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Title INTERACTIONS BETWEEN PAIRED CULTURES AS A BASIS FOR DIFFERENTIATING COMPATIBILITY GENOTYPES OF FOMES CAJANDERI KARST. FROM GLAZE-DAMAGED DOUGLAS-FIR

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A study was undertaken to determine the reliability of the formation of a line of demarcation between paired cultures of Fomes cajanderi Karst. as an indicator of dissimilar compatibility genotypes. The results were applied to an assay of the number and location of compatibility genotypes in each of four glaze-damaged, infected Douglas-firs.

Spores shed from sporophores formed in culture enabled development of pedigreed mono- and dikaryotic lines. Di-mon, and dikaryon pairings of known relationship were made in culture and data recorded as to the frequency of formation of the line of demarcation.

The results indicate the line of demarcation in paired culture to be a highly reliable diagnostic feature of the presence of dissimilar compatibility genotypes. Variations in the intensity of expression of

the line of demarcation occur when sibrelated dikaryons are paired, but when mycelia not closely related are paired there is little deviation from the distinct, dark line common to pairings of diverse relationship.

Four glaze-damaged Douglas-firs infected by F. cajanderi 22 years before initiation of the study, were examined to determine the number of compatibility genotypes present in the decay column of each tree. Sections of the bole were removed at intervals from along the decay column of each tree, placed in plastic sacks and incubated for five months. Isolations from mycelia growing on the surfaces of these sections were paired in culture to determine the relationships involved. On the basis of the line of demarcation in paired culture, the trees contained from one to eight compatibility genotypes, representing at least as many successful infections in each tree.

Variation in cultural appearance was found between ramets of the same compatibility genotype.

Observations indicated the presence of a zone of wood in the inner sapwood of the trees examined that is resistant or impervious to decay by organisms from either the heartwood or outer sapwood. However, "mycelial bridges", developed by F. cajanderi crossed this zone.

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INTRODUCTION

Western Oregon and Washington, often spoken of as the Douglas-fir subregion, contained about 26 million acres of forest land in 1963. Seedling, sapling and poletimber stands make up 37 percent (9. 6 million acres) and young-growth sawtimber stands occupy nearly 35 percent (9. 1 million acres) of this land (36).

As the future of the lumbering industry in the Pacific Northwest will ultimately depend heavily upon young- rather than old-growth timber, it is necessary to initiate studies pertinent to the diseases of young Douglas-fir.

The potential value of young-growth timber can be seriously affected by top-breakage resulting from adverse natural conditions. By their very nature broken tops occur in a portion of the young tree which is of interest to lumbermen at a future date, i. e., in that part of the young tree that will lie within the first through the third logs of the merchantable tree. Top-breakage of a young tree may greatly curtail height and diameter growth, if the broken tree survives at all. In trees that do survive, the break opens an avenue for potential decay of the bole which ultimately will reduce the scale and increase liability to further breakage. Most common among the decay fungi

associated with top rot is the basidiomycete Fomes cajanderi Karst. (Fomes subroseus (Weir) Overh.).

A recent study (17) of glaze-damaged Douglas-fir poles in western Oregon reported extensive top-breakage in particular geographical areas. Breaks one inch in diameter readily became infected; the frequency of infection increased with the diameter of the break up to three inches, at which point all breaks examined were infected. F. cajanderi was the major decay organism of the trees in this study. While this fungus may enter a tree through branch stubs along the bole, the most common point of entry is the exposed wood of a broken top resulting from glaze, snow and other storm damage, Figures 1 and 2.

F. cajanderi causes a brown cubical rot of the heartwood in the advanced stage of decay. In the incipient stage of decay the wood is stained a bluish-green. The decay column conforms to the cylindrical core of the heartwood and generally tapers irregularly to a central point at the lower extremity. Sporophores which regularly appear on exposed wood surfaces are woody, perennial brackets. They vary in size up to three inches in width and three-fourths inch thick at the base while tapering to a narrow edge. The upper surface is black, rough and concentrically zoned, while the pink, poroid undersurface is uniform in color, texture and topography.

Figure 1. Sporophore development on the surface of an unhealed 22 year old break. The new leader is seen tapering upward from the opposite side of the break. A cross section of this stem taken at the bottom of Figure 1, is shown in Figure 2.

Figure 2. A cross section of the mainstem near the top of the break showing an outline of the original leader (a) and the slab face of the break (b). The wound has not completely healed after 22 years.

Two distinct nuclear stages are involved in the life cycle of F. cajanderi, which is a typical wood-destroying basidiomycete. The single nucleate (monokaryotic) stage develops a mycelium from spores shed from a mature sporophore. Hyphal union of two compatible monokaryotic hyphae results in the formation of the binucleate (dikaryotic) stage, in which the component nuclei of the cell are not united, but act individually and in conjunction to control cell functions. Clamp connections, a diagnostic feature of the dikaryon, function to insure separation of conjugate nuclei during cell division. It is the dikaryotic mycelium that is found in decayed wood. The dikaryon, under favorable conditions, develops a fruiting body externally on the host within which the nuclei of young basidia unite just prior to spore development. To complete the life cycle, the 2n nucleus undergoes reduction division (meiosis) during the formation of four spores on the basidium. The four newly formed nuclei migrate one each through the four sterigmata into each of four single-nucleate spores.

F. cajanderi is a bipolar fungus with one pair of factors, Aa, controlling sexual compatibility. These segregate independently at meiosis to give rise to spores of only two mating types. This is a simpler situation than is found in some basidiomycetes that are tetrapolar in having two pairs of factors, AaBb, which segregate independently at meiosis to give rise to spores of two or four mating types depending on chromosome arrangement and constitution.

Practical implications of decay may be better understood with a fuller knowledge of the fungal interrelations involved in the decay process. Isolates of some basidiomycetes are known to react distinctively by the formation of a zone of discoloration along the line of mycelial contact when paired in culture. This zone is commonly referred to as "the line of demarcation". The formation of a line of demarcation in paired culture has been found useful in differentiating between unique fungal thalli (compatibility genotypes as they will be called in this thesis) of the same species in decay columns of conifers where more than one infection has occurred (15, 23, 39). The reliability of this reaction to differentiate between compatibility genotypes has not been specifically investigated, but its use appears promising for determining the number and location of dissimilar compatibility genotypes contributing to the decay column and hence the number of infections in Douglas-firs infected by the top rot fungus, F. cajanderi. However, before assuming that the formation of a line of demarcation between paired cultures invariably demonstrates the presence of dissimilar compatibility genotypes, it seems desirable to determine the reliability of the reaction and to understand something of its genetic foundation.

The purpose of this study is: 1) to ascertain the reliability of the line of demarcation as an indicator of dissimilar compatibility genotypes, and conversely, its absence as an indicator of like

compatibility genotypes, and 2) to apply paired culture tests to determine the number of compatibility genotypes naturally occurring in four glaze-damaged infected trees.

LITERATURE REVIEW

The meterology of the glaze phenomenon is extensively described by Bennett (4). His discussion includes as well, the effect of glaze upon forest trees, both conifers and angiosperms.

The name "glaze" was adopted in 1916 by the U. S. Weather Bureau in order to reduce the confusion of terminology surrounding the glaze phenomena, ". . . for the ice coating which forms when cold rain comes in contact with strongly chilled terrestrial objects" (1, p. 286). Such terms as "silver thaw", "ice storm", "sleet", "glazed frost", "glare ice" as well as "glaze" have been used interchangeably by many workers (4).

