NATURALLY INFECTED ROOT MATERIAL AS AN INOCULUM SOURCE FOR *PHELLINUS WEIRII* (MURR.) GILBERTSON.

by

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ABSTRACT:

Phellinus weirii (Murr.) Gilbertson is an important root rot of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) in western North America. The effect of site and tree species on the growth of P. weirii along roots can be measured by inoculation using naturally infected root material or P. weirii cultured on sterile wood. This thesis reports the development of an inoculation technique using naturally infected root material to infect Douglas-fir and western hemlock (Tsuga heterophylla (Raf.) Sarg.).

Intact naturally infected root sections of Douglas-fir used as an inoculum source resulted in P. weirii growing on approximately 87% of roots inoculated. Further inoculations were made using infected root sections split longitudinally with the exposed wood surface placed in contact with the host root.

Subsequently an attempt was made to evaluate the influence of xeric, submesic and hygric sites, within the Demonstration area of the UBC Research Forest, Maple Ridge, on inoculation and growth of P. weirii along roots of Douglas-fir and western hemlock. Dry soil conditions experienced during the summer of 1978 reduced the expected number of successful infections of host roots to approximately 20%, 26 weeks after installation.

All inoculations of a third series established after heavy rains in the late summer infected the roots of both Douglas-fir and western hemlock, verifying that the technique was successful when conditions were cool and moist.

P. weirii inoculum used was collected from two sources, Haney and Surrey. Laboratory studies indicated incompatibility between the two sources when raised on agar media and field results indicated a longer retention of viable P. weirii in inoculum blocks from the Surrey source.

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INTRODUCTION:

Phellinus weirii (Murr.) Gilbertson (Basidiomycetes, Polyporales), the yellow laminated root rot, is a facultative parasite of several conifer species. P. weirii is native to the Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) forests of Oregon, Washington and British Columbia (Childs, 1963) and has also been reported on western red cedar (Thuja plicata Donn) in Idaho and Alaska (Baxter, 1933). It is most destructive in immature Douglas-fir stands.

P. weirii spreads from diseased to healthy roots primarily by contacts and grafts. Although a rare occurrence, P. weirii can bridge diseased and healthy roots via other woody material, roots of minor vegetation and buried rock surfaces. The fungus grows ectotrophically on the outer bark surfaces of roots. The ectotrophic mycelia are usually white to mauve, form a thick sheath around the bark surface and may extend a few centimetres into the surrounding soil. Mycelium seldom develops on root surfaces in contact with the organic layers or decaying logs.

Penetration and death of bark occurs some distance behind the advancing ectotrophic front and the fungus invades the inner sapwood and outer heartwood. The advance of the fungus in the outer heartwood is accompanied by a red-brown stain. In later stages of development the wood becomes pitted and finally laminated.

Crown symptoms are often not observed until five to ten years after P. weirii has become established in a root system. Initially there is a reduction in leader growth with a subsequent thinning and yellowing of the foliage which is often followed by wind throw prior to death of the tree. Often disease symptoms are also expressed by a distress crop of smaller

than normal cones. Pockets of P. weirii mortality are characterized by wind-thrown trees with roots broken close to the root collar, producing root balls.

The annual mortality in Douglas-fir stands is estimated at one million cubic metres in British Columbia (Wallis, 1967) and 0.9 million cubic metres on the west side of the Cascade Range in Oregon and Washington (Childs and Shea, 1967) which represents approximately 10% and 5% of annual increment respectively.

The rate of spread of P. weirii in infection centres has been estimated by two methods. One method is to observe the spread of crown symptoms through time, the other is to determine the rate of growth of mycelium along roots. Nelson and Hartman (1975), using aerial photographs taken in 1946 and 1972 of a mixed conifer forest east of Oakridge, Oregon in the Cascade Range, studied ten infection centres common on both series of photographs. The rate of radial extension of the infection centres varied from 12 to 58 centimetres per year with the annual mean and standard deviation being 34 $\pm$ 15 centimetres.

Inoculation studies with P. weirii represent a method of observing the rate of spread on roots. Inoculation studies allow a detailed comparison of several factors that may influence fungal growth. These factors include direct observation of spread and host reaction as determined by tree age, species and ecological site resulting from root inoculation with one or more sources of P. weirii.

Early attempts to develop techniques for inoculating healthy trees with P. weirii met with limited success. Inoculation of Douglas-fir using wood chips or agar permeated with P. weirii failed to produce infection. When blocks permeated with P. weirii were placed in contact with previously

damaged roots only a low percentage of roots became infected (Buckland et al., 1954). Attempts by Wallis (1961) to infect Scots pine (Pinus sylvestris L.) with Fomes annosus (Fr.) Cke. using naturally infected root material resulted in low levels of infection of host roots. However, sterile beech (Fagus sylvatica L.) branch sections inoculated with F. annosus and placed in contact with healthy pine roots proved a satisfactory inoculation technique. This technique was later adapted for inoculation of Douglas-fir with P. weirii (Wallis and Reynolds, 1962). Subsequent studies have used sterile alder (Alnus rubra Bong.) stem sections inoculated with P. weirii as the inoculum source (Wallis and Reynolds, 1965, Wallis, 1976a). From one of these studies, Wallis and Reynolds (1962) reported average growth of mycelium away from inoculum blocks of deciduous hardwoods in contact with Douglas-fir roots of 21.6 $\pm$ 7.6 centimetres during a six month period from April to October. Mycelial growth over twelve months was not significantly different, averaging 18.8 $\pm$ 4.6 centimetres. This suggests that fungal activity virtually ceases during the winter or that food reserves of the inoculum block become depleted or too distant from the advancing mycelial front. Then if inoculum block site or distance from the advancing front influence the reduction of growth during the winter, then these figures tell little about the normal rate of spread along roots.

The only reported instance of the use of naturally infected material as the inoculum source is in a mass inoculation technique used for screening for resistance of Douglas-fir seedlings to P. weirii (Wallis and Reynolds, 1975). Naturally infected stem sections with late incipient to early-advanced decay were placed in close proximity to seedling roots. Infection of one and two year old seedlings occurred three months after establishment; mortality started after five months.

This thesis reports the development of a technique for inoculation of healthy trees with P. weirii using naturally infected root material. Initially, naturally infected intact root sections containing P. weirii were attached to healthy roots of Douglas-fir and western hemlock (Tsuga heterophylla (Raf.) Sarg.). Results suggested that high levels of infection of host roots were possible and that if such root sections were split and the infected root wood placed in contact with the healthy root, then the proportion of infections of the host roots would be greater than that achieved by using whole roots.

Having developed a promising inoculation technique it was decided to test the effect of site on the development of P. weirii. Three ecosystem unit types were chosen: a xeric, a submesic, and a hygric site. The major tree species were Douglas-fir and western hemlock. It was possible to delineate seven site-species combinations for comparison within the Demonstration area of the UBC Research Forest, Maple Ridge. Subsequently 206 inoculations were made on 116 Douglas-fir and 90 western hemlock roots using inoculum collected from within the Research Forest and from an area of forest in Surrey, B.C.

Seventy inoculations were removed ten weeks after installation. The proportion of successful inoculations was much lower than expected suggesting that the unusually dry weather conditions had likely had an effect on the success of inoculation, and following heavy rains shortly after, a subsequent series of inoculations were made to test the influence of the altered soil moisture conditions.

