.68
Non-sporulators	34	23.13

[a] Isolations from 147 2-2 white pine seedlings

[b] Expressed as percentage of total isolations from 2-2 white pine seedlings

Figure 2. Fusarium frequency on different age stock.

(Numbers in parentheses indicate total number of seedlings from which tissue plantings were made)

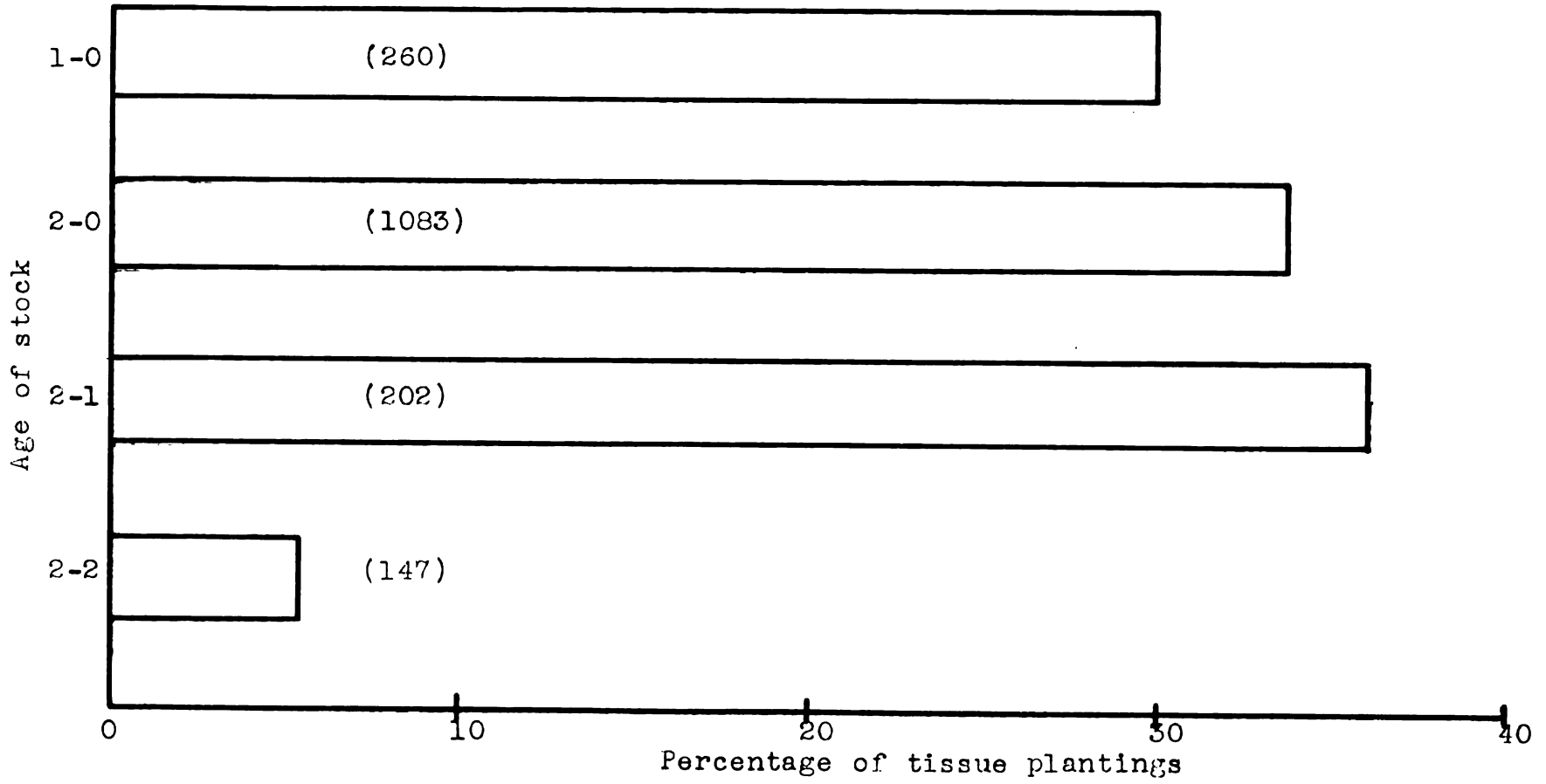


Figure 3. Non-sporulators frequency on different age stock.

(Numbers in parentheses indicate total number of seedlings from which tissue plantings were made)

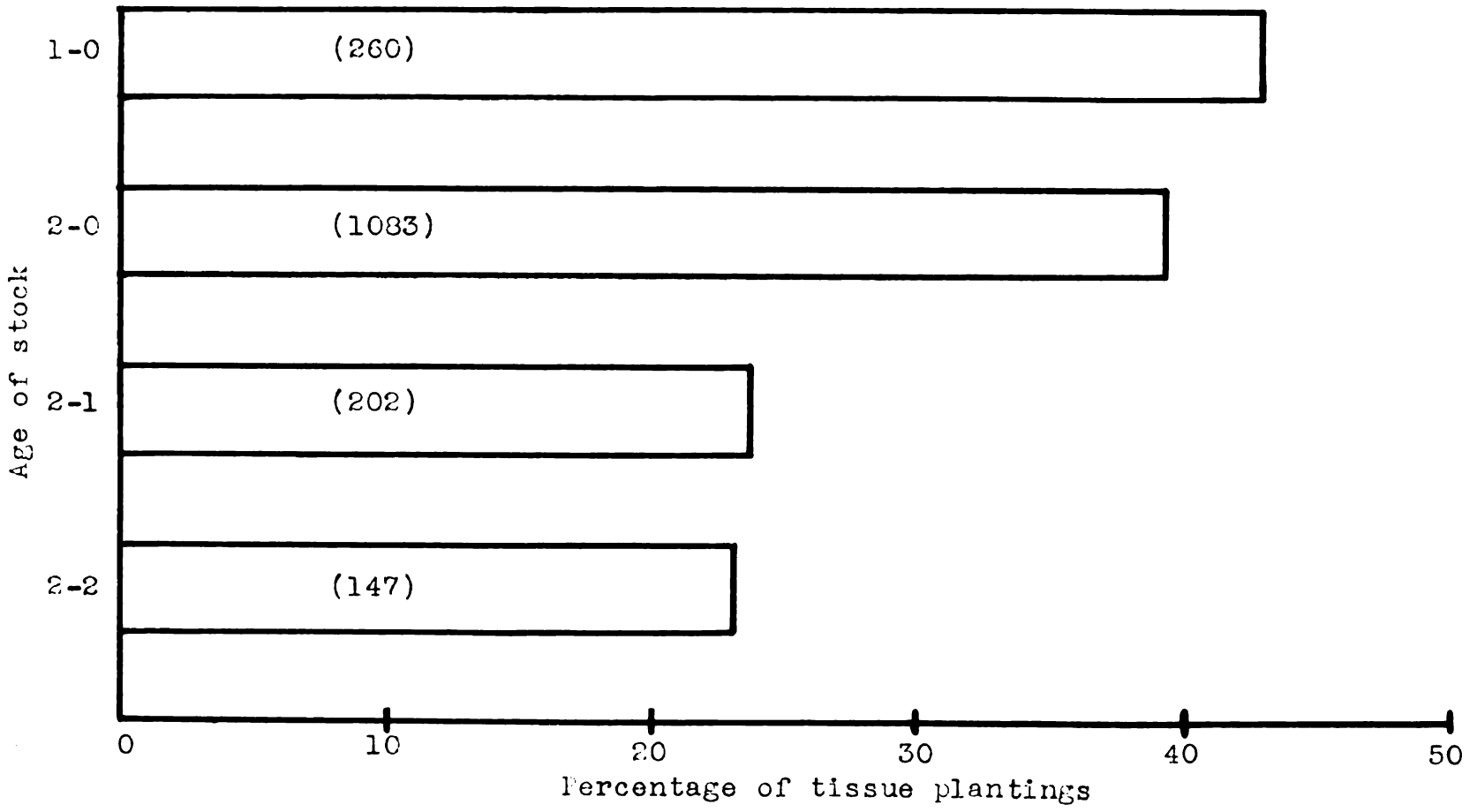


Figure 4. Trichoderma frequency on different age stock.

