

FORIA WEIRII ROOT ROT OF DOUGLAS-FIR:
SURVIVAL OF THE PATHOGEN AND
INFECTION OF THE HOST

by

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A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1962

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Date thesis is presented May 17, 1962

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ACKNOWLEDGMENTS

I wish to express my appreciation to Dr. L. F. Roth for his guidance in initiating the study, to Dr. F. H. Smith for his advice on preparing materials for microscopic examination, to Dr. H. K. Phinney for his advice concerning photographic technique, to Dr. R. O. Belkengren for his thorough criticism of the manuscript, and to other members of the faculty for their time and consideration on numerous matters.

Gratitude is expressed also to Dr. Ernest Wright and Mr. Max Halber of the Oregon Forest Research Center for their assistance in securing wood materials used in survival studies, and to the U. S. Forest Service for financial assistance.

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PORIA WEIRII ROOT ROT OF DOUGLAS-FIR: SURVIVAL OF THE PATHOGEN AND
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INTRODUCTION

Since it was first described in 1914 by Murrill (17), Poria weirii has been regarded as an important forest pathogen. In 1929 Poria weirii was reported to cause a destructive root rot of Douglas-fir (2). It has since been reported on almost all conifers in the Northwest and is responsible for greater losses of timber volume in the Douglas-fir subregion than is any other root pathogen.

As our population increases, natural resources become more valuable to us. We can no longer turn our backs on forest problems. Losses which were overlooked previously are now recognized as important. As older stands of timber are converted to more productive second-growth stands, root diseases become more common and deserve more attention from the researcher as well as the forest manager. Protection of stands from root disease will be necessary to maintain high yields of timber.

The ultimate goal of forest disease research is to realize practical solutions to the problems caused by forest pathogens. As an approach to the problem caused by Poria weirii, we must learn something about the pathogen itself, and what factors influence its activity. In attacking the problem, two avenues of research were selected: First, anatomical studies to determine the method of initial penetration of the host; and second, cultural studies to

determine factors affecting survival of the pathogen and how this survival is linked with the activities of other members of the soil microflora.

The objectives of this study are: First, to determine the ability of the pathogen to penetrate host tissues and to detect significant host reactions; second, to determine the effect of certain environmental and nutritional factors on the ability of Poria weirii to survive beneath the soil surface; and third, to demonstrate the antagonistic effects of certain soil fungi toward Poria weirii.

Results of this study may contribute to a better understanding of Poria weirii and the disease it causes, and could eventually lead to effective biological control.

LITERATURE REVIEW

According to information presented by the U. S. Forest Service (31, p. 123-128), there are 25,455,000 acres of commercial forest land in the Douglas-fir subregion of the Pacific Northwest (Washington and Oregon). This area supports 577 billion board feet of softwood saw-timber and 108 billion cubic feet of softwood growing stock. Essentially all of this volume is open to attack by Poria weirii, a pathogen which is now recognized to cause the most destructive root disease of timber within this subregion. "There is no question but that this disease, with a current annual growth impact of 454 million board feet, has become a major silvicultural problem in the Northwest" (31, p.207). The disease is not, however, restricted to Washington and Oregon. "The general distribution of the disease would suggest that it occurs throughout the northern range of Douglas-fir" (2).

Poria weirii was first described in 1914 by Murrill (17) from material Weir collected on western redcedar in Idaho. Notes by Weir are included in the publication. Overholts (20) further describes this species as it occurs on western redcedar and includes it in his key to common brown Porias. The difficulty of classifying members of the genus Poria may be realized from Overholts' statement: "This paper presents descriptions of five additional Poria species, not previously involved in my reports on this extremely difficult genus."

Resupinate species of Trametes, Fomes, and Polyporus complicate the taxonomy of the genus. Nobles (19, p.394-395) describes in detail the cultural characteristics of P. weirii.

It was thought for some time that Poria weirii occurred only on western redcedar; however, from observations made at Cowichan Lake Experiment Station in British Columbia it appeared that P. weirii was present on Douglas-fir in that locality prior to 1928 (2). In 1929, material taken from a 15- to 20-year-old stand of Douglas-fir in this area was identified as a brown Poria seeming to be P. weirii or a form of it occurring on Douglas-fir (15). Sporophores associated with the rot on Douglas-fir appeared identical with those from western redcedar; however, no sporophores older than one year were found on Douglas-fir. Poria weirii sporophores on western redcedar are often 5 or 6 years old. Wallis (32, p.13) cites an instance of a sporophore from western redcedar attaining an age of 17 years. Similarity of fungus cultures from rotted wood of Douglas-fir and from western redcedar as well as similarity of sporophores pointed up the probability of a single species of Poria being involved (15). Buckland, Molnar, and Wallis (7) later concluded that the Poria found on Douglas-fir was a variety of P. weirii or a distinct Poria species. They referred to the fungus occurring on Douglas-fir as annual P. weirii and that on western redcedar as perennial P. weirii.

Since that time Poria weirii has been found occurring on all northwestern conifers of commercial importance, none having appreciable immunity (8). The fungus has been reported specifically on Thuja plicata (western redcedar), Tsuga heterophylla (western hemlock),

Tsuga mertensiana (mountain hemlock), Abies concolor (white fir), Abies amabilis (silver fir), Abies grandis (grand fir), Picea sitchensis (Sitka spruce), Picea engelmannii (Engelmann spruce), Pinus ponderosa (ponderosa pine), Pinus contorta (lodgepole pine), Pinus monticola (western white pine), Larix occidentalis (western larch), Pseudotsuga menziesii (Douglas-fir), Libocedrus decurrens (incense-cedar), and a species of Chamaecyparis in the Northwest (2;7; 28, p.169;29;30). It may also occur on Thuja occidentalis (northern white-cedar) and on Chamaecyparis thyoides (Atlantic white-cedar) (5). Because Douglas-fir comprises the largest volume in the Douglas-fir subregion and because Douglas-fir appears as susceptible as any other species, the most serious losses could be expected within this species.

Poria weirii root rot has many similarities with the well-known root disease caused by Fomes annosus. Because of these similarities pertinent literature on both fungi is reviewed hereafter.

The host ranges of the two pathogens are broad. Fomes annosus attacks most conifers and some hardwoods throughout the world (25, p.365-371). Poria weirii attacks all conifers within its relatively narrow geographical range but has not yet been observed occurring on hardwoods.

Age of the host may influence the virulence of Fomes annosus. The fungus is most damaging to young stands growing on alkaline sites (26, p.14-15;27, p.237-244). Pine trees seem to develop resistance to killing by this pathogen at an age of about 25 years (27, p.225-229). Douglas-fir trees of all ages may be attacked

and killed by Poria weirii (32). It has been reported to occur naturally on Douglas-fir as young as 6 years (7).

Similarities in infection and local spread by Fomes annosus and Poria weirii are striking. Mycelia of these fungi grow over the surface of the root before actual penetration of the apparently healthy root tissues (27, p.221-224;32, p.8-10). While P. weirii has been observed on roots less than 1 mm. in diameter, it has also been known to extend to the root crown on major roots of trees. Both diseases have symptoms common to most root diseases of forest trees. Yellow laminated root rot caused by P. weirii typically occurs in patches of a few hundred square feet to over an acre in size. Killed trees may be standing or down. The roots of fallen trees are often broken off near the base of the trunk, exposing the typical rot. Living trees nearby may be leaning, displaying thin, ragged crowns, poor needle color, "distress" cones, and/or short lateral and terminal growth (9). These symptoms may vary with the degree of encroachment of the pathogen on the sapwood of the host (7;8). Symptoms may differ markedly among infected trees, even in the same infection center (8). Childs (10) states, "Most infected trees cannot be recognized by crown symptoms." Some infected trees may not exhibit symptoms because the destruction of main roots may be compensated for by callus tissue and by sufficient adventitious roots. These trees are very subject to windthrow (7).

Examination of crowns and excavated roots of infected trees showed that a tree infected by Poria weirii may continue normal growth

in height even though a large proportion of the root system has succumbed to disease. Trees weakened by P. weirii are predisposed to killing by bark beetles (9). Brown, crusty sporophores containing minute pores may form on the lower side of the trunk, or in root crotches of fallen trees in late summer or early fall, but usually remain too inconspicuous to be useful in detecting the disease (9). Wallis (32, p.3-4) found P. weirii fruiting abundantly on western redcedar in the interior of British Columbia but fruiting rarely on cedar on the coast. Conversely, fruiting of the fungus on Douglas-fir was more abundant on the coast.

In pine plantations infected for some time with Fomes annosus, needles are short or complements incomplete, roots become resinous, mycelium appears between bark scales, and, later, sporophores may appear at the base of the trunk. Usually growth decreases markedly one or two years before the death of the tree. Here, too, bark beetles are often secondary (25, p.368-370). Sporophores are produced the year round when the relative humidity is high and temperatures are favorable (26, p.1-5).

Damage caused by Poria weirii can usually be identified by the presence of characteristic decay exposed when trees fall or by cutting into roots or the lower bole of infected standing trees. The incipient decay is red-brown to brown in color. It appears on longitudinal sections of butts and main roots as streaks or broad bands, and in cross sections as circular, crescent-shaped or irregular areas (9).

The stain appears 2 to 6 feet in advance of later decay stages (2). Advanced decay develops, separating the annual layers of wood, and producing soft, flaky, yellowish to brownish wood residues with numerous small pockets about 0.02 inch in diameter and 0.04 inch in length. Velvety, white layers of mycelium or brown fungus threads usually are present in decayed spaces. Conks do not form until wood is in an advanced stage of decay. Eventually the decayed wood becomes a stringy mass and then completely disintegrates (2;9). Hubert (12, p.6-13) reported this rot to resemble closely that caused by Echinodontium tinctorium in grand fir; however, brown tufts of mycelium present in the layered wood distinguished the rot caused by Poria weirii from that caused by E. tinctorium. Weir, in correspondence with Murrill, states that P. weirii may continue heartwood destruction in fallen western redcedar throughout the tree. The fungus could be found in the sapwood and even in the bark (17).

Fomes annosus is not quite as easily recognized from the decay it causes as is Poria weirii; nevertheless, it can be identified readily by the conidia it produces. Characteristic conidiophores and conidia are produced in culture or on the surface of rotted wood during conditions of high relative humidity (23, p.369;26, p.1-5).

Wallis (33, p.25-27) found indications that Poria weirii was able to penetrate healthy bark of Douglas-fir roots. Rishbeth (25, p.381) states that Fomes annosus never or rarely infects trees through dead roots. Apparently healthy roots are susceptible to invasion. Mycelium may grow on the root surface before penetration,

especially in alkaline soils. Invasion itself is possible at any point (27, p.221-224). In some instances, invasion through mycorrhizal roots may take place (24).

Björkman (3) states that Fomes annosus, unlike most root rot fungi, seems able to grow as a saprophyte in the humus layer. Some growth in soil has been shown, and growth in sterile soil in the laboratory has been demonstrated (22). 22

Rishbeth (24) reports only feeble growth of Fomes annosus in unsterilized soil. The poor growth has been attributed to antagonism by such fungi as Trichoderma viride and various species of Penicillium (25, p.272-273). He further states that infection from this growth in the soil is unlikely.