Laboratory studies included the comparison of the rates of growth of the two sources of inoculum on agar and some observations on zone lines in roots sections infected with P. weirii and in excavated inoculum blocks.

METHODS AND MATERIALS:

FIELD:

Three separate trials were established in the UBC Research Forest, Maple Ridge, B.C. The trials were located within the Coastal Western Hemlock biogeoclimatic zone (Klinka, 1976).

Trial 1:

The initial inoculation study was established in a submesic ecosystem unit (Klinka, 1976) in and adjacent to a 22-year-old plantation of Douglas-fir. Inoculum was collected from the roots of recently dead Douglas-fir trees located within the plantation. The presence of P. weirii in the inoculum was confirmed by the presence of brown setal hyphae seven to ten days after culturing of samples on 2% malt-agar. Intact root sections 1.5 cm to 7 cm in diameter were cut into sections up to 30 cm in length and cleaned of surface soil and loose bark.

Twenty-two roots on a total of seven Douglas-fir trees were carefully excavated about one metre from the base of the tree, brushed vigorously to remove surface soil and loose bark taking care not to injure the root bark. A piece of infected root material was bound onto the healthy root with twine and the inoculation was covered with plastic sheeting to exclude soil and organic matter. The soil and/or organic matter was then replaced to its original depth. Three western hemlock trees were similarly inoculated on a total of eight roots at a second submesic site where western hemlock was interspersed with Douglas-fir. All 30 inoculations were removed and inspected nine weeks after establishment. The presence and extent of P. weirii mycelium on the inoculum blocks and host roots, necrosis of the host roots and the host root diameter were recorded.

Trial 2:

The results of the first trial indicated that naturally infected root material was a favourable inoculum source for P. weirii, and that by modifying the technique, results could be improved. It was apparent that when the infected root wood was exposed and placed in contact with the host root, a more copious mycelial growth occurred creating a greater chance of colonization of the host root.

To test the effect of site and species combinations on inoculation, seven site-species combinations were selected within the Demonstration area of the UBC Research Forest. These are described in Table 1.

Root sections of Douglas-fir naturally infected with P. weirii were collected from two sources for use as inoculum. One source of inoculum was from the Haney location used in Trial 1, and the other was from an area of Douglas-fir forest near the junction of Scott Road and Highway 10 in Surrey, B.C., which is in the Coastal Douglas-fir Biogeoclimatic zone (Krajina, 1965).

Root sections selected for use showed early-advanced decay with well distributed decay pockets on 20-50% of cut surfaces. The inoculum blocks were prepared by cutting root sections into six to nine centimetre lengths and then splitting them longitudinally. Paired roots of fifteen trees from each site-species combination were inoculated with both sources of inoculum. Roots to be inoculated were carefully excavated about one metre from the root collar and brushed free of soil and/or organic matter. Care was taken not to damage the living bark. The wood of freshly prepared inoculum blocks was trimmed in order to achieve close contact with the root

to be inoculated and held in place with polypropylene rope. Plastic sheeting to the width of the inoculum block covered the site of inoculation. The excavated soil and/or organic matter were replaced to their original depth. All inoculations were completed in the latter two weeks of May 1978. It was only possible to inoculate 13 suitable Douglas-fir trees growing on the predominantly western hemlock submesic site.

TABLE 1. Ecosystem units, predominant tree cover and species inoculated with *P. weirii* in Trial 2, May 1978.

Site[1]	Predominant Tree Cover	Species Inoculated
Xeric	Douglas-fir	Douglas-fir
Submesic	Douglas-fir	Douglas-fir
Submesic	Douglas-fir	Western hemlock
Submesic	Western hemlock	Douglas-fir
Submesic	Western hemlock	Western hemlock
Hygric	Douglas-fir/ Western hemlock	Douglas-fir/ Western hemlock

[1]The sites selected and their ecosystem unit were based on the classification by Klinka (1976).

The number of inoculations can be summarized thus:

Site-species combinations	7
Inoculum source	2
Trees inoculated on each site	15
	210
Less those inoculations not possible	4
Total number of inoculations	206

Evaluation of inoculations was made twice. Initially inoculations from five trees on each site-species combination were removed and inspected ten weeks after establishment. The balance of the inoculations were removed and inspected 26 weeks after establishment.

For each inoculation, the extent of P. weirii mycelium on the surface of the inoculated root and the presence and extent of root necrosis were recorded in the field. The inoculated root was considered colonized if P. weirii mycelium was present on the root surface.

Roots showing necrosis were taken into the laboratory and cultures were made on 2% malt-agar from dead and discoloured bark and wood to determine the presence and location of P. weirii. Inoculum blocks were also examined in the laboratory for the presence of visible surface crustose mycelia, and split to observe the presence of zone lines. To test the survival of P. weirii in each inoculum block, small pieces of each block were placed on 2% malt-agar and incubated at room temperature. P. weirii could be identified after seven to ten days microscopically or by the presence of characteristic brown setal hyphae.

Trial 3:

The degree of successful inoculation in Trial 2 was much less than in Trial 1. Observations suggested that unusually dry weather conditions had reduced the expected successful number of colonizations of host roots. To test this, a further 20 inoculations were established at the site used in Trial 1 after above average rains fell in August.

Ten Douglas-fir and ten western hemlock roots were inoculated using only inoculum from the Haney source. The blocks and inoculations were prepared as in Trial 2, and were removed after seven weeks. Colonization and necrosis of host roots were recorded.

LABORATORY:

Cultural Differences Between the Two Sources of Inoculum:

The inoculum collected from both locations was from recently dead Douglas-fir trees from a single infection centre at each site. These inocula were characterized by observing the rate of radial growth using 2% malt-2% agar and 2% malt-5% agar in continuous light or darkness. The differing amounts of agar in the media were used to observe the effect of higher matrix potential associated with higher agar content on mycelial growth P. weirii naturally grows under dark conditions and light and dark treatments were included to observe any differences of mycelial growth on the malt-agar substrates.

Cores 4 mm in diameter were taken from the advancing zone of cultures of the two sources previously raised on 2% malt-agar and transferred to the edge of the freshly made media in sterile petri dishes. The treatments requiring continuous light were placed on a laboratory bench at room

temperature and illuminated continuously by fluorescent ceiling lights. Dark treatments were placed in a cupboard for the duration of the growth period. The temperature of all treatments was near constant at room temperature ($22^{o} \pm 2^{o}C$).

Radial growth of six replicates of each treatment was measured after 14 days. The slower growing treatments raised under continuous light were remeasured at 18 days.

Four millimetre cores taken from the advancing fronts of cultures of each source were placed approximately three centimetres apart on a malt-agar plate to observe interaction between the mycelia from the two sources. This study was replicated on 2% malt-media with either 2% or 5% agar in continuous light or dark. Observations were made periodically.

Observation of Zone Lines:

Zone lines were frequently observed in inoculum blocks from Trial 2. Selected blocks were boiled in water for one hour and allowed to cool. Thin sections of areas containing zone lines were made using a razor blade or a sliding wedge microtome. Sections were stained with phloxene, observed and photographed using a Leitz Orthoplan large-field light microscope.

