

EPIPHYTOLOGY AND ETIOLOGY OF A
PHYTOPHTHORA-INDUCED ROOT ROT DISEASE
OF CHAMAECYPARIS IN OREGON

by

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TABLE OF CONTENTS

| | Page |
|--------------------------------------------------------------------------------------------------------------------------------------------|------|
| INTRODUCTION | 1 |
| LITERATURE REVIEW | 3 |
| Host Range and Geographic Distribution of <u>Phytophthora cinnamomi</u> Rands | 3 |
| Description and Classification of <u>Phytophthora cinnamomi</u> | 11 |
| Physiological Specialization of <u>Phytophthora</u> <u>cinnamomi</u> | 14 |
| Factors Influencing Disease Development and Spread | 16 |
| GENERAL METHODS | 20 |
| Isolation of the Pathogen | 20 |
| Culture of the Pathogen | 21 |
| Method of Inoculation | 22 |
| HOSTS ATTACKED BY <u>PHYTOPHTHORA CINNAMOMI</u> IN OREGON . . | 24 |
| HOST RANGE OF <u>PHYTOPHTHORA CINNAMOMI</u> | 31 |
| DISTRIBUTION OF OREGON NURSERIES INFESTED WITH <u>PHYTOPHTHORA CINNAMOMI</u> AND <u>P. LATERALIS</u> | 37 |
| MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF OREGON ISOLATES OF <u>PHYTOPHTHORA CINNAMOMI</u> | 39 |
| MORPHOLOGICAL AND CULTURAL COMPARISON OF OREGON ISOLATES OF <u>PHYTOPHTHORA CINNAMOMI</u> WITH ISOLATES FROM OTHER SOURCES | 43 |
| PHYSIOLOGICAL SPECIALIZATION OF <u>PHYTOPHTHORA</u> <u>CINNAMOMI</u> | 47 |
| FACTORS INFLUENCING DISEASE DEVELOPMENT AND SPREAD . . | 50 |
| Effect of Soil Texture on Disease Development and Spread | 50 |
| Effect of Temperature on Sporangial Production by <u>Phytophthora cinnamomi</u> and <u>P. lateralis</u> . . | 51 |
| Vertical Distribution of <u>Phytophthora</u> <u>cinnamomi</u> in the soil | 54 |

TABLE OF CONTENTS (continued)

| | Page |
|--------------------------------------|------|
| DISCUSSION AND CONCLUSIONS | 56 |
| SUMMARY | 63 |
| BIBLIOGRAPHY | 65 |

TABLES

Page

| | |
|-------------------------------------------------------------------------------------------------------------------------------------------|----|
| 1. Pathogenicity of <u>Phytophthora cinnamomi</u> isolates to the hosts from which they were isolated | 30 |
| 2. Susceptibility of various plant species to an isolate of <u>Phytophthora cinnamomi</u> from Lawson cypress | 32 |
| 3. Distribution of Oregon nurseries infested with <u>Phytophthora cinnamomi</u> and <u>P. lateralis</u> . . | 37 |
| 4. Comparison of chlamydospore and sporangial sizes of nine Oregon isolates of <u>Phytophthora cinnamomi</u> | 40 |
| 5. Growth after three days of nine Oregon isolates of <u>Phytophthora cinnamomi</u> on potato-dextrose-agar at various temperatures . . . | 42 |
| 6. Comparison of sporangial sizes of fourteen isolates of <u>Phytophthora cinnamomi</u> | 44 |
| 7. Growth after four days of fourteen isolates of <u>Phytophthora cinnamomi</u> on potato-dextrose-agar at various temperatures . . . | 46 |
| 8. Pathogenicity of fourteen isolates of <u>Phytophthora cinnamomi</u> to Alumi cypress, Irish yew and Douglas fir | 48 |
| 9. The effect of soil texture on the development and spread of <u>Phytophthora cinnamomi</u> root rot of Lawson cypress | 51 |
| 10. Effect of temperature on sporangial production by <u>Phytophthora cinnamomi</u> and <u>P. lateralis</u> | 53 |
| 11. Vertical distribution of <u>Phytophthora cinnamomi</u> under diseased Elwoodi cypress | 55 |

FIGURES

| | Page |
|-----------------------------------------------------------------------------------------|------|
| 1. Root rot of Alumi cypress caused by <u>Phytophthora cinnamomi</u> | 25 |
| 2. Root rot of English yew caused by <u>Phytophthora cinnamomi</u> | 27 |
| 3. Root rot of <u>Erica carnea</u> caused by <u>Phytophthora cinnamomi</u> | 29 |

EPIPHYTOLOGY AND ETIOLOGY OF A PHYTOPHTHORA-INDUCED
ROOT ROT DISEASE OF CHAMAECYPARIS IN OREGON

INTRODUCTION

Varieties of Lawson cypress or Port Orford cedar, Chamaecyparis lawsoniana Parl., are widely used as hedge and specimen plants in western Oregon landscape plantings. Lawson cypress is also used extensively in windbreak plantings, especially in Multnomah County. Because of their wide use Lawson cypress varieties are grown in many Oregon nurseries.

In the late 1930's a species of Phytophthora was found (40, 41) to be the cause of a root rot disease of Lawson cypress varieties in Oregon nursery and landscape plantings and was subsequently described and named (61) Phytophthora lateralis Tucker and Milbrath. P. lateralis has become widely disseminated and is now one of the major causes of economic losses in many Oregon nurseries.

Following preliminary work on this disease problem it was reported by the author (55, 56) in 1951 that Phytophthora cinnamomi Rands was the cause of a root rot disease of Lawson cypress with symptoms identical to those caused by P. lateralis. P. cinnamomi had not previously been reported as occurring in Oregon and has not as yet become widely distributed in the state.

In Oregon Phytophthora cinnamomi has not caused economic losses as great as those caused by P. lateralis. However, recognizing the fact that P. lateralis, with a host range limited to certain species of Chamaecyparis, has become a major cause of plant disease losses in Oregon, the possibility exists that P. cinnamomi, with a very wide host range, may become a more serious plant pathogen than P. lateralis.

If Phytophthora cinnamomi should become as important a pathogen in Oregon as it has elsewhere, information on the epiphytology and etiology of the diseases caused by this fungus would be needed in devising effective control measures. Studies to obtain this information were initiated shortly after the fungus was found in Oregon. The data obtained in these studies are presented in this thesis.

LITERATURE REVIEW

Host Range and Geographic Distribution of *Phytophthora cinnamomi* Rands

In 1922 Rands (50) reported that an undescribed species of *Phytophthora* was the cause of a bark canker disease of cinnamon, *Cinnamomum burmanni* Bl., in the west coast highlands of Sumatra. The causal organism was described and named *Phytophthora cinnamomi* n. sp. The fungus was not pathogenic to *C. zeylanicum* Bl., *C. camphora* Nees., *C. culilawan* Bl., *C. sintok* Bl., *Erythrina lithosperma* Miq., *Theobroma cacao* L., *Hevea brasiliensis* (Müll.) Arg. and *Carica papaya* L. As a result of these pathogenicity studies Rands believed that the cinnamon fungus was narrowly limited in its parasitism. However, the papers reviewed below show that *P. cinnamomi* is pathogenic to a large number of plant species in a number of tropical, subtropical, and temperate areas of the world.

Phytophthora cinnamomi was reported by Tucker (57) in 1929 as the cause of a root rot disease of avocado, *Persea americana* Mill., in Puerto Rico. It has subsequently been reported as occurring on avocado in South Africa (66), California (67, 75), Peru (14), Australia (47), Honduras (78), Mexico and Costa Rica (73). The disease has caused heavy losses in avocado plantings in many of these areas.

The first report of Phytophthora cinnamomi occurring in the United States was made by White (70) in 1930. He found that it was the cause of a wilt and root rot of Rhododendron ponticum L. and rhododendron hybrid seedlings in New Jersey. R. carolineanum Rehd., R. maximum L., R. catawbiense Michx., R. californicum Hook. and R. caucasicum Pall. were susceptible when artificially inoculated (69). Natural infections of R. maximum were not observed and artificial inoculations were erratic. R. mucronulatum Turca. has been reported (17) as a natural host in Maryland. P. cinnamomi has also been reported as the cause of a root rot of azalea in greenhouses in Missouri (60), of Rhododendron sp. and Azalea sp. in Argentina (23), of Azalea indica L. (R. indicum (L.) Sweet.) in Australia (49) and rhododendron and azalea in Maryland (27).

In 1930 Sideris and Paxton (52) reported that a fungus closely related to members of the genus Phytophthora was causing a stem or heart rot of pineapple, Annanas sativus Schult., in Hawaii and was also pathogenic to the roots of Allium cepa L. The causal fungus was named Pseudopythium phytophthoron. Mehrlich (36, 38, 39) subsequently showed that the fungus was actually Phytophthora cinnamomi. P. cinnamomi has also been reported on pineapple in

Australia (32, 33, 53) and probably is present in most of the pineapple growing areas of the world.

The first report of Phytophthora cinnamomi as the cause of a root rot of heather was made by Tucker (59), who reported in 1933 that the fungus had been isolated from Erica sp. in New York by Gill. In 1937, Oyler and Bewley (44) reported finding the fungus as the cause of a wilt and root rot of Erica hyemalis Nichols, E. nivalis Andr. and E. willmoreana Knowles and Westc. in English nurseries. The fungus was pathogenic to Calceolaria, beech seedlings, Antirrhinum, Schizanthus, stocks and Nicotiana glutinosa L. but not to China aster, Azalea, Begonia, carrot, Cineraria, Coleus, Erica cinerea L., E. tetralix L., Gloxinia, lavender, lilac, Nicotiana tabacum L., Pisum sativum L., potato, Primula obconica Hance, P. stellata Hort., Rhododendron ponticum, Solanum capsicastrum Link., strawberry, tomato and wallflower. The fungus has also been reported on Erica sp. in Australia (47) and E. regerminans L. in California (74).

