

**ASPECTS RELATING TO THE
SPREAD AND SURVIVAL OF PHELLINUS ROOT ROT**

by

DAVID ROBERT RUSCH

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ABSTRACT

Active and inactive edges of a *Phellinus weirii* root rot center were compared to see if the reason for the inactivation of part of the center could be explained. The inactivation could not be shown to be the result of differences in root contacts or soil factors. Hypovirulence caused by a dsRNA virus was also investigated as a possible cause for the inactivation of *Phellinus* root rot centers. A survey of several isolates of *P. weirii* from British Columbia and the United States failed to reveal the presence of dsRNA in any of the isolates. The possible role of zone lines in the survival of *P. weirii* was also investigated. The rate of moisture loss from Douglas-fir blocks with and without zone lines was compared to see if zone lines slow the rate of moisture loss from decayed wood. No significant differences ($\alpha = 0.05$) could be detected in the rate of moisture loss of blocks with and without zone lines.

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1.0 GENERAL INTRODUCTION

Phellinus weirii (Murr.) Gilbertson is the cause of laminated root rot, an important disease affecting a number of conifers throughout southern British Columbia and the northwestern United States. *P. weirii* was first described on western red cedar (*Thuja plicata* Donn) by Murril (1914). Later, Mounce et al. (1940) described it on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Subsequent studies have indicated that there are two distinct types of *P. weirii* designated the cedar type and the Douglas-fir type. The separation of *P. weirii* into two distinct types is based on physiological, morphological, and genetic differences as well as differences in host specificity (Angwin & Hansen 1989, Bae 1992, Larsen & Lombard 1989). The cedar type causes a butt rot of western red cedar and is of relatively minor economic importance compared to the Douglas-fir type. The Douglas-fir type, the cause of laminated root rot, has a number of conifer hosts. Douglas-fir, amabilis fir (*Abies amabilis* (Dougl. ex Loud.) Forbes), grand fir (*Abies grandis* (Dougl. ex D. Don) Lindl.), and mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.) are the most susceptible hosts to the Douglas-fir type. Subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), larches, spruces, and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) are moderately susceptible, pines are slightly susceptible, western red cedar is resistant, and hardwoods are immune to *Phellinus* root rot. This thesis deals exclusively with the Douglas-fir type of *P. weirii*.

Annual mortality to laminated root rot has been estimated at more than one million m³/yr (Wallis 1976). *P. weirii* can kill trees directly or indirectly by predisposing them to wind throw and attacks by bark beetles. The average incidence of *Phellinus* root rot on southeastern Vancouver Island is between 6 and 11 % (Beale 1992). The incidence of *Phellinus* root rot in other parts of B.C. is not well documented.

There are two stages in the life cycle of *P. weirii*: a spreading or parasitic stage, and a survival stage. The parasitic stage begins when the roots of a susceptible tree come into contact with the decayed roots or stump of a tree previously infected by *P. weirii*. Spore initiated infections are believed to be rare occurrences (Childs 1963).

After roots become infected, *P. weirii* spreads along the root surface by means of ectotrophic mycelium. Once the fungus becomes well established on the root surface it invades and penetrates the bark tissue. The fungus continues to spread along the root both ectotrophically and endotrophically. Ectotrophic mycelial fronts generally precede endotrophic mycelial fronts by 1.2 - 31.8 cm (Bloomberg & Reynolds 1982). The distance between ectotrophic and endotrophic mycelial fronts may be related to bark thickness (Wallis & Reynolds 1962). Ectotrophic mycelium is seldom observed on those portions of roots growing in the duff (Wallis & Reynolds 1965).

The first indication of decay by *P. weirii* is a red brown staining of the wood. The red brown staining is clearly evident in freshly cut stumps of trees infected by *P. weirii*. The red brown staining is followed by a pitted white rot. In the advanced stages of decay the wood separates along the annual rings giving the wood a distinct laminated appearance. The laminated decay and ectotrophic mycelium are the most reliable indicators of infection by *P. weirii*.

Above ground symptoms of Phellinus root rot, in order of appearance include: reduced height growth, a decrease in needle size and abundance, a decrease in diameter growth increment, yellowing of the crown, distress cones, and eventually death. Symptom expression occurs very soon after infection in young trees but several years can pass between the time of infection and symptom expression in older trees. In old trees *P. weirii* appears to be confined mainly to the inner heart wood and acts more like a butt rot than a tree killer. Other factors such as site quality, host resistance, pathogen virulence, and inoculum potential may also influence the length of time between infection and symptom expression. Host response to infection in older trees includes: barrier zone formation, the formation of tangential bands of

traumatic resin ducts, resin production, callusing, and adventitious root formation by infected roots (Fig. 1-3).

The fungus spreads from tree to tree by means of root to root contacts. Although some root to root spread occurs via endotrophic mycelium, the main method is via ectotrophic mycelium (Bloomberg & Reynolds 1982). The root to root spread of the disease results in the formation of root rot centers. A typical center might consist of an opening surrounded by dead fallen or standing trees that are in turn surrounded by trees showing symptoms of root rot (Fig. 4). Fallen trees often have very little in the way of support roots and lie in a crisscross pattern that indicates that decay and not wind was the major reason they fell over. Within centers, susceptible conifers are often replaced by western red cedar or hardwoods.



Fig. 1 *P. weirii* infected Douglas-fir root showing callus tissue (c), adventitious roots (a), and resin production (r).



Fig. 2 Cross section of a callus formed on a Douglas-fir root in response to an infection by *P. weirii*. Note the darkly stained barrier zone (b) between the healthy and decayed wood. Tangential bands of resin ducts (rd) can also be seen in the unstained wood.



Fig. 3 A longitudinal section through a callus formed on a Douglas-fir root in response to an infection by *P. weirii*. The arrow marks the boundary between dead and living tissue. b, barrier zone. rd, resin ducts.



Fig. 4 Douglas-fir trees along the edge of a *Phellinus* root rot center showing typical crown symptoms. The trees in the lower right have rounded crowns and sparse chlorotic foliage. The tree in the upper left appears to have a crop of distress cones.

Through its effects on stand structure and species composition *Phellinus* root rot can have a major impact on the spatial and temporal diversity of forest landscapes (van der Kamp 1991). The effect of *Phellinus* root rot on the forest landscape is particularly noticeable in the interior of B.C. where *P. weirii* commonly occurs together with another root pathogen, *Armillaria ostoyae* (Romagn.) Herink. The occurrence of these two root diseases results in large patches of mixed hardwoods and conifers where conifers would otherwise predominate.

Phellinus root rot centers expand outwards at a rate of about 0.35 m/yr (Bloomberg 1983, McCauley and Cook 1980, Nelson & Hartman 1975); however, considerable variation exists in the rate of spread of individual root rot centers and in the rate of radial spread in different directions within root rot centers (Bloomberg 1990, Hansen et al. 1983). In extreme cases, centers or parts of centers may stop spreading and become inactive. The rate of spread of *P. weirii* root rot centers is correlated with factors that influence the probability of root

contacts between trees. Such factors include diameter at breast height, stand density, and soil properties.

The survival stage in the life cycle of *P. weirii* begins when there are no more live susceptible hosts to which the fungus can spread. This is a critical stage for the continued survival of the fungus because considerable time may elapse before a susceptible host is present again. During this time the fungus survives as a saprophyte in decayed stumps and roots, sometimes for periods of 50 years or longer (Hansen 1979a). When it is no longer actively spreading, *P. weirii* forms brown crust like mycelial structures called zone lines in or on infected wood and bark (Fig. 5). These zone lines are composed of sheets of dark pigmented, thick walled hyphae (Nelson 1973). Zone lines are thought to play an important role in keeping out potential competitors and protecting *P. weirii* from conditions unfavorable for its survival. Eventually other organisms are able to penetrate the zone line and as this occurs a succession of new zone lines are laid down ahead of the advancing organisms.

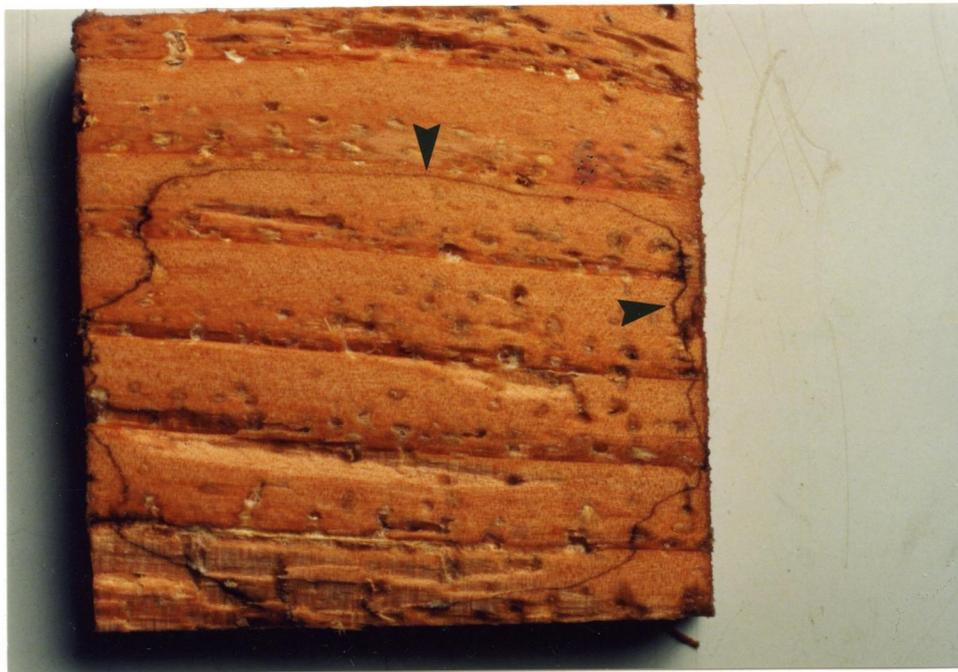


Fig. 5 Zone lines of *P. weirii* in a decayed Douglas-fir block.

The ability of *P. weirii* to survive for long periods is an important factor in determining

inoculum potential. Inoculum potential is the energy available for infection of the host at the point of infection (Garrett 1970). As stands become older, inoculum potential decreases as susceptible species are replaced and the energy supplies of the fungus are used up (Morrison et al. 1991). Inoculum potential and the probability of root contacts between susceptible host species are the most important factors in determining the incidence of Phellinus root rot. Where Phellinus root rot is ignored or goes unrecognized, standard silvicultural practices can lead to an increase in its incidence in second growth stands (Tkacz & Hansen 1982). It is common practice to plant high densities of single species of conifers within one or two years of harvest. If the species being planted is susceptible to Phellinus root rot, this results in a situation in which there is a high inoculum potential and high probability of root contacts.

This thesis deals with aspects of both the spread and survival of *P. weirii*. Part I is an investigation into possible causes for the inactivation of Phellinus root rot centers. Part II is an investigation of the possible roles zone lines may play in the long term survival of *P. weirii*.

2.0 PART I: POSSIBLE CAUSES FOR THE INACTIVATION OF PHELLINUS ROOT ROT CENTERS

2.1 Introduction

Phellinus root rot is currently controlled by planting alternate species or by removing infected stumps. In B.C., alternate species include pines, western red cedar, and hardwoods. White pine (*Pinus monticola* Dougl.) shows good resistance to *P. weirii* but is not generally planted because of its susceptibility to the white pine blister rust pathogen *Cronartium ribicola* Fisch. In most situations, planting with an alternate species is the best and most efficient way of dealing with the disease. On some sites however, pine or cedar are not preferred species, and planting with hardwoods may be uneconomical. Stump removal can be costly and is limited to flat areas with machine accessibility and a low potential for site degradation as a result of soil compaction or erosion. Control of Phellinus root rot by stump fumigation (Thies & Nelson 1987) and biological control by inoculation of stumps with *Trichoderma viride* Pers.:Fr (Nelson & Thies 1985) are also presently being tested in the United States. Stump fumigation will probably never be used on a widespread basis even though preliminary results appear promising because of cost and environmental and safety concerns. Inoculation of stumps with *T. viride* has been partially successful but significant improvements in stump colonization will be needed before *Trichoderma* can be used to achieve biological control (Nelson & Thies 1985).

The purpose of this study was to examine possible causes for the inactivation of Phellinus root rot centers. Determination of the factors responsible for the inactivation of root rot centers could potentially lead to new and innovative methods of controlling Phellinus root rot. Possible explanations for the inactivation of Phellinus root rot centers include:

- 1) a lack of root contacts between suitable hosts
- 2) various site factors (such as soil nutrients, pH, temperature, moisture, bulk density, etc.)

- 3) organisms antagonistic to *P. weirii* that prevent its spread
- 4) reduced virulence of the pathogen (hypovirulence)
- 5) host resistance.

The possibility that one or more of these factors may play a role in the inactivation of *Phellinus* root rot centers was investigated by studying a single root rot center with both active and inactive portions. The study began by investigating the possibility that the inactivation was due to lack of root contacts. After a lack of root contacts was ruled out as a probable cause of inactivation, the possibility that site factors or antagonists were responsible for the inactivation was investigated by comparing the growth of *P. weirii* on roots and branches when exposed to soil from active and inactive portions of the center. Hypovirulence was investigated by screening several isolates, including those from the root rot center in the Malcolm Knapp research center, for the presence of double-stranded RNA. Double-stranded RNA (dsRNA) is associated with hypovirulence in a number of plant pathogens. Host resistance was not directly looked at in this study.

2.2 Site Description

In 1957 a spacing trial involving Douglas-fir was set up at the Malcolm Knapp Research Forest in Maple Ridge, B.C. Two separate *Phellinus* root rot centers were started when roots from trees came into contact with old infected stumps left over from the previous stand. These centers later merged to form one larger center (Fig. 6). The date when each tree died was recorded on a metal stake located in the general area where each tree was located. The dates were based on observations made by Dr. Bart van der Kamp over several years. The center was actively spreading along its northern edge and the western part of its southern edge but had stopped spreading along its southeastern edge in 1992. A clearing created by snow press had prevented the disease from spreading west. For the purposes of this study, the edge of the center was divided into active and inactive parts (Fig. 7). The edge was defined as the halfway point between previously infected trees and trees that were uninfected at the start of

the study. Although the designation of what constituted an active and inactive edge was somewhat arbitrary, in all cases trees along the active edge had just begun or had not yet begun to show symptoms; while trees along the inactive edge had been dead for thirteen years or more (Fig. 7). The uniform spacing of the trees, known history of the center, and the fact that it contained an active and an inactive edge made it an ideal site for investigating the inactivation of *Phellinus* root rot centers. The size of the inactive edge also rules out host resistance as a possible cause for the inactivation since at least eight adjacent trees would have had to been completely resistant to *P. weirii* for resistance to have been the cause of the inactivation.

The root rot center was located just south of the Alouette River at approximately 5456 700m.N. and 531 700m.E. in zone 10 (Universal Transverse Mercator coordinates). The elevation at this location was 160 m above sea level. The terrain was smooth to gently rolling. The north side of the site was flat, while the south side was gently sloping (8 %) with a 110° aspect.

The area receives about 2166 mm annual water equivalent and the average temperature is about 9 °C (Reukema & Smith 1987). According to the system of biogeoclimatic ecosystem classification (Meidinger & Pojar 1991), the site is located in the dry maritime subzone of the coastal western hemlock zone. The soil moisture regime is fresh, and the soil nutrient regime is rich. The site belongs to the Cedar-Foamflower site association. There was a marked difference in the understory vegetation in the active and inactive portions of the root rot center. Along the active edge, the vegetation consisted mostly of mosses and *Lactuca muralis* (L.) Fresen (Fig. 8). There was a dense shrub and herb layer in the inactive part of the center (Fig. 9). The differences in understory vegetation in the active and inactive parts of the center can probably be attributed to the amount of light that reaches the forest floor. The soils in the area belong to the Capilano-Bose-Heron soil complex (Luttmerding 1980a).

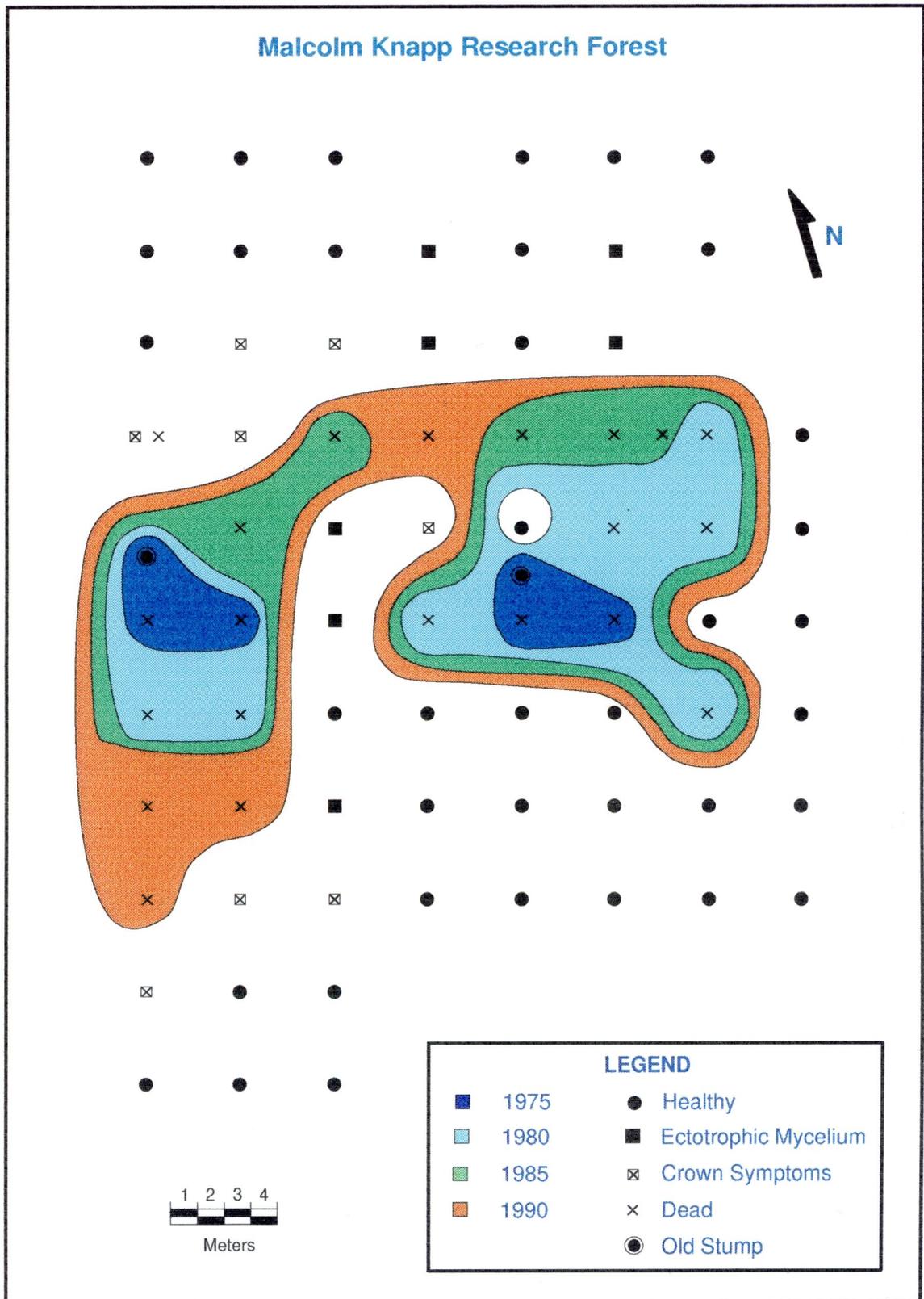


Fig. 6 A map of a *Phellinus* root rot center in the Malcolm Knapp Research Forest showing the extent of mortality caused by *P. weirii* in 1975, 1980, 1985, and 1990.