Other inoculum blocks were split longitudinally and radially to expose fresh P. weirii infected wood and placed over a water bath in a constant temperature cabinet ($20^{o} \pm 2^{o}C$) for several week. These conditions approach 100% relative humidity, conditions under which zone line formation has been reported (Lopez-Real, 1975). Periodic observations were made of mycelial development on the wood surfaces of of zone-line formation.

RESULTS AND DISCUSSION:

FIELD:

Trial 1:

An inoculation was considered successful when mycelia from the infected root section had extended on to and adhered to the host root. In this situation, I considered that P. weirii had colonized the host root surface. Of the 30 inoculations made using naturally infected root pieces, 26 colonized host roots. Necrotic bark under the mycelia was observed in 15 instances. P. weirii could often be isolated from the necrotic bark, indicating penetration of the host. There was little difference in the proportion of roots colonized or showing necrosis between western hemlock and Douglas-fir (Table 2).

Hyphae developed from the infected root sections both from the bark and from the exposed wood at the ends of the sections. In many cases the hyphal growth was profuse, with hyphae, extending 2 or 3 cm away from the contact with the host root. The mycelia were often white in colour at the advancing edge with the older mycelia developing the characteristic brown coloured setal hyphae. Figure 1 illustrates the mycelial development on a western hemlock root. This type of mycelial development on host roots is not typical of the natural infection. In natural infections the ectotrophic mycelia are grey-white to light mauve in colour and do not extend very far out from the root surface. The profusion of mycelia observed in Figure 1 is probably due to the free space provided between the inoculation and the covering plastic sheet. In subsequent trials the amount of plastic used was just sufficient to cover the width of the inoculum block.

Those inoculations where colonization failed to occur generally showed

TABLE 2. Colonization by *P. weirii* of roots of Douglas-fir (DF) and western hemlock (WH) nine weeks after inoculation with root sections of naturally infected material, February to April, 1978.

Species	Number inoculated	*P. weirii* on host roots	Necrotic bark at inoculation
DF	22	20	11
WH	8	6	4
Total	30	26	15

Figure 1a.

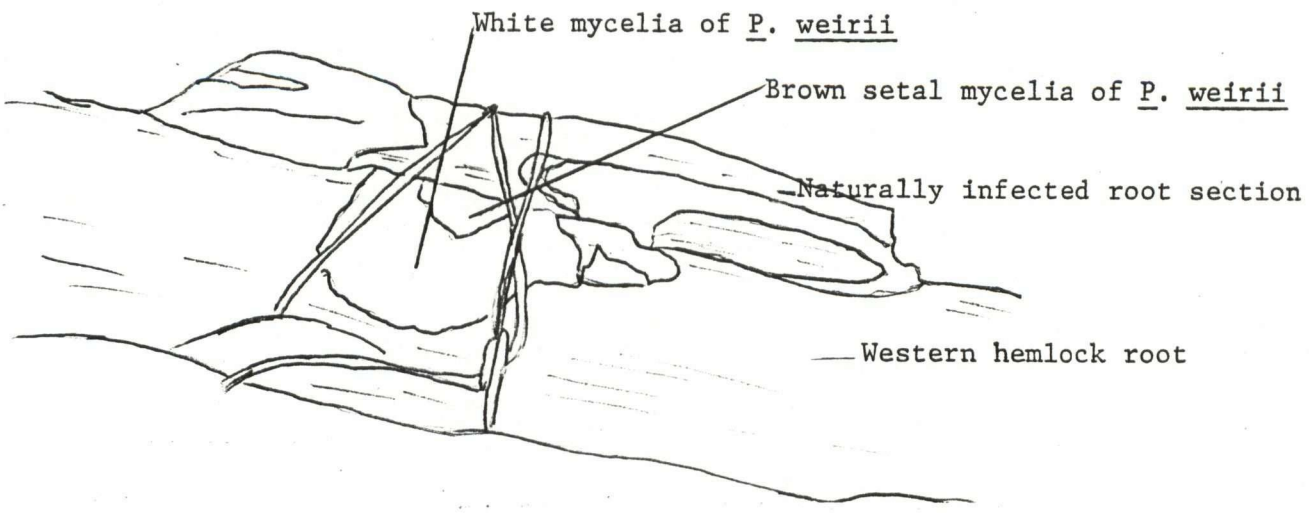

Figure 1b.

Figure 1. Photographic and schematic representation of the development of P. weirii on a western hemlock root nine weeks after inoculation with a section of naturally infected Douglas-fir root, (February-April 1978).

that there was very little contact between the inoculum block and the host root surface. In subsequent trials the inoculum used was split to expose the infected root wood and also pared to fit the host root.

Table 2 shows that there was little difference between colonization of Douglas-fir and western hemlock roots. Although the number of observations is small, the result is interesting. Mortality in western hemlock due to P. weirii in pure stands is not significant, but the disease development in mixed stands of western hemlock and Douglas-fir is more serious. Wallis (1976b) reports that although ectotrophic growth of P. weirii is similar on roots of both species, death of roots is less common in western hemlock.

Two roots of western hemlock trees inoculated with P. weirii developed a distinct zone surrounding the necrotic bark tissue. In Figure 2, the dark brown areas were necrotic regions from which P. weirii was isolated. The narrow lighter coloured zone surrounding the necrosis, and the living bark, were free of P. weirii.

Several anthocyanidin (cyanidin and pelargonidin) and non-anthocyanidin pigments have been isolated from secondary periderms formed in western hemlock stem bark (Mullick, 1969, Mullick and Jensen, 1973). The term necrophylactic periderm has been applied to these secondary periderms. The necrophylactic periderm forms an impervious barrier between living and adjacent dead bark cells as a non-specific response to agents causing injury, biotic and abiotic, and also in the absence of injury or disease (Mullick and Jensen, 1973, Mullick, 1975). Necrophylactic periderms have been observed on stems of many conifers and deciduous hardwoods (Soo, 1977), and preliminary observations have also indicated involvement of host reaction

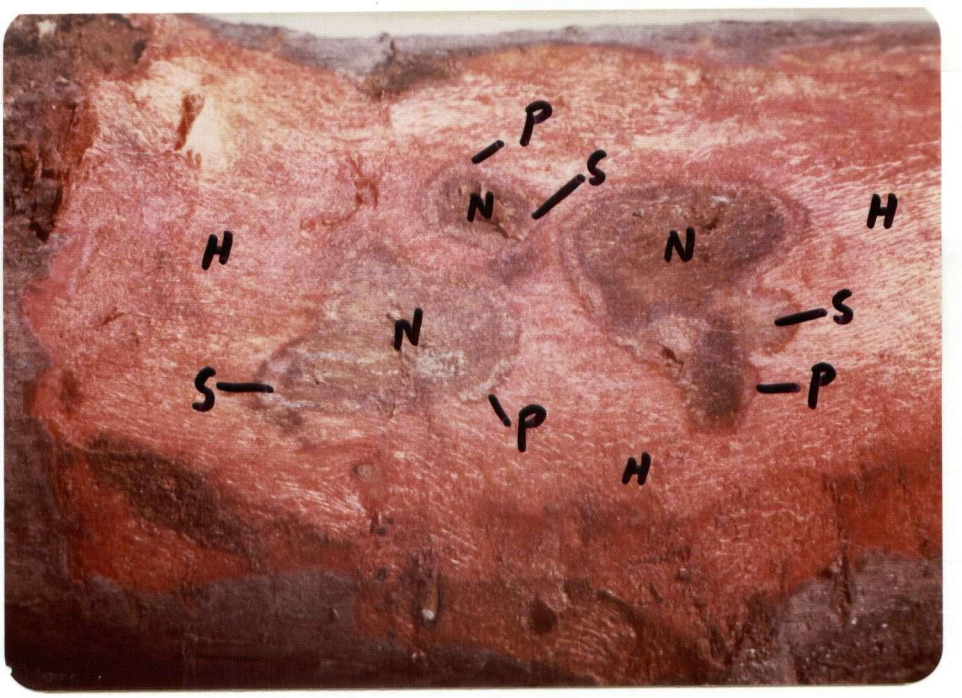

Figure 2. Necrosis of a western hemlock root inoculated with P. weirii. The fungus was isolated from the necrotic regions (N). Note (i) the distinct zone (S) surrounding the necrosis which did not contain P. weirii and (ii) the darker pigmented boundary (P) between the sterile zone and the healthy bark (H).

including necrophylactic periderm formation in the interaction between P. weirii and Douglas-fir (Mullick, 1977).