Coniferous trees as a class, are better adapted for resistance to glaze damage than are dicotyledonous trees. This resistance it appears, is due in part to the greater flexibility of coniferous wood over that of dicotyledonous wood allowing them to sustain a greater degree of bending before breakage of the stem, and in part to the smaller crowns of the conifer accumulating comparatively less ice and therefore being less susceptible to windthrow during glaze storms (4).

Glaze damage to conifers frequently results in exposure of the heartwood to wood-decay fungi. Fulcher (17) found the incidence of infection by Fomes cajanderi to become severe within ten years

after glaze damage to Douglas-fir poles in western Oregon.

Characteristics of F. cajanderi (21) are also published under the synonym F. subroseus by Campbell (13), Mounce and Macrae (24), Nobles (26), and Overholts (27), p. 58-59) summarizes information differentiating F. subroseus and the closely related F. roseus (Alb. & Schw. ex Fr.) Cke.

In the course of decay of coniferous and dicotyledonous woods, the host tissue is sometimes darkened along fungus-fungus and fungus-atmosphere interfaces. These darkened zones, commonly referred to as black lines or zone lines, have been of interest to the pathologist since the early 1900's.

Zone lines beneath the surfaces of a variety of woody tissues have been observed by many pathologists (8, 12, 20, 29, 30, 34). Their presence has been attributed to the production of "wound gum" (34, 40), oxidation products (19, 20, 29, 30), or masses of mycelia that are discolored or have discolored the wood (8, 12, 19, 19, 23).

Hubert (20) reported that zone lines formed between two fungi occupying the same wood substratum are of common occurrence, and that these lines are rare in the brown rots while being common in the white rots. Rhodes (29) noted the relative lack of zone line formation in coniferous woods as compared to dicotyledonous woods.

Host-parasite interaction is not an absolute requirement for the formation of zone lines in wood (29, 34). Rhodes (29) considers the

formation of brown decomposition products, i. e., "wound gum", in the wounds of living dicotyledonous trees as being due to an oxidation of the woody substance. White (40), however, attributes the deposition of wound gum in newly parasitized tissues to a secretion or a decomposition product of host origin produced in the relationship with the parasite.

Zone lines in wood, resulting from the action of a single mycelium, have been observed by many workers (8, 18, 29, 30, 33, 40). Rhodes (29) finds the brown substances occurring in dicotyledonous wood attacked by wood-rotting fungi to be indicative of the first stages of decay of the wood, i. e., representing the outermost limits of decay in the sense of Hiley (18) and White (40). Rhodes (29, p. 44), in summation observes, "The blackish zones are not constant in position since the decomposition products, which cause the discoloration, move forward with the advance of the decay in any part of the stem and ultimately disappear upon its completion within that part. The continual occurrence of the blackish zones between decayed and undecayed wood is due to the fact that the decomposition products are destroyed together with the wood while new ones are formed constantly from the wood as fast as it is attacked by the advancing fungus."

When two mycelia, which behave antagonistically towards one another, occupy the same substratum antagonism between them

can become evident in the form of zone lines in the host tissue. White (40, p. 154) has stated, "Black lines are encountered, but cultural or other evidence shows that when they do occur more than one species of fungus is at work. They are produced at the point of contact of two invading fungi in the case of many pairs of wood-destroying fungi, a phenomenon well known to pathologists."

Weir (39, p. 49) finds that antagonism of mycelia of different species is characteristic of many wood-rotting fungi, "Trametes pini seldom encroaches upon the wood of any conifer already occupied by Echinodontium tinctorium and vice versa. The rot of Fomes pinicola and that of F. fomentarious in the wood of the same birch tree is always sharply separated by a conspicuous black line although the decay produced by each species is quite characteristic. The same evident antagonism is often true in the case of purely saprophytic species when occupying the same substratum."

Sharples (33) working with Heva brasiliensis Mull. -Arg. wood decayed by Ustilina zonata (Lev.) Sacc. found the black lines of the fungus tissue appeared as the wood dries. Hubert's findings (20) agree with those of Sharples (33) in that a drying and oxidation of the decomposition by-products of the infested wood appears necessary for zone lines to become apparent.

Rhodes (29, p. 44) sums up the conditions which he feels are necessary for the formation of the brown decomposition products by

wood-destroying fungi: "(a) the presence of dead cells, (b) an optimum supply of moisture, and (c) a supply of oxygen sufficient to promote oxidation."

There is general agreement among pathologists concerning the color and width of the black lines found in wood. Brooks (8) reports the "black zones" formed after a wounding of the plant tissue, to be one thirty-second of an inch in diameter. Hubert (20) has found "zone lines" between the decay of Polyporous anceps Pk. and Lenzites sepiaria (Wulf ex Fr.) Fr. in Picea canadensis (L.) B. S. P. to be as large as one-fortieth of an inch wide. These lines had indistinct edges and were of a dull brownish color.

Some workers believe the black lines found in wood to result from a mass or matrix of vegetative hyphae. In this sense Campbell (12, p. 19) states, "Thus there is formed a body comparable to a sclerotium in that it is completely surrounded by a black rind of bladder hyphae and has a medulla of hyaline hyphae." The formation of the black line by Armillaria mellea (Vahl) Quel. is very similar to those formed by Xylaria polymorpha (Pers. ex Fr.) Grev. (12). Brooks (8, p. 159) compares the black lines of fungal hyphae in the host tissue to a "kind of sclerotic plate." He further states that, "... the difference between this aggregation and a typical sclerotium being that the latter does not include within it portions of the tissues of the host." Hiley (18, p. 156) found the black lines to be made up

entirely of hyphae which bear special short branches, "Both the branches and some of the original segments swell up into bladder-shaped bodies and their walls become tinted with a pale brown pigment."

Occasionally pairs of black lines have been observed. Hiley (18, p. 158) in studying the black lines formed by Armillaria mellea has found that "When two black lines approach each other they seldom unite, but cease to move when about 1 to 5 mm. apart. This causes the frequent phenomenon of a pair of black lines running parallel to each other." He does not indicate the number of individual mycelia involved in this observation.

Hubert (20) has noticed double zone lines between the rots of Polyporus anceps and Lenzites sepiaria in Picea canadensis, and Fomes applanatus (Pers. ex Wallr.) Gill. and Stereum frustulosum (Pers. ex Fr.) in Quercus sp.

As a diagnostic tool Hubert (20) believes zone line formation to be a valuable aid in the identification of decay fungi; but he cautions that additional evidence is usually necessary. Boyce (5, p. 350) however, states, "zone lines are of little diagnostic value and may even be misleading when a fungus causing the original decay is followed by another forming zone lines, which can happen with Fomes applanatus and Xylaria polymorpha."

Zone line formation appears to have a beneficial value for some fungi within the host tissue. Rishbeth (30, p. 20) has observed that zone line formation by Fomes annosus (Fr.) Cke. in a stump root as a result of partial desiccation acts as a barrier to Trichoderma viride Pers. ex Fr., Hypholoma fasciculare (Huds, ex Fr.) Quel. and certain "blue-stain" fungi. "These saprophytes cannot pass a recently formed zone line, however, and are temporary (sic) halted. When the root dries out again, they penetrate the zone-lines, possibly because it is damaged by expansion and contraction of the wood." Meanwhile, Fomes annosus forms another zone line which in turn again temporarily checks the advance of the saprophytes. In apparent agreement with Rishbeth, Nelson (25) believes that it is probable that zone lines tend to exclude antagonistic soil fungi from buried wood blocks inhabited by Poria weirii (Murr.) Murr.