No one has been able to demonstrate that Poria weirii is able to maintain itself in the soil in the absence of living or dead host tissues. Wallis (33, p.25-27) found numerous roots passing 20 to 50 mm. from adjacent infected roots without showing any signs of infection. In an attempt to control the spread of P. weirii by trenching, it was found that the fungus was unable to cross the trench even though it had been partially filled with accumulated soil and litter (34). In laboratory investigations rapid growth of P. weirii was demonstrated in glass tubes filled with sterilized forest soil or duff, but after 4 months the fungus could not be isolated from duff inoculated under forest conditions or from the blocks used in the inoculations (7).

It is known that Poria weirii can survive in wood residues beneath the soil. Buckland and Wallis (8) state that inoculum may remain viable in old, resinous stumps and roots for as long as 50 years. According to Childs (10) the organism may persist in dead root systems sometimes for more than a century. Where P. weirii growth is restricted to the heartwood, Douglas-fir trees have been considered somewhat resistant to yellow laminated root rot. According to Buckland, Molnar, and Wallis (7) it is these trees that play an important part in the long-term survival of P. weirii. The heartwood is resinous, frequently having bands of resin-impregnated wood encircling the decay. This may aid in the prevention of invasion by other saprophytes and slow the growth of P. weirii. Trees in which decay spreads readily are not regarded as important in long-term survival of the pathogen.

Fomes annosus may remain viable in stumps for 30 years (24;26, p.14-15), and is able to survive longer in these wood residues in alkaline soils. Survival in resinous wood is better than in non-resinous wood (24;26, p.14-20). The ability of F. annosus to survive is dependent upon the inability of other organisms to replace it. This replacement of F. annosus is dependent upon temperature, stage of decay, and/or soil reaction (24;25;26, p.14-15). Fomes annosus tends to survive longer in roots penetrating deep into the soil than in lateral roots (26, p.18-20). Formation of zone lines by F. annosus provides a temporary barrier against invasion by other fungi. Drying and swelling of the wood make these lines more easily penetrated by soil fungi (26, p.18-20). Among the common primary

invaders of wood colonized by F. annosus are Trichoderma viride, Torula ligniperda, and Peniophora gigantea followed by Hypholoma fasciculare, Melanospora sp. and various blue stain fungi (25;26, p.18-20). Many strains of T. viride can kill F. annosus and replace it in wood (24).

Apparently Poria weirii is quite resistant to desiccation. It has been isolated from wood stored for 18 months in a dry atmosphere. Sections made at this time indicated that only the fine hyphae had degenerated (7). Thus P. weirii, like F. annosus, can survive in appropriate substrates in the soil. Poria weirii is apparently little affected by low moisture levels when not in contact with soil, but may be adversely affected by low moisture levels when in the soil.

From the buried residues, hyphae of Poria weirii are apparently able to attack healthy roots of trees contacting the inoculum. Hubert (12, p.6-13) observed that in young stands containing remnants of old cedar trees destroyed by fire grand fir trees established after the fire developed considerable P. weirii decay. In a 15- to 20-year-old stand, two infected trees were found. Both of these trees were infected from the rotten stump of a diseased tree in the original stand (5). Once initial infection occurs from the old surviving inoculum, the fungus spreads along the root system of the tree and from tree to tree by root contacts. Grafting or contact of the xylem is unnecessary (32, p.14). The pathogen is able to move along the surface of the root as a brownish mantle of hyphae. This mantle may precede invasion of the xylem by 2.5 feet although cambial discoloration occurs within a few inches of bark mycelium. Rate of

surface growth is faster than that in the wood itself (32, p.9-10). Buckland et al. (7) state, "Since site and age of a stand determine the amount of root fusion which will occur, these two factors are of prime importance in spread of the disease." Fastest spread of the disease usually occurs in 20- to 60-year-old stands, well stocked and on good sites (7).

Rate of spread is not the same for all susceptible hosts. Wallis (33, p.25-27) found rate of spread in western redcedar negligible in comparison to that in Douglas-fir alone. On the other hand, western hemlock in contact with Douglas-fir showed no resistance to attack by Poria weirii and would not retard normal spread of P. weirii from infection centers. The percent of infection decreases with distance from a killed tree in pole-sized stands (9).

In addition to local spread in the above manner, there is a slight possibility of long-distance spread by basidiospores, probably initiating infection on or near butts of trees (9). On a scarred tree study, four hemlocks were found to be infected with P. weirii. One with a scar extending from 7.5 to 12 feet above ground level had decay induced by P. weirii extending from the 4- to the 13-foot level. There was no decay connecting the roots with the trunk, indicating a spore-initiated infection (36, p.141-146;37).

On the other hand, Fomes annosus is well known for its successful long-distance spread by spores. Spores are produced in large enough numbers to infect wide areas (24). Rishbeth was able to obtain a high percentage of pine stump infections by inoculating them with a basidiospore suspension during moist conditions (26, p.5-6). The

ability of F. annosus to infect stumps, however, decreases with the increasing age of the stump (26, p.8). Local spread of the fungus is similar to that of Poria weirii. Fomes annosus has been found on living roots at the point of contact with previously infected roots. The bark of larger roots is colonized to a considerable extent before penetration of the wood when soils are not too acid (24). Extensive growth over root surfaces is prevented in the acid soils by soil-borne antagonists such as T. viride (24;25, p.374-375). Rishbeth (26, p.14-15) measured growth rates of 7.3 mm. per day at 22.5° C. in pine roots and 2.8 mm. per day at 10° C. This would correspond to about one meter a year.

Damage caused by Poria weirii and Fomes annosus is not limited to mortality. Fomes annosus has been an important cause of cull in timber because of the butt rot it causes. For this same reason yellow laminated root rot caused by P. weirii has been regarded as an important disease of cedar since it was first collected by Weir (17;32, p.13). In a study of decay in western redcedar in British Columbia, Buckland (6, p.169-174) observed little direct loss in poles due to P. weirii, and butt rot caused by P. weirii was of minor importance in coastal cedar; however, losses in the interior ascribed to P. weirii were exceeded only by those caused by Poria asiatica.

In contrast to its behavior on western redcedar, the behavior of Poria weirii on other conifers is quite different. Here P. weirii is principally of importance as a killer. Actual loss of volume from rot is small since it is usually confined to the lower few feet of the trunk (9). Examination of a freshly cut 400-year-old stand

showed that the rot column can, on occasion, extend as much as 20 feet up the bole of the tree from the root collar (5). In the past certain losses in grand fir that were attributed to Echinodontium tinctorium should probably have been ascribed to P. weirii (12, p.6-13). Damage in specific localities can be very severe (7). Second-growth Douglas-fir productivity may be reduced by 5 percent. It has been stated that 454 million board feet of sawtimber and 96 million cubic feet of growing stock may be lost each year due to P. weirii. The pathogen causes the most destructive endemic root rot of conifers in the Douglas-fir subregion (29, p.5;31, p.207). Destruction occurs on all sites and in all age classes (8). The rate of damage from a particular center may double every 10-20 years (10). Actual damage is difficult to assess. Estimates of direct loss do not constitute a true picture. Irregular stocking caused by the disease may lead to snow and wind damage. Beetles may build up populations on weakened trees and attack and kill neighboring trees (8). Holes developing in a stand may be filled by more tolerant species and go unseen (7).

Studies of the histories of Douglas-fir stands in British Columbia indicate that fire and clear-cut logging methods tend to check the spread of Poria weirii root rot and reduce damage in succeeding rotations. There is more damage to succeeding stands if old trees are left, but a few pockets of P. weirii root rot will probably develop even if all old trees are taken (7). Childs (10) states that continuous rotations of Douglas-fir should result in increased damage from rotation to rotation.

Control of the disease has been scrutinized from several angles. Trenching, breeding resistant individuals, crop rotation, regulation of species composition, thinning, wide spacing, and severe burning have been proposed (8). Trenching has proved successful in stopping local spread but is not economically feasible under forest conditions (34). Breeding resistant Douglas-fir trees may hold future promise, but so far no immune or highly resistant trees have been located (8). Mixed stands are probably of little value in control since most species are susceptible and root contacts are common (32, p.14-15). Crop rotation would have the same difficulties. Thinning would have value only if severe enough to reduce root contacts appreciably (8). Since Poria weirii can colonize roots as small as 1 mm. in diameter, sufficiently wide spacing to reduce root contacts in plantations would lower stocking below minimum silvicultural standards (32, p.14-15). Incoming natural regeneration would be an added hazard. Severe burning would prevent fruiting but would not destroy buried inoculum (8). Childs (10) states, "Slash burning gives no protection." Since no actual control measure is yet practical, he suggests first cutting areas of damage where this is possible and salvaging killed trees if partial cutting is possible. Use of less susceptible species than Douglas-fir is also recommended (9). Until more sound measures of control are devised, we have no choice but to use the above measures.

Like Poria weirii, the local spread of Fomes annosus is difficult to control. Long-distance spread of F. annosus may be

lessened by treating freshly cut stumps, but the control of local spread of either disease cannot be achieved until more is learned through research.

ANATOMY OF ROOT INFECTION

Penetration of the outer host tissues by a fungus is the first step towards infection. At this stage of pathogenicity, there is also the first opportunity for the host to show signs of resistance. It is the objective of this study to demonstrate the ability of the fungus to penetrate the various host tissues and to record significant host reactions.

Procedure

Douglas-fir roots in the early stages of infection were obtained for microdissection by artificial inoculation of 5- to 8-year-old trees. Trees were carefully removed in January 1960 from a clear-cut area on Marys Peak in the Coast Range west of Corvallis, Oregon. The roots were washed and the trees transplanted into individual containers designed to give the least root disturbance during subsequent examination and treatment (Figure 1). Four planting treatments of 24 replications each were used:

1. Potting soil mixture; trees kept in the greenhouse.
2. "Permalite" (perlite-plaster aggregate); trees kept in the greenhouse.
3. Forest soil; trees kept in the greenhouse.
4. Forest soil; trees kept out-of-doors.



Figure 1. Two of 96 trees used in root inoculation trials. Boxes are constructed of cedar and "Masonite" reinforced with aluminum bands.

The trees planted in the soils were watered with tapwater. Trees planted in the perlite-plaster aggregate were watered with a dilute solution of White's major elements (35, p.74) and Nitsch's minor elements (18). About one cubic foot of planting medium was used for each tree. Mineral soil taken from the B horizon was used for planting treatments 3 and 4.

The trees remained in the boxes in the greenhouse for 4 to 8 weeks before being inoculated. The inoculum was prepared by inoculating blocks of sound Douglas-fir sterilized at 15 pounds per square inch for 30 minutes with pure cultures of Poria weirii growing on agar disks. Blocks of four sizes were employed: (1) 45 x 50 x 72 mm., (2) 44 x 22 x 52 mm., (3) 6 x 12 x 90 mm., and (4) 6 x 6 x 90 mm. The inoculated blocks were kept at room temperature in sterile glass jars for a period of 14 to 16 months.

