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Chamaecyparis lawsoniana, an extremely valuable timber tree species, is highly susceptible to a serious, soil-borne root disease caused by Phytophthora lateralis. Disease outbreaks of epidemic proportions are continually causing high mortality in natural stands of the host tree. Practical control measures rely on an understanding of the many factors of disease dynamics. One important factor is pathogen survival. The primary purpose of this investigation was to develop a better understanding of the survival capacity of P. lateralis in soil and organic debris.

Survival studies are dependent on techniques used to detect organism presence. A technique was developed whereby P. lateralis could be detected in the soil. The fungus could generally be detected only in an organic matter fraction of the soil. This organic fraction was separated from the mineral fraction and used as the substrate from which the fungus was baited. When baits of cedar foliage were

floated in water over the substrate, P. lateralis, if present, would infect and sporulate on the baits. Baits were considered infected only when sporangia of P. lateralis were observed.

The optimum temperature range for infection of baits was between 15 and 20 C; the optimum incubation period was determined as six days. Pre-flooding the organic substrate with distilled water or a cedar root extract for three or seven days prior to baiting was shown to increase the frequency of detection of P. lateralis over substrate which was baited immediately. Blending the organic substrate had no apparent effect on detection.

When an organic matter fraction of an infested soil was collected and stored at temperatures of 5, 10, 15, or 20 C for a period of five months, no apparent loss of infectivity occurred, indicating a high survival capacity of the fungus. P. lateralis was able to survive in organic material stored for four months at -5 C, 14 weeks at 25 C, or two weeks at 30 C.

Two naturally infested soils were each stored for periods of up to four months. Samples of each soil were stored at four different moisture levels at each of two temperatures (5 C and 20 C). Survival of P. lateralis was poor in soil stored at moisture levels equivalent to less than -15 bars matric water potential. Survival of the fungus was also decreased when soils were stored under saturated conditions at room temperature. P. lateralis stored in soils at moisture contents

between these two extremes lost little infectivity after four months.

Organic matter highly infective with P. lateralis was added to three soils, and each soil was subjected to various biological treatments. Soils were steamed, planted to cedar (host species), alder, and Douglas-fir (non-host species) and left untreated. The fungus was recovered from the organic matter from all treatments after six months. Survival of P. lateralis appeared best in soils planted to cedar seedlings. The higher infectivity was probably attributable to an increase in inoculum, not to a higher survival capacity of the fungus. Since six months was the longest period over which survival of P. lateralis was tested, further studies are needed to determine the upper limit of its survival capacity.

Survival of *Phytophthora lateralis* in An Organic Matter Fraction of the Soil

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William Dow Ostrofsky

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Professor of Botany and Plant Pathology
in charge of major

Redacted for Privacy

Chairman of Department of Botany and Plant Pathology

Redacted for Privacy

Dean of Graduate School

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THIS THESIS IS DEDICATED TO

MY PARENTS

Milton W. Ostrofsky

Luella D. Ostrofsky

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SURVIVAL OF PHYTOPHTHORA LATERALIS IN AN ORGANIC MATTER FRACTION OF THE SOIL

INTRODUCTION

Phytophthora lateralis Tucker and Milbrath is the primary causal agent of a severe root disease of Port-Orford cedar (Chamaecyparis lawsoniana (A. Murr.) Parl.). It has been known to exist in the natural range of the host tree since the early 1950's. Since that time, the fungus has steadily spread into previously uninfested forest sites. Devastation of Port-Orford cedar as a commercial forest tree species is probable unless forest management practices are undertaken which can effectively restrict the spread of this fungus.

Port-Orford cedar is a highly valued timber species. One of the three species of Chamaecyparis native to North America, it occurs naturally on the Pacific Coast in a narrow zone approximately from Coos Bay, Oregon to Eureka, California. At maturity, individuals commonly attain a height of over 40 meters and a diameter of over 1 meter. The species characteristically displays excellent bole form (Fowells et al. 1965). The root system is generally shallow and spreading, marked with a proliferation of surface-feeding humus strivers. Port-Orford cedar favors areas of both high soil and atmospheric moisture, and can grow well on a wide variety of soil types.

P. lateralis was first described in 1942 by Tucker and Milbrath. The optimum temperature range for mycelial growth in culture is between 15 and 20 C (Trione 1974). Growth is slow compared with most other members of the genus. Sporangia of P. lateralis form in the presence of free water. They are capable of either direct or indirect germination. Indirect germination involves the formation of motile zoospores which are fully developed within the sporangium. Chlamydospores are formed by mycelia in both solid and liquid media, and germinate by producing one to several germ tubes. Oospores are produced on natural cedar media and in host tissues (Trione 1959). The germination of oospores of P. lateralis has rarely been observed, but will apparently result in the formation of a single sporangium (Trione 1974).

P. lateralis is pathogenic only on species of the genus Chamaecyparis. Therefore, other tree species growing in association with Port-Orford cedar in forest stands are not affected. However, the fungus can attack and kill all sizes and ages of Port-Orford cedar under natural forest conditions.

Field observations suggest that the soil-moving activities of humans are largely responsible for the rapid spread of the fungus (Roth et al. 1972). An immediate control strategy is to prevent imminent losses by excluding the fungus from uninfested forest sites. The management of the root disease in the forest will require a

working knowledge of 1) the location and extent of the infested areas; 2) the mechanisms by which the pathogen is spread, both locally and over long distance; and 3) the survival capacity of the fungus in infested soil. The purpose of this investigation was to devise a method for detecting P. lateralis in soil, and to use the method to determine the survival of the pathogen in the soil.

LITERATURE REVIEW

Phytophthora Root Disease of Port-Orford Cedar

The destructive potential of *P. lateralis* was well known in the ornamental and nursery industries long before the fungus was introduced into the natural cedar forests. Hunt (1959) has stated that the earliest reports of the disease in the Pacific Northwest date back to 1923 and 1933, when affected cedar were found in areas of Portland, Oregon and Seattle, Washington. In 1938, Milbrath and McWhorter described the disease occurring in the Willamette Valley of Oregon. Here, the root disease rapidly became epidemic in nurseries and in ornamental plantings. The causal agent of the disease was first reported by Milbrath (1940) as a previously undescribed fungus belonging to the genus *Phytophthora*. In 1942, the fungus was described as a new species, *Phytophthora lateralis* (Tucker and Milbrath 1942). Pathogenicity trials established that the host range of *P. lateralis* is confined to the genus *Chamaecyparis*, with *C. lawsoniana* and its varieties highly susceptible (Torgeson 1952, Torgeson et al. 1954). Nursery production of *Chamaecyparis* species for ornamental plantings was largely abandoned by the mid-1950's.

P. lateralis was first isolated from diseased trees in the natural range of Port-Orford cedar in 1952 (Roth et al. 1957). However, it is believed that the fungus was introduced to the area some years prior to that time. The fungus rapidly spread, and the disease became epidemic within a few years. Disease spread was obvious and most rapid along roadways and waterways. Disease incidence was commonly associated with construction and logging operations and other soil-moving activities of humans. Less noticeable than it was 20 years ago, the epidemic is now occurring in more isolated forest areas, where localized outbreaks continue to cause heavy mortality (Roth et al. 1972).

The first intensive study of the pathology of P. lateralis under natural forest conditions was undertaken by Trione (1957, 1959). It was found that the localized spread of the pathogen occurred during the late winter and early spring seasons, when both cool temperatures and high soil moisture conditions prevailed. Trione (1959) also described the epiphytology of aerial infection, a previously unknown condition (Trione and Roth 1957). Aerial infections were found to depend on specific environmental conditions which occur only near the extreme coastal areas of the cedar range, and are therefore relatively uncommon.

Physiological studies of the life cycle of the fungus suggest that it is particularly well suited to initiate infections during the cool, wet

winter and spring seasons (Tucker and Milbrath 1942, Trione 1957, 1974, Englander 1972). These conditions are characteristic of the weather in the natural range of Port-Orford cedar.

Soil surface water has been shown to be a major factor influencing the localized spread of the fungus (Trione 1957). Evidence for this comes both from field experiments (Trione 1957) as well as from laboratory investigations (Atkinson 1967). Although the fungus is capable of infecting healthy trees from diseased trees via root grafts, stand conditions and topography are often not conducive to this manner of spread (Gordon 1974).

Long range spread of the fungus has been associated with human activity. Movement of nursery stock as well as roadbuilding, logging, and construction equipment have served to move quantities of infested soil to new areas (Roth et al. 1972). Thus, the fungus can be spread not only during the cool, wet seasons, but also during the dry summer months. Two spore forms of the fungus, the chlamydospore and the oospore, are believed to function as survival structures in infested soil during the warm, dry summer seasons (Trione 1957). Aerial photographs have also indicated that waterways such as streams and rivers can carry the fungus over long distances, probably by transporting contaminated debris.

Isolation of *Phytophthora* Species from Plant Tissues and Soil

Species of *Phytophthora* are often difficult to isolate from infected plant material or infested soil. Generally, these fungi have much slower growth rates than many common contaminants of such isolations. In unselective media, contaminants quickly overtake and mask any *Phytophthora* colonies present. In addition, detection in soil is difficult due to the characteristically low propagule numbers of most *Phytophthora* species in soil. Some progress has been made toward solving these problems by adapting isolation procedures to favor the detection of species of *Phytophthora*.