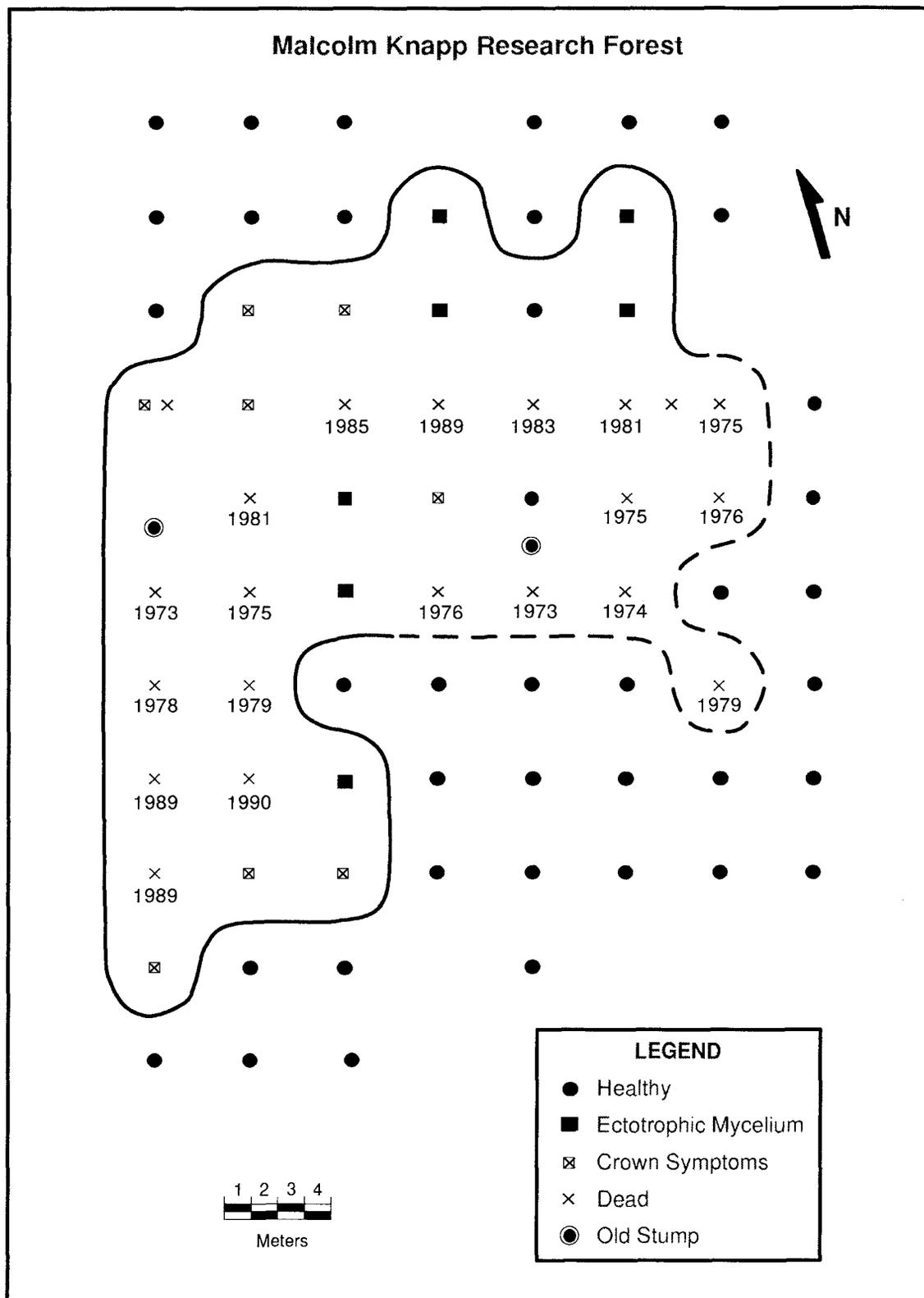


Fig. 7 A map of a *Phellinus* root rot center in the Malcolm Knapp Research Forest showing the active (solid line) and inactive (dashed line) edge. The years indicate when the trees died.



Fig. 8 Understory vegetation along the active edge of a *Phellinus* root rot center in the Malcolm Knapp Research Forest.



Fig. 9 Understory vegetation in the inactive part of a *Phellinus* root rot center in the Malcolm Knapp Research Forest.

The area was logged in 1955 and the slash was piled and burned (Reukema & Smith 1987). The logged stand consisted primarily of old growth Douglas-fir and some hemlock and western red cedar (Reukema & Smith 1987). In 1957 the site was replanted with 2+0 Douglas-fir stock. The trees were planted 3.7 m apart in a grid pattern. The north/south axis of the grid roughly corresponded to magnetic north (21°). The site index was estimated to be approximately 47 m (at a reference age of 50 years at breast height) using the site index curves of Mitchell & Polsson (1988). The mean basal area of the healthy portion of the stand was approximately 54.0 m²/ha in 1992. The estimated maximum mean annual increment using TIPSY (Mitchell et al. 1992) was 15.8 m³/ha/yr.

2.3 A COMPARISON OF THE NUMBER OF ROOTS FROM ADJACENT TREES AND SOIL PROFILES IN INACTIVE AND UNAFFECTED PARTS OF A PHELLINUS ROOT ROT INFECTED STAND

2.3.1 Introduction

The possibility that the inactivation was due to lack of root contacts between healthy and infected trees was investigated by comparing the number of roots thirteen years or older along the inactive edge to the number of such roots present between pairs of healthy trees bordering on the center. The purpose of this was to determine whether roots of the currently healthy trees were present in the neighborhood of the infected trees when they died. Soil profiles in different parts of the stand were also compared.

Reynolds & Bloomberg (1982) tested the effects of various root attributes such as number, length, cross-sectional area and volume of roots in a given volume of soil on the number of root contacts. They found the number of roots to be the most important overall root attribute examined. Factors important in determining the probability of root contacts between trees include inter tree distance, tree diameter, rooting depth, soil gravel content, and percent slope (Eis 1972, Reynolds & Bloomberg 1982). The probability of root contacts between trees increases with decreasing inter tree distance and rooting depth and increasing tree diameter, percent slope, and gravel content. Shallow rocky soils promote asymmetrical root systems with generally longer but fewer laterals (Eis 1974). In the root rot center being studied only tree diameter, soil gravel content and rooting depth varied. Inter tree distance and slope were both relatively constant.

2.3.2 Methods

Eight soil pits, 1 m long and extending down to the limit of root growth, were dug 0.5 m from where the last trees died along the inactive edge (Fig. 10). The number and location of all roots was recorded and the roots were numbered and collected so that they could later be aged by counting their annual rings. As a control, eight similar pits were dug between pairs of

healthy trees located along the perimeter of the center. Only roots extending towards the tree adjacent to the pit were counted.

Eight additional pits (four from the inactive edge and four between pairs of healthy trees) were also dug at a distance of 1.8 m. These additional pits were also used to classify the soil in different parts of the stand using the Canadian System of Soil Classification (Agriculture Canada Expert Committee on Soil Survey 1987). In the inactive part of the stand the pit locations were chosen at random from the eight possible locations. Between pairs of healthy trees however, the location of the pits (Fig. 10) was chosen in such a way as to gain soil information from as many different parts of the stand bordering the center as possible.

2.3.3 Results

Root contacts

The results of root excavations are summarized in Figs. 11 and 12. The number of roots thirteen years or older was generally quite low along both the inactive edge and the center perimeter. There was no significant difference ($p > 0.1$)¹ in the number of roots per pit present in 1979 at the 0.5 m or 1.8 m distances. The number of roots per pit as a function of depth in active and inactive portions of the center is shown in Fig. 13.

¹ p is the probability of a difference equal to or greater than that observed if there were no difference between the treatment means given the observed variation within treatments. Throughout this thesis a significance level of $\alpha = 0.05$ was used.

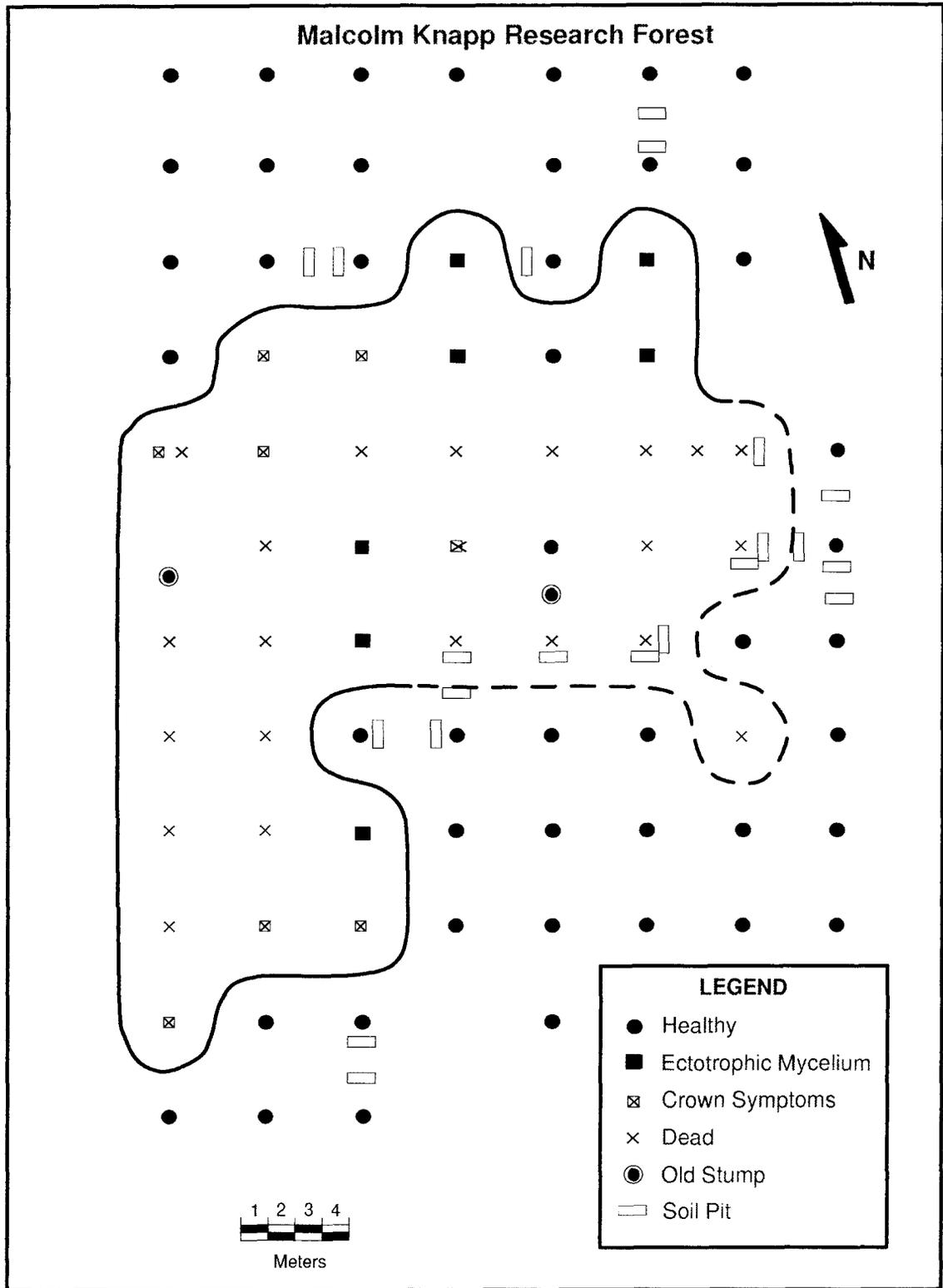


Fig. 10 A map of a *Phellinus* root rot center in the Malcolm Knapp Research Forest showing the location of soil pits.

Soil descriptions

Soil descriptions are given in Appendix 1. The soils throughout different parts of the stand were very similar. The main differences were in the degree of cementation of the Bfc, thickness of the Ah horizon, and whether or not an Ae horizon was present. Soil from all but two soil pits were classified as ortstein humo-ferric podzols. Ortstein humo-ferric podzols are podzols with a strongly cemented Bf horizon. The soils in the remaining two pits were classified as orthic humo-ferric podzols. The predominant humus form near soil pits (using the classification of Klinka et al. 1981) was a vermimull; however, mullmoders were common in areas outside the root rot center.

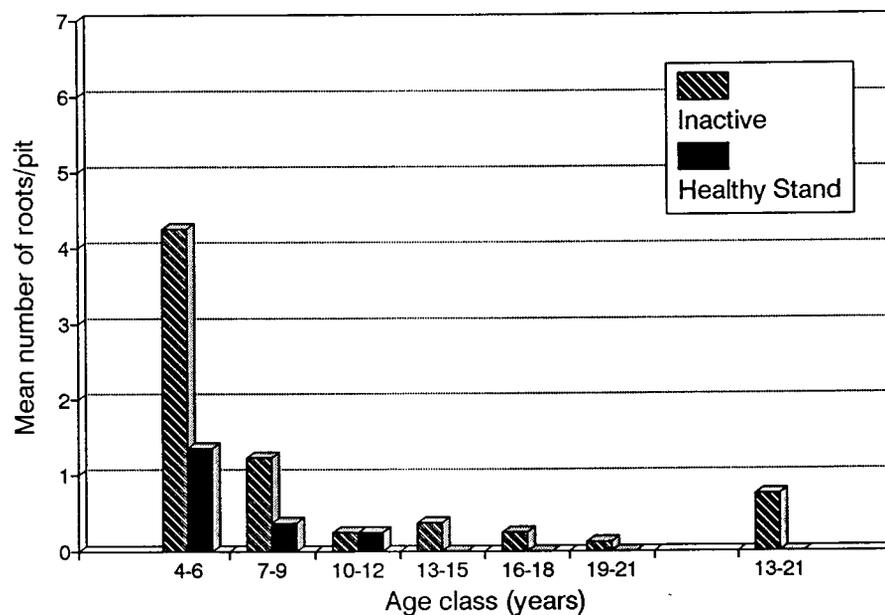


Fig. 11 Mean number of roots per pit, 0.5 m away from tree, by age class for trees bordering a *Phellinus* root rot center in the Malcolm Knapp Research Forest.

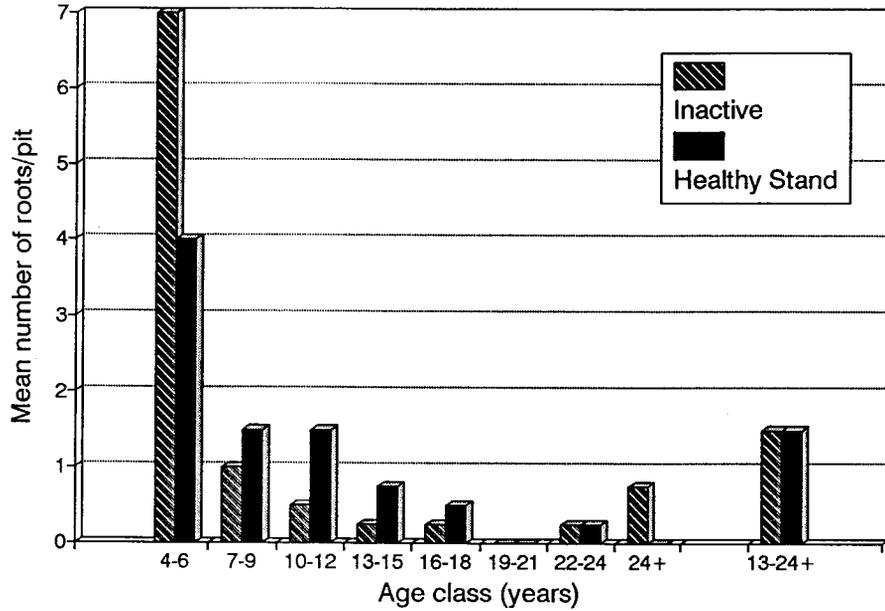


Fig. 12 Mean number of roots per pit, 1.8 m away from tree, by age class for trees bordering a *Phellinus* root rot center in the Malcolm Knapp Research Forest.

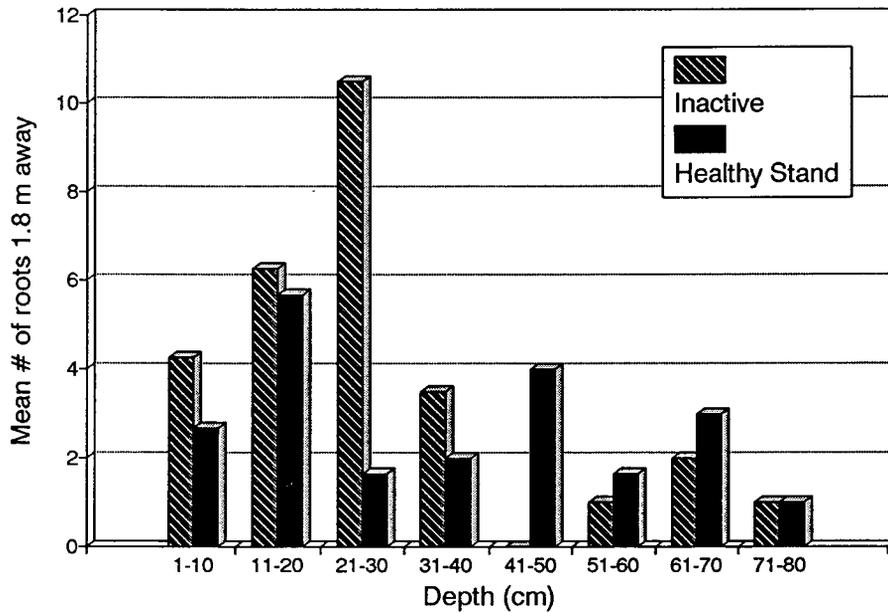


Fig. 13 Mean number of roots per pit, 1.8 m away from tree, by depth for trees bordering a *Phellinus* root rot center in the Malcolm Knapp Research Forest.

2.3.4 Discussion

Root contacts

The use of the number of roots older than a certain age and passing through a plane extending down into the soil as an estimate of the probability of past root contacts involved a certain number of assumptions. The first of these assumptions was that the roots could be accurately aged. It was sometimes difficult to determine the age of roots because they had poorly defined or discontinuous rings. A second assumption was that there were equal rates of root mortality in different parts of the stand.

There is no evidence to suggest that there was any difference in the likelihood of root contacts between the inactive edge and other parts of the stand. The number of roots decreased between the 1.8 m and 0.5 m distances around the center perimeter but stayed about the same along the inactive edge except for the 4-6 year age class. The larger drop in the number of roots around the center perimeter may be an indication that there was more competition in this part of the stand and the trees were deeper rooted. Deep rooted trees tend to have more but shorter laterals than shallow rooted trees. Also, the roots of deep rooted trees need to be longer to reach out the same distance horizontally as the roots of shallow rooted trees.

Soil descriptions

The soil descriptions match fairly closely with the general soil description given for soils belonging to the Capilano soil series. The soil descriptions differ from the general soil description of Capilano soils (Luttmerding 1980b) in having a thick Ah horizon and in not having a Bf horizon overlying the Bfc horizon.

The biggest difference between the active and inactive edges was the effective soil depth. With the exception of the areas surrounding the two trees killed along the westernmost part of the inactive edge, the effective soil depth along the inactive edge was only between 10 and 30 cm. A hard rocky cemented Bf horizon prevented or greatly inhibited root penetration below this depth. The effect of this layer on rooting depth can be seen in Fig. 13. There were very few roots located more than 30 cm below the soil surface along the inactive edge. All of the roots along the inactive edge deeper than 40 cm in Fig. 13 were from tree 33 (Appendix 2).

The discovery of a shallow rooting zone along the inactive edge was somewhat surprising given that shallow soils generally result in shallow dense root zones that are ideal for the spread of *Phellinus* root rot. The shallow soil could have indirectly stopped the spread of *P. weirii* if it inhibited the growth of the trees in this part of the stand to the point where they had smaller root systems than other trees in the stand. This may have been the case for the two trees along the northeast corner of the inactive edge but did not appear to be the case for other trees along the inactive edge. In 1964, the two trees along the northeast corner of the inactive edge had an average height of only 1.6 m compared to an average height of 4 m for the other trees located within the 1992 center boundary. In such shallow soil conditions the trees could also have formed asymmetric root systems that were not in widespread contact with the root systems of nearby trees.

Shallow soil could also have had an effect on the spread of *P. weirii* through its effects on soil moisture. Shallow soils may become slightly drier during periods of extended drought in the summer and would be wetter during the winter than deeper soils. Hansen et al. (1983) reported that ectotrophic growth of *P. weirii* along Douglas-fir branch segments buried in the soil was greatest at 15 % moisture content. In the field, they found that increasing or decreasing soil moisture from ambient levels reduced the extent of ectotrophic mycelium growth significantly. Beale (1992) found that the highest intensity of Phellinus root rot in the CDFmm and CWHxm subzones (Meidinger & Pojar 1991) of southeastern Vancouver Island occurred on mesic sites. In this study, the only part of the center where the soil was noticeably wetter after a heavy rain was the northeastern portion of the inactive edge which lies in a shallow trench.

2.3.5 Conclusions

Although it can not be proven that there were similar numbers of root contacts along the inactive edge and other parts of the stand, there was no evidence to suggest that there were fewer opportunities for root contacts along the inactive edge. The most notable soil difference along the active and inactive edges of the center was rooting depth. There was a strongly cemented upper Bf horizon throughout much (two thirds) of the inactive part of the center that served as a barrier to roots. By itself the difference in rooting depth does not seem adequate to explain why part of the center became inactive.