Inoculations of the trees were made by tilting the planting boxes slightly back, lifting the sliding front panel, carefully removing enough planting medium to expose the selected roots (using a spatula and wash bottle), placing the inoculum block against these roots, and packing it into place with the originally used planting medium. The front panel was then slid back into position and the box placed back on the greenhouse bench. Twenty-four of 48 boxes containing trees planted in forest soil were then placed outside, just west of the greenhouse.

Most of the trees were removed within 9 to 12 months whether or not infections were apparent. All removals were accomplished by lifting the front panel and washing away the planting medium,

using a garden hose lacking a nozzle. Roots in the vicinity of the inoculum block were removed, tagged, and placed in individual plastic bags along with the recovered block. Within a few hours the roots were examined under the binocular dissecting microscope and those containing Poria weirii mycelium were cut into short segments, placed in vials of Randolph's modification of Navashin's killing and fixing fluid, briefly subjected to partial vacuum and left for 12 hours. The segments were then washed in running tapwater for 24 hours and imbedded in paraffin.

The inoculum blocks recovered from each box were split open and cultures taken from various points. The presence of living Poria weirii within the blocks at the termination of inoculation attempts, especially near the outer surfaces of the blocks, was determined by culturing on malt agar containing 50 micrograms of streptomycin nitrate and 50 micrograms of thiamine per liter.

Sectioning of imbedded root material was begun as soon as a sufficient quantity was available. Sectioning of fresh material on a freezing microtome proved unsatisfactory and was abandoned. Imbedded material sectioned at 15 to 20 microns on a rotary microtome provided all the slides used in this study. This procedure proved satisfactory, although some tissue distortion and tearing occurred from hard, woody tissues. No attempt was made to keep serial sections. Haup's adhesive and 4 percent formalin solution were used to fix sections to the glass microscope slides. These slides were then oven-dried for 24 hours before staining with 1 percent safranin in 70 percent alcohol and fast green in 95 percent alcohol.

Stained material was mounted in H.S.R. resin. All sections which lacked the characteristic Poria weirii mantle with setal hyphae were discarded. Only hyphae which were associated with the mantle were recognized as P. weirii hyphae since no other identification character of the hyphae of P. weirii was known.

Results

Seventeen of the 96 inoculated trees showed Poria weirii mycelium on the root surfaces, but in only 12 of these trees were roots definitely penetrated by P. weirii hyphae. Macroscopic examination of washed roots at the time of their removal showed the outer mantle to extend up to 2 or 3 cm. beyond the point of contact of root and block although no direct measurements were made. Poria weirii was cultured from 21 of the 96 inoculation blocks at the time of their removal. Some survival of the fungus was recorded in all planting treatments, but only in blocks of the two largest sizes. No definite correlation was noted between survival in the blocks and root infection. It is possible that P. weirii survived in some blocks but not at the points from which cultures were taken. The fungus remained viable in some blocks for more than a year after burial.

Table 1.--The influence of planting medium and unit location on (1) infection of Douglas-fir roots by Poria weirii and (2) survival of the fungus in inoculum blocks

Planting medium and location	Blocks in which <u>Poria weirii</u> survived	Trees successfully inoculated
	<u>Number</u>	<u>Number</u>
In the greenhouse:		
Greenhouse potting mix	<u>1</u> / 2	2
"Permalite"	6	0
Forest soil	7	6
Out-of-doors:		
Forest soil	6	9
Total	21	17

1/ Out of a possible 24 units in each treatment.

Table 2.--The effect of the size of inoculum block on host infection and inoculum survival

Size of block	Blocks employed	Blocks with surviving <u>Poria weirii</u>	Blocks producing infection
	-----Number-----		
Small ^{1/}	24	0	2
Medium	55	17	11
Large	17	4	4
Total	96	21	17

^{1/} Includes blocks of the two smallest sizes of the four sizes used.

Examination of the mounted sections showed that apparently uninjured roots up to 5 mm. in diameter are susceptible to infection. A dense, brown mantle lies somewhat away from the root. This mantle does not stain to any appreciable extent. Inside this mantle a loose net of hyaline hyphae bridge the short gap between the outer mantle and the root surface and penetrate the root. These hyphae stain a blue-green color. Cork tissue of young roots does not seem to be an effective barrier against penetration. Hyphae in the vicinity of the outer cork layers permeate and deteriorate these layers. Once the hyphae have penetrated this weak barrier, there appears to be little resistance to their colonization of living phloem and, soon

thereafter, the xylem. Cells of the rays are invaded along with sieve elements, parenchyma, and finally fibers and tracheids of the xylem. Photomicrographs of root sections and fungus tissue are presented in Figures 2 through 8.



Figure 2. Section through mat of Poria weirii hyphae grown in pure culture on sawdust. (X 1500)

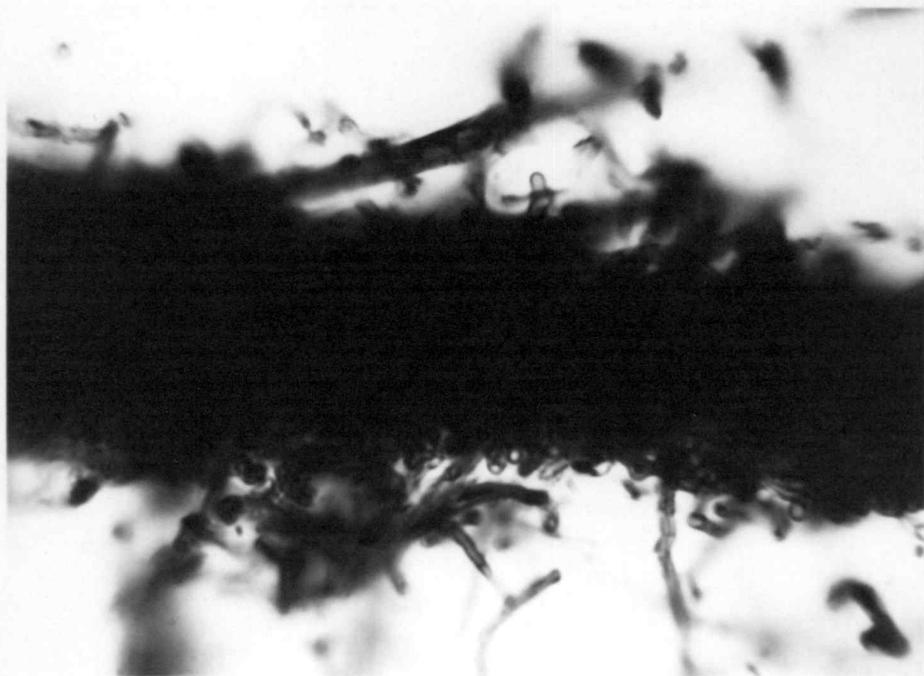


Figure 3. Section through mat of Poria weirii hyphae enveloping a portion of the root of an infected tree. (X 1500)

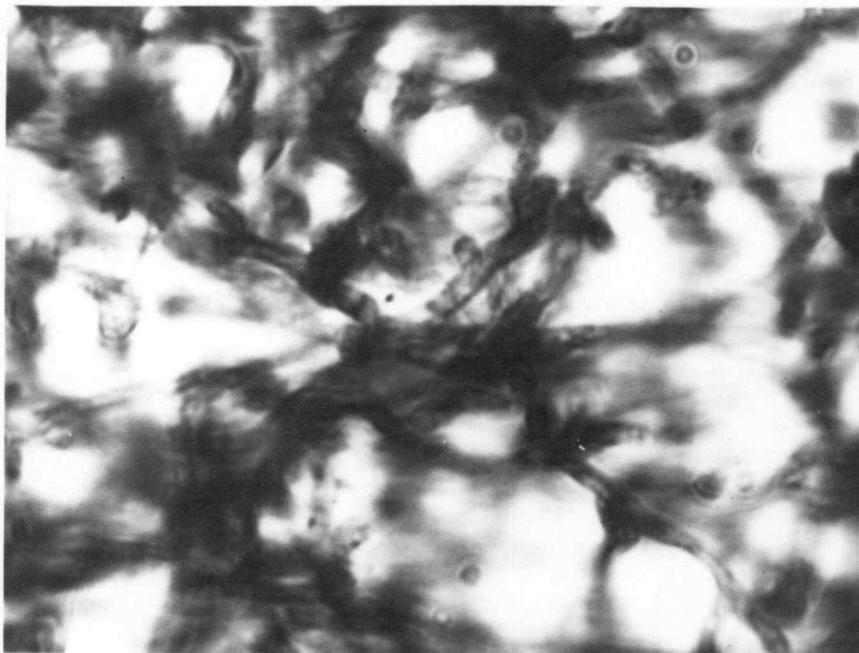


Figure 4. Section of loose hyphal network bridging the gap between the root surface and the outlying fungus mantle which surrounds the root. (X 1500)

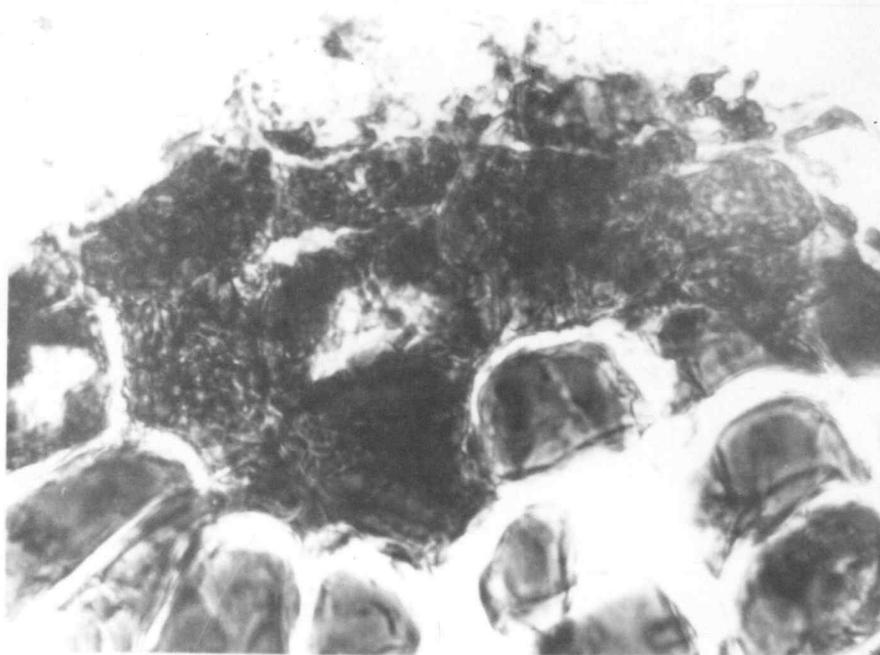


Figure 5. Section of infected root showing invasion of the thin cork layer of the root by hyphae of *Poria weirii*. (X 1500)