Wound gum formation by the host tissue is considered to be especially important in preventing decay of the wound area (34). The gum apparently acts as a barrier which is not easily penetrated by decay organisms.

On the other hand, if wound gum is considered as being a product of fungal invasion, upon further mycelial development in the host tissue it may become bleached or serve as a nutrient source for the fungus (40); in any event the remains of it cannot be detected by certain chemical tests (40).

Hiley (18, p. 157) feels that the significance of the black line formed in the host tissue by a single mycelium is obscure. He states, "It seems to indicate a stage of excessive vigour in the mycelium and though it does not directly cause marked changes in the character of the wood, it appears in some way to transform the wood into a state in which it is easily acted on by the hyphae behind" (18).

As has previously been noted, zone lines are rare in coniferous wood while being more prevalent in dicotyledonous wood. Zone line formation in dicotyledonous woods has been considered primarily from a morphological rather than a physiological or genetical viewpoint. Little emphasis has been placed toward comparing zone line formation in wood to similar appearing dark lines between paired, noncompatible mycelia in culture.

Reactions of paired mycelia in culture have been studied by many workers with dikaryons of the same species (14, 15, 23, 33, 38) with monokaryons of the same species (2, 10, 15, 24, 28, 35), and with dimon pairings (28, 38). Antagonistic reactions between paired mycelia have been considered as representing incompatibilities between mycelia possessing unlike compatibility factors and as such have been used as a means of differentiating mycelia of different origin (10, 14, 15, 23, 33, 38, 40).

In investigating the pairing reactions of Polyporous schweinitzii subcultures from a single isolation or single isolations from a single

source, Childs (15) found that hyphae of different cultures intermingled and antagonism did not occur. When isolations from four different sources were paired, however, a definite dark line formed between the mycelia in each case. Schmitz (32) also found this same expression of antagonism when cultures of Fomes pinicola (Sw. ex Fr.) Cke. from four different hosts, Douglas-fir, white fir, western hemlock and western white pine, Pinus monticola D. Don, were paired on artificial medium. Similarly, Mounce (23, p. 34) paired 35 diploid cultures of Fomes pinicola from one host species, Picea mariana (Mill.) B. S. P., and from one locality. She found the "line of aversion" to be the "usual reaction obtained when mycelium from sporophores or wood from various individuals of the same host species, and even from the same locality, are paired in culture."

In summary, Mounce (23, p. 34) notes the cultural circumstances under which "complete fusion" between two dikaryotic mycelia of Fomes pinicola will take place:

"(a) Whenever a mycelium is paired with itself.

(b) When mycelium from sporophore tissue is grown with mycelium from spores produced by the sporophore. A space or a white line of dense growth may occur under such circumstances.

(c) Usually when mycelium from infected wood of a tree is grown with mycelium from a sporophore which grew upon the tree.

(d) Usually when mycelium from spores or sporophore tissue is

grown with mycelium from spores or sporophore tissue from a second sporophore from the same tree."

Madhosingh (22) serologically compared dikaryotic, as well as monokaryotic isolates of F. cajanderi and found precipitin lines which were isolate specific. These results would appear to be correlated to the formation of the line of demarcation in paired culture, as found in F. pinicola (23).

Mounce (23) paired ten monokaryons from four different sources and found lines of aversion to be common in all pairs which were not sexually compatible. The color of the aversion lines ranged from white, through yellow, brown and black. In sexually compatible matings she found lines of aversion to be either absent or light in color, only occasionally did the line become brown. An exception to this was found in pairings of monosporous mycelia of F. pinicola from different geographical races. Clamp connections were still formed, yet often a distinct line of demarcation was formed in the contact zone of sexually compatible matings. Verrall's work (38) with monokaryons of Fomes igniarius (L. ex Fr.) Kickx is in general agreement with Mounce (23).

In contrast to these findings, Childs (15) did not find dark lines when monosporous mycelia of Polyporous schweinitzii of the same or different ancestry were paired in culture.

Di-mon isolates of Fomes igniarius were paired in culture by

Verrall (38). He found antagonism to result from these pairings, but he did not indicate whether or not the dikaryon diploidized the monokaryon.

Verrall's conclusions (38) in general, agree with those of Mounce (23), but he finds antagonism expressed as a dark line in di-mon pairings to be the rule rather than the exception.

Antagonistic effects between mycelia appear to be fairly independent of the composition of the culture medium. Mounce (23) experimented with many media to find a nutrient source allowing good development of the line of demarcation. Czapek's agar with peptone and dextrose, and potato agar with glycerine gave the best development of the line of demarcation as well as mycelial growth. She found that the black line of demarcation developed on any medium where Fomes pinicola formed even a thin mycelial mat.

Cayley (10) varied the depth of the medium, and the distance between inocula and found that mutual aversion of monospore mycelia of Diaportha perniciosa Marchal (an ascomycete) continued to be expressed irrespective of these changes.

Temperature and light may effect the rate of formation of the line of demarcation, but they do not inhibit it (23). Mounce (23) found the line of demarcation to develop earlier in cultures kept in the dark than in cultures in the light.

Hopp (19) found that he could induce coloration of hyaline mycelia

by exposure of the hyphae to large amounts of water and air. This reaction, however, was not related to antagonism of two mycelia, but was the effect upon a single mycelium and may be related to the matter of dark line formation in wood which was discussed earlier.

Thus, it appears that the potential of an antagonistic reaction to occur between two incompatible mycelia on an artificial substrate is little effected by the depth and composition of the medium, the placement of the antagonists, or temperature and light. The exposure of the aerial hyphae to water and air may enhance the formation of discolored hyphae.

In summary, Mounce (23, p. 38) has concluded that a line of aversion ". . . develops when mycelia from different genera and species of deciduous and coniferous hosts are paired, when mycelia from the same host species but from different localities are paired, and when mycelia from the same host species and the same locality are paired. It may even develop when mycelia from two sporophores growing on the same tree are used."

Verrall (38, p. 28) in his conclusions, stated, "When there is any apparent difference between isolates, even from the same tree, there is antagonism, and between isolates from different trees, whether similar or different in appearance, there is antagonism."

Verrall (38, p. 28) apparently sums up all the available information when he states, "It seems that through segregation and recombination almost every mycelium is genetically different with respect

to the factor or factors influencing compatibility."

The intermycelial reactions described above by many authors have much in common and may even be identical. To be sure variations occur but these appear exaggerated by the variety of terminology used in the literature to describe the phenomenon. "Barrage", "mututal aversion", "line of aversion", "line of demarcation", "black line", "discolored zone", and "zone line" have all been used to describe antagonistic reactions between paired mycelia.

Brodie (7, footnote p. 63) describes, as concisely as is possible in English, the meaning of the wood "barrage", "The French word 'barrage' is very difficult to render suitably in English to describe the phenomenon. The words 'aversion' and 'repulsion' translate part of the idea, but do not include the gap between mycelia as does the French word. In French, one may say that a 'barrage' exists between certain mycelia, meaning the material result of mutual aversion."