I suggest that the narrow sterile coloured zone surrounding the necrotic area is possibly a host hypersensitive reaction to fungal invasion and that the distinct, darker line surrounding this zone could conceivably be a necrophylactic periderm.

Trial 2:

Species composition, coverage and the number of stems per hectare of the tree species for the xeric, submesic and hygric site are presented in Appendix 1. Table 3 presents the ecosystem classification and soil types as described by Klinka (1976) and the average age and height of five trees at each site-species combination. The tree age was computed from ring counts of increment borings at 1.3 metres with correction for estimated early tree growth to reach that height. The corrections were obtained from the Forestry Handbook for British Columbia (Anon, 1975).

The three month period of May to July was unusually dry. A total of 16.46 cm of rain fell, and when compared to the 19-year average of 27.31 cm, was the third driest for the period. Only 8.71 cm fell during the first ten weeks after inoculation. The dry soil conditions were reflected in the behaviour of the inoculations removed at ten and twenty-six weeks. For the balance of the trial period a further 61.73 cm of rain fell. The period May to November was also the third driest for the 19 years that records have been kept at the UBC Research Forest.

Seventy inoculations were removed and examined ten weeks after establishment. The inoculation data for each site-species combination are

TABLE 3. Ecosystem unit, plant association, soil type and average tree age and height for each of the seven site-species combinations used in the second inoculation trial located in the UBC Research Forest, Maple Ridge.

Xeric Site:

Ecosystem unit:[1,2] Gaultheria-WH-DF.

Soil:[1] sandy loam Mini Humo-Ferric Podzol with mor humus.

Average tree age:[3] DF 106.

Average dominant tree height:[3] 24 m.

Submesic Site:

Ecosystem unit: Moss-WH.

Soil: loamy sand Mini Humo-Ferric Podzol with mor humus.

Predominantly western hemlock.

Average tree age: WH 96: DF 96.

Average dominant tree height: 37 m.

Predominantly Douglas-fir.

Average tree age: DF 103: WH 97.

Average dominant tree height: 33 m.

Hygric Site:

Ecosystem unit: Rubus-Polystichum-WRC.

Soil: sandy loam Gleyed Mini Humo-Ferric Podzol with mull humus.

Average tree age: DF 97: WH 91.

Average dominant tree height: 50 m.

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[1]Ecosystem units and soil types from Klinka (1976).

[2]A description of vegetation and stem numbers per hectare is presented in Appendix 1.

[3]Average of the five trees inspected at ten weeks after inoculation.

presented in Appendix 2, and are summarized for inoculum source, species inoculated and ecosystem unit in Tables 4, 5, and 6. Using a ratio estimation technique for comparing proportions (Choi, 1978, p 101.), tests of significance were computed.

Twenty-six weeks after establishment, 136 inoculations were removed and inspected. Observations and results of inoculations for each site-species combination are presented in Appendix 3 and summarized in Tables 4, 5, and 6.

Inoculum Source:

Results are summarized in Table 4.

(a) Ten weeks:

Three distinct differences between the two inoculum sources were evident after ten weeks. All blocks from the Haney source developed a dark brown mycelial crust on the exposed wood surfaces. Only 60% of blocks from the Surrey source showed the crustose mycelia. The second difference between the two sources was in the proportion of inoculations colonizing the host root surface. The proportion for the Haney source was double that of the Surrey source.

Zone lines were common in all blocks but there was a significantly lower proportion of blocks from the Haney source containing viable P. weirii.

(b) Twenty-six weeks:

Significant differences between the two inoculum sources were observed for the development of crustose mycelia and the presence of viable P. weirii in the blocks examined. The crustose mycelia developed on the majority of the blocks from the Haney source, but were absent on

TABLE 4. Frequency of zone lines, crustose mycelia, colonization and necrosis at ten and twenty-six weeks following inoculation of P. weirii on roots of Douglas-fir and western hemlock with respect to inoculum source, UBC Research Forest, May 1978.

	Number of inoculum blocks with:						Colonization of host roots		Necrotic bark
	Zone lines		Crustose[1] mycelia		Viable[2] inoculum		Total	Viable inoculum blocks	at inoculation
TEN WEEKS:									
Haney	(35)[3]	31a[4]	(34)	34a	(33)	19c	17a	9b	3
Surrey	(35)	33a		21c	(26)	25a	8b	7a[5]	1
TWENTY-SIX WEEKS:									
Haney	(68)	64a		55b		37c	14b	13a	7
Surrey	(68)	67a		30c		55b	12b	12a	8

[1]Crustose mycelia always associated with zone lines, except in one instance.

[2]Blocks containing viable P. weirii also contained zone lines, except in one instance.

[3]Number of observations unless results accompanied by a number in brackets.

[4]Values followed by the same letter are not significantly different at the 5% level (Comparison of proportions, Choi, 1978, pp. 101).

[5]One inoculum block was not tested for the presence of P. weirii.

more than half the blocks from the Surrey source. There were no significant differences in the frequency of zone lines between the two sources, but significantly more blocks from the Surrey source contained viable P. weirii.

A third difference between the sources noted at ten weeks was the proportion of root colonized. No significant difference was observed at twenty-six weeks.

(c) Comparison of results between ten and twenty-six weeks.

The comparison of viability of inoculum blocks at ten and twenty-six weeks showed a significantly lower proportion of blocks from the Surrey source contained viable P. weirii at the second observation while the proportion of blocks containing viable P. weirii from the Haney source remained unchanged. However, note that at 26 weeks the percentage of viable blocks from the Surrey source was still significantly higher than that for the Haney source.

Zone lines readily formed in inoculum blocks from both sources. However, it would appear that the effectiveness of the zone lines to maintain viability is greater in the Surrey blocks than in the Haney source. It is possible that the Surrey source of P. weirii has a greater ability to survive under dry conditions such as were encountered during Trial 2 than the Haney source. Average annual precipitation near where the Surrey inoculum was collected is approximately 125 centimetres compared to the Haney average of approximately 225 centimetres.

Heavy rains in the months of August and September did not enhance colonization of host roots. In fact the proportion of colonizations was significantly lower at twenty-six weeks than at ten weeks, primarily due to a significantly lower proportion of colonizations observed associated

with blocks from the Haney source.