(Numbers in parentheses indicate total number of seedlings from which tissue plantings were made)

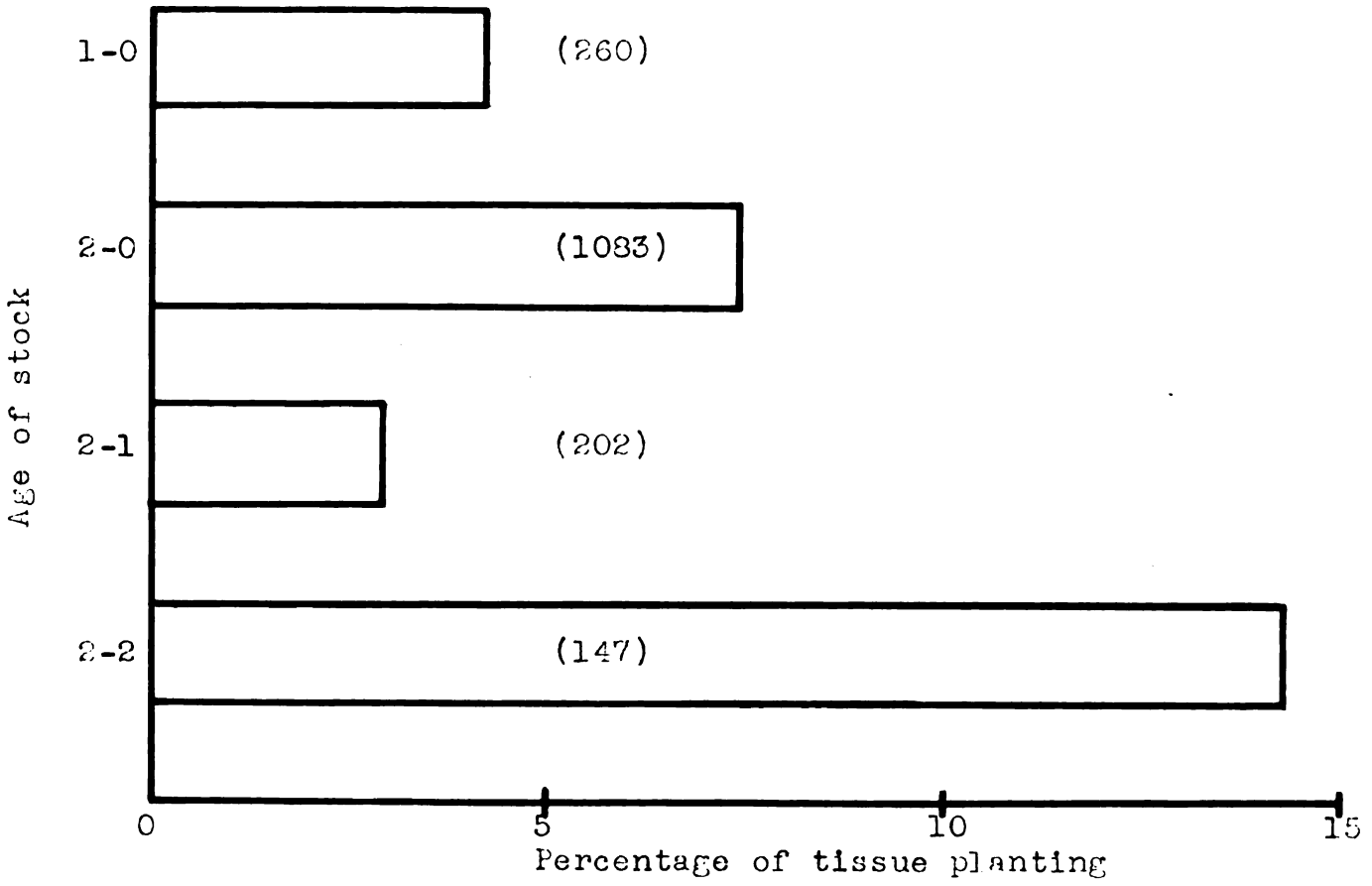


Figure 5. Penicillium frequency on different age stock.

(Numbers in parentheses indicate total number of seedlings from which tissue plantings were made)

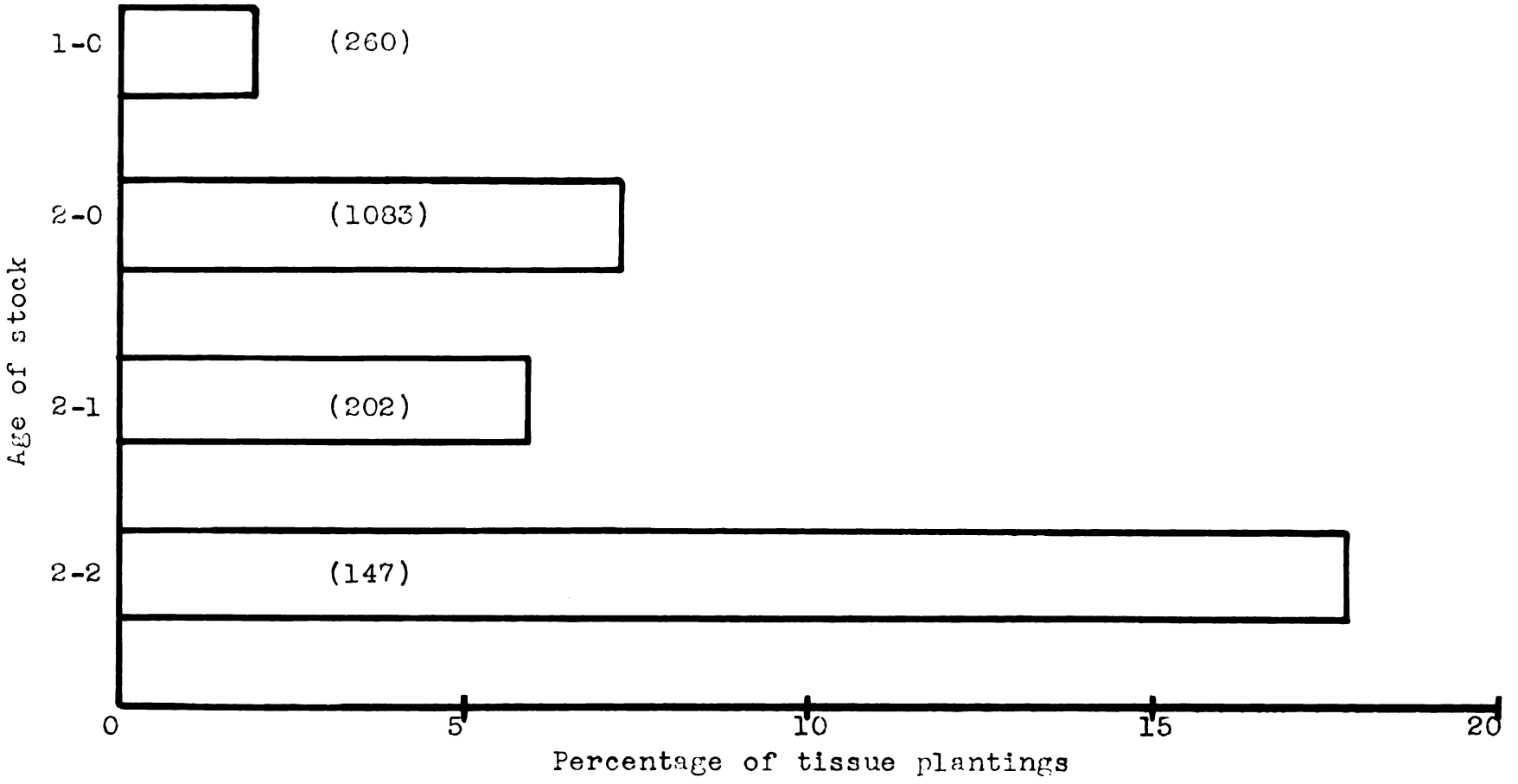


Figure 6. Rhizopus frequency on different age stock.

(Number in parentheses indicates total number of seedlings from which tissue plantings were made)