In 1932 Milburn and Gravatt (42) reported a Phytophthora, tentatively identified as Phytophthora cambivora (Petri) Buis., as the cause of a root rot disease of American chestnut, Castanea dentata (Marsh.) Borkh., in the southeastern part of the country. The causal fungus was later classified by Crandall, Gravatt and

Ryan (17) as P. cinnamomi and found to be the cause of a root rot of American chestnuts in Georgia, Maryland, Virginia, North and South Carolina, Tennessee, Mississippi and Alabama; of C. pumila (L.) Mill. in South Carolina; of C. crenata Sieb. and Zucc. in Louisiana; of C. ozarkensis Ashe in Arkansas and C. alabamensis Ashe in Alabama. The disease was believed to have been observed first in Georgia in 1824 (17). It was probably responsible for the recession of the American chestnut in the southeastern states and is known to have been responsible for the recession of the Ozark chinkapin, C. Ozarkensis, in the Ozarks. Field and greenhouse inoculation tests demonstrated that C. alnifolia Nutt., C. ashei Sudw., C. margaretta var. arcuata Ashe and C. sativa Mill. were also susceptible to the fungus, whereas, the Asiatic chestnuts, C. crenata Sieb. and Zucc., C. henryi (Skan) Rehd. and Wils., C. mollissima Blume and C. sequinii Dode showed a high degree of resistance.

Phytophthora cinnamomi was first reported as associated with the root rot disease of European chestnut, Castanea sativa Mill. in England in 1938 (20, 21). However, most European workers have attributed the root rot disease of chestnut to P. cambivora. Urquijo (62) working in Spain reported in 1942 preliminary cultural studies in which he compared his Phytophthora isolates

from chestnut and walnut with isolates from other sources and set up three provisional groups. In 1943 (63), he confirmed his earlier work and reported differences between his own isolates and those received as P. cambivora as well as differences among the P. cambivora isolates. He later (65) set aside part of the isolates as P. cambivora and placed the remaining isolates in a residual group. Urquijo (64) summarized his work in 1947 and concluded that chestnut root rot in Spain was caused by P. cinnamomi and not P. cambivora. The isolates from France and Italy alone were P. cambivora, while all other isolates were assigned to P. cinnamomi. In view of Urquijo's work it is probable that members of the Phytophthora A group of Pimintel (45), which were isolated from chestnut and several other hosts in Portugal, are P. cinnamomi and those of his Phytophthora B group, from chestnut in a single Portuguese nursery are P. cambivora. It is evident from these papers that P. cinnamomi has played a greater part in the chestnut root rot problem in southern Europe than was formerly realized. In addition to Castanea sativa, P. cinnamomi was also found to attack Juglans regia L., Pseudotsuga taxifolia (Poir.) Britton, Quercus robur L., Q. suber L., Betula alba L., Cedrus atlantica Manetti, Abies nordmanniana Spach., A. pectinata D. C., A. siberica Ledeb. and Castanea crenata

var. tamba.

Crandall and co-workers (15, 17, 26) found that Phytophthora cinnamomi was responsible for losses in seedbeds and plantings of various tree species in nurseries in the eastern and southeastern United States. The fungus was isolated from diseased Pinus resinosa Ait., P. sylvestris L., P. strobus L., Taxus cuspidata Sieb. and Zucc., T. baccata L., T. media Rehd., Picea abies Mill., P. pungens Engelm., Larix decidua Mill., L. leptolepis (Sieb. and Zucc.) Gord., Juglans nigra L., J. regia L., Quercus borealis Michx. f., Q. montana Willd., Q. alba L., Betula alba L., B. papyrifera Marsh., Platanus orientalis L., and Robinia pseudoacacia L. Pathogenicity was not proved in all cases. Several species that were slightly susceptible in the field were completely resistant in inoculation tests. The response of a number of other plants was also tested. Pinus mugo var. mughus (Scap.) Zenari, Fagus grandifolia Ehrh., F. sylvatica L., Lithocarpus cuspidatus (Thunb.) Nakai, Quercus agrifolia Nee, Q. coccinea Muench., Q. garryana Dougl., Q. macrocarpa Michx., Q. marilandica Muench., Q. palustris Muench., Q. phellos L., Q. prinus L., Q. rubra L., and Q. velutina Lam. were not susceptible to the fungus. Both Lithocarpus densiflorus (Rehd.) Rehd. and Pseudotsuga taxifolia (Poir.) Britton were susceptible.

Phytophthora cinnamomi was reported by Thompson (54) in 1940 as causing a root rot and wilt of Cinchona ledgeriana Moens and C. succirubra Pav. seedlings in Malaya. Crandall (13) has also reported the pathogen as the cause of a root rot of the Ledger form of C. officinalis L. seedlings in Peru. Darley and Flores (19) found that P. cinnamomi was the cause of a stripe canker of selections of C. pubescens Vahl. (C. succirubra), the Ledger form of C. officinalis and hybrids of the two species in Guatamala.

In 1938 Campbell (7) isolated Phytophthora cinnamomi from the roots of littleleaf-diseased shortleaf pine, Pinus echinata Mill. in the Southeast. During the last ten years this disease has caused losses of millions of dollars in that area. Campbell (8) subsequently reported that the fungus was present to a limited extent in the soil around the roots of healthy trees in stands free of littleleaf but that it was much more abundant in the root zones of littleleaf trees. He considered this strong evidence that P. cinnamomi is involved in the fine root necrosis known to accompany the disease. P. cinnamomi was later reported (6) as the only recognized pathogen among a large number of fungi isolated from shortleaf pine roots. Although the fungus was present in pine stands outside the littleleaf area it was present in relatively fewer plots and in smaller amounts than

in soil of plots in the littleleaf area (9, 10).

Phytophthora cinnamomi was first reported by Gill (24, 25) in 1948 as the cause of a root rot of camellia. The fungus was isolated from diseased Camellia japonica L. in Alabama and from plants from Louisiana and South Carolina. The fungus has also been reported on the same species in Australia (47) and California (74.) In Florida nurseries West (68) found that P. cinnamomi was causing losses of both C. japonica and C. sasanqua Thunb.

Phytophthora cinnamomi has also been reported as the cause of a root rot of tung, Aleurites fordii Hemsl., in Louisiana (46); foot rot of sour orange, Citrus aurantium L., in Brazil (22); crown rot of English walnut, Juglans regia, in Australia (12); root rot of Dicranopteris emarginata (Brack) Robinson in Hawaii (38); root rot of Cineraria stellata Hort. and C. grandiflora Hort. in England (43); root or crown rot of Castanea sativa, Chamaelaucium uncinatum Schau., Eriostemon lanceolatus Gaertn., Jacarandra mimosaeifolia D. Don, Amygdalus persica L. (Prunus persica Sieb. and Zucc.), Lilium philippense Baker and Olea europaea L. in Australia (47, 48); root or crown rots of Pinus radiata Don, Cupressus sp., Thuja sp., Plantanus orientalis L., Calycanthus floridus L., Salix carea L., Phaseolus lunatus L., Spirea cantoniensis Lour., Schinus molle L.,

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Sedum sp., Eucalyptus rostrata Schlecht., Ligustrum lucidum Ait., Pittosporum tobira Ait. and Echeveria gibbiflora D. C. in Argentina (23); possibly a root rot of Carica papaya L. in Peru (51); root rot of Japanese holly, juniper, viburnum and yew in Maryland (27); root rot of Chamaecyparis lawsoniana Parl. in Oregon (55, 56); and root rot of Cedrus deodara (Roxb.) Loud., Chamaecyparis lawsoniana var. elwoodi, Pinus radiata, P. canariensis C. Sm., Libocedrus decurrens L., Cupressus sempervirens L., Thuja compacta (T. occidentalis L. var compacta Carr.) and Myrtus compacta Ridley in California (74).

Description and Classification of *Phytophthora cinnamomi*

In 1922 Rands (50) described *Phytophthora cinnamomi* n. sp. as the cause of a bark canker disease of cinnamon. The fungus was described as follows (50, p. 52-53):

"*Phytophthora cinnamomi* n. sp. Mycelium permeating bark and outermost layers of wood; irregular, sparingly branched, inter- and intracellular, bearing occasionally chlamydospores in the tissues; aerial hyphae on oat agar hyaline, slender, 5-7 mikron in diameter, with age becoming thick walled and septate, haustoria not observed.

"Chlamydospores thin walled, globose to pyriform 28-60 mikron (most 31-50 mikron) in diameter average 41 mikron; terminal on short lateral branches; abundant in artificial cultures, frequently occurring in grapelike bunches or clusts of 3-10 spores; germinating by 3-11 germ tubes.

"Conidiophores undifferentiated, simple, or sympodially branched.

"Conidia (sporangia) not observed in nature nor in ordinary artificial cultures; but abundantly produced on mycelium transferred from nutrient solution to water; borne terminally and varying in shape from ovoid to ellipsoid or elongate, hyaline, thin walled, with broad, flat, inconspicuous papilla on end opposite point of attachment; size 25-100 x 18-43 mikron, mostly 38-84 x 27-39 mikron, average 57 x 33 mikron; secondary conidia produced on branches of conidiophore in successive proliferation through the empty ones; spore case partially collapsing after discharge, germinating in water generally by liberation of zoospores but occasionally by a tube developing hyphae or secondary conidia, conidial discharge in weak or contaminated cultures often abnormal, the zoospores failing to separate and the extruded mass disintegrating.

"Zoospores 8-40 from one conidium, more or less bean or kidney shaped with two flagella of unequal length attached to concave side; while swimming 11-10 mikron in diameter; germinating after about an hour by a tube.

"Oospores not observed."

The sexual stage was first observed by Ashby (3) who obtained oogonia with amphigynous antheridia in mixed cultures of Phytophthora cinnamomi and P. parasitica Dast. Ashby (4) later found oospores of P. cinnamomi in an old pure culture. The finding of oospores has also been reported by several other workers (43, 44, 58, 67), but their production was very sparse and sporadic. However, Zentmyer (71) and Zentmyer, Snyder and Hansen (77) have recently reported obtaining abundant production of the sexual stage of the fungus by using either sterile

avocado roots in water as a substrate or a water extract of avocado roots as a sexual reproduction stimulant. The homothallic and hermaphroditic nature of the fungus was demonstrated using either single hyphal tip or zoospore cultures.