Apparently, in response to lack of adequate terminology, modification of Vandendries and Brodie's original definition of the barrage phenomenon (37) has taken place since its conception. It is not my intention here to discuss all the possible variations of the original definition that have occurred in the intervening years, but the following examples will show to some extent the divergence that has come into use.

Papazian (28, p. 148) states, "The barrage region is not entirely devoid of hyphae, but these hyphae are thin, far apart, and rarely assume an aerial habit. Microscopically, hyphae in the barrage region are irregular, having numerous knobs and very short branches." Takemaru (35) has used the term barrage to describe conditions in which mycelial antagonism may be "barely distinguishable" to situations in which the barrage region is "some 7 mm. or so in width". He notes barrages as being formed in compatible matings of Collybia velutipes (Curt.) Fr. (up to 6.6 percent in frequency) and in common-B heterokaryotization (greater than 91.7 percent in frequency). Clamp connections are formed in both these matings indicating actual physiological contact between paired monokaryons.

Brodie (6) displays a picture of a barrage occurring between two dikaryotic mycelia of Corticium calceum Fr. His description is as follows: "Between them is a clearly defined narrow gap extending the entire length of the specimen (15 cm.). When examined under the microscope the gap is found to be almost entirely devoid of hyphae" (6, p. 188). He makes no mention of the dark line between the two mycelia in the picture.

A response apparently similar, if not identical to the barrage description (37) was used by Cayley (10) ten years earlier, in 1923. She called this response "mutual aversion" to signify the situation in which hyphae of two antagonistic strains did not meet when paired on

media. She describes (10, p. 355) the "aversion" as leaving a well-defined "line of demarcation" between colonies, and "A thick-walled stromatic protective growth of mycelium forms at the edge of each colony on either side along the line of demarcation, in some cases accompanied by discoloration of the medium."

There seems to be general agreement among pathologists as to the usage of "line of demarcation". Cayley (10), Childs (15), Mounce (23), Schmitz (32), Verrall (38) and Weir (39) have all used the term to denote the presence of antagonism between two mycelia on either natural or artificial substrates. This antagonism is expressed as a "space" or "gap" in the substrate between the two opposing colonies. Whether or not a darkening of the medium occurs at all with the line of demarcation appears not to be a critical consideration insofar as the usage of the term is concerned. It would seem, therefore, that the "barrage" of Vandendries and Brodie (37, as they used it in 1933) is in close agreement with the use of the earlier term "line of demarcation".

The early literature (8, 18, 29, 33, 40) describes antagonistic reactions in natural substrates by their most apparent characteristic; that is: "black lines", "zone lines" or "black zones". These darkened regions of the wood are described as having aggregations of darkened hyphae. Two investigators (18, 40) depict these "black lines" as moving through the substratum, and are therefore the

result of a host-fungus interaction, while others (8, 29, 33) find them resulting from interaction of two mycelia in the host tissue.

In summary, it seems that several terms have been used to describe antagonistic reactions occurring in natural and artificial substrate. Line of aversion, mutual aversion and line of demarcation appear to be identical terms for the same phenomenon as described by Cayley (10). Black line, zone line and black zone have a more restricted meaning and apply only to antagonistic relations involving a definite darkening of the host substrate.

The synonomy of terms related to mutual aversion, i. e., line of aversion and line of demarcation (10, 23) takes into account non-intermingling of opposing mycelia, i. e., the "barrage effect" described by Vandendries and Brodie (37). Later use of the barrage terminology by one of its authors (6) and others (28, 35) brings the usage of this term even more into line with the usage of the "mutual aversion" group. A true barrage is represented by a very specific type of fungal interaction, one in which an obvious space relatively free of hyphae exists between two colonies on an artificial substrate. The "mutual aversion" terminology as described by Mounce (23) better represents the variations in cultural antagonism encountered among closely related species. The term "line of demarcation", as adequately described by Mounce (23), to denote hyphal antagonisms in paired cultures is used in this thesis.

FORMATION OF THE LINE OF DEMARCATION AMONG MYCELIA OF KNOWN RELATIONSHIP IN PAIRED CULTURE

While an adequate terminology has been settled upon in the literature review to describe the phenomenon of interaction between mycelia of different colonies, a completely satisfactory terminology for the participants has not been achieved.

It must be borne in mind that the original dikaryotic genotype may change by mutation. The variations encountered in the cultural appearance of compatible mycelia isolated from various points of a decay column suggest that mutations of the original dikaryotic genotype take place fairly frequently under natural conditions in some thalli of F. cajanderi. Thus, each infection could ultimately lead to a number of slightly different genotypic hyphal masses comprising a decay column in whole or in part of compatible mycelia, Figure 3.

The problem here then is one of adequate and biologically correct terminology to describe as a group all the genotypes found in one tree possessing a single compatibility type in common. Childs (14) refers to all members (isolates) of a compatibility group as being of the same clone in studies with Poria weirii Murr. in Douglas-fir roots. As Childs (14) has pointed out the use of "genotype" to represent all compatible isolations originating from a single infection is not preferred because of variations occasionally found among the isolates.

In this study the interest is mainly in compatibility relationships,

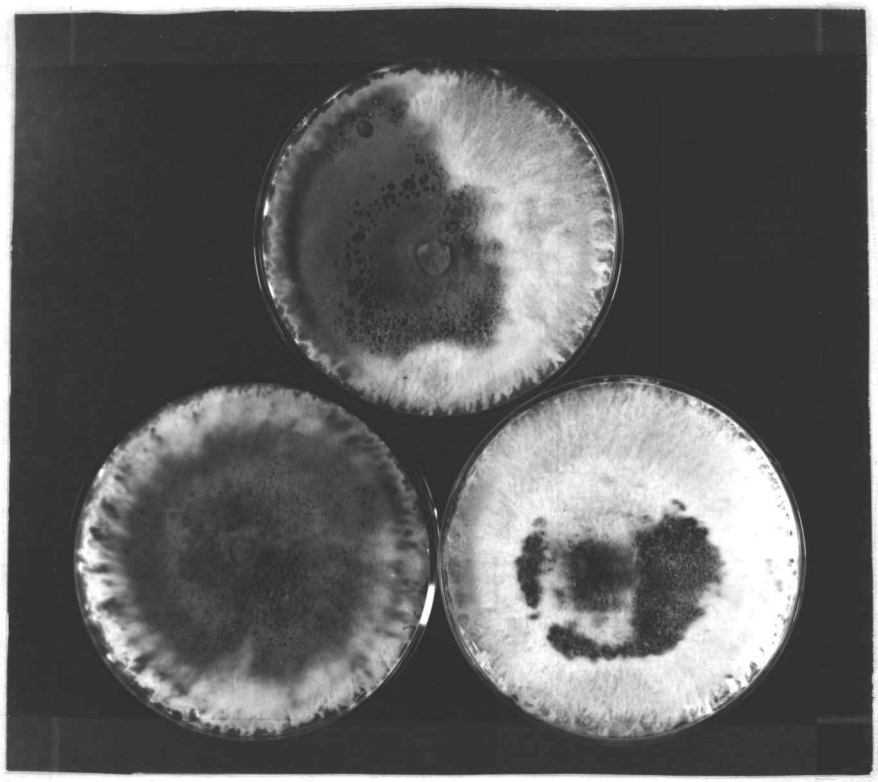

Figure 3. Individually plated isolates from tree I, demonstrating cultural variations occurring in the dikaryotic mycelia.

rather than cultural characteristics, among the individual isolates as evidence of common origin. Following this line of thought the isolates are then considered as being genotypically alike despite variations that might appear in other facets of their growth. Therefore, the term "compatibility genotype" is used here to describe any number of compatible isolates of Fomes cajanderi which in all probability have originated from the original infection binucleate cell.