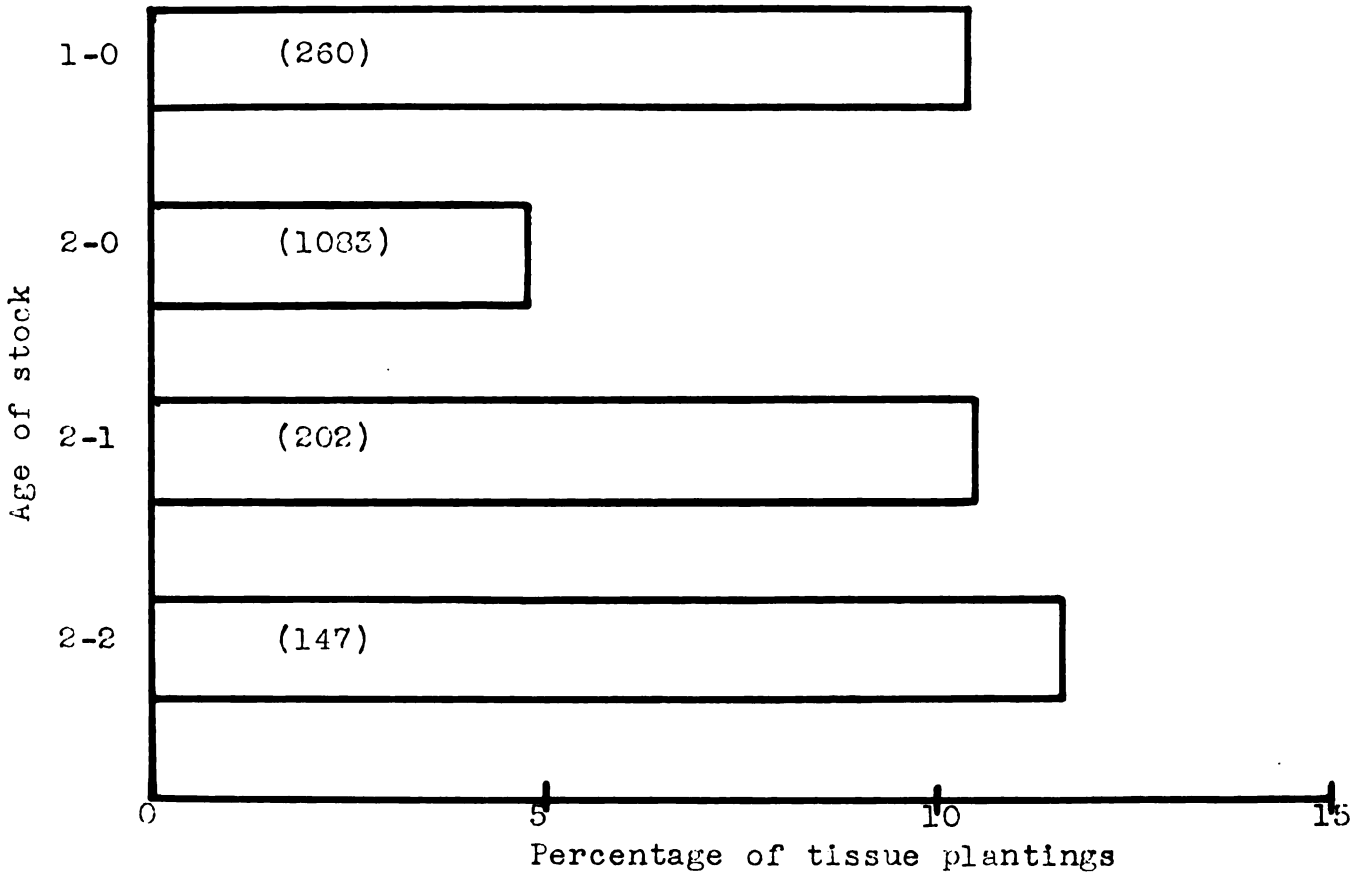


Figure 7. Bacteria frequency on different age stock.
(Numbers in parentheses indicate total number of seedlings from which tissue plantings were made)

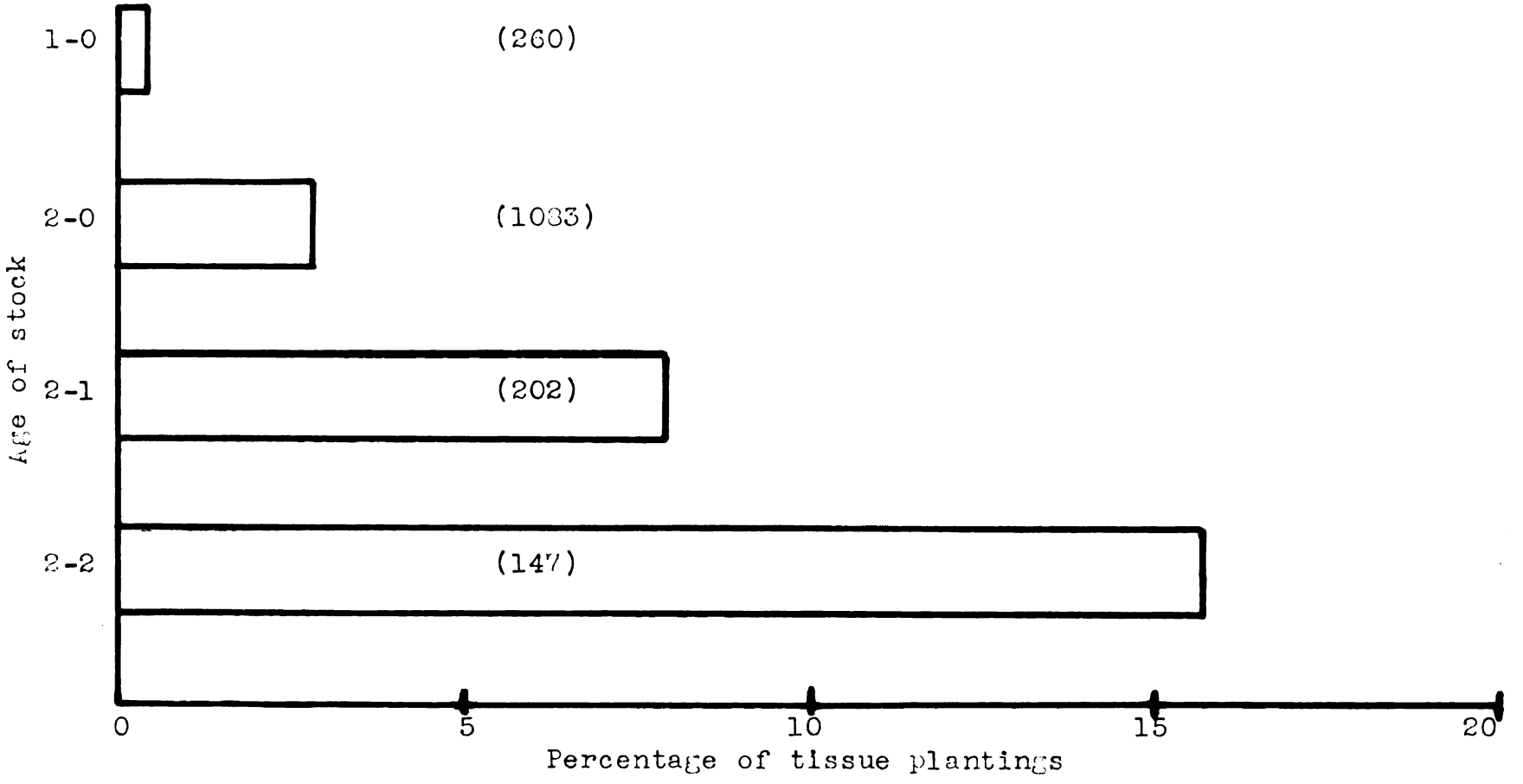


Table VII. Frequency of microorganisms isolated from white pine woodsplot seedlings.[a]

Microorganisms isolated	Number of times isolated	Percentage[b]
Penicillium	15	19.74
Fusarium	12	15.79
None (sterile)	9	11.84
Rhizopus	3	3.95
Bacteria	3	3.95
Trichoderma	2	2.63
Aspergillus	2	2.63
Alternaria	1	1.31
Non-sporulators	29	38.16

[a] Isolations from 76 seedlings

[b] Expressed as percentage of total isolations from white pine woodsplot seedlings

Figure 8. Frequency of microorganisms isolated from white pine woodsplot seedlings.