2.4 THE EFFECT OF SOIL FROM ACTIVE AND INACTIVE PARTS OF A PHELLINUS ROOT ROT CENTER ON FUNGAL GROWTH

2.4.1 Introduction

Two methods were used to determine whether chemical, physical, or biological soil properties played a role in inactivation. The first method involved measuring the growth of *P. weirii* along Douglas-fir sticks inserted into soil from active and inactive edges of the center in both the lab and the field. The second method involved measuring the growth of *P. weirii* along living Douglas-fir seedling roots packed in soil from these two sources. The inoculation method used in the experiments was a modification of a technique developed by George Reynolds and Rona Sturrock at the Pacific Forestry Center in Victoria. *P. weirii*-decayed red alder (*Alnus rubra* Bong.) blocks were used as inoculum sources. Wallis & Reynolds (1962) showed that alder is a better food base for *P. weirii* than autoclaved Douglas-fir.

2.4.2 Methods

2.4.2.1 Growth of *P. weirii* on sticks in the lab

Inoculum preparation

Red alder stems 4.5 to 5.5 cm in diameter were cut into 5-cm-long sections, leaving the bark intact, and autoclaved in bags for 90 minutes. The results of a previous experiment (Appendix 3) demonstrated that the moisture content of such freshly cut blocks (about 90 % of dry weight) was ideal for the development of *P. weirii*. A culture of *P. weirii* was obtained from an active and fast-spreading infection center in the Delta Watershed Park in Delta, B.C. The culture was grown on alder sawdust which was then used to inoculate the alder blocks by placing it in bags of sterilized alder blocks and shaking the bags to distribute the sawdust evenly. The inoculated blocks were incubated at room temperature (approx. 18 °C) in the dark for 10 weeks.

When the inoculum blocks were ready for use, each block was put into a small freezer bag. Douglas-fir branches were collected from a single tree and cut into sticks 22 cm long and

5 to 10 mm in diameter. Most sticks consisted of parts of the 3 to 5-year-old internodes. The sticks were fastened to the inoculum blocks (Fig. 14) and incubated at room temperature for 5 to 7 days to allow initial colonization of the sticks.

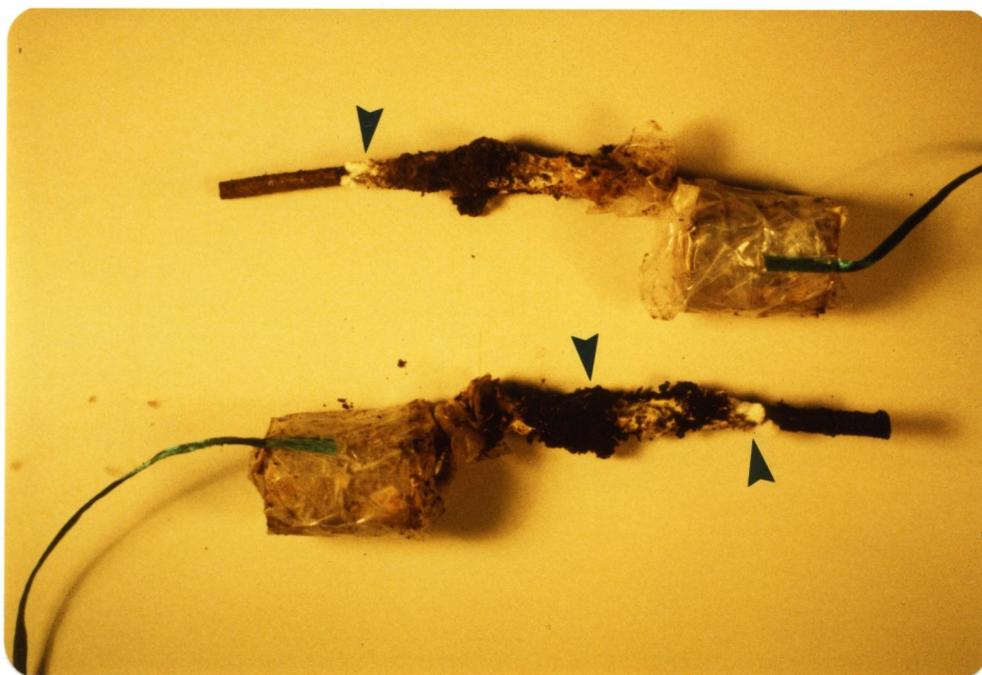


Fig. 14 Douglas-fir sticks fastened to alder inoculum blocks. The photo was taken following the completion of the experiment and shows the growth of ectotrophic mycelium along the sticks (arrows).

Experimental design

Composite soil samples were collected from a soil depth of less than 17 cm at several locations along the active and inactive edges (Fig. 15). The soil was sifted to remove large rocks and organic material, mixed, and put into 3 L pots. The soil was tamped down to give a bulk density of about 0.6 g/ml for the inactive soil and 0.7 g/ml for the active soil. Three sticks were inserted about 5 cm apart in each of ten pots containing active soil and ten pots containing inactive soil.

The soil moisture in the pots was maintained at field capacity (a water potential of -3 m) by adding water to the pots every 2 to 3 days until they were at the correct weight necessary to bring the gravimetric water content of the soil up to a level corresponding to field

capacity. In order to reduce evaporation, plastic bags were placed over the pots. The gravimetric soil water content corresponding to field capacity for both the active and inactive soil were determined prior to the experiment using a porous plate extractor. The active soil had a soil moisture content of 0.26 ml of H₂O/ml of soil at field capacity, and the inactive soil had a moisture content of 0.28 ml of H₂O/ml of soil at field capacity.

Measurement and analysis

After a period of 25 days, the distance the fungus grew along the sticks in cm was measured and single soil samples from both the active and inactive soil were sent to a soil testing lab and analyzed for pH, organic matter, nutrient content, and particle size distribution. The growth results were analyzed using both a simple t-test and ANOVA using a one-way nested design.

2.4.2.2 Growth of *P. weirii* on sticks in the field

Experimental design

The growth of the fungus along Douglas-fir branch segments was also tested in the field. Sticks attached to inoculum blocks were inserted into slots created in the soil using a tile shovel. The sticks were inserted into the soil to the point of initial contact with the inoculum block (17 cm) at 100 locations along the active edge and 100 locations along the inactive edge (Fig. 15). The inoculum blocks were covered to shade them from the sun.

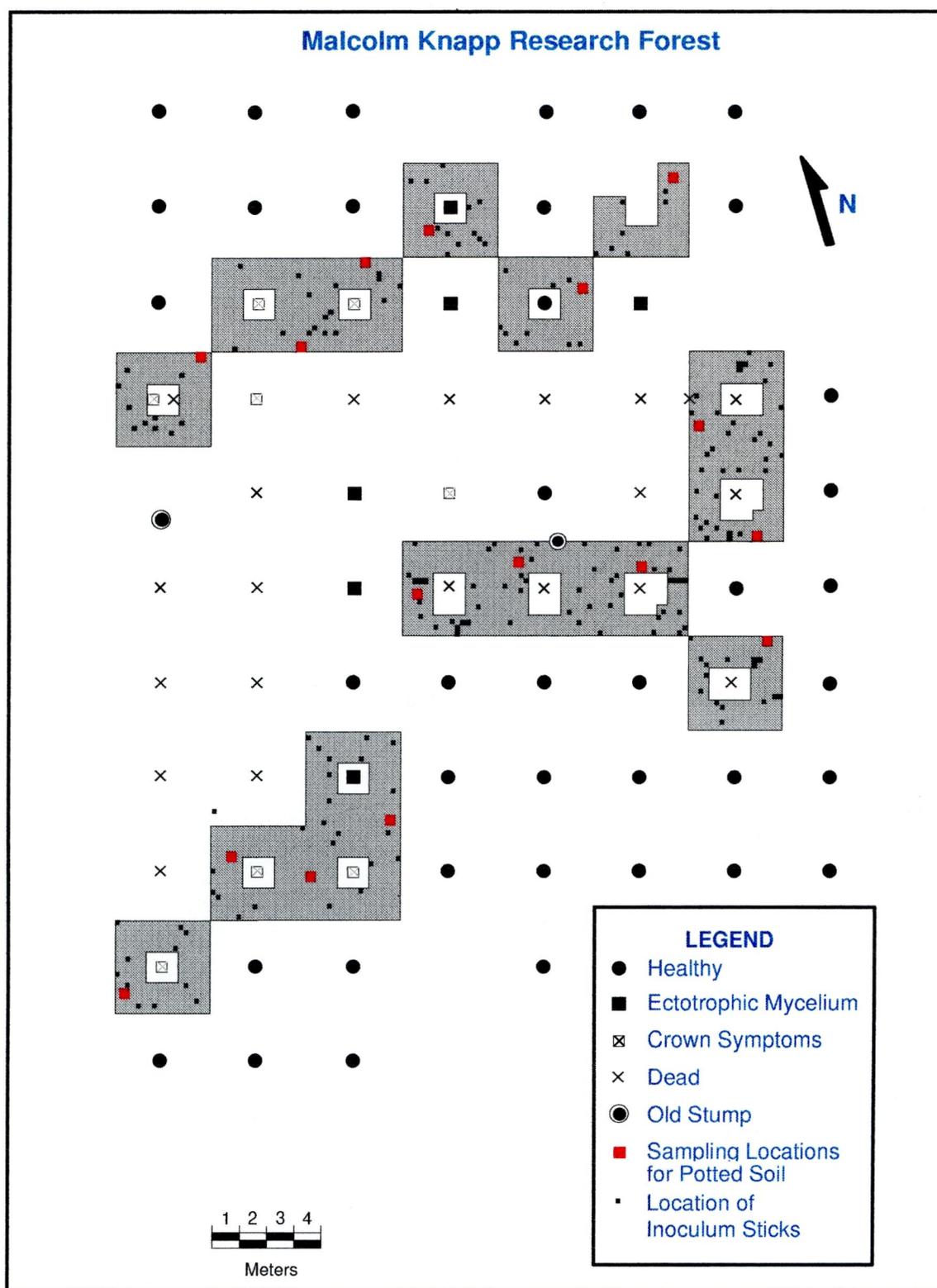


Fig. 15 Locations within a *Phellinus* root rot center in the Malcolm Knapp Research Forest where *P. weirii* inoculated sticks were inserted into the soil and soil samples were collected.

Soil temperature and bulk density

The soil temperature at a depth of 8.5 cm was measured at two locations within the active part of the center and two locations within the inactive part of the center during the course of the experiment. Bulk density and soil moisture were also measured during the course of the experiment. Bulk density was measured by carefully collecting soil from an approximately 10 cm³ volume of soil. The volume occupied by the soil was measured by determining the volume of water needed to fill a plastic bag placed in the soil cavity. The collected soil was then weighed, oven dried (110 °C), and reweighed to determine its dry weight and moisture content. The bulk density and soil moisture were measured at four locations along both the active and inactive edges of the center.

Measurement and analysis

On July 6, 3 weeks after the sticks were put in the ground, they were pulled out and the growth of the fungus along the sticks was measured. The results were analyzed using a simple t-test.

2.4.2.3 The effects of A and B horizon soil on the growth of *P. weirii* on sticks

To determine if there was a difference in the effect of A versus B horizon soil, the field experiment was repeated on a smaller scale. This time however, a nested design was used. Eight areas were randomly selected from the inactive portion and sixteen areas were randomly selected from the active portion (Fig. 16). Each area was subdivided into 36 smaller 20 x 20 cm areas and four of these smaller areas were then randomly selected from each of the larger areas. Only 20 x 20 cm areas that had not been used in previous experiments were selected. All of the Ah and Ae material was removed from 8 of the 16 areas along the active edge of the center prior to inserting the sticks into the soil. The B horizon in the inactive part of the stand was not tested for its effect on fungal growth because there was no rooting in the B horizon

throughout most of the inactive edge due to cementation of the upper Bfc horizon. The results of this experiment were analyzed using ANOVA for a one-way nested design.

2.4.2.4 Growth of *P. weirii* on seedling roots

Inoculation technique

Bareroot, 2-year-old Douglas-fir seedlings were planted in 3 L pots using a peat-sand-sawdust mixture. The pots were placed outside in sawdust beds to allow roots to grow outside the pots. Once the roots had grown to a sufficient length (approx. 30 cm) the roots outside the pots were packed in 3.2 cm diameter tubes made from ABS plumbing pipe. A 1.6 cm slot was cut out of the side of each tube so that the soil could be tamped down uniformly. Soil from either the active or inactive edge of the root rot center was tamped down to a bulk density of 0.6-0.7 g/ml of soil. The soil used was the same soil that had previously been used to test the growth of *P. weirii* on sticks in the lab. *P. weirii* decayed alder blocks prepared as described previously were attached to a short 7 cm Douglas-fir stick extending 2 cm past the end of the inoculum block. The roots at the distal ends of the soil tubes were placed in contact with the stick (Fig. 17).

Experimental design and analysis

A total of 14 seedlings were used. Due to a lack of suitable roots, it was not possible to use the same number of roots for each seedling. On four of the trees eight roots were used, on one of the trees four roots were used, and on the remaining seedlings six roots were used. In all cases, half of the roots were randomly assigned to soil from the active edge and the other half were assigned to soil from the inactive edge of the center.

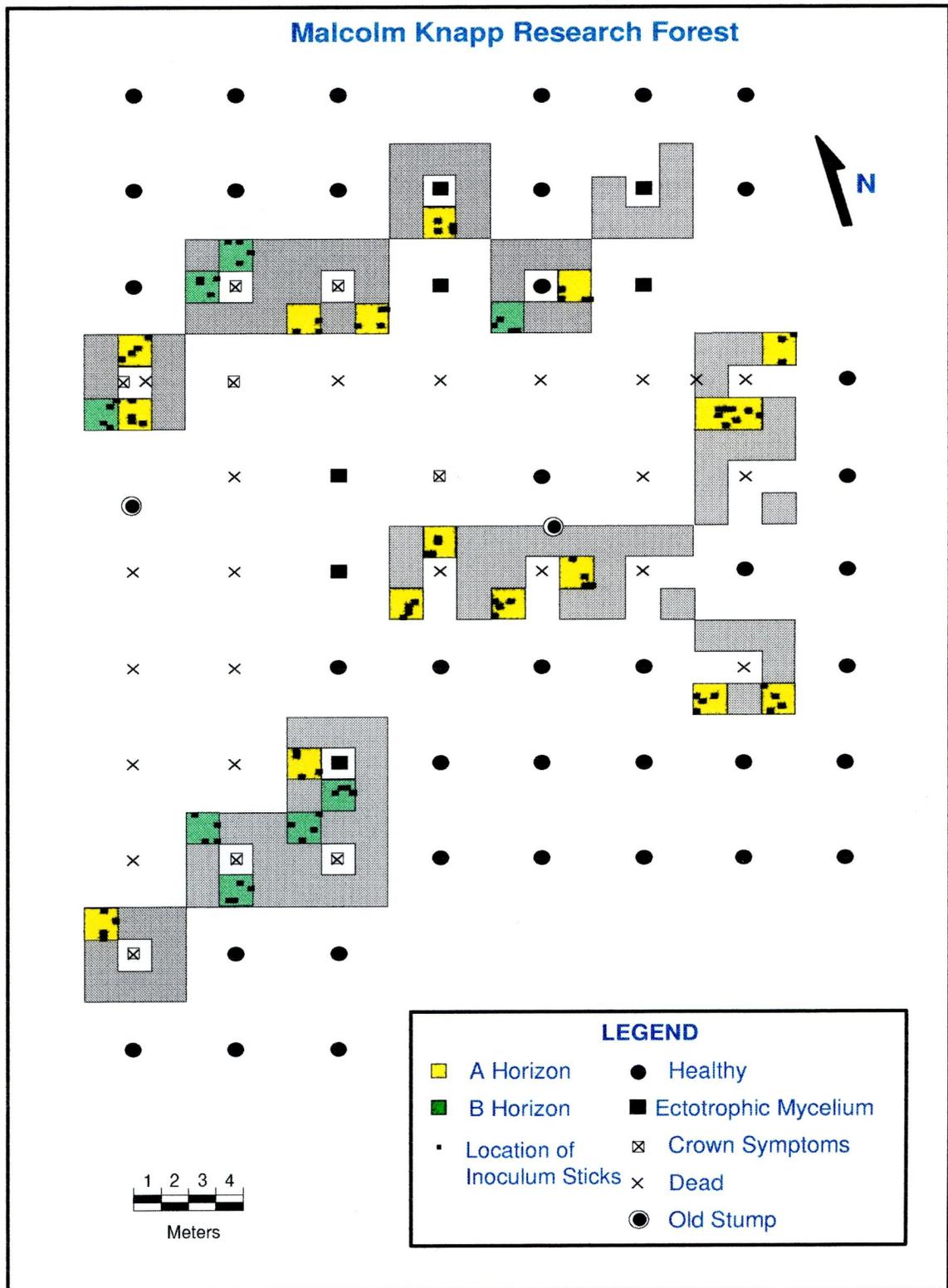


Fig. 16 Locations within a *Phellinus* root rot center in the Malcolm Knapp Research Forest where sticks were placed in the soil to test the effects of A vs. B horizon soil.

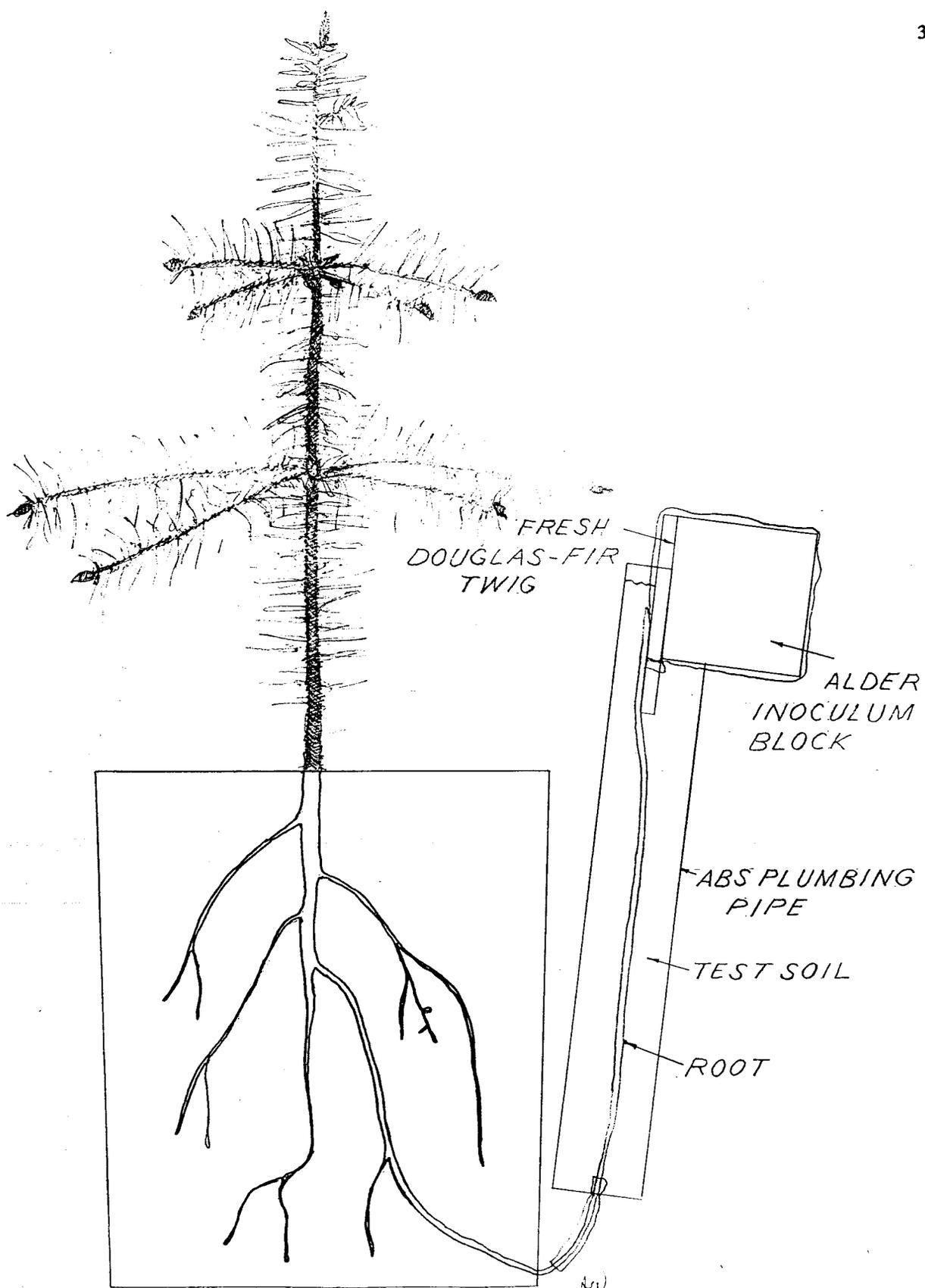


Fig. 17 A diagram showing the seedling root inoculation set-up.