The close relationship of Phytophthora cinnamomi to P. cambivora (Petri) Buis. was noted by Ashby (4) and Tucker (58). Tucker, however, separated P. cinnamomi from P. cambivora on the basis of abundant production of spherical vesicles on solid media, smaller oospores and decay of wounded potato tubers by P. cinnamomi. Leonian (29) also maintained the two species, but separated them on the basis of their differential tolerance of malachite green.

In 1932 Milburn and Gravatt (42) reported a Phytophthora tentatively identified as Phytophthora cambivora as the cause of a root rot of American chestnut. Crandall (15) later attributed the root rot of a number of broad-leaf and coniferous plants to P. cambivora with P. cinnamomi as a synonym. In 1936 Mehrlich (36), on the basis of a study of several isolates of the two species, concluded that the members of these two species represented physiological strains of one specific organism and suggested retaining them all under the name of P. cambivora.

White (69) followed this suggestion and classified the causal fungus of a wilt and root rot of rhododendron as P. cambivora. Crandall, Gravatt and Ryan (17) opposed this combination as the host ranges of the two species did not in general overlap. Urquijo (62, 63, 64, 65) and Pimentel (45) have recently clarified the situation in Europe, concluding that both P. cinnamomi and P. cambivora are present. Crandall (16) states that the two species may be readily distinguished in culture and it is only in published descriptions that overlapping characteristics are to be found.

In 1930, Sideris and Paxton (52) named the causal fungus of a stem or heart rot of pineapple as Pseudopythium phytophthoron. Mehrlich (36, 38, 39) later proved that the fungus was Phytophthora cinnamomi. Pseudopythium phytophthoron thus stands without description as a synonym of Phytophthora cinnamomi.

Physiological Specialization of Phytophthora cinnamomi

Mehrlich (36, 37) was the first worker to investigate the possible existence of physiological strains of Phytophthora cinnamomi. Of 8 isolates of P. cinnamomi tested, 5 were pathogenic to pineapple and 3 non-pathogenic. Mehrlich concluded, that with respect to

pathogenicity, the differences appeared to be of the magnitude recognized in the rust fungi as physiological strains.

White (69) using rhododendron and pineapple as differential hosts established three strains of the fungus, namely: (1) Isolates attacking European chestnuts: non-pathogenic to both rhododendron and pineapple, (2) Isolates attacking walnuts: pathogenic to rhododendrons but not pathogenic to pineapple, (3) Isolates from rhododendron and pineapple: pathogenic to both hosts. However, only the latter two strains were probably Phytophthora cinnamomi as Urquijo (64) found that isolates from the two sources, from which White obtained his European chestnut isolates, were P. cambivora. Differences in virulence between various isolates were also noted when comparative inoculations of avocado seedlings were made.

Crandall, Gravatt and Ryan (17), also, found that isolates of Phytophthora cinnamomi differed in their pathogenicity. Juglans nigra was susceptible to isolates from J. nigra and J. regia in Maryland, but not to isolates from Pinus resinosa in Maryland or Castanea dentata in Georgia. One of Rands' original isolates from cinnamon was only weakly pathogenic to P. resinosa, as compared with isolates from P. resinosa. However, twenty isolates from sixteen different hosts were all pathogenic to P. resinosa and Castanea sativa.

Factors Influencing Disease Development and Spread

In nearly all of the papers, concerned with the root rot diseases caused by Phytophthora cinnamomi, high soil moisture and heavy or poorly drained soils are mentioned as associated with the development and spread of the disease caused by this fungus. The effect of soil moisture upon the development and spread of these diseases has, however, been studied experimentally by only a few workers. White (69) found that the development of the root rot and wilt disease of rhododendron was more rapid and severe at high soil moisture levels. Crandall, Gravatt and Ryan (17), also, found that the disease incidence was higher for Pinus resinosa seedlings at high soil moisture levels or in greenhouse soil than for seedlings at low soil moisture levels or in a mixture of half greenhouse soil and half sand. Zentmyer and Richards (76), working with the root rot of avocado, demonstrated that in infested soil the disease appeared sooner and caused more rapid damage on trees of a group given weekly irrigations than on those of a group irrigated biweekly. No harmful effect was observed from frequent irrigation in the noninfested plots.

As wet poorly drained soils were known to have a limited oxygen-supplying power, Curtis and Zentmyer (18)

investigated the effect of oxygen supply on the root rot of avocado in nutrient solution. On the basis of their results they suggested that two types of injury to avocado trees in the field are possible, namely: (1) injury from root infections by Phytophthora cinnamomi under conditions of ample moisture and at any oxygen level from full aeration down to nearly total lack of oxygen, and (2) injury from nearly total exclusion of oxygen from the soil under severely water-logged conditions.

The nutrient salt concentration has been found by Anderson (1) to have an effect on the infection of pineapple roots by the fungus. The number of infected roots decreased as total nutrient salt concentration was increased up to about 1400 p.p.m. in "balanced" solutions. Infection was much less in "unbalanced" solutions of high potassium concentration than in "balanced" solutions of the same total concentration with a lower potassium concentration. Fertilization of pot cultures, based on the solution culture results, delayed or prevented severe root rot.

Growth of Phytophthora cinnamomi was found by White (69) to be inhibited at very acid pH levels on solid media. On the basis of this information an experiment was designed to demonstrate the influence of soil

acidity on the development of root rot of Rhododendron ponticum seedlings. After four months heavy mortality ranging from 60 to 100 per cent was present in those plots in which soil acidity ranged from pH 4.0 to 7.3. In the one plot below pH 4.0 only one plant out of fifteen showed evidence of disease and in the two plots above pH 7.3 only 33 per cent mortality occurred. However, plant growth was poor at the two high pH levels. In Queensland it has been observed (32) that pineapple wilt does not occur on any soil of greater acidity than pH 5.1. Crandall, Gravatt and Ryan (17) report that P. cinna-momi has been isolated from diseased nursery stock in soils ranging from pH 3.2 to 7.0. Field tests to control root rot of red pine and blue spruce by adjusting the soil acidity were unsuccessful. The fungus was active at pH readings as low as 3.3 at which point the host was injured by the acid condition. They postulated that once a plant was infected the pH would probably be more important as it affected the vigor of the host rather than the fungus.

Oyler and Bewly (44) found experimentally that heather plants wilted more quickly and the disease made more rapid progress at temperatures above 63° F. than at lower temperatures. White (69) observed that areas of infection in rhododendron beds enlarged more rapidly

during July and August and with the advent of cooler nights in September the extension of these areas was retarded. White, also, studied the influence of freezing on Phytophthora cinnamomi and concluded that exposure of the fungus to freezing temperatures for long periods of time was effective in completely eradicating the causal organism from infested soil. Zentmyer (72) found that the fungus forms sporangia only between temperatures of about 70° F. and 88° F. with a small temperature drop necessary to induce zoospore discharge. Zentmyer suggested that, as most of the infection in the field probably results from zoospores, the main infection period is in the late spring and early summer after the soil warms up sufficiently for zoospore production.

GENERAL METHODS

Isolation of the Pathogen

Because of the difficulty with which Phytophthora cinnamomi was isolated directly from diseased tissue the method of Tucker (58, pp. 56-57), using apple fruits as a differential medium, was adapted for use. Sections of diseased stems or roots were surface sterilized for 1 to 2 minutes in 20 percent commercial Chlorox, rinsed in sterile distilled water and inserted into opposite sides of sound apples. The points of inoculation were then covered with cellophane tape. The inoculated apples were incubated at room temperature. P. cinnamomi, if present, grew into the apple and within a week to 10 days had produced an area of firm dry rot around the point of inoculation. Aseptic transfers of decayed apple tissue were made from the margins of the brownish infected areas to plates of potato-dextrose-agar and incubated for 2 to 3 days. The plates were then examined for the presence of the fungus.

The pathogen was isolated from the soil using the method developed by Campbell (5) for isolating the fungus from the soil under littleleaf-diseased and healthy shortleaf pine. Soil samples were collected and a portion of each sample packed into a one-half inch hole

bored with a cork borer diagonally downward but not quite through an apple. The hole was filled within about one-half inch of the top and distilled water added until the soil was well saturated. The opening then was sealed with cellophane tape and the apples incubated at room temperature. Two apples were used for each soil sample. Phytophthora cinnamomi, if present, caused a firm dry rot of the apple tissue within five to ten days and was readily isolated in pure culture from the infected areas.

Culture of the Pathogen

All cultures of Phytophthora cinnamomi were maintained on potato-dextrose-agar slants. Potato-dextrose-agar was also used as the medium in studies of the effect of temperature on growth and for all isolations.

Pea broth was used as a medium in growing the fungus for inoculations or for the production of sporangia. Pea broth was prepared by boiling one pound of dried peas (split or whole) for 5 to 10 minutes, straining off the liquid and adding sufficient water to the liquid to make a volume of 3 liters.

The fungus was grown on water agar (20 grams of agar, one liter of distilled water) for the examination and measurement of chlamydospores.

As the fungus does not produce sporangia on standard media, a method developed by Mehrlich (34, 35) was used. The fungus was grown in pea broth for one week, the mycelia removed and washed in sterile distilled water, bits of the mycelia placed in a nonsterile soil leachate and incubated at 25° C.

Leonian's (28, p. 671) medium was used when the effect of malachite green on the growth of the fungus was studied. The basic medium was as follows:

| | |
|--------------------------------|-----------|
| Proteose peptone | 2.0 grams |
| Dihydrogen potassium phosphate | 0.5 gram |
| Magnesium sulphate | 0.5 gram |
| Succinic acid | 0.2 gram |
| Dextrose | 5.0 grams |
| Distilled water | 1.0 liter |

Various concentrations of malachite green were added to this medium.

Method of Inoculation

Inoculum was prepared in a Waring Blendor by macerating mycelia that had been growing in pea broth for a week to 10 days. Holes were made to a depth of 3 inches in the soil at opposite sides of the plant to be inoculated. An aliquot of inoculum was then poured into each hole and the hole subsequently filled with soil. The check plants were treated in the same manner except that sterile pea broth was used. The amount of inoculum used

per plant varied from experiment to experiment, but in any one experiment an equal amount of inoculum was added to each plant.

