SURVIVAL AND PATHOGENICITY OF *PHYTOPHTHORA CINNAMOMI* RANDS IN FOREST SOILS

by

ELMER GEORGE KUHLMAN

A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1961

APPROVED:

Redacted for privacy

Professor of Botany and Plant Pathology

In Charge of Major

Redacted for privacy

Chairman of Department of Botany and Plant Pathology

Redacted for privacy

Chairman of School Graduate Committee

Redacted for privacy

Dean of Graduate School

Date thesis is presented May 15, 1961

Typed by Gladys Averette

ACKNOWLEDGEMENTS

I would like to express my special appreciation to Dr. L. F. Roth for his counsel and encouragement during the course of this investigation. The assistance of Dr. R. A. Young in the preparation of this thesis is gratefully acknowledged. I would like to thank Dr. E. K. Vaughan and Dr. R. O. Belkengren for their constructive criticism of the manuscript. Miss Janet Nelson made the photographs.

Appreciation is expressed to the Bureau of Land Management for financial support and permission to use their land for field experiments.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

List of Tables (Contd.)

SURVIVAL AND PATHOGENICITY OF *PHYTOPHTHORA CINNAMOMI* RANDS IN FOREST SOILS

INTRODUCTION

The research reported here is part of an effort to evaluate the threat of *Phytophthora cinnamomi* Rands to Northwest forestry. Several considerations contribute to the timeliness of the investigation.

1. The fungus is pathogenic on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and other important timber trees. Douglas-fir was first found susceptible to the fungus in 1936 (20) by Crandall. Subsequently Pimentel (50) reported natural infection of Douglas-fir plantations in Portugal and Torgeson (61, p.38) reported that young Douglas-fir seedlings were killed readily by isolates of *P. cinnamomi*.

2. A vivid example of the destructive capacity of the genus *Phytophthora* confronts forestry people of the Northwest. *P. lateralis* caused extensive damage in southwestern Oregon to Port Orford cedar (*Chamaecyparis lawsoniana*) (65;66). Following its introduction into native stands in 1952, the fungus, aided by the moderate, moist marine climate, spread rapidly throughout the natural range of its host.

3. At the present time P. cinnamomi is a common enemy of nurserymen throughout the United States. The hosts of the fungus presently number well over one-hundred species (61;63;59). This large host range increases the potential danger by aiding in movement of the pathogen.

4. P. cinnamomi is not considered to occur now in our commercial forest lands. In 1955 Middleton and Baxter (43) stated that they had isolated P. cinnamomi along with three other Phytophthora spp. and several species of Pythium from soil in an undisturbed forest area of western Oregon. Subsequently two extensive surveys failed to substantiate the contention that P. cinnamomi is a natural inhabitant of Oregon forest soils. Loring (39) could not isolate P. cinnamomi from an area adjoining an infested nursery nor from under native rhododendrons in Douglas-fir stands. Although the fungus was prevalent in nurseries where infected plants were growing or had been growing, the fungus could not be isolated in even the most probable adjacent drainage areas. An extensive survey was undertaken in the autumn of 1958 and the spring of 1959 to determine if P. cinnamomi was present in Pacific Northwest forest soils (52). Samples were collected from over 1500 locations in the

autumn and spring from relatively undisturbed land and from recently disturbed land. Although the fungus appeared in 61% of samples from infested nursery soils used as controls, it was not found in any of the forest soil samples.

The objectives of this study were (1) to investigate the ability of P. cinnamomi to survive, move and infect Douglas-fir roots in natural forest soils, (2) to determine the influence of temperature, moisture and other soil organisms on survival, movement and pathogenesis, and (3) to assess the potential destructiveness of P. cinnamomi in Douglas-fir forests.

LITERATURE REVIEW

History of the Pathogen

Phytophthora cinnamomi Rands was first reported as a pathogen of the cinnamon tree of Southeast Asia in 1922 (51). The fungus has caused extensive damage to forests, agricultural crops and ornamentals around the world. The history of some of these diseases will give an idea of the potential of this fungus.

Chestnut trees (Castanea sativa Mill.) in Europe have been affected by a root rot called "ink disease" since 1838 (19, p.162). Phytophthora cambivora (Petri) Buis was first isolated as the causal agent (49) but later (66), P. cinnamomi was also found to be associated with the disease. In the United States records of chestnut trees in the southern Appalachians dying from Phytophthora infection date back to 1824. The capacity of the fungus to spread rapidly and to cause extensive damage can be gathered from reports made at the close of the Civil War (19;15). Trees throughout a valley died within a period of three years. Most probably the disease was introduced on exotic ornamentals imported from Asia for the luxurious gardens of the pre-war Southern estates. The American and European chestnut and chinquapin proved more susceptible than their Asian counterparts and the

disease spread progressively into the surrounding forests. At the turn of the century a similar epiphytotic moved through the Ozark chinquapins destroying all but a few trees at high elevations (19, p.163). The pathogen was first active in the bottom lands and at low elevations but is now attacking trees at the higher elevations.

A second major disease, the littleleaf disease of shortleaf and loblolly pine (Pinus echinata Mill and P. taeda L. respectively) is prevalent in 35% of the commercial growing areas of these species and causes an estimated annual loss of five million dollars (12, p.2-4). This disease was first recognized in 1934, but it was not until 1954 that P. cinnamomi definitely was established as the causal agent (12, p.1;19). The fungus attacks young feeder roots, greatly reducing the effectiveness of the root system (12, p.20-24). Jackson reported that 71% of the feeding roots of diseased trees were dead, compared with only 17% of the roots of healthy trees (33, p.92). The affected trees decline slowly, and the current year's needles and stems are stunted, giving a sparse and tufted appearance to the affected trees (12, p.5). The disease is most common in stands in the 30-50 year old age class. Trees younger than 20 years rarely have the disease (12, p.7).

A third major disease, the decline of avocados due to P. cinnamomi was first reported in 1929 from Puerto Rico and from California in 1940 (66;70). Excess soil water and P. cinnamomi were necessary to cause the disease. Crandall felt that this disease had been a limiting factor in the cultivation of avocados in Peru (18, p.123). Trees of all ages were affected. Normally the symptoms appeared as a die-back of the branches followed by loss of leaves. Death followed in the next dry season.

The fungus also causes serious losses in ornamental plantings. Nursery conditions are ideal for the pathogen since there is an abundance of succulent host tissue and a high soil moisture level through the warm part of the summer. Movement of lightly infected nursery stock to residential gardens provides a rapid means of long distance spread for the pathogen (89;75). Host range summaries have been compiled by Torgeson et al. (61;62; 63) and Thorn and Zentmyer (59). P. cinnamomi has been isolated from the following hosts in Oregon: Chamaecyparis lawsoniana, Taxus baccata L., Taxus baccata var. stricta Laws., T. cuspidata Sieb. and Aucc. var. nana Rehd., Rhododendron ponticum L., Erica carnea L., and Calluna vulgaris (L) Hull. var alba (West) Don. and aurea Don. (61, p.24).

The most recent report of a disease problem caused by P. cinnamomi infecting a woody plant comes from New Zealand (47), where it is especially destructive there in farm shelterbelts of Pinus radiata. Following periods of heavy rainfall in 1953 and 1956 great numbers of thirty to forty year old pines were killed. Many trees wilted and died rapidly while adjacent trees went into a slow decline similar to that reported for littleleaf disease.

There are scattered reports of P. cinnamomi being present in undisturbed soils. Mehrlich (41, p.25) found it not only in the pineapple fields of Hawaii but also in virgin soils at higher elevations than fruit cultures. Miller (44) reported a possible occurrence of P. cinnamomi in native California live oak, Quercus agrifolia Nee, along the coast. Zentmyer (85) isolated P. cinnamomi from avocado roots collected in a sparsely inhabited, forested area.

The Fungus

Phytophthora cinnamomi Rands forms a distinctive mycelium in culture. Irregular knobby projections called vesicles varying in size from small bumps to large globose swellings are found along the hyphae. The mycelium is generally coenocytic.

Sporangia are not formed on agar media. Transferring disks from actively growing colonies on potato dextrose agar or mycelium from pea broth cultures to a non-sterile soil filtrate will induce the production of sporangia (61, p.43-45;87;80). The sporangiophores are sympodially branched. Often the sporangia will give rise to smaller secondary and tertiary sporangia. The sporangia are ovate, obovate, or obpyriform with a papilla. On certain substrates, however, the sporangia are non-papillate. The sporangia germinate to produce zoospores, germ tubes or secondary sporangia. The zoospores are biflagellate and reniform in the motile stage.

Chlamydospores are produced on most aqueous media. Zentmyer (87) found V-8 agar best for chlamydospore production. The chlamydospores are terminal, lemon yellow to yellow brown, spherical to sub-spherical, and normally thin-walled. Germination is by a germ tube.

Ashby (4) in 1929 reported the production of sexual organs in pure cultures of P. cinnamomi kept at room temperature for six months. Oyler and Bewley (48) found oogonia in cultures on Quaker Oat agar when the mycelium touched the edge of the plate. In paired cultures of P. cinnamomi and P. cryptogea oospores were produced on maize and bean extract agar (57). There were some combinations purely of P. cinnamomi but generally the

antheridia were from P. cinnamomi and the oogonia were from P. cryptogea. When paired with P. dreschleri, P. cinnamomi formed oogonia but when paired with P. palmivora, formed antheridia. Waterhouse (71) pointed out there is good evidence to indicate that all species of Phytophthora are basically hermaphroditic. Biochemical stimulation rather than a heterothallic nature is demonstrated if two strains are essential to produce oospores. Buddenhagen (8, p.37-39) was unable to induce oospore formation when he separated isolates of P. cinnamomi and P. cryptogea by a cellophane strip. This may indicate that the stimulating substance is transferred only when the hyphae of the two species touch since oospores were formed in dual cultures without a cellophane strip. Recently Zentmyer (78) obtained oospores in 4-5 days in a water extract of Mexican avocado roots. Ashby (4, p.261) reported that the oospores have a mean diameter of 27.2 μ with a range from 19-36 μ. The average diameter of the oogonia is 32μ with a range of 21-42μ. The amphigynous antheridia have a 19 x 17μ average with a range of 15-25 x 13-19μ.

Movement Through the Soil

The capacity of a root-invading pathogen for moving to and infecting its host plant determines to a great

extent its potential for rapid spread. The fungus mycelium may grow through the soil to the host as in the case of Rhizoctonia and Pythium. The movement of zoospores in soil water may be the most common means of spread in the Phytophthora spp. In many instances a fungus does not grow through the soil and invade host roots, instead the host roots grow to the parasite and then are invaded. Some soil fungi must rely solely on spores for their dissemination.

There is some evidence that species of Phytophthora do not normally move through or survive in the soil as mycelium. Campbell and Copeland (12, p.23) were unable to infect pine roots with mycelium of P. cinnamomi. The method of study was not indicated. Legge (37, p.118) found that in non-sterile soil the hyphae of P. infestans, P. porri, P. syringae, P. cactorum, and P. megasperma decreased considerably as did the number of sporangia, whereas the number of oospores remained constant. When "baits" were put in the soil the Phytophthora spp. did not grow into them (37, p.96-98). Newhook (47, p.823) isolated P. cinnamomi commonly in the feeder root zone in a 6-10 foot radius from the trunk of trees but at greater distances its occurrence was very much limited. There is only one report (84) that mycelial growth was responsible for movement of the fungus. In a period of a year and a

half the fungus moved fifteen feet uphill in an avocado orchard. Zentmyer felt this movement was by mycelium in the roots and soil rather than zoospores.

Pine trees growing on the lowest, most poorly drained sites were the first to die from littleleaf (19). The fungus then spread from tree to tree from the lower area to the higher drier areas. This type of disease development follows Zentmyer's (84) observations. There are, however, indications that in many other Phytophthora-induced diseases the movement is more directly related to water movement. Lewcock (38) concluded that P. cinnamomi was disseminated to healthy pineapple plants by movements of surface water or heavy rain. Drainage water brought down from high lands to orchards was shown to spread P. cactorum. In Chamaecyparis lawsoniana nurseries P. lateralis was shown to move rapidly in the direction of surface water flow, but more slowly in that of ground water flow (53). The importance of transport of soil particles by workers was emphasized. The movement of P. cinnamomi in Indiana nurseries followed the drainage patterns (54).

Although drainage water seems to affect the spread of P. cinnamomi, attempts to isolate it from storage ponds and reservoirs containing water from diseased avocado orchards were unsuccessful. Even when the diseased trees were only 150-200 feet from the ponds the fungus could

not be isolated. The fungus was isolated from 3 out of 120 samples of mud from the edges of storage basins and drainage ditches (60). Blackwell (6), however, feels that some Phytophthora spp. can exist saprophytically as water molds since they have been isolated from ponds, brooks, and wells.

The Phytophthora spp. would seem to fit Garrett's (25, p.30) classification of root-inhabiting fungi. The general criteria which he applied were as follows:
1. The fungus is suppressed by saprophytes on agar plates and in sterilized soil. In culture it is more sensitive to pure antibiotics than are saprophytes.
2. The fungus does not grow through unsterilized soil in absence of a food base such as infected tissue. 3. Typically it only invades root tissues and invades dead tissue very infrequently. 4. Its distribution in the soil is limited to the area in which host plants are growing.

The difficulty that all workers have encountered in isolating most of the Phytophthora spp. may be due to suppression by soil saprophytes. In 1:1 and 4:1 dilutions with Penicillium expansum, P. cinnamomi could not be isolated (47, p.819). Members of the genus Phytophthora are most easily isolated from the most recently invaded tissue and therefore must be unable to suppress

the saprophytes. On agar plates they are rapidly overgrown (35). Legge was unable to demonstrate mycelial growth through non-sterile soil in the absence of a food base. Both Loring (39) and Newhook (47, p.816-818) found the distribution of P. cinnamomi limited to association with host plants.