The early methods of isolating species of *Phytophthora* by baiting from infected plant material with fruit were moderately successful (Tucker 1931, Klotz and Fawcett 1939). However, those methods have been replaced by direct isolation techniques based on the use of a variety of antibiotics. Generally, such antibiotics are added directly to the culture medium. Pimaricin, a polyene antibiotic suppressive to the growth of most non-Oomycetous fungi, has proven an invaluable aid in direct tissue isolations (Eckert and Tsao 1960, 1962, Eckert et al. 1961).

Antibiotics such as pimaricin have also been used for direct isolations from soil and in soil dilution plates. Other chemicals

commonly used in soil-plating include pentachloronitrobenzene (PCNB) (Haas 1964), streptomycin sulfate, mycostatin, and rose bengal (Kuhlman and Hendrix 1965, McCain 1967), vancomycin (Ocana and Tsao 1966, Tsao and Ocana 1969), gallic acid (Flowers and Hendrix 1969), chloramphenicol (Sneh 1972, 1974), and benomyl and thiabendazole (Ponchet et al. 1972).

Sieving techniques developed for the recovery of Phytophthora propagules have occasionally been used (McCain et al. 1967, Thomson and Allen 1974). Similarly, there have been few observations of propagules of Phytophthora species in soil (Legge 1951, Ko 1971, Sneh and McIntosh 1974). However, a new staining technique has given promising results, and direct observation attempts are now becoming more common (Tsao 1970, Mehrotra 1972, Gisi and Meyer 1973).

The most commonly used method of isolating species of Phytophthora from soil involves some form of a baiting technique. Different baits are used for different species of Phytophthora. Those species which have a wide host range, such as P. cinnamomi Rands, can be successfully recovered on a wide variety of baits.

A susceptible host plant, when used to bait a pathogen from a suspect soil, is often considered the ultimate test for the presence of a pathogen. This old, but very basic technique has been used most recently to recover P. cinnamomi (Zentmyer et al. 1960, Mircetich

and Zentmyer 1966), P. lateralis (Atkinson 1965), and P. nicotianae var. nicotianae (B. de Haan) Waterh. (Weststeijn 1973) from soil.

Development of the baiting technique began in 1907, when Butler devised the method whereby susceptible fruit, seeds, or other plant parts were floated on a water layer over soil suspected of being infested with Pythium species (Sewell et al. 1974). This baiting technique and its variations has found the widest acceptance as a method for detecting species of Phytophthora in the soil. Apples have been used to detect P. cinnamomi (Campbell 1949), P. cactorum (Leb. & Cohn.) Schroet., P. citricola Sawada, and P. syringae (Kleb.) Kleb. (Sewell et al. 1974) as well as many other species. P. cinnamomi, P. palmivora (Butl.) Butl., and P. parasitica Dast. have been isolated with pineapple crowns (Anderson 1951, Klemmer and Nakano 1962). P. citrophthora (R.E. Sm. & E.H. Sm.) Leonian, P. parasitica, and P. syringae have been isolated from soil with lemon fruits, and P. hibernalis Carne with orange fruits (Klotz and DeWolfe 1958, Tsao 1960). Zentmyer et al. (1960) have used avocado fruit to detect P. cinnamomi in soil. Pear fruits have been used to recover P. cactorum, P. megasperma Drechsler, P. cambivora (Petri) Buism., and P. drechsleri Tucker (McIntosh 1964, van der Scheer 1971) as well as P. citricola, P. cryptogea Pethybr. & Laff., and P. syringae (Sewell et al. 1974). Other fruits which have been used as baits include cacao pods for the isolation of P. meadii McRae,

P. nicotianae var. parasitica (Dast.) Waterh., and P. palmivora (Chee and Foong 1968, Dakwa 1974); green tomatoes for P. nicotianae var. nicotianae (Westeijn 1974); and various cucurbits such as cucumber and muskmelon for other species of Phytophthora (Banihashemi 1970).

Foliage and stem sections of various plants have been used as baits floated over soil or placed in soil slurries. P. lateralis was first isolated from soil with branchlets of Port-Orford cedar (Trione 1957). Pineapple leaf bases were as effective in isolating P. palmivora, P. parasitica, and P. cinnamomi as were the pineapple crowns (Anderson 1951, Klemmer and Nakano 1962). Nusbaum et al. (1952) recovered P. parasitica var. nicotianae from soil using tobacco stem sections. Potato stem sections have been used to isolate P. infestans (Mont) DeBy. (Zan 1956, 1962) as well as P. erythroseptica Pethybr. (Vujicic and Park 1964) from soil. Carnation petals have been reported as highly selective for the isolation of P. nicotianae var. parasitica from soil (Ponchet et al. 1972). Grimm and Alexander (1974) have isolated P. parasitica and P. citrophthora from soil using citrus leaf pieces as bait. Dance et al. (1975) have recently reported the use of needles of Pinus radiata and Cedrus deodara as bait for 13 different species of Phytophthora.

Germinated seeds and very young seedlings have also been used successfully as baits to detect certain species of Phytophthora in soil.

Blue lupin seedlings were first used for the recovery of P. cinnamomi by Chee and Newhook (1965). Several modifications of that technique have since been described (Podger et al. 1965, Pratt and Heather 1972, 1973, Gerrettson-Cornell 1973, 1974, Gerrettson-Cornell and Townsend 1974). A baiting technique using alfalfa seedlings for the recovery of P. megasperma from soil has recently been developed (Marks and Mitchell 1970, Pratt and Mitchell 1973). Similarly, Marks and Kassaby (1974) have used detached cotyledons of Eucalyptus sieberi to bait P. cinnamomi from forest soils in Australia.

Survival of Phytophthora Species in Plant Tissues and Soil

Species of Phytophthora may survive in soil for periods of over several months in the absence of a host (Critopoulos 1955, Apple 1963, Weststeijn 1973). Primary soil factors which influence survival include temperature, moisture, organic matter, and the associated microflora (Menzies 1963). Survival potential of several species of Phytophthora has often been related to the soil organic matter (Peries 1963, 1964, Zentmyer and Mircetich 1966, Waite and Diaz 1969, Zakharova 1972, Batini 1973). Although it is generally assumed that chlamydospores are the primary unit of survival for this genus

(Zentmyer and Erwin 1970, Schmitthenner 1970), notable exceptions are apparent and will be subsequently discussed.

Studies by Zentmyer and Mircetich (1966) suggest that moderate mycelial growth of P. cinnamomi can occur in the soil, and that the mycelium can persist for up to one month before lysis occurs (Mircetich and Zentmyer 1966, 1967). Other evidence indicates that mycelium of P. cinnamomi is completely lysed after a period of between 17 and 26 days, depending on the soil moisture availability (Reeves 1975). P. cinnamomi has also been shown to be a poor saprophytic competitor, unable to grow as mycelium in the soil (Kuhlman 1961, 1964). Even though hyphae can grow under extreme moisture stress in pure culture (Sommers et al. 1970), it is evident that other soil conditions can influence the survival of hyphae.

P. palmivora may be a strong competitor for fresh host organic matter (Turner 1965), and recent evidence indicates that it can persist in soil as mycelium much longer than as chlamydospores (Chee 1973). However, mycelium is not believed to be important for the survival of P. cactorum (Gisi and Meyer 1973, Sneh and McIntosh 1974), P. erythroseptica (Vujicic and Park 1964), P. parasitica (Tsao 1969), or other species (Zentmyer and Erwin 1970).

Many species of Phytophthora have been shown to form sporangia in soil (Legge 1951, Tsao 1969, Reeves 1975). These structures may play an extremely important role in relation to

infection and colonization of host tissues (Ko and Chan 1974). Few studies, however, have tested the survival of sporangia in soil. The information which does exist indicates a wide variation in the survival capacity between species, as discussed below.

Ho (1969) has found that lysis of P. megasperma var. sojae (Drech.) Hildebrand sporangia occurs within four to five days in soil. The sporangia of P. infestans apparently lose their viability within 77 days in the soil (Zan 1962). P. cactorum can survive as sporangia in soil for up to 84 days when subjected to temperature and moisture conditions similar to those which "naturally occur during a growing season" (Sneh and McIntosh 1974). Indirect evidence suggests that sporangia of P. parasitica can survive for as long as six months in the soil (Holdaway and Tsao 1971).

Both motile zoospores and cysts are generally considered to be short-lived propagules (Hickman 1970). Zoospores of P. infestans are thought to germinate within 24 hours in soil, but to retain their infectivity as germinated cysts or germ tubes for as long as 14 days (Zan 1956, 1962). Non-germinated zoospore cysts of P. megasperma var. sojae apparently survive in the soil for only one day (Mehrotra 1972), while those of P. drechsleri may persist for up to 15 days (Hickman 1970, Mehrotra 1972). This survival period is similar to that of P. cactorum. McIntosh (1972) found that zoospores of P. cactorum can persist in soil for only seven days in the absence of

a host, but for 28 days when host material in the form of germinated apple seeds was present. The cysts were noted to survive longer in relatively dry soils (-2 to -5 bars matric water potential) than in wet soils (-0.1 to -0.2 bars) (McIntosh 1972). Turner (1965) has made a similar observation with P. palmivora. He noted that zoospores or cysts were viable for ten days in a saturated, non-sterile soil, but could persist for as long as two years in soil maintained at 50% moisture holding capacity.