Figure 6. Radial section through phloem of invaded root showing *Poria weirii* hyphae in sieve elements and phloem ray cells. (X 1500)

Figure 7

Radial section of an infected Douglas-fir root showing typical penetration of xylem elements by hyphae of Poria weirii. (X 1500)

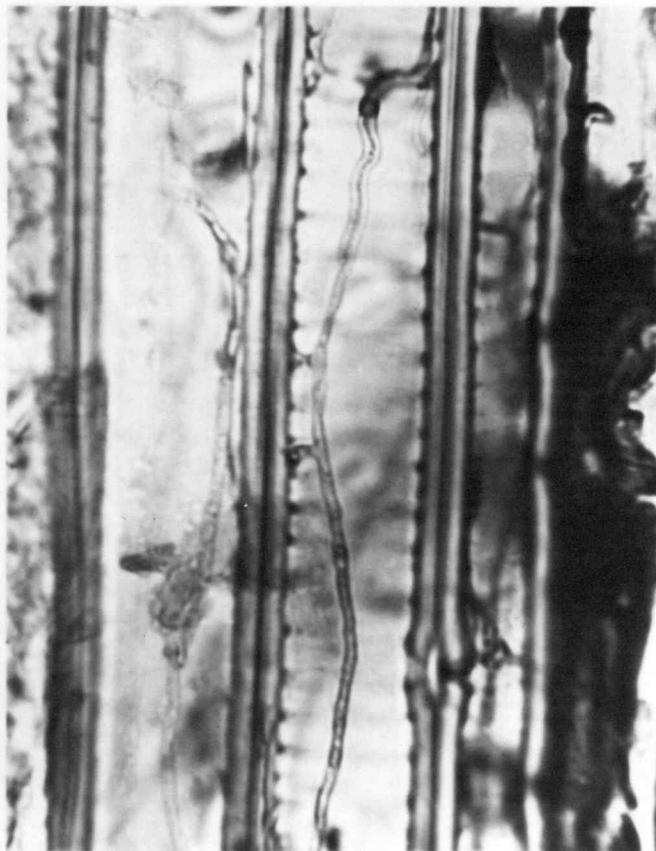
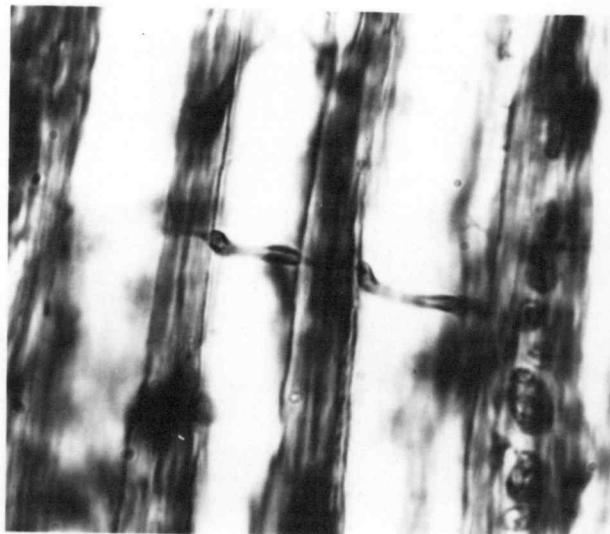


Figure 8

Radial section of root xylem infected by Poria weirii. Hyphae growing parallel with the long axis of the tracheids are not common. (X 1500)

Conclusions

Poria weirii is capable of attacking and infecting small roots of young Douglas-fir trees. Thin-walled hyphae lying between the dense mantle of hyphae and the surface of the root directly penetrate the root. Thin cork layers offer no appreciable resistance. Once entry is gained, P. weirii parasitizes and kills cells of the phloem and xylem as well as penetrating and destroying the older, thick-walled xylem elements.

SURVIVAL OF PORIA WEIRII IN SOILS DEVOID OF LARGE WOOD RESIDUES

There is doubt that Poria weirii is able to survive in forest soil in the absence of larger, colonized wood residues. This experiment will show survival of the fungus unassociated with large, woody residues or, if survival fails, then through negative results, to strengthen circumstantial evidence pointing toward the inability of P. weirii to survive in such a habitat.

Two similar experiments were conducted.

Procedure I

Soil from the B horizon obtained from MacDonald Forest in the Coast Range near Corvallis, Oregon was allowed to dry to a moisture content of 19 percent and screened through an 8-mesh screen. Sawdust screened through a 16-mesh screen was mixed on a volume basis with the soil in ratios of 100:0, 90:10, 75:25, and 50:50 parts of soil to sawdust.

The moisture contents of the soil-sawdust mixtures were determined after drying them in an oven at 100° C. Moisture content of the mixtures at field capacity was determined as follows: Three glass tubes, 36 inches in length and 22.5 mm. in inside diameter were lightly packed with mixture samples to a height of 30 inches, set in a vertical position and drenched with enough water to saturate the mixture. Rubber stoppers were placed in the tops of the tubes and the apparatus left for 48 hours. At the end of this period, a single sample was taken from the upper portion of each column, weighed,

dried at 100° C. for 24 hours and reweighed. Field capacity was then calculated as a percent of dry weight. This procedure was used for each of the soil-sawdust mixtures.

Twelve replicate 100-gram samples (on a dry weight basis) of each mixture were placed in small jars of about 260 ml. capacity and brought to field capacity by addition of the proper amount of distilled water. The jars were then closed with heavy plastic lids. Six jars of each mixture were autoclaved at 15 pounds per square inch for 30 minutes. All jars were then inoculated with masses of Poria weirii mycelium grown aseptically for 2 weeks in liquid culture. Half of the autoclaved and half of the nonautoclaved jars were incubated at 10° C., the remainder at 25° C. The jars were periodically weighed, and sterile, distilled water added if any moisture had been lost.

After 45 days five 1-gram samples were removed from each jar and placed in five sterile petri plates to each of which was added about 25 ml. of malt agar containing 50 micrograms of streptomycin nitrate and 50 micrograms of thiamine per liter. All plates were incubated at room temperature until the medium began to become crowded with mycelia. At this time all colonies resembling Poria weirii were isolated for positive identification. Only macroscopic identifications were necessary in most cases.

Results

Fungus colonies produced on the agar poured over sterilized soils were found to be Poria weirii in 80 of 110 cases. Fourteen plates from various jars showed no colonies and 6 plates were

contaminated. In addition, all 10 plates from two of the jars produced no colonies. This would indicate probable failure of the original P. weirii inoculations in the two jars. There appeared to be no correlation between the amount of sawdust in the mixture and survival although surface vegetative growth of the fungus appeared much more abundant on the higher percent sawdust mixtures.

No Poria weirii colonies were found on the same medium poured over nonsterilized soils.

Procedure II

The preparation of soil-sawdust mixtures, the amount of each mixture used in each replicate, and the method of determination of moisture contents in this experiment are the same as in the previous experiment. Larger, 450 ml. jars were used. Again half the samples were sterilized for 30 minutes at 15 pounds per square inch before inoculation, and half remained nonsterile. All the samples were inoculated with masses of Poria weirii mycelium grown in liquid culture for 30 days. The moisture content was held at 80 percent of field capacity by addition of enough sterile distilled water to maintain constant weight. No temperature control was used, but ambient room temperature was fairly constant at 21° C. throughout the experiment. After incubation for 54 days 1 gram of nonsterile soil was added to all but 3 of the autoclaved samples. Ninety days after the original inoculation with P. weirii (36 days after addition of the gram of soil) attempts were made to recover the fungus, using soil sampling tubes (16). The tubes, containing malt agar, oxgall agar,

peptone-dextrose agar, and synthetic acid agar (13), were placed in the soil in the jars immediately after opening ports of entry by puncturing the plastic tape with a hot dissecting needle. The tubes were removed 3 to 4 days after their introduction into the soil jars and transfers made from the sampling tube media to malt agar slants.

Results

Of the three sterilized mixtures inoculated only with Poria weirii, one had become contaminated, one produced no fungus colonies on agar, and one produced colonies of P. weirii exclusively. From the jars not sterilized and jars first sterilized but later contaminated from the added gram of soil, no P. weirii colonies developed. Colonies of Trichoderma were isolated most frequently.

Conclusions

Poria weirii, in the absence of other organisms, is capable of surviving in soils varying widely in organic content. Standard techniques can be used to isolate P. weirii from such soils. Poria weirii, unassociated with a substantial food base, grows poorly if at all in soils inhabited by other forest soil microflora. The actions of these organisms probably restrict the development of P. weirii in these soils.

SURVIVAL OF PORIA WEIRII IN BURIED WOOD BLOCKS

Poria weirii is able to survive from one rotation to the next in root systems of infected trees and to infect trees of the new stand from these residues (5;12, p.6-13). The factors affecting survival under these circumstances are not well known. The following study was conducted to determine the influence of several factors on fungus survival in buried wood blocks.

General Procedure

Experiments were conducted to determine the role of seven external factors in Poria weirii survival. These factors are:

1. Depth of burial
2. Stage of decay
3. Presence of sawdust
4. Amount of substrate
5. Soil temperature
6. Soil moisture
7. Soil reaction

The first four were investigated in the field near areas of P. weirii root-rot development in the Coast Range west of Corvallis, Oregon. A broad ridge somewhat above 2,000 feet in elevation was selected. The area supports a second-growth stand of small sawtimber on a clay-loam soil. The last three factors were studied in the laboratory, using containers of forest soil.

The 2-inch cubes used in this study were cut from decaying heartwood of a naturally infected Douglas-fir tree. These blocks were sorted for uniformity of decay as evidenced from external appearance and stored in a humid atmosphere at 5° C. until buried. The appearance of Poria weirii hyphae on the surface of these blocks just prior to burial was regarded as proof of viable P. weirii within the blocks at time of burial.

The blocks were recovered from the soil at 3-month intervals for 1 year. In addition, blocks used in the first 3 experiments were recovered after 20 months. Three replicates of each treatment and time were used. All recovered blocks were treated in the same manner. Each block was first scrubbed in running tapwater with a stiff brush to remove loose soil particles. It was then split along planes parallel to approximate radial and tangential faces, quartering the block. Cultures were made from points as illustrated in Figure 9. Malt agar slants containing 50 micrograms of streptomycin nitrate and 50 micrograms of thiamine per liter of medium were used for all cultures. After several days, the presence or absence of Poria weirii was determined and recorded. Isolates of all fungi were recorded by experiment, treatment, time of block recovery, replication number, and culture position. Fungi other than P. weirii were stored on malt agar slants at 5° C. for future use. The presence or absence of dark zone lines within the blocks was noted and recorded for all blocks recovered after the first 3 months.