HOSTS ATTACKED BY PHYTOPHTHORA CINNAMOMI IN OREGON

Phytophthora cinnamomi has been isolated from a number of plant species in Oregon nurseries and home plantings. The fungus was isolated from two naturally infected Lawson cypress or Port Orford cedar varieties, Alumi cypress, Chamaecyparis lawsoniana Parl. var. allumi (R. Smith) Beiss., and Elwoodi cypress, C. lawsoniana var. elwoodi; English yew, Taxus baccata L.; Irish yew, T. baccata var. stricta Laws. (T. baccata var. fastigiata Loud.); Japanese yew, T. cuspidata Sieb. and Zucc. var. nana Rehd.; heath, Erica carnea L.; and two varieties of heather, Calluna vulgaris (L.) Hull var. alba (West.) Don and C. vulgaris var. aurea Don. Plants of all ages appeared to be equally susceptible.

The symptoms of the root rot of Lawson cypress (Figure 1) caused by Phytophthora cinnamomi are identical to those of the root rot caused by P. lateralis. The fungus invades the roots of the plant and spreads into the lower part of the main trunk killing all tissues as it advances. If the outer portions of the bark at the crown of a diseased plant are removed a sharp line of demarcation between living and dead cells is apparent. About the time the fungus reaches the crown of the plant the foliar symptoms begin to develop. These involve

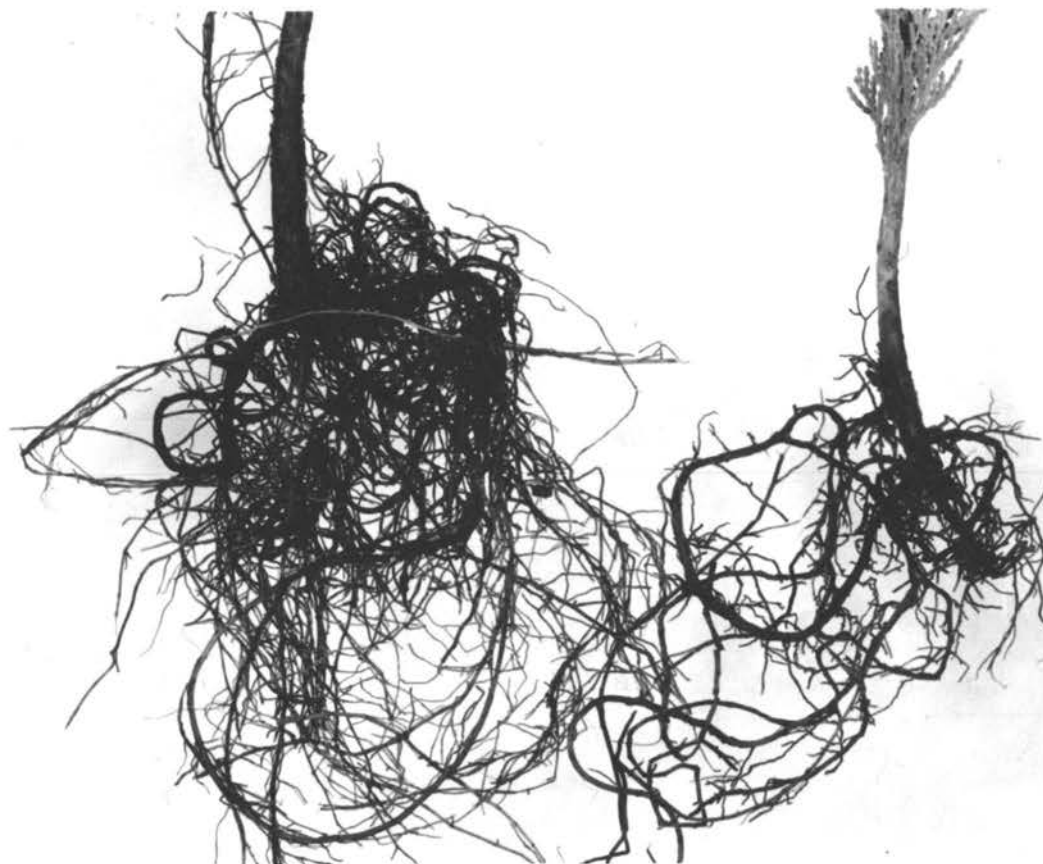


FIGURE 1. ROOT ROT OF ALUMI CYPRESS CAUSED BY PHYTOPHTHORA CINNAMOMI
Roots of healthy (left) and diseased (right) plants

gradual changes in color similar to those occurring when cypress trees die from transplanting injury. The first foliar symptom in the blue cypress varieties is the gradual disappearance of blue pigments until only the green under color remains. The color eventually fades to a tan or light brown and the foliage becomes crisp and dry. The only foliar symptom in the green varieties is a gradual fading of color until the plants are tan or light brown and dead. The first color changes are especially apparent if the plant is adjacent to a healthy plant. When the weather is cool and damp these color changes may develop over a period of several months, but if the weather is hot and dry the entire sequence may occur in 2 to 3 weeks.

The symptoms of the root rot of English, Irish and Japanese yew (Figure 2) are similar to those of the root rot of cypress. The fungus invades the small feeder roots of the plant, killing them as it advances, and eventually spreads into the main roots and on up the lower portions of the stem. The line of demarcation at the crown between living and dead tissues is not as distinct as in cypress. The dead areas are usually apparent as brown vertical streaks of varying width, which extend into the wood of the stem. Foliar symptoms begin to develop when all or most of the root system has been killed. The foliage of the diseased plant fades to a tan or light brown and

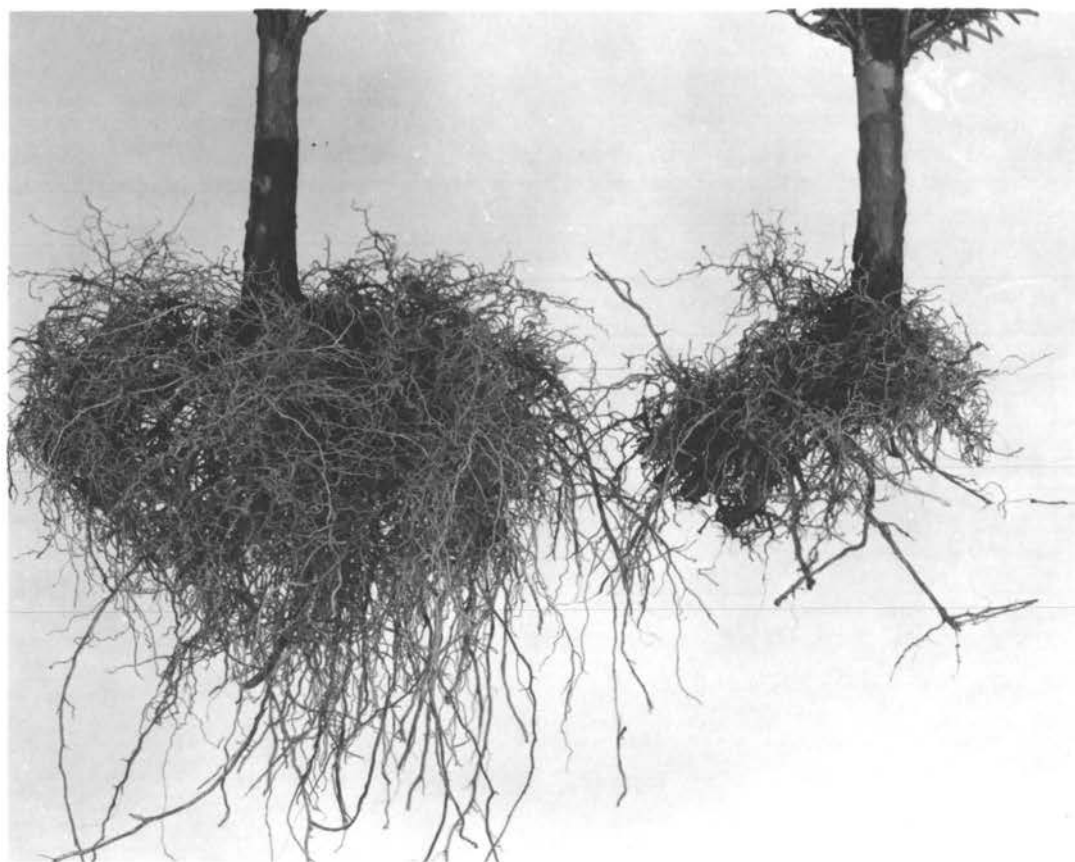


FIGURE 2. ROOT ROT OF ENGLISH YEW CAUSED BY PHYTOPHTHORA CINNAMOMI
Roots of healthy (left) and diseased (right) plants

becomes crisp and dry. This color change may be gradual or if the weather is hot and dry may occur in a few days.

The symptoms of the root rot of the heaths and heathers (Figure 3) differ from those of the root rots of cypress and yew in that there is usually a localization of the foliar symptoms. The fungus invades and kills the feeder roots, slowly advances into the main roots and may advance up the lower portions of the stems. The first foliar symptoms are the fading and dying of one or more branches. This condition spreads until the entire plant is dead. Several months may be required for this sequence of foliar symptoms to develop.

The pathogenicity of Phytophthora cinnamomi isolates to the hosts from which they had been isolated in Oregon was tested in the greenhouse. Plants growing in 6 inch pots were inoculated and isolations made when disease symptoms developed. All of the isolates were found to be pathogenic (Table 1).