Species of Phytophthora known to form chlamydospores under natural conditions include P. lateralis (Trione 1959), P. cinnamomi (Hendrix and Kuhlman 1965a), P. parasitica (Holdaway and Tsao 1971, 1972), and P. palmivora (Waite and Diaz 1969). Zentmyer and Erwin (1970) claim that the chlamydospore is the primary unit of survival for species of Phytophthora. Trione (1957) has postulated that the chlamydospores of P. lateralis function as resistant structures, allowing the organism to survive during the dry summer periods.

The potential of P. palmivora chlamydospores to infect and colonize host tissue has recently been demonstrated (Ko and Chan 1974). There are conflicting reports, however, as to the importance of chlamydospores in the long-term survival of the fungus. P. palmivora has been reported to survive for a year in infected host tissue placed on the soil surface (Waite and Diaz 1969). Other evidence indicates that chlamydospores persist for only four or five

weeks, and that the mycelium is more important in functioning as a survival structure (Chee 1973).

Chlamydospores of P. parasitica were found in naturally infected papaya residues. Attempts to germinate the spores failed, and it was concluded that their survival capacity was probably no longer than one month (Trujillo and Hine 1965). Later, it was suggested that if the moisture content of the soil was kept above 10%, and if the temperature was maintained between 16 and 34 C, the chlamydospores of P. parasitica could survive for "fairly long periods of time" (Trujillo and Hine 1967). Tsao and Bricker (1968) found that the spores would germinate only if exogenous nutrients were present. Survival of P. parasitica for four months in soil "near complete dryness," and for two years in moist soil has subsequently been reported (Tsao 1969).

Chlamydospores of P. drechsleri were formed in artificially infested root tissue only after the tissue was buried in soil for a minimum of seven days (Cother and Griffin 1973a). Spores produced in this manner remained viable for a period of at least ten months (Cother and Griffin 1973b). Germination of P. drechsleri chlamydospores can occur on agar with osmotic water potentials as low as -98.5 bars. It has been suggested that host infection by chlamydospores may occur at these low water potentials, when other competitive organisms are unable to grow (Cother and Griffin 1974).

The formation of chlamydospores of P. cinnamomi in either organic debris or in soil can occur under natural conditions (Hendrix and Kuhlman 1965a, 1965b, Mircetich and Zentmyer 1966). Investigations by Zentmyer and Mircetich (1966) have suggested that this organism can persist in soil and organic debris for periods longer than six years in the absence of a host. Dormant mycelium and oospores of P. cinnamomi have also been implicated as resistant structures, but chlamydospores are believed to be the major survival propagule (Zentmyer and Mircetich 1966). Although viable chlamydospores of this fungus have been recovered from naturally-infested host roots after a period of one year, such structures probably survive less than three months when the moisture content of the soil (sandy loam) is less than 3% (Mircetich and Zentmyer 1967). Exogenous nutrients were found necessary for chlamydospore germination, and various root diffusates were shown to fulfill this requirement (Mircetich et al. 1968). The resistance of the spore germ tubes to biological antagonisms has also been noted by Mircetich and Zentmyer (1970). Studies testing chlamydospore survival may actually be testing the survival of germ tubes.

Oospores are formed by a sexual process. They characteristically possess thick walls, and often do not germinate readily, even when subjected to conditions considered to be favorable. For these reasons, oospores are generally assumed to be resistant, or resting

structures able to survive adverse environmental conditions for long periods of time (Zentmyer and Erwin 1970). However, little is known about oospore survival in the genus Phytophthora.

Oospores of P. lateralis (Trione 1959), P. cinnamomi (Mircetich and Zentmyer 1966, 1967), and P. megasperma var. sojae (Slusher and Sinclair 1973) are known to be produced in naturally-infected host tissues. Oospores of P. cactorum (Gisi and Meyer 1973) and P. erythroseptica (Vujicic and Park 1964) have been noted to occur in soil.

Reeves (1975) has recently given evidence that antagonistic organisms, particularly Trichoderma viride Pers. ex. Fr., induce P. cinnamomi to produce oospores in soil. When mycelium of P. cinnamomi was placed in soil, oospores formed in 15 days (Mircetich and Zentmyer 1966, 1967). Although these spores were observed in a sandy-loam soil held at 3% moisture content, after three months they failed to germinate when placed on an agar medium. They were also unable to infect host plants under presumably favorable conditions. It was therefore postulated that the spores were important survival structures only in soils held above 3% moisture content (Mircetich and Zentmyer 1966, 1967). Oospores of P. cinnamomi have been implicated to survive in soil for six years (Mircetich and Zentmyer 1966). However, this has not been conclusively shown.

The survival capacity of P. cactorum oospores was investigated by Sneh and McIntosh (1974). Oospores added to the soil were viable after 14 weeks. Storage of the infested soil at either 10 or 20 C, or at moisture contents of -5, -0.5, or -0.2 bars matric water potential had no measurable effect on survival of the spores (Sneh and McIntosh 1974).

MATERIALS AND METHODS

Soils

Eight different soils were used in the course of this investigation. Six of the soils were collected from natural forest sites, and one soil was collected from an agricultural site. An artificially prepared loam was used to maintain vegetatively propagated cuttings of Port-Orford cedar in the greenhouse. Selected characteristics of these soils appear in Table 1.

Soil samples collected in the field were placed in plastic bags. The bags were tightly sealed, brought back to the laboratory, and stored at 5 C.

Plant Materials

Foliage used for baits or extracts was obtained from branchlets of mature Port-Orford cedar trees. Only the distal 10 to 25 cm of the branchlets were used, these being the youngest and most succulent.

Root material used for baits or extracts was obtained from rooted cuttings of Port-Orford cedar. Cedar cuttings were propagated in a sand bench with a regular schedule of mist irrigation, under greenhouse conditions.

Table 1. Characteristics of the eight soils used for detection and survival studies of Phytophthora lateralis.

Number	Stand characteristics	Soil source	Texture classification	pH	% O.M. (by wt.)
1	Alder	McDonald Forest Corvallis, Oregon	Witham silty clay loam	6.0	39[x]
2	Naturally infested cedar	Beaver Hill Rd. Coos County, Or.	Tahkenitch-Bohannon loam	5.4	12
3	Healthy cedar	Coos Co. Forest Coquille, Oregon	Coosbay-Templeton[y] silt loam	4.8	39[x]
4	Douglas-fir	McDonald Forest Corvallis, Oregon	Jory silty clay loam	5.8	2
5	Artificially infested cedar	Botany Farm, OSU Corvallis, Oregon	Chehalis silty clay loam	6.4	4
6	Rooted cedar cuttings	OSU Greenhouses Corvallis, Oregon	loam (artificial mix)	6.6	10
7	Naturally infested cedar	North-Coos Bay Glasgow, Oregon	Bandon sandy loam	5.5	8
8	Naturally infested cedar	Coos Co. Forest Coquille, Oregon	Coosbay-Templeton[z] silt loam	5.0	5

[x]Samples included duff material.

[y]Samples were taken to a depth of approximately 20 cm.

[z]Samples were taken below 20 cm (subsoil).

Port-Orford cedar wildlings up to four years old were collected from various locations in the Coos County area of southern Oregon. Seedlings were transplanted to a standard greenhouse potting soil and maintained in outside cold-frames. Port-Orford cedar seeds were obtained from the Coos County Forestry Department, and were used to produce germlings for various experiments. Seeds were stored in a ventilated container at 5 C until use.

Baiting Techniques

Baiting and Plating from Artificially Infested Soil

A method for the recovery of *P. lateralis* from artificially infested soil was tested. Cedar foliage baits were buried in saturated, infested soil. Infected baits were placed on media selective for the growth of *P. lateralis*.

Inoculum consisted of a suspension of chlamydospores and mycelial fragments of *P. lateralis* in sterile water. The inoculum was prepared as described by Englander (1972). Foliage baits were the entire, distal 5 cm of fresh Port-Orford cedar branchlets which, hereafter, are referred to individually as "baits." The baits were considered infected when their color changed from a bright green to a light- to dark-brown or black. This color change generally occurred

between one and two weeks after the baits were buried in the infested soil.

Two natural culture media were used. A root extract medium was prepared by boiling 50 grams of cedar root shavings in 600 ml of water for 10 minutes. The decoction was filtered twice through cheesecloth and adjusted to 1 liter with distilled water. Cholesterol (20 mg/liter) and agar (12 g/liter) were added to the medium (Englander 1972). The preparation was autoclaved at 15 lb pressure for 15 minutes. A foliage extract medium was prepared in a similar manner, using 50 grams of cedar foliage in place of the cedar root shavings. Pimaricin, penicillin, and polymixin were added to the autoclaved, cooled media in the ratio of 25:10:10 mg/liter by sterile filtration (Tuite 1969).