Perhaps it is more important to look at the saprophytic capacity of this root inhabiting fungus to learn whether it can compete with the common soil saprophytes and spread in this way. The competition between root inhabiting fungi and soil saprophytes is most critical on fragments of organic matter, at root surfaces, and on seed surfaces. The saprophytic organisms commonly produce antibiotics. Most workers consider the Phytophthora spp. to be poor saprophytes because they cannot be isolated with baits placed in or on the soil. Crop rotation has been used to eliminate some of the Phytophthora spp. making it seem unlikely that they can compete as saprophytes (37, p.132). Control of avocado root rot by adding alfalfa meal to the soil (79, p.65) suggests the buildup of antagonistic organisms.

Survival of the Fungus

In order for P. cinnamomi to remain a serious threat to Douglas-fir stands there must be evidence that it can survive unfavorable conditions encountered in forest soils. The fungus can survive as mycelium in infected plants and might survive also as resistant bodies (chlamydospores and oospores) or as a soil saprophyte. There have been no critical experiments to study the survival of P. cinnamomi in any stage of development. However, there have been observations on the survival of the fungus following infection. Several years after chestnut trees had died P. cinnamomi could still be isolated from soil in the root zone (28). In New Zealand Newhook (47, p.823) found that the amount of fungus in the soil decreased greatly in the first year or two following death of the host. After five years P. cinnamomi was still present although not in as great abundance as other Phytophthora spp. Drying the soil to a 1% moisture content killed this fungus (79). It is possible that the heat required to achieve this low moisture level might have been lethal. During comparatively dry periods the fungus survives in pineapple fields (3). Since a Mexican avocado root extract is presently the only medium on which oospores have been produced, it might be assumed

that this most resistant structure is not associated with the host plants studied.

Longevity studies on many of the Phytophthora spp. indicate that they have relatively low survival capacities (21;77;18;68). Long term survival is apparently dependent upon the production of resistant bodies. In those species that normally produce oospores the survival may be greatly increased (28;10;42;55), whereas mycelium and zoospores are relatively short-lived (37). In the previous section the saprophytic capacity of P. cinnamomi was discussed in relation to its effect on the movement of the fungus. Saprophytic activity could also play an important part in the survival of the fungus. The concept that P. cinnamomi is a poor soil saprophyte was further supported when Zentmyer (86) recovered P. cinnamomi readily in avocado roots and wheat stem pieces in sterile soil but poorly in non-sterile soil. A high soil moisture level (16%) regularly yielded higher recovery than a lower moisture content (8%).

The depth in the soil where the fungus is present has an effect on the environmental changes to which the fungus will be exposed. In Oregon, Torgeson (61, p.54-56;63, p.370) found the fungus most prevalent at about 7 inches below the surface, isolated it in 80% or more of the samples down to 13 inches, and occasionally as

deep as 27 inches. Campbell (14) reported that it was most abundant at 2 and 3 inch depths, fell rapidly to low levels at 6 and 7 inch depths and increased again at 8 and 9 inches. The fungus was most prevalent in the feeder root zone of various host plants (47, p.817;13).

Sporulation and Infection

Entrance of a fungus into a host plant is the most critical process in disease development. With the Phytophthora spp. infection is thought to be accomplished by zoospores. Campbell and Copeland (12, p.23) were unable to infect roots of shortleaf pines with mycelium of P. cinnamomi. Hickman and English (32) failed to get infection of strawberries with P. fragariae unless the soil water moved from the inoculum towards the host plant roots. All the evidence for zoospore infection is indirect but good. Excess soil moisture is always required for infection, movement of mycelium through the soil has not been demonstrated and the most rapid movement of the fungus is in the direction of soil water movement.

The feeder roots are first attacked. The fungus destroys the fine roots of the plant and then moves into the larger roots. Copeland (16) found that when 18 to 34% of the roots were dead trees did not continue

normal growth and showed littleleaf symptoms.

Zak and Campbell (76) grew shortleaf and loblolly pine in liquid culture in order to follow the initial penetration of the fungus. Three days after the inoculum was added to the nutrient solution containing the trees, the first centimeter of the root tip and the two adjacent centimeters were separately plated on corn meal agar. In shortleaf pine 61% of the root tips and 48% of the next two centimeters were infected. On each of the following days similar isolations were made. The percentage of root tips infected increased to a maximum after 6½ days and then decreased rapidly. Secondary invaders probably caused the decrease. Root tips and new roots were invaded before they became woody or had developed a mycorrhizal association.

Goode (26) made microscopic examinations of the infection of strawberry roots by P. fragariae. Equal numbers of zoospores encysted on the roots of susceptible and resistant varieties; however, the fungus did not infect roots of resistant varieties. The zoospores also encysted in large numbers on the roots of unrelated plants. Even roots that were killed in boiling water had a stimulatory effect which glass rods did not have on the encystment of zoospores. The greatest number of cysts occurred in the root hair and root tip zones. Few of the zoospores

encysted at the very apex or above the root hair region. The germ tubes in the root hair region were not directed into the root and penetration did not take place. In the root tip region the germ tubes penetrated the epidermal cells. Penetration occurred in all the plants tested but it was only in the susceptible varieties that growth continued. The epidermis was entered either between or directly through the cells. There was no distortion of the cells associated with the entrance. The fungus moved inter- and intracellularly in the root tip, through the cortex to the stele. Sex organs were formed three days after penetration.

Wounding seems to increase infection (46;71). It is not known whether this effect is due to stimulation of the fungus or to the weakening of host resistance to infection.

Age may have an effect on resistance. Although P. cinnamomi was as prevalent under Pinus radiata trees from 5 to 20 years old as it was under older trees, the former were not damaged (47, p.830). In shortleaf pine the 15 to 34 year age class had the smallest amount of disease. Symptoms usually appeared in 30 to 50 year old stands and losses increased with age (31). In greenhouse tests seedlings of both species were susceptible.

Factors that affect the production of zoospores by the fungus will have a profound influence on the infection

of the host. Sporangial production has been studied to some extent. Zentmyer and Marshall (79) found sporangia produced between 12°C and 30°C. Optimum production occurred at 24°C. Torgeson (61, p.51-52) reported sporangial production at 20°C, 25°C, and 30°C with no production at either 15° or 35°C. A microbial factor in a soil water extract is apparently necessary for sporangial production since attempts to produce sporangia in sterilized solutions were never as successful (80;87;8, p.23). Legge observed sporangia on the plant only when there was an excess of moisture (37, p.14). Fewer sporangia were formed in continuous light than either in continuous darkness or alternate light and dark periods (80). When there was a lack of oxygen in the surrounding medium the sporangia did not germinate (37, p.25). A small drop in temperature induced the discharge of zoospores (80).

Upon germination oospores of Phytophthora spp. may give rise to sporangia (37;56). Since the oospores are probably the most important overwintering body the factors affecting their formation and germination are of primary importance. Oospores of P. cinnamomi are formed readily on a water extract of Mexican avocado roots at temperatures between 60° and 77°F. (78). No reports have

been found of P. cinnamomi oospores in naturally infected tissues. In some species oospores are formed soon after infection has taken place (26;37, p.21). Germination of oospores can sometimes be induced by exposure to the soil microflora. Legge (37, p.120) found that one month old oospores of P. cactorum and P. megasperma germinated in three days in the soil. A gradual increase in the number of germinating oospores was obtained over a six month period in non-sterile soil. P. cactorum and P. erythroseptica oospores were germinated following passage through the garden snail (29). Sterile soil leachates have been used to germinate oospores of P. cactorum, P. megasperma, and P. infestans (37, p.126;56). P. cinnamomi oospores have not been germinated.

Influence of Environment on Infection

Soil Moisture

Excess soil moisture has been the primary environmental factor considered whenever a Phytophthora caused disease has developed. There is usually a close association between disease incidence and excess soil moisture from heavy rains, poor subsoil drainage or too frequent irrigation (70;66;7;72;24;84;12;23;61;38;47;69;32;17;3).

In Puerto Rico and California Phytophthora root rot was frequently found in heavy soils and on poorly drained locations. Young avocado trees on fairly steep slopes were also affected (66;69). In one instance the disease occurred on a site with loose top soil underlaid by a tight clay. Trees were able to reach a considerable size before being killed because the roots of the young trees were in the loose soil, but upon reaching the impermeable subsoil they became infected (66).

When avocado trees were grown in infested soil which was saturated for 2 to 4 days there was no injury. Waterlogging for 6-8 days in the presence of P. cinnamomi resulted in injury (82).

Campbell and Copeland (12, p.25) isolated P. cinnamomi in the same relative amounts from 20 different soil series but the incidence of littleleaf increased as the internal drainage became poorer. Exposure of heavily textured subsoils due to erosion of the lighter textured top soils favored the build-up of P. cinnamomi. Roots of trees on such sites may be infected in numerous places. Trees on loose, sandy, well-drained sites frequently remain unaffected for years.

Root rot of Pinus radiata caused by P. cinnamomi is most common and most severe on poorly drained sites but

is also present on well drained soil types and on fairly steep slopes (47, p.841).

Several of the experiments on the effect of soil moisture should be noted. Avocado trees in infested soil, watered weekly instead of biweekly, came down with symptoms in one growing season, while those watered biweekly did not show symptoms until the second year (83). Torgeson (61, p.50-51;63, p.368-369) noted that incidence of disease was much greater in a soil of moderate texture than in soils of a heavier or lighter texture. He theorized that zoospores cannot move well in heavy soils and that in light soils the water drains away before they can move. Crandall (19, p.176) found that when trees were given only enough water to stay alive, 25% of those in infested soil died, 60% of those given six times the minimal amount died. In White's (74) account soil moisture is said to have no effect on infection of roots by the causal organism. A series of potted plants were kept at soil moistures of 30 to 90 per cent of capacity. These plants were then inoculated through the roots with Phytophthora. The first evidence of wilting appeared in the higher soil moisture levels, but eventually wilting and death of plants occurred at all moisture levels. No methods for maintaining soil moisture levels or for inoculations were included.

Soil Temperature

The effect of soil temperature on infection has not been studied in detail. Since there is generally a cooling of the soil in the Pacific Northwest when the wet season starts this would seem to be an important factor for the disease in this region. If the minimum temperature requirements are above those that occur here during the wet season the disease would not have a good chance of developing. Epiphytotics of pine root rot in New Zealand take place only when the rainfall is above the fall average (April-June). The average soil temperature at plant diseases division, Auckland taken at 4 and 8 inches were April 15°-16°C; May 12.5°- 13.5°C; June 10°-11.5°C. Drought preceding wet autumns was connected with severe disease development. It was thought that cracking of the soil may have aided rewetting (47, p.830-836). If the ability to produce sporangia also includes the ability to produce zoospores, the assumption might be made that infection took place during April and May. During these two months the soil temperatures were above 12°C, the minimum temperature needed for sporangial production. Cultures of P. cinnamomi exposed to freezing temperatures for more than 120-200 hours lost their viability. By placing soil out-of-doors over winter in New Jersey the fungus was eradicated (73).

Mineral Nutrition

Mineral nutrition of the host has been reported to have varying effect on disease development. Bingham *et al.* (5) varied nitrogen, phosphorous and potassium and noticed no variation in disease development even at low concentrations. Littleleaf severity varied inversely with the total nitrogen in the soil but was not affected by other nutrients (12, p.28). Anderson (2) reported that high salinity and high potassium reduced the degree of infection by *P. cinnamomi*.

Soil Aeration

In nutrient solution all inoculated avocado plants died regardless of the oxygen level. Exposure to very low oxygen levels (0.5-0.05 ppm) was lethal to seedlings even in the absence of *P. cinnamomi* (22). Shortleaf pine is much less tolerant than loblolly pine to poor soil aeration (76). Loblolly pine has greater resistance to littleleaf which may be explained by its greater vigor.

Soil pH

White (74) felt that low pH and low temperature kept *P. cinnamomi* out of natural rhododendron stands in

New Jersey. His isolate did not grow below pH 4.25. The soil pH in that region went down to 2.9 and averaged 4.0. Crandall et al. (19, p.177) isolated P. cinnamomi from diseased nursery stock growing in soil with a pH range of 3.2 to 7.0. Copeland and McAlpine (17, p.640) stated that soil pH of 4.6 to 5.6 had no apparent effect on littleleaf. In culture Zentmyer (87) found sporangia on agar disks even if the pH of the soil extract was reduced to 4.0.

METHODS AND MATERIALS

General

The methods and materials section covers general techniques used throughout the investigations. Specific techniques are described with the results.

In order to provide as much variation as possible among the *P. cinnamomi* cultures used, isolates from the Southeast, British Columbia, and California as well as isolates from Oregon were used. A *Pythium* sp. isolated commonly from our study area in the Coast Range was used in several experiments.

P. cinnamomi cultures used in these studies were as follows:

Culture No.	Host	Source (location)	Isolated By
Pc 1	rhododendron	Portland, Oregon	Smithson
Pc 2	alumi cypress	Portland, Oregon	Smithson
Pc 3	heather	Oregon	Powelson
Pc 4	soil	Athens, Georgia	Zak
Pc 5	Port Orford cedar	California	Zentmyer
Pc 8	heather	Salem, Oregon	Smithson
Pc 9	avocado	California	Zentmyer
Pc 10	yew	Victoria, B.C.	Salisbury

Culture No.	Host	Source (location)	Isolated By
Pc 13	----	California	Zentmyer
Pc 18	noble fir	Corvallis, Oregon	Trione
Pc 21	soil	Oregon	Nelson
Pc 22	soil	Oregon	Baker
Pc 28	rhododendron	Oregon	Lambe
Pc 29	----	Oregon	Lambe
Pc 30	Port Orford cedar	Oregon	Kuhlman

Several different soils were used in various parts of this research. Willamette Valley soil was Amity silty clay loam from ornamental beds in Corvallis. The pathogen has been isolated from similar soils in the area. McDonald Forest and Maxfield Creek soils were both Aiken silty clay loams. The McDonald Forest area in which collections were made has a mature stand of Douglas-fir. The Maxfield Creek vegetation is composed predominantly of Acer macrophyllum, Rhus diversilosa and Alnus rubra.