The soil moisture was maintained at field capacity by keeping the tubes at a constant weight, as described previously. A plastic bag was placed over the top of each tube to slow the rate of water evaporation. The portion of each root between the pot to about 5 cm below the soil tube was kept moist by covering it with moist sawdust. The portion of the root between the sawdust and the soil tube was kept moist by wrapping it in plastic wrap. The results of the inoculation test were analyzed using the general linear model in Minitab (Minitab Statistical Software 1989).

2.4.3 Results

Growth on sticks

The average growth of *P. weirii* along sticks inserted into pots of soil from active and inactive edges of the center was 6.4 mm/day for the soil from the active edge and 5.7 mm/day for the soil from the inactive edge (Table 1). This did not represent a significant difference ($p = 0.335$). Sticks with less than 2.0 cm growth or on which *P. weirii* had died back were not included in calculations of mean growth along sticks or roots. Sticks on which zone lines were present or *Trichoderma* species were found were also excluded from calculations of mean growth. There was no significant pot effect on growth ($p = 0.62$). There was also no significant difference in the number of sticks with less than 2 cm growth or on which contaminants were found (Table 1).

Table 1: Growth rate, and proportion of sticks with less than 2 cm growth, zone lines, and contaminants present for growth of *P. weirii* along sticks or roots in soil from the active and inactive edge of a root rot center in the Malcolm Knapp Research Forest

Experiment	Soil Source	Sample size	Growth Rate (mm/day)	< 2 cm growth (%)	Contaminants present (%)
Sticks in potted soil	Active edge	30	6.4	17	17
	Inactive edge	30	5.7	13	30
Sticks in field	Active edge	100	4.9	8	6
	Inactive edge	100	5.3	6	8
Sticks in A or B horizon in field	Active edge; A horizon	32	4.2	16	9
	Active edge; B horizon	32	4.8	9	6
	Inactive edge; A horizon	32	4.4	19	9
Seedling roots	Active Edge	44	2.3	n.a.	18
	Inactive edge	43	2.8	n.a.	7

Contaminants were visually observed in all cases in which there was no initial growth of the fungus; but contaminants also occurred on a few sticks on which growth occurred. In most cases contaminants were observed on the alder inoculum block but in a few cases they were present on the sticks. Most of the contamination appeared to be caused by species of *Trichoderma*. In some cases in which there was good initial growth, *P. weirii* appeared to have died back even though there was no obvious signs of contamination.

The results of the soil tests are shown in Appendix 4. Both soils were sandy loams. The soil from the inactive edge had more organic matter, and a slightly lower pH than soil from the active edge (3.7 vs. 3.9). The inactive soil had higher levels of all nutrients except phosphorus and iron. The soil chemical analysis may not be a true reflection of conditions in the field because the soil was in the lab for three weeks at room temperature prior to being tested (i.e. some mineralization of nutrients may have occurred).

The average growth of *P. weirii* along sticks in the field was 4.9 mm/day along the active edge and 5.3 mm/day along the inactive edge. This did not represent a significant difference ($p = 0.25$). There was also no significant difference in the proportion of sticks with less than 2 cm growth or sticks with contaminants. Species of *Trichoderma* and/or zone lines appeared to be present on some but not all of the sticks on which there was less than 2.0 cm of growth at the time of measurement.

The soil temperature in both the active and inactive parts of the center was around 13 °C. Bulk density varied between 0.7 and 1.2 g/ml and soil moisture content varied between 0.23 and 0.72 ml H₂O/ml soil. There were no significant differences in the bulk densities or soil moisture contents in active and inactive parts of the stand.

In the experiment to compare the effect of A and B horizons on the growth of *P. weirii*, no significant differences ($p = 0.26$) could be detected in the mean growth of *P. weirii* in the A and B horizons of the active part of the center and the A horizon of the inactive part of the center. There was also no significant difference in the number of sticks along the active or inactive edge with more than 2 cm growth or on which contaminants were found.

Growth on seedling roots

Fig. 18 shows roots with ectotrophic mycelium growth on their surface. The dense mycelial growth around the root causes the soil to adhere to the root's surface.



Fig. 18 Ectotrophic growth of *P. weirii* on seedling roots as indicated by the adherence of soil to the root surface (arrows).

The average rate of growth of *P. weirii* on roots was 2.3 mm/day in the active soil and 2.8 mm/day in the inactive soil. There was no significant tree ($p = 0.478$), soil ($p = 0.943$) or interaction ($p = 0.928$) effects. Inoculation success was 70 % for the active soil and 76 % for the inactive soil. There was no significant difference in the number of roots with contaminants present. In some cases where the fungus failed to grow, the root was dead (38 % of unsuccessful inoculations) or *Trichoderma* was present but in other cases there was no obvious explanation for the failure of *P. weirii* to grow. Forty-four percent (4 of 9) of the dead roots had *Trichoderma spp.* associated with them.

2.4.4 Discussion

Effect of soil from the active and inactive edge on fungal growth

There did not appear to be any major differences in the effects of A or B horizon soil from the inactive and active edge on the growth of *P. weirii* on sticks or roots. The rate of growth was slightly faster in the lab than in the field, possibly as a result of the warmer temperature in the lab. *P. weirii* grows best at around 20 °C on artificial culture medium (Li et al. 1967). The growth of *P. weirii* on roots was considerably less than the growth on sticks. The faster growth on sticks may have been the result of a larger food base or a phytoplane community on Douglas-fir sticks that is not well adapted to soil conditions. The failure of *P. weirii* to grow was often associated with the presence of *Trichoderma*; however, *Trichoderma* appeared to be just as frequent in the active soil as it was in the inactive soil. *P. weirii* had formed zone lines on some of the sticks on which *Trichoderma* was seen. The discovery of zone lines in association with *Trichoderma* is consistent with the idea that zone lines tend to exclude antagonistic soil fungi (Rishbeth 1951). *P. weirii* did not appear to grow well on dead roots, and that may be the result of antagonism by soil saprophytes. *Trichoderma* was found more commonly associated with dead portions of roots than living roots.

Trichoderma spp. are commonly found in temperate forest soils and in association with wood decayed by other fungi (Nelson et al. 1987). *Trichoderma* has been found to be the principal fungus invading wood colonized by *P. weirii* (Nelson 1964, Nelson et al. 1987) and is strongly antagonistic to *P. weirii* in culture. Nelson (1976) found a significant inverse relationship ($\alpha = .05$) between *P. weirii* survival in buried alder blocks and the ability to isolate *Trichoderma* from the blocks. At least part of the antagonism of *Trichoderma* to *P. weirii* is a result of direct parasitism (Nelson 1964). Conditions that favor *Trichoderma* species while having a negative effect on *P. weirii* could greatly reduce the spread and survival of *P. weirii*. This may be one effect of stump fumigation. Roots from *P. weirii* infected stumps treated with fumigants are more frequently colonized by species of *Trichoderma* than untreated stumps (Nelson et al. 1987). Increased populations of *Trichoderma* species along with direct, sublethal effects of fumigation have resulted in disease control of *Armillaria mellea* (Vahl:Fr.) Kummer (Munnecke et al. 1981).

Soil chemical analysis

Most of the differences in organic matter and nutrients in the two soils can probably be attributed to the dense understory vegetation that has grown up along the inactive edge as a result of the openings created by Phellinus root rot. The creation of openings may also have caused a slight increase in temperature, and some soil mixing may have occurred as a result of wind throw of diseased trees. An increase in temperature and soil mixing are both factors that would tend to speed up the rate of decomposition. Cromack et al. (1991) looked at the effect of disturbance by *P. weirii* on decomposition and nutrient mineralization in a mountain hemlock forest. They found greater amounts of available soil nitrogen and increased rates of nitrogen mineralization in decomposing needle litter of stands in which *P. weirii* was present than they did in disease free stands.

Using the growth of *P. weirii* on sticks and seedling roots to study Phellinus root rot

The use of seedling roots and Douglas-fir branch segments to study the interactions between *P. weirii*, Douglas-fir, and the environment has several advantages over inoculating roots in the field. Both techniques are relatively quick and can be used inside under well controlled conditions. The rate of growth of *P. weirii* is also much faster on inoculated seedling roots and twigs than on inoculated tree roots. The average rate of growth on inoculated seedling roots was about 1.8 cm/week compared to average total (proximal + distal) rates of 0.7 cm/week (Wallis & Reynolds 1962) and 0.3 cm/week (Hansen et al. 1983) on inoculated roots in the field.

One of the problems encountered with the seedling inoculation technique was the low inoculation success rate. Although the inoculation success rate was within the range of 25 - 100 % reported for field inoculations (Hansen et al. 1983, Kellas 1985, Wallis & Reynolds 1962) it will need to be improved before the technique can be considered reliable. The number of successful inoculations could be improved by reducing root mortality. Some damage to the roots probably resulted during the weighing of the soil tubes. A way of controlling soil moisture that does not involve moving the tubes would probably increase the proportion of successful inoculations.

The usefulness of the measuring the growth of *P. weirii* on sticks and seedling roots also depends on whether or not the behavior of *P. weirii* on sticks and seedling roots relates to its behavior on large roots. The growth patterns of *P. weirii* on young roots and old roots appear to be quite similar; however, roots go through a number of physiological and morphological changes associated with secondary growth and necrophyllactic periderm formation that could have an effect on the infection process. It is not clear what role very young roots play in the natural infection process. The resistance of trees may also change over time. The time between infection and death is much shorter in young trees infected with *P. weirii* than it is in older trees.

2.4.5 Conclusions

The inactivation of a portion of the root rot center could not be shown to be the result of differences in the soils from the active and inactive edges of the root rot center.

Trichoderma was commonly associated with poor growth of *P. weirii*, but was found with almost the same frequency in active and inactive portions of the center. The seedling inoculation technique used in this experiment has several advantages over other methods involving the inoculation of roots in the field. With further refinement and testing this technique could be useful in future studies involving *Phellinus* root rot.

2.5 A COMPARISON OF ISOLATES FROM ACTIVE AND INACTIVE PARTS OF A PHELLINUS ROOT ROT CENTER

2.5.1 Introduction

The pairing of fungal isolates in culture to determine whether they were vegetatively compatible has been a commonly used in the past for determining whether two heterokaryotic root rot isolates of the same species had a common origin. Isolates that were vegetatively compatible were assumed to be derived from the same "clone", where the term clone was used to denote isolates which were derived vegetatively from the same binucleate cell but may have had slight genetic differences as a result of somatic mutations (Childs 1963). Isolates that are vegetatively incompatible form lines of demarcation between them when they are paired whereas vegetatively compatible isolates grow together smoothly without any demarcation. The zone where the line of demarcation occurs in vegetatively incompatible reactions is characterized by degenerating dying hyphae and aborted heterokaryotic cells (Glass & Kuldau 1992, Rayner & Todd 1979).

From studies involving ascomycetes it appears that proteinaceous factors or cell wall interactions may be involved in incompatibility (Glass & Kuldau 1992). Genetic studies on vegetative compatibility in ascomycetes have revealed distinct vegetative compatibility groups within species. Both allelic and nonallelic vegetative incompatibility systems have been identified in ascomycetes (Glass & Kuldau 1992). In allelic incompatibility systems, incompatibility occurs as a result of hyphal fusion between two fungal strains that have different alleles at one or more loci. In nonallelic incompatibility systems, incompatibility occurs as a result of hyphal fusion between two fungal strains that have genetic differences at two separate and distinct loci.

From the few wood-decaying basidiomycetes in which vegetative compatibility has been studied in detail, it appears as though basidiomycetes have a multiple allelic incompatibility system (Rayner & Todd 1979). The intensity of the vegetative compatibility interaction depends on how closely the isolates are related, being less intense for closely related isolates and more intense for distantly related isolates (Rayner & Todd 1979). The relationship between genetic variability and vegetative compatibility has not been explored in *Phellinus* or other basidiomycetes that do not form clamp connections but vegetative compatibility seems to be a very sensitive indication that isolates differ (Hansen 1979b, 1979c). If the isolates are vegetatively compatible it may indicate that they are heterokaryons belonging to the same clone or that they are sexually compatible single spore isolates; however, to date all isolates collected from decayed wood have been heterokaryons (Hansen 1979b). In this study vegetative compatibility was used as a means of comparing isolates from the active and inactive edge.

2.5.2 Methods

Three isolates from or near the active edge (isolates from trees 9W, 19, and 41 in Appendix 2) and one isolate from the inactive part of the center (an isolate from tree 33N) were collected. The isolates were grown and paired on Difco® malt agar in order to compare their rates of growth and cultural characteristics and to determine if they were vegetatively compatible.

2.5.3 Results

There were no obvious differences between isolates from active and inactive parts of the center. The isolates looked similar in culture and did not show any signs of vegetative incompatibility when paired.

2.5.4 Discussion and Conclusions

The similarity in culture morphology and compatibility between isolates from active and inactive portions of the centers suggests that the isolates may have had a common origin. If the isolates did have a common origin then it is possible that they could still show differences in virulence as a result of somatic mutations but they would likely show similar virulence. The possibility that hypovirulence caused by a dsRNA virus might be responsible for the inactivation was investigated as part of a larger study which looked at a number of different isolates (including those from the root rot center being studied). This study is described in the following section.

2.6 A SEARCH FOR HYPOVIRULENT STRAINS OF PHELLINUS WEIRII

2.6.1 Introduction

Hypovirulence is a reduction in the ability to cause disease. The most likely causes of hypovirulence are double-stranded RNA (dsRNA) particles or mycoviruses. Viruses have been reported in a large number of fungi including the root rot fungus *Armillaria ostoyae* (Reaves et al. 1988). While most fungal viruses do not appear to have any obvious debilitating effects on their hosts, some cause altered cultural characteristics such as:

- 1) changes in pigmentation
- 2) non concentric growth
- 3) slower or in some cases faster than normal growth,
- 4) a reduction in the frequency or a complete absence of sporulation.

Double-stranded RNA (dsRNA) viruses comprise the largest group of known fungal viruses. Transmissible dsRNA particles that are not associated with any coat protein or lipoprotein (and hence do not fit the definition of a virus) are also known in all major classes of fungi (Nuss & Koltin 1990). Since large quantities of dsRNA are not normally found in fungal cells that do not contain dsRNA viruses or particles, dsRNA viruses or particles can be detected by the presence of dsRNA in the host fungus.

There are a number of plant pathogenic fungi in which dsRNA has been suggested to cause hypovirulence, but for many of these the evidence is weak and sometimes contradictory. In *Rhizoctonia solani* Kuehn it has even been suggested that dsRNA may be required for virulence (Nuss & Koltin 1990). In most instances the best evidence for attributing hypovirulence to dsRNA or dsRNA mycoviruses is based on observations that virulent strains of pathogenic fungi become hypovirulent when dsRNA or dsRNA mycoviruses are transmitted by hyphal anastomosis and that the loss of dsRNA or mycovirus is associated with a recovery of virulence (Buck 1988). Some plant pathogenic fungi in which there is fairly good evidence

linking hypovirulence to the presence of dsRNA are listed in Table 2. DsRNA was recently proven to be the cause of hypovirulence in *E. parasitica* by showing that transformation of virulent strains with cDNA copies of dsRNA encoded genes confers hypovirulence (Choi & Nuss 1992).

Table 2: Some plant pathogenic fungi in which dsRNA has been associated with hypovirulence

<u>Fungus</u>	<u>Disease</u>	<u>Reference</u>
<i>Endothia parasitica</i> (Murr.) P.J. & H.W. Anderson	Chestnut Blight	Choi & Nuss 1992
<i>Ophiostoma ulmi</i> (Buisman) Nannf.	Dutch Elm Disease	Rogers et al. 1988
<i>Gaeumannomyces graminis</i> var. <i>tritici</i> Walker	Wheat Take All	Naiki & Cook 1983
<i>Ustilago maydis</i> (D.C.) Corda	Corn Smut	Koltin 1988
<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary	diseases of vegetables	Boland et al. 1992
<i>Mycosphaerella pinodes</i> (Berk. et Blox) Stone	Mycosphaerella Blight of Pea	Yamada et al. 1991

The possibility that hypovirulent strains of *P. weirii* might exist was suggested after the observation of some virus-like symptoms in some cultures of *P. weirii*. These symptoms included sectoring and slow, irregular, appressed growth. Serial subculturing from the edges of affected cultures led to normal cultures; while subculturing from the older parts led to a retention of abnormal characteristics.

In an effort to establish whether dsRNA or dsRNA viruses occur in *P. weirii*, 31 isolates representing 17 sites in B.C. and 30 isolates from Washington and Oregon were screened for dsRNA using a variation of the technique of Morris & Dodds (1979) developed at the Agriculture Canada Vancouver Research Station. It was decided to approach the question of whether hypovirulence in *P. weirii* exists by looking for dsRNA rather than by looking for

hypovirulence directly because it is relatively easy to screen fungi for dsRNA and at present there are no good methods for comparing the virulence of *P. weirii* isolates.

All of the isolates were collected from infected Douglas-fir with the exception of two isolates that were collected from white pine and one that was collected from western red cedar. The isolate collected from western red cedar is probably the cedar type of *P. weirii* (as opposed to the Douglas-fir type).

As well as looking for dsRNA an attempt was also made to isolate virus particles from two of the isolates. All of the isolates were examined in culture for the presence of any virus like symptoms.

2.6.2 Methods

dsRNA extraction

P. weirii mycelia were grown in flasks containing P-YE-G (1% peptone- 1% yeast extract- 2% glucose) liquid media. The flasks were inoculated by one of two methods. In the first method two PDA cultures (approx. 1 week old) were blended for 5 seconds in 40 ml of sterile distilled water in a sterile Waring® blender. The blended mycelium was then poured into four 250 ml flasks each containing 50 ml of liquid media. The second method was similar to the first except that the PDA cultures were added directly to 200 ml of media, blended for 5 seconds in a 950 ml Waring® blender, and then poured into four 250 ml flasks. The flasks were incubated on a rotary shaker at room temperature for approximately 1 week before the mycelium was harvested.

Pleurotus pulmonarius (Fr.) Quèl, *Ustilago maydis*, and *S. sclerotiorum* isolates known to contain dsRNA were used as controls. *U. maydis* and *S. sclerotiorum* were grown in P-YE-G medium. Tissue for extraction of dsRNA from *P. pulmonarius* was obtained from fruiting bodies frozen at -20 °C.

The mycelia were separated from the media by centrifuging at 10,000 rpm for 10 min. in a GSA rotor at 4 °C. The mycelium was washed by resuspending the pellet in 2x STE (100 mM NaCl, 50 mM Tris-HCl, 1mM EDTA) and centrifuging for another 10 min.

Twenty grams (wet weight) of mycelia per sample were extracted in two volumes of 2x STE, 1.5 volumes of STE saturated phenol, 0.02 volumes of 2-mercaptoethanol, and 0.5 volumes of 10 % sodium dodecyl sulfate. Tissue from all samples except for those of *P. pulmonarius* was homogenized using a Polytron®. *P. pulmonarius* tissue was powdered in liquid nitrogen prior to extraction.

The phenol and aqueous phases were separated by centrifuging at 10,000 rpm for 20 minutes in a GSA rotor. The aqueous phases were pipetted into tubes and made up to 16 % ethanol by the addition of 0.2 volumes of 95 % ethanol. Two and one half grams of CF-11 Whatman® cellulose were then added to each sample. The tubes were shaken briefly and centrifuged at 3500 rpm for 2 minutes in a Baxter® megafuge to pellet the cellulose. The cellulose was washed by repeatedly resuspending the pellet in fresh STE-ethanol and recentrifuging. The cellulose was poured into a column and the bound nucleic acid eluted with three 5 ml aliquots of STE.

The eluant was then digested with DNase I (2 units/ml) and T1 RNase (10 units/ml) to digest DNA and single-stranded RNA, respectively. After the digestions had been carried out the samples were adjusted to 20 % ethanol, 0.3 g of cellulose APX (Serva®) was added per sample, and the samples were shaken for 20 minutes. The solutions were then poured into columns, washed with 30 ml of STE-ethanol and the dsRNA eluted with 2.5 ml of STE.

The eluant was collected in 1.5 ml eppendorf tubes and centrifuged at 10,000 rpm in an eppendorf microfuge to remove any cellulose that washed through the column. Any dsRNA present in the eluant was precipitated using 2.5 volumes of a 95 % ethanol: 0.3M sodium acetate solution. The tubes were stored at -20 °C overnight.