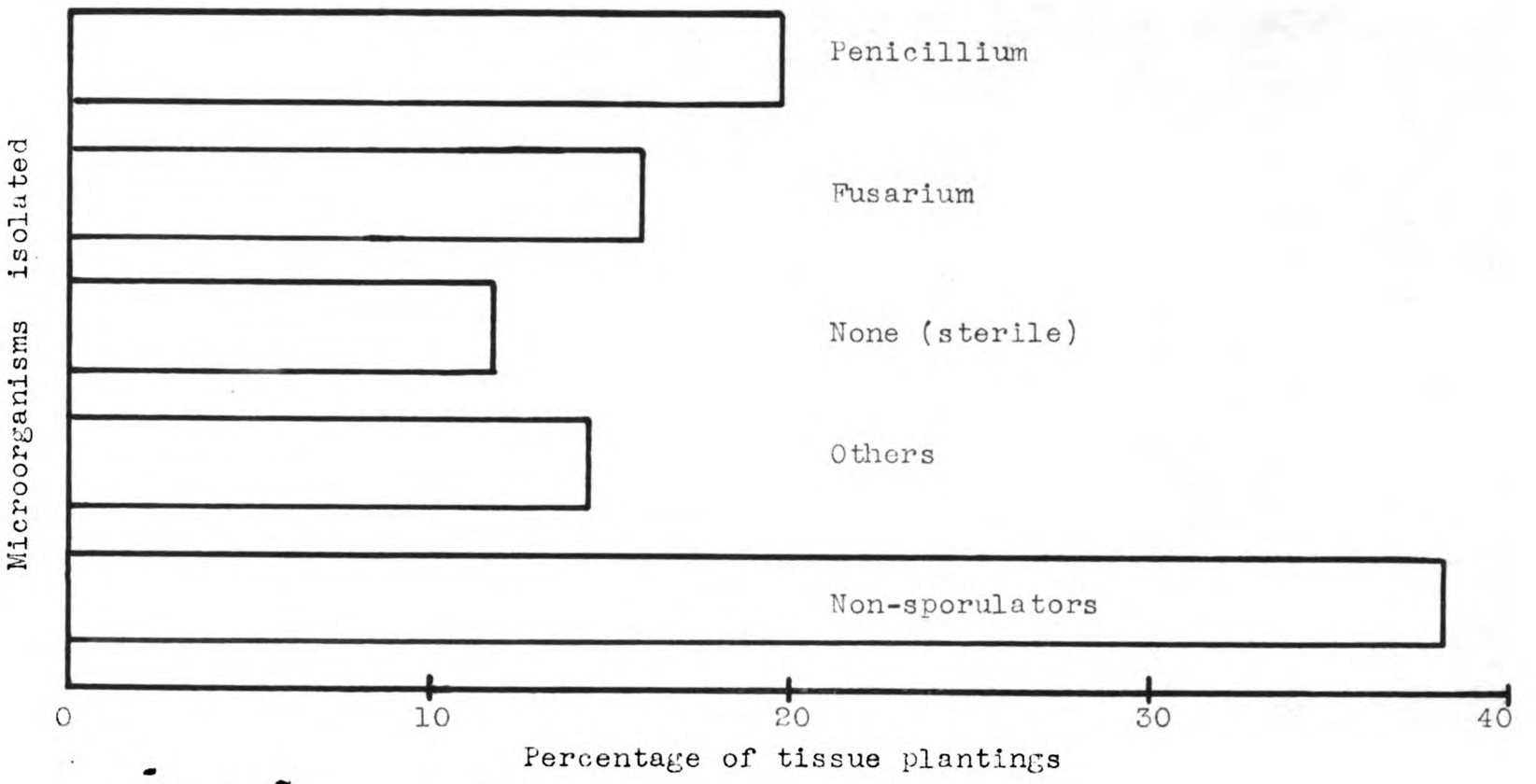


Table VIII. Frequency of microorganisms isolated from white pine woodsplot seedlings transplanted into the nursery transplant beds.[a]

Microorganisms isolated	Number of times isolated	Percentage[b]
Fusarium	24	37.50
Penicillium	18	28.12
Zygorhynchus	4	6.25
Trichoderma	3	4.69
Rhizopus	3	4.69
Bacteria	2	3.12
None (sterile)	1	1.56
Non-sporulators	9	14.06

[a] Isolations from 64 white pine seedlings

[b] Expressed as percentage of total isolations from white pine woodsplot seedlings transplanted into the nursery

Figure 9. Frequency of microorganisms isolated from white pine woodsplot seedlings transplanted into nursery transplant beds.

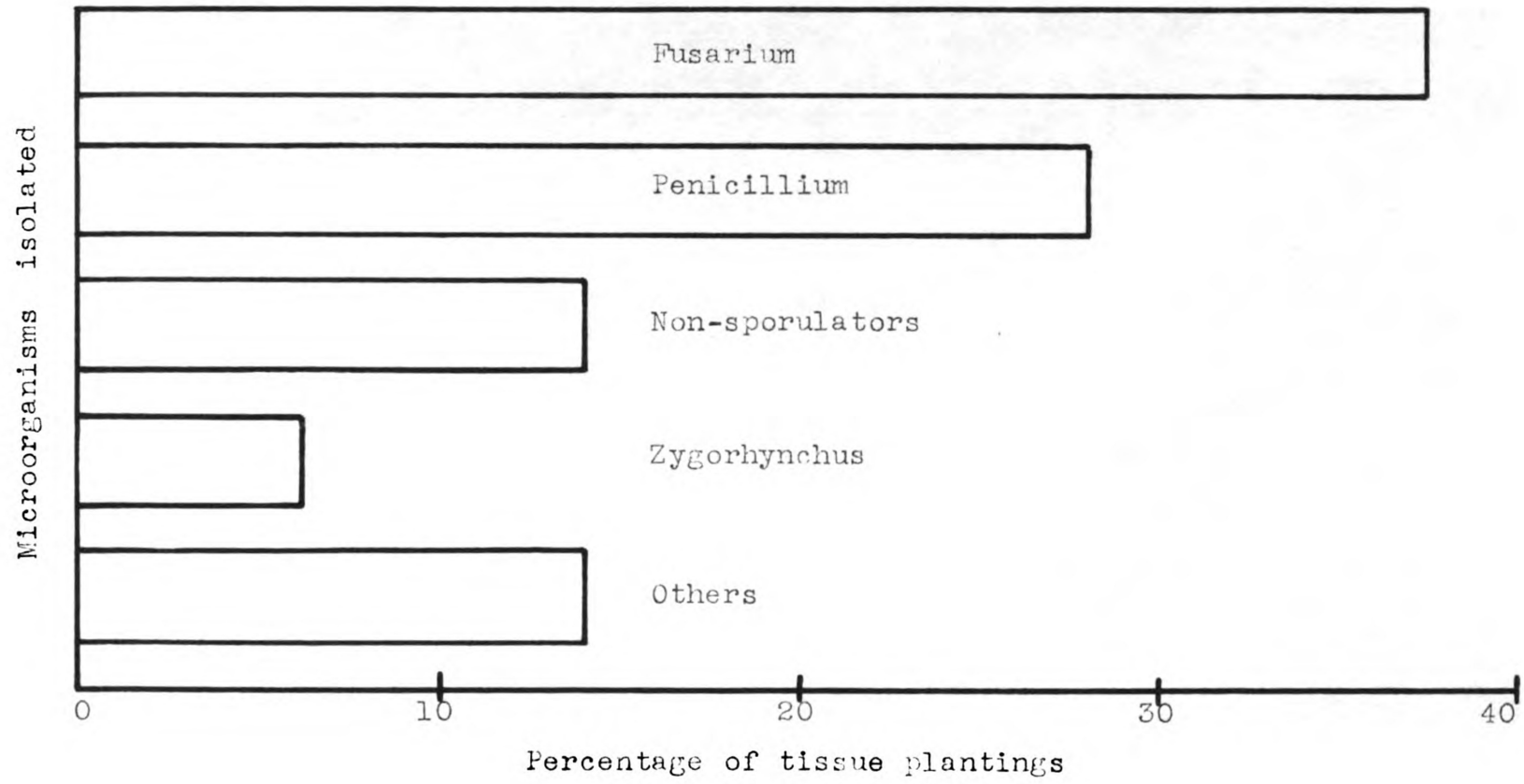


Table IX. Frequency of microorganisms reisolated from white pine seedlings grown in soil in the first and second greenhouse series.

Source of inoculum	Infestations									Controls				
	33	42	46	49	62	MM[a]	LFM[b]	Total	Percentage	PLM[c]	CCV[d]	CNM[e]	Total	Percentage
No. of plants used for reisolation	101	90	105	103	87	107	33	626	--	100	65	169	334	--
Micro-organisms isolated														
Fusarium	90	79	89	93	79	96	14	540	86.26	34	38	24	96	28.74
Others[f]	11	11	16	10	3	11	19	86	13.74	66	27	145	238	71.26

[a] Isolate numbers 33,42,46,49, and 62 grown on synthetic liquid medium and their mycelial mats combined and used as inoculum

[b] Liquid from five Fusarium isolates as above grown on synthetic liquid medium

[c] Check containing the unplanted synthetic liquid medium

[d] Check containing the unplanted cornmeal vermiculite medium

[e] Control without any medium or inoculum added

[f] Includes the following microorganisms with their respective percentages for the infected plants: Penicillium 3.51, Verticillium 2.40, None (sterile) 4.79, Non-sporulators 1.12, Bacteria 0.64, Trichoderma 0.32, Rhizopus 0.32, Alternaria 0.32, and Aspergillus 0.32
Includes the following microorganisms with their respective percentages for the control plants: Penicillium 26.95, None (sterile) 17.06, Non-sporulators 9.28, Verticillium 8.38, Trichoderma 3.59, Bacteria 2.40, Rhizopus 1.20, Aspergillus 0.90, and Botrytis 1.50

Figure 10. Frequency of microorganisms reisolated from white pine seedlings grown in soil in first and second greenhouse series.

