

THE PHYSIOLOGY AND PATHOLOGY OF PHYTOPHTHORA LATERALIS
ON NATIVE CHAMAECYPARIS LAWSONIANA

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THE PHYSIOLOGY AND PATHOLOGY OF PHYTOPHTHORA LATERALIS
ON NATIVE CHAMAECYPARIS LAWSONIANA

INTRODUCTION

In 1952, the serious root disease of Chamaecyparis caused by Phytophthora lateralis Tucker and Milbrath, was discovered in the native range of Chamaecyparis lawsoniana (Murr.) Parl. Previous work, on ornamental plantings of Chamaecyparis dating back to 1937, had established the extreme susceptibility of this tree to the pathogen. Thus, a serious fungus disease threatened the tree in the only existing native habitat. This study was initiated, in the summer of 1954, to gather information on the spread and survival of the pathogen under natural forest conditions. The results of studies on the epiphytology of the disease as well as basic physiological data, which had been largely omitted by previous workers, are presented in this thesis.

The host of primary concern in this study, Chamaecyparis lawsoniana, is known locally as Port Orford cedar or Lawson cypress. Port Orford cedar is known for its beauty and has been cultivated as an ornamental tree in America and abroad for many years. This tree grows naturally only along the Pacific Coast of North America from Coos Bay in Oregon, southward into Humboldt County of northern California. Relatively pure stands of limited extent occur but more often Port Orford cedar is intermixed with Sitka spruce (Picea sitchensis (Bong.) Carr.), western

hemlock (Tsuga heterophylla (Raf.) Sarg.), and Douglas fir (Pseudotsuga menziesii (Mirb.) Franco.) (16, p. 246-250).

Port Orford cedar of commercial importance is confined to a strip varying from a few miles to 30 miles wide along the seaward slope of the Coast Range Mountains in southwestern Oregon. The easily accessible virgin forests have been logged, and at present the tree is being cut from the more mountainous parts of its range. Young growth forests and regeneration on the cut-over lands vary in age up to 60 years and usually occur in rather even aged groups.

The pathogen, Phytophthora lateralis, is a fungus which grows best in relatively cool, wet soils. The moderate, moist, marine climate which exists throughout most of the Chamaecyparis range is well suited to the growth of this pathogen. Therefore, in 1954, with the pathogen widely distributed throughout the susceptible Chamaecyparis range, the stage was set for a serious epiphytotic disease.

LITERATURE REVIEWTHE PATHOGEN, PHYTOPHTHORA LATERALIS

An apparently undescribed species of Phytophthora causing a serious root disease of Chamaecyparis in Oregon was reported by Milbrath and McWhorter (38, p. 1) in 1938. Milbrath (37, p. 788) reported extensive losses in commercial and private ornamental plantings of Chamaecyparis caused by the same Phytophthora in 1940. Studies on the morphology and physiology of this Phytophthora from Chamaecyparis by Tucker and Milbrath in 1942 demonstrated that the isolates formed a well-defined taxonomic unit differing in various respects from the previously described species of this genus (56, p. 96). These workers named the fungus Phytophthora lateralis sp. nov. describing it as follows:

"Hyphae continuous when young, becoming septate with age, usually smooth, but sometimes gnarled or tuberosus.

Sporangia none on agar media but developing fairly abundantly on washed mycelial mats transferred from 7-day pea broth cultures (20° C.) to sterile distilled water and incubated 7 days at 20° C.; sporangia borne sympodially on sporangio-phores resembling vegetative hyphae, mostly ovate, obovate or obpyriform, occasionally elongate, hyaline to lemon yellow, nonpapillate, with apical refringent plug very thin and often indistinguishable, 26-60 x 12-20 micra, averaging about 36 x 15 micra. Zoospores fully differentiated within the sporangium, biciliate, reniform in motile stage, spherical in nonmotile phase, 10-12 micra in diameter.

Chlamydozoospores abundant in agar cultures and liquid media, usually subspherical to spherical, occasionally ovate to irregular, contents densely and often coarsely granular, lemon yellow to light brown; wall usually thin, sometimes thick (6-7 micra); terminal or intercalary, the latter developing as lateral swellings of the hyphae, often appearing sessile at maturity, 20-77 micra in diameter, averaging about 40 micra; germination by germ tubes.

Oogonia, antheridia and oospores unknown."

Tucker and Milbrath also reported (56, p. 101) that the type of mycelial growth on Difco corn-meal agar at 20° C. had no distinguishing characters. Growth was radiating, regularly circular, submerged and frequently slightly rhizoidal in appearance (56, p. 101). Sympodial as well as the proliferating type of sporangial development was reported. Intercalary chlamydozoospores arose as lateral outgrowths from the hyphae. As the chlamydozoospores matured they often appeared to be sessile on the hyphae or supported by two hyphae attached to the wall in juxtaposition. Frequent searches for sexual organs were made in liquid cultures and in potato dextrose agar and oatmeal agar cultures up to 6 months old. Chlamydozoospores were abundant in old cultures but oogonia and antheridia were not found (56, p. 99).

The reported physiological studies on Phytophthora lateralis were concerned primarily with the effect of temperature on mycelial growth and sporangial formation. Tucker and Milbrath (56, p. 99) reported the optimum

temperature for growth on Difco corn-meal agar (pH 6.1) as 20° C. They further found that growth was slower at temperatures lower than 20°, was restricted at 25° and was inhibited entirely at 30° C. These findings were verified by Salisbury (46, p. 7). Tucker and Milbrath (56, p. 99) observed sporangia only when tufts of mycelium were transferred from pea broth cultures, washed, and incubated in sterile distilled water. They did not observe sporangia on agar media. Torgeson (49, p. 52-53) reported sporangial production only at 20° C. when mycelial mats were transferred from pea broth cultures, washed, and incubated at five temperatures in a non-sterile soil filtrate.

THE PATHOLOGY OF PHYTOPHTHORA LATERALIS
ON NATIVE CHAMAECYPARIS LAWSONIANA

The disease of Chamaecyparis caused by P. lateralis is well reported in the literature. In the late 1930's, attention of pathologists at the Oregon Agricultural Experiment Station was directed to a root rot disease of Chamaecyparis in western Oregon nursery and landscape plantings (38, p. 1). The pathogen causing this trouble is now widely distributed through Oregon, Washington, and British Columbia (44) but has not been reported outside the Pacific Northwest. Neither the origin of P. lateralis nor the time of its first appearance in the native Port Orford

cedar region are known. While the fungus was first definitely observed and isolated in Coos County in Oregon in 1952 (49, p. 38; 44), 15 years after it had been found in the inland valleys, there is limited evidence that it was there prior to 1952 (44). Some observers consider that the fungus is endemic and has served to limit the natural range of Port Orford cedar. However, Roth et al. (44) state that the scarcity of early records of abnormal death of Port Orford cedar, the lack of acquired resistance in native trees and the present epiphytotic behavior all suggest recent introduction of the pathogen.

Host range studies have been limited to the family Cupressaceae. Phytophthora lateralis is reported only on species of the genus Chamaecyparis. Torgeson, Young and Milbrath (51, p. 13) reported the following plants susceptible to root rot caused by P. lateralis: Chamaecyparis lawsoniana and its varieties alumi, cerula, elwoodi, erecta aurea, erecta glauca, erecta veridis, lutea, minima glauca, nestoides, stewarti, wisseli and C. obtusa var. crippsii. In addition to this list Milbrath and McWhorter (38, p. 1) found the disease on C. obtusa var. gracilis. Salisbury (46, p. 11) reported C. nootkatensis to be susceptible.

Plants tested in Oregon by Torgeson et al. (51, p. 14) and stated to be "resistant" to root rot caused by

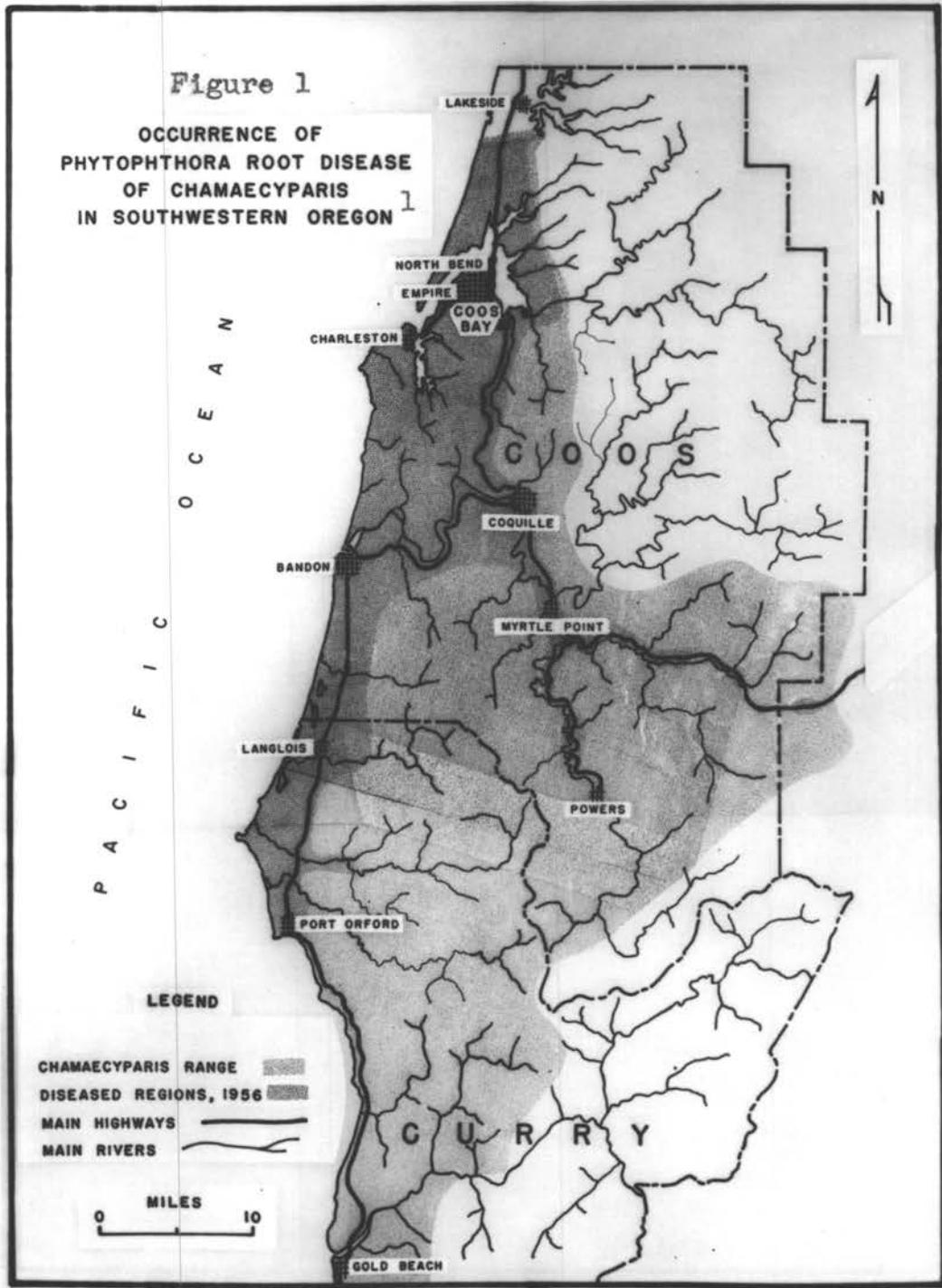
P. lateralis included: Chamaecyparis nootkatensis var. compacta, C. obtusa varieties ericoides, tetragona aurea, and torulose, C. pisifera varieties aurea nana, filifera, plumosa, plumosa aurea, squarrosa and squarrosa nana, C. thyoides, Juniperus excelsa, J. horizontalis, Thuja occidentalis var. fastigiata, T. orientalis var. aurea conspicua, and T. plicata. Salisbury (46, p. 11) reported Taxus baccata var. stricta "resistant" to P. lateralis.

Milbrath and McWhorter (38, p. 1) reported that in 1937 the disease was present in a majority of the nurseries in northern Oregon and suggested that the disease had been present for some time before it was recognized. In the 20 years following this initial report the pathogen has been widely observed throughout the Pacific Northwest (46, p. 6; 51, p. 37-38; 45, p. 640).

Torgeson (49, p. 38) and Roth et al. (45, p. 640) first observed P. lateralis in the native Chamaecyparis range in 1952. At that date diseased trees were found only in a small area near the city of Coos Bay. By the spring of 1954 scattered trees with root disease symptoms could be seen for 75 miles along the coastal shelf through the heart of the native cedar range (45, p. 640). The distribution of trees with root infections was often associated with water courses or with disturbed soil. Within the cedar range, infected trees were numerous around the margins of

lakes, along streams and along the roadsides (45, p. 640). Phytophthora lateralis is now epiphytotic on Chamaecyparis lawsoniana in many of the young forests in the commercial cedar range from Lakeside, Oregon on the north to Port Orford on the south (44).

Symptoms of Phytophthora root rot in ornamental Chamaecyparis have been described by various workers (38, p. 2; 46, p. 4; 51, p. 5; 56, p. 94-95; 44). Symptoms in native Chamaecyparis stands were reported by Roth et al. (44). Descriptions of the root disease agree that Phytophthora lateralis invades the roots of Port Orford cedar and spreads into the lower part of the main trunk, killing the inner bark as it advances. If the outer bark at the base of a diseased tree is removed a sharp line of demarcation is apparent between living tissues and dead areas invaded by the fungus. There is also general agreement that foliar symptoms begin to develop about the time the fungus reaches the base of the tree. These are reported to progress as follows: the foliar pigments slowly lose their intensity and eventually fade to a tan or light brown, and the foliage becomes crisp and dry. The first color changes are especially apparent if the diseased trees are adjacent to healthy trees. Roth et al. (44) reported that foliar symptoms of the root disease in native Chamaecyparis stands developed in a few weeks when the



¹ This map illustrates the results of a Phytophthora root disease survey conducted in the summer of 1956 by Dr. John Hunt, Associate Pathologist, Pacific Northwest Forest and Range Experiment Station, Portland, Oregon.

weather was hot and dry, but that a longer period was required when the weather was cool and damp.

Dissemination of P. lateralis from one area to another appeared to occur primarily through the movement of diseased plants or infested soil, Torgeson et al. (51, P.9). Within an area the pathogen is readily spread by water movement (44). Local spread of the fungus through the soil from tree to tree is accomplished by the movement of zoospores through the soil water (51, p. 9). Torgeson (49, p. 61) further reported that since soil temperature must be about 70° F. before motile spores are produced, dissemination by this means probably occurs only in late spring or summer.

Control of this Phytophthora root disease appears difficult if not impossible. At present no treatments are known that will save plants after they have become infected (51, p. 16). The best means of preventing losses from cedar root rot is to follow practices designed to avoid the disease organism. Growing disease-free propagating stock in new soil or soil known to be free from the root rot fungus is at present the only sure means of avoiding the disease. Soil treatment is not practical with the chemicals that are presently available (51, p. 16). The

use of disease resistant rootstocks has not proven feasible due to incompatibilities between Port Orford cedar varieties and rootstocks of resistant species (51, p. 18).

GENERAL METHODS AND MATERIALS

The initial steps of this investigation included a disease survey of the commercial Port Orford cedar range. From the result of this survey areas were chosen for subsequent study in which natural infection was well established in dense Port Orford cedar stands. Mingus Park, an area of about 30 acres in the city of Coos Bay, was selected as the most promising area for study. The major part of the park is undeveloped for recreational activities and contains one of the earliest centers of infection in the Port Orford cedar region. In June 1954, two years after the disease was first discovered in the Chamaecyparis range, symptoms of root infection could be seen on groups of trees in several general areas within the park. These areas were mapped in detail and the disease progress was followed during the subsequent three year period.

In addition to the centers of natural infection, areas free from the disease were chosen as locations for artificial soil inoculations. The purpose of these inoculations was to determine the ability of the pathogen to become established, survive, and spread through the soil of Port Orford cedar stands under various conditions. These inoculations were made in natural cedar stands on the property of the Coos Bay Pulp Corporation near Charleston, Oregon.