Baiting with Cedar Branchlets in the Field

Another baiting method used was a modification of the branchlet technique described and employed by Trione (1957). Branchlets of Port-Orford cedar were cut to approximately 20 cm in length. All the side foliage was stripped from the main axis, and the bark was shaved from one side to expose the cambium. These baits were placed vertically in the soil to a depth of 15 cm. After two weeks, the baits were collected and cut into 1 to 5 mm pieces and placed in 9 cm

diameter, glass petri plates. Enough water was added to flood the substrate bait, and secondary cedar foliage baits (refer to the following section) were added. The plates were incubated at 18 C for six days. After incubation, the secondary baits were examined for sporangia of P. lateralis.

Baiting over Organic Material

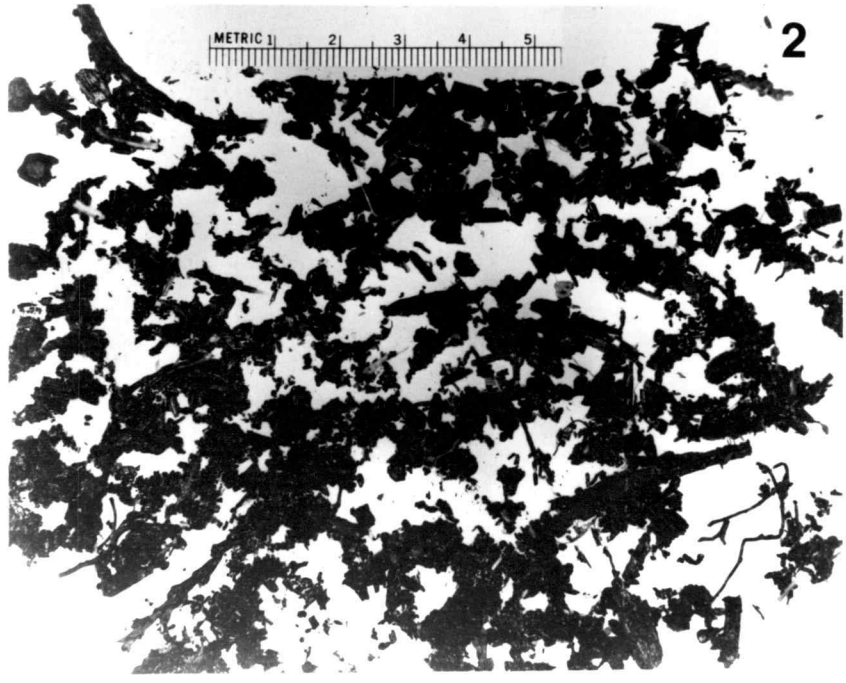
The majority of the experimentation reported in this thesis depended on a direct method of baiting P. lateralis from soil. These experiments used an organic matter fraction (OMF) of the soil as the substrate which was baited. The OMF was collected by sieving the soil sample through a 5 mm mesh screen. One to two liters of the sample were then placed in a 10 liter container of water and thoroughly mixed. When the soil-water mixture became an even slurry with no soil aggregates remaining, it was filtered through a double layer of cheesebloth. The fine sand, silt, and clay particles passed through the filter, while the larger sand particles and an OMF remained (Figure 1). This material was then washed on the cheesecloth with additional water.

Large sand particles were separated from the organic material by stirring 0.25 to 0.5 liters of the collected mixture with water in a 2 liter glass beaker. After stirring, the sand rapidly settled to the bottom of the beaker. The supernatant containing the organic material

Figure 1. Procedure for collecting the organic matter fraction from a soil. From left to right; soil is sieved, slurried in water, and poured through a cheesecloth filter.

Figure 2. Detail of the organic matter fraction retained by the cheesecloth filter.


1


METRIC 1
2
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2

was again filtered through cheesecloth. Several such washings were sometimes necessary to obtain an OMF which was free of the mineral components of the soil (Figure 2).

Excessive moisture was removed from the collected OMF by wringing. Organic material which was not to be assayed immediately was stored at 5 C in tightly sealed plastic bags unless otherwise noted.

To test for the presence of P. lateralis, an aliquot of up to 15 grams of the OMF was placed in a petri plate. This substrate was covered with water, and five cedar foliage baits were added. The plates were then incubated according to the particular experiment. The baits were prepared by stripping all the side foliage from the central axis of the branchlet. These stripped sections were then cut into 3 to 4 cm long baits. Only the distal ends of the branchlets were used for baits; woody tissue was excluded (Figure 3).

After incubation with the OMF, the baits were placed in water in the bottom half of a clean petri plate, covered with a glass slide, and examined under low magnification (50 X) with a light microscope. Sporangia of P. lateralis, when present, were readily identified (Figure 4). The presence of a single sporangium of P. lateralis indicated infection of that bait.

Clearing and Staining Organic Material

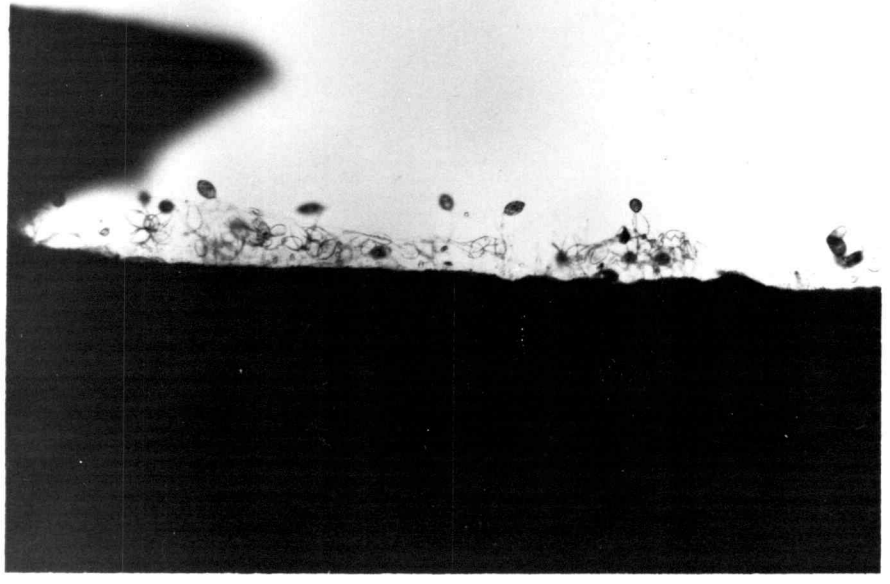
Tissue clearing and staining was done in an attempt to determine

Figure 3. The procedure for the preparation, use, and microscopic examination of Port-Orford cedar foliage baits.

Figure 4. Sporangia of Phytophthora lateralis on infected foliage bait as seen microscopically (50 X).

3

4



the fungal structures which accounted for the infectivity of the OMF. The method described by Phillips and Hayman (1970) was followed. Briefly, this involved heating the organic material in 10% KOH for two hours, followed by bleaching with H_2O_2 until the plant pigments were cleared. Staining was done by simmering the cleared material in a 0.05% solution of trypan blue in lactophenol. The organic material was then washed in clear lactophenol and examined under a light microscope for the presence of fungal structures.

RESULTS

Comparison of Baiting Techniques

The literature indicates that the most successful method of detecting species of Phytophthora in soil is by baiting with susceptible plant parts. This generalization has also been supported by experiences with P. lateralis, which was previously recovered from soil only by baiting with cedar branchlets (Trione 1957). Several methods were tested and compared for their efficiency to detect P. lateralis in soil.

Baiting and Plating from Artificially Infested Soil

Soil artificially infested with P. lateralis was used in initial baiting experiments. A large sample of soil 3 was collected and sieved through a 5 mm mesh screen. Approximately 20 cc of raw (unsterilized) soil were placed in each of 54 plastic cups (125 ml). An additional 30 cups of sterilized soil were set up as controls. Soil was sterilized with propylene oxide (Tuite 1969).

Inoculum was added to soil in one of two concentrations; 1,000 or 500 chlamydospores per ml of sterile, distilled water. The inoculum, however, was not entirely free of mycelial fragments.

Twenty-four of the raw soil samples and 12 of the sterilized soil samples were each inoculated with 1,000 chlamydospores. An equal number of samples were each inoculated with 500 chlamydospores. Six samples of uninoculated raw soil, and six samples of uninoculated sterilized soil were included as controls. Tight-fitting plastic lids were put on all the cups, and the cups were placed in a 25 C incubator. The lids maintained the soil moisture content at approximately 35% (w/w).

Samples were baited after storage periods of 0 (control), 2, and 4 days, and 1, 2, 3, 4, 6, 8, 10, 14, and 18 weeks. After the appropriate storage period, two raw soil samples and one sterilized soil sample of each inoculum concentration were baited. The soil samples of two uninoculated soil cups (one sterilized, one raw) were baited after each alternate storage period. The samples to be baited were saturated with distilled water, and two cedar foliage baits were buried in the soil. Lids were replaced on the cups, and the samples were incubated at 15 C for a period of up to two weeks.

When the baits appeared infected, they were removed from the soil, washed in tap water, blotted dry, and plated on one of the two antibiotic media previously described. Plates were incubated at room temperature (20 C) and examined microscopically (50 X) for colonies of P. lateralis at approximate intervals of four days for a

period of two weeks. No Phytophthora colonies were found on any of the plates at any time.