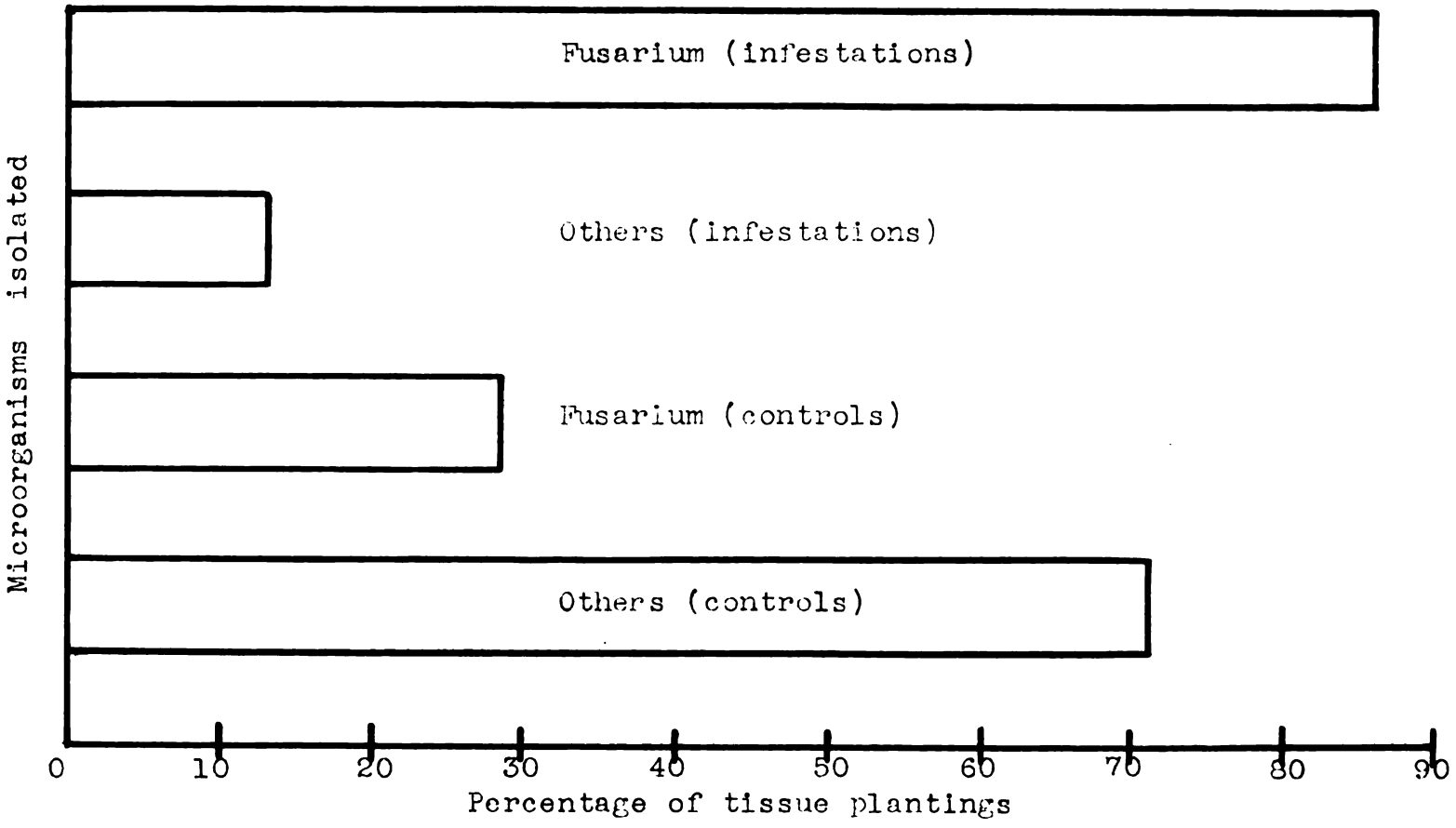


Table X. Frequency of microorganisms reisolated from white pine seedlings grown in soil in the first greenhouse series.

Source of inoculum	Infestations									Controls				
	33	42	46	49	62	FMM[a]	LFM[b]	Total	Percentage	PLM[c]	CCV[d]	CNM[e]	Total	Percentage
No. of plants used for reisolation	34	30	36	39	25	41	33	238	--	39	6	46	91	--
Microorganisms isolated														
Fusarium	29	24	35	35	22	37	14	196	82.35	14	4	9	27	29.67
Others[f]	5	6	1	4	3	4	19	42	17.65	25	2	37	64	70.33

[a] Isolate numbers 33,42,46,49, and 62 grown on synthetic liquid medium and their mycelial mats combined and used as inoculum

[b] Liquid from the five Fusarium isolates as above grown on synthetic liquid medium

[c] Check containing the unplanted synthetic liquid medium

[d] Check containing the unplanted cornmeal vermiculite medium

[e] Control without any medium or inoculum added

[f] Includes the microorganisms with their respective percentages for the infected plants: Penicillium 5.88, None (sterile) 5.46, Non-sporulators 2.52, Verticillium 2.10, Rhizopus 0.84, Alternaria 0.42, and Bacteria 0.42
Includes the following microorganisms with their respective percentages for the control plants: Penicillium 38.46, None (sterile) 9.89, Verticillium 3.79, Non-sporulators 6.59, Bacteria 4.39, Trichoderma 1.10, and Aspergillus 1.10

Figure 11. Frequency of microorganisms reisolated from white pine seedlings grown in soil in the first greenhouse series.

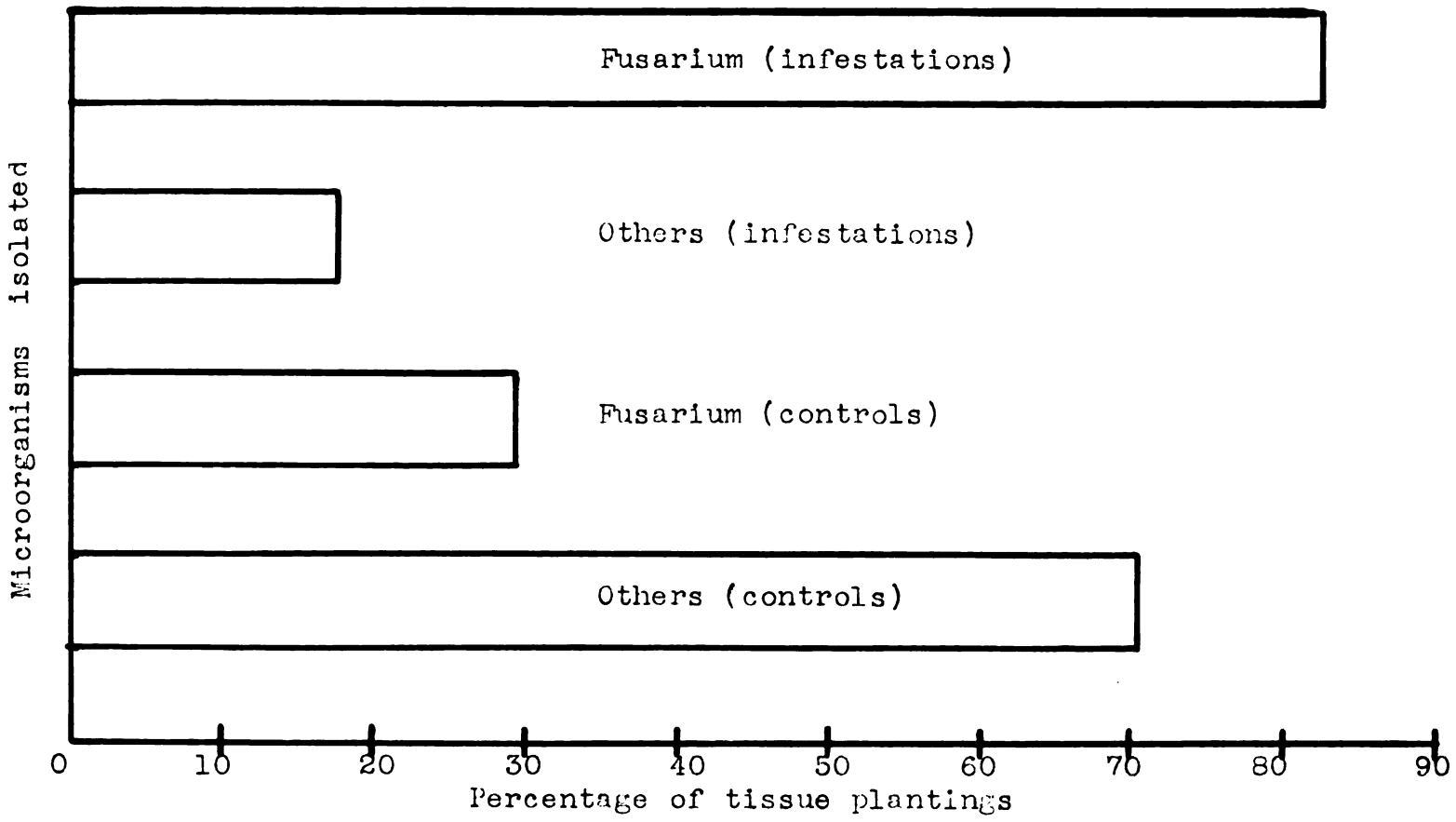


Table XI. Frequency of microorganisms reisolated from white pine seedlings grown in soil in the second greenhouse series.