The air-borne stage of the disease cycle, which forms a part of this study, was first observed in September 1954 and was especially common in the southern part of the native cedar range near Langlois, Oregon. The development and spread of natural air-borne infections was followed in the region between Langlois and the Elk River. Aerial inoculations were made in disease free areas near Langlois. These inoculations afforded a means of following the rate of spread of aerial infections as well as the over-all developmental pattern of the disease.

ISOLATION

Isolations from branches, stems, and large roots were made by the tissue transfer technique. Small sections, taken from the active transition zone between diseased and healthy tissue in the cambial region, were transferred to potato dextrose agar in Petri dishes. The fungus grew from the tissue into the agar in two days at room temperature. Branches, stems, and roots smaller than one inch in diameter were washed, surface-sterilized in 10 per cent commercial Clorox for two minutes, and rinsed in sterile distilled water before exposing the cambial region by peeling back the bark. Surface sterilization was not necessary when diseased parts were greater than one inch in diameter. Phytophthora lateralis was isolated easily from the cambial region of fresh tissue but was difficult to isolate from

tissue which had dried somewhat or had been held at temperatures above 25° C. for several hours. Isolation attempts were rarely successful from the outer cortex or the cambial region which had been diseased for some time.

Isolations from foliage and small roots were also made by the tissue transfer technique. The plant material was surface-sterilized in 10 per cent commercial Clorox for two minutes, and rinsed twice in sterile distilled water. The diseased parts were then cut in cross section and small tissue sections transferred to potato dextrose agar.

Phytophthora lateralis is difficult to isolate from the soil during most of the year. Attempts to isolate this organism from soil throughout the fall and winter were usually unsuccessful. In the mild, wet spring, however, isolations were readily made. A selective method using healthy, freshly cut Port Orford cedar twigs proved the most satisfactory. The twigs, about four inches long, were placed in or on the wet forest soil and allowed to remain undisturbed for four weeks. Successful isolations were obtained when the temperature ranged between 10° and 20° C. during the incubation period. The presence of P. lateralis in the soil was evidenced by infection of the twigs and production of the typical color symptoms of early foliar infections. Positive identification of P. lateralis was

easily obtained by placing the infected twigs in a Petri dish with distilled water and incubating at 20° C. for two days. Abundant sporangiophores bearing sporangia of P. lateralis grew from the infected twigs into the water.

CULTURE

Petri dish cultures as well as stock cultures were maintained on potato dextrose agar (200 g. fresh potatoes, 15 g. dextrose, 15 g. agar per liter). The stock cultures were kept on agar slants at 5° C.

The effect of cedar extracts and growth stimulating substances on the morphology of mycelial colonies of P. lateralis was studied by incorporating these substances into a V-8 agar medium (see Growth in Culture for preparation of V-8 agar). Alcoholic extracts of Port Orford cedar foliage and phloem were prepared by allowing 10 g. of fresh tissue to remain in 100 ml. absolute ethanol for 24 hours at room temperature. The extracts were reduced to 10 ml. with vacuum distillation and then added to the V-8 agar medium. Difco yeast extract and Difco casamino acids were used due to their high concentrations of organic nitrogen in the form of amino acids. The prolonged effect of γ (indole-3) - n - butyric acid was also of interest.

The indole butyric acid was dissolved in ethanol, as were the plant extracts. These solutions were considered sterile and were added to the agar medium with a sterile pipette. To sterilize the yeast extract and the casamino acids the exact amounts were weighed, dried at 80° C. for 24 hours and incorporated directly into the agar medium. The following amounts of plant extracts and growth substances were added to 100 ml. of sterile warm V-8 agar: (1) 2 ml. ethanol (as a control); (2) 1ml. foliage extract; (3) 10 ug. indole butyric acid (in 1 ml. ethanol) plus 1 ml. foliage extract; (4) 1 ml. phloem extract; (5) 1ml. foliage extract plus 1 ml. phloem extract; (6) 500 mg. Difco yeast extract; (7) 500 mg. Difco casamino acids; (8) 500 mg. casamino acids plus 1 ml. foliage extract; (9) 500 mg. casamino acids plus 1 ml. phloem extract. Quadruplicate Petri dishes containing these nine media were inoculated with P. lateralis and incubated at 20° C. for 20 days. The final appearance of the morphology of the resulting colonies was recorded photographically.

The most satisfactory method for the production of sporangia and zoospores required initial growth in pea broth. Pea broth was also used as the medium when growing the fungus for soil inoculations. Pea broth was prepared by autoclaving 150 g. of dried split peas in one liter of tap water for three minutes at 15 pounds pressure. The

clear pea broth was immediately decanted, placed in Erlenmeyer flasks and autoclaved for 10 minutes at 15 pounds pressure. The sterile pea broth was then poured into sterile Petri dishes (15 ml. per dish) and inoculated. The inoculum consisted of many small hyphal fragments. After four days incubation at 20° C. many small mycelial colonies developed. When the pea broth medium was replaced by distilled water numerous sporangia developed at the periphery of the colonies within 24 hours. These colonies grew attached to the bottom of the Petri dish and remained in place during the removal of the pea broth and subsequent distilled water rinses. This method of obtaining sporangia and zoospores was both rapid and efficient.

The best culture medium for the production of chlamydo spores consisted of dilute commercial V-8 juice (containing juices of tomatoes, carrots, celery, beets, parsley, lettuce, spinach and watercress) with dextrose added. To 100 ml. of V-8 juice, which had been strained through cheesecloth, was added 15 g. of dextrose and enough tap water to bring the volume to one liter. This V-8 dextrose broth was then autoclaved for 10 minutes at 15 pounds pressure. It was subsequently poured and inoculated as described above for pea broth. After seven days incubation at 20° C. chlamydo spores were abundant at the periphery of the colonies.

The search for a medium favoring oospore production included media prepared from the following sources: commercial Pablum (cereal mixture); carrots; peas; lentils; commercial V-8 juice; potatoes; alfalfa; and ten mixed frozen vegetables. These media were prepared by placing the designated amount of plant material (50 g. Pablum; 200 g. fresh carrots; 100 g. dried peas; 100 g. dried lentils; 100 ml. V-8 juice; 200 g. fresh potatoes; 50 g. dried alfalfa; and 200 g. frozen mixed vegetables) in one liter of water and adding 15 g. of agar. After autoclaving for 10 minutes at 15 pounds pressure the media were poured into sterile Petri dishes. Hansen and Snyder's method (19, p.370) of sterilizing plant material with propylene oxide was used for the sterilization of host tissue. Port Orford cedar foliage, bark, roots and seeds were dried at 80° C. for 12 hours, placed in a propylene oxide atmosphere for 24 hours and then added to sterile melted water agar (12 g. agar per liter tap water). Media were prepared employing these cedar plant parts separately and in all combinations. A total of 5 g. of dried plant material was added to 100 ml. of sterile water agar. A decoction of cedar foliage was also prepared by steaming 200 g. of fresh cedar foliage in 500 ml. tap water at 95° C. for one hour, decanting the liquid, adding 15 g. of glucose, 15 g. of agar and enough water to bring the volume to one liter.

INOCULATION

The root dip method of inoculation was used in pathogenicity trials in the greenhouse. Inoculum was prepared by combining, in distilled water, mycelial mats grown in pea broth for seven days with mycelia grown on potato dextrose agar. The mixture was macerated slightly in a Waring Blendor. The roots of three year old Port Orford cedar seedlings growing in six inch pots were gently freed from the loose soil and dipped immediately into the inoculum suspension. The seedlings were then carefully repotted and watered. The checks were treated in the same manner except that a sterile agar suspension was used as the dip. No noticeable transplanting injury occurred. The plants were kept warm (18° - 23° C.) and moist during the incubation period.

In the field inoculations concerned with the root disease phase of this study three different methods were used but only one of the methods was used to inoculate each point. The methods employed were:

1. Mycelia grown in pea broth for seven days at room temperature were washed and placed in dilute soil filtrates. The mycelial mats were broken up by thorough mixing with a stirring rod. A 300 ml. aliquot of the mycelial suspension

was poured into a small hole (5 inches deep) at each point so inoculated. Check points received the same amount of filtrate without the pathogen.

2. Main root inoculations were made on large established trees by cutting through the bark and inserting a small piece of mycelium growing on potato dextrose agar into the cambial region of the root. The wounds were closed with adhesive tape. Controls received the same treatment except that sterile potato dextrose agar was substituted for the mycelial culture.

3. Equal amounts (800 mls.) of infested soil in which Port Orford cedar seedlings had been recently killed by Phytophthora root disease were placed in the soil, watered well and covered lightly. Check points were treated in the same manner with non-infested soil.

Inoculations employed in studying the aerial phase of the disease included both stem and foliage inoculations. Stem inoculations were made by cutting through the bark and inserting small pieces of mycelium growing on potato dextrose agar into the cambial region of the stem. The wounds were closed with adhesive tape.

In order to study the rate of increase of foliar infections and the pattern of development of the aerial phase, inoculations of foliage were made at single points. Tips of foliage were moistened with distilled water followed by

several drops of an active zoospore suspension. The inoculations were made six feet above the soil on cedar trees with foliar crowns extending to the ground.

DISSEMINATION

The rate of development of root infections was followed in the naturally infected areas in Mingus Park. Maps were drawn to include infected trees and the surrounding healthy trees. The maps were constructed with the aid of a staff compass, an engineer's tape and a per cent abney. The plots were divided into blocks 10 feet square by string transects and the exact position of the trees and seedlings falling in each block were recorded. Six plots with a total area of four acres and including 952 Port Orford cedar trees and 371 other conifers were mapped in this manner. All Port Orford cedars in these plots were numbered with aluminum tags so that observations on individual trees could be accurately and readily recorded. A tree was considered diseased when symptoms of the fungus were detected in the inner bark at the base of the tree. The number of diseased trees as well as the foliar symptoms were followed from September 1954 until September 1956.

The horizontal and vertical distribution of the pathogen in the soil of infested areas was recorded using

the soil isolation technique described above. To determine the advance of the fungus in the surface layers of the soil, healthy, freshly cut cedar twigs were placed in the soil at six foot intervals in a 30 foot grid. The grid was located in a diseased area to include both healthy and infected trees. Infection of the twigs after four weeks incubation indicated the presence of the pathogen in the soil. Twigs not infected by P. lateralis remained healthy and normal after a four week period.

The vertical distribution of the pathogen in the soil was studied by sampling the soil at six inch intervals to a depth of 24 inches. Four replicate samples taken at each depth were placed in separate Petri dishes and covered with distilled water. Healthy, freshly cut cedar twigs were placed in this wet soil mixture and incubated seven days at 20° C. Infected twigs indicated the presence of the pathogen in the soil sample.

The spread of aerial infections through the foliage was recorded by periodic photographs. The rate of development of stem cankers was recorded by measuring the canker margins at monthly intervals. Such measurements were made on stems varying from one to four inches in diameter at the canker locus.

MORPHOLOGY OF PHYTOPHTHORA LATERALIS

The description of the vegetative mycelium and asexual reproductive stages of Phytophthora lateralis given by Tucker and Milbrath (56, p.97) is very accurate. However, certain additional observations on the vegetative and asexual stages, together with the description of the newly discovered sexual stage are of interest.

MYCELIUM

The diameter of young and vigorously growing hyphae is 5 μ . The hyphae are continuous and smooth when young but often become septate and gnarled with age. The morphology of the vegetative colony depends upon the substrate. The nitrogen level of the medium seems to exert the greatest influence. Thin, spreading colonies develop on media with low nitrogen levels, whereas dense, compact colonies develop on high nitrogen media (Figure 2). All isolates (about 120) of P. lateralis examined were morphologically indistinguishable on potato dextrose agar, as were 30 single zoospore isolates.

SPORANGIA

Sporangia typical of this species are shown in Figure 3. Simple and forked sporangiophores are often observed bearing sympodial as well as the proliferating

sporangial types. Unlike many other species of Phytophthora the mature sporangia of P. lateralis usually remain firmly attached to the sporangiophores. The sporangia germinate directly by germ tube formation and indirectly by zoospores. Zoospores are always fully developed within the sporangium, and each sporangium gives rise to 25 to 40 zoospores.

The original workers (56, p.99) on this species did not observe sporangia on agar substrates but in this investigation sporangia were frequently seen on many agar substrates of low nutritional value (see Sporulation). Aerial sporangia do not occur even though aerial hyphae are common on media favorable for sporangial production.

OOSPORES

Oospores are observed most frequently on agar containing decoctions of Port Orford cedar foliage (see Sporulation). A single oospore fills the oogonial cavity of each oogone (Figure 4). The granular protoplasm of the oospore contains one or more reserve globules. The oospores are spherical with a smooth wall 6 μ . thick. The diameters of 100 oospores formed on cedar foliage agar varied between 34.5 μ and 45.3 μ with an average of 40 μ . Oospore color depends upon the medium. In alfalfa agar oospores are amber, whereas in cedar foliage agar they are

cinnamon brown. Brown intramatrical oospores were observed in naturally infected cedar foliage and cortex of young twigs. Colonies resulting from single zoospore isolates of P. lateralis produced oospores, thus demonstrating the homothallic nature of this fungus.

The oogonia are smooth, spherical and terminal and the antheridia are paragynous. The origin of the antheridium is proximal to the oogonium, and only one antheridium forms per oogonium.

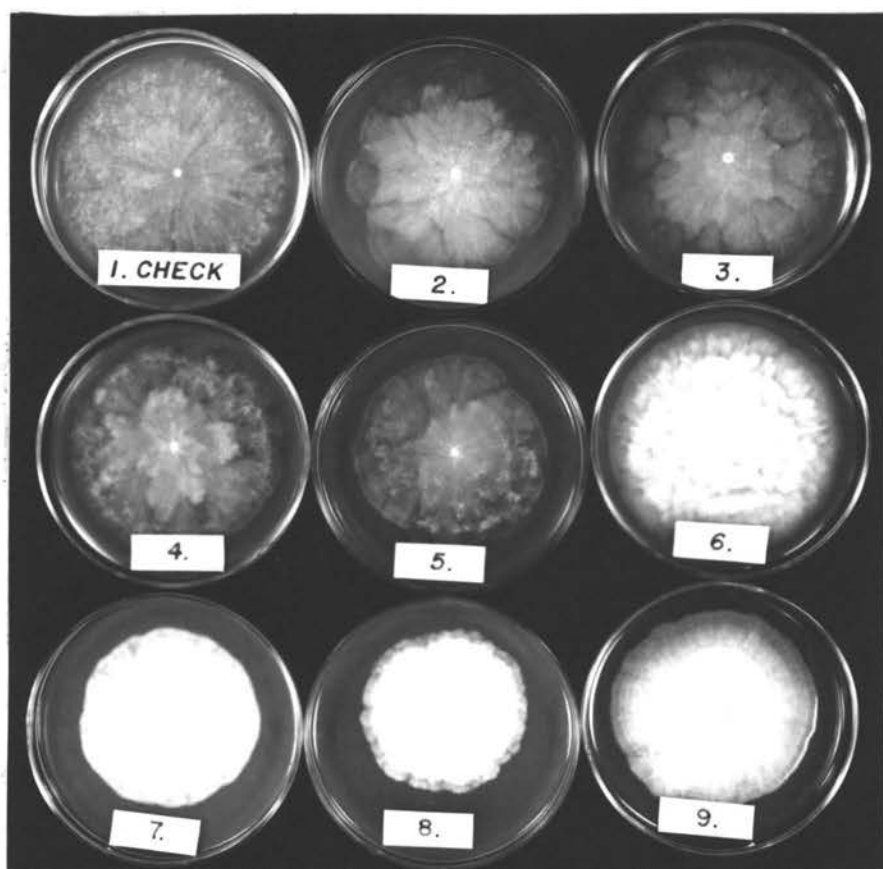


Figure 2. The morphology of vegetative colonies of Phytophthora lateralis on nine agar media after 20 days incubation at 15° C.