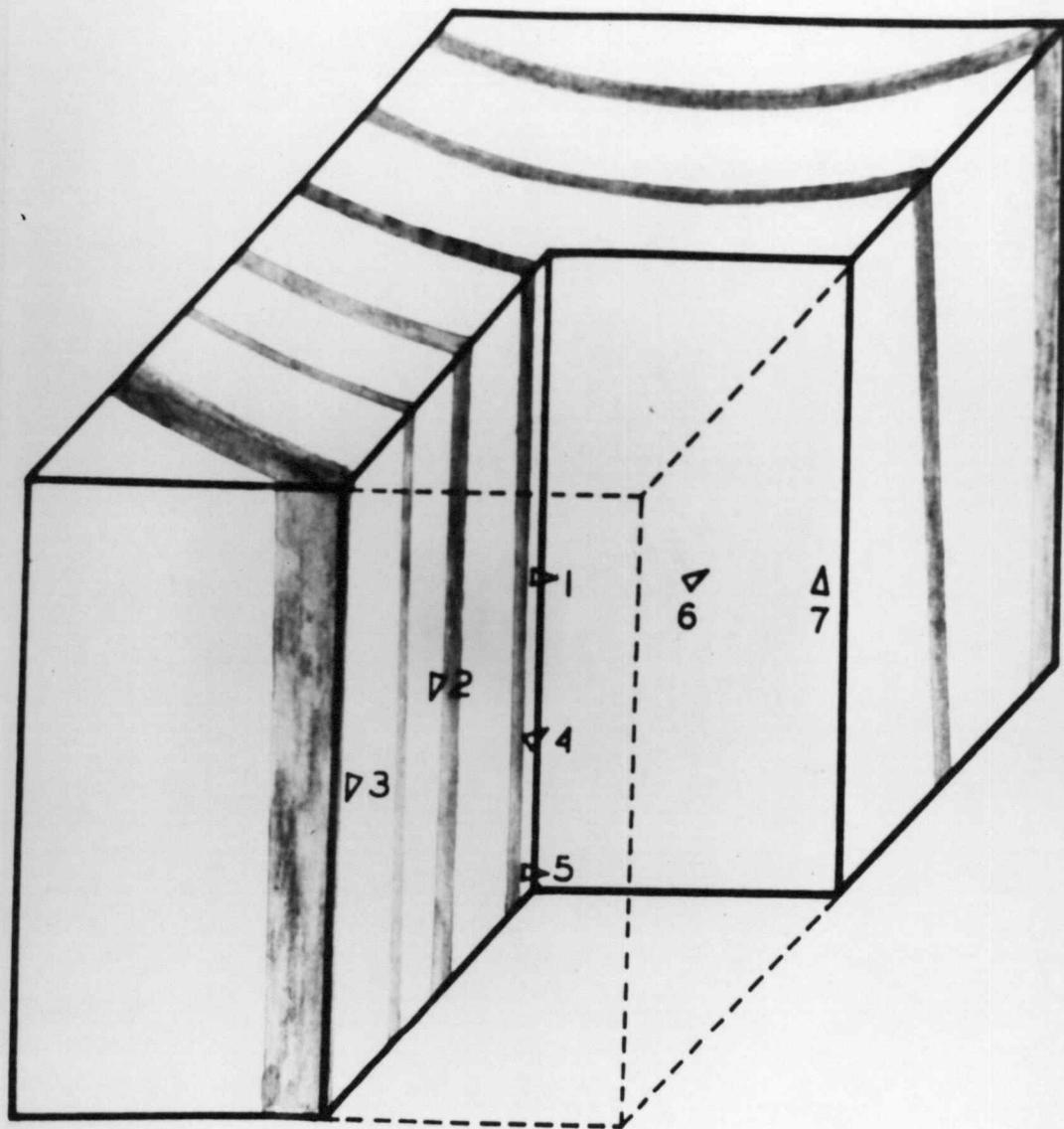


Figure 9. Diagram of a block used in *Poria weirii* survival studies. Points from which tissue transplants were removed for culture are represented by small numbered triangles. This system of numbering isolation points was employed in all blocks. Light bands represent grain.

Field Experiments

The experiments conducted in the field considered the effects of various depths of burial, stages of decay, amounts of surrounding sawdust and amounts of inoculum. All these variables were tested in the same area and all had procedures in common.

Depth of Burial. Depth in the soil will influence other factors such as temperature, organic content, moisture, etc. Probably the most important factor dependent upon soil depth is the composition of the microflora. All these, but especially the last, may influence the survival of Poria weirii.

Procedure. In June 1960, blocks 90 to 100 percent surface-stained by Poria weirii and showing advanced decay pockets over less than 10 percent of the surface of the block were buried randomly at depths of 3, 6, 12, and 24 inches on a grid (8 x 12 feet) divided into 96 squares. A table of random numbers was used to select positions on the grid. Holes were bored with a 3-inch soil auger, blocks placed at the proper depths, and the soil cores returned to their original positions. Care was taken to prevent contamination with soil from other levels, but some disturbance was inevitable.

Results. Survival of Poria weirii as determined from cultures taken from recovered wood blocks is indicated in Table 3.

Table 3.--Survival of Poria weirii in wood blocks after given intervals of burial at four depths in the soil

Depth of burial	All blocks (months buried)						Blocks with zone lines (months buried) ^{1/}					
	3	6	9	12	20	Total	6	9	12	20	Total	
<u>Inches</u>	<u>Percent</u>						<u>Percent</u>					
3	^{2/} 81	33	67	33	33	50	100	100	50	100	83	
6	43	19	33	24	33	30	57	100	72	50	66	
12	72	67	57	33	14	49	67	86	33	100	61	
24	86	33	81	52	10	52	100	81	79	14	66	
Total	70	38	60	36	23	45	76	89	54	51	68	

^{1/} An independent tabulation was made from only those blocks with zone lines (Figure 10). Values are based on the 7 points in each of 0 to 3 blocks.

^{2/} Values are based on isolations from 7 points in each of 3 replicate blocks.

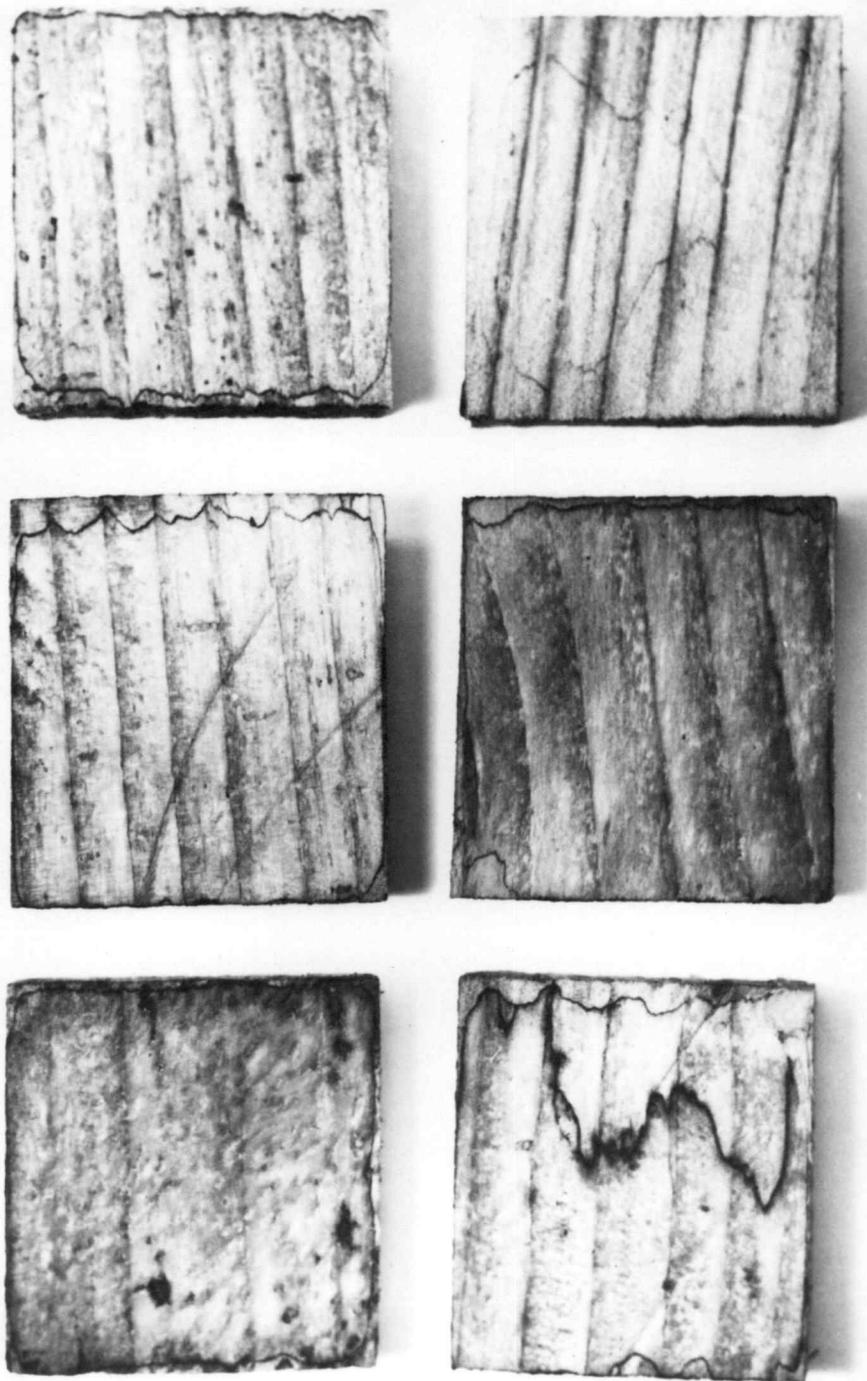


Figure 10. Exposed radial faces showing prominent zone lines when blocks used in Poria weirii survival studies are split open.

Some survival was found in each treatment. Variation in recovery of Poria weirii is large among replicates. Often blocks were found to contain no active P. weirii or active P. weirii at all points sampled. The apparent importance of zone lines in enhancing survival of P. weirii greatly contributed to this variation. Differences among treatments might be lessened with use of more replications. The unexpected high recovery of P. weirii at 9 months stands out.

Stage of Decay. A cubic unit of wood in an advanced stage of decay is partially exhausted of food. It seems reasonable that if substrate is an important factor in survival, the more advanced the decay becomes, the shorter should be the survival period. The ability of the organism to maintain itself in the presence of competing organisms could also conceivably depend upon stage of decay. Either the physical nature of the wood or the amount of fungus tissue within might be important.

Procedure. This experiment was begun in June 1960. The blocks used were:

Advanced-- 100 percent advanced decay on surface.

Incipient--100 percent surface stained.

Partial-- 10 to 25 percent of surface without dark, stained appearance; 75 to 90 percent stained.

Blocks were buried at an 8-inch depth on a grid (8 x 9 feet) divided into 72 squares. Positions on the grid were determined using a table of random numbers. Holes were bored with a 3-inch soil auger, blocks placed at the selected depth, and the soil cores returned to their original positions.

Results. Survival of Poria weirii determined as in the previous experiment is indicated in Table 4.