Isolation

All Phytophthora spp. are relatively slow growing. In isolating this group from infected tissue or infested soil it is necessary to provide conditions adequate for their growth and less favorable for other fungi.

Phytophthora cinnamomi was isolated from infected tissues most successfully when the transition zone between healthy and diseased tissue was used. If the disease was in its early stages only the young roots were infected. Small sections of these roots at the transition zone readily yielded the fungus. The root sections were washed in running tap water, then placed on corn meal agar or potato dextrose agar. Growth of most other fungi is restricted on corn meal agar so chances of identifying P. cinnamomi are improved. In the advanced stages of disease development the transition zone is present in the young vascular tissue of the main roots or at the root collar. Larger root pieces were thoroughly washed and the bark was peeled back, exposing the sapwood. Small pieces of the sapwood and young phloem at the transition zone were removed aseptically and either placed directly on agar or surface sterilized in 70% alcohol for 3-5 minutes before being placed on agar. Some success was obtained when the infected tissue was inserted into holes bored in apples in a modification of Campbell's technique (11).

Isolation of Phytophthora cinnamomi from the soil is more difficult since an abundance of other soil microorganisms are present. A modification of Campbell's (11) apple technique was used. Two holes were bored into each

apple at angles to each other so that they met at the center. One hole was filled with the soil sample, the other hole was supplied daily with distilled water. When rot appeared the apple was split open and small pieces from the margin of the rot were removed and put on agar. Potato dextrose agar was used at first but 2% water agar proved more effective since it slowed the growth of saprophytic fungi. Campbell reported 40% of his apples gave positive results (11).

A number of methods were tried in an effort to increase the effectiveness of the soil isolation technique. Because a Pythium sp. was commonly isolated, a method of inhibiting it was sought. In culture P. cinnamomi grew at pH 3.5 whereas the Pythium sp. was inhibited completely at pH 3.75. The soil has such a tremendous buffering capacity that all attempts to adjust small soil samples to these narrow limits proved exceedingly difficult. Another method that was tried was the use of baits. Root pieces, stem pieces, and leaves of Port Orford cedar and Douglas-fir were placed on top of saturated soil in petri dishes. When P. cinnamomi was present in large quantities it showed up readily as sporangia on the surface of the tissue. However, in field soil Fusarium sp. and Pythium sp. were rapid invaders of the host tissue. The presence of P. cinnamomi

could be detected only by very close observation of the tissue under the microscope or by putting the tissue through the apple technique. A modification of Anderson's (1) host infection technique was also tried. Anderson suspended rooted pineapple crowns or slips in water containing 1 or 2 tablespoons of soil. P. cinnamomi produced brown lesions within 4 days and then produced mycelium and sporangia. Rooted Port Orford cedar cuttings and Douglas-fir seedlings were suspended in pint jars containing water and a small amount of soil. Douglas-fir is very intolerant of low oxygen levels and was killed whether or not the fungus was present. Port Orford cedar, although more tolerant of low oxygen levels, nevertheless, did not readily show the presence of the fungus.

Culture Methods

Several media were used in isolation and culture of P. cinnamomi. Stock cultures as well as inoculum were maintained on potato dextrose agar (PDA).

PDA: Mixed broth from 200 gm. potatoes in 500 cc. water with 20 gm. agar, 20 gm. dextrose, and 500 cc. water. Autoclaved 20 minutes at 15 pounds pressure.

Corn meal agar: 50 gm. of corn meal in 500 cc. water heated to 70°C; kept at 60°-70°C for 1 hour, filtered, 20 gm. agar, 20 gm. dextrose and 500 cc. water added, autoclaved 20 minutes.

Pea broth: 150 gm. of dried split peas autoclaved 3 minutes in 1 liter water, decanted, and autoclaved 20 minutes.

Alfalfa meal agar (AMA): 50 gm. alfalfa meal in 1 liter water heated to 70°C; kept at 60°-70°C for 1 hour, filtered through cheesecloth; 500 cc. of filtrate mixed with 20 gm. agar, 20 gm. dextrose, and 500 cc. water, autoclaved.

Milk agar: 20 cc. of milk, 1 liter water, 20 gm. agar, autoclaved.

Alfalfa sand meal: Alfalfa meal at the rate of 5% of the weight of the sand, mixed thoroughly. Watered to make a moist but well aerated mixture. Autoclaved 30 minutes.

P. cinnamomi was grown on pea broth, milk agar, PDA, and AMA in attempts to induce the formation of sporangia. In addition, pieces of various host tissue were sterilized in a propylene oxide-air gas mixture (30, 90), and placed on the surface of actively growing

cultures of P. cinnamomi on PDA. Following a period of growth, disks of agar or pieces of the substrata containing the fungus were placed in erlenmyer flasks or petri dishes containing 25 ml. of soil filtrate. The soil filtrate was prepared by mixing 100 gm. of soil in a liter of distilled water and filtering through Whatman No. 1 filter paper (40;80;87).

Inoculations

In all the constant soil temperature tank studies the inoculum consisted of two week old cultures of P. cinnamomi. Cultures grown on both PDA and AMA were macerated in distilled water in a Waring Blendor. One hundred milliliters of the macerated culture suspension were poured into a hole six inches deep midway between the trees in each pot. The checks were inoculated with 100 cc. of an AMA-PDA mixture. Field inoculations were made with infested soil which was prepared by mixing macerated two week old cultures grown on PDA and AMA into the soil at the rate of 1-100 by volume.

The Study Area

Field plots were established in order to study the fungus under field conditions after it was felt the

fungus was not a rigorous pathogen. The disease may be introduced into the forest through logging disturbances, therefore a cut-over area was selected. This land also offered a smaller chance of starting a large disease outbreak. The Bureau of Land Management permitted field plots to be established on land located in the W½ of section 19, T10S, R5W. The area is in the valley of a tributary of Maxfield Creek. It is a typical mountain valley with steep sides extending up from the stream. Vegetation in the study area is primarily Acer macrophyllum, Rhus diversilosa, and Alnus rubra. There are a few Douglas-fir seed trees present. Five experimental plots at an elevation of 1250-1500 feet were selected to include a number of different environmental conditions. Plot one is on a south exposure on a small bench. Plots two and three are also on a south exposure; however, plot two is on a very steep slope with no shade and plot three is in a small drainage area so that throughout the winter water flows through it. Plots four and five have northern exposures and are shaded. Plot four is on level ground and plot five on a steep slope. All of the plots were fenced to prevent disturbance by animals. The plots were 15 feet square and each contained three rows each of Douglas-fir, noble fir and Port Orford

cedar seedlings. The rows were 12 inches apart with 24 inch intervals between the different species. Most of the first plantings died from drought and were replaced with Port Orford cedar and Douglas-fir.

RESULTS

Symptoms of the Disease

The disease has only been observed on Douglas-fir saplings in an ornamental planting and under greenhouse conditions. The symptoms on the saplings in ornamental plantings closely resembled those of the littleleaf disease of shortleaf and loblolly pine in the Southeast. The current year's needle and twig growth was very much stunted and gave a tufted appearance. Usually an affected tree would not last through more than one growing season in this condition. The entire tree slowly dried up and became brittle. In the greenhouse disease development was so rapid that there was no chance for development of the littleleaf symptoms. The foliage simply lost color and turned brown.

The symptoms on the roots were quite characteristic. The fungus started at the root tips and moved up into the larger roots. Finally the fungus progressed into the root collar and death soon followed. Diseased cambium and phloem have a reddish-brown color. The transition from diseased to healthy tissues was very striking since the healthy tissue was white.

Growth or Movement of the Pathogen

The pathogenic potential of P. cinnamomi on Douglas-fir would in part be determined by the speed with which it can move through the soil in nature. Mycelial growth, zoospore movement, or movement of fungal parts in soil water are possible means of spread for this fungus. A pathogen is often aided by the disturbances of man. Under normal circumstances P. cinnamomi is confined to the area occupied by its hosts. Legge has shown with other Phytophthora spp. that mycelial growth does not occur in non-sterile soil (37, p.98,102).

Movement in a Controlled Environment

Growth of mycelium and movement of zoospores were followed under controlled conditions. In earlier experiments it had been noted that when agar disks containing mycelium were placed in sterile soil mycelium grew out into the soil profusely, whereas disks placed in non-sterile soil produced no mycelium. Soil from McDonald Forest collected from the upper eight inches of the soil profile was mixed with distilled water in order to bring the soil to a moisture content of 43% which is slightly above field capacity. A similar lot of the same soil

was raised to 58% moisture content. Portions of the soil from both moisture levels were autoclaved for 20 minutes at 15 pounds pressure. Upon cooling the soil was spread evenly to a depth of one-half inch in petri dishes. Non-sterile soil was spread in petri dishes in the same manner. PDA disks containing the fungus were placed in the center of each dish. Five replications of both sterile and non-sterile soil were incubated at 15°, 20°, and 25°C for three weeks. After this time soil samples were taken one inch from the inoculum. Two apples were inoculated with the soil samples from each petri dish. The fungus was not recovered from non-sterile soil at any of the temperatures or moisture levels although it was recovered at all levels in sterile soil (Table 1).

Table 1. Growth of *P. cinnamomi* mycelium in sterile and non-sterile forest soil.

Soil Moisture Content (%)	Temperature (°C)	Number of positive recoveries of fungus from soil one inch from inoculum[1]	
		Sterile Soil	Non-sterile
43	15	10/10[1]	0/10
58	15	10/10	0/10
43	20	10/10	0/10
58	20	10/10	0/10
43	25	10/10	0/10
58	25	10/10	0/10

[1] Denominator indicates number samples tested, numerator number of times *P. cinnamomi* recovered.

The movement of zoospores through the soil under their own power and with the aid of soil water movement is apparently the chief means of spread of this pathogen. Zoospores readily germinate on Douglas-fir root tips and the developing hyphae immediately form sporangia. Thus zoospore movement can be detected by placing excised root tips at various distances from sporangial inoculum. The zoospores move to the root tips and form sporangia on them. The presence of the distinctive sporangia is a positive indication that the fungus has moved to the root.

Zoospore movement was followed in a soil filtrate. Disks of AMA containing 16-day-old P. cinnamomi mycelium were placed in a soil filtrate. After a week the disks were checked microscopically for the presence of sporangia. Each disk was put on the point of a thumb tack. The tack was held in place by inserting the head under a rubber band which was wrapped around a microscope slide. Douglas-fir root tip sections approximately one inch long were held at 1, 2, 3, and 4 inch distances from the inoculum by inserting them under rubber bands wrapped around slides. Controls were included which had root tips and no inoculum. The slides were placed in deep petri dishes and sufficient soil filtrate was added

to submerge both the inoculum and host tissue. Care was taken to insure the minimum of water movement. The cultures were maintained at room temperature. After three days the root tips were examined under a microscope for the presence of sporangia. The zoospores readily swam two inches, at a distance of three inches only one out of twenty roots was infected and none of those at four inches were infected (Table 2).

Table 2. Movement of zoospores in a non-sterile soil filtrate.

Distance (inches)	No. of root tips with sporangia (out of twenty)
1	11
2	9
3	1
4	0

Movement in the Field

Movement of the pathogen through soil was studied at Maxfield Creek. Two week old cultures of the fungus were mixed with Amity soil from the Willamette Valley. In previous studies the recovery of *P. cinnamomi* was always higher from this soil. Three treatments were used: in the first the infested soil was put in 15 six inch pots, in the second lot of 15 six inch pots two year old Douglas-fir seedlings were planted in the

infested soil at the time it was taken to the field, and in the third two year old Douglas-fir seedlings were grown in the infested soil in the greenhouse for three weeks so that some infection could occur before being taken to the field. Three single pot replications of each of the treatments were established in each of the 5 plots at Maxfield Creek in January, 1960. The soil was removed from the pots and carefully buried in a compact ball. Sampling to determine spread of the fungus was carried out once every two months. One sample was collected at the center of the infested soil, one was collected at the edge of the infested soil, and two were gathered two inches uphill and downhill from the infested soil with a 15 mm. cork borer.

The results are summarized in Tables 3 and 4 by plots and by treatments. The scattered movement of the fungus probably can be attributed to rodents which seemed to abound in the area. The death of the fungus in the infested soil is rapid as the soil dries out. In the driest plots (1,2,3) the recovery was already greatly reduced in June. A reduction in the recovery of the fungus was expected during the drier months but the rate of recovery should have increased again when the wet season started if there were any resistant structures present.

Table 3. Influence of soil type and microflora on survival of *P. cinnamomi*.

	Recovery of *P. cinnamomi* after different monthly intervals																							
Plot	Center of plot						Edge of plot						Two inches downhill						Two inches uphill					
No.	2	4	6	8	10	14	2	4	6	8	10	14	2	4	6	8	10	14	2	4	6	8	10	14
1	7[1]	8	3	0	0	0	6	7	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
2	7	9	1	0	0	0	8	9	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
3	8	9	1	0	0	0	7	9	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	9	9	4	0	0	0	8	8	7	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
5	7	9	8	0	2	0	8	9	6	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0

[1] No. of times *P. cinnamomi* recovered (out of 9 times).

Table 4. Influence of the presence of infected or uninfected host tissue in infested soil on survival of *P. cinnamomi*.

	Recovery of *P. cinnamomi* after different monthly intervals																							
Treat-	Center of plot						Edge of plot						2" downhill						2" uphill					
ment	2[1]	4	6	8	10	14	2	4	6	8	10	14	2	4	6	8	10	14	2	4	6	8	10	14
Soil alone	13	15	6	0	1	0	13	14	5	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Infected host	13	15	5	0	1	0	12	14	8	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0
Host in soil	12	14	6	0	1	0	12	14	8	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

[1] No. of times *P. cinnamomi* recovered (out of 15).

Samples were collected at the center and edge of the infested soil in order to see if the forest soil inhibited the P. cinnamomi mycelium in any way. Recovery in the two places was not different.