One may quickly conclude from the literature and from consideration of the genetic variates set forth in the preceding paragraph that variation is to be expected in form and intensity of production of the line of demarcation between paired mycelia in culture. Variation does occur and in this thesis the differing reactions are distinguished and symbolically designated "+", "-", and "o". Considerable variation in the degree of discoloration of the line of demarcation in noncompatible pairings occurred during the course of these experiments, figure 4(a). The intensity of the discoloration in the contact zone between noncompatible ramets was rated either "+" or "-" to denote this variation for its possible significance in the overall conclusions. The "+" indicates the occurrence of a strong discoloration of the medium and hyphae between paired colonies, without free intermingling of aerial hyphae. The "-" represents an intermediate situation in which the medium and hyphae in the contact zone between paired mycelia were not discolored as intensely as in

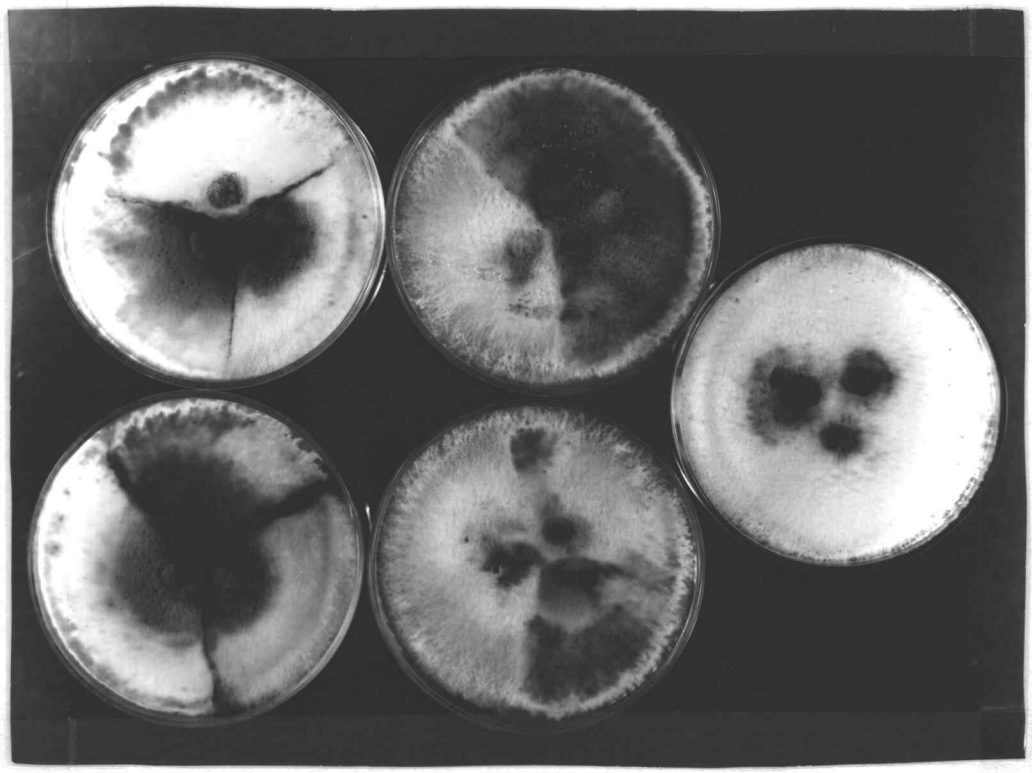

Figure 4. Noncompatible and compatible isolations from tree III. Noncompatible pairings in plates at the left (a) show examples of variation in the intensity of the line of demarcation. Compatible ramets differing in cultural characteristics are shown in the center plates, and similar appearing, compatible ramets in the plate on the right(b).

"+" pairings. The "o" represents pairings in which a line of demarcation did not form, with consequent free intermingling of aerial hyphae between mycelia, Figure 4(b).

Materials and Methods

Fomes cajanderi like other species of Fomes (23, 40) has been noted in random observations to form the line of demarcation between paired cultures. However, the consistency of line formation has not been established as in F. pinicola (23). Twenty-three dikaryons isolated from western North America were assembled in order to learn the consistency of occurrence of the line of demarcation in F. cajanderi. These cultures are listed in Table 1 along with the host and geographic origin of each. Their responses when paired in all possible combinations appear in the section on results.

Results of the above pairings were informative but were less than fully satisfying because the inheritance of the participating cultures was unknown.

The genetic relationships involved in formation of the line of demarcation in paired culture have been little studied. To investigate the effect of inheritance on line formation four dikaryotic cultures of F. cajanderi, numbers 184, 185, 187 and 188 were found

Table 1. Numerical designation, host and origin of 23 dikaryons of F. cajanderi from western North America.

Culture Number	Host	Origin of Culture
71	Pseudotsuga menziesii	Benton Co., Oregon
73	" "	Humboldt Co., California
87	Picea glauca	Aleza Lake, B. C., Canada
88	Pseudotsuga menziesii	Merritt, B. C., Canada
89	" "	Otero Co., New Mexico
102	Unknown	Linn Co., Oregon
117	Pseudotsuga menziesii	Umatilla Co., Oregon
119	Unknown	Benton Co., Oregon
127	Pseudotsuga menziesii	Josephine Co., Oregon
133	" "	Oregon
138	" "	Coos Co., Oregon
139	Unknown	Coos Co., Oregon
157	Pseudotsuga menziesii	Cochise Co., Arizona
168	Pinus banksiana	Enterprise, N. W. T., Canada
171	Psuedotsuga menziesii	Pima Co., Arizona
179	Conifer wood	Clearwater Co., Idaho
180	Pinus ponderosa	Larimer Co., Colorado
181	Psuedotsuga menziesii	Benton Co., Oregon
183	Pinus ponderosa	Graham Co., Arizona
184	Pseudotsuga menziesii	Benton Co., Oregon
185	" "	Benton Co., Oregon
187	" "	Benton Co., Oregon
188	" "	Benton Co., Oregon

naturally occurring in two glaze-damaged Douglas-firs from western Oregon; 184, 185 and 187 were isolated from one tree, while 188 was isolated from a second tree.

Cultures 184 and 187 from the same Douglas-fir, when paired on media, grow together with free intermingling of both submerged and aerial hyphae thereby appearing mutually compatible. These cultures are dissimilar in mycelial appearance however, raising a question as to their relationship. These matters will be described in detail later. Culture 185 isolated from the same tree as 184 and 187 is not culturally compatible with either. Culture 188 is non-compatible with 184, 185 and 187. These relationships are summarized below.

	184	185	187	188
184		+	o	+
185	+		+	+
187	o	+		+
188	+	+	+	

Before pedigreed lines of F. cajanderi could be developed for study, methods were required to accomplish sporophore production in culture. Fortunately this is easily accomplished with F. cajanderi.