Each Petri dish in Figure 2 contains 20 ml. V-8 dextrose agar in addition to the following plant extracts and growth-promoting substances:

1. Check, 0.4 m. ethanol
2. 0.2 ml. cedar foliage extract
3. 0.2 ml. cedar foliage extract plus 2 ug. indole butyric acid
4. 0.02 ml. cedar foliage extract plus 0.02 ml. cedar phloem extract
5. 0.2 ml. cedar foliage extract plus 0.2 ml. cedar phloem extract
6. 100 mg. yeast extract
7. 100 mg. casamino acids
8. 100 mg. casamino acids plus 0.2 ml. cedar foliage extract
9. 100 mg. casamino acids plus 0.2 ml. cedar phloem extract

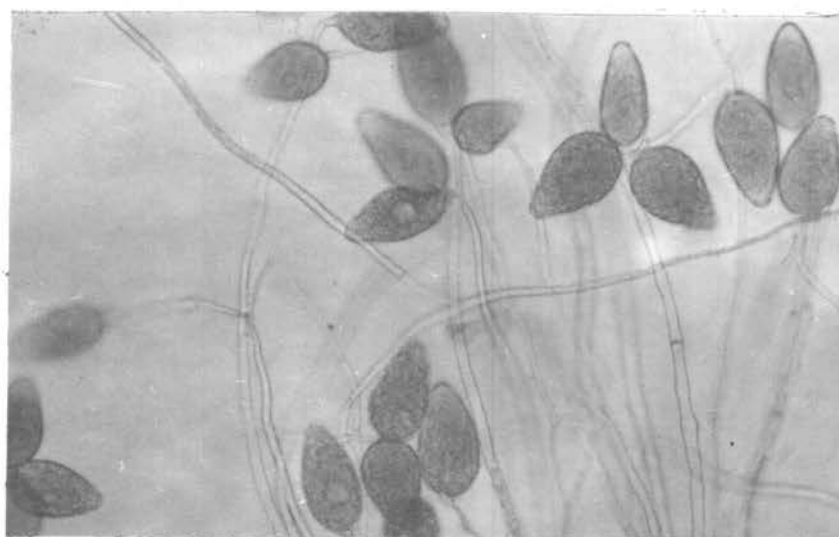


Figure 3. Sporangia of Phytophthora lateralis

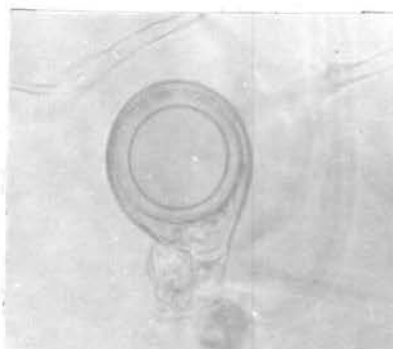


Figure 4. Oospore of Phytophthora lateralis

PHYSIOLOGY OF PHYTOPHTHORA LATERALIS

Parasitism is concerned with the interaction of two biological entities. At the onset of this study the gross physiology of the host was largely known but little was known of the physiology of the pathogen. After the airborne stage was discovered and a resistant overwintering stage suspected, the physiological characteristics of this pathogen took on new significance. After the growth, sporulation and germination characteristics of a pathogen are known the pathologist is better able to understand the development of the disease in the field.

GROWTH IN CULTURE

Much general information and orientation can be obtained from the study of a fungus on various substrates. Results from this simple technique often provide guidance for subsequent detailed and specialized studies. This procedure is one of the best means of studying the organism in the laboratory as a biological entity, and it is a very useful method for observing fungus behavior in response to the environment.

Growth is a process which takes place in time and is best studied by making many growth measurements during incubation. Many of the potentialities of the fungus can be

discovered only by prolonged observation. Both the rate and the amount of mycelium produced during incubation are of interest.

1. Growth on Plant Media

Growth studies are frequently made using only one type of substrate and reported for only one incubation period. Such studies are incomplete and fail to demonstrate the physiological capacity of the organism to grow under differing conditions.

The growth rates of fungi on agar media are extremely variable. Phytophthora lateralis has a low growth rate compared with many Phytophthora species. The possibility of increasing this growth rate by altering the substrate and incubation temperature would be of interest.

Growth on six types of plant media was compared with the growth on standard Difco potato dextrose agar. The six plant materials chosen were lentils, dried split peas, wheat germ, fresh potato tubers, fresh Port Orford cedar foliage and commercial V-8 juice. These media were prepared by adding the designated amount of plant material (100 g. lentils, 100 g. dried peas, 50 g. wheat germ, 200 g. potato tuber, 200 g. Port Orford cedar foliage, 125 ml. V-8 juice) to one liter of tap water and heating in an Arnold steamer at 95° C. for one hour. The mixtures were then

filtered through cheesecloth and the filtrate restored to one liter. To each liter of these plant decoctions were added 15 g. of glucose and 15 g. of agar. Difco potato dextrose agar was prepared according to the Difco directions. After autoclaving for 10 minutes at 15 pounds pressure the media were dispensed into sterile Petri dishes (15 ml. per dish). All media were prepared at the same time and all growth studies were carried out simultaneously.

The inoculum used in this growth study was taken from the periphery of actively growing seven day old potato dextrose agar cultures of Phytophthora lateralis. Agar discs, three ml. in diameter, were transferred to the center of the Petri dishes. Five replicate Petri dishes were incubated at each of the following constant temperatures: 5°, 10°, 15°, 20°, 25° C. Diameters of the colonies were recorded every other day for 20 days and final dry weights of the colonies were determined. The procedure for drying the colonies involved removal of the mycelial mats from the substrate after the agar had been melted, followed by two washes in hot water. The mycelial mats were dried at 80° C. and weighed.

Growth rates were constant under all conditions except at 25° C. Most colonies did not grow at 25° C. but the few that did grow enlarged slowly with irregular rates which showed no relation to the media.

In general, growth rates increased linearly as the temperature rose from 5° to 20° C. (Figure 5). However, the growth on lentil agar and V-8 agar was nearly the same at 15° and 20° C. Growth based on the final dry weight rises sharply at temperatures above 10° C. (Figure 6). Thus, both the rate and amount of mycelium produced can be varied by altering the substrate and/or the incubation temperature. Under all conditions tested P. lateralis would be considered a slow-growing fungus.

TABLE 1. THE EFFECT OF TEMPERATURE ON THE GROWTH RATE
AND THE FINAL DRY WEIGHT OF PHYTOPHTHORA LATERALIS ON PLANT DECOCTION MEDIA

Substrate	Initial pH	Growth at Four Incubation Temperatures							
		5°		10°		15°		20° C.	
		mm. ^a	mg. ^b	mm.	mg.	mm.	mg.	mm.	mg.
Peas	6.0	0.4	4	1.5	15	3.1	55	4.1	98
Wheat Germ	6.1	0.4	3	1.3	14	2.6	21	3.8	63
Potato	5.9	0.2	2	0.5	6	1.5	12	3.5	81
Lentils	6.1	0.4	4	1.6	15	2.7	68	3.0	123
Difco PDA	5.7	0.1	1	0.8	3	2.2	12	3.0	25
V-8 Juice	5.1	0.4	3	1.2	6	2.5	22	2.5	46
Cedar Foliage	5.2	0.2	1	0.8	3	1.7	7	2.3	10

mm.^a Average diameter increase per day (millimeters).
mg.^b Average dry weights after 20 days (milligrams).

Figure 5. Effect of temperature on the growth rate of Phytophthora lateralis on seven plant media.

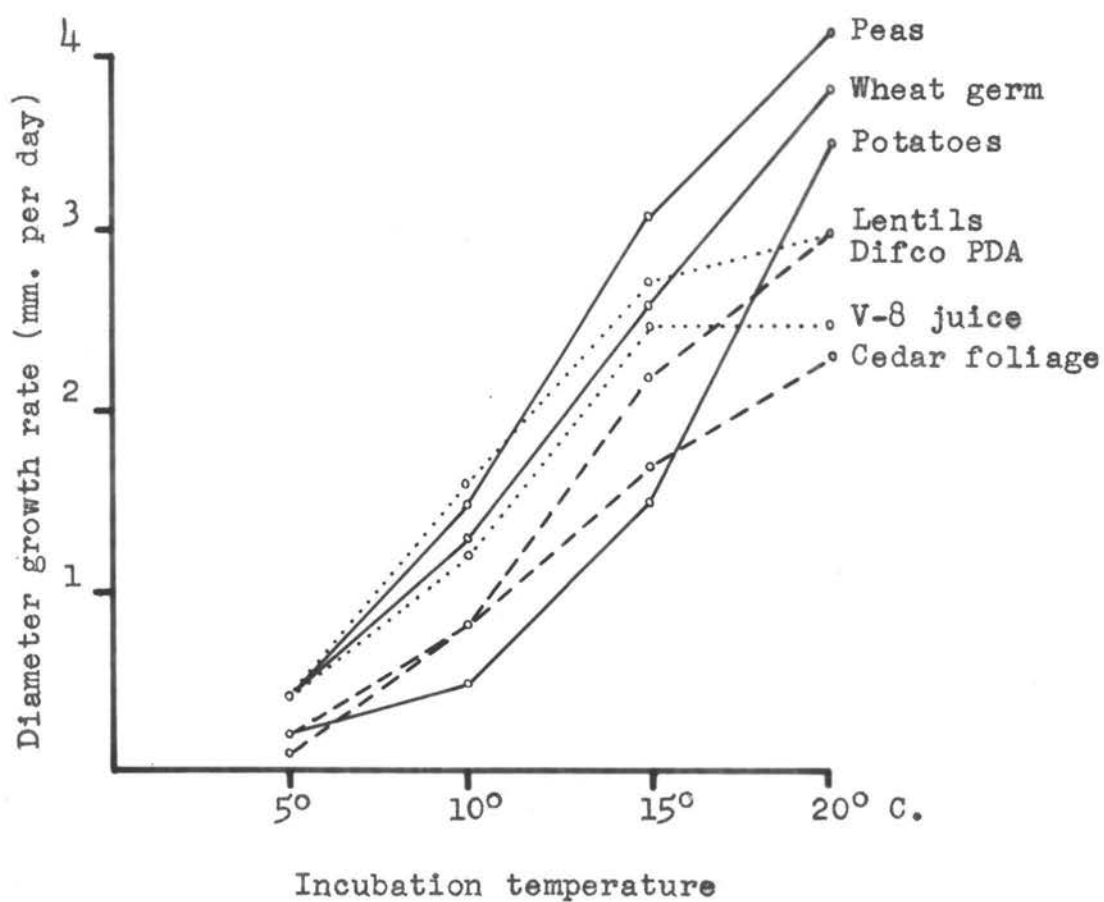
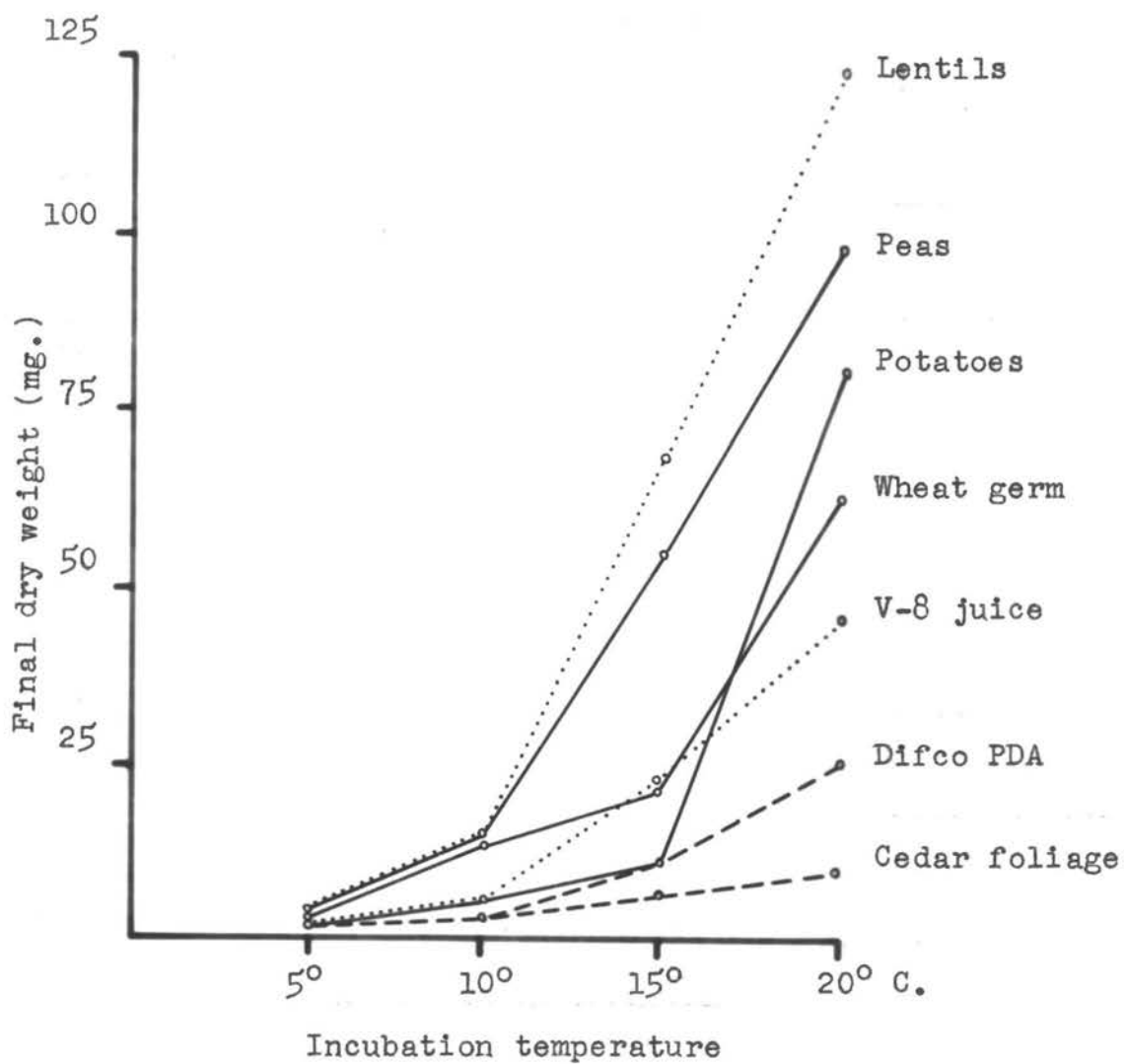


Figure 6. Effect of temperature on the dry weight of vegetative colonies of Phytophthora lateralis after 20 days incubation.



2. Growth in Chemically Defined Media

Knowledge of the exact nutritional requirements of a pathogen enables preparation of selective media having many applications in pathological research. Also the growth of an organism on chemically defined media is frequently required in detailed physiological and biochemical studies.

Phytophthora species were not successfully cultivated on synthetic media until thiamine was proved to be essential to their nutrition (42, p.274). Several synthetic solutions have subsequently been described (59, p.232; 30, p. 752) and it is interesting to note that thiamine appears to be the only vitamin required by Phytophthora species. Attempts to grow P. lateralis in these previously reported chemically defined media were unsuccessful. However, the following synthetic solution which supported growth of P. lateralis was developed in this study: 10 millimoles KH_2PO_4 , 0.05 mM. CaSO_4 , 2 mM. MgSO_4 , 0.12 mg. Fe (as the ethylenediaminetetraacetic acid salt), 1.0 ml. Hoagland's minor element solution (21, p.31), 4.97 g. dl-asparagine, 40 g. glucose, 1.0 mg. thiamine and distilled water to make one liter. The pH was adjusted to 6.0 with 1N NaOH and 1N HCl.

Lopatecki and Newton (30, p.753) reported that when glucose was autoclaved with the other nutrient constituents, growth was retarded. Thus, it seemed advisable to try

several methods of sterilization: (1) all constituents (salts, amino acid, sugar and vitamin) were mixed together and autoclaved, (2) salts, amino acid and vitamin were autoclaved separately from the sugar and mixed together when cold, (3) salts autoclaved; amino acid, sugar and vitamin filtered through a sterile Seitz asbestos filter and mixed with the salts when cold, (4) salts autoclaved; amino acid, sugar and vitamin dried at 80° C. for 24 hours and mixed with the salts when cold.