In order to ascertain whether the forest soil microflora was a limiting factor in the spread of the fungus, Amity soil from the Willamette Valley was substituted for the forest soil at Maxfield Creek in ten small 18" x 18" x 8" plots. In each corner of the plots healthy Port Orford cedar seedlings were planted. A diseased Port Orford cedar seedling was planted in the center of each plot. The diseased trees had portions of their root systems infected but the foliage was still green. Five of the plots were supplied with water from an irrigation system during the summer. The water was continuously dripped onto the center of the plot. After one year five of the center trees were dead, and two more had die-back at the tip. Four out of the five dead trees were in the non-watered group. Nine of the other trees were dead and six of these were in the non-watered group. Isolations from the soil yielded P. cinnamomi in only two of the plots. Disease development apparently slowed down following transfer to the field. Trees in the irrigated plots probably were killed by the fungus. The trees in

the non-watered plots may have died from the summer drought.

Inhibition by the Soil Microflora

P. cinnamomi is greatly inhibited by many organisms in culture. Many contaminants such as Penicillium and Trichoderma spp. completely prevent growth of P. cinnamomi within several centimeters of the mycelium of the contaminant. It was felt that the forest soil might have a severe fungistatic effect on P. cinnamomi which would be limiting to its growth and survival. The fungistasis of some soils to mycelium or spores of certain fungi has been demonstrated by Nils Molin and Jackson (45;34).

The technique consists of placing disks of agar containing fungal mycelium on cellophane in contact with the soil. The rate of growth on sterile soil is compared with that on non-sterile soil. Soils from Maxfield Creek, McDonald Forest, and the Willamette Valley were collected. Half of the soil from each area was steam sterilized for 20 minutes at 15 pounds pressure. Two level tablespoons of soil were placed in each petri dish and 10 ml. of sterile distilled water was added. Fifteen replications of both sterile and non-sterile soil were made for each soil type. Cellophane disks of the same diameter as the petri dishes were sterilized in distilled water in the

autoclave to remove any water proofing substances and were then spread over the surface of the soil under aseptic conditions. Disks of PDA were cut from poured plates with a flamed number 15 cork borer, 22 mm in diameter. A flamed spatula was used to remove the disks and to place them on the cellophane. The inoculum disks, 4 mm in diameter, of P. cinnamomi growing on PDA were placed in the center of the agar disks. The plates were incubated at 25° C for two days or until the mycelium reached the edge of the disks. Three measurements of each colony diameter were averaged to determine the average growth per colony. The amount of inhibition for a particular soil was determined as the ratio of the average growth for the 45 colonies on non-sterile soil to the average growth for the 45 colonies on sterile soil expressed as a percentage. Samples were processed periodically for a year (Table 5). The forest soils were never as inhibitory as the valley soil. There appears to be no period during the year when the soil would be fungitoxic to P. cinnamomi since some growth always occurred.

Inhibition of P. cinnamomi was compared on non-sterilized soil, steam sterilized soil, and propylene oxide sterilized soil. When these results were subjected

to an analysis of variance test, there was no significant difference between the mean diameters of colonies on non-sterile and propylene sterilized soils whereas the diameters of colonies on steam sterilized soil were significantly greater at the 1% level.

Thus the fungistatic compounds were produced by the soil microflora prior to incubation and were not due to the incubation. At two different times the rate of inhibition of P. cinnamomi was compared with the inhibition of some other fungi. The data presented in Table 6 indicates that most fungi are affected by antibiotics produced in the soil. The method has certain limitations and may not show the important antibiotic effects or the effects of non-diffusible antibiotics.

Table 5. Fungistasis in soils towards *P. cinnamomi* as indicated by inhibition of mycelial growth.

Source of Soil	Mycelial growth on soil samples collected on:*					
	8/59	9/59	11/59	1/60	3/60	6/60
Willamette Valley	37.7*	3.2	32.9	38.4	68.6	51.5
McDonald Forest	44.5	33.6	43.9	50.5	80.4	57.1
Maxfield Creek	65.7	31.2	41.2	52.4	88.3	47.5

* Expressed as percentage of growth on sterile soil.

Table 6. Fungistasis towards several species of fungi as indicated by inhibition of mycelial growth.

Name of fungus	Mycelial growth[1] on soil samples from:	
	Willamette Valley	McDonald Forest
Pythium sp. (isolated at Maxfield Creek)	64.86	83.8
P. cinnamomi	37.70	44.5
Helminthosporium sp.*		39.1
Fusarium sp.*		55.1
Rhizoctonia sp.*		65.4
*P. cinnamomi**		63.6

* Tested in Nov., 1960; the first two were tested in August, 1959.

[1] Expressed as percentage of growth on sterile soil.

Survival of the Pathogen

Survival of P. cinnamomi in the forest situation is important not only on a long term basis but also on a year to year basis. The annual summer drought provides very severe conditions for the fungus although production of resistant spores by the fungus would increase its chances of survival. After death of the host plant it is very difficult to isolate P. cinnamomi because of the rapid invasion of the host tissues by soil saprophytes. Whether P. cinnamomi is killed or simply overgrown by these fungi is not known. Long term survival studies can be an aid to control of the disease by demonstrating possible susceptible periods in the life cycle of the fungus.

The effects of soil microflora, soil type, presence or absence of host tissue, and soil moisture level on P. cinnamomi survival were studied over a two and a half year period. The soil treatments used were as follows: 1) sterile soil, 2) sterile soil infested with Pythium sp., 3) Amity soil from the Willamette Valley, 4) Aiken soil from Maxfield Creek, 5) Aiken soil from McDonald Forest. Each sample consisted of soil packed to within one-half inch of the top of French square bottles, $2\frac{1}{2}$ inches square and 6 inches tall with screw caps.

Sub-treatments for soil moisture were to be maintained by watering one set of thirty samples, allowing one set to vary with the season, and capping one set so that its moisture level would drop throughout the experiment. Heavy rains the first winter supersaturated all the open bottles so that they remained moist all year long. The results of these two sub-treatments were combined on the data sheet. A severe cold spell during the first year cracked many of the open bottles adding another variable. In the fourth sub-treatment a Douglas-fir seedling was planted in each bottle. All of the soil was infested with macerated cultures of P. cinnamomi at the rate of 100 parts soil to 1 part fungus. Once every two months the soil in two bottles from each sub-treatment was checked for the presence of P. cinnamomi using the apple technique. During the first year four apples were used per bottle but only two were used for the remainder of the time.

The results are reported as the per cent recovery of P. cinnamomi in Tables 7 and 8 and Figures 1-5. The figures illustrate the poor survival of this fungus. After a year the recovery rate dropped to a very low level. There seems to be 3 plateaus. Sporangia, mycelium,

Table 7. Influence of soil type and microflora on survival of *P. cinnamomi*.

Treatment *P. cinnamomi* in:	*Recovery of *P. cinnamomi* after different monthly intervals														
	2	4	6	8	10	12	14	16	18	20	22	24	26	28	31
Sterile soil	42.8	59.4	25	15.6	15.6	43.7	6.3	12.5	0	6.3	0	0	0	6.3	0
Sterile soil + *Pythium*	13.2	28.1	0	3.1	12.5	6.3	3.1	0	0	0	0	0	0	0	0
Amity soil from the Willamette Valley	61.1	72	68.8	34.4	21.4	12.5	6.3	0	18.7	12.5	0	0	0	0	0
Aiken soil from Maxfield Creek	31.2	34.4	18.7	0	6.3	0	0	6.3	0	0	0	0	0	0	0
Aiken soil from McDonald Forest	37.4	50	31.2	3.1	15.6	47.5	6.3	12.5	0	6.3	0	0	0	0	0

* Recovery expressed as the percentage of positive isolations.

Table 8. Influence of variation in soil moisture and presence or absence of host tissue on survival of *P. cinnamomi*.

Treatment	*Recovery of P. cinnamomi after different monthly intervals														
	2	4	6	8	10	12	14	16	18	20	22	24	26	28	31
Open containers	25	50	18	7.5	15	5	5	5	5	5	0	0	0	0	0
Closed containers	50	33	25	20	17	10	7	15	5	5	0	0	0	0	0
Host tissue present	46	55	45	12.5	7.5	32	5	5	5	0	0	0	0	5	0

* Recovery expressed as the percentage of positive isolations.

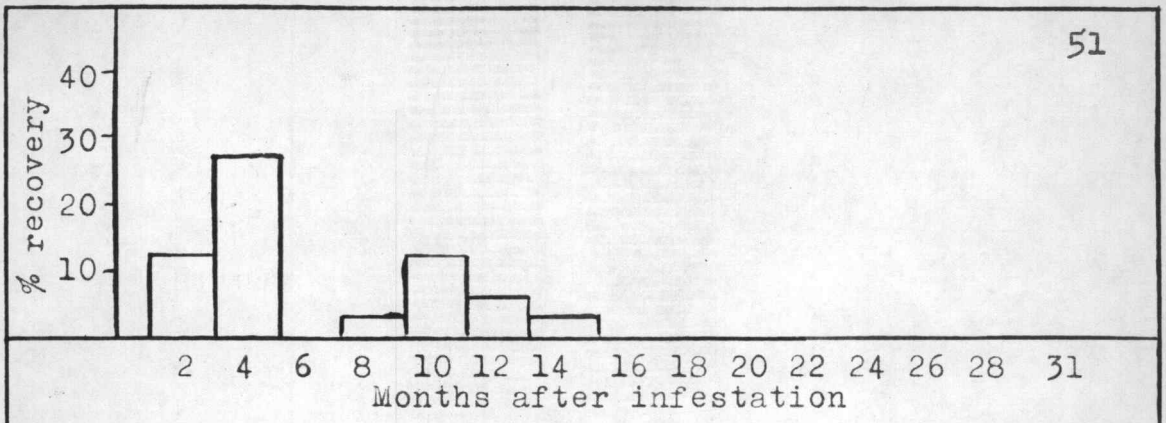

Figure 1. Recovery of P. cinnamomi from sterile soil infested with P. cinnamomi and Pythium sp. after different monthly intervals.

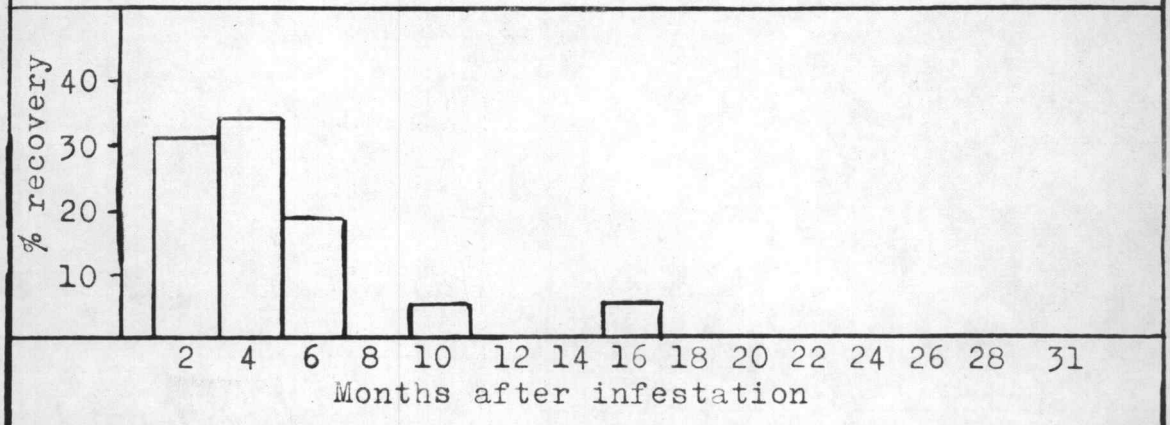

Figure 2. Percentage recovery of P. cinnamomi from infested Aiken soil from Maxfield Creek after different monthly intervals.

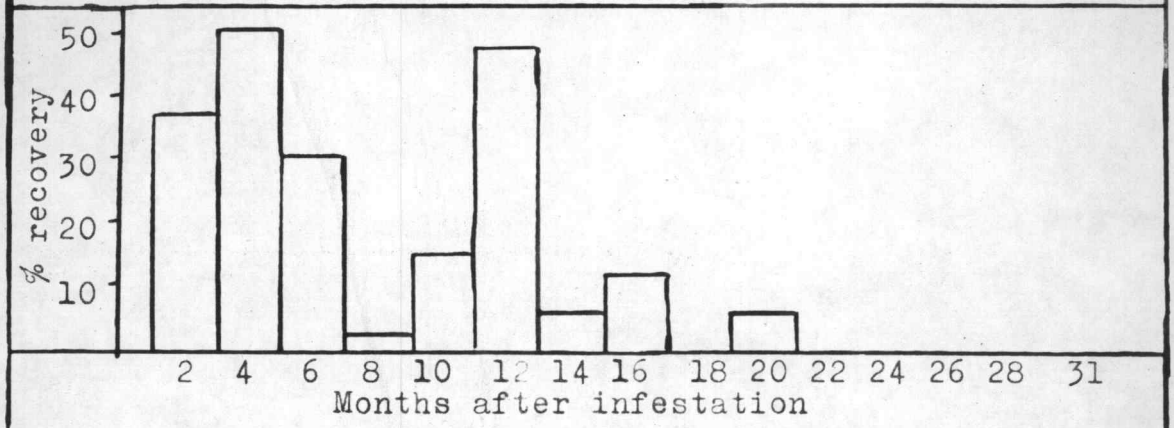

Figure 3. Percentage recovery of P. cinnamomi from infested Aiken soil from McDonald Forest after different monthly intervals.