Badcock's (3) accelerator mixture, consisting of maize meal, bone flour, potato starch, sucrose and wood ash, was used as the basis of a medium for inducing sporophore formation in culture. A liter of medium contained 7. 14 g of the accelerator, 30 g of malt

extract, 3 g of yeast extract, and 18 g of flake agar. The medium was autoclaved 15 minutes to melt the agar and added in 28 ml lots to 4 g of Douglas-fir sawdust in a 2.5 by 20 cm test tube. The accelerator becomes present, by weight, to an amount equaling five percent of the sawdust. The tubes were plugged and autoclaved for 20 minutes at 15 pounds pressure. Upon cooling, this medium was solidified into a long slant.

The parental stock cultures were inoculated onto the above medium in slant culture, the slant placed upright and the inoculum incubated at room temperature. Mycelial development was allowed to continue with the tubes upright until sporophore development began near the top of the slant, about 15 days after inoculation. The slants were then laid with the sporophore surface facing down on a 30 degree incline, the plugged end of the tube uppermost. Spores were shed onto the inner glass surface of the tube 22-25 days after the initial inoculum transfer to the slants.

The spores were washed from the tube surface and dispersed in a dilution series. One ml of each dilution was pipetted onto a separate plate of three percent malt extract medium and swirled to spread the spore suspension evenly over the surface of the medium. Germinating spores formed visible colonies after 48 hours incubation at room temperature. Isolated single spore colonies were removed with the aid of a dissection microscope and a flattened needle and

placed on three percent malt extract in slant culture for incubation and subsequent use. Single spore cultures obtained by this method were later examined microscopically for the presence of clamp connections. The latter are characteristically found on the dikaryons of F. cajanderi. Those cultures lacking clamp connections were considered to be monokaryons and retained for subsequent use as the monokaryon itself and in the production of dikaryons.

Fifteen monokaryons from each parent culture were paired in all possible combinations to develop relationships among the monokaryons and to synthesize sibcomposed dikaryons of known relationship among themselves and with their constituent monkaryons. These and all subsequent pairings were made on malt-yeast extract medium containing 30 g malt extract, 3 g yeast extract, and 18 g flake agar per liter. Each plate was poured to contain about 25 ml of medium.

The mating pattern obtained when 15 sib monokaryons of culture 184 were paired in all possible combinations is shown in Figure 5. Determination of mating type, based on the presence of clamp connections, was made by microscopically examining mycelia removed from the contact zone between each pair of monokaryons. Where clamp connections were formed a dikaryon was thus indicated and the paired monokaryons were recognized to be of opposite mating types; lack of clamp connections indicated that mycelia of like mating type had been paired. A "+" in the figure indicates the presence of

	1	4	5	7	11	13	15	2	3	6	8	9	10	12	14
1	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
4	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
5	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
7	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
11	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
13	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
15	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
10	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
14	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-

Figure 5. Mating pattern obtained when 15 sib monokaryons of culture 184 were paired in all possible combinations. A "+" indicates the formation of clamp connections and a "-" their absence.

clamp connections and a "-", their absence. This pattern is typical of the mating patterns found in similar pairings of sib monokaryons of cultures 185, 187 and 188, which are not shown. Dikaryon formation always developed as a "sector" between two monokaryons of dissimilar mating type. Compatible and noncompatible mating type responses are illustrated in Figure 6.

The monkaryons of each of the four parental cultures were divisible into two groups, i. e., a bipolar mating pattern, on the basis of mycelial pairings. Clamp connections formed in all cross-pairings between members of the two groups, but not in pairings between members of the same group.

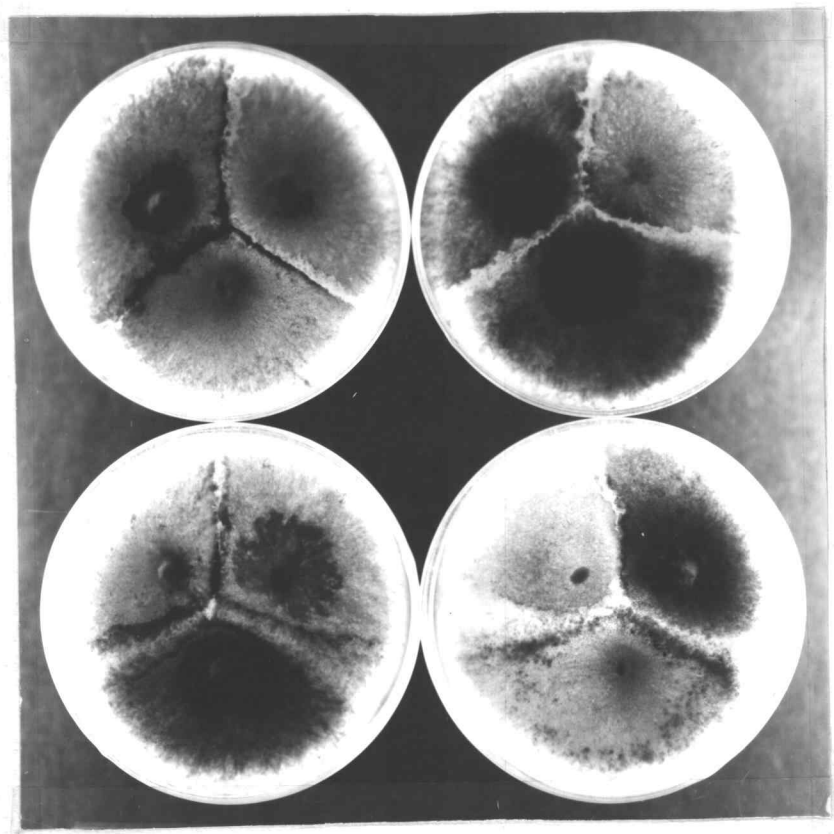

Figure 6. Reactions in culture between compatible and noncompatible monokaryons. The two uppermost plates show lines of demarcation between monokaryons of the same mating type. Dense lines of demarcation have formed in the left hand plate, while very thin lines have formed in the right hand plate. The two lower plates show dikaryon formation arising as a sector between two compatible monokaryons.

Results

Figure 7 summarizes the reactions found upon pairing in culture the 23 dikaryons isolated in western North America. The controls, i. e., the self-pairing of ramets, did not display any indications of a line of demarcation. While the formation of definite lines of demarcation predominated in the other pairings, the intensity of discoloration commonly regarded as typical was not as fully expressed in a few of the latter pairings, which were regarded as intermediates.

The results appearing in Figure 1 establish the line of demarcation as a highly consistent feature of dikaryons of F. cajanderi in paired culture. Cultures 184 and 187 were exceptions to all others in that they were mutually compatible, however, they showed no tendency toward compatibility with any of the other isolates. Controls were compatible without exception. These results indicate the universality of the line of demarcation between distantly related dikaryons in paired culture, but do not guarantee the reliability of line formation for differentiating between naturally occurring genotypes of F. cajanderi occurring in close physical association.