Baiting with Cedar Branchlets in the Field

A branchlet baiting technique was tested under field conditions and compared with a detection method based on baiting over an OMF of the soil. The site selected for this study was a small stand of young Port-Orford cedar (10 to 15 years old). The trees were planted and maintained for experimental purposes at the Oregon State University Botany Farm in Corvallis. The site was uniformly infested with P. lateralis several years ago, and tree conditions now range from healthy to dying and dead.

Two separate plots were established. The plots were 180 cm square, and each plot center was marked by a recently killed cedar. Sampling points were placed 30 cm apart around the center point. The plot was, therefore, described by seven rows each of seven sampling points, with the center sampling point occupied by the diseased tree.

Cedar branchlets were prepared as previously described, and placed at alternate sampling points. The baits were left in the field for a period of two weeks during late spring. Air temperatures generally remained below 20 C, and several rain

showers occurred during the two week period. A 250 cc sample of soil was collected at each of the remaining sampling points at the time the cedar branchlets were collected.

The OMF was collected from each of the soil samples, placed in separate petri plates, and baited with five cedar foliage baits. Similarly, each of the branchlets served as the substrate for five secondary cedar foliage baits. Preparation of the branchlets as a baited substrate was described in a previous section. The plates were incubated at 18 C for six days.

The results obtained from both plots are shown in Table 2. Recovery of the fungus was more frequent when the OMF rather than the branchlet technique was used. The mean number of baits infected by P. lateralis differed significantly (P = 0.05) between detection methods in both plot trials.

Table 2. Comparison of baiting over branchlets subjected to infested soil and baiting over the organic matter fraction of the infested soil to detect Phytophthora lateralis.

Plot	Baited substrate	
	Cedar branchlets	Organic matter
1	0.083 a[z]	0.875 b
2	0 a	0.333 b

[z]Values indicate mean number of baits infected with P. lateralis of five baits in each of 24 replicate plates. Values followed by the same letter within any trial (plot) are not significantly different (P = 0.05) by Duncan's new multiple range test (Steel and Torrie 1960).

Baiting with Port-Orford Cedar Seedlings

Live seedlings were used as bait for the detection of P. lateralis in soil. Samples were collected from soils 2, 7, and 8 during the late winter season. Ten plastic pots (800 cc) of each soil were prepared, and one cedar seedling was transplanted into each pot. Ten pots of uncontaminated, artificially-mixed loam with one seedling per pot served as controls. Seedlings were maintained under greenhouse conditions for a period of 20 weeks. Plants were watered with tap water daily in an attempt to provide optimum conditions for disease development.

After 20 weeks, the root system of each tree was inspected for symptoms indicative of infection by P. lateralis. The taproot of only one seedling (soil 8) expressed disease symptoms. Several tissue pieces 0.5 cm long were cut from this root, surface sterilized in 1% NaOCl, rinsed in sterile, distilled water, damped dry, and plated on a standard cornmeal agar medium supplemented with pimaricin (20 mg/ml). P. lateralis was not isolated after incubation of the plates for five days at 20 C. Isolations were not attempted from any of the other plants, since no other root systems appeared diseased.

Baiting with Port-Orford Cedar Tissue Sections Buried in Soil

Cedar root and foliage sections were used as baits to detect P. lateralis in infested soil. Foliage baits were prepared as previously described. Cedar root baits were prepared from small (2 mm diameter) roots. The roots were washed in tap water and cut into sections 3 to 4 cm long. Samples from the previous collection of soils 2, 7, and 8 were used to prepare 20 125-ml plastic cups of each soil. Holes were made in all cups to allow for adequate drainage. Five foliage baits were buried in each of ten cups of each soil, and five root baits were buried in each of the remaining cups. The cups were maintained under greenhouse conditions and watered with tap water daily for a period of two weeks. The baits were then recovered, rinsed in distilled water, and examined for sporangia of P. lateralis. Sporangia were found on only one bait, a foliage bait from soil 7.

Baiting over Flooded Soil

Four types of bait were tested for their efficiency to detect P. lateralis in samples of each of the three soils used in the preceding experiment. The baits included cedar foliage, cedar roots, cedar germlings, and hemp seeds. Port-Orford cedar seeds were germinated on moist filter paper. Germlings were used as bait when they

had grown 1 to 2 cm in length. Hemp seeds were cut in half before use as baits.

Ten to 15 grams of soils 2, 7, or 8 were placed in petri plates and flooded (15 ml) with distilled water. Each bait was tested in ten replicate plates of each soil. Five baits were placed in each plate. The plates were placed in an incubator at 18 C for a period of six days. After incubation, all baits were examined for sporangia of P. lateralis. Sporangia were found on a total of six baits, including three cedar foliage baits and three cedar germlings (Table 3).

Baiting over Flooded Organic Matter

The OMF was collected from samples of each of the soils previously collected (2, 7, and 8). Ten to 15 grams of the OMF were placed in a petri plate and flooded with distilled water. The four bait types used in the preceding experiment were tested in each of ten replicate plates of organic matter. All plates were placed in an incubator at 18 C for six days. The baits were then examined for sporangia of P. lateralis.

The results of this and the two preceding experiments are shown in Table 3. Since the difference between the assay methods appeared obvious, only the final assay method was statistically analyzed. P. lateralis was recovered most frequently from the OMF of soils 7 and 8. There was no significant difference ($P = 0.05$) in any soil

Table 3. Comparison of three baiting methods used for the detection of Phytophthora lateralis in soil.

Bait	Baiting method		
	Baiting over organic matter	Baiting over soil	Burial of baits
	Soil 2		
Cedar foliage	0.3 a,b[z]	0.3	0
Cedar roots	0 a	0	0
Cedar germlings	0.3 a,b	0.3	
Hemp seeds	0 a	0	
	Soil 7		
Cedar foliage	1.2 b,c	0	0.1
Cedar roots	0.5 a, b	0	0
Cedar germlings	1.2 b,c	0	
Hemp seeds	0 a	0	
	Soil 8		
Cedar foliage	1.0 a, b, c	0	0
Cedar roots	0.3 a,b	0	0
Cedar germlings	1.6 c	0	
Hemp seeds	0 a	0	

[z]Values indicate the mean number of baits infected with P. lateralis of five baits in each of ten replicate plates or cups. Values followed by the same letter are not significantly different (P = 0.05) by Duncan's new multiple range test.

between the number of foliage baits and germling baits infected. Root sections were less effective as baits in all three soils than either foliage or germling baits, but were more effective than halved hemp seeds. P. lateralis was never found on any hemp seed baits. The hemp baits were difficult to examine microscopically because of their thickness and the presence of many contaminating organisms. Foliage baits were the easiest to prepare and examine, and were relatively free of other organisms.

Localization of Infectivity

Results of the comparative baiting experiments suggested that the source of inoculum for infection of baits by P. lateralis was localized in an OMF of the soil. This premise was tested more critically by further experimentation. An attempt was also made to determine the type of fungal structures present in the OMF which could account for this infectivity.

Localization of Infectivity in the Soil Organic Matter

An 800 gram sample of soil 6 was sieved through a 5 mm mesh screen and divided into two 400-gram aliquots. The OMF was removed from one aliquot by a variation of the standard collection method, as follows. A two-liter plastic beaker was filled with 1850 ml

of tap water. A double layer of cheesecloth was tied over the top of the container. The cloth was allowed to dip approximately 2 cm below the surface of the water. Soil was placed on the cloth and lightly agitated by hand until the mineral fraction had passed through the filter. The OMF remaining on the cloth was discarded. The mineral fraction was stirred into an even slurry, and 50 ml were added to each of 45 (20 mm deep) petri plates. Five cedar foliage baits were added to each plate, and the plates were placed in an incubator at 18 C for six days. After incubation, the baits were examined for sporangia of P. lateralis.

The second 400 gram aliquot of soil was used for the control. An even slurry was prepared by stirring the soil in 1850 ml of tap water. The OMF was not removed. Plates were then prepared as in the treatment.

The experiment was repeated using a sample of soil 7. However, only 1600 ml of tap water were used to make the slurries with each 400 gram aliquot. Therefore, only 40 treatment and 40 control plates were set up.

The results are shown in Table 4. The difference between the control and treatment means was significant ($P = 0.01$) for both trials. The removal of the OMF from soils 6 and 7 resulted, respectively, in a 2.8 and 15-fold decrease in the mean number of baits infected with P. lateralis.

Table 4. The effect of the removal of an organic matter fraction of the soil on the detection of Phytophthora lateralis.

Organic matter soil source	Soil with organic matter present	Soil with organic matter removed
6	2.20 a[z]	0.78 b
7	2.25 a	0.15 b

[z]Values indicate mean number of baits infected with P. lateralis of five baits in each of 40 (soil 7) or 45 (soil 6) replicate plates. Values followed by the same letters within the same trial (soil source) are not significantly different (P = 0.01) by Duncan's new multiple range test.

Fungal Structures Present in the Organic Matter

Organic material collected from soil 6 was examined microscopically for fungal structures of P. lateralis. Several grams of the organic matter were prepared for observation by the clearing and staining technique previously described. Examination of the prepared tissue revealed round, chlamydospore-like structures in relatively few of the tissue pieces. Structures which appeared to be fungal hyphae were also found occasionally in the organic material. The chlamydospore-like structures were not believed to be those of an endomycorrhizal fungus because of the presence of thick spore walls and the smaller size (approximately 40 μ) of the spores observed. These characteristics were similar to those of the chlamydospores of P. lateralis.