Source of inoculum	Infestations								Controls				
	33	42	46	49	62	FMM[a]	Total	Percentage	PLM[b]	CCV[c]	CNM[d]	Total	Percentage
No. of plants used for reisolation	67	60	69	64	62	66	388	--	61	59	123	243	--
Microorganisms Isolated													
Fusarium	61	55	54	58	57	59	344	88.66	20	34	15	69	28.40
Others[e]	6	5	15	6	5	7	44	11.34	41	25	108	174	71.60

[a] Isolate numbers 33,42,46,49, and 62 grown on synthetic liquid medium and their mycelial mats combined and used as inoculum

[b] Check containing the unplanted synthetic liquid medium

[c] Check containing the unplanted cornmeal vermiculite medium

[d] Control without any medium or inoculum added

[e] Includes the following microorganisms with their respective percentages for the infected plants: None (sterile) 4.38, Verticillium 2.58, Penicillium 2.06, Bacteria 0.77, Trichoderma 0.51, Aspergillus 0.51, Alternaria 0.26, and Non-sporulators 0.26 Includes the following microorganisms with their respective percentages for the control plants: Penicillium 22.63, None (sterile) 19.75, Non-sporulators 10.28, Verticillium 8.23, Trichoderma 4.53, Botrytis 2.06, Rhizopus 1.65, Bacteria 1.65, and Aspergillus 0.82

Figure 12. Frequency of microorganisms reisolated from white pine seedlings grown in soil in the second greenhouse series.

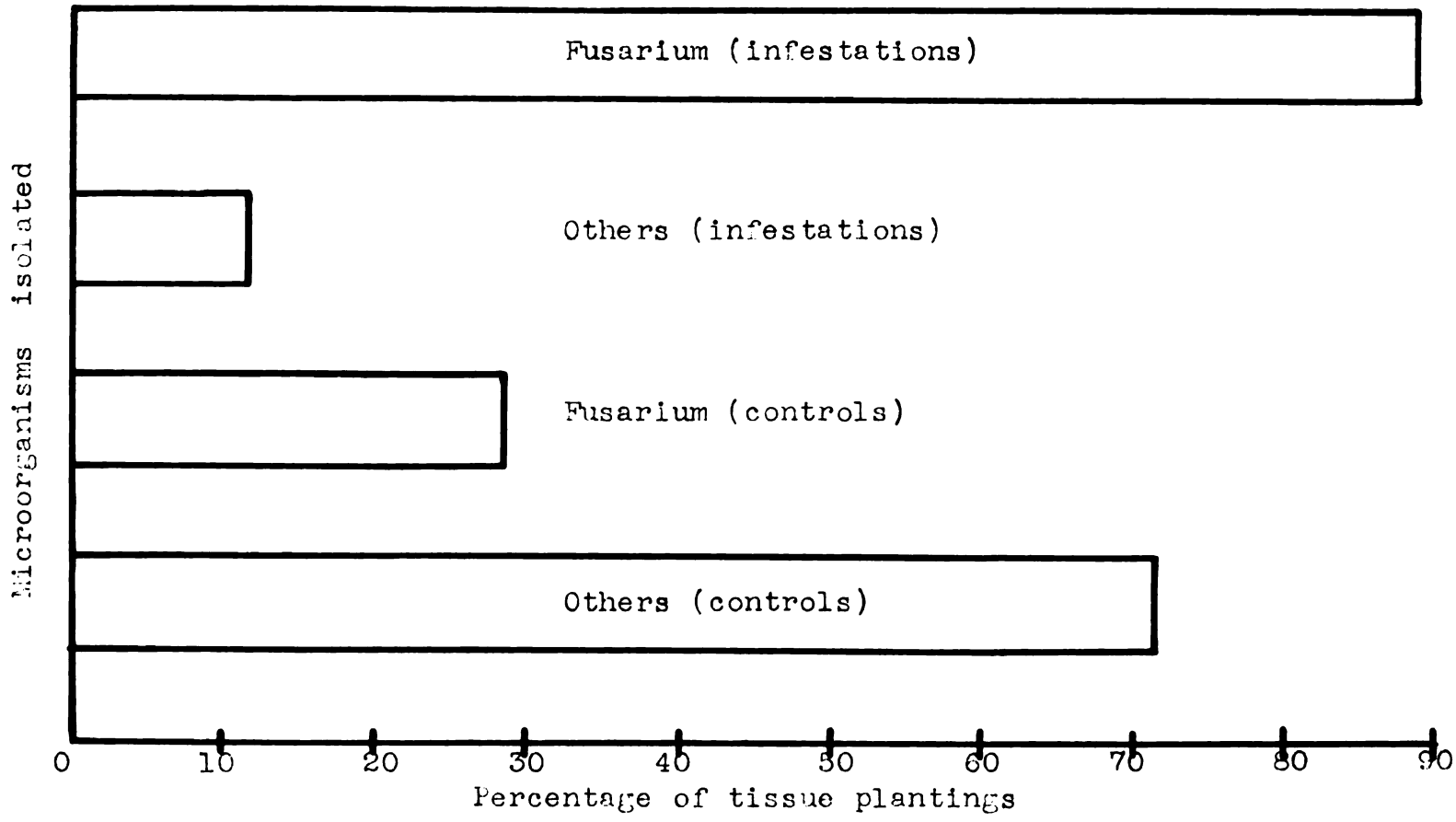
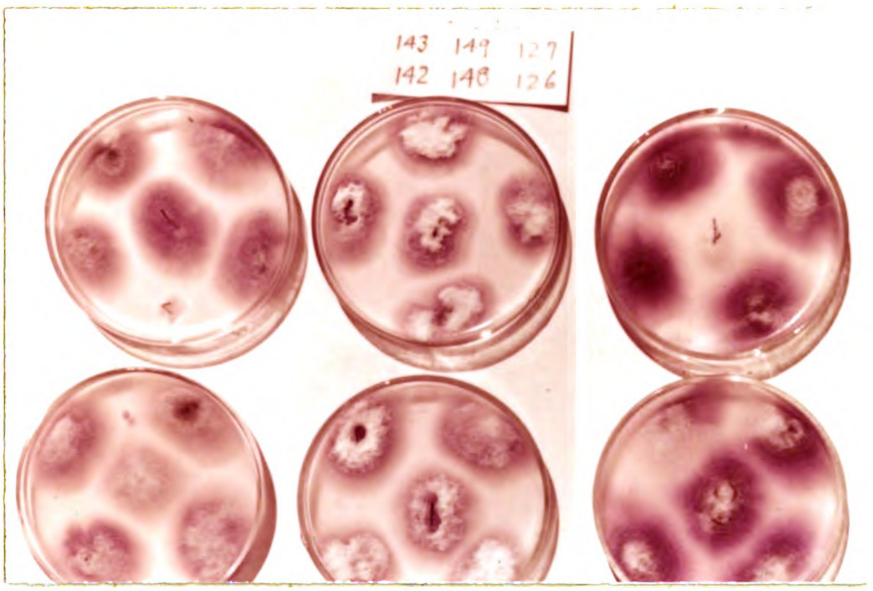


PLATE VIII



Growth on PDA medium of Fusarium reisolated from white pine seedlings of the second greenhouse series.

Table XII. Frequency of microorganisms reisolated from white pine seedlings grown in soil in the third greenhouse series.

Source of inoculum	Infestations[a] Fusarium	Fusarium and Nematodes[b]	Total	Percentage	Controls CCV[c]	CNM[d]	Total	Percentage
No. of plants used for reisolation	5	7	12	--	6	5	11	--
Micro-organisms isolated								
Fusarium	3	7[e]	10	83.33	0	0	0	0
Others[f]	2	0	2	16.67	6	5	11	100

[a] A Verticillium isolate obtained from a seedling in the second series from Fusarium 46 flat was infested into the soil to test its pathogenicity. Verticillium was not reisolated from the tissue plantings

[b] Six plants used for reisolation for the nematode treatment only gave the following: Penicillium 2, Trichoderma 2, Non-sporulators 1, Rhizopus 1, and nematodes 9

[c] Check containing the unplanted cornmeal vermiculite medium

[d] Control without any medium or inoculum added

[e] Two nematodes were recovered

[f] Includes the following microorganisms with their respective percentages for the infested plants: Rhizopus 8.33, Non-sporulators 8.33
Includes the following microorganisms with their respective percentages for the control plants: Rhizopus 45.45, Non-sporulators 36.36, and Zygorhynchus 18.18

Figure 13. Frequency of microorganisms reisolated from white pine seedlings grown in soil in the third greenhouse series.