There was no evidence on or in any inoculum block that P. weirii had re-invaded the areas beyond a zone line. In several cases a second zone line had formed inside the first as the P. weirii retreated into the centre of the blocks. Cultures made from blocks in the laboratory failed to detect the presence of P. weirii beyond the innermost zone line. It should be noted that zone lines very rarely formed in the wood under a surface that supported any crustose mycelia. In fact the two structures merged to form a continuous barrier.

Species Inoculated:

Results are presented in Table 5.

(a) Ten weeks:

Zone line formation, inoculum block viability and colonization of host roots were not significantly different between the two species inoculated, Douglas-fir and western hemlock. However, crustose mycelia were observed more frequently on blocks associated with western hemlock. The nature of rooting in western hemlock is such that most roots inoculated occurred in the organic layers or near the soil/humus interface and these locations may initially favour mycelial development of P. weirii, compared to the environment offered in the mineral soil layers where most of the inoculations were made on Douglas-fir.

(b) Twenty-six weeks:

No significant differences were observed between inoculations on Douglas-fir and western hemlock for block viability, crustose mycelial development, zone line formation of colonization of host roots after twenty-six weeks.

TABLE 5. Frequency of zone lines, crustose mycelia, colonization and necrosis at ten and twenty-six weeks following inoculation of P. weirii on roots of Douglas-fir and western hemlock with respect to species inoculated, UBC Research Forest, May 1978.

	Number of inoculum blocks with:						Colonization of host roots		Necrotic bark
	Zone lines		Crustose[1] mycelia		Viable[2] inoculum		Total	Viable inoculum blocks	at inoculation
TEN WEEKS:									
Douglas-fir	(40)[3]	35a[4]	(39)	27b	(33)	23a	12ab	7c	2
Western Hemlock	(30)	29a		28a	(26)	21a	13ab	9bc[5]	2
TWENTY-SIX WEEKS:									
Douglas-fir	(76)	73a		47b		48a	13a	12ab	6
Western Hemlock	(60)	58a		38b		44a	13a	13a	9

[1]Crustose mycelia always associated with zone lines, except in one instance.

[2]Blocks containing viable P. weirii also contained zone lines, except in one instance.

[3]Number of observations unless results accompanied by a number in brackets.

[4]Values followed by the same letter are not significantly different at the 5% level (Comparison of proportions, Choi, 1978, pp. 101).

[5]One inoculum block not tested for the presence of P. weirii.

(c) Comparison of results between ten and twenty-six weeks.

The only characteristic that was significantly different when the two series of observations were compared was the significant reduction in the proportion of western hemlock roots colonized at twenty-six weeks compared to ten weeks. The majority of western hemlock roots inoculated in Trial 2 were located in the organic layer or at the interface between the organic layer and the mineral soil surface. This zone, at the mineral soil surface, supports one of the most diverse populations of living organisms of all ecological niches (Spurr and Barnes, 1973) and contains many saprophytes that could compete with P. weirii for occupation of the surface of the host roots, and possibly replace P. weirii after it had become established.

Site:

Results are presented in Table 6.

(a) Ten weeks:

Viability of inoculum blocks after ten weeks in the soil showed no significant differences between sites. However, the frequency of crustose mycelia on blocks on the xeric site was significantly lower than that on the other two sites. The same trend was observed for colonization of host roots. Without mycelial development away from the surface of the inoculum block, no contact can be made between the fungus and the root surface and hence no colonization occurs.

(b) Twenty-six weeks:

No significant differences were observed for the characteristics measured at twenty-six weeks in Trial 2 between the three sites used.

TABLE 6. Frequency of zone lines, crustose mycelia, colonization and necrosis at ten and twenty-six weeks following inoculation with P. weirii on roots of Douglas-fir and western hemlock with respect to ecosystem unit used, UBC Research Forest, May 1978.

	Number of inoculum blocks with:						Colonization of host roots		Necrotic bark
	Zone lines		Crustose[1] mycelia		Viable[2] inoculum		Total	Viable inoculum blocks	at inoculation
TEN WEEKS:									
Xeric	(10)[3]	10a[4]		5c	(8)	5a	1b	1a	1
Submesic	(40)	34b	(39)	33a	(32)	22a	15a	8b[5]	2
Hygric	(20)	20a		17ab	(19)	17a	9a	7a	1
TWENTY-SIX WEEKS:									
Xeric	(20)	20a		10c		16a	3b	2a	0
Submesic	(76)	71ab		49c		48a	15b	15a	9
Hygric	(40)	40a		26bc		28a	8ab	8a	6

[1]Crustose mycelia always associated with zone lines, except in one instance.

[2]Blocks containing viable P. weirii also contained zone lines, except in one instance.

[3]Number of observations unless results accompanied by a number in brackets.

[4]Values following by the same letter are not significantly different at the 5% level (Comparison of proportions, Choi, 1978, pp. 101).

[5]One inoculum block was not tested for the presence of P. weirii.

(c) Comparison of results between ten and twenty-six weeks:

There was a significant reduction in the number of colonizations observed on the submesic site (5% level) and the hygric site (10% level) between ten and twenty-six weeks after establishment of the inoculations. Viability of inoculum blocks associated with colonization of host roots differed also between the two observation periods. At twenty-six weeks all but one of the inoculum blocks associated with colonization of roots were viable, whereas only 16 of 24 inoculum blocks associated with colonization at ten weeks were viable. Blocks in which viable P. weirii could not be detected, originated from the Haney source.

It would appear that for successful colonization, that the inoculum must remain viable until the fungus has become established in the host root.

Trial 3:

The twenty inoculations were removed seven weeks after establishment. All had developed mycelia onto the host roots. The amount varied from one or two small pockets two millimetres in diameter to instances where the inoculum block was firmly attached to the host root along the entire length and width of the block. Necrotic bark was observed on three Douglas-fir and five western hemlock roots.

This trial showed further, that given suitable moisture conditions, excellent results can be obtained using the inoculation procedure used throughout this study.

LABORATORY:

Rate of P. weirii growth on agar media:

The radial growth of mycelia cultured from the Haney and Surrey sources was measured on media containing 2% malt and either 2% or 5% agar, raised in light or dark conditions. The results are presented in Table 7.

The radial growth of cultures grown in the dark was significantly greater for both sources than those cultures raised in continuous light, and there were also differences in appearance. Dark grown cultures exhibited raised, evenly textured white to cream coloured aerial hyphae whilst mycelia on the light grown cultures were not elevated and showed a mottled appearance due to the presence of brown setal hyphae (Figure 3). There is no explanation for the differences in mycelial appearance other than a response to light inducing the production of setal hyphae.

There was no significant difference in the rate of radial growth between the two sources of mycelia when raised on the same medium in the dark. However, when results are compared between the two media used, growth on the 5% agar was significantly greater than on 2% agar.

The trends observed for radial growth of light grown cultures differed from the dark grown ones. Growth on 5% agar was significantly less than that observed on 2% agar. Also significant was that the rate of growth of the Haney source was greater on both media than that of the Surrey source.

The reason for the reversal of trends for radial growth on the two media for dark versus light conditions is not obvious. Similarly, why the Haney source should grow more rapidly than the Surrey source on the same medium in the light is unknown.

TABLE 7. Lineal growth of *P. weirii* from two sources raised on media containing either 2% or 5% agar under conditions of continuous light or dark.