Table 4.--Survival of *Poria weirii* in wood blocks in different stages of decay after given intervals of burial

Stage of decay	All blocks (months buried)							Blocks with zone lines (months buried) ^{1/}				
	3	6	9	12	20	Total	6	9	12	20	Total	
	-----Percent-----							-----Percent-----				
Advanced ^{2/}	95	91	62	24	0	55	91	62	24	0	45	
Incipient	38	5	62	43	19	32	--	93	64	57	74	
Partial	29	57	38	29	0	31	86	57	43	--	63	
Total	54	51	54	32	6	39	89	69	41	14	55	

^{1/} An independent tabulation was made from only those blocks with zone lines (Figure 10). Values are based on the 7 points in each of the 0 to 3 blocks.

^{2/} Values are based on isolations from 7 points in each of 3 replicate blocks.

The influence of zone lines in Poria weirii survival is again evident. Initial high survival and later decline of P. weirii in blocks in advanced stage of decay can be seen. Survival in blocks in intermediate stage of decay follows a consistent trend when zone lines are present. Blocks partially decayed behave in much the same way.

Presence of Sawdust. The nature of surrounding materials may play an important role in the survival of Poria weirii. This influence would probably be linked with the microorganisms associated with a particular kind of environment. Others have shown that addition of organic matter to soil is instrumental in shifting populations of soil microflora (20, p. 117).

Procedure. In June 1960, blocks showing 75 to 100 percent incipient decay with 0 to 25 percent advanced decay (usually less than 10 percent) were buried at an 8-inch depth on a grid (8 x 15 feet) divided into 120 squares. Positions on the grid were selected using a table of random numbers. The holes were bored with a power auger. No attempt was made to return the soil to its original position. The blocks were enclosed by a cushion of 1.5 pints of forest soil-sawdust mixture within a No. 4 paper bag just prior to burial. Coarse, Douglas-fir sawdust was used. The amount of sawdust varied: 100, 50, 25, 10, and 0 percent sawdust on a volume basis were used.

Results. Survival of Poria weirii as determined from cultures taken from recovered wood blocks is indicated in Table 5.

Table 5.--Survival of *Poria weirii* in wood blocks surrounded by mixtures of soil and sawdust at given intervals of burial

Percent sawdust in mixture	All blocks (months buried)							Blocks with zone lines (months buried) <u>1/</u>				
	3	6	9	12	20	Total	6	9	12	20	Total	
	-----Percent-----							-----Percent-----				
100	<u>2/</u> 95	95	95	5	0	58	95	95	5	0	49	
50	95	100	95	33	48	68	100	95	33	48	69	
25	100	100	95	38	0	73	100	95	38	0	59	
10	86	100	52	62	0	61	100	52	62	0	54	
0	95	91	67	57	24	67	91	67	57	25	60	
Total	94	97	81	39	15	65	97	81	39	15	58	

1/ An independent tabulation was made from only those blocks with zone lines. Values are based on the 7 points in each of 3 replicate blocks.

2/ Values are based on isolations from 7 points in each of 3 replicate blocks.

Survival of Foria weirii in this experiment was generally higher than in the preceding two experiments. This is probably because zone lines formed within all blocks used in the experiment. Survival after 20 months, however, dropped to a relatively low level.

The combined data for all naturally infected blocks buried in the field are summarized in Table 6 and presented graphically in Figure 11.

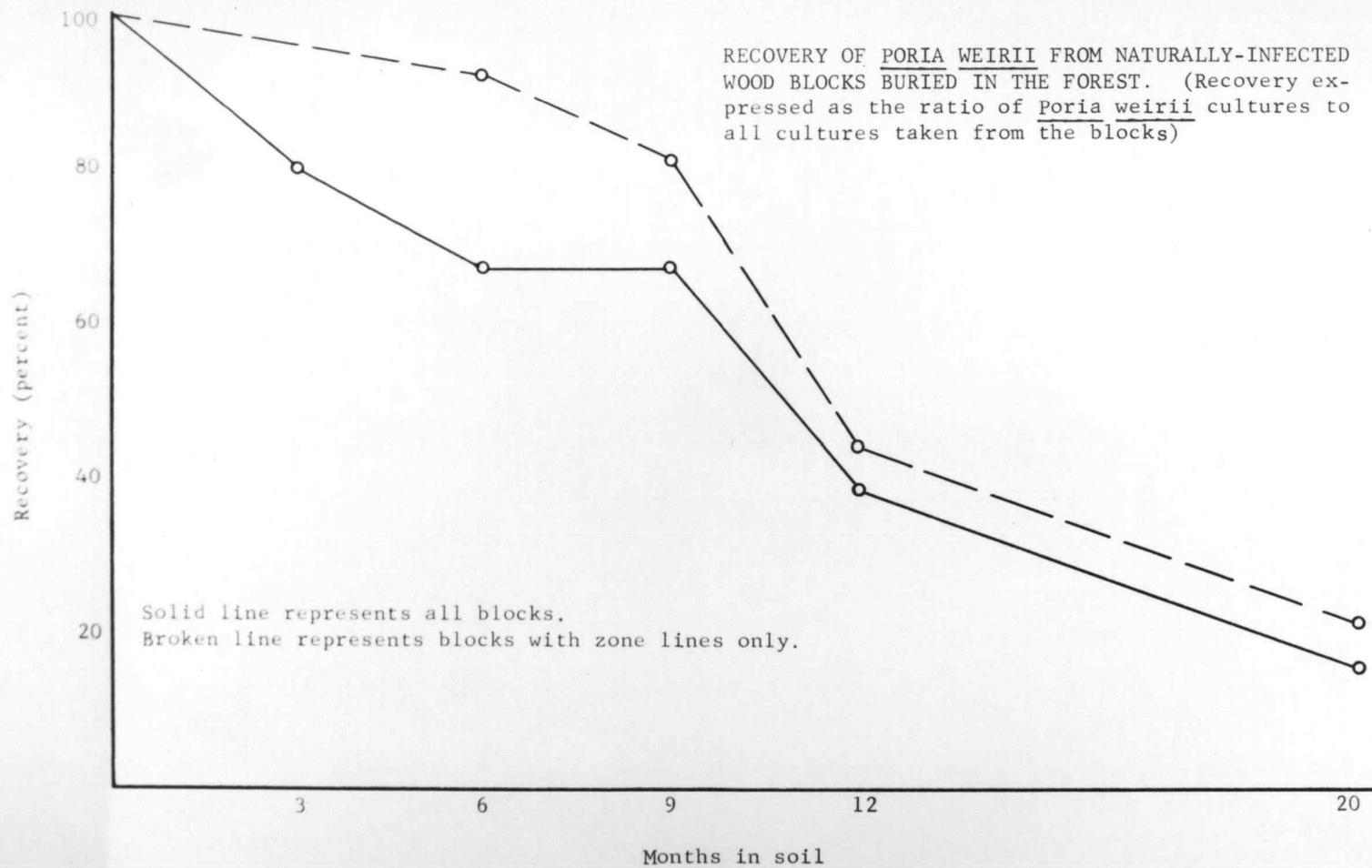


Figure 11

The combined results show a more orderly pattern in Poria weirii survival over the 20-month period, especially considering only those blocks having zone lines. The sharpest decline in survival seems to come between 9 and 12 months.

Amount of Substrate. If survival is largely dependent upon substrate depletion, there should be better survival in larger substrates. If survival is dependent upon the ability of the fungus to maintain itself in a hostile environment, larger blocks would enable the organism to survive longer because the antagonist would have to invade to a greater depth in order to colonize the entire block.

Procedure. In July 1960, blocks were cut from sound Douglas-fir heartwood into 2-, 1.5- and 1-inch cubes and autoclaved in water at 15 pounds per square inch for 30 minutes. The blocks were then inoculated in the same manner as those used in the root inoculation experiments presented earlier. When their surfaces were entirely covered with the mycelium of Poria weirii, the blocks were buried 8 inches deep. Position on the grid was determined using a table of random numbers.

Results. Survival of Poria weirii determined as in preceding experiments is indicated in Table 7.

Table 7.--Survival of Poria weirii in wood blocks of three different sizes after given intervals of burial

Size of block	Months buried			
	3	6	12	Total
	-----Percent-----			
Small	<u>1/</u> 29	5	0	11
Medium	67	95	43	68
Large	24	33	0	19
Total	40	44	14	33

1/ Values based on isolations from 7 points in each of 3 replicate blocks.

Zone lines were not distinguishable within any of the blocks. Hyphae of Poria weirii were encrusted on the surface of some blocks, however, and may have been effective in resisting fungus invasion. Survival was consistently higher in the 1.5-inch blocks than in either of the other sizes.

Laboratory Experiments

Laboratory experiments studied the effects of soil temperature, moisture, and pH on survival of Poria weirii. All three experiments were conducted in the same general manner. Naturally infected blocks in an incipient stage of decay were placed in soil in containers which were covered with thin polyethylene. The desired moisture levels were maintained by frequent weighing and, when necessary, addition

of distilled water. Incubation was at room temperature (about 21° C.) except where temperature was the variable being tested. A mixture of A and B horizons of forest soil was used.

Soil Temperature. Soil temperatures were maintained at 5° C., 15° C., and 25° C. The moisture level in this experiment was maintained at 75 percent of field capacity. Moisture content at field capacity was about 44 percent.

Soil Moisture. The soil was maintained at 16, 24, 32, and 40 percent moisture content. Moisture content at field capacity was about 44 percent.

Soil Reaction. Soil was adjusted to pH values of 4, 5, 6, and 7 by the addition of the required amount of calcium hydroxide or sulfuric acid. The initial pH value of the soil used was about 5. Moisture content of the soils was maintained at 90 percent of field capacity. Respective pH values at the end of 12 months had not changed.

Survival of Poria weirii in 2-inch naturally infected wood blocks maintained under laboratory conditions was poor, ranging from none at all in the soil reaction experiment to 10 percent in the soil temperature experiment based on isolations from all blocks. Absence of zone lines was noted in almost all blocks in all three experiments.

Conclusions

Buried under forest conditions, Poria weirii is able to survive in very small wood residues for at least 20 months. Survival decreases with increasing length of time the residues are buried in the soil. Formation of zone lines within the wood greatly enhances survival of P. weirii, although these barriers are only temporary. Zone lines are formed more readily in wood in an advanced stage of decay, but they are more easily penetrated than those formed in wood in less advanced stages of decay. Under experimental conditions, survival of P. weirii seems to depend upon the action of soil fungi rather than substrate depletion.

ANTAGONISTIC EFFECTS OF SOME FUNGI AGAINST PORIA WEIRII

Survival of Poria weirii in buried wood substrates is the key factor in infection of subsequent stands. The ability of P. weirii to spread from these residues is responsible for the continuation of the disease. Survival seems to be dependent upon the effects of the soil microflora. From observations on survival of P. weirii in wood blocks it was observed that several organisms were capable of invading wood substrates which had been colonized previously by P. weirii. If these organisms are capable of destroying P. weirii, or in some way containing it, they may have value in biological control of the pathogen. Obviously, highly effective biological control of P. weirii does not occur under all forest conditions, but if conditions are known under which soil organisms may prove to be strongly antagonistic to P. weirii there could be a possibility of modifying present conditions to better suit the requirements of the antagonists.