The following day the tubes were centrifuged at 14,000 rpm to pellet any dsRNA. The pellets were dried and resuspended in TAE loading buffer (a 40 mM Tris, 40 mM acetate, 2

mM EDTA buffer solution containing 0.04% bromophenol blue, 0.04% xylene, and 30% glycerol). The dsRNA bands were examined by electrophoresis in a 1 % agarose gel for 2 hours at 60 V. TAE was used as the electrophoresis buffer. The gels were stained with ethidium bromide and examined under ultra violet light for the presence of dsRNA bands.

Virus purification

An attempt was made to purify virus particles from two *P. weirii* isolates. One of the isolates was from a large active center and the other was from a nearby small, apparently inactive center.

Approximately 300 g (wet weight) of tissue from each of the isolates was homogenized with a Polytron® in 5 ml/g of 0.1 M di-sodium orthophosphate buffer (pH 7.0) and 0.1 % 2-mercaptoethanol. The homogenized tissue was shaken for 1 hour at 4 °C, and then centrifuged for 20 minutes at 10,000 rpm in a GSA rotor. The supernatant was centrifuged at 30,000 rpm for 2 hours in a Ti 50.2 rotor.

The pellets were resuspended in one tenth of the original volume of phosphate buffer, homogenized briefly with the Polytron®, and shaken overnight at 4 °C. The samples were then centrifuged at 10,000 rpm for 20 min. The aqueous phase was collected and centrifuged at 30,000 rpm for 2 hours in a Ti 50.2 rotor.

Following resuspension of the pellets, each sample was divided into two equal portions. Chloroform (0.2 volumes) was added to one of the portions. The portion to which the chloroform was added was then shaken for 15 min and centrifuged at 10,000 rpm for 20 minutes to separate the aqueous phase from the chloroform.

The resuspended pellets were centrifuged separately at 40,000 rpm for 2 hours in a Ti 50.2 rotor. The resulting pellets were then resuspended in Na₂HPO₄/KH₂PO₄ phosphate buffer (pH 7.0) and shaken overnight at 4 °C. Cesium chloride was added to all of the resuspended pellets (4 g CsCl/ml) and then they were centrifuged at 60,000 rpm for approximately 16 hours in a Ti 70 rotor. Bands visible in the CsCl gradients were collected

with a syringe. Phosphate buffer was added to the samples and then they were centrifuged for 1.5 hours at 55,000 rpm in a Ti 70 rotor. The pellets were resuspended in phosphate buffer and viewed under the electron microscope.

Rate of growth

The rate of growth of 28 isolates of *P. weirii* from 14 centers was measured on Difco® malt agar (approx. 12.5 ml/petri plate). The number of isolates per center varied from one to five. Plates were inoculated with an agar disk that was removed from the actively growing edge of a colony with a #1 cork borer. The plates were randomly stacked and incubated in the dark at 20 °C. Measurements were taken after 2 and 7 days. Five plates were measured per isolate. The vegetative compatibility of isolates from the same site was tested and cultures were examined for any virus like symptoms.

2.6.3 Results

dsRNA extraction

dsRNA was not detected in any of the *P. weirii* isolates, but was detected in the control fungi (Fig. 19). The aqueous phase that was obtained following phenol extraction was quite viscous and made elution of the cellulose column more difficult but did not appear to interfere with the extraction of dsRNA. Mixing of *U. maydis* and *P. weirii* tissue prior to extraction had no effect on the ability to detect dsRNA in *U. maydis*.

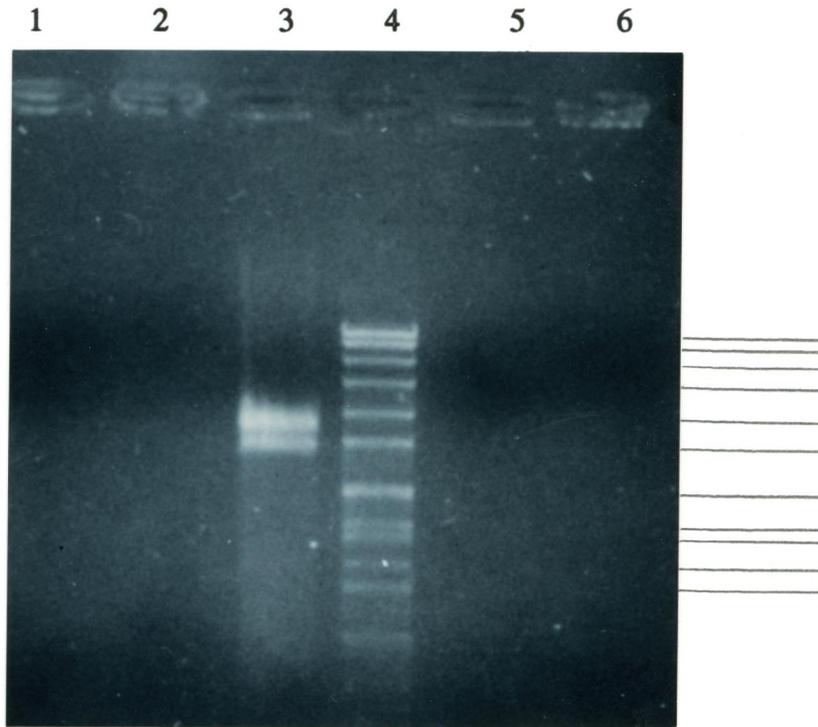


Fig. 19 A gel showing the results of a dsRNA extraction of *P. weirii* isolates. Lanes 1,2,5 and 6 are dsRNA extracted *P. weirii* samples, lane 3 contains dsRNA extracted from *U. maydis*, and lane 4 contains molecular markers (EcoR1 digested SPP-1). The sizes of the molecular marker bands from top to bottom are 8, 7.1, 6, 4.8, 3.5, 2.7, 1.9, 1.5, 1.4, 1.15, 1.10 ($\times 10^3$ b.p.).

Virus purification

Large diffuse bands were collected from near the middle of the CsCl gradients, and very faint bands were collected near the top and bottom of the gradients. When these bands were viewed under the electron microscope, they were found to contain a large amount of cellular debris. No virus particles could be detected in any of the bands.

Rate of growth and cultural characteristics

The average rate of growth of isolates based on the average for each center was 6 ± 1 mm/day.² The distribution in the rate of growth of isolates from different centers was tested for normality using a chi-squared test. The distribution in growth rate was not significantly different from what would be expected from the normal distribution ($p = 0.53$).

An analysis of variance showed significant differences ($p < 0.001$) in the rates of growth of *P. weirii* isolates from different centers (Appendix 5). There were also significant differences ($p < 0.05$) in the within center rates of growth of isolates from two of the six centers with more than one isolate (Appendix 5).

Only isolates collected from the same areas were vegetatively compatible. All vegetatively compatible isolates were considered to be from the same center. Cultures of one of the isolates showed virus-like symptoms in the form of dark pigmentation and nonconcentric reduced growth (Fig. 20). Subsequent attempts at sub culturing yielded normal looking cultures in which no dsRNA could be detected.

2.6.4 Discussion

There does not appear to be evidence that dsRNA is present in *P. weirii* despite the fact that viral infections of fungi are common (Buck 1988, Ghabrial 1980, Nuss & Koltin 1990). It is possible that in fungi like *P. weirii* in which successful spore infections are rare the opportunity for the spread of viruses may be limited. Vegetative incompatibility may also prevent virus transmission (Caten 1972).

²Upper and lower bounds indicate a 95% confidence interval

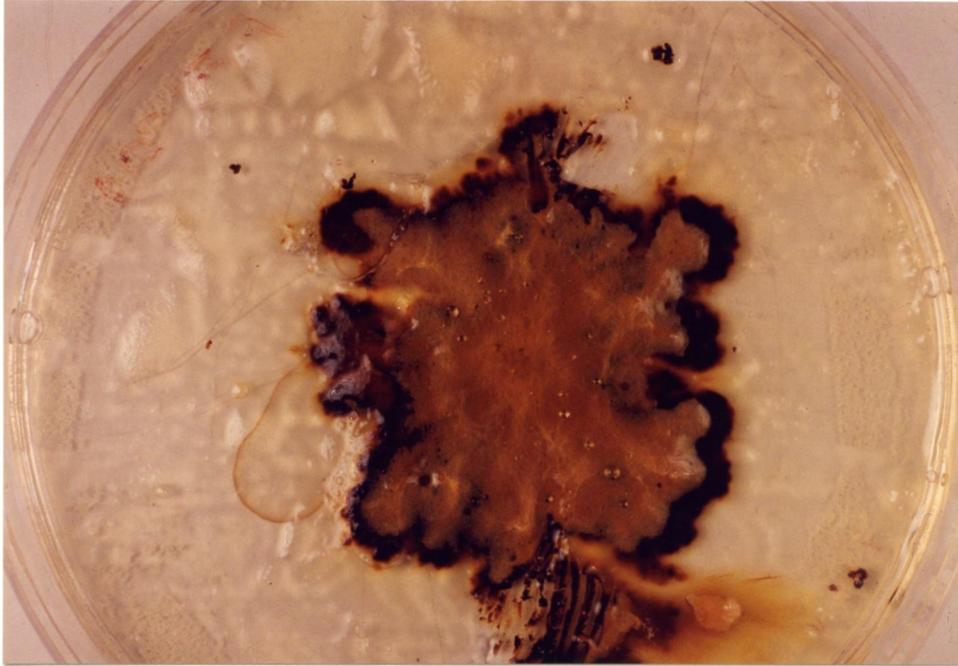


Fig. 20 Dark pigmentation and nonconcentric reduced growth in a *P. weirii* culture.

There was considerable variation in the rate of growth of different isolates; however, there is no reason to suspect that any of the isolates had abnormally slow rates of growth. The differences in the rates of growth of isolates from the same center suggests that vegetatively compatible isolates although derived from the same clone are not necessarily genetically identical. Genetic differences were detected within the same infection center using restriction fragment length polymorphism analysis (Bae 1992). These genetic differences were attributed to somatic mutations.

The temporary appearance of virus-like symptoms in cultures of one of the isolates may represent a switch in mycelial type. Distinctive mycelial types (a fast growing, white, effuse type and a slow growing, appressed, pigmented type) have been described in fungi belonging to species of *Phellinus*, *Hymenochaete*, and *Inonotus* (Sharland et al. 1986). Sharland et al. (1986) suggested that distinctive colony types may be regulated by endogenous mechanisms controlling differential gene expression.

2.6.5 Conclusions

There was no evidence found to indicate that dsRNA or dsRNA viruses are responsible for the inactivation of Phellinus root rot centers or that they even occur in *P. weirii*.

2.7 CONCLUSIONS

The inactivation of part of the root rot center could not be shown to be due solely to a lack of root contacts, differences in soil factors, soil antagonists, or differences in pathogen virulence. It is possible that a combination of lack of root contacts and soil factors may have resulted in the inactivation of part of the root rot center.

3.0 PART II: ZONE LINES AND THEIR POSSIBLE ROLES IN THE SURVIVAL OF *PHELLINUS WEIRII*

3.1 Introduction

Zone lines are structures formed by wood decay fungi, that appear as thin dark lines in sections of decayed wood (Fig. 2). Although they appear as lines in section, they are actually part of a continuous outer boundary that surrounds a volume of wood. Zone lines show many similarities to sclerotia in terms of their structure and function (Campbell, 1934). In recognition of their similarity to sclerotia and in order to distinguish zone lines from reaction zones formed as part of the compartmentalization process that takes place in wood (Shigo & Marx 1977); some workers prefer to use the term pseudosclerotial plate (PSP) to describe zone lines.

Zone line or pseudosclerotial plate formation occurs in at least 44 species of Basidiomycetes and 10 species of Ascomycetes (Lopez-Real, 1975). Rayner & Todd (1979) are of the belief that zone lines occur in most if not all decay fungi.

While zone line formation has been reported in many decay fungi; it has only been studied in a few fungi. The best information on zone line formation exists for *A. mellea* (Campbell 1934, Hansson & Seifert 1987, Lopez-Real 1975, Lopez-Real & Swift 1975, 1977).

3.2 Stages in Zone Line Formation

In the fungi in which zone line formation has been studied, the first stage of zone line formation involves hyphal proliferation (Lopez-Real 1975). In wood showing early stages of decay, hyphal proliferation is mostly confined to the cell lumen (Campbell 1934). Hyphal cells in the zone of proliferation are often swollen and bladder like and have thickened walls (Campbell 1933, Campbell 1934, Campbell & Munson 1936, Hopp 1938, Lopez-Real 1975, Nelson 1973). Setal hyphae appear to form an integral part of the zone lines formed on the surface of *P. weirii* in sawdust culture. Setal hyphae are pointed, large, thick walled,

pigmented hyphae that are readily formed in culture and look like reddish brown wiry whiskers to the naked eye. The final stage in zone line formation in many fungi involves a progressive darkening of hyphal cell walls by pigments (Campbell 1933, Campbell 1934, Campbell & Munson 1936, Hopp 1938, Lopez-Real 1975).

Li (1983) found that the pigments of *Phellinus weirii* were tyrosine-melanin in nature based on visible, ultra violet and infrared absorption spectra, degradation products, and other properties. Melanins can protect hyphae against microbial lysis and can inhibit growth of some antagonistic microorganisms (Li, 1983).

3.3 Factors that trigger zone line formation

Zone lines are formed as a result of interspecific and intraspecific antagonism between fungi or in response to environmental factors. Zone lines are also formed by *Ustulina deusta* (Fr.) Petrak at reaction zone margins (Pearce 1991) and by *Hymenochaete corrugata* (Fr.:Fr.) Lev. when different colony types derived from the same isolate are paired (Sharland et al. 1986).

Antagonism

It has long been recognized that wood decay fungi form zone lines in response to interspecific antagonism (Rayner & Todd 1979). Nelson (1973) was able to induce the formation of zone lines in sawdust cultures of *P. weirii* by adding non sterile soil to them. When he added sterile soil to the cultures, no zone lines were formed.

Many decay fungi also form zone lines as a result of intraspecific competition. Zone lines formed as a result of intraspecific competition are particularly common in fungi such as *Coriolus versicolor* (L. ex Fr.) Quèl. and *Stereum hirsutum* (Wild. ex Fr.) S.F. Gray (Rayner & Todd 1979). The zone lines formed due to intraspecific competition are often double, each mycelium being separated by its own zone line, and the wood around such zone lines is often hard and undecayed (Rayner & Todd 1979). Within a single tree it is often possible to map out

pockets of decay occupied by separate isolates of the same species of decay fungi based on the location of zone lines.

Abiotic factors

Wood moisture, relative humidity, air entry, mechanical injury, temperature, and stage of decay, have all been suggested as important factors that may trigger zone line formation (Campbell 1934, Lopez-Real & Swift 1975, Nelson 1973). The results of experiments to determine the effects of environmental factors on zone line formation differ depending on the fungus and methods used. The factors important in triggering zone line formation may be different for different species of fungi.

Hopp (1938) concluded that relative humidity had only an indirect effect on zone line formation in *Fomes applanatus* (Pers.) Gill. (*Ganoderma applanatum* Pat.) and *Fomes igniarius* (L.:Fr.) Cooke (*Phellinus igniarius* (L.:Fr.) Quèl.). Rishbeth (1951) found that zone lines formed at 70 % relative humidity (R.H.) but not at 100 % R.H in roots decayed by *Fomes annosus* (Fr.) Karst. (*Heterobasidion annosum* Bref.). He interpreted the results as evidence that zone lines are formed in response to the drying out of decayed wood. Lopez-Real & Swift (1975) found that zone lines were only produced by *Stereum hirsutum* (Willd.:Fr.) S.F. Gray at 100 % R.H., but that *A. mellea* formed zone lines over a range of relative humidities (85-100 %) so long as the wood moisture remained greater than 40 % of oven dry weight. Lopez-Real & Swift (1975) concluded that their experiments excluded moisture loss as a stimulating factor in zone line formation. Although the experiments of Lopez-Real & Swift (1975) demonstrate that zone lines can be formed in the absence of any moisture loss, their experiments do not rule out the possibility that moisture loss may be able to trigger zone line formation.

Hansson & Seifert (1987) found that oxygen concentrations of 5 % or less (in nitrogen) inhibited zone line formation by *A. mellea*. This was in contrast to Lopez-Real & Swift (1977) who found good zone line formation at oxygen concentrations as low as 0.5 % oxygen.

However, in the studies by Lopez-Real & Swift (1977) decayed wood blocks were grown under atmospheric gas concentrations prior to exposure to low oxygen concentrations, whereas Hansson & Seifert exposed veneers 3 mm or less in thickness to low oxygen concentrations, from the time of inoculation.

Data of both Hansson & Seifert (1987) and Lopez-Real & Swift (1977) indicate that a high carbon dioxide concentration (20%) causes some inhibition of zone line formation. Hansson & Seifert (1987) reported a delay in zone line formation at concentrations of 5 % and 10 % carbon dioxide. Lopez-Real & Swift (1977) found that a high concentration of carbon dioxide caused zone lines of *A. mellea* and *S. hirsutum* to form closer to the surface of blocks. In a mixture of 10 % carbon dioxide and 1 % oxygen in nitrogen, *S. hirsutum* did not form zone lines at all, even though it produced zone lines at oxygen concentrations as low as 0.5 % oxygen in the absence of carbon dioxide.

All of the work by Lopez-Real & Swift (1975, 1977) on the effects of physiological factors on zone line formation involved wounding of the fungus by cutting. Based on their work, Lopez-Real & Swift (1977) concluded that moisture content, carbon dioxide concentration, and oxygen concentration were not responsible for the initial stages of zone line formation and hypothesized that wounding was necessary for zone line formation. Such a mechanism could also explain why zone lines are formed in response to antagonism. While wounding may trigger zone line formation, the work of Hansson & Seifert (1987) in which there was no wounding and personal observations with *P. weirii* suggest that direct wounding is not a necessary prerequisite for zone line formation.

Nelson (1973) stated that zone lines produced by *P. weirii* form more readily at temperatures between 2 and 15 °C. He suggested that this was due to the effect of temperature on the ability of *P. weirii* to compete with other soil organisms. Nelson (1967) also observed that zone lines produced by *P. weirii* form more readily in wood showing advanced decay (decay pockets well distributed on cut surfaces) than in wood showing earlier stages of decay.

3.4 Possible Roles of Zone Lines in the survival of *Phellinus weirii*

3.4.1 Introduction

Zone lines presumably benefit the survival of *P. weirii* and other fungi that produce them. The extent to which zone lines aid in the survival of wood decay fungi however, is still largely unclear. A greater understanding of the roles of zone lines would enhance our current understanding of the biology of *P. weirii* and root rot fungi in general. There are at least three ways in which zone lines could benefit the survival of *P. weirii*.

- 1) By providing a barrier against competitive and antagonistic soil microorganisms
- 2) By preventing desiccation
- 3) By limiting gas exchange and in so doing slowing the rate of decomposition and enabling *P. weirii* to persist for a greater period of time.

It is widely accepted that zone lines provide a barrier to antagonistic fungi. This idea is based mostly on the observation that zone lines often separate volumes of decay in wood occupied by different decay fungi. The idea that zone lines are barriers to antagonistic fungi is also supported by the work of Rishbeth (1951). Rishbeth found that when inoculum blocks containing *Torula ligniperda* (Willk.) Sacc. or *Trichoderma viride* Pers.:Fr. were placed in contact with the ends of *F. annosus* infected roots having disrupted zone lines there was extensive replacement of *F. annosus*. *F. annosus* was not replaced in roots with intact zone lines. Nelson (1967) was able to demonstrate a strong correlation between zone line formation and the ability to reisolate *P. weirii* from decayed wood blocks that previously contained the fungus.

If zone lines are barriers to the invasion of soil fungi, they may also prevent the outward movement of *P. weirii*. The question of whether zone lines prevent the outward spread of *P. weirii* has important implications in terms of the infectivity of old stumps. In 50-year-old stumps, *P. weirii* is often confined to a "small interior island within zone lines"

(Hansen 1979a). Whether or not new infections can arise from such stumps is not presently known.

There has been very little consideration paid to other possible functions of zone lines. Campbell (1934) believed that one of the main functions of zone lines was to protect against drying, while Rishbeth (1951) thought that zone lines were formed in response to drying. Lopez-Real and Swift (1975) were unable to detect any differences in the rate of moisture loss from beech disks with and without external zone lines on their surface.

If zone lines prevented the drying out of decayed wood, this could greatly enhance the survival of *P. weirii* in decayed wood exposed to the air. The benefits to *P. weirii* in buried roots would probably not be as important since the moisture content of most soils would have to drop below the permanent wilting point in order for the moisture content of the wood to drop below the fiber saturation point (Siau 1984). Most fungi can survive in wood with a moisture content above the fiber saturation point.

Transport of water through wood occurs via bulk flow and diffusion. Diffusion is the principle means of transport in wood during drying. There are two types of diffusion that occur in wood: gaseous diffusion through the pits and lumens, and diffusion of bound water through the cell wall. Pit openings probably have little effect on diffusion rates at higher wood moisture contents (Siau 1984).