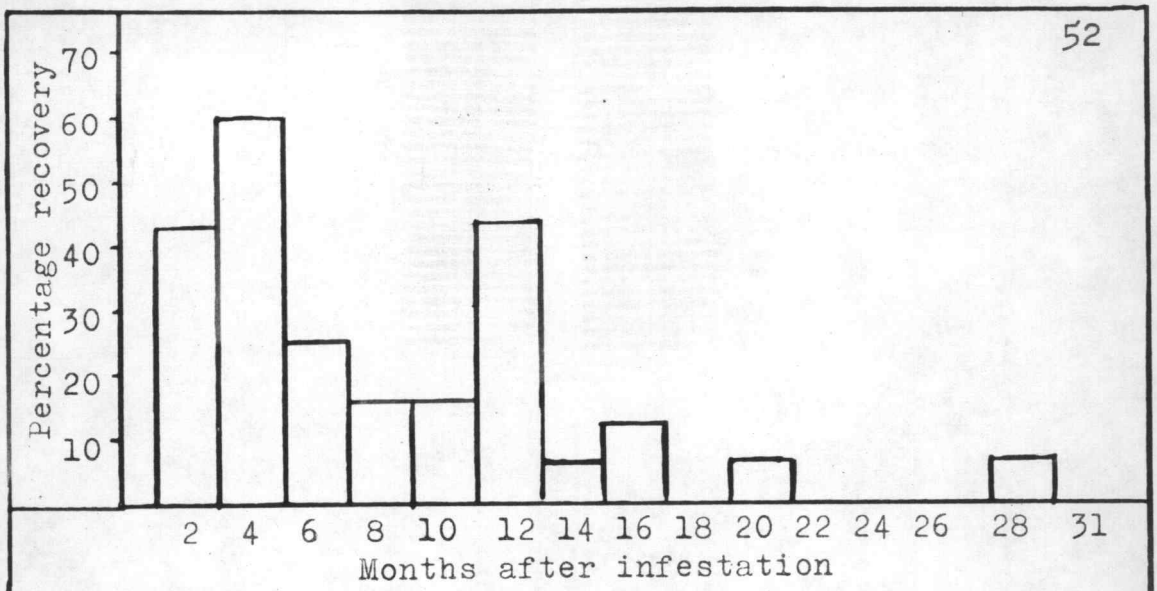

Figure 4. Percentage recovery of P. cinnamomi from sterile soil infested with P. cinnamomi after different monthly intervals.

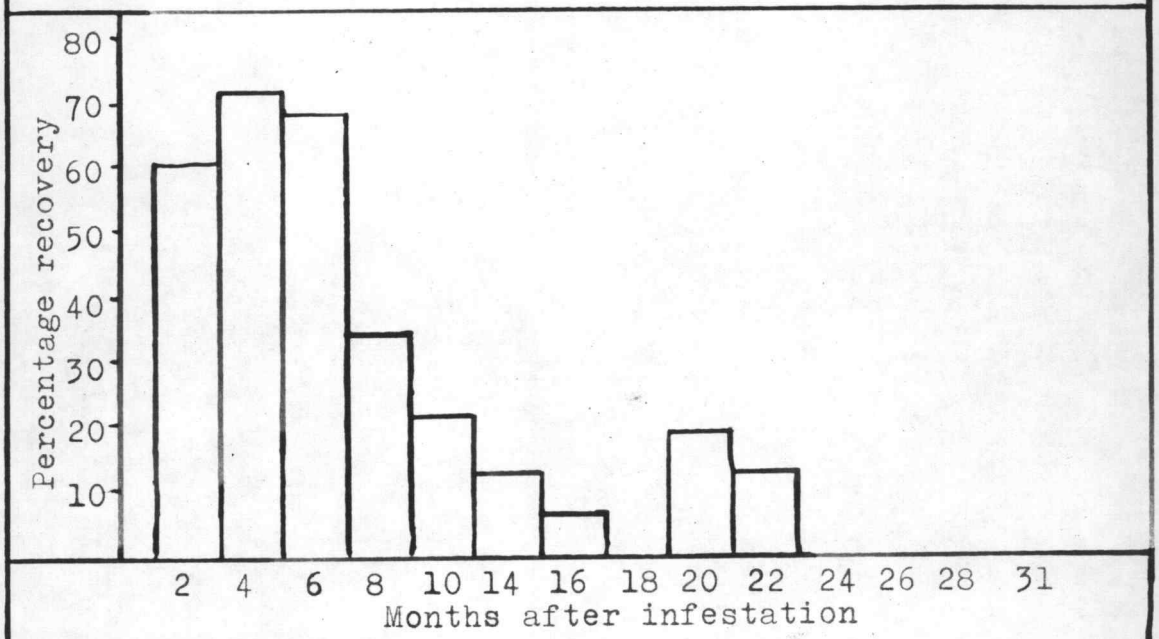

Figure 5. Percentage recovery of P. cinnamomi from infested Amity soil from the Willamette Valley after different monthly intervals.

and chlamydospores were probably all functional during the first six months. During the next twelve months chlamydospores probably were the only surviving form of the fungus. The sporadic recovery during the last year indicates that only a very few chlamydospores are functional over an extended period of time. The two soil treatments that gave the poorest recovery contained the Pythium sp. which was isolated in previous field studies at Maxfield Creek to a greater degree than P. cinnamomi. It is possible that the Pythium sp. has nutritional requirements similar to P. cinnamomi and by growing faster gets there first. The Amity soil was used because the disease had occurred in it and recovery from it was always better. Since the survival time was not increased in this soil it appears survival in forest soils is not markedly different. The increased recovery in the 12th month from Aiken soil from McDonald Forest and the infested sterile soil is difficult to explain. High temperatures and an adequate supply of water provided ideal conditions for recovery of the fungus and for growth from the host tissue since the increase shows up there as well. The rapid invasion of infected tissue by soil saprophytes would cause a decrease in recovery of P. cinnamomi. Failure of the other treatments to respond

similarly may be due to the lack of uninfected host tissue since a considerable number of the seedlings died.

As the soil dries the recovery of P. cinnamomi becomes increasingly difficult if not impossible. Rewetting the soil reactivates the fungus to some extent but after a longer period of drying recovery of the fungus is not possible. The following experiment was set up to determine how long the soil had to remain dry in order to kill the fungus. Aiken soil from McDonald Forest was infested with macerated PDA and AMA cultures of P. cinnamomi at the rate of 100 cc per can of soil. Four cans of soil were kept moist while the others were allowed to dry out at greenhouse temperatures. After each sampling two of the cans that had been allowed to dry were rewetted. Twenty samples from each treatment were checked each month. Moisture content in the dried soil ranged from 5.5 to 11%. After two months of drying the fungus could no longer be recovered. The results are given in Table 9.

Table 9. The effect of drying the soil on recovery of *P. cinnamomi*.

Treatment	Recovery of *P. cinnamomi* at monthly intervals*			
	0	1	2	3
Moistened checks	75*	30	35	20
Dried 1 month then rewet	--	35	30	0
Dried 2 months then rewet	--	--	0	0
Dried 3 months	--	--	--	0

* Recovery expressed as the percentage of positive isolations.

Saprophytic Ability

Survival of many root pathogens is facilitated by their capacity to invade dead tissues as saprophytes. This capacity is determined in part by speed of growth, competitive ability, and inoculum level of the fungus. *P. cinnamomi* has been shown to be slow growing. Two experiments were conducted to determine the competitive saprophytic ability of the fungus and the inoculum level necessary for saprophytic invasion to occur. In the first experiment dried Douglas-fir twigs varying in diameter from 1-4 mm. were cut into 2 inch sections and buried at 4-6 inch depths in infested soil containing Douglas-fir seedlings killed by *P. cinnamomi*. The soil

was in pots in temperature tanks maintained at 24°, 28°, and 32°C. There were 45 twigs at each temperature. After one month the twigs were removed and half inch sections from the ends were plated out as previously described. Only one twig at 24°C yielded P. cinnamomi. The most common saprophytes present were species of Pythium, Fusarium, Pencillium, and Mucoraceae.

The inoculum level at which P. cinnamomi can compete with soil saprophytes was determined in the following experiment. The fungus was grown in alfalfa meal-sand for one month. The inoculum was diluted with non-sterile McDonald Forest and Willamette Valley soils at rates of 100/0 (inoculum/soil); 90/10; 70/30; 50/50; 25/75; 12.5/87.5; 6.25/93.75; 3/97; 0/100. Two replications of each soil type at each dilution were made, using number 10 cans. Ten two-inch long pieces of dried Douglas-fir twig were buried in each can at an approximate depth of 4 inches. After incubation for one month in the greenhouse with periodic watering, the twigs were removed from the soil, washed in tap water, cut into four ½-inch sections, surface sterilized in 70% alcohol and plated on corn meal agar. In the first trial the inoculum was contaminated so that the results were not consistent. In the second trial the inoculum was thoroughly mixed before being diluted and the results were much better. At very

low levels P. cinnamomi is a poor saprophyte in either soil (Table 10). It is likely that the microflora in the forest soil is better adapted to the invasion of woody tissues and therefore the competition is greater resulting in a lower incidence of invasion by P. cinnamomi in this soil.

Table 10. The effect of inoculum level on saprophytic invasion of Douglas-fir twigs by P. cinnamomi.

Dilution inoculum/ soil	Number of twigs yielding P. cinnamomi out of 20 in: McDonald Forest soil	Willamette Valley soil
100/0	17	19
90/10	18	20
70/30	14	17
50/50	5	12
25/75	1	6
12.5/87.5	1	2
6.3/93.7	0	3
3.1/96.9	0	0
0/100	0	0

Survival studies reported here, and those of other workers, indicate that P. cinnamomi generally has a shorter survival time in the soil than other Phytophthora spp. Although chlamydospores have been commonly reported for

this species, oospores have rarely been seen. Oospores probably are responsible for long term survival. No reports of microscopic examination of infected tissue have been found in the literature. Extensive hand sectioning of infected rhododendron roots showed a great amount of mycelium. The mycelium was not particularly characteristic of P. cinnamomi but was finer and did not have vesicles. There were a few round bodies somewhat resembling chlamydospores in some epidermal cells of the root. Freezing microtome sections of Douglas-fir roots stained with rose-bengal showed scattered vesicular bodies in some of the cells. Pieces of avocado and Port Orford cedar root tissue which were inoculated with the fungus and then hand sectioned yielded more positive results. Chlamydospores were present in the cedar tissue and a few oospores were observed in the avocado tissue.

Infection of the Host

Movement and survival of the pathogen are essential for infection of the host plant. Douglas-fir is susceptible to this pathogen but the conditions under which it is susceptible are not known. Most workers have concentrated on the moisture requirements for infection. There is general agreement that abundant moisture is required and trees on poorly drained sites are the first to be

affected (70;66;7;72;24;84;12;23;61;38;47;69;32;17;3). For part of the year moisture requirements in western Oregon would be quite adequate for infection, however, during that time the soil temperature may be a limiting factor.

Constant soil temperature tanks at 12°, 16°, 20°, 24°, 28°, and 32°C were used to determine the range over which infection would occur. Port Orford cedar seedlings were used in the first trial since they were available and were not as susceptible to injury from excess water as is Douglas-fir. Two trees were planted in each of the 12 pots in each temperature tank and were allowed to become established for a month before being inoculated. The soil mixture used consisted of: 4 parts greenhouse soil, 1 part sand, 2 parts leaf mold, 1 part sterilized garden soil, and commercially prepared ammonium phosphate fertilizer (1 pint/8 buckets of soil). The trees were watered lightly four times a week but no specific control of the moisture levels was attempted since each pot had a drain. After one month half of the pots were inoculated with P. cinnamomi. Three months after inoculation the trees were removed from the soil and the roots examined for necrotic and discolored areas. Eight sections were plated out from each tree. Results are given in Table 11. Douglas-fir seedlings were used in the second trial over the same

Table 11. The effect of temperature on infection of Port Orford cedar by *P. cinnamomi*.

Temp. (°C)	Infested (I) or Uninfested (U) soil	Number of trees out of 12			No. of times *P. cinnamomi* was isolated
		Affected	Killed	With disc. vascular tissue	
32	U	2	2	0	0
32	I	9	7	9	5
28	U	0	0	0	0
28	I	9	6	9	4
24	U	0	0	0	0
24	I	9	4	9	5
20	U	0	0	0	0
20	I	6	0	6	4
16	U	0	0	0	0
16	I	0	0	0	0
12	U	0	0	0	0
12	I	0	0	0	0

temperature range. The results of this experiment are given in Table 12. In both of these experiments the highest incidence of infection occurred at the three highest temperatures. At 32°C the Douglas-fir roots were damaged by the high temperature in both the infested and uninfested soil (Fig. 9). The roots were confined to the upper two inches of soil where the soil temperature was probably a few degrees lower. These roots were short and club-like. At 24°C and 28°C there were great differences between the inoculated and the uninoculated trees (Fig. 8). The pathogen affected most of the roots and soon girdled the main root. The 20°C tank did not maintain as stable a temperature as the other tanks because it leaked and water had to be added continuously. It varied ±3°C whereas the others varied ±1°C. Thermographs were used to record the temperature in the tanks for a month. Symptoms varied from only a few dead roots such as the middle seedling in Figure 7 to those with a reduced root system and subsequent reduction in foliage as that on the right. Absence of infection at 16°C is the most striking result (Fig. 6). There were no dead roots on either the inoculated or the uninoculated seedlings. Root production on both Douglas-fir and Port Orford cedar appeared to be very good at the lower temperatures. The one occurrence of infection was due to a pot that was

Table 12. The effect of temperature on infection of Douglas-fir by *P. cinnamomi*.

Temp. (°C)	Infested (I) or Uninfested (U) soil	Number of trees out of 12			No. of times *P. cinnamomi* was isolated
		Affected	Killed	With disc. vascular tissue	
32	U	4	4	0	0
32	I	11	8	3	0
28	U	1	1	0	0
28	I	11	7	4	1
24	U	1	0	0	0
24	I	12	7	5	4
20	U	2	0	0	0
20	I	4	2	2	3
16	U	0	0	0	0
16	I	1	1[1]	0	1
12	U	0	0	0	0
12	I	2[2]	2	0	0

[1] Pot was out of the water several inches more than the other pots were.
[2] Both were watersoaked, due to excess soil water.