Procedure

Six hundred isolates of fungi from the wood blocks used in the survival studies covered previously were paired with a single isolate of Poria weirii on malt agar in individual petri plates. Inoculation with P. weirii at one edge of the plate preceded inoculation at the far edge of the plate with the fungus to be tested by 5 to 6 days. At the time the second fungus was introduced, the colony of P. weirii had extended about one-third of the way across the plate. This insured establishment of P. weirii on the agar and was

more nearly comparable to the situation in nature where P. weirii has first established itself in tree roots. Plates were incubated in the dark at room temperature (about 21° C.). Observations and measurements were made at weekly intervals until the degree of antagonism was established. Reactions between the organisms and P. weirii were grouped as follows:

1. Poria weirii overgrows the isolate tested.
2. Poria weirii is overgrown by the isolate tested.
Growth of P. weirii is stopped. Dark staining often occurs.
3. A definite inhibition zone devoid of fungus hyphae is formed.
4. Fungi contact one another but neither fungus is capable of overgrowing the other.

Those isolates falling into the first category above were discarded. The others were stored in soil-tube culture for further experimentation and identification. Fungi were also isolated from 12 sterilized wood blocks buried in forest soil for 4 months to compare microorganisms inhabiting these blocks with those inhabiting the blocks previously colonized by P. weirii.

Results

About 25 percent of the 600 isolates tested showed no antagonistic effects toward Poria weirii and were overgrown. The remainder fell into categories 2, 3, and 4. The only fungi tested which produced permanent inhibition zones were three isolates of

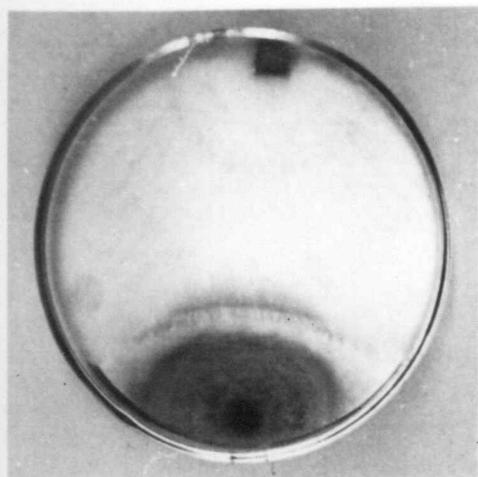
Aspergillus. Antagonists falling into the second category were the most prevalent. These consisted of 199 isolates of the genus Trichoderma, 151 of which produced green colonies and 48 of which produced white colonies. Six isolates of a species of Gliocladium also fell into this category. These two genera along with the three isolates of Aspergillus comprised the only isolates showing strong tendencies of antagonism. Species of Penicillium fell into categories 1 and 4. One hundred seven isolates were in the fourth group showing some antagonism. Narrow inhibition zones were usually produced but they were only temporary. Only two other fungus types were isolated frequently and both fell into the fourth category. Sixty-eight isolates of a single Mucor species or closely related fungus and 56 isolates of a yet unidentified imperfect fungus showed weak antagonistic tendencies, being very slowly overgrown by P. weirii, or, for the most part, holding their own. These last two fungi were frequently isolated together or with other antagonists from the same point within a wood block.

The remainder of the antagonists isolated fell into the fourth category. Because of their lack of definite signs of antagonism toward Poria weirii in culture and their infrequent isolation, these organisms have not been considered further.

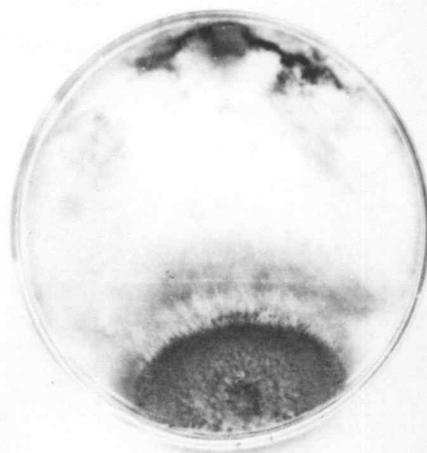
Based on the petri plate tests, only those isolates falling into the second category appeared to show real promise in biological control. There seemed to be no correlation of specific organisms

isolated with treatment or the length of time the blocks remained in the soil. Some of the antagonistic effects of the isolates plated can be observed in Figures 12, 13, and 14.

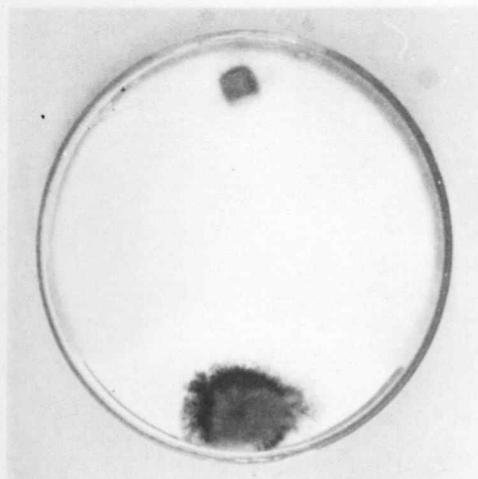
Only 29 of the 84 culture points from the 12 sterilized buried blocks produced cultures of fungi on malt agar slants, 55 being blank. Of these 29, seven were isolates of Trichoderma. The remainder were various other fungi which appeared to be representative of those isolated from the blocks initially colonized by Poria weirii. Only one of the 12 blocks had been penetrated to its center by invading soil fungi during the 4 months the blocks were buried.



A



B



C



D

Figure 12. Interaction of *Poria weirii* and certain saprophytic fungi isolated from buried wood blocks. Cultures are on malt agar at room temperature. *Poria weirii* is at the top of each plate. A, B. *Penicillium*, isolate 232, after 10 and 30 days, respectively; C, D. *Aspergillus*, isolate 349, after 10 and 30 days, respectively.

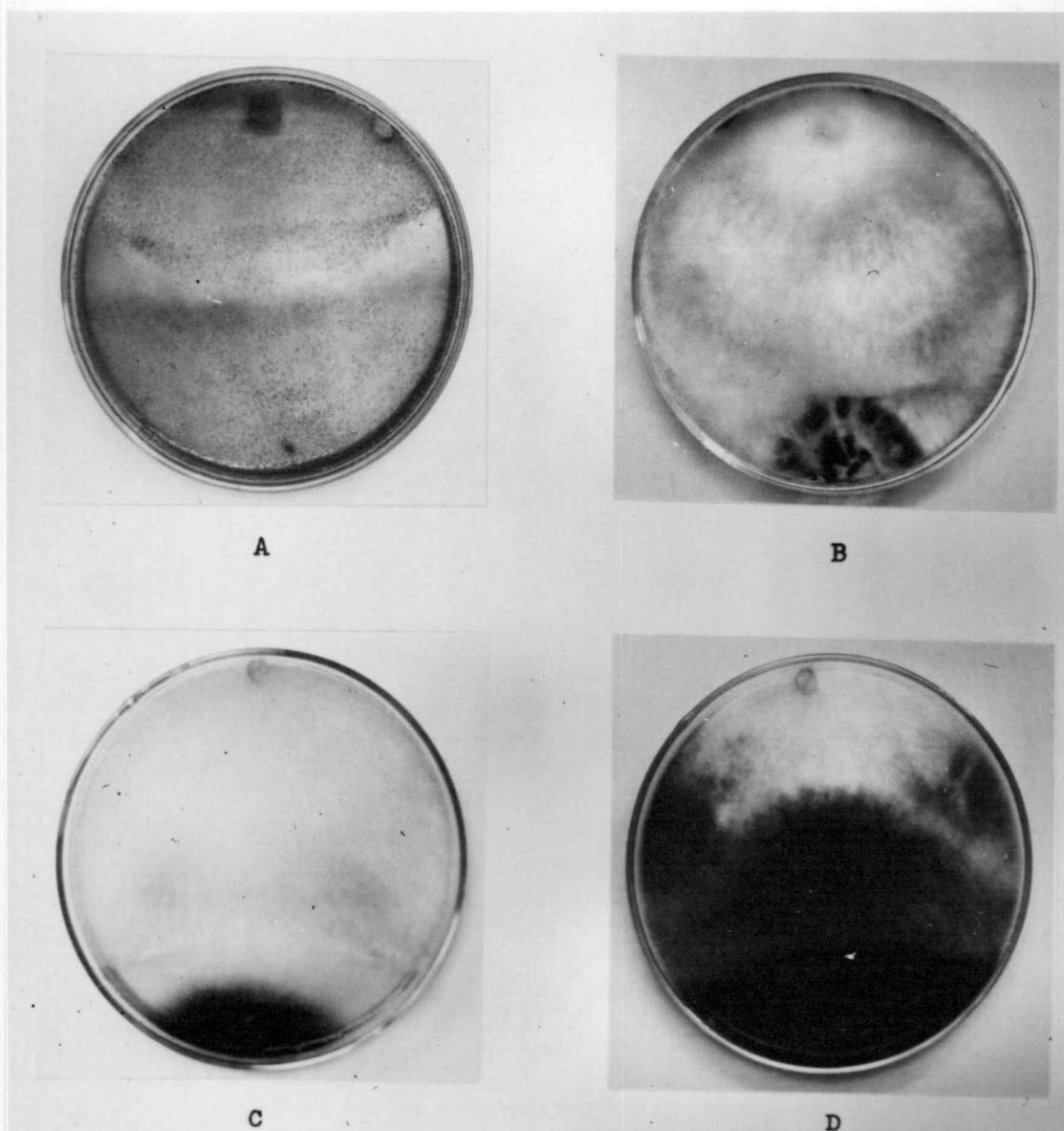
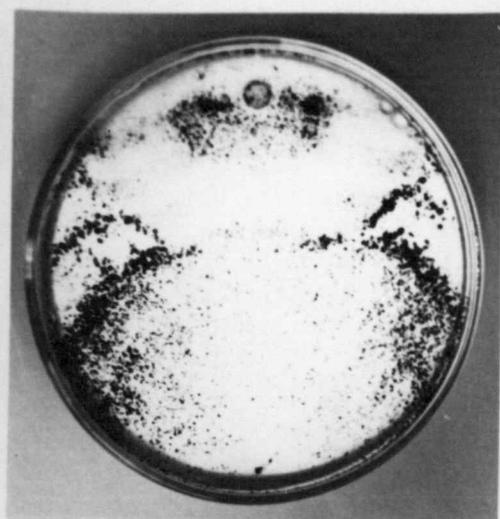


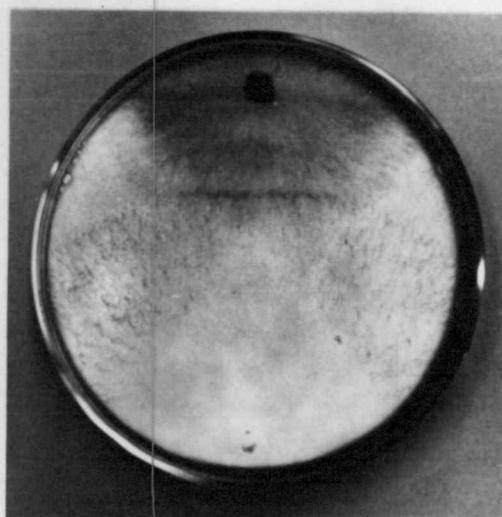
Figure 13. Interaction of Poria weirii and certain saprophytic fungi isolated from buried wood blocks. Cultures are on malt agar at room temperature. Poria weirii is at the top of each plate. A. Gliocladium, isolate 390, after 30 days. The Poria weirii colony is completely overgrown. B. Mucor sp., isolate 318. After 30 days the fungus is all but overgrown by Poria weirii. C, D. Unidentified stain-causing fungus, isolate 358, after 10 and 30 days, respectively. Note the advancing stain beneath the Poria weirii colony after 30 days.