The possibility that zone lines might slow the rate of decomposition by slowing the flow of gases has never been studied. If zone lines are not effective in slowing the movement of water vapor, it is very unlikely that they would slow the flow of oxygen or carbon dioxide.

Of the three possible roles zone lines could play in the survival of *P. weirii*, the second (the effect of zone lines on the rate of water loss from wood) is the easiest to test. A series of experiments were set up to determine the effect of zone lines on the movement of water in *Phellinus*-decayed Douglas-fir blocks.

3.4.2 Methods

P. weirii decayed Douglas-fir blocks (5 cm per side) were cut from Phellinus root rot infected trees that had blown over. Zone line formation was induced in the blocks by burying them in soil for a period of 2 to 3 months. The permeability of *P. weirii* zone lines to water was tested by soaking the blocks in water containing food coloring. After 4 or 5 days the blocks were cut open to see if the dye had penetrated the zone lines. The permeability of zone lines to water was also tested by placing drops of water containing food coloring on the surface of external zone lines formed in culture.

The effect of zone lines on the rate of moisture loss was tested by comparing the difference in the rate of moisture loss between 5 cm cubical blocks with intact zone lines, blocks with disrupted zone lines, and blocks without zone lines. Just prior to the start of the experiment, the blocks without zone lines were cut from the same tree as the blocks that were buried 2 months earlier in order to induce zone line formation. Fifty-two blocks with zone lines were paired on the basis of the amount of decay, orientation of the grain, and wet weight. The zone lines of one block from each pair were then disrupted by drilling a 6.4 mm diameter hole longitudinally through the center of each block. Fifty-two blocks without zone lines were also paired and a hole was drilled in one block from each pair. Pairs of blocks with zone lines were then paired with pairs of blocks without zone lines. The wettest blocks with zone lines were paired with the wettest blocks without zone lines which had similar amounts of decay and similar orientation of the grain. Each set of four blocks was then placed together about 4.5 cm apart on a bench top. The blocks were covered with 35 x 27 x 11 cm cardboard boxes to remove the effect of air currents. The weight of the blocks and the temperature was recorded at 3 to 17 hour intervals for a period of 8 days. At the end of the experiment the blocks were oven dried and weighed. The blocks with zone lines were then cut into 4 sections and the zone lines mapped out. The blocks were given a zone line rating out of 12. A zone line rating of 0 indicated no zone lines had formed, while a zone line rating of 12 indicated complete zone line formation in all sections of the block.

For each block, regression was used to estimate the rate of moisture loss as a function of moisture content (MC) using the following regression equation.

$$\text{rate of water loss}_{(i-i+1)} = b_0 + b_1X_i + b_2X_i^2 + e_i$$

$$\text{where } X_i = (MC_i + MC_{i+1})/2$$

The regression equations were then used to predict the rate of moisture loss (g/hr) of each block at a moisture content of 0.2 g/cm³. Moisture content was expressed on a volume basis rather than on a dry weight basis because of the variation in block dry weight. A moisture content of 0.2 g/cm³ was chosen because it represented an intermediate value of moisture content for the blocks being tested and almost all the blocks were at 0.2 g/cm³ moisture content at one stage during the drying cycle. The predicted rates of moisture loss were divided by the block volume to determine the rate of water loss per cubic centimeter of wood. The predicted rate of water loss on a per volume basis was used to determine whether blocks with intact zone lines had reduced rates of moisture loss compared to blocks with disrupted zone lines or blocks without zone lines.

A similar experiment was done under conditions of controlled relative humidity. One hundred twenty blocks were paired on the basis of amount of visual decay, orientation of the grain, and wet weight. One block from each pair was buried in the ground to induce zone line formation and the remaining blocks (the controls) were frozen.

After two months the buried blocks were dug-up and the frozen blocks were thawed out. The blocks were matched according to their original pairings and placed in an incubator containing 26 % sulfuric acid solutions. A sulfuric acid concentration of 26 % corresponds to a relative humidity of approximately 80 %. The sulfuric acid solutions were changed after one week. The temperature remained around 30 °C except for the second day of the experiment in which the temperature rose to 39 °C due to an incubator malfunction. The block weight was recorded once a day for a period of two weeks.

The rate of water loss as a function of moisture content was calculated using regression equations of the same form as were used in the previous experiment. The rates of moisture loss of blocks with and without zone lines was tested at several moisture contents.

3.4.3 Results

Zone lines appear to be effective in preventing the bulk flow of water (Fig. 21). The photograph of the block shows that the dye was only able to penetrate the zone line at one location where there was a break in the zone line (lower arrow in Fig. 21). The break occurred immediately above a decay pocket where there was no wood to provide structural support to the zone line. The external zone lines formed in culture were also effective in keeping out water.

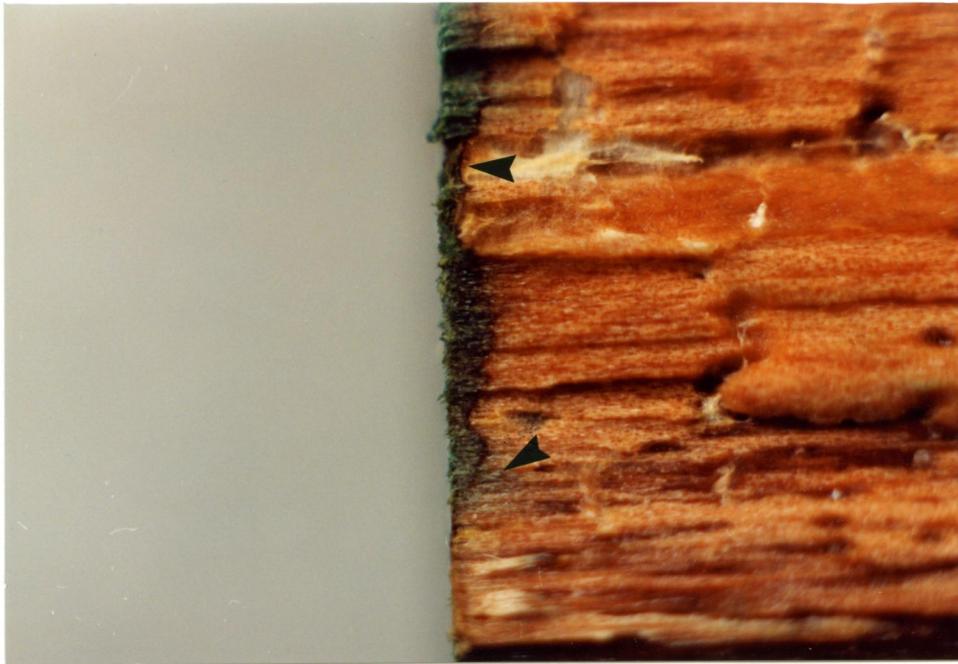


Fig. 21 Section of a block with a zone line formed by *P. weirii* showing the movement of food coloring in relation to the zone line (arrows).

In the first experiment to test the ability of zone lines to slow the rate of water loss, blocks with intact and disrupted zone lines were classified as having good or poor zone line

formation based on their zone line rating. Blocks with zone line ratings of 7 to 12 were classified as having good zone line formation, while blocks with zone line ratings of 0 to 6 were classified as having poor zone line formation.

There were significant differences ($p < 0.1$) in the initial moisture and specific gravity of different categories of blocks (Table 3) based on ANOVA. The mean initial moisture contents were separated into three groups using Tukey's multiple range test: blocks with good zone line formation, blocks with poor zone line formation, and control blocks. The mean specific gravity of control blocks and blocks with good zone line formation was significantly different using Tukey's method but the specific gravity of blocks with poor zone line formation was not significantly different ($p > 0.1$) from either group. There was no relationship between the initial water content of the blocks and their specific gravity (Fig. 22). The initial rate of water loss of the blocks was directly proportional to their initial water content (Fig. 23).

The mean standard error of the estimate and coefficient of determination for the regression equations for predicting rate of water loss as a function of moisture content were 0.018 and 0.747, respectively. A number of hypothesis tests were performed (Table 4). None of the tests were significant.

Table 3: Number of blocks, average zone line rating, initial moisture content, specific gravity, and mean predicted rate of moisture loss at 0.2 g/cm³ moisture content for different categories of blocks in the uncontrolled relative humidity experiment

Category	n	ZLR ^a	Initial Moisture (g/cm ³)	Specific Gravity (g/cm ³)	Rate of Moisture Loss ^b (g/hr/cm ³)
1. Good ^c zone lines; no hole	14	11.0	0.41	0.43	0.0015 ± 0.0001
2. Good zone lines; hole	12	10.6	0.40	0.44	0.0016 ± 0.0001
3. No zone lines; no hole	26	0.0	0.23	0.55	0.0012 ± 0.0001
4. No zone lines; hole	26	0.0	0.23	0.55	0.0012 ± 0.0002
5. Poor ^d zone lines; no hole	13	2.2	0.30	0.49	0.0016 ± 0.0001
6. Poor zone lines; hole	13	3.1	0.32	0.49	0.0016 ± 0.0001

^a Zone line rating

^b Mean rate of moisture loss at a moisture content of 0.2 g/cm³ (upper and lower bounds indicate a 95 % confidence interval)

^c Zone line rating ≥ 7

^d Zone line rating ≤ 6

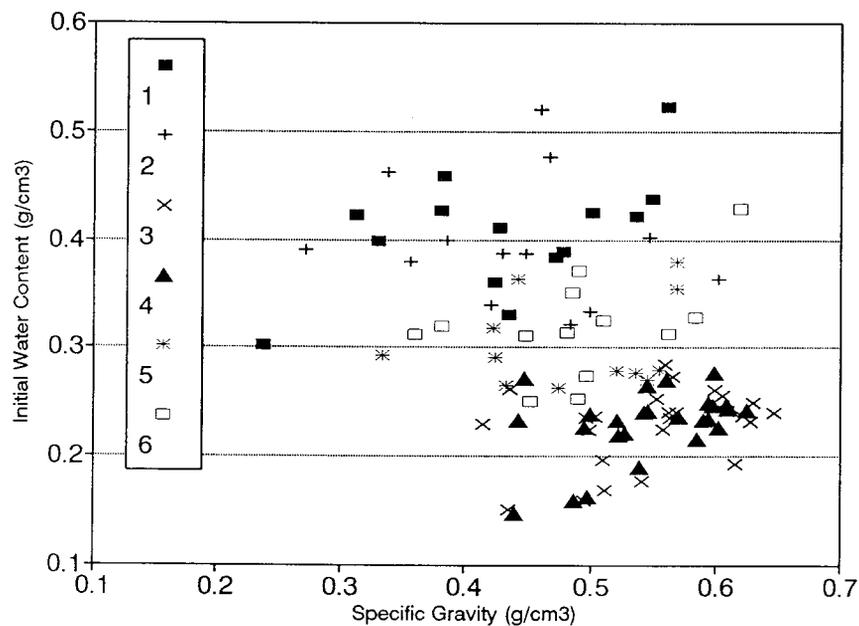


Fig. 22 Initial block moisture content versus specific gravity for *P. weirii* decayed Douglas-fir blocks with and without zone lines in the uncontrolled relative humidity experiment. The six categories of blocks in the legend refer to the six categories in Table 2.

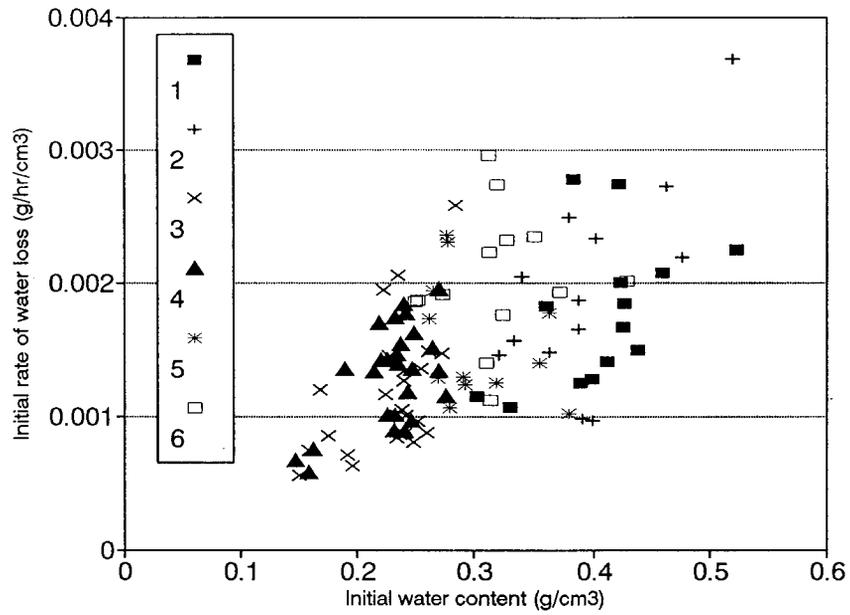


Fig. 23 Initial rate of moisture loss versus initial moisture content for different categories of *P. weirii* decayed Douglas-fir blocks in the uncontrolled relative humidity experiment. The six categories of blocks in the legend refer to the 6 categories in Table 2.

Table 4: Null and alternative hypotheses tested to determine whether zone lines affect the rate of moisture loss in *P. weirii* decayed Douglas-fir blocks in the uncontrolled humidity experiment. R_{ij} refers to the rate of water loss of the j th block belonging to the i th category (from Table 2). R_i refers to the mean rate of water loss of blocks belonging to the i th category.

zone line rating has no effect on rate of moisture loss in blocks without holes	H0: $R_1 - R_5 \geq 0$ H1: $R_1 - R_5 < 0$	
zone line rating has no effect on rate of moisture loss in blocks with holes	H0: $R_2 - R_6 \geq 0$ H1: $R_2 - R_6 < 0$	
zone lines have no effect on rate of moisture loss in blocks without holes	H0: $R_1 - R_3 \geq 0$ H1: $R_1 - R_3 < 0$	H0: $\Sigma(R_{1j} - R_{3j})/n \geq 0$ H1: $\Sigma(R_{1j} - R_{3j})/n < 0$
zone lines have no effect on rate of moisture loss in blocks with holes	H0: $R_2 - R_4 \geq 0$ H1: $R_2 - R_4 < 0$	H0: $\Sigma(R_{2j} - R_{4j})/n \geq 0$ H1: $\Sigma(R_{2j} - R_{4j})/n < 0$
holes have no effect on rate of moisture loss in blocks with zone lines	H0: $R_1 - R_2 \geq 0$ H1: $R_1 - R_2 < 0$	H0: $\Sigma(R_{1j} - R_{2j})/n \geq 0$ H1: $\Sigma(R_{1j} - R_{2j})/n < 0$
holes have no effect on rate of moisture loss in blocks without zone lines	H0: $R_3 - R_4 \geq 0$ H1: $R_3 - R_4 < 0$	H0: $\Sigma(R_{3j} - R_{4j})/n \geq 0$ H1: $\Sigma(R_{3j} - R_{4j})/n < 0$

The moisture contents and specific gravities of the different categories of blocks in the controlled humidity experiment (Table 5) were significantly different ($p < 0.1$) using ANOVA. All two way comparisons of mean moisture content were significantly different ($p < 0.1$) but no significant differences ($p > 0.1$) could be detected between two way comparisons of mean specific gravity using Tukey's multiple range test. The moisture content of the blocks in the freezer dropped an average of 0.01 g/cm^3 while they were in the freezer. The moisture content of the buried blocks increased by an average of 0.11 g/cm^3 while they were buried. The relative moisture content of the blocks remained the same. The blocks with

the highest moisture contents prior to burial to induce zone line formation also had the highest moisture contents after burial (Fig. 24). There was very little zone line formation in the buried blocks. Only 19 of the 60 blocks contained zone lines and of these only 7 had zone line ratings greater than 6.

Table 5: Number of blocks, average zone line rating, specific gravity, initial moisture content, and mean predicted rate of moisture loss at 0.2 g/cm³ moisture content for different categories of blocks in the controlled relative humidity experiment

Category	n	ZLR ^a	Initial Moisture (g/cm ³)	Specific Gravity (g/cm ³)	Rate of Moisture Loss ^b (g/hr/cm ³)
1. Good ^c zone lines	7	9.7	0.50	0.50	0.0012 ± 0.0001
2. Poor ^d zone lines	12	2.3	0.38	0.47	0.0012 ± 0.0001
3. Buried ^b ; no zone lines	41	0.0	0.33	0.50	0.0009 ± 0.00003
4. Controls	58	0.0	0.24	0.50	0.0013 ± 0.0001

^a Zone line rating

^b Mean rate of moisture loss at a moisture content of 0.2 g/cm³ (upper and lower bounds indicate a 95 % confidence interval)

^c Zone line rating ≥ 7

^d Zone line rating ≤ 6

There was no obvious relationship between the specific gravity and initial moisture content of the blocks in the controlled humidity experiment (Fig. 25). As in the uncontrolled humidity experiment, the initial rate of moisture loss was proportional to the initial water content (Fig. 26). The stage of drying also influenced the predicted rate of moisture loss. At moisture contents less than or equal to 0.2 g/cm³, initial moisture content did not have a strong influence on the predicted rate of moisture loss (Fig. 27) but at initial moisture contents greater than 0.2 g/cm³, blocks with low initial water contents had higher predicted rates of moisture loss than blocks with high initial water contents (Fig. 28).

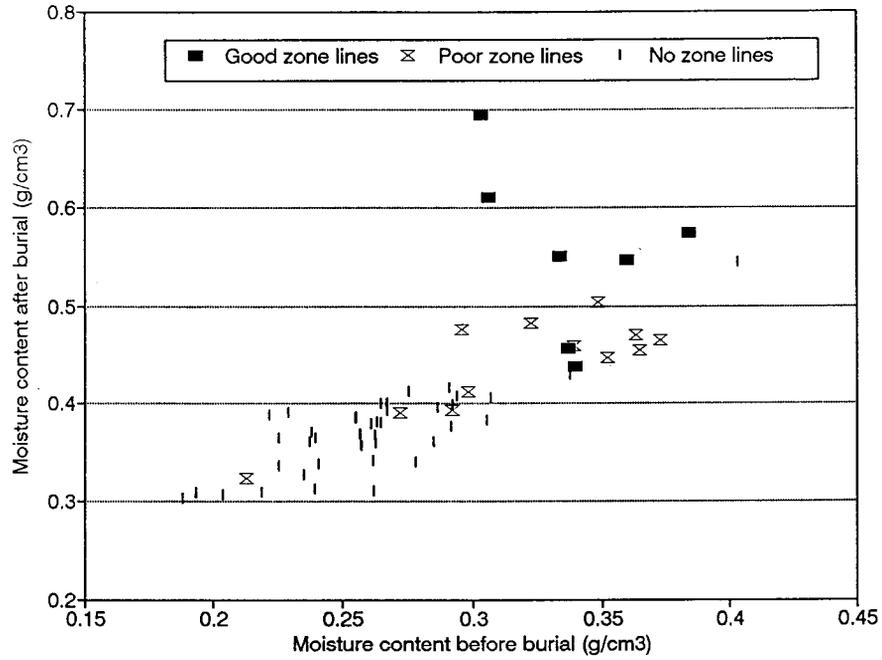


Fig. 24 Moisture content after burial to induce zone line formation in *P. weirii* decayed Douglas-fir blocks versus moisture content prior to burial.

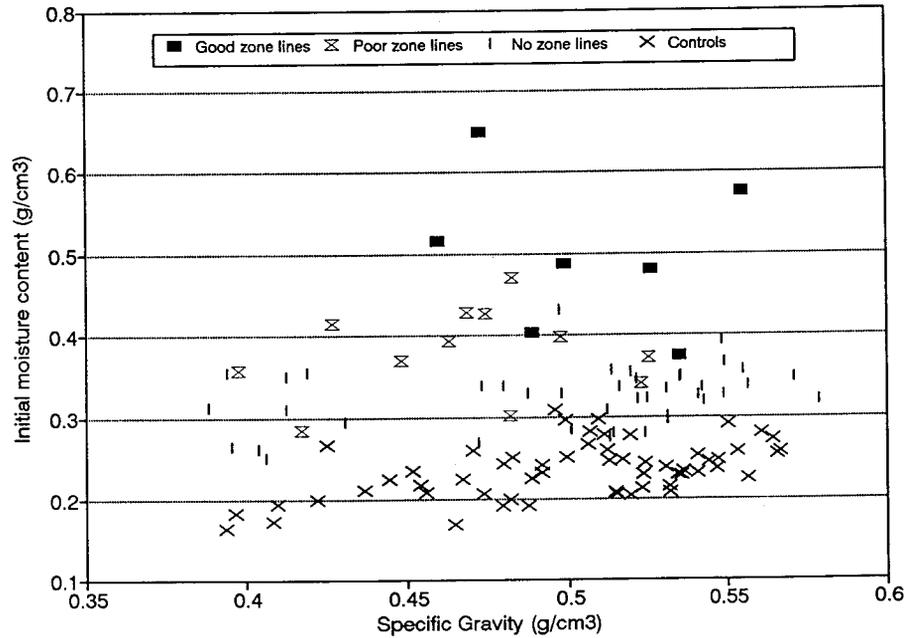


Fig. 25 Initial block moisture versus specific gravity of *P. weirii* decayed Douglas-fir blocks with and without zone lines in the controlled relative humidity experiment.