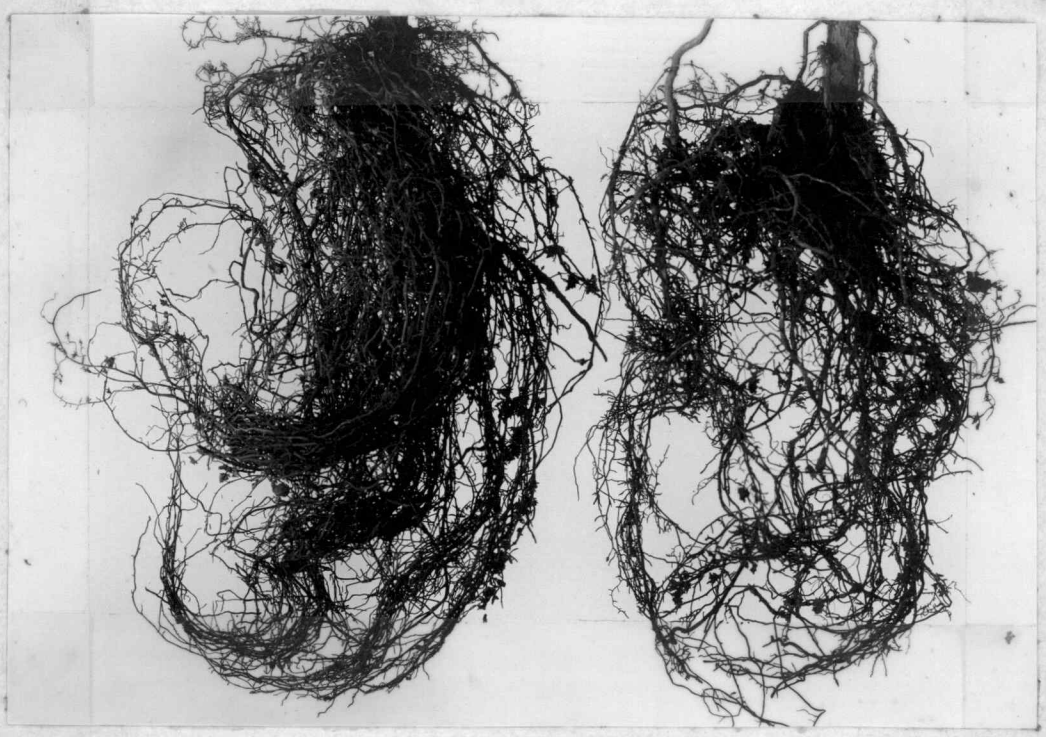

Figure 6. Roots of Douglas-fir seedlings grown in uninfested soil (left) and infested soil (right) at 16°C.

Figure 7. Roots of Douglas-fir seedlings grown in uninfested soil (left) and infested soil (right) at 20°C.

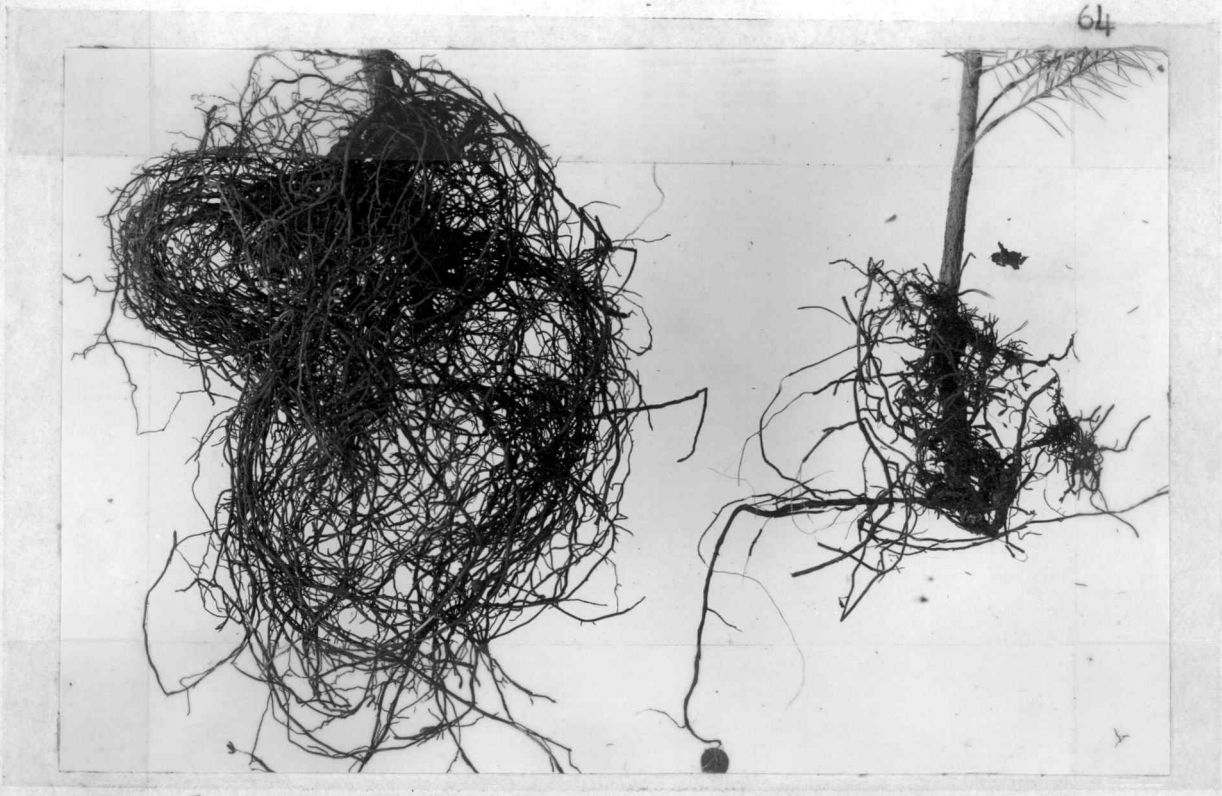

Figure 8. Roots of Douglas-fir seedlings grown in uninfested soil (left) and infested soil (right) at 24°C.

Figure 9. Roots of Douglas-fir seedlings grown in uninfested soil (left) and infested soil (right) at 32°C.

set higher out of the water than the other pots. The temperature gradient cone that goes from air temperature to soil temperature several inches below the soil surface probably extended farther in this case. At 12°C there was no infection.

Douglas-fir seems quite intolerant of continuous high soil moisture. Some trees in both infested and uninfested soil died because of poor drainage. The young vascular tissue of trees killed in this way is blackened and often watersoaked and therefore is not readily confused with the red-brown discoloration caused by P. cinnamomi.

Temperature tanks were next set up at 15°, 17.5°, and 20°C in order to determine more exactly the minimum temperature at which infection would occur. All conditions were the same as in the previous experiment except the pots had no drains and there were 52 trees at each temperature. Glass tubes were inserted to the bottom of the pots and the excess water was removed with a vacuum. Results are summarized in Table 13. Infection occurred at 17.5°C and 20°C. The 17.5°C tanks varied by +2°C while the other tanks varied only ±1°C. Temperatures above 16°C are required for infection of Douglas-Fir by P. cinnamomi.

Table 13. Study of the minimal temperature required for infection of Douglas-fir.

Temp. (°C)	Infested (I) or Uninfested (U) soil	Number of trees out of 26			
		Affected	Killed	With disc. vascular tissue	No. of times *P. cinnamomi* was isolated
20	U	4	4	0	0
20	I	13	7	10	0
17.5	U	0	0	0	0
17.5	I	5	1	4	0
15	U	0	0	0	0
15	I	2*	2	0	0

* Watersoaked

Sporulation and Germination

Sporangial formation and spore discharge are of great importance in infection since the zoospores are the most effective means of infection. Without sporangial formation there would be little spread of the pathogen and the disease problem would be greatly reduced. Sporangial formation is affected by substrate, temperature, moisture, and microflora.

Sporangial Formation and Discharge

P. cinnamomi was grown on a number of different substrates in order to find one on which it would regularly produce an abundant supply of sporangia. Pea broth has commonly been reported as a good media for sporangial production. After seven days growth on pea broth the mycelial mats were transferred to a soil filtrate or distilled water, and sporangia were formed in a couple days. These sporangia were round rather than papillate as are those formed on host tissue. Papillate sporangia were formed on sterilized roots of Douglas-fir, Port Orford cedar, and avocado placed in distilled water and inoculated with P. cinnamomi. Zentmyer's method (80;87) of placing agar disks from two week old PDA cultures in a non-sterile soil filtrate for several days produced good

results. The most abundant sporangial production occurred when disks from 16 day old AMA cultures were placed in a non-sterile soil filtrate for a week.

The transfer of mycelium to a non-sterile soil filtrate seems to have two effects, a reduction in nutrient level and a biological stimulation from the soil organisms. When mycelial mats grown in pea broth were transferred to distilled water or .01M KNO_3 solution the sporangial production was less than in a non-sterile soil filtrate. However, immediately following germination of zoospores on Douglas-fir root tips sporangia are formed. Soil filtrates were made from Aiken soil from McDonald Forest, Amity soil from the Willamette Valley, and greenhouse soil. Five AMA disks containing the fungus were placed in 25 ml. of filtrate. There were five replications of each filtrate and the experiment was repeated once. After a week at room temperature (20°-25°C) two counts per disk were made of the number of sporangia at 100x magnification under a grid disk. The number of sporangia that had discharged was determined by counting the number of empty sporangia. The average of the fifty counts was used to determine the number of sporangia/mm^2. The grid disk enclosed .3844 mm^2. An analysis of variance of the results (Table 14) showed the differences were not

Table 14. The effect of soil filtrate on sporangial formation and discharge.

Source of soil	Total number of sporangia (per mm^2)	Number discharge (per mm^2)
Willamette	9.55	1.72
"	11.39	1.51
McDonald Forest	15.04	2.78
" "	15.41	2.74
Greenhouse mix	13.04	1.66
" "	14.05	1.72

significant. The soil filtrates from these soils have the same effect on sporangial formation and discharge.

Temperature requirements for sporangial formation have been found by other workers (87;61), but temperatures at which sporangial discharge occurs have not been reported. The same techniques were used as reported above except only greenhouse soil was used. The cultures were incubated for a week at 10°, 15°, 20°, 25°, 30°, 35°C. The experiment was repeated once and the combined data appear in Table 15. Some sporangia discharged at all temperatures at which they had formed including 15°C.

Table 15. The effect of temperature on sporangial formation and discharge.

Temperature (°C)	Total number of sporangia (per mm^2)	Number discharged (per mm^2)
10	0	0
15	3.75	0.16
20	27.58	0.47
25	25.55	1.29
30	8.01	0.22
35	0	0

Moisture Effect

To determine moisture levels at which sporangia would form, water was added to greenhouse soil to bring it to various moisture levels above field capacity and the soil was placed in glass jars which were sealed to prevent rapid drying. Agar disks were buried in the soil for a week and then were washed out of the soil. Results were never consistent although at different times sporangia were found at all moisture levels that did not absorb water from the agar disks.

Oospore Production and Germination

Oospore production by P. cinnamomi has previously been observed on Mexican avocado roots and extracts (91) and in old cultures on oatmeal agar (49). A medium was sought on which P. cinnamomi would produce an abundance of oospores. Roots of Port Orford cedar, Douglas-fir, rhododendron and avocado (California and Mexican varieties) sterilized in propylene oxide were placed in sterile distilled water and in 0.75% water agar and were inoculated with P. cinnamomi (30). A few oospores developed in the avocado tissue. In another treatment sterilized roots were crushed with a mortar and pestle in distilled water and the crushed tissue was added to cooled agar before it was poured. Oospores developed most abundantly on macerated Mexican avocado roots. The roots were first sterilized in propylene oxide, then placed in distilled water at a ratio of 5 ml. of distilled water per gram of sterilized root and blended in a Waring Blendor for 30 seconds. The extract was poured through cheesecloth, autoclaved and poured into petri dishes to be inoculated. Oospores developed on mycelium growing in close proximity

to the inoculum disks and did not occur on hyphae growing in the medium.

Oospore germination was attempted in the following ways: A host-soil leachate was prepared by pouring distilled water on the soil around potted host plants and the first 100 ml. of leachate coming out the bottom of the pot was collected (57). The non-sterile leachate was passed through Whatman No. 1 filter paper. Mycelium containing oospores was placed in leachates from Mexican avocado, Port Orford cedar, and Douglas-fir. When the cultures were examined three weeks later most of the oospores were infected with Rhizophydium carpophilum, a Chytrid. Oospores were placed in both sterile and non-sterile marsh water. In another test oospore cultures were smeared on slides and allowed to dry at room temperature for a week, then placed in a soil filtrate. Both sterile and non-sterile soil leachates from avocado trees were used on some cultures. Cultures were frozen at -10°C for two days and then were put in a non-sterile soil filtrate. Cellophane strips containing oospore smears were buried in the soil for two weeks, the strips were then placed in a soil filtrate. Some oospores were treated with dilute hydrochloric acid in an attempt to digest away the outer walls. The protoplasm was destroyed by this treatment.

None of the oospores germinated. Either oospores produced in culture are nonviable or they require conditions for germination other than those provided in the experiments.

DISCUSSION

Phytophthora cinnamomi has been recognized as a potentially serious threat to the Douglas-fir forests of western Oregon and Washington. The studies reported here attempted to determine how well this fungus could grow and survive in Oregon forest soils and under what conditions it would infect Douglas-fir.

Mycelium of some *Phytophthora* spp. does not grow through non-sterile soil (37). *P. cinnamomi* grew readily in sterile soil at moisture levels above field capacity but did not grow at all in non-sterile soil. In field studies only very sporadic movement of the fungus was found and this may have been due to animals in the soil. Spread of *P. cinnamomi* usually is in the direction of soil water movement (38;54). Zentmyer and Richards (83) reported that *P. cinnamomi* moved 15 feet uphill in an avocado orchard and suggested the movement was the result of mycelial growth along the roots. Zoospore movement might be a more likely possibility even for uphill movement since zoospores in soil filtrates were able to move up to three inches and easily moved one or two inches. Sporangia are formed rapidly after zoospores germinate. After three days root tips exposed to zoospores had some sporangia that had proliferated two or

three times. If the zoospores can germinate and produce sporangia at any point on the root or in other organic matter, spread of P. cinnamomi along the root will be quite rapid.