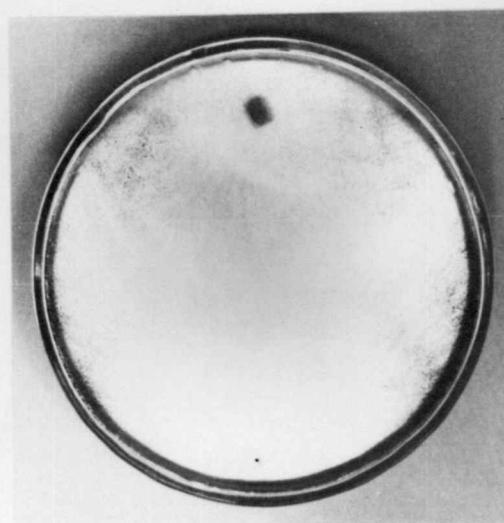
A



B



C



D

Figure 14. Four isolates of Trichoderma grown on malt agar with an isolate of Poria weirii. All of the Trichoderma isolates have overrun the colonies of Poria weirii within 10 days after the introduction of the antagonists. Poria weirii colonies are at the top of each photo. A. Isolate 22, B. Isolate 518, C. Isolate 113, and D. Isolate 448.

Conclusions

Because the tests of antagonism which were conducted in the laboratory may not be accurate indicators of antagonism in nature, further experimentation should be carried out to determine the antagonistic effects of these fungi in nature and the implications of these antagonisms. It is apparent, however, that Trichoderma is a major component of the total fungus population invading wood residues colonized by Poria weirii, and that this fungus is highly antagonistic to P. weirii in laboratory tests. Trichoderma, as well as certain other fungi, seems capable of eliminating P. weirii from small wood residues in forest soil. How this capability may be affected when bark or resin is present is not known.

DISCUSSION

Poria weirii is capable of penetrating apparently healthy roots of young Douglas-fir trees from inoculum placed in contact with the roots. Once the fungus mantle has formed, the roots are penetrated with little difficulty. The thin cork layers at the surface of the small roots do not appear to be effective barriers against penetration. Living tissues of the phloem and xylem are attacked by the fungus. The relatively poor success (18 percent) in inoculation of the trees most probably was due to the inability of P. weirii to survive in most of the inoculum blocks.

The ability of the fungus to survive in some form in the soil, then, could be of utmost importance in the continuation of the disease. Experiments conducted show that Poria weirii is able to maintain itself in sterilized soils mixed with no sawdust or as much as 50 percent sawdust by volume at either 10° C. or 25° C. The soil plate technique and a soil tube technique employed were effective in obtaining the fungus from the soil when it was not competing with other soil organisms. If P. weirii occurred in an active state in the soil, it is probable that one of these techniques would be occasionally successful in isolating it. It is interesting to note that even when P. weirii was allowed to colonize sterilized soils for as long as 54 days, it could not be isolated by the soil tube technique if other organisms were introduced sometime before the attempted isolation.

Survival of Poria weirii in small, wood blocks buried in the soil was determined for various depths of burial, stages of decay, amounts of ambient sawdust, amounts of substrate, soil temperatures, levels of soil moisture, and levels of soil acidity. It was found that P. weirii survival in those tests conducted in the field was much higher than in those tests conducted in the laboratory. This would seem to indicate the presence of one or more factors acting to reduce P. weirii survival under laboratory conditions. The possibility of restricted gas exchange or some other environmental factor causing this difference in survival is not unlikely. Table 4 and Figure 11 show the influence of zone lines formed by P. weirii on its ability to survive in the buried blocks. The positive effect of these lines on survival is less effective as time passes. The sharpest drop in the survival of P. weirii occurred between 9 and 12 months. This coincided with the time of year when the soil temperature was rising and moisture was still at a high level, mid-March to mid-June. Rishbeth (23, p.18-20) states that Fomes annosus zone lines may provide temporary barriers against invasion by other fungi and that entrance by these other fungi may be gained through the zone lines when they are broken by swelling or drying of the wood. This possibly may be the case with P. weirii under the conditions of the field experiments. It is also possible that the higher populations of soil microflora resulting from rising temperatures or improved aeration may be directly responsible for penetration of the barrier.

It has been noted that formation of distinct zone lines rarely occurred in the laboratory experiments. Hopp (11, p.614-619) states that high moisture content and ample oxygen are necessary for formation of zone lines. It is possible that the oxygen in these laboratory experiments was restricted to a point below that necessary for zone line formation. This, in turn, would account for a much poorer survival than occurred in the field.

The rise in recovery of Poria weirii at 9 months in the experiment considering depth of burial cannot be explained. One might expect survival to be correlated with depth of burial because survival seems to be dependent upon competition with other organisms. It is known that populations of soil organisms decrease as depth increases. Increased survival at the 3-inch depth cannot be logically attributed to lower populations of microorganisms. The blocks buried at that depth are within the zone of influence of the unusually large numbers of microflora in the F layer of the soil where competition is very high. Here the antagonists of P. weirii may themselves be held in check to a point where they are not able to attack the buried, infected blocks.

The data from the experiment testing the effect of the stage of decay indicate that zone lines were more easily formed when the blocks were in an advanced stage of decay; however, these zone lines were more easily penetrated than zone lines formed in less-decayed blocks. Initial survival was therefore best in blocks in an advanced stage of decay, survival after 12 months better in less-decayed blocks. The greater mass of fungous tissue in blocks in

advanced stages of decay might easily account for the faster formation of zone lines. Likewise, a fungus which has utilized a large proportion of its substrate may be more vulnerable to attack than one which has ample substrate remaining.

Data from the experiment testing the effect of ambient sawdust show high survival of Poria weirii in all treatments. Zone lines were formed in all blocks buried in this experiment. This unusually high incidence of zone lines might be explained by the procedure used in burying the blocks. Use of a power auger and packing the blocks first in a bag of wood chips and soil would both tend to create conditions for at least a temporary increase in aeration. This is, as mentioned earlier, one of the conditions necessary for zone line formation. There seems to be little difference in survival among treatments within the experiment.

One might expect longer survival in larger substrates because of the greater food supply. Results of the experiment on the effect of the amount of substrate indicate that this is not necessarily the case. Survival in the smaller blocks may be less than the two larger sizes partly because each respective point of isolation is closer to the block faces than in the larger blocks. This would enable an invading organism to colonize the entire block faster. Lower survival in the larger blocks might be explained by the fact that complete surface colonization of the inoculated block by Poria weirii takes longer and therefore penetration of the blocks at the time of burial is shallower in some places than it would be in smaller-size blocks.

The data resulting from this experiment are probably distorted by the experimental procedure. Repetition of many of the experiments would be desirable, using more replications than before. Survival in all three laboratory experiments was so low that no conclusions could be drawn within the individual experiments.

The ability of soil fungi to compete successfully for substrate seems to be of importance in the survival of Poria weirii. If the organism invading wood colonized by Poria weirii is capable of penetrating the substrate, spreading throughout the substrate, and causing lysis of P. weirii within the substrate, biological control of this root rot seems promising.

Initial tests of antagonism indicate that those isolates of Trichoderma and Gliocladium tested are able to fill these requirements at least in part. Their rapid rate of growth on malt agar indicates a potential for rapid growth where conditions are favorable. Whether or not these requirements would be met in buried wood is not known. In light of the number of times it was isolated from the blocks in the survival study and from the buried, sterilized blocks, Trichoderma seems to be able to penetrate wood easily. How much this penetration may be effected in nature where bark becomes a factor is not known. The tests conducted here may be considered only as rough approximations of what might happen in nature. The presence of organisms antagonistic to the antagonists of P. weirii might reverse the situation. Substrate differences and other conditions of the physical environment also might affect antagonistic tendencies. Further tests need to be conducted to determine the

antagonistic effects of soil fungi under more nearly natural conditions and to determine the mechanisms involved. It might also be of value to determine antagonistic effects between the principal antagonists of P. weirii and other members of the soil community.

Trichoderma has been reported as a parasite of various fungi. It is likely that invasion of Poria weirii-inhabited blocks is not restricted to parasitic relationships of the two fungi since Trichoderma was also found in sterilized, buried, wood blocks. According to some reports, the strong cellulose-decomposing ability of Trichoderma is questionable (20, p.135,252). The exact nutrition of this fungus growing in wood would be desirable if further studies on the nature of the antagonism are to be made.

It would seem that the greatest hope for control of this disease now lies in reducing the survival of the fungus in buried wood residues. The role of antagonistic organisms in contributing to reduction of Poria weirii survival in nature is not known to any extent. Research in this direction seems to hold promise from indications of laboratory results. There is, however, a large amount of work to be done to determine the practical application of results.

SUMMARY

1. Douglas-fir trees were inoculated with blocks containing Poria weirii. From these inoculations it was found that P. weirii is capable of infecting small roots of Douglas-fir trees by direct penetration of the roots through the thin cork layer. The fungus is fully capable of parasitizing and killing living tissues of the phloem and xylem.
2. Experiments conducted in the laboratory in containers of soil indicate that although Poria weirii is capable of surviving in sterilized forest soils containing varying amounts of sawdust and can be isolated from these soils, using standard techniques, the fungus appears unable to survive in soils inhabited by other microflora. It is probably the action of these soil organisms that is responsible for the inability of P. weirii to survive in unsterilized soils when unassociated with a substantial food base.
3. Experiments conducted in the laboratory and in the field showed that Poria weirii is able to survive buried under forest conditions for at least 20 months, even in very small residues. Survival in these residues is enhanced by formation of zone lines within the wood, although these barriers are only temporary. The lines are formed more easily in wood in advanced stages of decay; however, once formed, the lines are more easily

penetrated by soil fungi in these blocks than in blocks in less advanced stages of decay. Survival seems to depend upon the action of soil fungi rather than substrate depletion.

4. Soil fungi isolated from the wood blocks colonized by Poria weirii were plated on malt agar to determine antagonistic effects which might occur. Trichoderma was isolated frequently and, based on the petri plate tests, proved to be highly antagonistic toward P. weirii. How the effects of Trichoderma and other antagonists might be affected in nature by the presence of bark or resin along with the wood is not known, but such factors should be considered in further studies.

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