FIGURE 3. ROOT ROT OF ERICA CARNEA CAUSED BY PHYTOPHTHORA CINNAMOMI
Roots of healthy (left) and diseased (right) plants

TABLE 1. PATHOGENICITY OF PHYTOPHTHORA CINNAMOMI
ISOLATES TO THE HOSTS FROM WHICH THEY WERE ISOLATED

| Plant | Inoculated | | Not inoculated | |
|----------------------------------------------------------------|------------|--------------|----------------|--------------|
| | No. | No. infected | No. | No. infected |
| <u>Calluna vulgaris</u> var. <u>alba</u> | 4 | 4 | 2 | 0 |
| <u>C. vulgaris</u> var. <u>aurea</u> | 4 | 4 | 2 | 0 |
| <u>Chamaecyparis lawsoni-</u> <u>ana</u> var. <u>allumi</u> | 10 | 9 | 6 | 0 |
| <u>C. lawsoniana</u> var. <u>elwoodi</u> | 10 | 10 | 7 | 0 |
| <u>Taxus baccata</u> var. <u>stricta</u> | 6 | 5 | 3 | 0 |

HOST RANGE OF PHYTOPHTHORA CINNAMOMI

Shortly after discovering that Phytophthora cinna-
momi was the cause of a root rot of several ornamentals
in Oregon, tests were initiated to determine the host range
of an Oregon isolate of the fungus. The isolate used had
originally been isolated from Lawson cypress. Suscepti-
bility of 47 plant species or varieties to this isolate
was tested during the past three years. The plants tested
were selected either because they had previously been
reported as hosts of the fungus or were commonly grown in
Oregon nurseries. The plants were obtained as one or 2
year old stock from nurseries thought to be free from P.
cinnamomi and were planted either in clay pots or in
benches. After the plants were well established they were
inoculated and kept well watered. When the plants showed
root rot symptoms they were removed and isolations made.

The results of the pathogenicity trials are summar-
ized in Table 2. Twenty-seven of the 47 plant species or
varieties tested were found to be susceptible to this
fungus.

Chamaecyparis thyoides and the varieties of C.
obtusa, C. pisifera and C. nootkatensis were not sus-
ceptible to this fungus. No root damage was apparent on
any of the plants 3 to 4 months after inoculation. All

TABLE 2. SUSCEPTIBILITY OF VARIOUS PLANT SPECIES
TO AN ISOLATE OF PHYTOPHTHORA CINNAMOMI FROM LAWSON CYPRESS

| Plant species | Inoculated | | Not inoculated | |
|-------------------------------------------------------------------------------------------------|------------|--------------|----------------|--------------|
| | No. | No. infected | No. | No. infected |
| <u>Betula alba</u> L. | 8 | 8 | 4 | 0 |
| <u>Brunkenthalia spiculifolia</u> (Salisb.) Reichenb. | 9 | 9 | 3 | 0 |
| <u>Calluna vulgaris</u> (L.) Hull var. <u>alba</u> (West) Don | 9 | 6 | 3 | 0 |
| <u>C. vulgaris</u> var. <u>aurea</u> Don | 9 | 9 | 3 | 0 |
| <u>Castanea dentata</u> (Marsh.) Borkh. | 8 | 8 | 4 | 0 |
| <u>Cedrus deodara</u> (Roxb.) Loud. | 6 | 0 | 4 | 0 |
| <u>Chamaecyparis lawsoniana</u> Parl. var. <u>allumi</u> (R. Smith) Beiss. | 6 | 6 | 4 | 0 |
| <u>C. lawsoniana</u> var. <u>elwoodi</u> | 6 | 6 | 4 | 0 |
| <u>C. lawsoniana</u> var. <u>fletcheri</u> | 6 | 6 | 4 | 0 |
| <u>C. lawsoniana</u> var. <u>lutea</u> (R. Smith) Beiss. | 7 | 7 | 3 | 0 |
| <u>C. lawsoniana</u> var. <u>nestoides</u> | 6 | 6 | 4 | 0 |
| <u>C. nootkatensis</u> (Lamb.) Spach. var. <u>compacta</u> Beiss. | 8 | 0 | 5 | 0 |
| <u>C. obtusa</u> (Sieb. and Zucc.) Endl. var. <u>crippsi</u> (Cripps) Rehd. | 7 | 0 | 4 | 0 |
| <u>C. pisifera</u> (Sieb. and Zucc.) Endl. var. <u>filifera</u> (Senecl.) Hartw. and Ruempl. | 7 | 0 | 4 | 0 |
| <u>C. pisifera</u> var. <u>plumosa</u> (Carr.) Otto | 7 | 0 | 5 | 0 |
| <u>C. pisifera</u> var. <u>squarrosa</u> (Endl.) Beiss. and Hochst. | 6 | 0 | 4 | 0 |
| <u>C. thyoides</u> (L.) B.S.P. | 8 | 0 | 8 | 0 |
| <u>Daboecia cantabrica</u> (L.) K. Koch var. <u>alba</u> (D. Don) Dipp. | 9 | 9 | 3 | 0 |

TABLE 2 (Continued)

| Plant species | Inoculated | | Not inoculated | |
|-------------------------------------------------------------------------------------------------|------------|--------------|----------------|--------------|
| | No. | No. infected | No. | No. infected |
| <u>Daphne cneorum</u> L. | 9 | 0 | 3 | 0 |
| <u>D. odora</u> Thunb. | 9 | 0 | 3 | 0 |
| <u>Erica arborea</u> L. var. <u>alpina</u> Bean | 9 | 9 | 3 | 0 |
| <u>E. carnea</u> L. King George variety | 18 | 18 | 6 | 0 |
| <u>E. ciliaris</u> L. | 9 | 5 | 3 | 0 |
| <u>E. cinerea</u> L. | 9 | 9 | 3 | 0 |
| <u>E. mediterranea</u> L. | 9 | 6 | 3 | 0 |
| <u>E. terminalis</u> Salisb. (<u>E. stricta</u> Andr.) | 9 | 9 | 3 | 0 |
| <u>E. tetralix</u> L. | 9 | 9 | 3 | 0 |
| <u>E. vagans</u> L. | 9 | 6 | 3 | 0 |
| <u>Juglans regia</u> L. | 8 | 8 | 4 | 0 |
| <u>Juniperus chinensis</u> L. var. <u>pfitzeriana</u> Spaeth | 6 | 0 | 4 | 0 |
| <u>J. excelsa</u> Bieb. var. <u>stricta</u> Gord. | 6 | 0 | 4 | 0 |
| <u>J. sabina</u> L. | 6 | 0 | 4 | 0 |
| <u>J. squamata</u> Lamb. var. <u>meyeri</u> Rehd. | 6 | 0 | 4 | 0 |
| <u>Picea abies</u> (L.) Karst. (<u>P. excelsa</u> Link) | 6 | 0 | 4 | 0 |
| <u>Pinus mugo</u> Turra var. <u>mughus</u> (Scop.) Zenari | 6 | 0 | 4 | 0 |
| <u>Pseudotsuga taxifolia</u> (Poir.) Britton | 10 | 5 | 10 | 0 |
| <u>Quercus borealis</u> Michx. f. | 11 | 0 | 4 | 0 |
| <u>Rhododendron molle</u> (Bl.) D. Don (<u>Azalea mollis</u> Bl.) | 9 | 9 | 3 | 0 |
| <u>R. obtusum</u> (Lindl.) Planch. (<u>Azalea</u> <u>obtusum</u> Lindl.) Hinodegiri variety | 9 | 0 | 3 | 0 |
| <u>R. ponticum</u> L. | 9 | 9 | 3 | 0 |

TABLE 2 (Continued)

| Plant species | Inoculated | | Not inoculated | |
|----------------------------------------------------------------------------------------------------------------|------------|--------------|----------------|--------------|
| | No. | No. infected | No. | No. infected |
| <u>Taxus baccata</u> L. var. <u>stricta</u> Laws. (<u>T. baccata</u> var. <u>fastigiata</u> Loud.) | 9 | 9 | 3 | 0 |
| <u>T. cuspidata</u> Sieb. and Zucc. | 9 | 9 | 3 | 0 |
| <u>Thuja occidentalis</u> L. var. <u>aureo-</u> <u>variegata</u> Henk. and Hochst. | 6 | 0 | 4 | 0 |
| <u>T. occidentalis</u> var. <u>fastigiata</u> Jaeg. (<u>T. occidentalis</u> var. <u>pyramidalis</u> Hort.) | 6 | 0 | 4 | 0 |
| <u>T. occidentalis</u> var. <u>woodwardii</u> Spaeth. | 6 | 0 | 4 | 0 |
| <u>T. orientalis</u> L. var. <u>aurea</u> Senecl. | 6 | 0 | 4 | 0 |
| <u>T. orientalis</u> var. <u>compacta</u> Beiss. | 6 | 0 | 4 | 0 |

of the 5 varieties of Lawson cypress, C. lawsoniana, were susceptible, although they differed in degree. Six months after inoculation neither C. lawsoniana var. nestoides or C. lawsoniana var. lutea had been killed by the fungus but the roots were severely damaged and top growth was markedly reduced, whereas, the other 3 varieties were killed in 1 to 3 months.

The 12 species and varieties of 4 genera of heathers or heaths tested were found to be susceptible to the fungus. However, there was considerable variation in the degree of susceptibility of the different species and varieties. Calluna vulgaris var. aurea was much more susceptible to the fungus than C. vulgaris var. alba. This substantiated observations that had previously been made in the field. Brunkenenthalia spiculifolia, Calluna vulgaris var. aurea, Daboecia cantabrica, Erica arborea var. alpina, E. carnea, E. cinerea, E. terminalis and E. tetralix appeared to be more susceptible than C. vulgaris var. alba, E. ciliaris, E. mediterranea and E. vagans.

Other plants found to be susceptible were Betula alba, Castanea dentata, Juglans regia, Pseudotsuga taxifolia, Rhododendron molle, R. ponticum, Taxus baccata var. stricta and T. cuspidata. Daphne cneorum, D. odora, Picea abies, Pinus mugo var. mughus, Quercus borealis,

Rhododendron obtusum, Juniperus chinensis var. pfitzeriana, J. excelsa var. stricta, J. sabina, J. squamata var. meyeri, Thuja occidentalis var. aureo-variegata, T. occidentalis var. fastigiata, T. occidentalis var. woodwardii, T. orientalis var. aurea and T. orientalis var. compacta were not susceptible.

Plants found susceptible that had not previously been reported as hosts of P. cinnamomi were Brunkenthalia spiculifolia, Calluna vulgaris var. alba, C. vulgaris var. aurea, Chamaecyparis lawsoniana var. allumi, C. lawsoniana var. fletcheri, C. lawsoniana var. lutea, C. lawsoniana var. nestoides, Daboecia cantabrica, Erica arborea var. alpina, E. carnea, E. ciliaris, E. cinerea, E. mediterranea, E. terminalis, E. tetralix, E. vagans and Rhododendron molle.