Roots of Port-Orford cedar known to be infected with P. lateralis were also examined for fungal structures. The fine, fibrous roots were sampled from several symptomatic cedar. The smaller (1 mm) diameter roots were cleared and stained. Hyphae and chlamydospore-like structures were also found in this material (Figure 5).

Factors Affecting the Detection of Phytophthora lateralis in Organic Matter

Accuracy of the data collected on the survival of P. lateralis is ultimately dependent on the technique used to assay for its presence. Several experiments were conducted in an effort to improve the detection technique of baiting over the OMF of a soil.

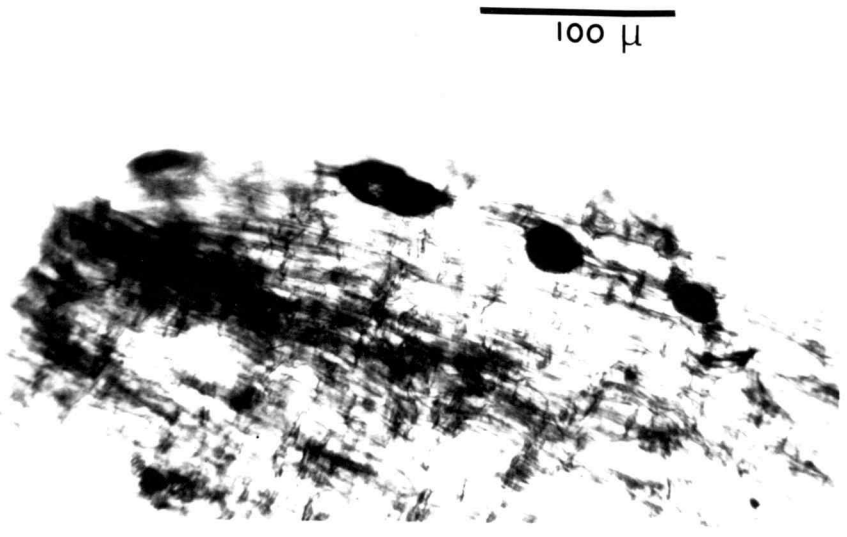
Optimum Temperature of Incubation

The effect of incubation temperature on the recovery of P. lateralis was investigated. The baiting technique was tested at 5, 10, 15, 20, 25, and 30 C. Three amounts of the OMF substrate were tested at each of the temperatures, since excessively high or low infectivity could have resulted in obscuring the relative temperature effects.

The OMF was collected from soil 6, thoroughly mixed, and placed in standard petri plates in aliquots of 1, 5, or 10 grams. Six replicate plates of each amount were set up for each temperature

Figure 5. Chlamydospore-like structures and mycelium in Port-Orford cedar root tissue. The root tissue was taken from a tree known to be infected with Phytophthora lateralis. Similar structures were occasionally found in the organic matter of infested soil.

5



treatment. The OMF was flooded with distilled water, and five cedar foliage baits were added to each plate. The plates were placed in incubators at their respective treatment temperatures for six days. After incubation, the baits were examined for sporangia of P. lateralis.

Results are shown in Table 5. The optimum incubation temperature for the detection of P. lateralis ranged between 15 and 20 C. A few baits incubated at 25 C were also infected. P. lateralis was not observed on any of the baits incubated at temperatures of 5, 10, or 30 C.

The experiment was repeated with the OMF collected from a sample of soil 7. The infectivity of the OMF was very low, and the fungus was recovered from only four of the 108 plates. Infected baits were found in one plate incubated at 10 C, two plates incubated at 15 C, and one plate incubated at 20 C.

Optimum Period of Incubation

The use of the baiting technique required a knowledge of the time period necessary for bait infection and subsequent fungus sporulation. The OMF was collected from a sample of soil 6 and thoroughly mixed. Five grams of this OMF substrate were added to each of 120 petri plates. The substrate was flooded with distilled water, and five cedar foliage baits were added to each plate. Plates were incubated at 18 C.

Table 5. The effect of incubation temperature and amount of organic matter substrate on the detection of Phytophthora lateralis.

Grams organic matter per plate	Temperature of incubation (°C)					
	5	10	15	20	25	30
1	0 a[z]	0 a	3.7 d	2.3 c	0.5 a, b	0 a
5	0 a	0 a	1.3 b,c	0.5 a, b	0.3 a, b	0 a
10	0 a	0 a	0.8 a,b	0.8 a, b	0.8 a, b	0 a

[a]Values indicate mean number of baits infected with P. lateralis of five baits in each of six replicate plates. Values followed by the same letter are not significantly different (P = 0.05) by Duncan's new multiple range test.

The experiment was conducted over a period of 12 days. Ten plates were removed from the incubator at 24 hour intervals, and the baits examined for sporangia of P. lateralis. The experiment was repeated with organic matter collected from a sample of soil 5.

The results of both trials are shown in Table 6. Bait infection and fungus sporulation took place after a minimum incubation period of three days. There was no significant increase ($P = 0.05$) in the number of baits infected after five days.

Optimum incubation time was considered that period long enough to allow for initial infection, yet short enough to preclude infection of new baits from infected baits. A second cycle of infection would bias the estimate of the infectivity in the organic matter being tested. Therefore, six days at 18 C became the standard incubation period of the OMF recovery technique.

Effect of Amendments over Time on Detection

The ability of two amendments to increase the number of baits infected with P. lateralis from the OMF was tested. The experiment was conducted four times. The OMF from a sample of each of soils 2 and 7 was collected. Enough organic material was collected from each source to run the experiment twice.

Table 6. The effect of incubation time on infection of cedar foliage baits by Phytophthora lateralis.

Incubation time at 18 C (days)	Soil source of organic matter fraction	
	5	6
1	0 a[z]	0 a
2	0 a	0 a
3	0 a	0.1 a
4	0.8 a, b	4.0 b
5	1.6 a, b	4.8 c
6	1.1 a, b	5.0 c
7	0.7 a, b	5.0 c
8	1.5 a, b	5.0 c
9	1.4 a, b	4.8 c
10	2.1 b	5.0 c
11	1.6 a, b	5.0 c
12	2.3 b	5.0 c

[z]Values indicate mean number of baits infected with P. lateralis of five baits in each of ten replicate plates. Values followed by the same letter within any trial (soil source) are not significantly different (P = 0.05) by Duncan's new multiple range test.

A cedar root extract amendment was prepared by blending 300 grams of fine (2 mm diameter) Port-Orford cedar roots in 500 ml of distilled water in a Waring blender for one to two minutes. The resulting mixture was expressed through cheesecloth. Approximately 350 ml of the extract was recovered. The root tissue material left after extraction was discarded. The extract was diluted with 1450 ml of distilled water.

The cedar foliage extract amendment was prepared in the same way, but used 300 grams of fresh cedar foliage in place of the root material. The 350 ml of recovered extract was diluted with 1050 ml of distilled water.

Five grams of organic material were placed in each of 90 petri plates. Thirty plates each received 15 ml of root extract, 30 received 15 ml of foliage extract, and 30 received 15 ml of distilled water as a control. Five cedar foliage baits were added to each of ten plates in each treatment, and all 90 plates were placed in an incubator at 18 C. After three days, foliage baits were added to ten additional plates in each treatment, and after seven days foliage baits were added to the remaining 30 plates. Plates remained in the incubator for six days after the addition of the baits. Baits were then examined for sporangia of P. lateralis.

The results are shown in Table 7. Flooding the substrate in the root extract amendment or in distilled water for a period of three or

Table 7. The effect of pre-treating the organic matter substrate with water, a cedar foliage extract amendment, or a cedar root extract amendment on detection of Phytophthora lateralis.

Time of bait addition	Amendment	Soil source of organic matter fraction			
		2	7	2	7
Immediately	Foliage	0 a[z]	0 a	0 a	0 a
	Root	0 a	0 a	0 a	0.5 a
	Distilled water	0 a	0 a	0.2 a	0.9 a
At 3 days	Foliage	0 a	0 a	0 a	0 a
	Root	0.3 a, b	1.0 b, c	0.1 a	0.2 a
	Distilled water	1.1 b, c	1.5 c	0.6 a, b	0.5 a
At 7 days	Foliage	0 a	0.3 a, b	0.6 a, b	0 a
	Root	0.6 a, b	0.2 a	1.5 b	1.1 a
	Distilled water	1.6 c	0.1 a	0.5 a, b	0.5 a

[z]Values indicate the mean number of baits infected with P. lateralis of five baits in each of ten replicate plates. Values followed by the same letter within any trial are not significantly different (P = 0.05) by Duncan's new multiple range test.

seven days prior to the addition of baits significantly increased ($P = 0.05$) the mean number of baits infected by P. lateralis. This increase was significant in three of the four trials. The foliage extract amendment was not effective in increasing the number of baits infected at any time in any of the trials.