Source of *P. weirii*	Agar medium	Lineal growth in millimetres per day[1]	
		Dark grown	Light grown
Haney	2%	4.73b[2]	3.07c
Haney	5%	5.29a	2.35e
Surrey	2%	4.70b	2.79d
Surrey	5%	5.16a	2.09f

[1]Mean of six replicates.

[2]Means followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

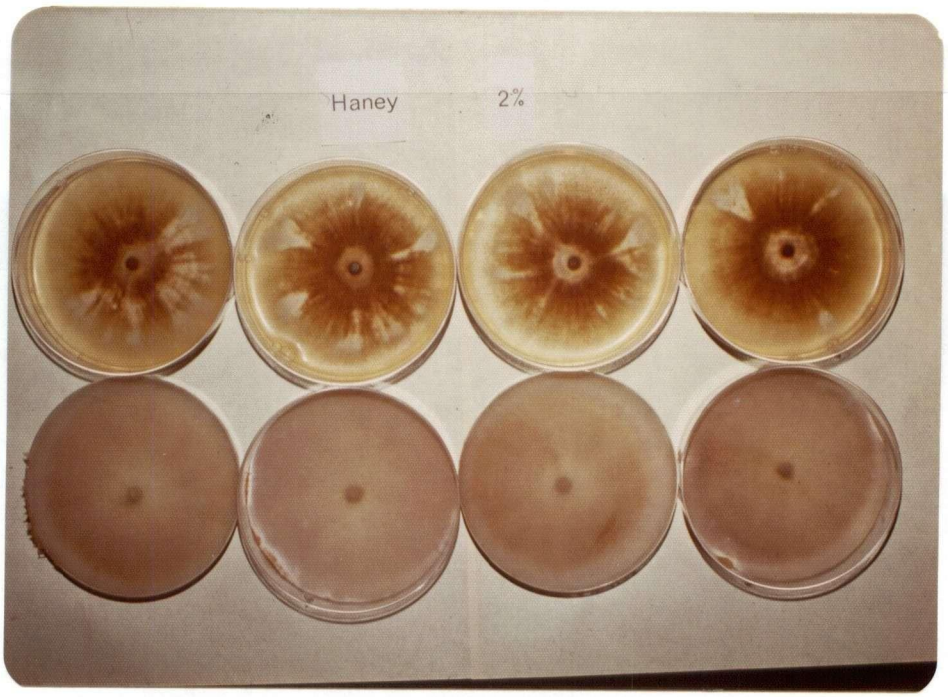

Figure 3. Comparison of the aerial hyphal characteristics of P. weirii collected from Haney and raised on 2% malt-agar for 16 days under conditions of continuous light (upper plates) or darkness (lower plates).

The reason for using media with two agar concentrations was to compare the effect of moisture availability from the substrate on the growth of P. weirii. The only conclusion is that under dark conditions both sources are stimulated on the medium containing the higher agar concentration and retarded on the media containing the lower agar concentration, and that the reverse occurs in light conditions.

Edgecombe (1941) examined the rate of growth of six wood-inhabiting basidiomycetes on media composed of sucrose, potato extract, sodium chloride and peptone together with agar concentrations of one, two and three percent. Edgecombe found that the fungal growth increased as agar concentration was reduced, and was independent of the lighting regime. Results presented here for light raised cultures agree with Edgecombe's findings but disagree with respect to the effect of lighting. The relevance of exposure to light is questionable. Under normal conditions P. weirii grows in the dark and is not visible above ground except when fruiting.

Interaction between P. weirii sources on agar media:

A reaction between the two inoculum sources when grown in close proximity was evident. Cultures raised in the light exhibited a clear zone devoid of hyphae between the advancing fronts from each source. The reaction observed in the dark was more pronounced. In cultures containing 5% agar, the advancing fronts of each source stopped abruptly when they met. A thin line separated the two sources and a distinct brown line was evident when the plates were viewed from beneath (Figure 4).

The reaction between the two sources was more pronounced on cultures raised on media containing 2% agar. The aerial hyphae at the boundary of

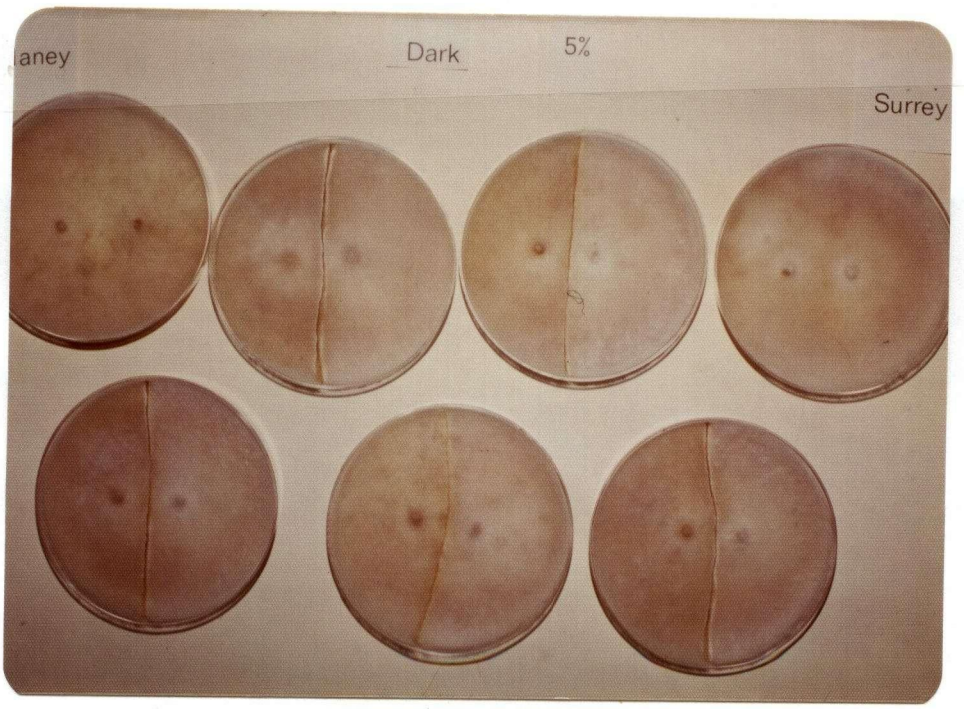

Figure 4a. Top view.