CHAS. L. BROWN 2000

DISTRIBUTION OF OREGON NURSERIES INFESTED WITH
PHYTOPHTHORA CINNAMOMI AND P. LATERALIS

During the past 3 years an effort was made to determine the statewide distribution of Phytophthora cinnamomi and P. lateralis. Isolations were made from diseased plants collected from nurseries and home plantings throughout the state. Members of the Bureau of Nursery Service of the Oregon State Department of Agriculture cooperated in the survey and collection of diseased plant specimens. The number of nurseries found infested in each county is listed in Table 3.

TABLE 3. DISTRIBUTION OF OREGON NURSERIES
INFESTED WITH PHYTOPHTHORA CINNAMOMI AND P. LATERALIS

| County | Number of nurseries infested | |
|------------|------------------------------|--------------------------|
| | With <u>P. lateralis</u> | With <u>P. cinnamomi</u> |
| Benton | 1 | 0 |
| Clackamas | 3 | 1 |
| Columbia | 1 | 0 |
| Coos | 1 | 0 |
| Lane | 1 | 0 |
| Linn | 2 | 0 |
| Marion | 1 | 2 |
| Multnomah | 11 | 1 |
| Washington | 2 | 0 |
| Total | 23 | 4 |

Phytophthora lateralis was isolated from diseased plants from 23 nurseries located in 9 counties, whereas, P. cinnamomi was isolated from diseased plants from only 4 nurseries in 3 counties. The largest number of infested nurseries was found in counties in which a large number of nurseries are located i.e. Multnomah and Clackamas.

With the exception of one nursery in Coos County all of the infested nurseries are located in the Willamette Valley. Phytophthora lateralis had not previously been found in the native Lawson cypress area of southwestern Oregon.

Phytophthora lateralis was also found causing damage to windbreak, home and landscape plantings of Lawson cypress through the Willamette Valley. P. cinnamomi was isolated from diseased Irish yew from one home planting in Benton county.

MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF OREGON
ISOLATES OF PHYTOPHTHORA CINNAMOMI

All Oregon isolates of Phytophthora cinnamomi were indistinguishable morphologically. The mycelia were irregularly branched, hyaline, variable in diameter (3 to 9 microns), becoming septate with age and covered with irregularly shaped protrusions or swellings. Spherical, thin walled chlamydospores of variable diameter were produced terminally on short lateral branches. The chlamydospores frequently occurred in grape-like clusters. The sporangiophores were simple or sympodially branched. Thin walled, nonpapillate or inconspicuously papillate, ovoid to ellipsoid sporangia were produced terminally. Sporangial proliferation was frequently observed. The sporangia germinated directly by the formation of germ tubes or indirectly by zoospores. The sexual stage of the fungus was not observed. Rather extensive morphological and cultural comparisons were made of 9 isolates from several different hosts.

Measurements were made of sporangia, produced in a nonsterile soil filtrate, and chlamydospores, produced on one percent water agar. The average of the measurements for each isolate is given in Table 4. The differences between the average sporangial and chlamydospore sizes of the 9 isolates were very small.

TABLE 4. COMPARISON OF CHLAMYDOSPORE AND SPORANGIAL SIZES OF NINE OREGON ISOLATES OF PHYTOPHTHORA CINNAMOMI

| Source of isolate | Average diameter of 50 chlamydospores in microns | Average length and width of 100 sporangia in microns |
|------------------------------------------|--------------------------------------------------|------------------------------------------------------|
| English yew | 37 | 50 x 32 |
| Irish yew | 39 | 43 x 32 |
| Irish yew | 33 | 44 x 31 |
| Irish yew | 36 | 46 x 33 |
| Japanese yew | 35 | 42 x 33 |
| Alumi cypress | 36 | 52 x 36 |
| Elwoodi cypress | 29 | 45 x 33 |
| <u>Calluna vulgaris</u> var. <u>alba</u> | 27 | 48 x 35 |
| <u>Erica carnea</u> | 35 | 53 x 37 |

The pathogenicity of the 9 Oregon isolates to Netted Gem potato tubers was tested. Sound potato tubers of equal size were washed, surface sterilized in 15 percent Chlorox and allowed to dry. The tubers were then inoculated by making a surface cut with a sterile scalpel, inserting the inoculum growing on potato-dextrose-agar in the wound and sealing the wound with cellophane tape. Checks were treated similarly but were inoculated with sterile potato-dextrose-agar. Three tubers were used for each isolate and all tubers incubated at room temperature. After 10 days they were examined and all tubers inoculated with Phytophthora cinnamomi found to be partially decayed, whereas, the checks were sound. The fungus was reisolated from the advanced edges of the decayed portions of the inoculated tubers.

The temperature-growth relationships of the Oregon isolates were studied. The 9 isolates were grown on potato-dextrose-agar in petri plates for 5 days at 25° C. Discs 5 millimeters in diameter were cut out of the cultures with a cork borer and one disc transferred to the center of each of 35 plates of potato-dextrose-agar. Five plates of each isolate were incubated at 7 different temperatures and the mycelial growth measured after 3 days. The results are given in Table 5.

The growth responses of the isolates to the various temperatures were very similar. All isolates failed to grow at 5° C. and 35° C., the fungus being dead after 3 days exposure. Growth was negligible at 10° C., moderate at 20° C., and usually slightly greater at 25° C. and 30° C.

TABLE 5. GROWTH AFTER THREE DAYS OF NINE OREGON ISOLATES OF
PHYTOPHTHORA CINNAMOMI ON POTATO-DEXTROSE-AGAR AT VARIOUS TEMPERATURES

| Source of Isolate | Diameters* in millimeters of mycelial growth at | | | | | | |
|-------------------------|-------------------------------------------------|--------|--------|--------|--------|--------|--------|
| | 5° C. | 10° C. | 15° C. | 20° C. | 25° C. | 30° C. | 35° C. |
| English yew | 0 | t** | 17 | 29 | 28 | 34 | 0 |
| Irish yew | 0 | t | 24 | 40 | 42 | 45 | 0 |
| Irish yew | 0 | t | 26 | 35 | 39 | 39 | 0 |
| Irish yew | 0 | t | 26 | 34 | 40 | 42 | 0 |
| Japanese yew | 0 | t | 23 | 36 | 41 | 37 | 0 |
| Alumi cypress | 0 | t | 17 | 37 | 41 | 46 | 0 |
| Elwoodi cypress | 0 | t | 22 | 46 | 45 | 45 | 0 |
| <u>Calluna vulgaris</u> | 0 | t | 29 | 47 | 68 | 45 | 0 |
| <u>Erica carnea</u> | 0 | t | 29 | 58 | 60 | 52 | 0 |

* - average of 5 plates

** - trace, growth too slight for measurement

MORPHOLOGICAL AND CULTURAL COMPARISON OF OREGON ISOLATES
OF PHYTOPHTHORA CINNAMOMI WITH ISOLATES FROM OTHER SOURCES

Isolates of Phytophthora cinnamomi from areas other than Oregon were compared with Oregon isolates in order to determine if they were as morphologically and culturally homogeneous as the Oregon isolates. Isolates of the fungus were obtained from several workers in this country and Hawaii. The 25 isolates obtained appeared to be indistinguishable from the Oregon isolates. Five Oregon isolates and 9 from other sources were selected for rather extensive morphological and cultural comparisons.

Average sporangial measurements of the 14 Phytophthora cinnamomi isolates from Oregon and elsewhere are given in Table 6. There appeared to be no marked differences between the average sporangial sizes of the different isolates.

Using the method described previously, the pathogenicity of the 14 isolates to Netted Gem potato tubers was tested. All of the isolates were found to be pathogenic.

The effect of different concentrations of malachite green on growth has been used to differentiate several of the species of Phytophthora. An experiment was, therefore, set up to determine if there were differences in the effect of malachite green on the growth of different isolates of

TABLE 6. COMPARISON OF SPORANGIAL SIZES OF
FOURTEEN ISOLATES OF PHYTOPHTHORA CINNAMOMI

| Source of isolate | | Average length and width of 100 sporangia in microns |
|-------------------------|-------------------|------------------------------------------------------------|
| Host | Location | |
| Avocado | California | 56 x 39 |
| <u>Calluna vulgaris</u> | Oregon | 50 x 34 |
| <u>Camellia</u> | Alabama | 47 x 33 |
| Chestnut | Georgia | 45 x 33 |
| English yew | Oregon | 51 x 34 |
| <u>Erica carnea</u> | Oregon | 49 x 35 |
| Heather | California | 45 x 32 |
| Irish yew | Oregon | 46 x 28 |
| Japanese yew | North Carolina | 53 x 36 |
| Japanese yew | Oregon | 46 x 32 |
| Japanese yew | Maryland | 51 x 33 |
| Pineapple | Oahu, H.I. | 54 x 34 |
| Shortleaf pine | Southeastern U.S. | 47 x 33 |
| Yew | California | 52 x 36 |

Phytophthora cinnamomi. Culture tubes containing 5 milliliters of Leonian's medium at two concentration levels of malachite green, 1:8,000,000 and 1:4,000,000, were prepared. With a cork borer discs 5 millimeters in diameter were cut out of 5 day old cultures of the 14 isolates growing in petri plates on potato-dextrose-agar. One disc was transferred to each of three tubes of medium at the two malachite green concentration levels. The inoculated tubes were incubated at room temperature and after one week examined for mycelial growth. All 14 isolates had grown at the lower malachite green concentration level, but at the higher concentration level the isolates failed to grow.

The temperature-growth relationships of the 14 isolates were also studied. The method described previously was used except that 6 different temperatures and only 4 plates per isolate at each temperature were used. The results are given in Table 7. The growth responses of the isolates to the different temperatures were similar to those previously obtained for the 9 Oregon isolates.