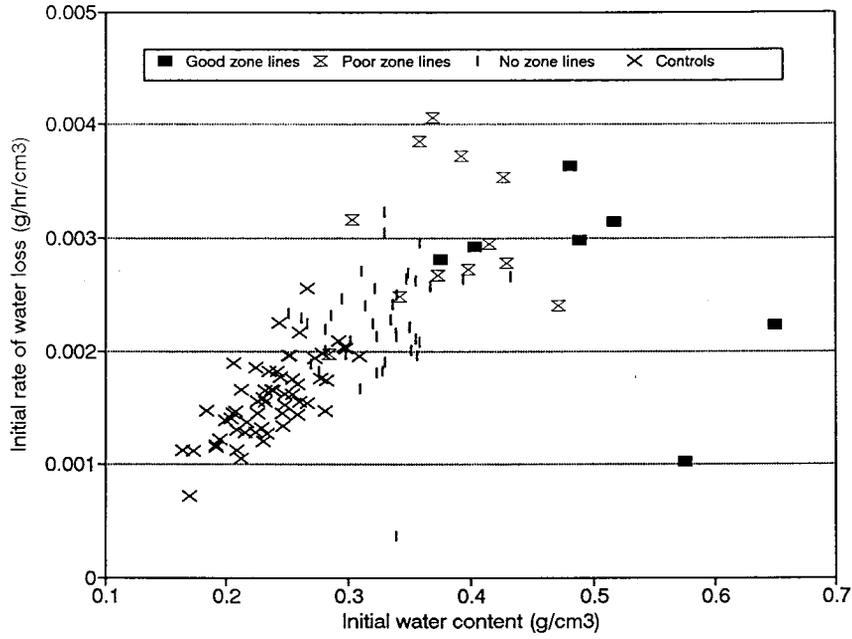


Fig. 26 Initial rate of moisture loss versus initial moisture content of *P. weirii* decayed Douglas-fir blocks with and without zone lines in the controlled humidity experiment.

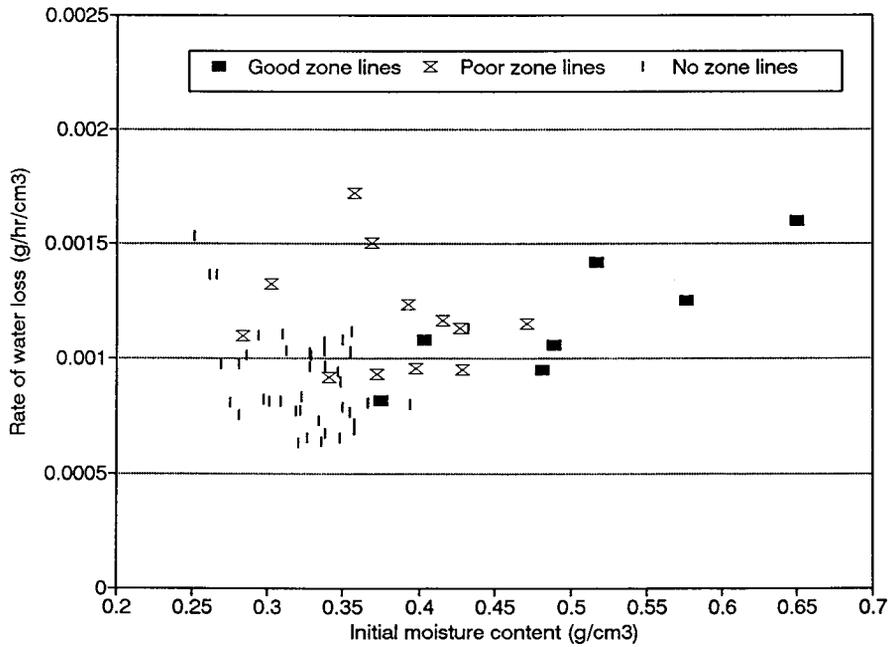


Fig. 27 Predicted rate of water loss of *P. weirii* decayed Douglas-fir blocks with and without zone lines at 0.2 g/cm³ moisture content as a function of initial moisture content.

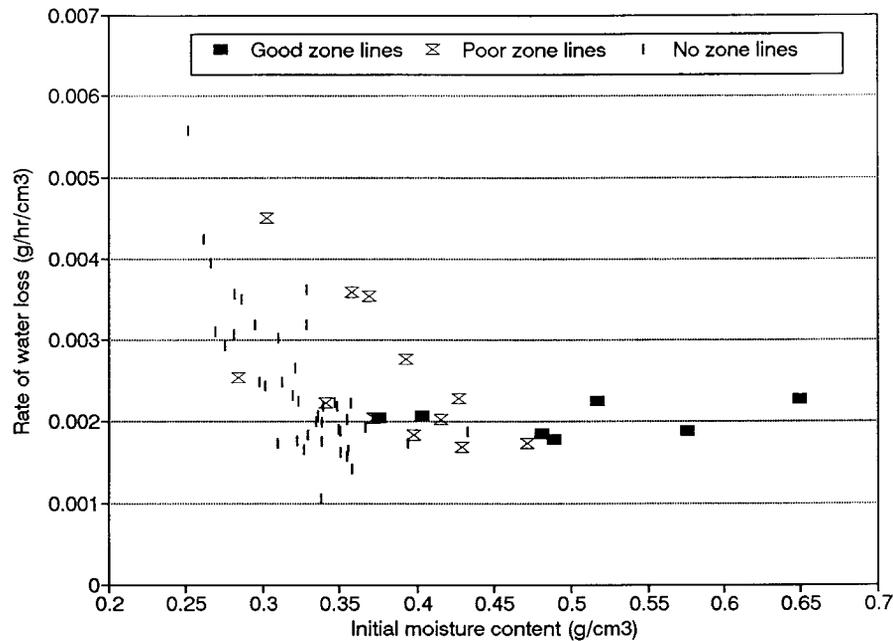


Fig. 28 Predicted rate of water loss of *P. weirii* decayed Douglas-fir blocks with and without zone lines at 0.3 g/cm³ moisture content as a function of initial moisture content.

The mean standard error of the estimate and coefficient of determination for the regression equations were 0.014 and 0.936, respectively. No significant differences could be shown in the estimated rate of moisture loss of blocks with and without zone lines at 0.2 g/cm³. In fact, the blocks with good zone line formation had a slightly higher estimated rate of moisture loss at 0.2 g/cm³ than the blocks without zone lines.

3.4.4 Discussion

The blocks with higher moisture contents at the time of burial formed zone lines more readily than blocks with lower initial moisture contents. The moisture contents of the blocks went up after they were buried but their relative moisture contents remained the same (Fig. 25) indicating that zone line formation did not have an effect on the moisture content of the blocks. The formation of zone lines may be a reflection of the amount of living mycelium in the blocks (i.e. blocks with a large amount of viable mycelium probably form zone lines less

blocks (i.e. blocks with a large amount of viable mycelium probably form zone lines less readily than blocks with a low amount of viable mycelium). Alternatively, *P. weirii* may only readily form zone lines above a minimum moisture content. This would explain why zone lines formed more readily in wetter blocks. There was no obvious relationship between initial block moisture and the specific gravity of the blocks.

The rate of moisture loss at 0.2 g/cm^3 water content was used to test the effect of zone lines on the rate of moisture loss rather than the initial rate of moisture loss because initial moisture content has a strong influence on the rate of moisture loss (Figs. 23 & 26). The amount of time that the blocks have been drying also influences the rate of moisture loss. The graph of rate of estimated moisture loss at 0.3 g/cm^3 moisture content versus initial moisture content (Fig. 28) shows a curvilinear relationship indicating that blocks with a lower initial moisture content had faster rates of estimated moisture loss than blocks with a higher initial moisture content. Presumably this was due to the fact that blocks with initially high moisture contents had drier surfaces after a period of drying than blocks with initially low moisture contents that were just beginning to dry out. At a moisture content of 0.2 g/cm^3 all the blocks had been drying out for a period of time and the effect of initial block moisture could no longer be seen (Fig. 27).

The fit of the regression curves was much better in the controlled humidity experiment but fewer of the buried blocks formed zone lines. The blocks with zone lines in the controlled humidity experiment probably did not contain any living mycelium because the temperature increase that occurred was probably sufficient to kill any living *P. weirii* mycelium that was present in the blocks. Nelson & Fay (1974) found that *P. weirii* was unable to tolerate $39 \text{ }^\circ\text{C}$ for even 1 day. The increase in temperature that resulted from the malfunctioning of the incubator would have had little effect on the relative humidity. For a relative humidity of approximately 80 %, the increase in relative humidity would be in the order of 0.3 % (Solomon 1951).

The absence of zone line formation in some blocks and the differences in moisture between blocks with and without zone lines made interpretation of the results more difficult. Nevertheless, when the effect of initial moisture content was removed there was no indication that zone lines have a major effect on the rate of moisture loss in wood blocks.

3.4.5 Conclusions

Zone lines are a barrier to the mass flow of water but do not appear to be effective in slowing the rate of moisture loss from decayed wood exposed to drying conditions. Blocks with high moisture contents are more likely to form zone lines than blocks with low moisture contents.

3.5 Conclusions

The structure and formation of zone lines has been studied in a number of fungi. The factors that trigger zone line formation are not fully understood at present. Zone lines prevent the mass flow of water but are not a barrier to diffusion. The inability of zone lines to slow the movement of water vapor precludes the idea that they may function to slow the rate of decomposition by slowing gas exchange. Most evidence to date supports the idea that the main function of zone lines is to keep out other organisms.

4.0 GENERAL CONCLUSIONS

The first part of this thesis examined possible causes for the inactivation of a root rot center in the Malcolm Knapp Research Forest. The inactivation of a portion of the *Phellinus* root rot center could not be explained solely by lack of root contacts, differences in soil properties, soil antagonists, or differences in pathogen virulence based on the results of these experiments. This does not mean that these factors could not have been the cause of the inactivation, only that they were no longer evident at the time these experiments were performed or that they were not detectable with the methods employed. The inactivation of *P. weirii* root rot centers may be due to a combination of factors rather than any one single factor. The causes of root rot inactivation may also differ for different centers. Future studies should focus on identifying other inactive centers in order to study the phenomenon of root rot inactivation more thoroughly. To study the effects of soil from the active and inactive edges of the root rot center on the growth of *P. weirii*, a method of inoculating seedlings roots was devised based on the inoculation method developed by George Reynolds at the Pacific Forestry Center in Victoria. This technique may be of some use in future studies involving interactions between *P. weirii*, its hosts, and the environment.

The second part of the thesis investigated some possible roles of zone lines in the survival of *P. weirii*. Specifically, whether or not zone lines slow the rate of drying in decayed wood. Zone lines did not have a significant effect on the rate of moisture loss from blocks of decayed Douglas-fir. The inability of zone lines to stop the gaseous flow of water makes it unlikely that zone lines effect the rate of decay by restricting gas flow. Very little research has been done on zone lines due to the difficulty in designing and setting up experiments involving zone lines. Zone lines probably play an important role in the survival of *P. weirii* and other root decay fungi and an understanding of zone lines is essential for interpreting the population and community structure of decay fungi in wood (Rayner & Todd 1979). The importance of

zone lines to decay fungi and the mechanisms involved in their formation are definitely areas that deserve further research.

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Appendix 1 Descriptions of soil in a *P. weirii* root rot center in the Malcolm Knapp Research Forest

Soil Perviousness Classes

- R (Rapidly pervious), M (Moderately pervious), S (Slowly pervious)

Soil Drainage Classes

- VR (Very rapidly drained), R (Rapidly drained), W (Well drained), MW (Moderately well drained), I (Imperfectly drained), P (Poorly drained), VP (Very poorly drained)

Color

- Hue Value/Chroma (according to Munsell)

Texture

- H (heavy), C (clay), Si (silt or silty), S (sand or sandy), L (loam or loamy)

Mottles

- color, size (mm), % of matrix or external ped surface occupied by mottles, M (Matrix) or E (external ped surface)

Structure

- 1A (single-grained), 1B (massive), 2A (angular blocky), 2B (subangular blocky), 2C (granular), 3A (platy), 4A (prismatic), 4B (columnar)
- f (fine), m (medium), c (coarse), v (very coarse)
- W (weak), M (moderately), S (strong)

Consistence

- W (Wet), M (Moist), D (Dry)
- S (Stickiness), P (Plasticity), F (Friability), H (hardness)
- WS0 (Nonsticky), WS1 (Slightly sticky), WS2 (Sticky), WS3 (Very sticky)
- WP0 (Nonplastic), WP1 (Slightly plastic), WP2 (Plastic), WP3 (Very plastic)
- MF0 (Loose), MF1 (Very friable), MF2 (Friable), MF3 (Firm) MF4 (Very firm)
- DH0 (Loose), DH1 (Soft), DH2 (Slightly hard), DH3 (Hard), DH4 (Very hard), DH5 (Extremely hard), DH6 (Rigid)

Roots

- V (very few), F (few), P (plentiful), A (abundant)
- b (very fine), c (fine), d (medium), e (coarse)

Coarse Fragments

- stone:cobble:gravel (% volume)

NAME: D. RUSCH
 PEDON: 76/WEST/1.8 m
 LAT.: 122 35 W LONG.: 49 18 N ELEV.: 160 m SLOPE: NIL
 LOCALITY: ALOUETTE RIVER VEGETATION: CEDAR-FOAMFLOWER
 STAND AGE: 37 SITE INDEX: 45 m BASAL AREA: 54 m2/ha MAI: 17.3 m3/ha
 STAND HISTORY: LOGGED 1955/ SLASH PILED & BURNED
 LANDFORM: FLUVIAL PARENT MATERIAL: GLACIAL
 DRAINAGE CLASS: R PERVIOUSNESS: R
 PIT DEPTH: 80 cm ROOTING DEPTH: 70 cm
 WATER TABLE: COMPACT TILL: 26 cm BEDROCK:
 SOIL TEMPERATURE:
 SOIL CLASSIFICATION: ORTSTEIN HUMO-FERRIC PODZOL
 UNIFIED CLASS: GC HUMUS FORM: VERMIMULL

<u>Horizon</u>	<u>Depth (cm)</u>	<u>Color</u>		<u>Texture</u>	<u>Mottles*</u>	<u>Structure</u>	<u>Consistence</u>
		<u>m</u>	<u>d</u>				
LFH	2-0						
Ah1	0-14	5YR 3/2	10YR 5/3	CL	10YR 5/8 10,10,M	2BfW	WP1,WS1,MF2,DH1
Ah2	14-20	2.5YR 2.5/2	10YR 3/2	CL		2BfW	WP1,WS1,MF2,DH2
Aecj	23-26	5YR 2.5/2	10YR 5/1	CL	10YR 3/1 70,10,M 10YR 6/4 1,5,M 10YR 5/1 25,20,M	2BfW	WP1,WS1,MF3,DH3
Bfc(>1/3)	26-76	7.5YR 4/4	10YR 6/4	SL	7.5YR 5/8 60,50,M 10YR 5/8 40,40,M	2BfW	WP0,WS0,MF1
BC	76-	2.5Y 5/4	2.5Y 7/4	S		1A	WP0,WS0,MF0

<u>Horizon</u>	<u>Roots</u>	<u>Special Features</u>	<u>Coarse Fragments</u>	<u>Boundary</u>	<u>Thickness Range (cm)</u>	<u>pH (Test Kit)</u>
LFH						
Ah1	FbPcPdVe		0:0:15	clear smooth		4.5
Ah2	FbPcPdFe		0:0:15	abrupt smooth		5
Aecj	VbVcVdVe		0:0:25	abrupt wavy		5.5
Bfc(>1/3)	VbVcVdVe	strongly cemented	0:15:40	clear smooth		6.5
BC			0:10:30			7

*Mottles are due to differences in organic matter rather than gleying

NAME: D. RUSCH
 PEDON: 57/EAST/1.8 m
 LAT.: 122 35 W LONG.: 49 18 N ELEV.: 160 m SLOPE: NIL
 LOCALITY: ALOUETTE RIVER VEGETATION: CEDAR-FOAMFLOWER
 STAND AGE: 37 SITE INDEX: 45 m BASAL AREA: 54 m²/ha MAI: 17.3 m³/ha
 STAND HISTORY: LOGGED 1955/ SLASH PILED & BURNED
 LANDFORM: FLUVIAL PARENT MATERIAL: GLACIAL
 DRAINAGE CLASS: R PERVIOUSNESS: R
 PIT DEPTH: 22-35 cm ROOTING DEPTH: 18 cm
 WATER TABLE: COMPACT TILL: 18 cm BEDROCK:
 SOIL TEMPERATURE:
 SOIL CLASSIFICATION: ORTSTEIN HUMO-FERRIC PODZOL
 UNIFIED CLASS: GC HUMUS FORM: VERMIMULL

<u>Horizon</u>	<u>Depth (cm)</u>	<u>Color</u>		<u>Texture</u>	<u>Mottles*</u>	<u>Structure</u>	<u>Consistence</u>
		<u>m</u>	<u>d</u>				
LF	1-0						
Ahc	0-18	5YR 3/2	10YR 3/3	CL		2BfW	WP1,WS0,MF2,DH2
Bfc(>1/3)	18-	10YR 4/6	2.5Y 7/4	SL		1A	WP0,WS0,MF1,DH0

<u>Horizon</u>	<u>Roots</u>	<u>Special Features</u>	<u>Coarse Fragments</u>	<u>Boundary</u>	<u>Thickness Range (cm)</u>	<u>pH (Test Kit)</u>
LF						
Ahc	PbPcPdVe		0:25:35	abrupt smooth	17-19	5.5
Bfc(>1/3)		strongly cemented	0:50:25			7

*Mottles are due to differences in organic matter rather than gleying

NAME: D. RUSCH
 PEDON: 57/NORTH/1.8 m
 LAT.: 122 35 W LONG.: 49 18 N ELEV.: 160 m SLOPE: NIL
 LOCALITY: ALOUETTE RIVER VEGETATION: CEDAR-FOAMFLOWER
 STAND AGE: 37 SITE INDEX: 45 m BASAL AREA: 54 m2/ha MAI: 17.3 m3/ha
 STAND HISTORY: LOGGED 1955/ SLASH PILED & BURNED
 LANDFORM: FLUVIAL PARENT MATERIAL: GLACIAL
 DRAINAGE CLASS: R PERVIOUSNESS: R
 PIT DEPTH: 50 cm ROOTING DEPTH: 12cm
 WATER TABLE: COMPACT TILL: 10 cm BEDROCK:
 SOIL TEMPERATURE:
 SOIL CLASSIFICATION: ORTSTEIN HUMO-FERRIC PODZOL
 UNIFIED CLASS: GC HUMUS FORM: VERMIMULL

<u>Horizon</u>	<u>Depth (cm)</u>	<u>Color m</u>	<u>d</u>	<u>Texture</u>	<u>Mottles*</u>	<u>Structure</u>	<u>Consistence</u>
LF	2-0						
Ahc	0-10	5YR 3/2	10YR 5/3	CL		2BfW	WP3,WS1,MF1
Bfc(>1/3)	10-	10YR 4/6	2.5Y 7/4	SL	5YR 4/6 60,100,M 10YR 5/6 40,50,M	2BfW	WP0,WS1,MF0

<u>Horizon</u>	<u>Roots</u>	<u>Special Features</u>	<u>Coarse Fragments</u>	<u>Boundary</u>	<u>Thickness Range (cm)</u>	<u>pH (Test Kit)</u>
LF						
Ahc	PbPcPdVe		0:0:20	clear wavy	10-13	6
Bfc(>1/3)	VbVcVdVe	strongly cemented	0:20:50		>35	7

*Mottles are due to differences in organic matter rather than gleying

NAME: D. RUSCH
 PEDON: 33/NORTH/1.8 m
 LAT.: 122 35 W LONG.: 49 18 N ELEV.: 160 m SLOPE: NIL
 LOCALITY: ALOUETTE RIVER VEGETATION: HEMLOCK FLAT MOSS
 STAND AGE: 37 SITE INDEX: 45 m BASAL AREA: 54 m²/ha MAI: 17.3 m³/ha
 STAND HISTORY: LOGGED 1955/ SLASH PILED & BURNED
 LANDFORM: FLUVIAL PARENT MATERIAL: GLACIAL
 DRAINAGE CLASS: V PERVIOUSNESS: R
 PIT DEPTH: 80 cm ROOTING DEPTH: 80 cm
 WATER TABLE: COMPACT TILL: BEDROCK:
 SOIL TEMPERATURE:
 SOIL CLASSIFICATION: ORTSTEIN HUMO-FERRIC PODZOL
 UNIFIED CLASS: GC HUMUS FORM: VERMIMULL