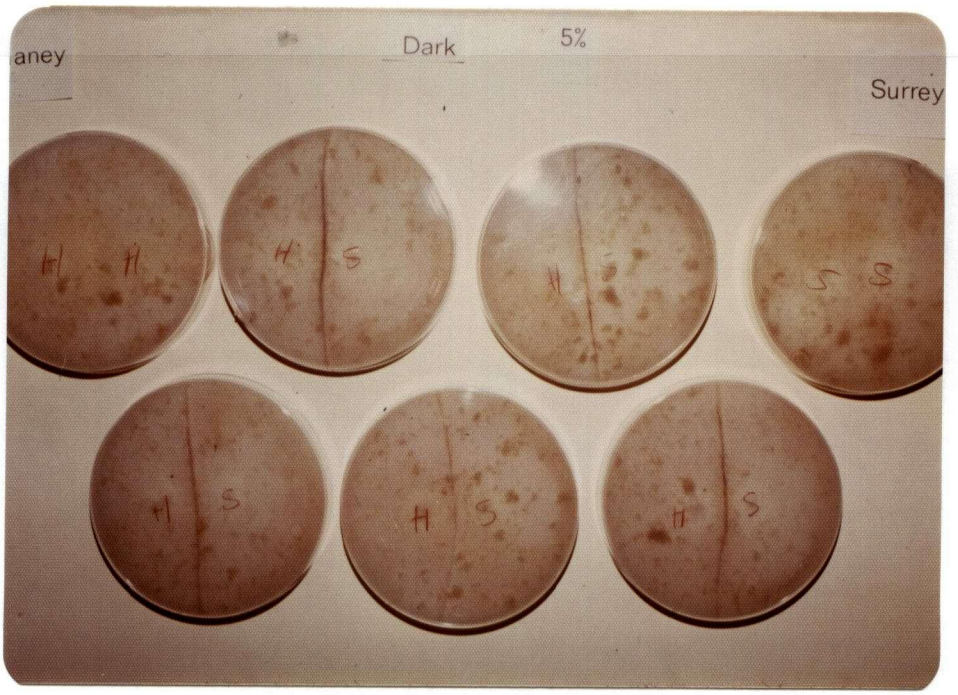

Figure 4b. Bottom view.

Figure 4. Reaction between the advancing front of the Haney source (left of plates) and the Surrey source (right of plates) of P. weirii dark grown on 5% agar-2% malt for 18 days. Note that no reaction occurs when two samples of the Haney source (top left) or the Surrey source (top right) were raised together.

the two sources were brown and microscopic examination showed that these were setal hyphae. As shown in Figure 5a, setal hyphae often extended over the entire surface of the aerial hyphae from the Haney source. The under side of the plates showed a more distinct boundary between the two sources (Figure 5b) than was observed on the medium containing 5% agar. Setal hyphae were identified in the boundary zone submerged in the agar.

These results indicate incompatibility between the two sources. When cores of the same source were placed on the same medium no reaction occurred when the advancing fronts met (Figures 4 and 5). Differences between sources of P. weirii have been previously reported (Buckland et al. 1954, Childs, 1963). It should be noted that these types of interactions on culture media can be observed between P. weirii inocula from infection centres in close proximity to each other (Childs, 1963).

Zone Lines:

Zone lines are composed of thickened hyphae accompanied by dark pigments in the cells of wood infected by many basidiomycetes and some ascomycetes. They are formed by the fungus to act as a barrier between itself and the external environment. Zone lines can form rapidly. For instance when Armillaria mellea (Vahl.) Quel. is raised in sawdust culture, zone lines form in two to three days (Lopez-Real, 1975). Zone lines in wood infected by P. weirii can be induced by several factors including temperature, soil moisture and soil microflora (Nelson, 1973). Zone lines were found in many inoculum blocks and at the conclusion of Trial 2 all blocks that contained P. weirii showed zone lines. However, zone lines do not guarantee retention of viability. In many instances, P. weirii could not be detected from blocks containing zone lines. Another feature

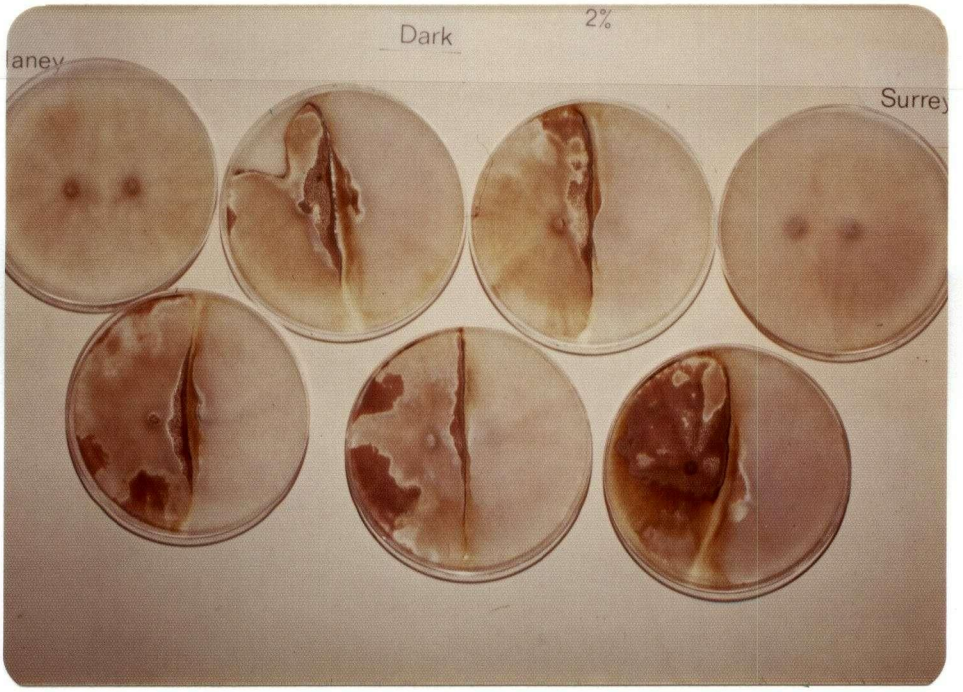

Figure 5a. Top view.

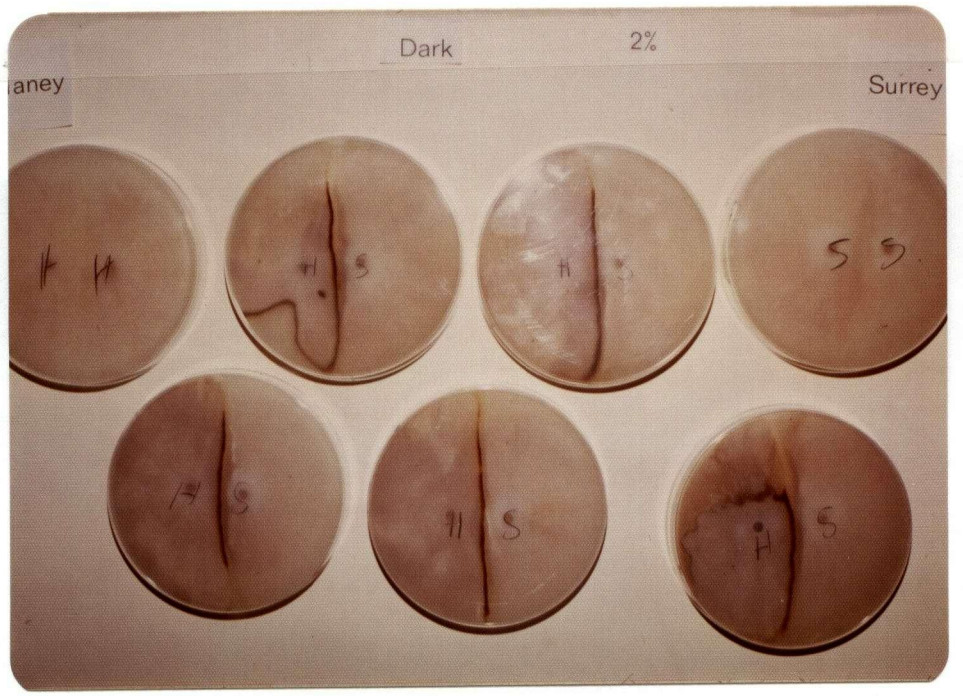

Figure 5b. Bottom view.