Pairing combinations used in analyzing mycelial relationships involved in the formation of a line of demarcation in paired, pedigreed cultures of F. cajanderi, are given in Table 2. Synthesized dikaryons and monokaryons, were paired among themselves and with their

	71	73	87	88	89	102	117	119	127	133	138	139	157	168	171	179	180	181	183	184	185	187	188
71	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
73	+	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
87	+	+	o	-	+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
88	+	+	-	o	+	-	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+
89	+	+	+	+	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
102	+	+	-	-	+	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
117	+	+	-	-	+	+	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
119	+	+	+	+	+	+	+	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
127	+	+	+	-	+	+	+	+	o	+	+	+	+	+	+	+	+	+	-	+	+	+	+
133	+	+	+	+	+	+	+	+	+	o	+	+	+	+	+	+	+	+	+	+	+	+	+
138	+	+	+	-	+	+	+	+	+	+	o	+	+	+	+	+	+	+	-	-	+	+	-
139	+	+	+	-	+	+	+	+	+	+	+	o	+	+	+	+	+	+	+	+	+	+	+
157	+	+	+	+	+	+	+	+	+	+	+	+	o	+	+	+	+	+	+	+	+	+	+
168	+	+	+	+	+	+	+	+	+	+	+	+	+	o	+	+	+	+	+	+	+	+	+
171	+	+	+	+	+	+	+	+	+	+	+	+	+	+	o	+	+	+	+	+	+	+	+
179	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	o	+	+	+	+	+	+	+
180	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	o	+	+	+	+	+	+
181	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	o	+	+	+	+	+
183	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	o	+	+	+	+
184	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	o	+	o	+
185	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	o	+	+
187	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	o	+	o	+
188	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	o

Figure 7. Interactions of 23 cultures of F. cajanderi from western North America with respect to formation of the line of demarcation when paired in all possible combinations.

Table 2. Genetic constitution of various cultures combined to determine the influence of genetic relationship on formation of the line of demarcation.

Experiment Number	Pedigree of Cultures and Pairing Combination	Symbol Explanation
1	$A^xA^y \times A^1$ [1/]	parent dikaryon paired with its progeny monokaryons
2	$B^xB^y \times A^1$	parental dikaryon paired with monokaryons of another parent
3	$A^1A^3 \times A^1$	sibcomposed dikaryons paired with monokaryons of the same parent, one factor in common
4	$A^2A^3 \times A^1$	as in (3), but no factors in common
5	$A^1A^2 \times A^3A^4$ and $A^1A^3 \times A^2A^4$	pairing and cross-pairing of sibcomposed dikaryons from the same parent, no factors in common
6	$A^1A^2 \times A^1A^3$	pairing of sibcomposed dikaryons from the same parent, one factor in common
7	$A^xA^y \times A^1A^2$	pairing of sibcomposed dikaryons with the parent of the component monokaryons
8	$B^xB^y \times A^1A^2$	pairing of the sibcomposed dikaryons with nonparental dikaryons

[1/] A^xA^y and B^xB^y represent the parental stock cultures 184, 185, 187 and 188, and A^1 and A^1A^2 represents their mono- and synthesized dikaryon progeny.

parental and nonparental cultures in a series of eight experiments. The results are presented in Table 3 and Figure 8.

Progenies from four, rather than one parent were used in all experiments in this study. Results of each experiment are the cumulative results of all four groups. It was felt that more reliable results could be obtained with some variety in the parental stock.

In the pairing of monokaryons with their parent dikaryon, Experiment 1, or with a nonparental dikaryons, Experiment 2, the formation of a line of demarcation occurred at a frequency of greater than 95 percent in both experiments. The line of demarcation did not form in the remainder of these pairings.

In di-mon pairings of mycelia possessing one factor in common, Experiment 3, hyphal intermingling freely occurred in 52 percent of 132 pairings. Strong discoloration (+) of hyphae and medium in the contact zone was found in just eight percent of the pairings, while intermediate expressions of discoloration (-) were found in 40 percent of the pairings. In Experiment 4, di-mon pairings of sib-related mycelia which unlike those of Experiment 3 possess no factors in common, the line of demarcation was noted in 81 percent of 200 pairings; of these 34 percent were rated strong (+) and 47 percent were rated intermediate (-). Compatible mycelia were found in 19 percent of the pairings.

Experiments 5 and 6 are concerned with the interaction of sibcomposed dikaryons among themselves. Sibcomposed dikaryons having no factors in common are paired in Experiment 5 and those dikaryons which share a single factor are paired in Experiment 6.

Sibcomposed dikaryons with no factors in common showed varying degrees of discoloration in the contact zone between paired mycelia in 83 percent of 354 pairings, while in those dikaryons sharing a common factor a line of demarcation appeared in 65 percent of 177 pairings. The percent pairings of sibcomposed dikaryons of either Experiment 5 or 6 in which a line of demarcation was not present is 17 and 35 percent, respectively.

Experiments 7 and 8 together compare the effects of parental-sibdikaryon relationship upon the expression of the line of demarcation in paired culture. In Experiment 7 sibcomposed dikaryons are paired with the parent of their constituent monokaryons, while in Experiment 8 sibcomposed dikaryons are paired with a nonparental dikaryon.

Sibcomposed dikaryons paired with their parental dikaryon were compatible in 41 percent of 66 pairings, as compared to the complete lack of compatible mycelia in 98 pairings in which the paired mycelia were of mixed origin. The remaining 59 percent of the parental-sibcomposed pairings of Experiment 7 were divided with 24 percent showing a strong expression, and 35 percent showing an intermediate

expression of the line of demarcation.

In general, the data indicate the formation of a demarcation line in paired culture of unrelated mycelia to be very strong evidence of paired unlike compatibility genotypes. They fully support use of the presence of a line of demarcation between paired cultures as grounds for concluding the participants to be of discrete genetic origin, and where the paired cultures are from the same tree as evidence of two or more infections. With this concept in mind, the boles of four glaze-damaged Douglas-firs were examined to determine the relationships among the fungi contributing to the respective rot columns.

Table 3. Summary of results from the pairings in Table 8 presented as percentages of the total pairings of each experiment falling into each response class.

Expt. No.	Pairing Combination	No. of Pairings	Expression of development of the line of demarcation by response class[1]			
			+	±	-	o
			percent			
1	$A^xA^y \times A^1$	50		96[2]		4
2	$B^xB^y \times A^1$	41		95[2]		5
3	$A^1A^3 \times A^1$	132	8	(48)	40	52
4	$A^2A^3 \times A^1$	200	34	(81)	47	19
5	$A^1A^2 \times A^3A^4$	354	36	(83)	47	17
6	$A^1A^2 \times A^1A^3$	177	20	(65)	45	35
7	$A^xA^y \times A^1A^2$	66	24	(59)	35	41
8	$B^xB^y \times A^1A^2$	94	99	(100)	1	0

[1] "+" strong discoloration, "-" intermediate, and "o" no discoloration of the medium or hyphae in the contact zone, "±" strong and intermediate totals combined.

[2] combined totals only (+ and -) were recorded for these two experiments.