The fungus was cultured in 125 ml. Erlenmeyer flasks containing 20 ml. nutrient solution and incubated for 10 days at 20° C. Only chemically clean glassware was used. The final mycelial mats were rinsed twice in distilled water, dried at 80° C., and weighed. The mean weight of mats of quadruplicate cultures was recorded as the measure of growth. Weight variation between replicates was very slight. Average mycelial weights of colonies grown for 10 days in the same synthetic solution when sterilized by the four methods described above were as follows: (1) 37 mg., (2) 46 mg., (3) 11 mg., (4) 63 mg. Media sterilized by method four supported the best growth and was used in subsequent experiments on synthetic solutions.

Few attempts were made to alter the composition of the effective synthetic solution. It was found, however,

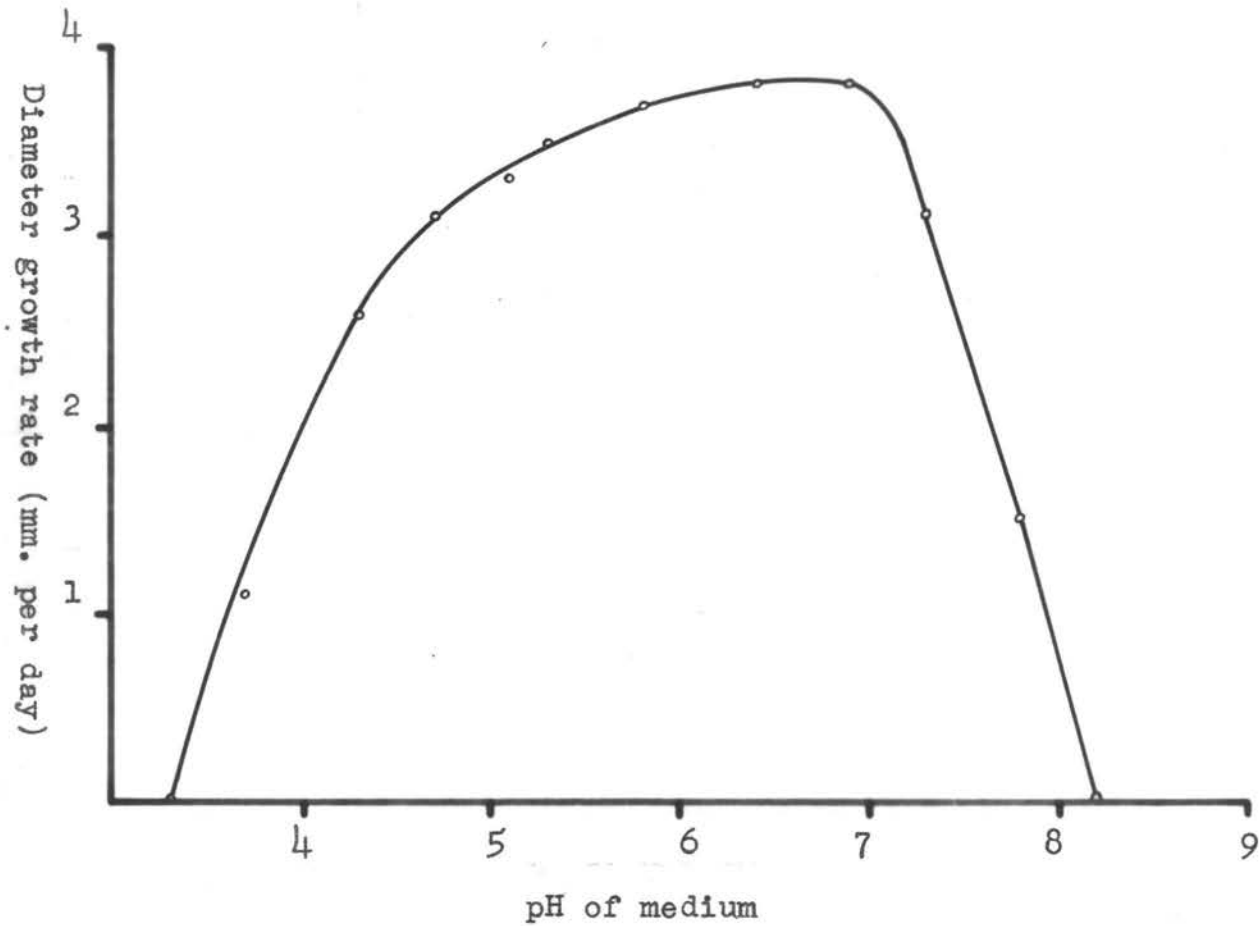
that P. lateralis grew equally well when l-glutamic acid was substituted for dl-asparagine (at the same nitrogen level) and/or sucrose was substituted for glucose (at the same carbon level). Growth did not occur when organic nitrogen was replaced by the inorganic nitrogen salts, NaNO_3 or NH_4Cl , at the same nitrogen level. Growth in the synthetic solution containing organic nitrogen was about 75 per cent as good as the growth in common pea broth (average dry weights: 65 mg. compared to 89 mg.). Since this fungus does not grow as well in the chemically defined medium as in the natural pea broth it would seem that the constituents of the synthetic medium were not properly balanced or that specific metabolites, not present in the synthetic medium, are capable of increasing the growth of this organism.

3. The Effect of pH on Growth Rate

Knowledge of the effect of hydrogen ion concentration on the growth of a soil pathogen is an important aid in predicting the capacity of the pathogen to grow at different soil pH values. The hydrogen-ion concentrations permitting growth were determined by measuring the growth rate of P. lateralis on a series of phosphate buffered, potato dextrose agar media.

Media of different pH values were prepared by combining appropriate quantities of phosphates (H_3PO_4 , NaH_2PO_4 , Na_2HPO_4 , Na_3PO_4) with potato dextrose agar. The quantity of phosphate at each pH level was 33 millimoles per liter. Four replicate Petri dishes at each level of hydrogen-ion concentration were inoculated as in studies described above and incubated at 20° C. Colony diameters were measured every other day for 10 days. Initial and final pH values of the media were almost identical; the greatest pH shift being 0.15 units toward the acid side at pH 8.3. The rate of increase of colony diameter at each pH level was constant. Growth rate increased rapidly (Figure 7) as pH increased from 3.7 to 5.3, a maximum was reached between pH 5.8 and 6.9, and the growth rate decreased rapidly between pH 7 and 8. Growth did not occur at pH 3.3 or pH 8.2 which appear to be near the acidic and alkaline limits of growth for this fungus.

Figure 7. Effect of pH on the growth rate of *Phytophthora lateralis* in phosphate buffered potato dextrose agar at 20° C.



SPORULATION IN CULTURE

The life of an individual fungus is often relatively short and the continuance of the strain may depend upon the production of asexual or sexual spores. Spore production is extremely important in the build-up of inoculum required to bring about an epiphytotic disease. Hence, information on the events and conditions which control the production of fungus spores is very valuable. Phytophthora lateralis produces sporangia, zoospores, chlamydospores and oospores. The results presented in this section are primarily concerned with the effect of substrate, temperature and moisture on sporulation. Sporulation within and from the surface of host tissue is discussed under the section on epiphytology.

1. Formation of Sporangia

Tests were made of the capacity of many liquid solutions and agar media to support sporangial formation. The most notable feature associated with the formation of sporangia in cultures of P. lateralis was the low nutritional level of the medium. Good vegetative growth and sporangial formation were never associated. The induction of sporangia usually occurred only when vegetative growth ceased or was reduced to a minimum. Moist conditions were

essential for the production of sporangia and the presence of excess water increased their abundance. Sporangial formation occurred equally well under conditions of light or darkness.

Sporangia are produced under many conditions both in liquid and on agar media. Sporangia, numerous enough for identification purposes, are easily obtained on many agar media low in nutrients. For example, one liter of water agar containing 10 ml. commercial V-8 juice, or 25 g. of fresh cedar foliage, or 25 g. of corn meal or 20 ml. whole milk, will induce sporangial formation. When, in the preparation of these minimal media, Hoagland's mineral solution number 1 (21, p.31) was used in place of distilled water, the production of sporangia was greatly increased.

Initial culture in pea broth proved to be the greatest stimulus to sporangial development. Sporangia are not formed in pea broth, but when mycelial colonies are washed and covered with distilled water or Hoagland's mineral solution number 1 after four days cultivation in pea broth at room temperature, abundant sporangia develop. The fruiting response is prompt on small mycelial colonies. On large mycelial colonies, well supplied with reserve nutrients, sporangial production is rare and is limited to the periphery of the colony.

The effect of temperature on the rate of sporangial formation was studied using cultures from pea broth. Five replicate Petri dishes containing small mycelial colonies in non-sterile distilled water were maintained at constant temperatures. Periodic observations were made during the initial 6 to 72 hours of incubation. Sporangia were produced most abundantly at 15° and 20° C.; maximum numbers formed after 24 hours (Table 2). Longer periods were required at temperatures lower than 15° C. This experiment was repeated using a final incubation in non-sterile, five per cent soil filtrate instead of non-sterile distilled water. The results were identical to those presented in Table 2. It is noted that Torgeson (49, p.53) reported sporangial production of *P. lateralis* only at 20° C. The study presented here demonstrates that sporangia can be formed from 5° to 25° C.

TABLE 2. THE EFFECT OF TEMPERATURE ON THE RATE OF SPORANGIAL FORMATION IN NON-STERILE DISTILLED WATER.

Incubation time (hours)	Relative abundance of sporangia at five temperatures				
	5°	10°	15°	20°	25° C.
6	0 ^a	0	1	2	0
12	0	0	3	4	1
24	0	2	4	4	1
48	2	2	4	3	1
72	2	3	3	2	1

^a 0 indicates no sporangial production, 1 indicates rare, 2 common, 3 numerous, and 4 abundant.

2. Formation of Chlamydo-spores

Production of chlamydo-spores was closely associated with vegetative growth of P. lateralis. Abundant chlamydo-spore production was dependent upon high food reserves in the medium and incubation temperatures of 15° to 25° C. Chlamydo-spores were produced on practically all types of media which supported vegetative growth but were most numerous in V-8 broth (100 ml. V-8 juice, 15 g. glucose, 900 ml. distilled water). Incubation for five to seven days in V-8 broth at 20° C. yielded abundant chlamydo-spores at the periphery of the mycelial colonies.

Chlamydo-spores which formed in the cedar foliage agar (see Growth in Culture) were visible to the unaided eye due to the intense cinnamon brown pigments which accumulated in the cell walls of the spores and to a lesser extent in their cytoplasm. Pigmentation was greatest at 15° C.

3. Formation of Oospores

Prior to this study, oospores of Phytophthora lateralis had not been described. Many species of Phytophthora are reported as homothallic and it is well known that oospores are produced only when the fungi are grown upon the proper nutrient media.

Some cultures of P. lateralis were incubated at constant temperatures, others at varying temperatures from 10° to 20° C. on many types of media containing incorporated plant materials or plant decoctions (see Methods). Observations for oospores were made at two week intervals over a period of two to three months. Growth was good on all media but oospores were produced only on the media containing cedar foliage or decoctions of cedar foliage, and on the medium containing alfalfa tissues. When the amount of cedar foliage per liter was doubled or halved, oospores were not produced.

Single zoospore isolates of Phytophthora lateralis also produced oospores on cedar foliage agar, thus demonstrating the homothallic nature of this organism. P. lateralis isolates from 10 different points in Oregon were paired in all possible combinations on cedar foliage agar. Oospores were produced by all isolates but their numbers were not increased as a result of the pairing.

4. Comparative Sporulation of Ten Isolates of P. lateralis

It has been found that Phytophthora species often show variation in the abundance of spores produced by different isolates (29, p.402). Ten isolates of P. lateralis from various parts of Oregon were chosen for a comparative

study of their sporulation capacities. The pea broth method was used to check sporangial formation. Cultivation in V-8 broth was used for chlamyospore formation. Oospore production was compared on cedar foliage agar. Six replicate cultures of each isolate were observed for each treatment. Sporangia were produced abundantly by all isolates except isolate 2, which produced few sporangia (Table 3). The number of chlamyospores varied from few in cultures of isolates 5 and 8, to many in cultures of isolates 2 and 3. All isolates produced oospores equally well but few in number. Thus, the isolates varied greatly in the number of chlamyospores which they were capable of producing.

TABLE 3. COMPARATIVE SPORULATION OF TEN ISOLATES OF PHYTOPHTHORA LATERALIS ON MEDIA MOST FAVORING SPORULATION.

Isolate Number	Source of Isolate	Relative abundance of spores formed		
		Sporangia (Pea broth)	Chlamydo spores (V-8 broth)	Oospores (Foliage agar)
1	Portland, Ore.	4 ^{a)}	2	2
2	Salem, Ore.	2	4	2
3	Corvallis, Ore.	4	4	2
4	Hauser, Ore.	4	2	2
5	Coos Bay, Ore.	4	1	2
6	Charleston, Ore.	4	2	2
7	Bandon, Ore.	4	2	2
8	North Langlois, Ore.	4	1	2
9	Langlois, Ore.	4	3	2
10	Harbor, Ore.	4	3	2

a) 1 indicates rare spore production, 2 indicates common, 3 numerous, and 4 abundant.

GERMINATION IN CULTURE

All fungus diseases must begin with infection and since the germination of the spore is often the precursor of infection, knowledge of the conditions which favor or inhibit spore germination is of great practical importance. Spore germination represents a change from an inactive to an active phase in the life cycle of Phytophthora, and since it involves the first stages of growth many of the factors which influence vegetative growth also affect spore germination.

1. Germination of Sporangia

Sporangia of Phytophthora lateralis are capable of germinating by two different methods; directly by the formation of a germ tube, and indirectly by the formation of zoospores. Direct germination of sporangia was most frequently associated with lack of free water and/or incubation temperatures above 20° C. Sporangia in agar media usually germinated directly due to the lack of water. When water was present and the temperature was 20° C. or lower the sporangia germinated indirectly by formation of actively swimming zoospores. In addition to its influence on the method of germination, temperature also affects the time required for germination and the per cent of germination.

Mature sporangia of P. lateralis remain attached to the sporangiophores at the periphery of the colonies and new sporangia are continually being formed. Thus, quantitative data on the time required for germination and the per cent germination was not obtained. In general, however, at 15° to 20° C. indirect germination of mature sporangia was virtually completed within four hours. Indirect germination occurred at all temperatures from 5° to 25° C., with maximum germination (about 90%) at 15° C., but direct germination in aqueous solutions occurred only at 25° C.

2. Germination of Zoospores

After a period of motility, zoospores of P. lateralis come to rest and round up. Germination then occurs by means of a germ tube. Since zoospores are probably the forerunners of most foliage and root infections, information on their germination characteristics is of interest. The effect of temperature on germination and on germ tube elongation was determined at five temperatures by employing the standard glass slide technique (1, p.627-629). Actively swarming zoospores, which had been formed at 15° C., were suspended in non-sterile distilled water on glass slides. Quadruplicate slides were maintained at each temperature. In studies of percentage germination, 100

zoospores were observed on each slide, and in studies of germ tube elongation, 25 germ tubes were measured on each slide.

The rate of germination of zoospores increased with a rise in temperature up to 20° C., then decreased (Table 4). The number of zoospores germinating after 12 hours was essentially the same at all temperatures between 5° and 20° C. Germination was slightly less at 25° C. The growth rate of germ tubes increased with temperature up to 25° C. (Table 5). This growth pattern parallels the response of vegetative growth to temperature except that very little or no mycelial growth occurs at 25° C.

TABLE 4. THE EFFECT OF TEMPERATURE ON ZOOSPORE GERMINATION IN DISTILLED WATER.