TABLE 7. GROWTH AFTER FOUR DAYS OF FOURTEEN ISOLATES OF
PHYTOPHTHORA CINNAMOMI ON POTATO-DEXTROSE-AGAR AT VARIOUS TEMPERATURES

| Source of isolate | | Diameter* in millimeters of mycelial growth at | | | | | | |
|-------------------------|-------------------|------------------------------------------------|--------|--------|--------|--------|--------|--------|
| Host | Location | | 10° C. | 15° C. | 20° C. | 25° C. | 30° C. | 35° C. |
| Avocado | California | t** | 26 | 49 | 67 | 71 | 0 | |
| <u>Calluna vulgaris</u> | Oregon | t | 42 | 60 | 84 | 40 | 0 | |
| Camellia | Alabama | t | 32 | 48 | 81 | 68 | 0 | |
| Chestnut | Georgia | t | 26 | 44 | 82 | 76 | 0 | |
| English yew | Oregon | t | 23 | 31 | 69 | 71 | 0 | |
| <u>Erica carnea</u> | Oregon | t | 42 | 69 | 82 | 73 | 0 | |
| Heather | California | t | 21 | 38 | 76 | 73 | 0 | |
| Irish yew | Oregon | t | 27 | 36 | 84 | 78 | 0 | |
| Japanese yew | North Carolina | t | 30 | 39 | 70 | 65 | 0 | |
| Japanese yew | Oregon | t | 37 | 59 | 88 | 60 | 0 | |
| Japanese yew | Maryland | t | 37 | 52 | 71 | 59 | 0 | |
| Pineapple | Oahu, H.I. | t | 30 | 47 | 83 | 85 | 0 | |
| Shortleaf pine | Southeastern U.S. | t | 28 | 36 | 81 | 71 | 0 | |
| Yew | California | t | 23 | 38 | 52 | 46 | 0 | |

* - average of 4 plates

** - trace, growth too slight for measurement

PHYSIOLOGICAL SPECIALIZATION OF PHYTOPHTHORA CINNAMOMI

Several workers have shown that physiological strains of Phytophthora cinnamomi probably exist. Because the existence of a large number of physiological strains would have a bearing on control measures, an attempt was made to determine the extent of physiological specialization in the species, particularly among the Oregon isolates.

The 14 isolates of Phytophthora cinnamomi used in the morphological and cultural studies were tested for differences in pathogenicity using Alumni cypress, Irish yew and Douglas fir as differential hosts. Nine rooted Alumni cypress cuttings were planted in each of 15 22 inch x 22 inch sections of greenhouse bench. Rooted Irish yew cuttings were planted in the same manner. Water movement between sections was prevented by lining the sections with heavy waterproof paper. Four 2-year Douglas fir seedlings were planted in each of 72 12 inch clay pots. When the plants were well established each section of Alumni cypress and Irish yew and each 5 pot lot of Douglas fir was inoculated with a different isolate of the fungus. Approximately 3 months after inoculation the plants were removed and examined for root rot. The results are given in Table 8.

TABLE 8. PATHOGENICITY OF FOURTEEN ISOLATES OF
PHYTOPHTHORA CINNAMOMI TO ALUMI CYPRESS, IRISH YEW AND DOUGLAS FIR

| Source of isolate | | Plants inoculated | | |
|-------------------------|-------------------|-------------------|--------------|--------------|
| | | Alumi cypress | Irish yew | Douglas fir |
| | | (9 plants) | (9 plants) | (20 plants) |
| Host | Location | No. diseased | No. diseased | No. diseased |
| Avocado | California | 5 | 7 | 20 |
| <u>Calluna vulgaris</u> | Oregon | 6 | 4 | 20 |
| Camellia | Alabama | 0 | 0 | 18 |
| Chestnut | Georgia | 0 | 5 | 0 |
| English yew | Oregon | 5 | 6 | 19 |
| <u>Erica carnea</u> | Oregon | 8 | 7 | 18 |
| Heather | California | 5 | 4 | 18 |
| Irish yew | Oregon | 8 | 6 | 20 |
| Japanese yew | North Carolina | 6 | 8 | 17 |
| Japanese yew | Oregon | 9 | 4 | 17 |
| Japanese yew | Maryland | 4 | 9 | 19 |
| Pineapple | Oahu, H.I. | 4 | 7 | 18 |
| Shortleaf pine | Southeastern U.S. | 4 | 8 | 18 |
| Yew | California | 6 | 4 | 19 |
| Check (no inoculum) | | 0 | 0 | 0 |

On the basis of the pathogenicity of 14 isolates of Phytophthora cinnamomi to Alumi cypress, Irish yew and Douglas fir only 3 physiological strains of P. cinnamomi could be differentiated. Twelve of the 14 isolates tested were pathogenic to the 3 plant species used as differential hosts. The 5 Oregon isolates were among those pathogenic to the 3 hosts. An isolate from camellia in Alabama was pathogenic to Douglas fir but not to Alumi cypress and Irish yew, whereas, the chestnut isolate from Georgia was pathogenic to Irish yew but not to Alumi cypress or Douglas fir.

FACTORS INFLUENCING DISEASE DEVELOPMENT AND SPREAD

Effect of Soil Texture on Disease Development and Spread

The effect of soil texture on the development and spread of Phytophthora cinnamomi root rot of Lawson cypress was studied experimentally in the greenhouse. A greenhouse bench was divided into 3 blocks of 4 22 inch x 22 inch sections. A section of each of the 3 blocks was then filled with one of the following soil mixtures: heavy clay loam, 3 parts heavy clay loam to 1 part sand, 1 part heavy clay loam to 1 part sand or 1 part heavy clay loam to 3 parts sand. Three rows of 3 Lawson cypress were then planted in each bench section. When the plants were well established one of the corner plants in each bench section was inoculated with an isolate of P. cinnamomi, which had originally been isolated from Lawson cypress. Following inoculation the soil in all sections was maintained at a moisture level of approximately field capacity. About 11 months after inoculation the plants were removed from the bench sections and examined for root rot. At this time the inoculated plant in all sections showed root rot symptoms. The results are given in Table 9.

TABLE 9. THE EFFECT OF SOIL TEXTURE ON THE DEVELOPMENT AND SPREAD OF PHYTOPHTHORA CINNAMOMI ROOT ROT OF LAWSON CYPRESS

| Soil mixture | | Number of diseased plants in each replication of 9 plants | | | Average per- centage of plants diseased |
|--------------|------|-----------------------------------------------------------------|-------|-------|-----------------------------------------------|
| Clay loam | Sand | No. 1 | No. 2 | No. 3 | |
| % | % | | | | |
| 100 | 0 | 1 | 1 | 1 | 11.1 |
| 75 | 25 | 7 | 5 | 6 | 66.6 |
| 50 | 50 | 1 | 2 | 2 | 18.6 |
| 25 | 75 | 1 | 1 | 2 | 14.8 |

The spread and development of root rot away from the point of inoculation was nil or slight in the heavy clay loam, the 1 part heavy clay loam to 1 part sand and the 1 part heavy clay loam to 3 parts sand. However, in the 3 parts heavy clay loam to 1 part sand there was considerable spread and development of root rot away from the point of inoculation, an average of 66.6 percent of the plants being diseased.

Effect of Temperature on Sporangial Production by *Phytophthora cinnamomi* and *P. lateralis*

The major development and spread of the root rot diseased caused by *Phytophthora cinnamomi* and *P. lateralis* in Oregon has been thought to occur during the winter months when the soil frequently becomes saturated with

water. Zentmyer (72), however, believed that, as P. cinnamomi forms sporangia only between temperatures of about 70° F. and 88° F. and as most of the infection in the field probably results from zoospores, the main infection period in California is in the late spring and early summer after the soil warms up sufficiently for sporangial formation. Using Oregon isolates of P. cinnamomi and P. lateralis the effect of temperature on sporangial production by the two species was studied in an effort to determine the possible influence of soil temperature on the development and spread of the root rot diseases caused by these two fungi in Oregon.

Washed mycelia of isolates of the two species were transferred to 125 ml. flasks containing 50 ml. of a non-sterile soil filtrate. Two flasks of each isolate were then incubated at each of five temperatures. After 2 days, portions of the mycelia were examined microscopically for the presence of sporangia. The results obtained are given in Table 10.

TABLE 10. EFFECT OF TEMPERATURE ON SPORANGIAL PRODUCTION
BY PHYTOPHTHORA CINNAMOMI AND P. LATERALIS

| Species | Temperature | | | | |
|---------------------|-------------|--------|--------|--------|--------|
| | 15° C. | 20° C. | 25° C. | 30° C. | 35° C. |
| <u>P. cinnamomi</u> | 0 | + | ++ | + | 0 |
| " | 0 | + | + | + | 0 |
| " | 0 | + | ++ | + | 0 |
| " | 0 | + | ++ | + | 0 |
| " | 0 | + | ++ | + | 0 |
| " | 0 | + | + | + | 0 |
| <u>P. lateralis</u> | 0 | + | 0 | 0 | 0 |
| " | 0 | + | 0 | 0 | 0 |
| " | 0 | + | 0 | 0 | 0 |

0 - no sporangial production
+ - moderate sporangial production
++ - heavy sporangial production

There was no sporangial production by the 6 isolates of Phytophthora cinnamomi at 15° C. and 35° C., but all of the isolates produced sporangia at 20° C., 25° C. and 30° C. Sporangial production, however, was more profuse at 25° C. than at 20° C. or 30° C. P. lateralis produced sporangia only at 20° C.

Vertical Distribution of *Phytophthora cinnamomi* in the Soil

Soil fumigation has frequently been suggested as a possible method for the eradication of *Phytophthora cinnamomi* from infested nursery soils. Eradication by this method would be successful only if the fungus was present in the upper foot or less of soil as it is impossible to get adequate penetration of the soil below that depth with any of the soil fumigants available on the market at the present time. Therefore, the vertical distribution of *P. cinnamomi* in the soil under diseased Elwoodi cypress in a planting at Salem, Oregon was determined in August, 1951.

Soil samples were taken from under 20 Elwoodi cypress, 2 to 3 feet in height, showing root rot symptoms. The samples were taken at scattered points in the planting. A pit approximately 3 feet deep was dug immediately to the side of each of the plants under which samples were to be taken. The side of the pit face, 3 to 4 inches from the trunk of the diseased plant, was made vertical and soil samples taken at 0.3 foot intervals to a depth of 2.4 feet. Only a few decayed roots were found at the 2.4 foot level. Each of the samples was subsequently used to inoculate 2 apples and the presence of *Phytophthora cinnamomi* verified by isolating from the rotted tissues that developed in the inoculated apples. The

percentage of samples from which P. cinnamomi was isolated at the different depths is given in Table 11.