Effect of Blending the Organic Matter Substrate on Detection

Infection of baits by P. lateralis from blended and unblended organic matter was compared. A sample of 100 grams of the OMF was collected from each of soils 2, 5, and 7. The sample was thoroughly mixed and divided into two 50-gram aliquots. One aliquot was blended in a Sorvall Omnimixer (model 0.-115, Ivan Sorvall, Inc., Norwalk, Connecticut) with an equal volume of distilled water for one to two minutes. After blending, additional water was added until the final volume of the blended slurry was 200 ml. Twenty ml of the slurry were poured into each of ten petri plates. An additional ten plates were prepared using the second aliquot of the OMF as a control. Five grams of the unblended organic matter were added to each of ten petri plates. The organic substrate was then flooded with distilled water. Five cedar foliage baits were added to all plates and incubated at 18 C for six days. After incubation, the baits were examined for sporangia of P. lateralis.

The results of the four trials are shown in Table 8. Differences between control and treatment means were not significant in any trial.

Table 8. The effect of blending the organic matter substrate on the detection of Phytophthora lateralis.

Soil source of organic matter	Blended organic matter	Non-blended organic matter
2	0[z]	0.3
5	0	0.4
7	0.5	2.0
7	0	0.2

[z]Values indicate mean number of baits infected with P. lateralis of five baits in each of ten replicate plates. Values are not significantly different (P = 0.05) within any trial by Duncan's new multiple range test.

Survival of Phytophthora lateralis in Organic Matter

Temperature, moisture, and biological interactions greatly influence the ability of an organism to survive in the soil. The survival capability of P. lateralis was tested under a variety of physical and biological conditions. Presence of the fungus was assayed using the direct OMF recovery method.

Effect of Temperature on Survival in Isolated Organic Matter

The survival of P. lateralis was tested over a five month

period. The OMF was collected from a sample of soil 6 and divided into seven 100-gram samples. Each sample was placed in a tightly sealed plastic bag and stored at one of seven temperatures. Storage temperatures of -5, 5, 10, 15, 20, 25, and 30 C were used. Samples were stored at moisture contents of 20% [(g water/g dry soil) x 100].

At two week intervals, ten grams of organic matter were removed from each sample. One gram was placed in each of ten replicate petri plates, flooded with distilled water, and baited with five cedar foliage baits. Plates were incubated for six days at 18 C. Baits were then examined for sporangia.

Results are shown in Table 9. The infectivity of the OMF stored at either 5, 10, or 15 C did not significantly ($P = 0.01$) change over the five month period. Infectivity remained very high throughout this time, indicating a high survival potential of the fungus when subjected to these temperatures. However, a slight, steady decrease in infectivity occurred after 16 weeks when the OMF was stored at 20 C. In this experiment, P. lateralis was shown to survive a maximum of 16 weeks at -5 C, 14 weeks at 25 C, and only two weeks at 30 C.

Effect of Temperature and Moisture on Survival in Soil

Field soils infested with P. lateralis were stored up to four months under various conditions of moisture and temperature. The

Table 9. The effect of temperature on the survival of Phytophthora lateralis in organic matter.

Storage time (weeks)	Temperature (°C)						
	-5	5	10	15	20	25	30
2	1.3 b, c, d[z]	5.0 g	4.9 g	4.7 g	5.0 g	4.3 f, g	0.2 a
4	0.6 a, b	5.0 g	4.8 g	5.0 g	4.9 g	1.8 d	0 a
6	0.5 a, b	5.0 g	4.7 g	4.8 g	4.7 g	1.8 d	0 a
8	0 a	4.9 g	5.0 g	5.0 g	4.6 f, g	1.6 c, d	0 a
10	0 a	4.9 g	4.9 g	4.9 g	4.9 g	0.8 a, b, c	0 a
12	0.6 a, b	5.0 g	5.0 g	4.9 g	5.0 g	1.6 c, d	0 a
14	0.1 a	4.9 g	5.0 g	4.6 f, g	4.5 f, g	0.4 a, b	0 a
16	0.1 a	4.9 g	5.0 g	4.8 g	4.2 f, g	0 a	0 a
18	0 a	4.9 g	4.8 g	5.0 g	3.7 f	0 a	0 a
20	0 a	4.6 f,g	4.8 g	4.8 g	2.8 c	0 a	0 a

[z]Values indicate the mean number of baits infected with P. lateralis of five baits in each of ten replicate plates. Values followed by the same letter are not significantly different (P = 0.01) by Duncan's new multiple range test. Time "0" control value was 4.7 baits infected (of ten replicate plates).

experiment was conducted once with OMF collected from soil 5, and repeated with OMF collected from soil 7.

Approximately 72 liters of the appropriate soil were collected, sieved through a 5 mm mesh screen, and thoroughly mixed. The soil was then divided into four 18-liter aliquots. One aliquot was saturated with tap water. Two aliquots were spread evenly on a greenhouse bench and allowed to dry, each for a different period of time (Table 10). The moisture content of the fourth aliquot was not adjusted. The aliquots, therefore, represented four moisture treatments.

Soil 5 had an approximate moisture content of 37% (-0.3 bars matric water potential) when collected, 35% (-0.3 bars) when dried for one hour, and 9% (-25 bars) when dried for 24 hours. Soil 7 had an approximate moisture content of 45% (-0.1 bars) when collected, 22% (-0.1 bars) when dried for six hours, and 19% (-0.1 bars) when dried for 24 hours. The water potentials of the soils were determined by a standard pressure-plate technique.

The soil at each moisture level was divided into nine 2-liter samples, and each sample was placed in a tightly sealed plastic bag. Four samples from each moisture treatment were stored at 5 C, and four were stored at room temperature (20 C). One sample from each moisture treatment was assayed after every storage interval. Assaying involved collecting the OMF, placing five grams of the material in

Table 10. The effect of temperature and moisture on the survival of Phytophthora lateralis in soil.

Moisture treatment	Storage time (weeks)								
	0	2		4		8		16	
		5 C	RT[x]	5 C	RT	5 C	RT	5 C	RT
				Soil 5					
Saturated	2.7 b-h[z]	3.3 d-j	2.5 b-g	1.4 b	3.5 e-j	4.1 h-j	0.1 a	4.3 i, j	0 a
37%[y]	3.9 g-j	2.4 b-f	2.7 b-h	3.2 d-j	2.9 d-h	3.1 d-i	1.9 b-d	4.5 j	3.3 d-j
35%	2.6 b-g	4.3 i, j	3.9 g-j	2.8 c-h	3.0 d-i	1.5 b, c	2.1 b-e	3.7 f-j	3.3 d-j
9%	0.2 a	0 a	0 a	0 a	0 a	0 a	0 a	0.1 a	0.2 a
				Soil 7					
Saturated	0.3 a, b	1.0 a, b	0 a	0 a	0 a	0.6 a, b	0 a	0.2 a	0 a
45%	0 a	0.8 a,b	0.6 a, b	2.0 c	0.2 a, b	0.1 a.b	0.4 a, b	1.0 a-c	0.7 a, b
22%	1.2 b, c	0.8 a,b	1.2 b, c	0.1 a, b	0.5 a, b	0.5 a, b	0.4 a, b	0.5 a, b	0 a
19%	0 a	0.5 a,b	0 a	0 a	0.5 a, b	0 a	0.3 a, b	0.8 a, b	0 a

[x]Room temperature (20-22 C).

[y]Moisture content determined by the gravimetric method [(g water/g dry soil) x 100].

[z]Values indicate mean number of baits infected with P. lateralis of five baits in each of ten replicate plates. Values followed by the same letter within the same treatment are not significantly different (P = 0.05) by Duncan's new multiple range test.

each of ten replicate plates, and flooding with distilled water. Five cedar foliage baits were added to each plate, and all plates were placed in an incubator at 18 C for six days. Baits were then examined for sporangia of P. lateralis.

Table 10 shows the results of the two trials. Storage of soil 5 for eight weeks at either temperature, and at moisture contents of either 37% or 35% had no apparent effect on the survival of P. lateralis in the OMF. Drying the soil to a moisture content of 9% prior to storage, however, decreased survival of the fungus drastically. Although data indicate survival for 16 weeks at this low level of moisture, additional experiments should be conducted to verify this.

Survival of P. lateralis in the OMF was good during storage in saturated soil at 5 C, but apparently decreased in saturated soils stored at room temperature. The results of both trials indicated this trend. The OMF from soil 7 was relatively lower in infectivity than the OMF of soil 5. Variability of the results obtained from the OMF of soil 7 restricts analysis to general observations. P. lateralis was apparently able to survive equally well in soil 7 under all conditions except as noted above.

Effect of Biological Environment on Survival in Soil

Survival of P. lateralis in an OMF subjected to various

biological environments was tested over a six month period. Samples of soils 1, 3, and 4 were collected and sieved through a 5 mm mesh screen. Thirty clay pots (800 cc) were filled with soil 1, 50 with soil 3, and 30 with soil 4. Ten pots of each soil type received one of the three following treatments: untreated (raw), steamed (four hours at 5 lb pressure), planted to its native tree species with one seedling per pot. The remaining 20 pots of cedar soil were each planted with one seedling of either alder (ten pots) or Douglas-fir (ten pots). Thus, 11 different biological environments were each "replicated" ten times (Table 11).

A large quantity (1300 grams) of the OMF was collected from soil 6 and thoroughly mixed. Ten grams of this material were completely mixed into the soil of each pot. The OMF was added to all pots before seedlings were planted, but after treatment when soil was steamed. All pots were watered daily and maintained under standard greenhouse conditions.