Time (hours)	Per cent germination of zoospores at five temperatures				
	5°	10°	15°	20°	25° C.
2	1	12	15	31	14
4	21	26	38	56	32
8	46	51	57	69	46
12	83	83	80	86	71

TABLE 5. THE EFFECT OF TEMPERATURE
ON GERM TUBE ELONGATION IN DISTILLED WATER.

Time (hours)	Average germ tube length (microns) at five temperatures				
	5°	10°	15°	20°	25° C.
2	9	12	18	26	31
4	15	27	50	66	114
8	31	78	104	122	160

3. Germination of Chlamydo spores and Oospores

Chlamydo spores were rarely seen to germinate. Germination was observed only after a dormant period of a week or more at 3° C. Usually more than one germ tube arose from a germinating chlamydo spore. The germ tubes continued to elongate giving rise to a mycelial colony. Oospores were also rarely seen to germinate and again germination occurred only after a prolonged period (several months) at low temperature. Germinating oospores each produced a single germ tube which in turn gave rise to a single sporangium. The germination of the sporangia produced by germinating oospores was not observed.

THE PATHOLOGY OF PHYTOPHTHORA LATERALIS
ON NATIVE CHAMAECYPARIS LAWSONIANA

PATHOGENICITY

Fungus disease studies frequently begin by proving the pathogenicity of the fungus on the host concerned. This traditional approach has been followed here. About 120 isolates² of the pathogen, Phytophthora lateralis, were isolated from diseased Port Orford cedar trees in various parts of Oregon in the initial phase of this study. All isolates appeared to be morphologically alike when cultured on potato dextrose agar. From this group ten isolates were chosen representing the geographical distribution of the fungus in the state. Three were from the Willamette Valley and seven were from the native Chamaecyparis range. The comparative pathogenicity of these isolates was studied on cedar roots and foliage.

Each isolate was used to inoculate (see Methods) five, three year old Port Orford cedar seedlings. Ten control plants were similarly treated in the absence of the pathogen. The plants were kept warm (18° to 23° C.) and well watered during the incubation period. The foliar

²Twenty isolates were received from Mr. Walter J. Tolmsoff of the Plant Disease Clinic at Oregon State College.

symptoms that accompany root damage began to appear on some plants within one month. Ninety per cent of the inoculated plants had severely infected roots when examined six weeks after inoculation. The roots of the remaining 10 per cent of the plants were moderately infected. Most of the root systems were completely invaded and the pathogen had advanced above the soil line on the main stem. Small sections of the roots or main stems including the transition zone between healthy and diseased tissue were excised, placed in distilled water and incubated at 20° C. External hyphae and sporangiophores bearing sporangia were apparent on all the tissue sections after two days. These sporangia confirmed the presence of the pathogen in the root systems of all inoculated plants (Table 6). The roots of all control plants were healthy.

The pathogenicity of the same 10 isolates to Port Orford cedar foliage was studied by placing the tips of 10 healthy, detached foliar twigs in a non-sterile active zoospore suspension of each isolate. The twigs remained in the zoospore suspension for eight hours and were then transferred to humidity chambers at 20° C. where their petioles were placed in Hoagland's mineral solution (21, p.31). Ten control twigs were similarly treated in the absence of the pathogen. Symptoms of foliar infection began to appear after three days. After six days all 10 twigs inoculated

with each isolate showed infection (Table 6). The presence of the pathogen in the host tissue was confirmed by covering the twigs with distilled water and incubating at 20° C. External hyphae and sporangiophores bearing sporangia were observed on all twigs after two days. All control twigs remained healthy and turgid.

TABLE 6. PATHOGENICITY OF *P. LATERALIS* ISOLATES
ON PORT ORFORD CEDAR ROOTS AND FOLIAGE.

<u>Isolate number</u>	<u>Source of isolate</u>	<u>Number of Plants</u>		<u>Number of Twigs</u>	
		<u>inoculated</u>	<u>infected</u>	<u>inoculated</u>	<u>infected</u>
1	Portland, Ore.	5	5	10	10
2	Salem, Ore.	5	5	10	10
3	Corvallis, Ore.	5	5	10	10
4	Hauser, Ore.	5	5	10	10
5	Coos Bay, Ore.	5	5	10	10
6	Charleston, Ore.	5	5	10	10
7	Bandon, Ore.	5	5	10	10
8	N. Langlois, Ore.	5	5	10	10
9	Langlois, Ore.	5	5	10	10
10	Harbor, Ore.	5	5	10	10

FIELD SYMPTOMS

1. Symptoms of Root Disease

Symptoms of root infection on the individual trees in a Port Orford cedar stand resemble those described for ornamentals (38, p.1; 46, p.6; 51, p.5; 56, p.94-95). However, conditions of the natural stand modify the usual symptom pattern and the rate and manner of spread through the stand may be distinctive (44, p.296).

The most characteristic feature of this root disease in cedar is the uniform manner in which the entire foliar crown is affected. This uniform disturbance reflects the drastic malfunctions occurring in the root system. To one familiar with the healthy, erect, turgid foliage of Port Orford cedar the earliest symptom expression of root infection is a loss of turgidity, a slight wilting. The erect nature of the foliage is lost; the younger twigs become more pendant. Color changes, which reflect more drastic physiological disturbances, soon follow. The foliar color changes do not appear until the base of the tree has been girdled by the pathogen.

The early color changes of the foliage are often difficult to detect because they are within the broad, normal color range found among healthy native trees. In general, however, the green pigments are gradually

destroyed and the foliage takes on a bronze cast. The bronze shade gradually changes to a typical light brown color of the dead foliage. As these color changes occur the foliage dries, but trees killed by this disease do not defoliate until one to two years after their death.

The rate of development of symptoms is related to water availability. In the warm, dry summer months the foliar crowns of root diseased trees often receive less water than they require because of nonfunctional roots damaged by the pathogen. In such cases the trees decline and die within a month of the onset of symptoms and the foliage assumes a bright rust-brown color which later fades to the typical light brown of dead foliage. Trees which do not experience a severe water stress tend to decline slowly over a period of a year or more and never take on the bright rust-brown color of rapidly killed trees. Green foliar crowns, showing only a slight degree of wilt, have been observed occasionally on trees which have been girdled at the base for more than two years. Although it is unknown, it is likely that root grafts from adjacent healthy trees may be aiding the root systems of such diseased trees.

Root symptoms are seldom seen in the forest and the point of entry of the pathogen into the root system is unknown. It is known, however, that the fungus girdles the base of the tree soon after its advanced growth reaches the

root crown. The linear rate of development of the fungus along the major roots and the base of the tree varies with tree size and the amount of water available to the root system but generally is from 6 to 24 inches per year. Mycelial extension in large roots with abundant water supplies is fairly rapid but is much slower in small roots growing in drier soil.

Root infected trees expressing foliar symptoms also show symptoms of the fungus invasion at the base of the tree. When the outer bark is removed the cinnamon-brown discoloration of the infected inner bark contrasts sharply with the pinkish-white color of the healthy inner bark (Figure 8). The transition zone between diseased and healthy tissue is irregular and is higher on that side of the tree first entered by the pathogen. The fungus advances more rapidly in the cambial region than in the phloem. The positive proof of infection in a tree suspected of Phytophthora root disease is the brown discolored inner bark at the base of the tree.

2. Symptoms of Aerial Infection

Symptoms of aerial infection caused by Phytophthora lateralis are readily distinguished from the foliar symptoms of the root disease. As described above, the entire foliar crown of a cedar having root infection wilts,

discolors and dies uniformly. Death from foliar and branch infections is localized and progressive, and several years may be required for complete destruction of an infected crown.

Individual infections may be scattered through the tree but usually they occur on one side, frequently on low hanging foliage near the ground. The diseased area increases in size spreading from the lower branches upward and laterally in an irregular triangle (Figure 9). The average upward spread during the period 1954-1956 approximated 4 to 6 feet a year although several instances of annual extensions of 10 to 20 feet were observed. Infections old enough to appear triangular have a group of dead defoliated branches in the center near the base. Above and to the sides there occur successive zones varying in symptom expression. The innermost consists of dead, light brown foliage. The middle zone contains dry newly killed foliage, rust to brown in color. Infected foliage of the outer zone is moist and pliable with a color range from bronze through grayish green to a water-soaked green. The width and continuity of the zones is determined primarily by the density of the foliar crown.

Diseased areas enlarge by secondary infections from zoospores and by mycelial extension from established infections. Under ideal conditions the first symptoms of

foliar infection develop within three days after the zoospores come into contact with the foliage. As the infection progresses, the leaves farther from the growing point are invaded in succession. At first they appear water-soaked, then light grayish green, and later a darker grayish green. With drying, the color changes to bronze and finally to a light brown typical of the dead foliage. Penetration by mycelium from established infections back into the foliar crown results in a blighted appearance when many small twigs and branches are involved.

When larger branches and main stems are invaded consequent to foliar infections, cankers form. Cankers may also form when inoculum penetrates the bark through open wounds and also may occur occasionally by direct infection of branches striking the ground. Cankers are difficult to detect on dry stems and are conspicuous only on fast-growing, smooth-barked stems where the cankered area becomes slightly depressed and darker brown than the surrounding tissue. When the stems are wet, the cankers are seen as a chocolate brown discoloration of the bark. Limits of the cankers in the inner bark are conspicuous, the cinnamon-brown infected tissues contrasting sharply with the pinkish-white healthy bark. Old cankers may be easily located by color changes in the associated drying foliage.



Figure 8. The base of a diseased Port Orford cedar tree (22 inches in diameter). The outer bark has been partially removed to show the discoloration of the inner bark caused by Phytophthora lateralis. The base of this tree is completely girdled by mycelium and the foliar crown is nearly dead.

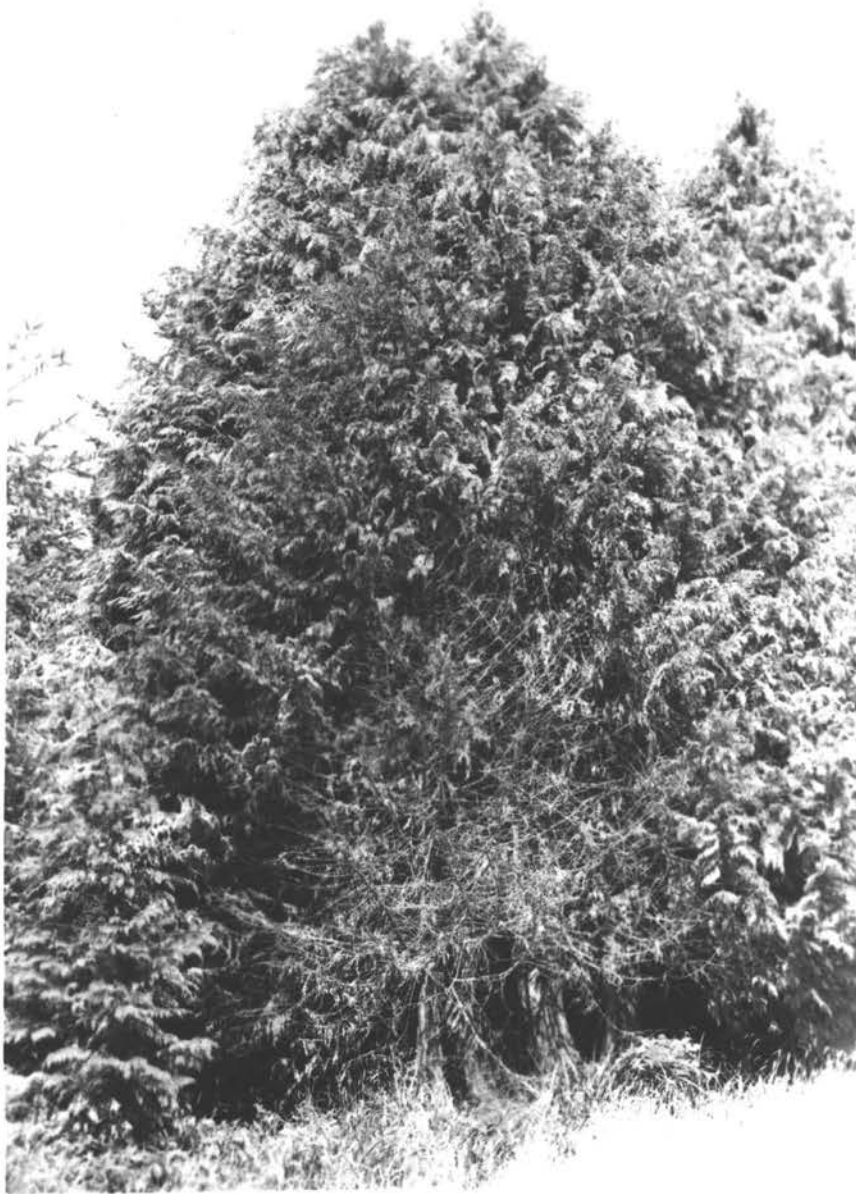


Figure 9. A large triangular diseased area resulting from cedar foliar infections.



10a. June and
September 1956.



10b. December 1956.



10c. March 1957.



Figure 11. Stem canker on a young Port Orford cedar tree (3 inches in diameter). Outer bark has been removed to show the discoloration of the inner bark.

EPIPHYTOLOGY OF AERIAL INFECTION

Disease spread involving infection of the above-ground parts (referred to as aerial infections) of Port Orford cedar began in the fall after a prolonged period of wet weather. The aerial spread of the disease was very slow throughout the cool, wet, fall and winter seasons but new aerial infections increased greatly as the temperatures began to rise in the spring. Aerial infections did not occur in the warm, dry, summer months.

1. Foliar Infection

Trees with aerial infections frequently were found near trees having Phytophthora root disease. In much of the epiphytotic area aerial infections were associated with infested soil. The aerobic pathogen, P. lateralis, was present in the surface layers of the water-saturated, poorly-aerated soil during the winter and early spring. It was observed that foliar infection often began in the spring where low hanging foliage brushed against infested soil during periods of wet, windy weather. Trees lacking foliage near the ground escaped aerial infection unless inoculum was carried to them by contact with diseased neighboring trees. External sporangia of P. lateralis formed on the newly infected foliage only in the presence of free water and were most abundant

near the tips of the foliar twigs. Spread through the lower branches and to adjacent trees with interlacing branches progressed as weather conditions were suitable for sporangial production. The prolonged growth of Port Orford cedar foliage, along with the lack of protective bud scales, maintains the foliar tips in a susceptible conditions over long periods. Water droplets bearing zoospores accumulate at these pendant foliar tips, enabling infection.

The development of foliar infections from a single tip infection was studied in a disease-free area near Langlois, Oregon. Tip inoculations were made six feet above the soil in five trees having full, foliar crowns extending to the ground. Inoculations were made in April and periodic photographs were taken throughout the subsequent year to record development of the infections. The infection pattern which developed from a single initial infection on a typical tree is illustrated in Figure 10. Warm, dry periods occurred in April and May soon after the initial inoculations and restricted the establishment of new infections at that time. The onset of foliar infections was not evidenced again until December. The major development of new infections occurred between December and March. By the end of March many new infections were apparent, as were enlargements of old infections. It is noteworthy that new infections became established only below the initial point

of inoculation, whereas, foliar infections which start naturally at the ground level characteristically move up the tree (Figure 9).

Aerial spread was influenced primarily by climatic conditions. The time required for the development of sporangia from infected foliage was largely regulated by temperature. This observation was supported by experiments employing infected, excised, Port Orford cedar twigs. The twigs were surface-sterilized in 1:10 commercial Clorox for 30 seconds, to remove any external sporangiophores or sporangia, washed thoroughly and placed in Petri dishes containing Hoagland's mineral solution number one (21, p.31). Ten infected twigs were thus incubated at the following temperature intervals: 3°, 5°, 10°, 15°, 20°, 25° and 30° C. The production of sporangia was observed periodically for 84 hours following surface-sterilization. Sporangia began to appear on infected twigs after 12 hours at temperatures of 10°, 15° and 20° C. (Table 7).