A fungistatic effect was demonstrated in forest soils and in a cultivated soil that has supported the pathogen. The inhibitory effect appeared equally great in all soils tested. The method of placing agar disks on cellophane on the soil did not show the complete fungistatic effect that was noted when mycelial disks were placed in the soil. This may be analagous to growth from diseased tissue where P. cinnamomi mycelium will be among the first to grow from the tissue, but will be subsequently overgrown by other fungi.

Survival studies indicated that P. cinnamomi is short-lived in the soil even in the presence of host tissue. After twelve months, recovery of the fungus dropped to a very low level and after twenty months there was only one recovery. The fungus survived even more poorly when subjected to severe drying and was almost completely killed as the soil dried in summer. In greenhouse studies the fungus was killed after the soil had been allowed to dry for only two months. Microscopic examination of infected Douglas-fir roots disclosed the presence of no resistant structures of the

fungus. Formation of oospores and chlamydospores was induced in artificially inoculated sections of avocado and Port Orford cedar respectively. Newhook (47) reported that recovery of P. cinnamomi dropped considerably one or two years after the death of the host, Pinus radiata, and although still present after five years it was never recovered in the same quantity as other Phytophthora spp.

Experimental results may not be conclusive because a fungus grown in culture may lose some of its capacity for competition. For example, isolates grown in culture for a long time may be less pathogenic than recent isolates. In the same way isolates kept in culture very long have shorter survival capacities. The absence of resistant spores greatly reduces the survival chances of this fungus since the mycelium and sporangia are more sensitive to environmental changes than are either chlamydospores or oospores. Mycelium in older host tissue might be expected to last longer because of the protection the tissue would offer to environmental changes.

Saprophytic invasion of organic matter by a fungus can provide a means of survival in the absence of living hosts. P. cinnamomi was able to colonize twigs of Douglas-fir consistently only when more than half of

the soil volume was an alfalfa meal-sand mixture infested by P. cinnamomi. Butler (9) would classify P. cinnamomi as having a low sparophytic ability. In forest soil the mycelium was less aggressive than in cultivated soil. This may be an indication of greater competition in the forest soil, such as that offered by the Pythium sp. at Maxfield Creek. The other soil organisms grow more rapidly and P. cinnamomi is suppressed.

With the evidence thus far presented P. cinnamomi can be classified as a root-inhabiting fungus according to Garrett's criteria since it: 1) is inhibited by organisms in the soil; 2) does not grow through unsterilized soil; 3) invades living tissue readily but only rarely invades dead tissues; 4) is limited in distribution in the soil to those areas occupied by host plants. The importance of factors affecting infection are made more important by the recognition of P. cinnamomi as a root-infecting fungus, since the fungus is behaving normally in the forest soil when it dies rather rapidly, does not move through the soil, and does not invade dead Douglas-fir tissue.

Sporangial formation and zoospore discharge occurs from 15°C to 30°C. Sporangial formation was 7.5 times greater at 20°C than at 15°C and zoospore discharge was three times greater. This reduction in inoculum level

may account for the absence of infection at 16°C even though P. cinnamomi can form zoospores at this temperature. No effect of filtrate microflora could be shown. The biological stimulus necessary for sporangial formation was present in all soils tested.

Attempts to germinate oospores were unsuccessful. Legge (37) has demonstrated that upon germination oospores of other Phytophthora spp. give rise to sporangia. This might start the disease cycle following an unfavorable period. Since oospores apparently are not involved in the disease cycle on Douglas-fir this study was of academic rather than practical interest.

At 16°C and below no infection occurred and root growth was prolific. Temperatures above 16°C (61°F) are necessary for infection and maximum disease development occurred at and above 24°C. At 32°C Douglas-fir roots were killed by the heat as well as the fungus. Most of the trees were killed within 3 months at the higher temperatures but at 20°C symptom expression was confined principally to the roots. This relatively warm temperature requirement for infection may well be a limiting factor in the spread of this pathogen from ornamental nurseries into forests. Irrigation in nurseries during the warm summer months would seem to be an aid to disease development.

This pathogen may be restricted in three ways: 1) competitive inhibition by the forest soil microflora, 2) soil moisture levels that are not adequate when the temperature is high enough, and 3) soil temperatures too low for infection. Since P. cinnamomi apparently produces no resistant spores the other soil organisms could so overrun any substrate that P. cinnamomi would be completely suppressed. High soil moisture levels are necessary for infection although the exact requirements are not known. Generally during the wet winter and spring months soil temperatures do not get above 60°F. so root rot would not develop. Table 16 presents some soil temperatures for spring and fall at Maxfield Creek. On the exposed and therefore drier sites temperatures above 62°F probably can be expected until the second week of October and again after the last week of April. On the shaded sites no temperatures above 60°F were recorded in the transitional periods between wet and dry seasons. Conditions in the region probably closely approximate those in New Zealand where Newhook (47, p.830-833) feels that the disease is most severe when extreme summer droughts are followed by heavy early autumn rains. The soil cracks during the summer and the rain is able to permeate the soil much faster. In Oregon unless there are warm spring rains or heavy warm fall rains the

chances of disease development are greatly reduced. Summer rains especially along the coast may come during warmer weather and start small pockets of infection.

Any fungus with as extensive a host range as this fungus has may become better adapted to its environment. Therefore all precautions against spread should not be abandoned. Forest nurseries must continue to guard against this pathogen because of the widespread dissemination of their planting stock. Nursery stock could create extensive incipient infections which under proper conditions might develop into a large scale epiphytotic. The general public should continually be made aware of the possibility of introducing diseases of this type through movement of diseased planting stock. All foresters and forest pathologists should realize that, although the temperature requirements for infection will generally exclude this organism from the Douglas-fir forests, any exceptionally wet warm periods may introduce the problem on a large scale if the pathogen is not excluded from forests.

SUMMARY

1. *Phytophthora cinnamomi* mycelium grew through sterile forest soil at two moisture levels, 43 and 58% and at temperatures of 15°, 20°, and 25°C, but did not grow in non-sterile soils at the same moisture and temperature levels.
2. Zoospore movement was followed in a non-sterile soil filtrate up to three inches. The zoospores readily moved one and two inches and formed sporangia following germination on Douglas-fir roots.
3. In the field the fungus moved in only very limited amounts from infested soil and infected host plants.
4. Inhibition of *P. cinnamomi* mycelial growth was demonstrated throughout the year in both forest soils and cultivated soil. Other fungi were similarly inhibited although a *Pythium* sp. isolated from forest soil was less inhibited than the others.
5. Survival of *P. cinnamomi*, as measured by recovery, dropped to a low level during the first 6 months in soils containing a *Pythium* sp., remained at a very low level for the next 14 months, and was not recovered after 19 months. In the other soils *P. cinnamomi* was recovered frequently for 6 months, rarely during the next 14, and only once in the last 11 months.

Frequency of recovery of the fungus was greatest from a cultivated soil but length of survival was no greater than in forest soils. The presence of Douglas-fir seedlings did not lengthen survival time.

6. Low soil moisture levels were very injurious to the fungus. In field inoculations summer drought killed the fungus on several occasions. In greenhouse studies the fungus was killed after the soil was allowed to dry for two months.
7. P. cinnamomi is a poor competitive soil saprophyte. When alfalfa-meal sand infested with P. cinnamomi was diluted with forest soil and cultivated soil, the fungus readily invaded Douglas-fir twigs only when the concentration of P. cinnamomi infested sand was above 50%.
8. Microscopic examination of infected Douglas-fir roots showed no resistant structures were present. A chemical stimulus present in Mexican avocado roots seems to be necessary for oospore formation.
9. Oospores produced in cultures could not be germinated after a number of treatments.
10. A biological effect needed for sporangial production was present in several different soil filtrates.

11. Sporangia were produced and zoospores discharged at temperatures of 15°, 20°, 25°, and 30°C. Sporangia were not formed at 10°C or 35°C.

12. Sporangia were produced at all moisture levels that did not desiccate the mycelium.

13. Infection of Douglas-fir and Port Orford cedar seedlings did not occur at soil temperatures of 16°C and below. Maximum infection was at 24° and 28°C. At 32°C Douglas-fir roots were injured by the heat.

14. P. cinnamomi should not be considered a threat to the Douglas-fir forests of the Pacific Northwest under normal climatic conditions. Both high soil moisture and high soil temperature are required for disease development and these normally do not occur simultaneously in this region. Indiscriminate introduction of the pathogen into the forest could constitute a hazard if environmental conditions became adequate for the fungus or the fungus became adapted to the environment.

BIBLIOGRAPHY

1. Anderson, E. J. A simple method for detecting the presence of Phytophthora cinnamomi Rands in soil. Phytopathology 41:187-189. 1951.

2. Anderson, E. J. Effect of nutrient salt concentration on infection of pineapple roots by Phytophthora cinnamomi. Phytopathology 41:2. 1951.

3. Anderson, E. J. The Phytophthora cinnamomi problem in pineapple fields of Hawaii. Phytopathology 41: 1-2. 1951.

4. Ashby, S. F. The production of sexual organs in pure cultures of Phytophthora cinnamomi Rands and Blepharospora cambivora Petri. Transactions British Mycological Society 14:260-263. 1929.

5. Bingham, F. T., G. A. Zentmyer and J. P. Martin. Host nutrition in relation to Phytophthora root rot of avocado seedlings. Phytopathology 48:144-147. 1958.

6. Blackwell, E. Species of Phytophthora as water moulds. Nature 153:496. 1944.

7. Boyce, A. M. Research and avocado root rot. California Citrograph 43:20-21. 1957.

8. Buddenhagen, I. W. Natural and induced variability in Phytophthora cactorum (L. & C.) Shroet. and Phytophthora cinnamomi Rands. Ph.D. thesis. Corvallis, Oregon State College, 1957. 114 numb. leaves.

9. Butler, F. C. Saprophytic behavior of some cereal root rot fungi. I. saprophytic colonization of wheat straw. Annals Applied Biology 40:284-297. 1953.

10. Cairns, H. and A. E. Muskett. Pink rot of the potato. Annual Applied Biology 20:381-403. 1933.

11. Campbell, W. A. A method of isolating Phytophthora cinnamomi directly from the soil. Plant Disease Reporter 33:134-135. 1949.

12. Campbell, W. A. and O. L. Copeland Jr. Littleleaf disease of shortleaf and loblolly pines. 1954. 41 p. (U. S. Dept. of Agriculture. Circular 940)

13. Campbell, W. A. The occurrence of Phytophthora cinnamomi in the soil under pine stands in the southeast. Phytopathology 41:5. 1951.

14. Campbell, W. A. Vertical distribution of Phytophthora cinnamomi in the soil under littleleaf-diseased shortleaf pine. Plant Disease Reporter 35:26-27. 1951.

15. Clinton, G. P. Chestnut bark diseases. In: Report of the Station Botanist, 1911-1912. Part 5 of the annual report of Connecticut Agricultural Experiment Station for 1912. Storrs, 1912. p. 359-453.

16. Copeland, O. L. Root mortality of shortleaf and loblolly pine in relation to soils and littleleaf disease. Journal of Forestry 50:21-25. 1952.

17. Copeland, O. L. and R. M. McAlpine. The interrelations of littleleaf, site index, soil, and ground cover in Piedmont shortleaf pine stands. Ecology 36:635-641. 1955.

18. Crandall, B. S. Phytophthora cinnamomi root rot of avocados under tropical conditions. Phytopathology 38:123-130. 1948.

19. Crandall, B. S., G. F. Gravatt and M. M. Ryan. Root diseases of Castanea species and some coniferous and broadleaf nursery stocks, caused by Phytophthora cinnamomi. Phytopathology 35:162-180. 1945.

20. Crandall, B. S. Root diseases of some conifers and hardwoods caused by Phytophthora cambivora (P. cinnamomi). Plant Disease Reporter 20:202-204. 1936.

21. Critopoulos, P. D. Root rot of tomato incited by Phytophthora capsici. Bulletin, Torey Botanical Club 82:168-182. 1955.

22. Curtiss, D. S. and G. A. Zentmyer. Effect of oxygen supply on Phytophthora root rot of avocado in nutrient solution. American Journal Botany 36:471-474. 1949.

23. Day, W. R. Root rot of sweet chestnut and beech caused by species of Phytophthora. Forestry (Great Britain) 12:101-126. 1938.

24. Frezzi, M. J. Phytophthora cinnamomi y su relacion con la muerta de los Paltos en Urundel (Salta), Argentina, Revista Argentina Agronimea 9:214-219. 1952. (Abstracted in Review of Applied Mycology 34:532. 1955)

25. Garrett, S. D. Biology of root infecting fungi. Cambridge, University Press, 1956. 293 p.

26. Goode, P. M. Infection of strawberry roots by zoospores of Phytophthora fragariae. Transactions, British Mycological Society 39:367-377. 1956.

27. Gravatt, G. F. Potential danger to the Persian walnuts, Douglas-fir and Port Orford cedar of the Pacific coast from the cinnamon Phytophthora. Plant Disease Reporter 38:214-216. 1954.

28. Grente, J. and G. Solignat. La maladie de l'encre du châtaigner et son evolution. (Chestnut ink disease and its development). Académie d'agriculture de France, Paris. Comptes rendus des séances 38:126-129. 1952. (Abstracted in Review Applied Mycology 32:106-107. 1953)

29. Gregg, M. Germination of oospores of Phytophthora erythroseptica. Nature 180:150. 1957.

30. Hansen, H. N. and W. C. Snyder. Gaseous sterilization of biological materials for use as culture media. Phytopathology 37:369-371. 1947.

31. Hepting, G. H. and J. W. Cruikshank. Littleleaf disease in South Carolina as determined by the forest survey. Journal of Forestry 48:837-839. 1950.

32. Hickman, C. J. and M. P. English. Factors influencing the development of red core in strawberries. Transactions, British Mycological Society 34:223-236. 1951.

33. Jackson, L. W. R. Root defects and fungi associated with the littleleaf disease of southern pines. Phytopathology 35:91-105. 1945.