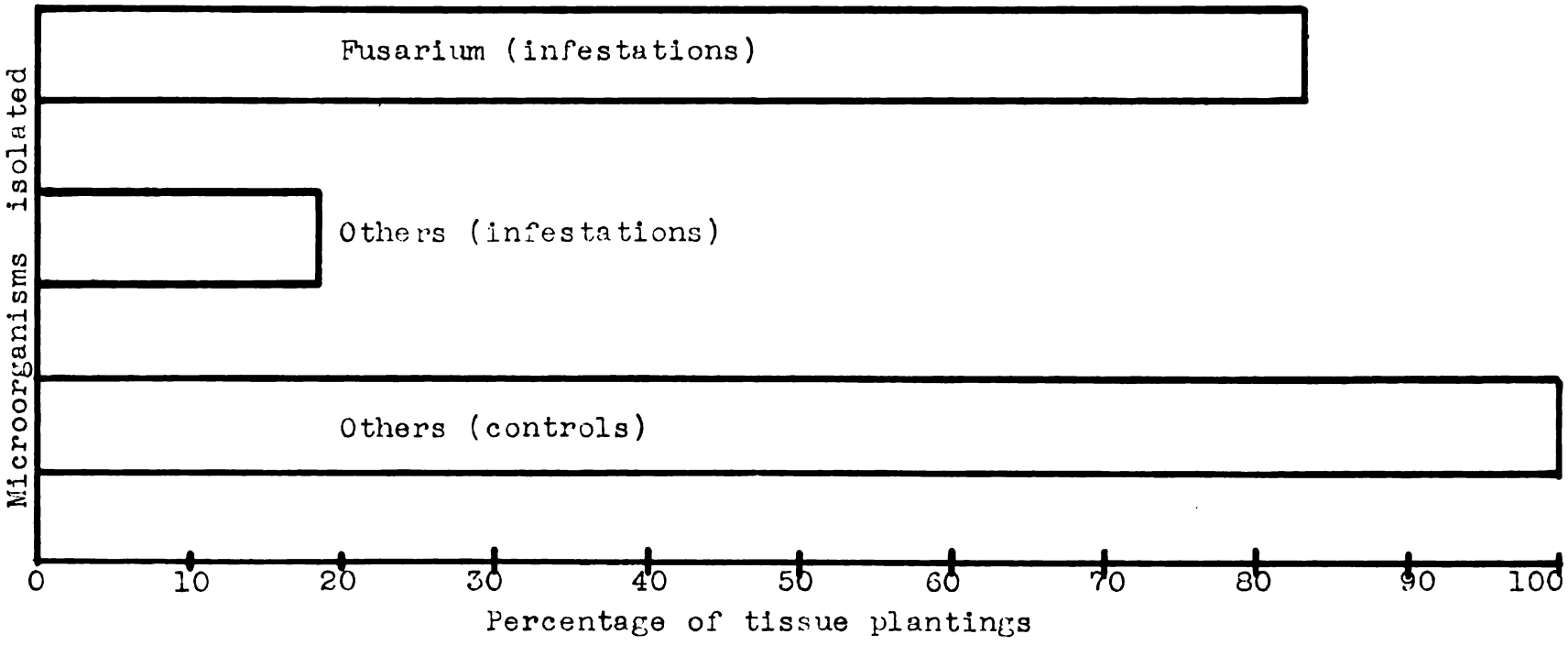


Table XIII. Genera of nematodes associated with white pine seedlings in the Chittenden Nursery in 1950.[a]

Genera found	Genera found
Acrobeles	Metarhabditis
Acrobeloides	Panagrolaimus[b]
Aphelenchoides	Paraphelenchus
Aphelenchus	Plectus
Ditylenchus	Seinura
Dorylaimus	Tylenchus
Metaphelenchus[b]	

[a] Determinations by Dr. G. Steiner (2)

[b] Numerous

Table XIV. Species of nematodes associated with white pine seedlings and nursery soil in the Chittenden Nursery in 1950.[a]

Species found	Species found
Leptonchus granulosus	Acrobeles sp.[b]
Tylenchorhynchus dubius	Chiloplacus sp.[b]
Ditylenchus sp.	Aphelenchus avenae[b]
Aphelenchoides sp.	Nygolaimus brachyurus[b]
Tylencholaimus proximus	Mononchus papillatus[b]
	Monochus n. sp.[b]

[a] Determinations by Dr. G. Thorne and Dr. C. E. Dieter

[b] Saprophytes not considered plant parasites

Table XV. Frequency of nematodes extracted from soil samples collected from nursery plots, the woodsplot, and a white pine plantation near the Chittenden Nursery.

Sample location	Number of samples	Nematodes per sample[a] 24 hour extraction[b] Range	Mean	72 hour extraction[c] Range	Mean
Block I, Section 2	14	18-364	171	--	--
Block IV, Section 2	1	--	--	1355	1355
Block VIII, Section 11, Bed 8	6	15-1525	616	--	--
Bed 10	6	34-1225	504	--	--
Block VIII, Section 12, Bed 1	5	18-1812	931	--	--
Bed 3	6	175-1250	666	--	--
Woodsplot	5	--	--	25-637	294
White pine plantation	5	9-150	48	--	--

[a] 250 ml. (by volume) soil sample used

[b] Culture tube containing nematodes removed from the inverted bottle after 24 hours

[c] Culture tube containing nematodes removed from the inverted bottle after 72 hours

Table XVI. Frequency of nematodes extracted from the roots of diseased white pine seedlings collected at various locations in the Chittenden Nursery.

Sample location	No. of samples	No. of roots per sample	Nematodes per sample: 24 hour extraction[a] Range	24 hour extraction[a] Mean	72 hour extraction[b] Range	72 hour extraction[b] Mean
Block I,	9	11	25-254	114	--	--
Section 2	6	13	--	--	19-82	36
Block IV, Section 2	2	8	--	--	6-164	35
Block III, Section 8	1	10	--	--	222	222

[a] Culture tube containing nematodes removed from the inverted bottle after 24 hours

[b] Culture tube containing nematodes removed from the inverted bottle after 72 hours

DISCUSSION AND CONCLUSIONS

Isolations from the advanced margin of lesions on white pine affected with root rot in various degrees produced a number of microorganisms (Table I). Fusarium was the predominant microorganism isolated from the root tissue, being found on 30.8 percent of the tissue plantings. Examination of Table I shows that Fusarium was isolated nearly three times as often as the three next highest identifiable fungi, namely Penicillium, Trichoderma, and Rhizopus. Since it has been established by others that some Fusarium species are able to cause damping-off and root rot of various plants, and since Fusarium was isolated more commonly than other fungi, it was desirable to test its pathogenicity on white pine seedlings.

The frequency of isolation of Fusarium species from white pine seedlings from different age stocks was about equal except for the 2-2 stock (Tables III-VI). Fusarium species were found on 30.0, 33.7, 36.1, and 5.4 percent respectively of the 1-0, 2-0, 2-1, and 2-2 stock examined. The low frequency of Fusarium in the 2-2 stock may be due to such factors as age and vigor of the host, or elimination of more susceptible plants. Still another possible contributing factor may be a rhizosphere effect, that is, the influence of various factors in the soil adjacent to the roots (4). Examination of Tables III-VI shows that Trichoderma was isolated from 14.3 percent of

the 2-2 stock, but only from 4.2, 7.4, and 2.9 percent of the 1-0, 2-0, and 2-1 stock respectively. It has been established that various Trichoderma species, for example, T. viride, inhibit parasitic fungi (20).

The data listed in Tables III-VI also indicate that as the age of the nursery stock increased, there was a progressive decrease in percentage of non-sporulators isolated. Approximately 43.1 percent of all microorganisms isolated from the 1-0 white pine stock and 23.1 percent of those isolated from the 2-2 stock failed to sporulate on the culture medium used. Figures 5 and 7 indicate that Penicillium species and bacteria increased in percentage as the age of the nursery stock increased. The reason for these changes is not known. The increase in saprophytes may be due to an increase in the dead tissue normally sloughed off by the older roots, and to the difficulty in disinfecting the surface of the older larger root sections.

Sixteen percent of all the microorganisms isolated from the woodsplot seedlings were Fusarium species (Table VII and Figure 8). This was considerably less than obtained from the main nursery. When the 2-0 woodsplot seedlings were transplanted into the nursery transplant beds, the percentage of Fusarium isolated showed a definite increase (Table VIII and Figure 9). Root rot does not occur in the woodsplot, and even though Fusarium was recovered there, the seedlings did not show the typical

symptoms which are associated with root rot. Because of this circumstantial evidence, it is believed that the Fusarium species in the woodsplot were saprophytic forms. The increase in percentage of the Fusarium isolation in the nursery must then be due to the additional pathogenic Fusarium species there. Other microorganisms which were isolated from these two areas, such as Penicillium and Rhizopus, remained relatively constant. There was a greater number of non-sporulators isolated from the woodsplot seedlings as compared to those which were transplanted into the nursery (Tables VII and VIII). The woodsplot contains more organic matter than plots in the nursery, and this may have had an influence on the percentages of non-sporulators in these two areas.