TABLE 7: EFFECT OF TEMPERATURE ON SPORANGIAL FORMATION OF PHYTOPHTHORA LATERALIS FROM INFECTED, SURFACE-STERILIZED, PORT ORFORD CEDAR TWIGS IN MINERAL SOLUTION

Temperature (°C)	Hours following sterilization						
	12	24	36	48	60	84	
3	0 ^a	0	0	0	0	0	
5	0	0	0	0	0	+	
10	+	+	++	+++	+++	+++	
15	+	+	+++	+++	+++	+++	
20	+	+	++	+++	+++	+++	
25	0	0	0	0	0	0	
30	0	0	0	0	0	0	

^a0 indicates no sporangial production, + indicates trace, ++ moderate, and +++ abundant.

After 36 hours abundant sporangia had developed at the optimum temperature (15° C.). The production of sporangia was moderate at 10° and 20° C., which appear to lie near the minimum and maximum temperatures for sporangial formation. Zoospores were subsequently released from these sporangia at 10°, 15° and 20° C., and direct germination of the sporangia by germ tubes did not occur. Since the temperatures prevailing at the time of foliar infections in the field are between 10° and 20° C. these data indicate that inoculum for aerial spread consists mainly of zoospores.

Field and laboratory observations suggest that sporangia of Phytophthora lateralis usually are not air-borne.

Sporangia rarely developed from infected foliage held in a saturated atmosphere when no free water was present, but abundant sporangia were formed when infected foliage was wet. The mature sporangia, however, remained attached to the sporangiophores. The zoospores which result from sporangial germination are transported in water droplets. Zoospores quickly perish in the absence of free water. Thus, water on the surface of the foliage is essential from the onset of sporangial development until the zoospore germ tubes have penetrated the surface of the leaf. Driving rains and hard winds appear important in transporting the zoospores to susceptible foliage. However, in being blown and washed over the foliage the concentration of the zoospore suspension is greatly reduced.

Foliar infection as well as sporangial formation was influenced by temperature. Healthy, foliar, cedar twigs were placed in Petri dishes containing active zoospore suspensions of P. lateralis. Ten inoculated twigs and four uninoculated twigs were incubated at each of seven temperature intervals from 3° to 30° C. Observations were made every other day for 12 days. At the more favorable temperatures (10° to 20° C.) symptoms of infection occurred within four to six days (Table 8). Longer periods were required at temperatures lower than 10° C. The occurrence of infection at 25° C. is noteworthy in that vegetative

fungus growth is very poor at that temperature. However, zoospores of P. lateralis germinate well and their germ tubes elongate rapidly at 25° C. No infection occurred at 30° C. which is above the maximum for growth of this fungus. All of the uninoculated control twigs remained healthy.

TABLE 8. EFFECT OF TEMPERATURE ON INFECTION OF EXCISED PORT ORFORD CEDAR TWIGS BY ZOOSPORES OF PHYTOPHTHORA LATERALIS.

Temperature (°C)	Days following inoculation						
	2	4	6	8	10	12	
3	0 ^a	0	0	0	1	1	
5	0	0	0	0	4	5	
10	0	0	8	10	10	10	
15	0	9	10	10	10	10	
20	0	9	9	9	9	9	
25	0	6	7	8	8	8	
30	0	0	0	0	0	0	

^aNumber of twigs infected out of 10 tested at each temperature.

Zoospores have been demonstrated to be the primary forerunners of foliar infection. Microscopic observations have established that zoospores die quickly as the free water evaporates from the foliar surface. Hence, the minimum time required, for zoospore contact with cedar foliage, to induce infection was of interest. The tips of healthy, excised, Port Orford cedar twigs were immersed in active zoospore suspensions at 15° C. for various time intervals up to 12 hours. Since, in fungus diseases the

degree of infection is often proportional to the inoculum concentration, two concentrations of zoospore suspensions were used; an active dense suspension and a tenfold dilution. After the initial incubation in zoospore suspensions, the twigs were placed in an atmosphere at 22° C. and 43% humidity. The petioles of the twigs were constantly immersed in Hoagland's mineral solution number one (21, p.31). The free water on the surface of the twigs evaporated rapidly and after 12 hours at the low humidity the twigs were placed in a moist atmosphere at 20° C. The number of infected twigs was recorded after seven days.

TABLE 9. EFFECT OF ZOOSPORE CONCENTRATION AND ZOOSPORE FOLIAGE CONTACT TIME ON THE NUMBER OF FOLIAR INFECTIONS AT 20° C.

Zoospore-foliage contact time (hours)	Zoospore concentration	
	Dilute	Dense
0.1	0 ^a	0
2	2	10
4	1	10
6	3	10
8	2	10
10	4	10
12	3	10

^aNumber of twigs infected out of 10 tested at each incubation period.

The results (Table 9) indicated that the minimum time of foliage-zoospore contact, required to induce foliar infections, was greater than six minutes and less than two

hours. Beyond two hours infection was independent of the zoospore-foliage incubation period. The rate of zoospore germ tube elongation at 20° C. is of interest in relation to this study. The germ tube length after two hours incubation at 20° C. was found (Table 5) to average 26 microns (maximum length 42 μ). The average germ tube length increased with time but the per cent infection showed little or no increase with time. Hence, zoospore germ tube lengths of 26 to 42 μ were sufficient to induce foliar infections at 20° C., and longer germ tubes did not appear to increase the per cent infection.

The results (Table 9) also indicated that the number of infected twigs increased greatly as the zoospore concentration increased. Thus, foliar infections may occur if viable zoospores are in contact with cedar foliage for two hours or more at 20° C., but the chance of infection occurring increases as the number of zoospores in contact with the foliage increases. As zoospores are washed about in water droplets under natural forest conditions their concentrations are greatly reduced. This decreases the potency of the zoospore inoculum.

2. Canker Development

Spread within the foliar crown from established infections occurred also by mycelial growth back into the

twigs and branches. A canker formed whenever the fungus grew from a branchlet into a branch. The canker soon girdled the branch and the host tissues distal to the canker gradually declined and finally died.

Branch and stem cankers, not associated with mycelial extension from foliar infections, appeared to arise only when the pathogen gained entrance to the inner bark through an open wound. Many attempts were made to induce canker formation by placing active zoospore suspensions on suberized stems with numerous lenticels. The zoospore suspensions were placed in a trough formed from masking tape attached to the stem. No infections resulted from these inoculations.

The rate of canker development in woody stems was of interest and was determined by measuring the mycelial extension from a point inoculation. Stems two inches, three inches and four inches in diameter were inoculated by inserting a piece of mycelium into the inner bark through a small wound. The wound was closed with tape. Periodic measurements of canker development were recorded. This study was carried out during the cool, wet, fall and winter. The linear rate of mycelial growth was approximately two inches per month (Table 10). Growth down the stem was slightly faster than growth up the stem. Stems two inches

in diameter were girdled in two months, whereas five months were required to girdle stems four inches in diameter.

TABLE 10. RATE OF DEVELOPMENT OF STEM CANKERS
IN SMALL PORT ORFORD CEDAR TREES

Diameter and number of stems	Average mycelial extension (inches) from point of inoculation				Time re- quired to girdle the stem
	1 month		3 months		
	up	down	up	down	
2 inches (10 stems)	2	2	4	7	2.5 months
3 inches (8 stems)	2	2	5	6	4 months
4 inches (3 stems)	2	2	5	6	5 months

After a stem had been girdled the distal growth of the mycelium was soon restricted, but the proximal development continued. The continued mycelial growth in the inner bark permitted the fungus to overwinter in the mycelial form in branches and stems not directly exposed to the warm sun. At the onset of the rainy season in the fall, sporangiophores bearing sporangia of Phytophthora lateralis were often seen to emerge through the lenticels of small stems, near the margins of cankers.

3. Resistant Spores in Host Tissue

In the late spring, as the rainy season ended and the temperatures rose, foliar infections stopped. Cankers in branches and stems continued to increase, but infected foliage dried rapidly. Tissue sections from the infected, dried foliage revealed numerous chlamydo spores and oospores. These spores are resistant to unfavorable environmental periods and presumably act as an important overwintering stage of this pathogen. Laboratory studies have indicated that these spores germinate only after a prolonged cold period. Therefore, these resistant spores would not be expected to germinate until late fall or early winter. It is interesting to note that this is precisely the period of the year when the inoculum for the aerial stage of this disease begins to increase rapidly. Thus, a correlation is postulated between germination of resistant spores and the increase of inoculum.

EPIPHYTOLOGY OF ROOT INFECTION

The root disease of Port Orford cedar caused by Phytophthora lateralis occurs much more frequently and is found over a much larger area than the aerial stage of the disease. At this early phase of the epiphytotic in the

Chamaecyparis range, the economic loss which results from this disease is due, almost entirely, to the root disease stage.

1. Distribution and Seasonal Development of
Phytophthora lateralis in the Soil

The method by which the fungus enters the root system of a cedar tree is unknown. However, after root infections become established the pathogen continues to grow proximally, by mycelial extension through the inner bark toward the trunk of the tree. The rate of mycelial extension along main roots was found to vary from 6 inches to 24 inches a year. Trees were girdled at the base soon after the pathogen reached the soil line. The foliar symptoms which developed as a result of root infections were closely associated with mycelial girdling at the base of the trees. Occasionally, however, slight wilting occurred before the trees were completely girdled, but foliar color changes did not occur until girdling was complete. In several rare cases foliar color changes were not expressed until one to two years after the trees (diameters 10 to 12 inches) were girdled.

The distribution of the pathogen in the surface soil between diseased and healthy trees was studied by the soil isolation method, employing healthy detached cedar twigs. A plot (30 feet x 30 feet) was established including three

trees which had been diseased less than one year at one side and three healthy trees at the opposite side. In November 1956, healthy detached cedar twigs were placed in the forest soil at six foot intervals throughout the plot. Periodic observations and twig replacements were made until March 1957.

In December 1956, active growth of the fungus in the surface soil was limited to a small localized area surrounding one of the diseased trees. By March 1, 1957, the fungus was active around two of the diseased trees and throughout one-half of the total plot area, adjacent to the diseased trees. However, a few isolated points within this "infested area" did not indicate the presence of the pathogen. By March 30, 1957, the pathogen was actively growing around all of the diseased trees and completely covered about two-thirds of the plot adjacent to the diseased trees. The pathogen was never active in the area surrounding the healthy trees.

The limited development of the actively growing stage of this pathogen in the surface soil in December 1956, suggested that the mycelium in the surface layers did not survive the dry, warm summer. This limited activity in the fall and the high activity of the pathogen in the soil in the spring was analogous to the development of the pathogen in the aerial stage of the disease. Cedar litter

was abundant on the floor of the cedar forest, and when the newly fallen foliar twigs contacted infested soil, they often became infected. Resistant spores (chlamydozoospores and oospores) developed within the infected cedar debris. Thus, it appears that the soil inoculum as well as the aerial inoculum follows a seasonal cycle in which resistant spores play an important role in the overwintering stage. The rapid build up of soil inoculum and aerial inoculum in late winter presumably are correlated with the germination of the resistant spores. The mild temperatures and moist soil which exist in late winter and early spring are ideal for vegetative growth enabling the rapid build up of P. lateralis.

The immediate local spread of this organism in the soil is due to mycelial growth, but dissemination to new soil regions is associated with spore transport and soil movement. Soil filtrates were found to stimulate the formation of sporangia and chlamydozoospores in culture. The sporangia germinated indirectly to produce zoospores. Spores may be transported to new soil regions by the movement of surface water. The movement of infested soil, or surface litter bearing resistant spores, also aids this pathogen in becoming established in new areas.

During the winter and spring 10 attempts were made to determine the vertical distribution of P. lateralis in infested soil. Soil samples were collected at different depths from areas where the fungus was known to be active in the surface layers. The samples were placed in Petri dishes containing distilled water and healthy, detached, cedar foliage. Very few foliar twigs were infected after 10 days incubation at 20° C. Many samples of surface soil known to contain actively growing mycelia gave negative results by this method. Hence, negative results were often due to failures of the method to detect the fungus, rather than absence of the pathogen in the soil sample. An adequate method of recovering P. lateralis from soil samples gathered in the field was not found. Thus, the vertical distribution of the pathogen in the soil was not determined.

2. Rate of Root Disease Development

The annual increase in the number of diseased trees around points of natural infection was studied in Mingus Park in the city of Coos Bay, Oregon. The park has a total area of about 30 acres and is covered by a dense stand of timber, about 75 per cent of which is Port Orford cedar. The cedar, which is growing in association with western hemlock, Douglas fir and Sitka spruce, constitutes

an even-aged stand, 50 to 55 years old. The recreational activities in Mingus Park are limited to one corner and the major portion of the park consists of an undisturbed, uniform, natural forest site.

The root disease caused by P. lateralis was first observed in Mingus Park in 1952 (44, p.296). In the summer of 1954, when this study was initiated, diseased cedar trees could be seen in several general areas within the park. Six plots, each including the diseased trees of the infected area and the surrounding healthy trees, were established (Figure 12). The cedar trees for which records were kept varied in diameter (breast height) from 3 to 30 inches and averaged 10 inches. The six plots with a total area of four acres, included 952 Port Orford cedar trees. The increase in the number of diseased trees within these plots was followed for two years.

Concern of city officials over the rapid dying of cedar in Mingus Park, attributed to cedar bark beetles (Phloeosinus spp.), prompted the selective cutting of many beetle infested trees immediately prior to the establishment of the disease plots. An inspection trip into the recently cut areas in May 1954 established that many of the beetle attacked trees had advanced symptoms of root disease. The clearing operations associated with the selective cutting of dead trees considerably disturbed the surface soil

in three of the six disease plots subsequently established. The establishment of plots in disturbed and undisturbed areas provided a basis for comparing the progress of the disease in these two areas.

The annual development of the disease is illustrated in Plates 1 to 6 (Appendix). Newly infected cedars were usually adjacent to trees which had been infected for some time. Occasionally, however, trees remained healthy even though surrounding trees became diseased (Figure 13). Healthy cedar seedlings were frequently present in areas where the larger trees had died. These healthy trees standing in "diseased areas" probably were disease escapes rather than trees resistant to the pathogen.

The results of this study on the annual development of the disease are presented in table form (Table 11) and summarized graphically (Figure 14). The plots (1, 2, and 6) in which the surface layers of the soil were disturbed by the clearing operations in Spring 1954 show a greater annual increase in disease development than the plots (3, 4, and 5) which were undisturbed. The movement of infested soil, associated with the dragging of logs, undoubtedly established the pathogen over a larger area than it would have normally occupied. The average increase in the number of diseased trees in the disturbed plots was 13

per cent per year compared to 7 per cent per year in the undisturbed plots. Therefore, the rate of infection in plots having surface disturbance was almost twice the normal rate. It is interesting to note that when the lines presented in Figure 14 are extrapolated to zero per cent infection, the intercept falls between 1952 and 1953 which is precisely the time the pathogen was first observed in the Coos Bay area (44, p.295).