Figure 5. Reaction between the advancing front of the Haney source (left of plates) and the Surrey source (right of plates) of P. weirii dark grown on 2% agar-2% malt for 18 days. Note that no reaction occurs when two samples of the Haney source (top left) or the Surrey source (top right) were raised together.

was that in all but one instance where colonization occurred at twenty-six weeks, the inoculum blocks contained zone lines and viable P. weirii.

Freshly exposed surfaces of root sections and inoculum blocks containing P. weirii placed over water in a constant temperature cabinet, developed either crustose mycelium or zone lines just below the surface in such a manner that the two structures formed a continuous layer. No instances were observed where mycelia developed beyond a previously formed zone line. This observation was also noted in the field and raises questions about the re-infection of new stands of Douglas-fir growing on old P. weirii infection centres. P. weirii can remain viable in dead root systems for at least 50 years (Buckland et al., 1954, Wallis and Reynolds, 1965) but the mycelia are often surrounded by sound wood heavily impregnated with resin, with no indication that P. weirii could invade this zone. In addition, the margin of viable P. weirii is frequently marked by zone lines (Hansen, 1976), probably making the P. weirii within these barriers ineffective in creating new infections in the regenerating forest. Hence, the potential for new infections would depend on the longevity of ectotrophic mycelia associated with the bark. This period can exceed 20 years (Hansen, 1976). The spread of P. weirii in the absence of ectotrophic mycelia is doubtful. Roots from the advancing regeneration would have to penetrate the wood and zone lines surrounding the residual P. weirii or produce an exudate that would stimulate the advance of P. weirii towards the healthy root. In both instances, the zone line barrier surrounding viable P. weirii would be broken allowing possible replacement by other saprophytes and limiting the possibility of new infection.

CONCLUSIONS:

1. Root material naturally infected with P. weirii is a satisfactory inoculum for artificial inoculation of healthy roots of Douglas-fir and western hemlock.

2. High rates of infection with such inoculum were only achieved during moist cool weather as experienced during the spring and autumn of 1978 at the UBC Research Forest, Maple Ridge.

3. Differences in behavior were observed between P. weirii collected from Haney and Surrey. Differences included incompatibility when the two sources were raised together on agar media and longer retention of viable P. weirii in inoculum blocks from the Surrey source.

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APPENDIX 1.

TABLE 1. Vegetation description of sites used in Trial 2 for inoculation study, UBC Research Forest, Maple Ridge.

Species	Ecosystem unit			
	Xeric	Submesic Predominantly Douglas-fir	Submesic Predominantly Western hemlock	Hygric
TREES				
Pseudotsuga menziesii	8	8	5	7
Thuja plicata	4	4	4	4
Tsuga heterophylla	5	5	8	8
SHRUBS AND HERBS				
Acer circinatum				3
Oplopanax horridus				1
Vaccinium parvifolium	1	1	1	1
Gaultheria shallon	8	5	6	
Polystichum munitum		1		3
Vaccinium ovalifolium		1		
Rubus spectabilis				1
Dryopteris austriaca				1
Goodyera oblongifolia				1
Trillium ovatum				1
Achlys triphylla				1
Menziesia ferruginea				1
BRYOPHYTES				
Hylocomium splendens	5	4	4	3
Rhizomnium glabrescens		1	1	1
Plagiothecium undulatum	3	3	3	3
Rhytidiadelphus loreus	1		1	1
Stokesiella oregana	3	3	3	3
Isothecium stoloniferum	1			1
Dicranum fuscescens	1		1	

Species significance, total for site:

1, sparse, .3-1%; 2, 1-2%; 3, 2-5%; 4, 5-10%; 5, 10-25%; 6, 25-33%; 7, 33-50%; 8, 50-75%; 9, 75+%.

APPENDIX 1.

TABLE 2. Stem numbers per hectare for Douglas-fir, western hemlock and western red cedar on sites used in Trial 2, for inoculation study, UBC Research Forest, Maple Ridge. Figures calculated from 30m x 10m plots at each site.

Species	Stems per hectare			
	Xeric	Submesic Predominantly Douglas-fir	Submesic Predominantly Western hemlock	Hygric
Douglas-fir				
dominant-codominant	1000	634	467	367
suppressed	100	0	0	0
Western hemlock				
dominant-codominant	100	100	767	300
suppressed	0	100	133	167
Western red cedar				
dominant-codominant	433	133	133	67
intermediate	900	0	0	0
suppressed	800	233	67	33
TOTALS	3333	1200	1567	934

APPENDIX 2. Extent of colonization and root necrosis, and inoculum block characteristics of seventy inoculations made on Douglas-fir (DF) and western hemlock (WH), ten weeks after inoculation with *Phellinus weirii* on xeric, submesic and hygric sites in the UBC Research Forest, Maple Ridge.

Site-species combinations	Inoculum source	Zone lines present in blocks	Crustose mycelia on blocks	Viable inoculum blocks	Colonization of host roots		Necrotic bark at inoculation
					Total	Viable inoculum blocks	
Xeric DF	Haney	5	5	3	1	1	1
	Surrey	5	0	2 (3)[1]	0	0	0
Submesic DF	Haney	2	5	2	2	0	0
	Surrey	5	3	2	2	2	0
Submesic DF							
Inoculated WH	Haney	5	5	2	1	0	1
	Surrey	4	4	3	1	1	0
Submesic WH	Haney	5	5	5	3	3	0
	Surrey	5	5	3 (3)	2	1[2]	0
Submesic WH							
Inoculated DF	Haney	4	4 (4)	2	4	1	1
	Surrey	4 (4)	2	3	0	0	0
Hygric DF	Haney	5	5	4 (4)	3	3	0
	Surrey	5	3	5	0	0	0
Hygric WH	Haney	5	5	3	3	1	0
	Surrey	5	4	5	3	3	1
TOTALS		64	55	44	25	16	4

[1]Number of observations if less than five.

[2]One inoculum block not tested for presence of *P. weirii*.

APPENDIX 3. Extent of colonization and root necrosis and inoculum block characteristics of 136 inoculations made on Douglas-fir (DF) and western hemlock (WH), twenty-six weeks after inoculation with Phellinus weirii on xeric, submesic and hygric sites in the UBC Research Forest, Maple Ridge.

Site-species combinations	Inoculum source	Zone lines present in blocks	Crustose mycelia on blocks	Viable inoculum blocks	Colonization of host roots		Necrotic bark at inoculation
					Total	inoculum blocks	
Xeric DF	Haney	10	8	7	2	1	0
	Surrey	10	2	9	1	1	1
Submesic DF	Haney	7	4	0	0	0	0
	Surrey	10	5	10	6	6	5
Submesic DF							
Inoculated WH	Haney	9	6	1	0	0	0
	Surrey	9	4	10	1	1	1
Submesic WH	Haney	10	10	9	3	3	2
	Surrey	10	7	7	3	3	1
Submesic WH							
Inoculate DF	Haney (8)[1]	8	8	6	2	2	0
	Surrey (8)	8	5	5	0	0	0
Hygric DF	Haney	10	10	5	2	2	1
	Surrey	10	5	6	0	0	0
Hygric WH	Haney	10	9	9	5	5	4
	Surrey	10	2	8	1	1	1
TOTALS		131	85	92	26	25	15

[1]Number in brackets represents number of observations if less than ten.