<u>Horizon</u>	<u>Depth (cm)</u>	<u>Color</u>		<u>Texture</u>	<u>Mottles*</u>	<u>Structure</u>	<u>Consistence</u>
		<u>m</u>	<u>d</u>				
LFH	1-0						
Ahcc	0-8	5YR 3/2	10YR 4/2	L		2BfW	WP0,WS1,MF2,DH2
Ae	8-10	10YR 3/2	10YR 5/1	CL		2BfW	WP3,WS1,MF2
Bfc1 (>1/3)	10-49	5YR 4/6	10YR 5/4	SL	5YR 5/6 50,50,M 10R 3/4 50,80,M	2BfW	WP0,WS1,MF0
Bfc2 (>1/3)	49-	5YR 3/4	10YR 6/4	SL		2BfW	WP0,WS1,MF0

<u>Horizon</u>	<u>Roots</u>	<u>Special Features</u>	<u>Coarse Fragments</u>	<u>Boundary</u>	<u>Thickness Range (cm)</u>	<u>pH (Test Kit)</u>
LFH		charcoal, SH, <2,7				
Ahcc	VbPcPdVe		0:15:30	abrupt smooth	4-18	5.5
Ae	VbVcVdVe		0:5:30	abrupt wavy	2-3	5.5
Bfc1 (>1/3)	VbPcPdVe	strongly cemented	5:15:35	gradual smooth	18-22	6
Bfc2 (>1/3)	VbVcFdVe		0:15:35		18-30	6.5

*Mottles are due to differences in organic matter rather than gleying

NAME: D. RUSCH
 PEDON: 20/WEST/1.8 m
 LAT.: 122 35 W LONG.: 49 18 N ELEV.: 160 m SLOPE: NIL
 LOCALITY: ALOUETTE RIVER VEGETATION: HEMLOCK FLAT MOSS
 STAND AGE: 37 SITE INDEX: 45 m BASAL AREA: 54 m²/ha MAI: 17.3 m³/ha
 STAND HISTORY: LOGGED 1955/ SLASH PILED & BURNED
 LANDFORM: FLUVIAL PARENT MATERIAL: GLACIAL
 DRAINAGE CLASS: VR PERVIOUSNESS: R
 PIT DEPTH: 80 cm ROOTING DEPTH: 80 cm
 WATER TABLE: COMPACT TILL: BEDROCK:
 SOIL TEMPERATURE:
 SOIL CLASSIFICATION: ORTHIC HUMO-FERRIC PODZOL
 UNIFIED CLASS: GC HUMUS FORM: VERMIMULL

<u>Horizon</u>	<u>Depth (cm)</u>	<u>Color</u>		<u>Texture</u>	<u>Mottles*</u>	<u>Structure</u>	<u>Consistence</u>
		<u>m</u>	<u>d</u>				
LFH	2-0						
Ah	0-3	5YR 2.5/2	10YR 4/2	CL		2BfW	WP1,WS1,MF2
Aej	3-5	10YR 2/1	10YR 4/1	CL		2BfW	WP1,WS1,MF2
Bf	5-16	5YR 3/4	10YR 5/4	SL	2.5YR 2.5/2 30,50,M 2.5YR 3/6 30,50,M 5YR 4/6 40,50,M	2BfW	WP0,WS0,MF2
BC1	16-37	10YR 4/4	2.5Y 5/4	SL		1A	WP0,WS0,MF0
BC2	37-	10YR 4/4	2.5Y 6/4	SL		1A	WP0,WS0,MF0

<u>Horizon</u>	<u>Roots</u>	<u>Special Features</u>	<u>Coarse Fragments</u>	<u>Boundary</u>	<u>Thickness Range (cm)</u>	<u>pH (Test Kit)</u>
LFH						
Ah	VbPcPdVe		0:5:10	abrupt smooth	2-5	5
Aej	VbVcVdVe		0:0:10	abrupt wavy	0-3	5
Bf	VbPcPdVe		0:20:25	gradual wavy	5-13	5.5
BC1	VbFcFdVe		0:20:30	diffuse smooth	17-23	6.5
BC2	VbFcFdVe		0:20:35			7

*Mottles are due to differences in organic matter rather than gleying

NAME: D. RUSCH
 PEDON: 54/NORTH/1.8 m
 LAT.: 122 35 W LONG.: 49 18 N ELEV.: 160 m SLOPE: NIL
 LOCALITY: ALOUETTE RIVER VEGETATION: HEMLOCK FLAT MOSS
 STAND AGE: 37 SITE INDEX: 45 m BASAL AREA: 54 m²/ha MAI: 17.3 m³/ha
 STAND HISTORY: LOGGED 1955/ SLASH PILED & BURNED
 LANDFORM: FLUVIAL PARENT MATERIAL: GLACIAL
 DRAINAGE CLASS: VR PERVIOUSNESS: R
 PIT DEPTH: 80 cm ROOTING DEPTH: 80 cm
 WATER TABLE: COMPACT TILL: BEDROCK:
 SOIL TEMPERATURE:
 SOIL CLASSIFICATION: ORTHIC HUMO-FERRIC PODZOL
 UNIFIED CLASS: GC HUMUS FORM: VERMIMULL/MULLMODER

<u>Horizon</u>	<u>Depth (cm)</u>	<u>Color</u>		<u>Texture</u>	<u>Mottles*</u>	<u>Structure</u>	<u>Consistence</u>
		<u>m</u>	<u>d</u>				
LFH	1-0						
Ah	0-10	2.5YR 2.5/2	10YR 3/3	L		2BfW	WP1,WS1,MF2,DH1
Ae	10-13	7.5YR 3/2	10YR 5/2	L		2BfW	WP1,WS1,MF2,DH1
Bf	13-66	5YR 3/4	10YR 5/4	SL	5YR 5/8 35,30,M 7.5YR 5/8 30,30,M 2.5YR 3/4 35,30,M	2BfW	WP0,WS0,MF1
BC	66-	10YR 5/4	10YR 7/4	LS		1A	WP0,WS0,MF0

<u>Horizon</u>	<u>Roots</u>	<u>Special Features</u>	<u>Coarse Fragments</u>	<u>Boundary</u>	<u>Thickness Range (cm)</u>	<u>pH (Test Kit)</u>
LFH						
Ah	VbPcFdVe		0:5:20	abrupt wavy	0-12	5
Ae	VbPcFdVe		0:0:20	abrupt smooth	0-4	4
Bf	VbPcFdVe		0:20:40	clear wavy	35-50	6
BC	VbFcVdVe		0:25:45			6.5

*Mottles are due to differences in organic matter rather than gleying

NAME: D. RUSCH
 PEDON: 77/NORTH/1.8 m
 LAT.: 122 35 W LONG.: 49 18 N ELEV.: 160 m SLOPE: NIL
 LOCALITY: ALOUETTE RIVER VEGETATION: CEDAR-FOAMFLOWER
 STAND AGE: 37 SITE INDEX: 45 m BASAL AREA: 54 m²/ha MAI: 17.3 m³/ha
 STAND HISTORY: LOGGED 1955/ SLASH PILED & BURNED
 LANDFORM: FLUVIAL PARENT MATERIAL: GLACIAL
 DRAINAGE CLASS: R PERVIOUSNESS: R
 PIT DEPTH: 80 cm ROOTING DEPTH: 70 cm
 WATER TABLE: COMPACT TILL: 24 cm BEDROCK:
 SOIL TEMPERATURE:
 SOIL CLASSIFICATION: ORTSTEIN HUMO-FERRIC PODZOL
 UNIFIED CLASS: GC HUMUS FORM: VERMIMULL

<u>Horizon</u>	<u>Depth (cm)</u>	<u>Color</u>		<u>Texture</u>	<u>Mottles*</u>	<u>Structure</u>	<u>Consistence</u>
		<u>m</u>	<u>d</u>				
LF	2-0						
Ah	0-9	5YR 3/2	10YR 5/3	CL		2BfW	WP1,WS1,MF2,DH1
Ahj	9-17	10YR 4/3		CL		2BfW	WP2,WS1,MF2,DH2
Aecj	17-24	10YR 4/2	10YR 5/2	CL		2BfW	WP3,WS1,MF2,DH2
Bfc(> 1/3)	24-55	5YR 3/4	10YR 5/4	SL	2.5YR 2.5/4 20,50,M 5YR 3/4 40,100,M 5YR 4/6 40,150,M	2BfW	WP0,WS0,MF1
BC	55-	7.5YR 4/4	2.5Y 6/4	S		1A	WP0,WS0,MF0

<u>Horizon</u>	<u>Roots</u>	<u>Special Features</u>	<u>Coarse Fragments</u>	<u>Boundary</u>	<u>Thickness Range (cm)</u>	<u>pH (Test Kit)</u>
LFH						
Ah	FbPcPdFe		0:0:20	clear smooth	8-10	5
Ahj	FbPcPdFe		0:0:15	abrupt smooth	0-8	5.5
Aecj	VbPcVdVe		0:0:15	abrupt smooth	0-7	4.5
Bfc(> 1/3)	VbVcPdVe	strongly cemented	0:30:35	clear smooth	28-32	6
BC	VbVcFdVe		0:20:20			6.5

*Mottles are due to differences in organic matter rather than gleying

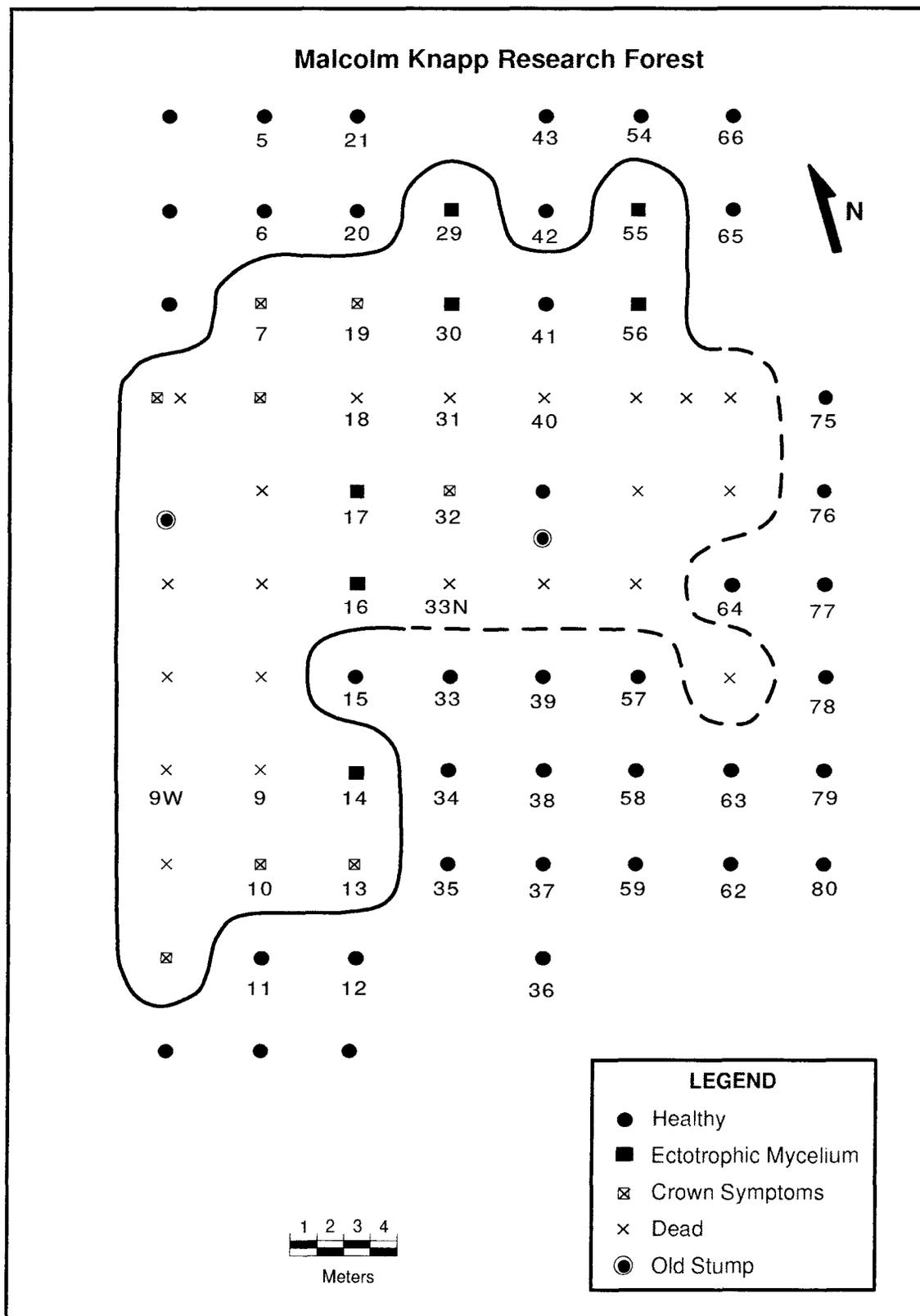
NAME: D. RUSCH
 PEDON: 12/SOUTH/1.8 m
 LAT.: 122 35 W LONG.: 49 18 N ELEV.: 160 m SLOPE: NIL
 LOCALITY: ALOUETTE RIVER VEGETATION: HEMLOCK FLAT MOSS
 STAND AGE: 37 SITE INDEX: 45 m BASAL AREA: 54 m²/ha MAI: 17.3 m³/ha
 STAND HISTORY: LOGGED 1955/ SLASH PILED & BURNED
 LANDFORM: FLUVIAL PARENT MATERIAL: GLACIAL
 DRAINAGE CLASS: R PERVIOUSNESS: R
 PIT DEPTH: 60 cm ROOTING DEPTH: 53 cm
 WATER TABLE: COMPACT TILL: 28 cm BEDROCK:
 SOIL TEMPERATURE:
 SOIL CLASSIFICATION: ORTSTEIN HUMO-FERRIC PODZOL
 UNIFIED CLASS: GC HUMUS FORM: VERMIMULL

<u>Horizon</u>	<u>Depth (cm)</u>	<u>Color</u>		<u>Texture</u>	<u>Mottles*</u>	<u>Structure</u>	<u>Consistence</u>
		<u>m</u>	<u>d</u>				
LFH	3-0						
Ahc1 (>1/3)	0-11	10YR 2/2	10YR 3/3	CL		2BfW	WP1,WS1,MF2,DH2
Ahc2 (>1/3)	11-21	10YR 3/2	2.5Y 4/2	CL		2BfW	WP1,WS2,MF2,DH3
Bfc (>1/3)	21-52	10YR 3/4	2.5Y 5/4	SL	5YR 3/1 2,10,M 5YR 5/8 40,50,M 7.5YR 5/8 50,50,M	2BfW	WP1,WS1,MF1
BC	52-	10YR 4/6	2.5Y 6/4	LS		1A	WP0,WS0,MF1

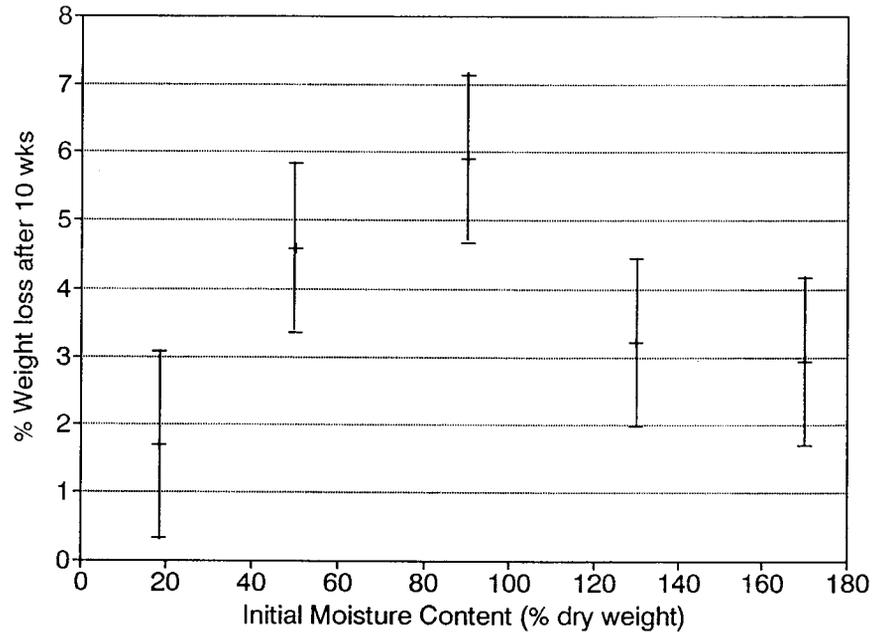
<u>Horizon</u>	<u>Roots</u>	<u>Special Features</u>	<u>Coarse Fragments</u>	<u>Boundary</u>	<u>Thickness Range (cm)</u>	<u>pH (Test Kit)</u>
LFH						
Ahc1 (>1/3)	VbPcPdVe		0:0:20	abrupt smooth	10-14	5
Ahc2 (>1/3)	FbVcFdVe		0:5:20	abrupt smooth	7-11	5
Bfc (>1/3)		strongly cemented & O.M. rich intrusion	5:15:40	abrupt smooth	25-30	5.5
BC		layer with roots	0:10:35			6.5

*Mottles are due to differences in organic matter rather than gleying

Appendix 2. Numbering system for trees in a *Phellinus* root rot center in the Malcolm Knapp Research Forest



Appendix 3 Percent weight loss of *P. weirii* decayed alder blocks as a function of moisture content



Appendix 4 Soil analyses of soil from the active and inactive edge of a *P. weirii* root rot center in the Malcolm Knapp Research Forest

	<u>Active Edge</u>	<u>Inactive Edge</u>
pH	3.9	3.7
Organic Matter ^a (%)	9.4	10.8
Total Nitrogen (%)	0.21	0.27
<u>Available Nutrients (ppm)</u>		
Phosphorus	18	11
Potassium	280	320
Calcium	85	185
Magnesium	110	180
Copper	0.55	0.7
Zinc	2.7	4.9
Iron	75	45
Manganese	25	47
Boron	0.1	0.1
<u>Particle Size (% wt. < 2.00 mm)</u>		
sand (0.05 - 2.00 mm)	74.3	71.8
silt (0.002 - 0.05 mm)	17.5	17.8
clay (<0.002 mm)	8.2	10.4

^a Based on carbon content

Appendix 5 ANOVA and means for the growth of *P. weirii* isolates in cultureBetween Center Analysis of Variance

Source	DF	SS	MS	F	p
Center	5	720.8	144.2	12.53	0.000
Error	14	161.0	11.5		
Total	19	881.8			

Location	Mean rates of growth of isolates within centers (mm/day)	Mean rate of growth of all isolates within a center ^a (mm/day)
Delta	7.4, 7.7, 8.3, 7.5, 6.9	7.6a
Haney	7.3, 7.8, 8.0, 7.8, 6.0	7.4a
Lyons Bay	5.6, 6.5	6.1
Brackendale	4.1, 4.8	4.4b
Cowichan Lake	5.3, 4.8, 3.9	4.7b
Kamloops	6.2, 7.3, 7.6	7.0a

^a values followed by different letters are significantly different using Tukey's test

Within Center Analysis of Variance - Delta

Source	DF	SS	MS	F	p
Center	4	126.96	31.74	5.05	0.006
Error	20	125.6	6.28		
Total	24	252.56			

Within Center Analysis of Variance - Haney

Source	DF	SS	MS	F	p
Center	4	332.64	83.16	23.63	0.000
Error	20	70.40	3.52		
Total	24	403.04			

Within Center Analysis of Variance - Lyons Bay

Source	DF	SS	MS	F	p
Center	1	48.4	48.40	6.91	0.030
Error	8	56.00	7.00		
Total	9	104.40			

Within Center Analysis of Variance - Brackendale

Source	DF	SS	MS	F	p
Center	1	22.40	32.40	6.00	0.040
Error	8	43.20	5.40		
Total	9	75.60			

Within Center Analysis of Variance - Cowichan

Source	DF	SS	MS	F	p
Center	2	118.09	59.5	3.21	0.077
Error	12	222.04	18.5		
Total	14	340.03			

Within Center Analysis of Variance - Kamloops

Source	DF	SS	MS	F	p
Center	2	145.73	72.87	15.08	0.001
Error	12	58.00	4.83		
Total	14	203.73			