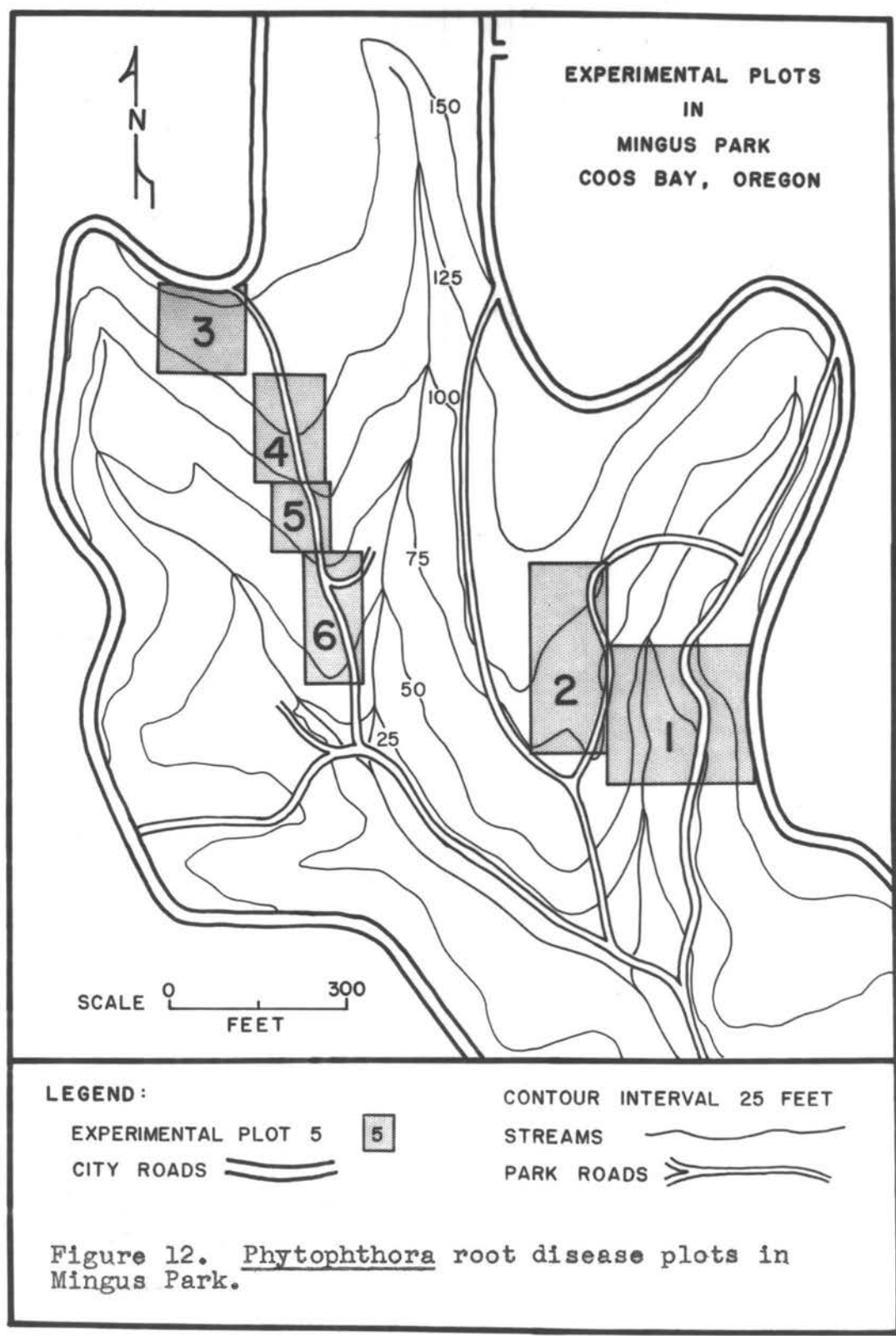




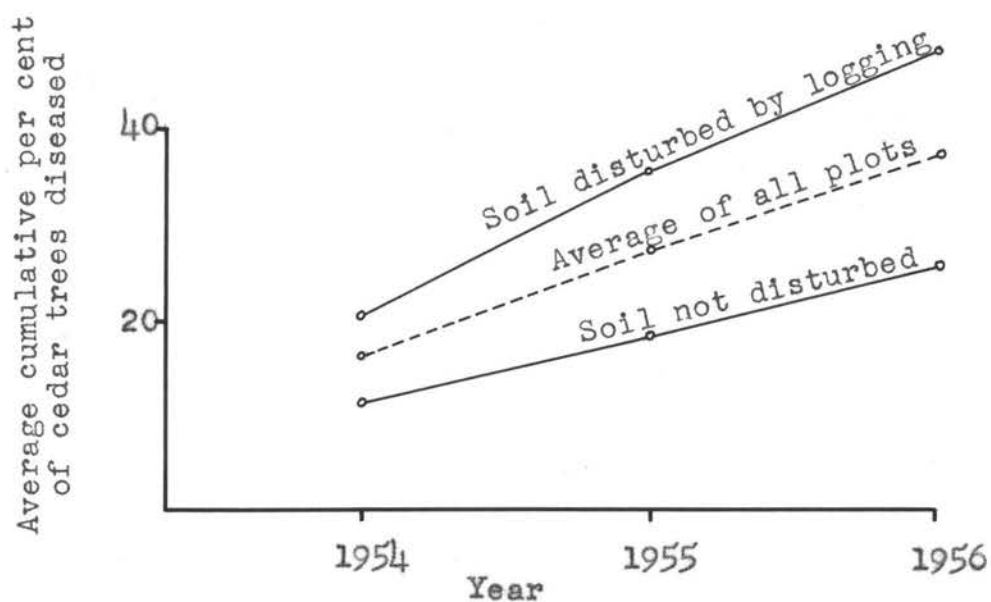
Figure 13. Colored aerial photo of Phytophthora root disease plots one and two in Mingus Park, Coos Bay, Oregon. Note dead and dying cedar trees. Dark green trees are Douglas firs.

TABLE 11. ANNUAL INCREASE OF PHYTOPHTHORA ROOT DISEASE IN A 50 YEAR OLD PORT ORFORD CEDAR STAND.

Plot Number	Plot area (acres)	Cedar trees per plot	Cumulative per cent cedar trees diseased by year		
			1954	1955	1956
1 ^a	1.32	271	36	54	63
2 ^a	0.88	232	13	28	42
3	0.52	112	20	23	31
4	0.50	142	8	19	28
5	0.27	72	4	11	17
6 ^a	0.51	123	10	24	35

^a Surface layers of the soil were disturbed by logging operations in spring 1954.

FIGURE 14. AVERAGE ANNUAL INCREASE OF PHYTOPHTHORA ROOT DISEASE IN A 50 YEAR OLD PORT ORFORD CEDAR STAND.



3. Survival of Phytophthora lateralis in the Soil

The capacity of Phytophthora lateralis to become established, survive, and spread through the soil was studied under natural forest conditions on three cedar sites. The sites included a sparsely stocked area with a dense brush cover, a medium stocked area with medium brush cover and a densely stocked cedar stand with essentially no cover beneath the forest canopy. The Port Orford cedars growing on these sites were 50 years old and averaged 8 to 12 inches in diameter. In the fall of 1954, P. lateralis was introduced into these disease-free areas near Charleston, Oregon on land owned by the Coos Bay Pulp Corporation. Three methods of inoculation were employed (see Methods): (1) mycelial suspensions were poured directly into the soil, (2) infested soil containing the pathogen was placed in the forest soil and (3) mycelium was placed directly into wounded roots. Throughout this general area 35 different points were inoculated, but each point received only one type of inoculation. Each method of inoculation was replicated two to five times on each cedar site.

Disease resulting from the 35 inoculations could be seen only on those trees which had direct root inoculations. The nine trees which were directly inoculated were all dead or dying two and one-half years after the initial

inoculation, but adjacent cedar trees were healthy and showed no evidence of the disease. Likewise, cedar trees surrounding the points of soil inoculation (inoculation methods 2 and 3) showed no evidence of infection. Soil samples from these inoculation points, were collected in the spring of 1957 and returned to the laboratory for isolation purposes. These attempts to recover the pathogen from the soil were unsuccessful. This soil isolation technique (see Methods) subsequently proved to be highly inconsistent and inadequate to establish the absence of the pathogen in the soil.

In the fall of 1954, after the inoculum had been placed in the soil, several warm, dry periods occurred before the rainy season began. These unfavorable climatic conditions may have destroyed much of the inoculum before it became established, but it is probable that the infested soil used as inoculum (inoculation method 2) contained resistant spores which permitted the pathogen to survive the warm, dry periods. However, two and one-half years after the introduction of P. lateralis into the three cedar sites, this pathogen could be observed only on those trees directly inoculated. It is possible that roots may be infected in many trees which have not as yet expressed symptoms.

DISCUSSION

The physiological characteristics of Phytophthora lateralis were investigated to gain information on the capacity of this pathogenic fungus to survive and spread under different climatic conditions. P. lateralis was found to be a slow-growing, "cool-weather" fungus. In culture the growth rate increased from 3° to 20° C. then decreased at 25° C. which was near the maximum temperature allowing growth of the mycelium. Therefore, in the forest, mycelial growth probably occurs only in the cool and mild seasons of the year and is often restricted by the warm temperatures in the summer.

Temperature was also found to affect the sporulation of this organism. Sporangia were produced from 5° to 25° C. in culture and from 5° to 20° C. on infected foliar twigs. The formation of sporangia, both in culture and on host tissue, was slow and sparse below 10° C. However, abundant sporangia were formed in 24 to 36 hours at 15° to 20° C. The sporangia are capable of germinating indirectly to produce motile zoospores from 5° to 25° C.

In addition to the proper temperature this pathogen also requires abundant moisture to carry on its vital processes of growth, sporulation and germination. Moisture was essential for the formation of sporangia and zoospores. Sporangia rarely developed from infected twigs held in a

humid atmosphere, but sporangia developed abundantly from infected twigs when free water was present on the surface of the foliage. Sporangia were found to produce zoospores only in the presence of free water. Zoospores as well as sporangia perished very quickly as the water surrounding them evaporated. Thus, the sporangia and zoospores, so intimately related to the aerial stage of this disease, were functional only when free water was present on the surface of the infected foliage. Experimental results indicate that at 15° C. free water must be present on the foliar surfaces for 24 to 36 hours before new infections will be initiated. At temperatures lower than 15° C. sporulation, germination and subsequent infection were slowed down. Hence, at low temperatures free water must be present on foliar surfaces for longer periods than are required at the more optimum temperatures of 15° to 20° C. If the water evaporates from the surface of the foliage at any time between the onset of the formation of sporangia and the penetration of the foliar surface by the germ tubes of the zoospores, the infection sequence is stopped.

In contrast to the sporangia and zoospores which required abundant moisture for development and germination, the chlamydospores and oospores produced by this fungus appeared to live through the warm, dry summer periods. Laboratory observations of chlamydospores and oospores

growing in pure culture under many conditions of temperature and moisture suggested that a prolonged cold period was a prerequisite to the germination of these resistant spores.

The nutritional requirements of P. lateralis were defined by the development of a simple synthetic solution which supported good growth. Thiamine appeared to be the only vitamin required for growth. The simple nature of the nutrient medium which supports good growth suggests that the limited host range of this pathogen is based on factors other than the nutritional requirements.

There are two main stages in the disease of Chamaecyparis lawsoniana caused by P. lateralis: the aerial stage and the root stage. The aerial stage of the disease begins in the fall with the onset of mild, rainy weather. During these initial rainy periods the mycelium which overwintered in association with cankers on small branches grows through the lenticels and produces external sporangia. Zoospores resulting from the sporangia are washed about and produce the initial foliar infections. The mild, rainy weather in the early fall is very similar to the climatic conditions of the spring, but the establishment of new aerial infections in the fall is very limited. This presumably is due to a relatively small production of

zoospores which are necessary to establish new foliar infections. It was found that the frequency of foliar infections is proportional to the number of zoospores in contact with the foliage. Thus, it appears that the limited production of zoospores in the fall season limits the number of new foliar infections. The activity of the pathogen in the surface layers of the soil during the late fall season is also very limited even though mild, moist conditions prevail throughout this season.

During the early winter season the increase of inoculum for both the aerial and root stages of this disease is very slight. The winter inactivity is due primarily to the static influence of the low temperatures.

Heavy rains occur throughout the late winter and early spring and as the temperatures begin to rise the aerial and soil inoculum builds up rapidly. Many new foliar infections are started on succulent twigs as a result of abundant sporulation from adjacent infections. Aerial infections show their greatest development during this ideal climatic period. The low temperature requirements (10° - 20° C.) of this pathogen for growth, sporulation, germination and infection agree well with the spring nature of the disease.

As periods of warm, dry weather become more frequent in the late spring, the rate of development of the aerial

stage is visibly slowed. Foliar infections cease entirely when the rainy season ends and infected foliage dries. The inactivity of the pathogen throughout the summer and even after several months of mild, moist weather in the fall suggests that much of the fungus mycelium is killed by the warm, dry summer. Phytophthora lateralis may live through this unfavorable summer period in several ways: (1) as perennial mycelium in the soil, (2) as mycelium within the tissues of a root, stem or branch of Port Orford cedar, or (3) as resistant spores. The fungus presumably utilizes all three methods of overwintering.

Resistant chlamydospores and oospores are present in dried, infected, foliar twigs. In laboratory studies these spores appeared to require a prolonged cold period before germinating. The slow development of inoculum in the fall followed by a rapid build up in the late winter suggests that the resistant spores are a very significant phase of the life cycle of this organism. It is postulated that the resistant spores function as an important overwintering stage of the pathogen, both in the cedar litter on the forest floor and in the dried infested foliage in the cedar trees. If such is the case, the germination of these resistant spores is essential to the large and rapid build up of inoculum which occurs in early spring.

Aerial infection of Port Orford cedar is most common near Langlois in the southern part of the commercial cedar range. This local occurrence cannot be explained by a special physiological strain of the pathogen, for isolates from other parts of Oregon were also demonstrated to cause aerial infections on Port Orford cedar foliage. Thus, it appears that a local environment favoring aerial infection exists in that region. Aerial spread is aided primarily by mild temperatures and moist conditions and is retarded by high or low temperatures and dry weather. The causal organism is very sensitive to climatic conditions. Thus, the disease severity of the aerial stage can be expected to vary with the climatic conditions.

Symptoms of aerial infection caused by Phytophthora lateralis can be readily distinguished from the foliar symptoms accompanying root disease. The entire foliar crown wilts, discolors and dies in the case of the root disease, whereas death from foliage and branch infections is localized and progressive.

The annual rate of root disease development in a local area was studied under natural forest conditions on a total area of four acres which included 952 Port Orford cedar trees. Disease development in this area greatly increased when the surface soil was disturbed by logging operations. The rate of increase of root disease in such

disturbed areas was about twice as great as in similar undisturbed areas. The movement of infested soil and infested cedar debris undoubtedly established the pathogen over a much larger area than was occupied prior to the logging operations. It is presumed that the increase in the rate of disease development in the disturbed areas was due to the greater dispersion of the pathogen throughout the area.

Research on the physiological characteristics of this pathogen has shown that 15° to 20° C. is optimum and 25° C. maximum, for most infection processes: sporulation, germination, reinfection and growth within the host. Thus, Phytophthora lateralis has been demonstrated to be a "cool weather" fungus which is well adapted to live along the cool, moist coast of southwestern Oregon. On the basis of these physiological and pathological studies it appears that the epiphytotic disease of Port Orford cedar caused by Phytophthora lateralis which now plagues the Chamaecyparis range will continue to increase. The entire Chamaecyparis range is seriously threatened by this pathogen.

SUMMARY

1. Phytophthora lateralis is a "cool weather" fungus which shows limited growth at 3° C., has an optimum between 15° and 20° C. and grows only slightly at 25° C.
2. Both the growth rate and the amount of mycelium produced can be altered by varying the substrate and/or the incubation temperature. A chemically defined medium which supported good growth of P. lateralis was formulated. This fungus grows on phosphate buffered media from pH 3.7 to 7.8 with best growth from pH 5.3 to 6.9.
3. Sporangia, zoospores, chlamydospores and oospores are produced in the natural life cycle of this pathogen.
4. Under natural conditions sporangia can develop at temperatures between 5° and 20° C. and germinate within that temperature range by forming zoospores.
5. Zoospores are the primary precursors of foliar infection and can germinate by germ tube formation from 5° to 25° C. Germ tube elongation increases with time and with temperature up to 25° C. Foliar infections resulting from zoospore inoculations occur from 3° to 25° C.

6. Resistant chlamydospores and oospores are produced in culture and in host tissue. The chlamydospores and oospores presumably function as an important over-summering stage of this pathogen and apparently germinate in the fall or winter only after a prolonged period of cold weather.
7. The first description of the oospores of P. lateralis is presented in this thesis.
8. Ten isolates, representing the geographical distribution of the pathogen in Oregon, were extremely pathogenic to Port Orford cedar roots and foliage.
9. An aerial stage, previously unknown in the history of this pathogen, is reported. Symptoms of aerial infection are readily distinguished from root disease symptoms. Aerial infections are localized and progressive, whereas the entire foliar crowns of root diseased trees decline uniformly.
10. In the establishment of a new aerial infection, free water on the surface of the foliage appears essential from the onset of sporangial development until zoospore germ tubes have penetrated the foliar surface.
11. The seasonal development of aerial inoculum and soil inoculum is similar and parallel. Disease development of the aerial and soil stages of this disease is very restricted in the fall and winter but increases

rapidly in the spring. The warm, dry summer, however, destroys much of the fungus mycelium. The cool, wet fall then starts the disease cycle anew.