TABLE 11. VERTICAL DISTRIBUTION OF PHYTOPHTHORA CINNAMOMI IN THE SOIL UNDER DISEASED ELWOODI CYPRESS

| <u>Depth of samples</u> Feet | <u>Incidence of P. cinnamomi</u> Percentage of 20 samples |
|---------------------------------|--------------------------------------------------------------|
| 0.3 | 60 |
| 0.6 | 100 |
| 0.9 | 95 |
| 1.2 | 80 |
| 1.5 | 40 |
| 1.8 | 20 |
| 2.1 | 40 |
| 2.4 | 10 |

Phytophthora cinnamomi was more abundant in the top 1.2 feet of soil than at the greater depths. The percentage of samples from which the fungus was isolated was highest at the 0.6 and 0.9 foot depths. Although the frequency with which the fungus was found gradually decreased at the greater depths the fungus occurred to a limited extent even at a depth of 2.4 feet.

DISCUSSION AND CONCLUSIONS

Surveys made to determine the distribution of Oregon nurseries infested with Phytophthora cinnamomi and P. lateralis showed that the latter species was present and causing plant losses in 23 nurseries in 9 counties, whereas, the former species was present and causing losses in only 4 nurseries in 3 counties. Most of the major growers of Lawson cypress varieties were included in the 23 nurseries in which P. lateralis was found. P. lateralis appears to have become widely distributed in the 15 years since the fungus was first found in the state. In view of the extensive distribution of P. lateralis in the last 15 years, it is probable that the limited distribution of P. cinnamomi, which has a much larger host range, will become greatly extended in a decade or so.

Phytophthora cinnamomi was found to be the cause of root rot of a number of plant species in Oregon nurseries, including 5 species or varieties not previously reported as hosts. Because of its wide host range P. cinnamomi can cause great losses as was well demonstrated in one nursery in which the fungus was found attacking and causing losses of Alumi and Elwoodi cypress, English and Irish yew, heath and heather throughout the nursery.

The results of pathogenicity trials, conducted to determine the susceptibility of a number of commonly grown

nursery plants, showed that 27 of the 47 plant species or varieties tested were susceptible to Phytophthora cinnamomi. A number of the plants found susceptible had not previously been reported as hosts of the fungus. Several rather incongruous results were obtained. The isolate of P. cinnamomi used was not pathogenic to Quercus borealis which has been reported as being susceptible but was pathogenic to Erica tetralix and E. cinerea which have been reported as not being susceptible. This disagreement is perhaps due to physiological specialization of different isolates of the fungus. The large number of plant species and varieties found susceptible is indicative of the plants the fungus would attack in a nursery or landscape planting where a large number of different kinds of plants are grown.

The results of experiments designed to determine the existence of physiological strains of Phytophthora cinnamomi indicate that 3 strains of the fungus were present among the 14 isolates tested. Using Alumi cypress, Douglas fir and Irish yew as differential hosts the 3 strains were differentiated as follows: Strain 1. Pathogenic to Alumi cypress, Douglas fir and Irish yew; Strain 2. Pathogenic to Douglas fir but not to Alumi cypress and Irish yew; Strain 3. Pathogenic to Irish yew but not to Alumi cypress or Douglas fir. As the isolates tested were from a number of different hosts and

geographic areas it appears that although physiological strains exist in P. cinnamomi their number is not great. If additional differential hosts had been used it is possible that the number of strains differentiated would have been increased.

Morphologically and culturally the Oregon isolates of Phytophthora cinnamomi appeared to be identical. The same homogeneity of morphological and cultural characteristics was found when Oregon isolates were compared with isolates of the fungus obtained from other workers. The average chlamydospore sizes of 9 Oregon isolates varied only slightly. Since chlamydospore size is not commonly used in differentiating species of Phytophthora this characteristic was not employed in comparing Oregon isolates with isolates from other sources. Sporangial size within a species has been found to be variable (30, 31) and is used by Tucker (58) and Leonian (29) only as a secondary characteristic in their keys to the species of Phytophthora. However, as there was little variation in average sporangial size of isolates of P. cinnamomi from Oregon and elsewhere it appears that sporangial size is a rather constant characteristic in this species and is perhaps of taxonomic value. Tucker (58) used pathogenicity of P. cinnamomi to potato tubers as a means of separating this species from P. cambivora.

This separation has been questioned (36, 69) but on the basis of the results obtained, with the isolates studied, Tucker's separation appears to be valid. Leonian's (28, 29) use of malachite green in separating P. cinnamomi from several other species of Phytophthora was also substantiated. Tucker (58) found that the temperature-growth relationships of Phytophthora species were constant and used them as a major characteristic in separating many of the species of Phytophthora. Although differences were noted between isolates in their rate of growth at a specific temperature the temperature maximum and minimum at which growth did not take place was constant for all isolates studied and appeared to be a valid characteristic for separating P. cinnamomi from other species. Both Tucker (58) and Leonian (29) had available for study only a few isolates of P. cinnamomi when preparing their keys to the species of Phytophthora, but, as all of the characteristics used by them were found to be constant for the P. cinnamomi isolates studied here, their separations appear to have been valid.

As the symptoms of the root rot diseases of Lawson cypress caused by Phytophthora cinnamomi or P. lateralis are identical, it is impossible to determine in the field which fungus is the causal organism. However, the two species are readily distinguishable in the laboratory.

P. lateralis has an optimum temperature for growth in the vicinity of 20° C. and does not grow at 30° C., whereas, P. cinnamomi has a temperature optimum of between 25° C. and 30° C. and will not grow at 35° C. In addition P. cinnamomi has larger sporangia and grows at a much faster rate on artificial media than P. lateralis.

The effect of soil moisture on disease development and spread was not studied experimentally, but observations made in the field and greenhouse indicate soil moisture is an important factor. In nurseries the disease was usually observed in areas that were poorly drained or kept very well irrigated. Plants inoculated in the greenhouse appeared to develop root rot at a faster rate when they were well watered.

Spread of the disease was much greater in a soil of moderate texture than in soils of a heavier or lighter texture. It is believed that in soils of heavy texture the zoospores are unable to move freely with the result that spread of the disease is not great. Likewise spread is slight in soils of very light texture because of the rapidity with which the water necessary for zoospore dissemination drains away. A soil of moderate texture is apparently the optimum for conditions of proper moisture and ease of movement necessary for zoospores to move and infect roots of susceptible plants.

Most of the infection of susceptible plants by Phytophthora cinnamomi probably does not take place in Oregon until late spring or early summer when the soil has warmed up sufficiently for sporangial production. P. cinnamomi was found to produce sporangia only in a temperature range of about 20° C. to 30° C. P. lateralis produced sporangia only at 20° C., indicating that zoospore infections may occur only within a very restricted range of soil temperature. As the primary means of infection by both species of Phytophthora is probably by zoospores very little infection apparently takes place during the winter when soil temperatures are below the minimum for sporangial production.

In the soil under diseased Elwoodi cypress, Phytophthora cinnamomi was found to a depth of 2.4 feet and was most abundant in the upper 1.2 feet of soil. Campbell (11) found that P. cinnamomi was most abundant at 2 and 3 inch depths but occurred to a limited extent at a depth of 12 inches in infested shortleaf pine stands. Anderson (2) reported that in Hawaii this fungus had been recovered to a depth of 27 inches in very compact subsoil. These differences in the vertical distribution of P. cinnamomi in the soil are probably due to differences in moisture content and texture of the soil and the depths

to which roots of host plants have penetrated. Because of the depths at which P. cinnamomi may occur in the soil chemical eradication of the fungus does not appear to be feasible.

On the basis of these studies and those of other workers it appears that Phytophthora cinnamomi is potentially a very serious plant pathogen in Oregon. The pathogen attacks a wide range of ornamental plants and experimentally it was shown that 5 Oregon isolates were pathogenic to Douglas fir seedlings. Although the pathogen has not been shown to damage large Douglas fir trees this aspect is perhaps worthy of further study because of the economic importance of Douglas fir in Oregon.

The constant movement of nursery stock from nursery to nursery and from nursery to landscape plantings and the wide host range of this fungus will probably result in the pathogen becoming widely disseminated throughout the more temperate areas in the state. These factors plus the lack of any practical means of controlling the diseases caused by this fungus may result in Phytophthora cinnamomi becoming as important a plant pathogen in Oregon as it has elsewhere.

SUMMARY

1. Phytophthora cinnamomi was found to be the cause of root rot of Alumi and Elwoodi cypress, Irish yew, Japanese yew, English yew, heath and heather in Oregon nurseries and home plantings.

2. An isolate of Phytophthora cinnamomi was pathogenic to 27 of the 47 plant species or varieties tested. Seventeen of the plant species or varieties found susceptible had not previously been reported as hosts of P. cinnamomi.

3. Phytophthora lateralis which has been present in Oregon for at least 15 years was found in 23 nurseries in 9 counties, whereas, P. cinnamomi which first was reported in Oregon in 1951 was found in only 4 nurseries in 3 counties.

4. Oregon isolates of Phytophthora cinnamomi were compared and found to be morphologically and culturally indistinguishable.

5. Oregon isolates of Phytophthora cinnamomi were also found to be morphologically and culturally indistinguishable from isolates of P. cinnamomi from other sources in the United States and Hawaii.

6. Three physiological strains were differentiated among the 14 isolates of Phytophthora cinnamomi tested

using Alumi cypress, Irish yew and Douglas fir as differential hosts.

7. Soil of a moderate texture appeared to be more favorable for the development and spread of root rot caused by Phytophthora cinnamomi than lighter or heavier textured soils.

8. The major development and spread of the root rot caused by Phytophthora cinnamomi and P. lateralis in Oregon probably occurs after the soil warms up to about 20° C. in the late spring or early summer.

9. Because Phytophthora cinnamomi may be present in the soil to at least a depth of 2.4 feet soil fumigation does not appear practical as a method of eradicating the fungus from infested soil.

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