34. Jackson, R. M. An investigation of fungistasis in Nigerian soils. Journal of General Microbiology 18: 248-258. 1958.

35. Kristic, M. Note on the inhibitory activity of some microorganisms against Phytophthora cinnamomi. Plant Disease Reporter 38:879. 1954.

36. Legge, B. L. A Phytophthora crown rot of Campanula. Transactions, British Mycological Society 34:300. 1951.

37. Legge, B. L. Studies of species of Phytophthora. Ph.D. thesis. Birmingham, University of Birmingham, 1953. 120 numb. leaves.

38. Lewcock, H. K. Top rot of pineapples and its control. Queensland Agricultural Journal 43:145-149. 1935.

39. Loring, L. B., State plant pathologist, Salem, Ore. Severity of Phytophthora cinnamomi within infested nurseries and adjoining areas. Willamette Valley - Winter 1956-57. Typewritten research note.

40. Mehrlich, F. P. Non-sterile soil leachate stimulating to zoosporangia production by Phytophthora sp. Phytopathology 24:1139-1140. 1934.

41. Mehrlich, F. P. Pathogenicity and variation in Phytophthora species causing heart rot of pineapple plants. Phytopathology 26:23-43. 1936.

42. Mes, M. M. A wilt of snapdragon (Antirrhinum majus) in South Africa. South African Journal of Science 31:281-287. 1934.

43. Middleton, J. T. and D. V. Baxter. The occurrence of Phytophthora and Pythium species on roots of native plants in northern California and southern Oregon. (Abstract) Phytopathology 45:694. 1955.

44. Miller, P. A. Bleeding canker disease of California trees. Proceedings of the Eighth Western Shade Tree Conference, 1941, p. 39-45.

45. Molin, N. A study on the infection biology of Fomes annosus. Stockholm Statens Skogsforskningsinstitut Meddelanden 47 (3):36. 1957.

46. Müller, K. O. Wound healing of the potato tuber in relation to infection by Phytophthora infestans. Australian Journal of Biological Science 10:189-196. 1957.

47. Newhook, F. J. The association of Phytophthora spp. with mortality of Pinus radiata and other conifers. I. Symptoms and epidemiology in shelterbelts. New Zealand Journal of Agricultural Research 2:808-843. 1959.

48. Oyler, E. and W. F. Bewley. A disease of cultivated heaths caused by Phytophthora cinnamomi Rands. Annals of Applied Biology 24:1-16. 1937.

49. Petri, L. Ricerche sulla morfologia e biologia della Blepharospora cambivora, parassitta del castagno. Atti della R. Accademia Nazionale dei Lincei. Rendiconti. Cl. di Sci., Fis., Mat., e Nat. Roma. (Ser V)26 (II):297-299. 1917. (From Crandall et al. Phytopathology 35:162-180. 1945)

50. Pimentel, A. A. L. Novas observacoes sobre a morfologia, biologia e fiscologia di dois fungos de genero Phytophthora deBary, parasitas do castanheiro. Agron. Lusit. 7:337-353. (From Gravatt, G. F. Plant Disease Reporter 38:214-216. 1954)

51. Rands, R. D. Streepkander van kaneel, veroorzaakt door Phytophthora cinnamomi n. sp. Department Landbouw. Nijverheid Handel, Mededeelingen van het Buitenzorg Instituut von Plantenziekten 54:1-53. 1922. (From Crandall et al. Phytopathology 35: 162-180. 1945)

52. Roth, L. F. Phytophthora cinnamomi is not prevalent in Pacific Northwest forest soils. Unpublished report to the forest disease committee. Corvallis, Oregon, Oregon State Agricultural Experiment Station, 1959.

53. Salisbury, P. J. Parasitism of Phytophthora spp. isolated from root rot of Port Orford cedar in British Columbia. Bi-monthly Progress Report, Division of Forest Biology, Department of Agriculture Canada 2:3-4. 1955.

54. Schreiber, L. R. and R. J. Green, Jr. Die-back and root rot disease of Taxus spp. in Indiana. Plant Disease Reporter 43:814-817. 1959.

55. Smith, H. C. Red-core (Phytophthora fragariae) of strawberries. New Zealand Journal of Science and Technology 33:49-56. 1951.

56. Smoot, J. J. et al. Production and germination of oospores of Phytophthora infestans. Phytopathology 48:165-171. 1958.

57. Stamps, D. J. Oospore production in paired cultures of Phytophthora species. Transactions, British Mycological Society 36:255-259. 1953.

58. Sutherland, C. F., F. J. Newhook and J. Levy. The association of Phytophthora spp. with mortality of Pinus radiata and other conifers. II. Influence of soil drainage on disease. New Zealand Journal of Agricultural Research 2:844-858. 1959.

59. Thorn, W. A. and G. A. Zentmyer. Hosts of Phytophthora cinnamomi Rands. Plant Disease Reporter 38:47-52. 1954.

60. Thorn, W. A., G. A. Zentmyer and P. P. Wong. Irrigation water tests to isolate the avocado root rot fungus. California Avocado Association Yearbook 43:104-106. 1959.

61. Torgeson, D. C. Epiphytology and etiology of a Phytophthora induced root rot disease of Chamaecyparis in Oregon. Ph.D. thesis. Corvallis, Oregon State College, 1952. 72 numb. leaves.

62. Torgeson, D. C., R. A. Young and J. A. Milbrath. Phytophthora root rot of lawson cypress and other ornamentals. Corvallis, 1954. 18 p. (Oregon Agricultural Experiment Station. Station Bulletin 537)

63. Torgeson, D. C. Root rot of lawson cypress and other ornamentals caused by Phytophthora cinnamomi. Contributions of the Boyce Thompson Institute 17: 359-373. 1954.

64. Trione, E. J. The pathology of Phytophthora lateralis on native Chamaecyparis lawsoniana. Phytopathology 49:306-310. 1959.

65. Trione, E. J. The physiology and pathology of Phytophthora lateralis on native Chamaecyparis lawsonia. Ph.D. thesis. Corvallis, Oregon State College, 1957. 107 numb. leaves.

66. Tucker, C. M. Report of the plant pathologist Mayaguez, 1929. p. 29-35. (Puerto Rico. Agricultural Experiment Station. Report for 1928)

67. Tucker, C. M. and J. A. Milbrath. Root rot of Chamaecyparis caused by a species of Phytophthora. Mycologia 34:94-103. 1942.

68. Valleau, W. H. Longevity of the tobacco black shank fungus in the soil in the absence of tobacco. Plant Disease Reporter 35:453-454. 1951.

69. Wager, V. A. The dying back of avocado trees in southern California. California Avocado Association Yearbook 24:40-43. 1940.

70. Wager, V. A. Phytophthora cinnamomi and wet soil in relation to the dying-back of avocado trees. Hilgardia 14:517-532. 1940.

71. Waterhouse, B. M. Identification of Phytophthora spp. by means of oospores produced in dual cultures. In: Proceedings of the Seventh International Botanical Congress, Stockholm 1950, p. 413-414.

72. Welsh, M. F. Studies of crown rot of apple trees. Canadian Journal of Research C20:457-490. 1942.

73. White, R. P. Rhododendron wilt and root rot. New Brunswick, 1937. 32 p. (New Jersey. Agricultural Experiment Station. Bulletin 615)

74. White, R. P. Summary of nine years' experience with rhododendron wilt. Plant Disease Reporter 20:204-207. 1936.

75. Young, R. A. Dissemination of plant pathogens on nursery and ornamental plants. Plant Disease Reporter 38:417-420. 1954.

76. Zak, B. and W. A. Campbell. Susceptibility of southern pines and other species to the littleleaf pathogen in liquid culture. Forest Science 4:156-160. 1958.

77. Zan, F. Persistence and movement of Phytophthora infestans in soil. (Abstract) Transactions, British Mycological Society 39:385. 1956.

78. Zentmyer, G. A. A substance stimulating sexual reproduction in Phytophthora cinnamomi. Phytopathology 42:24. 1952.

79. Zentmyer, G. A. Avocado root rot. California Avocado Association Yearbook 34:63-67. 1950.

80. Zentmyer, G. A. and R. A. Marshall. Factors affecting sporangial production by Phytophthora cinnamomi. Phytopathology 49:556. 1959.

81. Zentmyer, G. A., J. D. Gilpatrick, and W. A. Thorn. Methods of isolating Phytophthora cinnamomi from soil and from host tissue. (Abstract) Phytopathology 50:87. 1960.

82. Zentmyer, G. A. and L. J. Klotz. Microorganisms in avocado tree decline. California Citrograph 31:436-437. 1946.

83. Zentmyer, G. A. and S. J. Richards. Pathogenicity of Phytophthora cinnamomi to avocado trees and the effect of irrigation on disease development. Phytopathology 42:35-37. 1952.

84. Zentmyer, G. A. and L. J. Klotz. Phytophthora cinnamomi in relation to avocado decline (Abstract) Phytopathology 37:25. 1947.

85. Zentmyer, G. A. Phytophthora cinnamomi on avocado in Mexico and Costa Rica, and other avocado diseases in Mexico. Plant Disease Reporter 36:31. 1952.

86. Zentmyer, G. A. Phytophthora root rot of avocado. Proceedings of the Sixth Pacific Coast Research Conference on Soil Fungi, 1959, p. 28.

87. Zentmyer, G. A. Spore stages of the avocado root rot fungus, Phytophthora cinnamomi. California Avocado Association Yearbook 43:110-112. 1959.

88. Zentmyer, G. A. and F. T. Bingham. The influence of nitrite on the development of Phytophthora root rot of avocado. Phytopathology 46:121-124. 1956.

89. Zentmyer, G. A., K. F. Baker and W. A. Thorn. The role of nursery stock in the dissemination of soil pathogens. (Abstract) Phytopathology 42:478. 1952.

90. Zentmyer, G. A., W. C. Snyder, and H. N. Hansen. Use of natural media for inducing sporulation in fungi. Phytopathology 40:971. 1950.

APPENDIX

Table 16. Maximum (M) and minimum (m) soil temperatures (°F) recorded at a 4" depth on three sites at Maxfield Creek in spring and fall of 1957 and 1958. The sites were: 1) an unshaded south exposure, 2) a slightly shaded south exposure, and 3) a heavily shaded north exposure.

	1957 Site						1958 Site					
	1		2		3		1		2		3	
Date	M	m	M	m	M	m	M	m	M	m	M	m
Sept.												
20	77	60	70	60	60	60						
21	78	60	70	60	59	58						
22	81	63	74	60	60	59						
23	82	61	75	62	59	56						
24	77	64	70	62	57	57						
25	74	64	69	62	58	56						
26	67	56	64	60	57	57						
27	66	56	64	58	57	55						
28	75	59	62	60	57	57						
29	74	60	67	60	57	56	71	58	65	59	52	51
30	66	60	68	62			72	59	65	59	52	50
Oct.												
1							73	60	67	60	54	52
2							73	60	67	60	55	52
3							69	60	67	60	55	52
4							62	60	68	62	54	53
5							69	58	68	64	54	53
10	63	55	57	56	55	54	68	56	62	58	52	51
11	55	55	58	54	54	54	66	56	60	56	51	49
12	62	55	55	55	56	55	64	56	61	60	52	50
13	54	54	59	55	55	53	66	56	63	59	52	50
14	56	54	55	54	52	51	60	57	61	59	51	50
15	60	53	55	54	54	51	59	54	60	58	52	50
16	64	56	55	54	54	54	58	50	62	58	51	50
17							59	48	58	58	51	50
18							58	50	60	57	49	48
24							58	48	54	52	47	45
25							60	48	55	51	47	46
26							60	50	56	53	47	45
27							60	50	55	52	47	44
28							60	50	54	52	47	45
29							60	50	55	53	46	44
30							57	50	55	55	48	46

Table 16 (contd.)

Date	1957 Site 1 M	1957 Site 1 m	1957 Site 2 M	1957 Site 2 m	1957 Site 3 M	1957 Site 3 m	1958 Site 1 M	1958 Site 1 m	1958 Site 2 M	1958 Site 2 m	1958 Site 3 M	1958 Site 3 m
April												
13	54	48	51	50	49	49						
14	54	48	52	49	50	49						
15	54	46	53	47	50	47						
16	54	48	56	50	52	49						
17	53	46	54	49	51	50						
18	50	44	57	50	50	50						
19	54	50	53	50	50	45						
20	59	47	58	48								
21	54	49	55	50								
22	56	45	56	49								
23	53	47	54	50								
24	58	48	57	50								
30	60	56	60	58	56	56						
May												
1	60	56	60	54	54	52	72	65	60	56	50	47
2	60	50	66	55	55	51	72	67	62	57	47	45
3	64	51	68	60	58	54	75	66	57	54	49	47
4	66	52	71	61	59	56	68	53	56	55	47	46
5	69	55	72	62	57	56	70	56	62	57	49	47
6							60	40	63	55	50	48
7							59	54	64	57	50	47
8							72	59	64	58	49	48
9							78	60	59	56	50	48
10							80	60	59	53	49	46
11	58	58	60	60	55	55	75	59	61	53		
12	60	56	62	59	56	56	62	50	64	57		
13	64	58	63	59	56	56	62	50	64	57		
14	60	58	62	60	55	55	70	50	68	56		
15	57	58	64	56	55	55	72	58				
16	55	50	57	55	56	54						
17	58	52	60	55	56	55						
18	64	52	59	56	56	55						
26							68	56	64	63	43	41
27							60	58	67	62	43	42
28							64	58	62	59	42	40
29							70	60	67	62	42	40
30	69	56	67	57	59	59	67	59	64	61	42	39
31	70	56	68	57	59	58	59	57	63	61	43	41

Table 16 (contd.)

	1957 Site						1958 Site					
	1		2		3		1		2		3	
Date	M	m	M	m	M	m	M	m	M	m	M	m
June												
1	72	58	60	59	58	58	62	58	63	60	43	42
2	74	60	71	60	58	57	64	56	66	60	44	43
3	76	64	73	62	59	58	76	64	73	62	44	43
4	75	62	72	62	59	58	65	59			47	45