12. Soil isolations are most successful in the wet spring when the pathogen is very active in the surface layers of the soil.
13. The local spread of the root disease is fairly rapid and is greater in areas with soil disturbance than in nondisturbed areas.
14. Phytophthora lateralis is well adapted to spread throughout the native Chamaecyparis range and the disease of Port Orford cedar caused by this pathogen is expected to increase.

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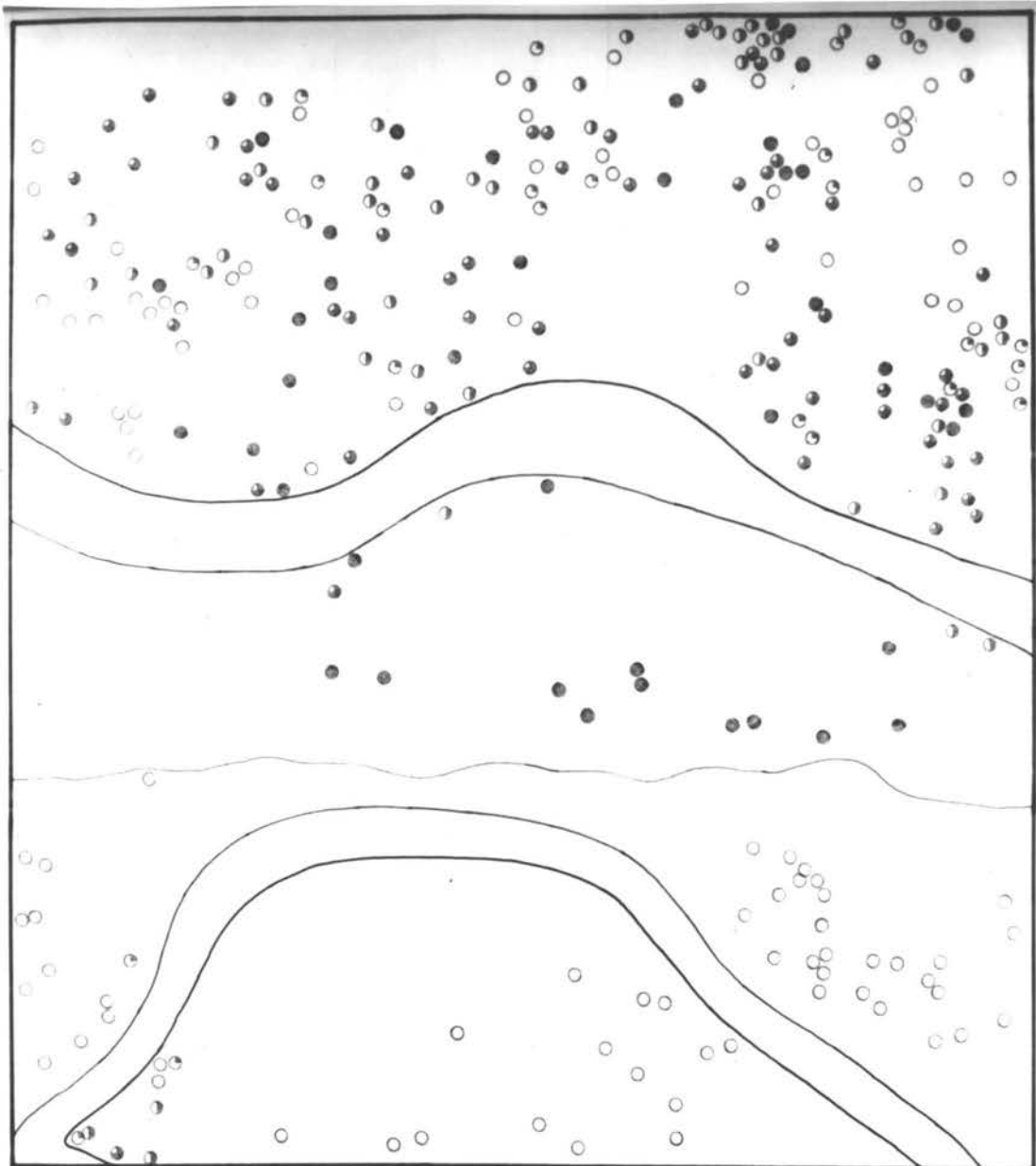
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APPENDIX

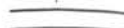

The plates presented in the Appendix represent the six experimental plots in Mingus Park in the city of Coos Bay, Oregon. These plots illustrate the rate of root disease development over a three year period in a natural stand of 50 year old Port Orford cedar.



LEGEND

CEDAR TREES :

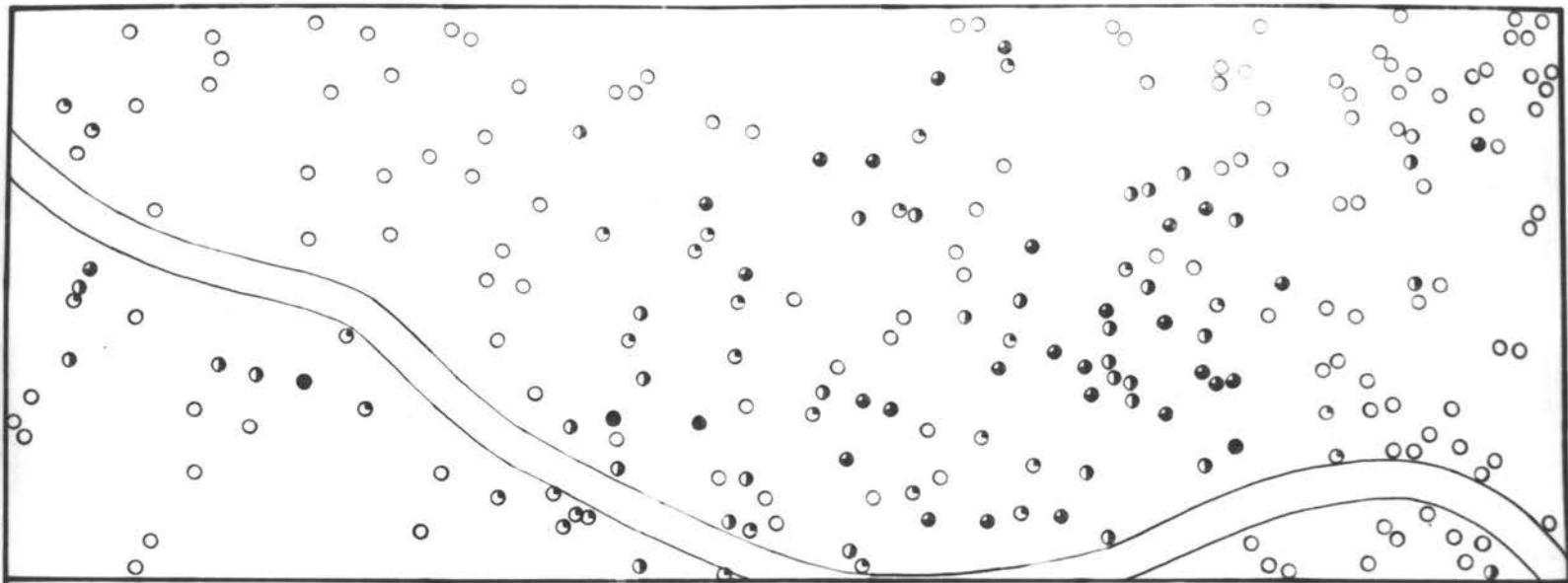
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- DISEASED SEPT. 1955 ○
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- HEALTHY SEPT. 1956 ○

- PARK ROADS 
- STREAM 

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Plate 1. Root disease plot number one.

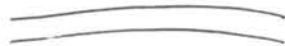


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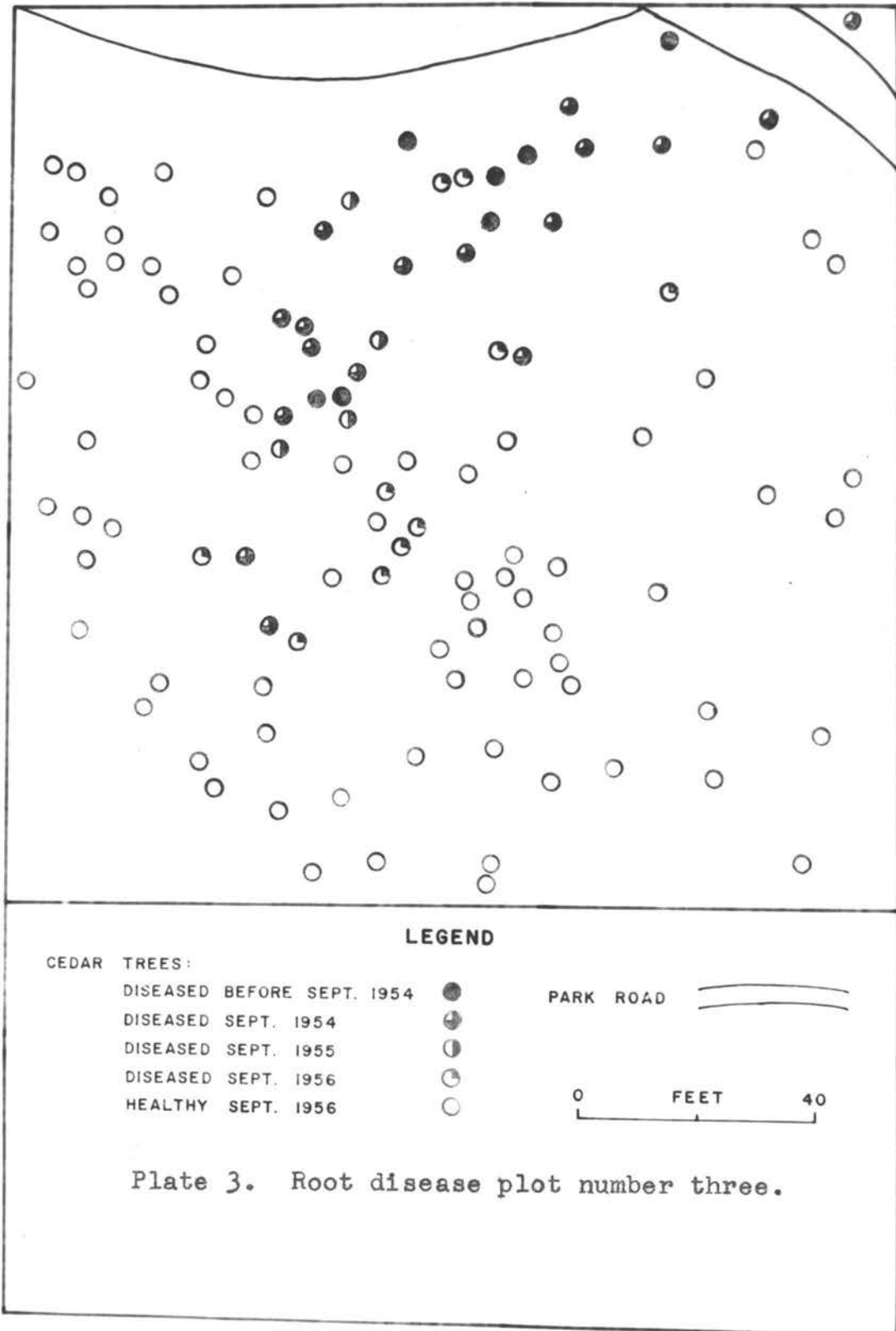
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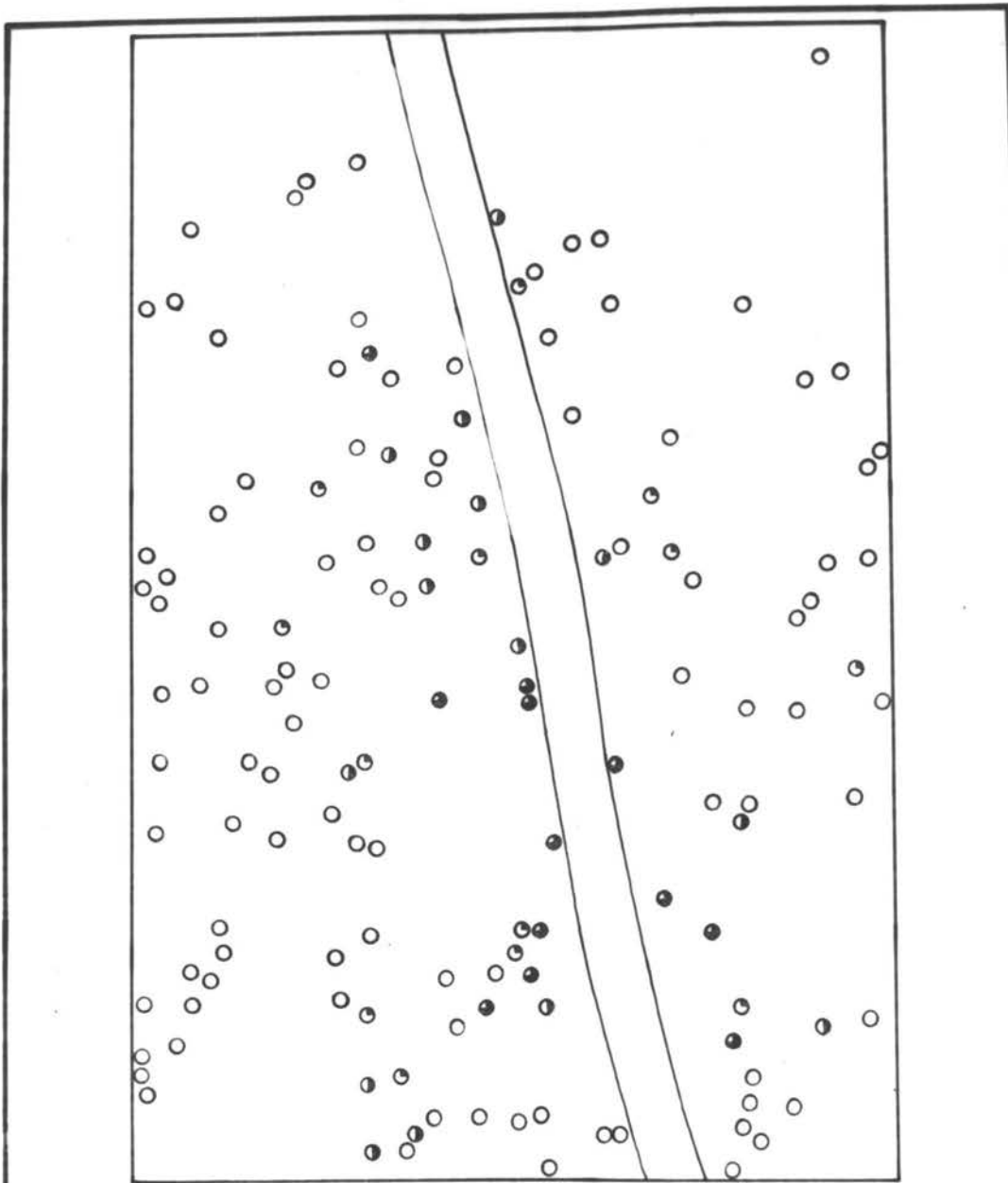
PARK ROAD



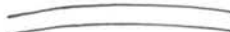
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Plate 2. Root disease plot number two.





LEGEND

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| DISEASED SEPT. 1954 | ◐ | |
| DISEASED SEPT. 1955 | ◑ | |
| DISEASED SEPT. 1956 | ◒ | |
| HEALTHY SEPT. 1956 | ○ | |

0 FEET 40

Plate 4. Root disease plot number four.