The data in Table IX and Figure 10 clearly indicate that Fusarium species were the principal microorganisms reisolated from seedlings of the first two greenhouse series. These seedlings were grown in soil to which cultures of Fusarium were added. Examination of Table IX shows that 626 seedlings were used for reisolation from the Fusarium infested flats, and 86.26 percent of the seedlings gave cultures of Fusarium (Figure 10). The Fusarium reisolation rate from control seedlings was only 28.74 percent. Apparently Fusarium spread into the control flats from the nearby infested flats or was brought in by other agencies such as insects and air currents. When 28.74 percent is used as a common value of Fusarium

infestation for all other flats, the amount of Fusarium reisolated and due solely to the infestation with the Fusarium isolates is 57.52 percent. Since the control treatment gave very few isolates of Fusarium, it appears that the inocula used were pathogenic.

When the liquid from the five Fusarium mats (LFM) was used as inoculum, it was noted that Fusarium was reisolated from fourteen of thirty-three plants (Table X). It is believed that this high recovery resulted from some spores and possible mycelial fragments not completely filtered out of the medium. Browning of the roots and reduced vigor of seedlings were more apparent in the treatment containing the Fusarium mycelial mats (FMM) than in the treatment containing the liquid from these mats (LFM). It had been assumed that the staling products of Fusarium isolates growing on the synthetic liquid medium would be toxic to the white pine seedlings, but apparently this assumption was not valid for the case.

Tables X-XI and Figures 11-12 list the microorganisms reisolated from the white pine seedlings in the three greenhouse series. In the first series 82.35 percent of the reisolations from those plants grown in Fusarium infested soil were Fusarium. In the flats containing the controls, ninety-one seedlings were used for reisolation, and the percentage of Fusarium recovery was 29.67, which means that the difference of 52.68 percent must be accounted for by the deliberate infestation of

the Fusarium isolates (Table X and Figure 11). The corresponding difference due to the inoculation for the second greenhouse series was 60.26 percent (Table XI and Figure 12).

In the third series of Fusarium infestations, as shown in Table XII and Figure 13, the Verticillium isolate used did not appear to be pathogenic. Further examination of the table reveals that ten of twelve reisolations where Fusarium was infested in the soil proved to be Fusarium. The percentage recovery of Fusarium therefore was 83.33 percent. When Fusarium alone was used as inoculum, three of five reisolations were Fusarium, but all the reisolations from the Fusarium plus nematode infestation were Fusarium. Two of the treatments in the third greenhouse series included nematodes, and eleven nematodes were reisolated from these treatments. More care was taken to avoid cross contamination , and no Fusarium was isolated from the seedlings grown in the control flats.

The exact role played by nematodes in the white pine root rot disease is not known. Although time did not permit a full classification of the nematodes extracted from the soil and plant samples, it had been shown previously that several parasitic species of nematodes were present in the Chittenden Nursery soil and diseased white pine seedlings (Table XIV). Of the nematodes found: "The first five listed were considered as causing injury to the white pine seedlings." (Personal communication from Dr.

C. E. Dieter, Dow Chemical Company, to Mr. E. D. Clifford, Superintendent of the Chittenden Nursery, March 2, 1951).

Table XV shows that many nematodes were extracted from soil samples collected throughout the nursery. The proportion of parasitic to saprophytic nematodes is not known. A further examination of this table shows that fewer nematodes were extracted from the soil samples collected from the woodsplot and from a white pine plantation near the nursery than from soil samples collected in the nursery. Since the root rot disease is not present in the woodsplot seedbed or in plantations of white pine consisting of stock from the Chittenden Nursery, the data in Table XV strongly suggest that nematodes are a contributing factor to the root rot disease in the nursery. Further evidence in support of this observation was found in greenhouse tests, where there was a greater number of Fusarium diseased plants in combination Fusarium-nematode infestations than in treatments with either Fusarium isolates or nematode isolates alone. Table XVI shows that many nematodes were extracted from the roots of diseased white pine seedlings, which still further strengthens the possibility of nematodes as a contributing agent in the root rot disease.

Although many permanent slides were prepared from apparently healthy and diseased white pine seedlings, few of the sections showed fungus hyphae. It was observed on the slides showing fungus hyphae that the fungus was

present in the epidermis and cortical regions. In a few cases, hyphae were found in the stelar region of the roots.

SUMMARY

The following points are summarized from this investigation.

1. Fusarium species comprised 30.8 percent of all the microorganisms isolated from diseased white pine seedlings studied at the Chittenden Nursery.

2. Fusarium species comprised 86.26 percent of all the reisolations from the first and second series of infestation tests conducted in the Plant Science Greenhouse at Michigan State University using soil infested with several Fusarium isolates obtained from the Chittenden Nursery. In the control flats, Fusarium species, which was considered due to contamination by air and insect borne Fusarium, comprised 28.74 percent of the reisolations. This leaves a difference of 57.52 percent of Fusarium reisolations which is due solely to the infestation of the soil.

3. The percentage recovery of Fusarium from the first series of greenhouse tests above the number of Fusarium recovered from the control flats was 52.68 percent and from the second series 60.26 percent.

4. In the third greenhouse series of tests, when both Fusarium and nematode isolates were used to infest the soil, there was an increase in the number of diseased plants due to Fusarium over that obtained with either Fusarium or nematodes separately. Although the study on the role of the nematodes was not completed, there is

evidence that the nematodes may increase the number of white pine seedlings killed by the Fusarium species.

5. A greater number of nematodes were extracted from soil samples collected in the nursery than from soil samples collected in the woodsplot or in a white pine plantation near the nursery. Since root rot does not appear in the woodsplot and little if any appears in the plantation, there is additional circumstantial evidence that nematodes are a contributing factor.

LITERATURE CITED

1. ANDERSON, R. L., D. D. SKILLING, and E. D. CLIFFORD. 1956. Nursery root disease control by soil fumigation. Lake States For. Exp. Sta. Tech. Notes No. 449.

2. CLIFFORD, E. D. 1954. Synopsis of past work on white pine root rot at the Chittenden Nursery. (mimeographed)

3. ELIASON, E. J. 1938. Buckwheat as a factor in the root rot of conifers. (Abs.) Phytopathology 28:7.

4. GARRETT, S. D. 1956. Biology of root-infecting fungi. Cambridge Univ. Press, London. 293 p.

5. GIFFORD, C. M. 1911. The damping-off of coniferous seedlings. Vt. Agr. Expt. Sta. Bul. 157: 141-171.

6. GILL, D. L. 1958. Effects of root-knot nematodes on Fusarium wilt of mimosa. Plant Disease Reptr. 42: 587-590.

7. HANSBROUGH, T., and J. P. HOLLIS. 1957. The effect of soil fumigation for the control of parasitic nematodes in the growth and yield of loblolly pine seedlings. Plant Disease Reptr. 41: 1021-1025.

8. HARTLEY, C., T. C. MERRILL, and A. S. RHOADS. 1918. Seedling diseases of conifers. J. Agr. Res. 15: 521-558.

9. HENRY, B. W. 1953. A root rot of southern pine nursery seedlings and its control by soil fumigation. Phytopathology 43: 81-88.

10. HOLTZMANN, O. V. 1955. Organisms causing damping-off of coniferous seedlings and their control. Diss. Abstr. 15: 2392.

11. HOPPER, B. E. 1958. Plant parasitic nematodes in the soil of southern forest nurseries. Plant Disease Reptr. 42: 308-314.

12. JACKSON, L. W. R. 1948. Deterioration of shortleaf pine roots caused by a parasitic nematode. Plant Disease Reptr. 32: 192.

13. JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill Book Co., New York. 523 p.

14. MAKI, T. E. and B. W. HENRY. 1951. Root-rot control and soil improvement at the Ashe Forest Nursery. Southern For. Exp. Sta. Occas. Paper No. 119. 23p.

15. RATHBUN, ANNIE E. 1922. Root-rot of pine seedlings. Phytopathology 12: 213-220.

16. RAWLINGS, T. E. 1933. Phytopathological and botanical research methods. John Wiley, New York. 156p.

17. SPAULDING, P. 1907. A blight disease of young conifers. Science. n. s. 26: 220-221.

18. TINT, H. 1945. Studies in the Fusarium damping-off of conifers. II. Relation of age of host, pH, and some nutritional factors to the pathogenicity of Fusarium. Phytopathology 35: 440-457.

19. VAARTAJA, O. 1953. Seedling disease of conifers in Saskatchewan. Bi-m Progr. Rep. Div. For. Biol. Dep. Agric. Can. 9: 2.

20. VAARTAJA, O. 1958. Effects of Trichoderma on tree seedlings and their pathogens. Bi-m Progr. Rep. Div. For. Biol. Dep. Agr. Can. 13: 1.

21. VAARTAJA, O., and W. H. CRAM. 1956. Damping-off pathogens of conifers and of Caragana in Saskatchewan. Phytopathology 46: 391-397.

22. WESTVELD, R. H. 1949. Applied silviculture in the United States. John Wiley, New York. 590 p.