Infectivity of a 25 gram sample of the OMF was tested immediately after collection as a time control. One gram of material was placed in each of 25 petri plates and flooded with distilled water. Five cedar foliage baits were added to each plate, and plates were incubated at 18 C for six days. After incubation, the baits were examined for sporangia of P. lateralis.

Table 11. The effect of various biological environments on the survival of Phytophthora lateralis.

Soil treatment	Time period (months)	Soil 3	Soil 4	Soil 1
Untreated	3	0.72 a, b[z]	0.48 a, b	0.92 a, b
	6	1.16 a, b, c	0.60 a, b	0.68 a, b
Steamed	3	0.44 a, b	0.48 a, b	0 a
	6	0.56 a, b	0.32 a, b	0.44 a, b
Planted to native tree	3	3.68 f	1.72 c, d, e	0.32 a, b
	6	2.64 e, f	1.80 c, d, e	0.36 a, b
Planted to alder	3	1.20 a, b, c		
	6	2.48 d, e, f		
Planted to Douglas-fir	3	1.08 a, b, c		
	6	2.84 e, f		

[z]Values indicate mean number of baits infected with P. lateralis of five baits in each of 25 replicate plates. Control (time - 0) mean was 4.7 based on 25 replicate plates. Values followed by the same letter are not significantly different (P = 0.05) by Duncan's new multiple range test.

After three months (March through May, 1975), five replicate pots in each treatment were individually assayed. The OMF of each pot was thoroughly mixed. Half of the material was discarded, and the other half was divided equally into five plates. By dividing the OMF in this way, the infectivity of the OMF in the treatment plates (five pots, five plates per pot) was comparable to the infectivity of the OMF in the 25 control plates.

The remaining 55 pots were placed in an outside cold-frame and shaded with a double layer of cheesecloth for the duration of the experiment (June through August, 1975). At the end of August, these pots were processed as described above.

Results of this experiment appear in Table 11. P. lateralis was recovered from the organic matter from 10 of the 11 treatments after three months, and from all treatments after six months. There was no significant difference between the mean number of baits infected by P. lateralis from OMF stored in steamed soil or raw soil. P. lateralis from the OMF of soils planted to cedar seedlings, however, was able to infect more baits than the inoculum in soils 1 and 4 planted to non-host species. No other obvious treatment differences were found.

DISCUSSION

The Baiting Technique

The development of a technique for the detection of P. lateralis in soil was prerequisite to investigating the survival of the fungus in the soil. A suitable technique was developed during the course of this investigation. The analysis which follows considers the advantages and disadvantages of this technique, and will permit a more sound interpretation of the dependent survival data.

The use of organic material as a substrate which is baited with cedar foliage has proven to be superior to all other techniques tested specifically for the detection of P. lateralis in soil (Tables 2 and 3). Assays can be set up rapidly. The OMF can be collected from a soil sample of two to five liters in less than 15 minutes, the processing time being influenced more by the number of individual samples than the quantity of each sample. The method of collecting the OMF is simple in application, and requires no specialized equipment. Fresh cedar foliage baits are effective, easily prepared, and available in all seasons.

Assay results are obtained within six days, rather than the two weeks or longer required by the branchlet technique or the planted seedling assay. The separation of the OMF from the mineral

fraction has the effect of concentrating the infested material. Therefore, the same amount of soil can be assayed with fewer plates than are necessary for baiting directly over the soil. In addition, concentration of the OMF can possibly increase the propagule density, in any single replicate, to above the threshold density necessary for bait infection to occur.

Another important advantage of the OMF baiting technique is its ability to recover P. lateralis from infested soil during any season of the year. The fungus has been recovered from several different soils during the summer months.

There are, however, several disadvantages of this baiting technique. The technique does not appear to be suitable, in its present state of refinement, for use in determining the presence or absence of the pathogen in soil prior to symptom expression of diseased trees. P. lateralis probably occurs in small, isolated areas in newly infested forest stands. An intensive sampling of the forest site may be necessary to identify these areas. Bait examination resulting from such intensive sampling may be too time consuming to warrant the use of this technique, as it takes approximately one hour to examine 50 foliage baits. This problem may best be handled by the use of a detection method similar to Trione's (1957) branchlet technique. It must be recognized that the use of the branchlet technique is restricted

to cool, wet seasons and requires a long (two month) period of time before results are obtained.

The OMF baiting technique is considered to be conservative in establishing the presence of P. lateralis. The technique relies on positive bait infection. Further, infected baits on which sporangia are not produced cannot be distinguished from uninfected baits. This problem could be overcome by plating suspect baits on an antibiotic medium to obtain Phytophthora colonies which could then be identified (Pratt and Heather 1972).

The OMF baiting technique, made somewhat quantitative by using five baits in each replicate plate, is less accurate if secondary infection cycles are allowed to occur. Infection of the cedar foliage baits, and the subsequent sporulation of P. lateralis was found to require a minimum of three to four days of incubation (18 C). These results agree with those reported by other investigators using similar techniques (Chee and Newhook 1965, Dance et al. 1975). The assumption was made that secondary infection cycles of P. lateralis would take a minimum of six days. The technique cannot detect the presence of P. lateralis if the fungus is present in a form which is unable to either directly or indirectly infect, and sporulate on cedar foliage baits within six days. It is conceivable that oospores, and possibly chlamydospores of P. lateralis require a longer incubation period at 18 C before germinating (Trione 1974). When longer incubation

periods were tested (Table 7), detection of the fungus was more frequent. This could be indicative of a greater amount of spore germination, but there is no conclusive evidence. Further research is needed to clarify this problem.

Survival of *Phytophthora lateralis* in Soil

Phytophthora species other than *P. lateralis* have been implicated to survive principally in the infested organic matter of the soil. Batini and Cameron (1971) have shown that the spread of *P. cinnamomi* by natural means is much slower than its spread by "the transport of soil containing infected root material" by humans. Gerrettson-Cornell (1973) has made a specific point of collecting "soil-and-root samples" when assaying for *P. cinnamomi*. Ho (1969) has concluded that "it is most probable that *P. megasperma* var. *sojae* persists in the soil within remains of diseased soybeans." Results presented in this thesis show that the inoculum in soils infested with *P. lateralis* is, with little doubt, present largely in infested foliage and root debris of Port-Orford cedar.

P. lateralis has been demonstrated to survive for four months in naturally infested soils (Table 10). Studies of the survival of *P. lateralis* under extreme laboratory conditions and field conditions support this observation (Tables 9 and 11). Results also suggest that the fungus is able to survive for periods much longer than those for which it was tested.

Survival of P. lateralis was significantly affected by time, temperature, and a time-temperature interaction (Table 9). Temperatures between 5 and 20 C favored long-term survival. This closely reflects the temperature range in which P. lateralis can actively grow (Trione 1974). Although the fungus was shown to survive at an extremely cold (-5 C) temperature for up to 16 weeks, a temperature of 30 C limited survival to two weeks. The extent to which the fungus is protected from temperature extremes by the organic matter is not known.

Survival is greatly decreased when soils are dried to less than -15 bars of matric water potential. This is thought to be the case for most soil fungi (Menzies 1963). Soil physical properties greatly influence the survival of microorganisms by influencing water availability. The moisture contents of soils 5 and 7 (Table 10) were decreased by 28% and 26%, respectively, when dried for 24 hours. However, the moisture availability of soil 7 was not noticeably changed (-0.1 bars matric water potential), while that of soil 5 decreased to a level well below (-25 bars) those favorable for fungal growth and survival. This relationship obviously has important control implications (Batini 1973).

P. lateralis survived better in saturated soils stored at 5 C than at room temperature. Two explanations which may account for this difference are 1) since the metabolic rate of microorganisms

increases with increasing temperature, an increase in the oxygen consumption could have created anaerobic conditions more quickly, resulting in the death of the aerobes, and 2) higher temperatures could have favored the growth of antagonistic microorganisms.

The results of investigations testing the survival of P. lateralis under a variety of biological conditions support those of previous survival studies (Zentmyer and Mircetich 1966, Westeijn 1973, Apple 1963). P. lateralis was recovered from soil after a period of six months (Table 11) when the soil was maintained under approximate field conditions of temperature and moisture characteristic of the spring and summer seasons. The ability of P. lateralis to survive in steamed soils did not apparently differ from its ability to survive in raw soils. These results suggest that P. lateralis is present in the OMF in an inactive form (chlamydospore or oospore). A rapid colonization of the steamed soil by an aggressive microbial population may have taken place within a few days after treatment. Evidence by Reeves (1975) and others indicates that such organisms would be more detrimental to the active growth forms (mycelium and zoospore) than to the inactive forms.

Recovery of the fungus from soils planted to cedar seedlings was significantly higher than from soils planted to non-hosts. The presence of the host plant probably caused an increase in inoculum of P. lateralis during this time interval.

Only one cedar seedling succumbed to the fungus before termination of the experiment. This indicates, as did the trial which used planted seedlings as bait (Table 3), that a long period of time is often necessary for host infection and subsequent tree death to take place.

Recovery of P. lateralis was slightly higher from cedar soils after six months than after three months in three treatments (untreated soil, soil planted to Douglas-fir, soil planted to alder). This suggests that the fungus may have some true saprophytic ability under certain, specific conditions.

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