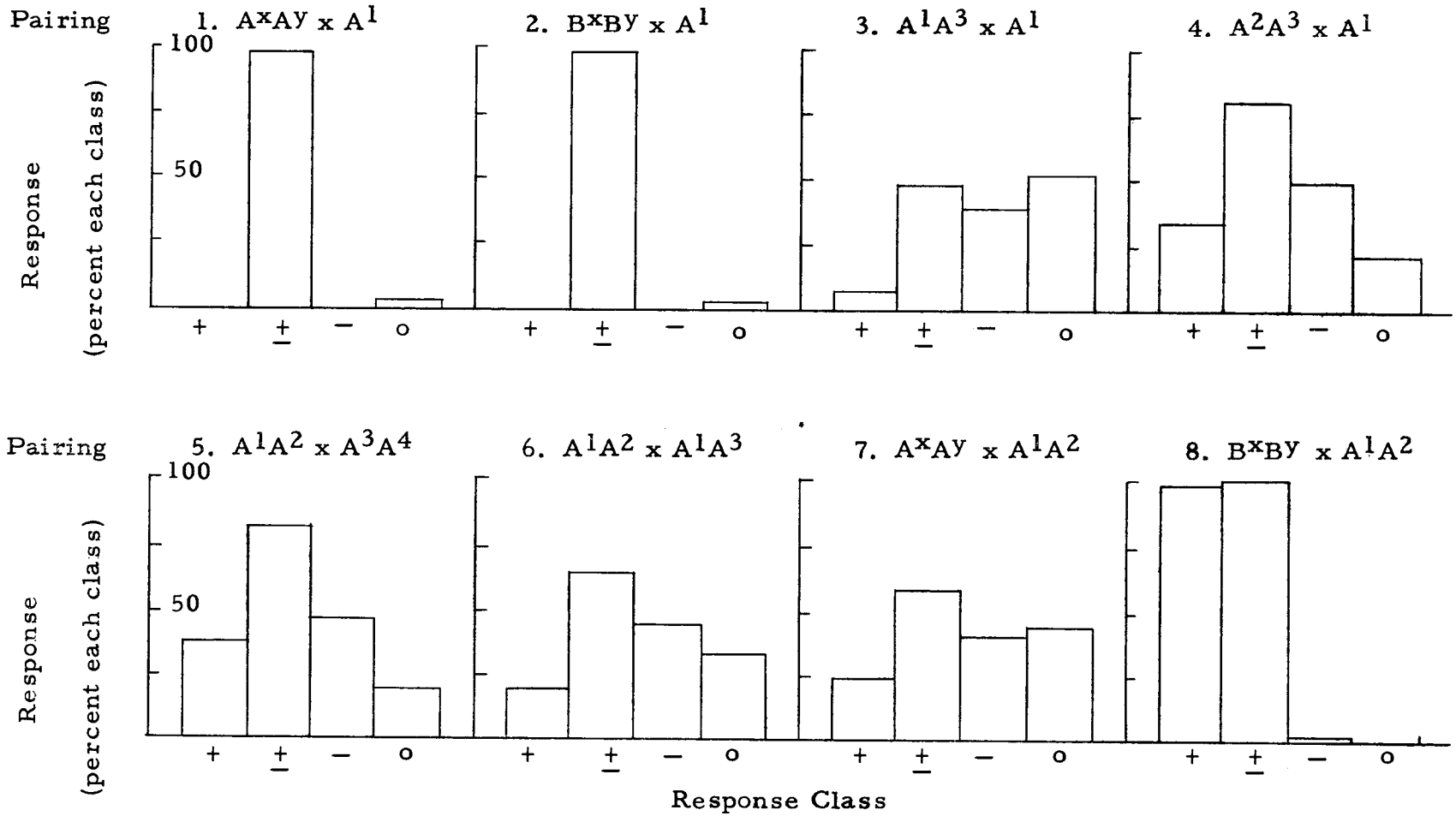

Figure 8. Responses in the formation of a line of demarcation in paired culture as affected by relationship. The symbols "+, ±, −, and o" designate respectively: strong, strong and intermediate totals combined, intermediate, and no discoloration of the medium in the contact zone between paired mycelia.

SEPARATION OF COMPATIBILITY GENOTYPES FOUND OCCURRING NATURALLY IN GLAZE-DAMAGED DOUGLAS-FIR

Four glaze-damaged Douglas-firs infected with F. cajanderi were felled and two inch thick cross-sections of the infected boles were incubated in the laboratory for five months. Mycelia isolated from the mats which covered the exposed heartwood of these sections following incubation, became the basic experimental material for determining the number of fungal compatibility genotypes naturally occurring in individual glaze-damaged, infected trees.

Materials and Methods

The experimental trees were located on the Starker Tree Farm in the Westwood district of Benton County, Oregon. The 70 year old site III stand is located on the north drainage of Greasy Creek and has a southwest aspect. Douglas-fir dominates the stand, only occasionally are western hemlock, Tsuga heterophylla (Raf.) Sarg., and lowland white fir, Abies grandis (Dougl.) Lindl., encountered.

Trees of merchantable size were selected for study. All had tops broken from a glaze storm in 1941 and were classified as codominants and intermediates. Many codominants in this stand maintain this relatively high position because of initial top breakage to all trees in their immediate area and to lack of recurrent breaks up to the time of this study. Trees in the intermediate class generally have had

recurrent breaks since 1941.

Two codominant and two intermediate trees were selected for determination of the identity and distribution of fungal genotypes within their boles. Descriptive statistics of each of the four trees are given in Table 4.

Samples consisted of cross sections, 1.5 to 2.0 inches long, of the upper bole cut from the interwhorles of each tree. Branch whorles were generally avoided because of the difficulties in subsampling at these points. To enable subsequent orientation of the sections upon removal a line was extended along the bole of each felled tree and the sections were notched on their upper surface along this line. Sampling locations were recorded as consecutively numbered points, varying from 1.0 to 2.5 feet apart, along the bole away from the broken top (Table 5). One section, the last, was taken from each tree below the lowest point of visible decay.

Sections were cut from the felled trees with a chain saw, the bark removed with a knife and each section individually placed in a plastic bag. Each bag was closed with a rubber band and the bag marked with identifying numbers denoting the location and orientation of each section.

Within three days after collection, each section was removed from its bag and all exposed sapwood swabbed with 70 percent alcohol to retard possible contamination of the heartwood surface by

Table 4. Descriptive statistics of the four trees analyzed for compatibility genotypes of F. cajanderi comprising the rot columns.

	Tree			
	I	II	III	IV
Crown class (I - intermediate, CD - codominant)	I	I	CD	CD
Tree height (feet)	96	78	109	105
Diameter outside bark at breast height (inches)	12.1	14.2	19.0	19.0
Height of break (feet)	65.0	58.5	76.0	72.0
Length of break (inches)	unkn.	4	10	12
Diameter inside bark at time of break (inches)	4.9	2.7	2.4	2.8
Diameter inside bark at time of falling (inches)	10.1	10.5	11.7	10.0
Length of advanced decay column (feet)	30.1	7.3	11.1	8.1
Extent of incipient decay beyond advanced decay (feet)	----[1/]	1.9	2.7	----[1/]
Total extent of decay (feet)	30.1	9.2	13.8	8.1
Years since break occurred to time of study (1964)	22	22	22	22

[1/] Incipient decay was not visible in Trees I and IV.

Table 5. Location of trunk sections serving as samples of each of four infected Douglas-firs.

Section Number	Tree Number I	II	III	IV
	distance below the break in feet			
1	2.4	0.4	1.5	0.4
2	3.6	2.0	2.3	1.0
3	4.5	2.7	3.0	1.9
4	5.5	3.7	3.7	3.0
5	6.5	4.7	4.6	4.4
6	7.8	5.8	5.3	5.2
7	9.1	7.2	6.3	6.1
8	10.8	8.2	7.3	7.1
9	12.5		8.1	8.1
10	14.3		9.0	9.0
11	16.3		10.0	
12	18.2		11.1	
13	19.9		12.0	
14	22.5		12.8	
15	25.0		13.8	
16	27.5			
17	29.5			
18	31.5			

microorganisms from the surface of the sapwood. One surface of the heartwood was saturated with an atomized spray of sterile distilled water and the sections returned to clean plastic bags. Incubation of the mycelia within the heartwood took place at room temperature with the sections standing on their narrow sides.

Maximum mycelial development on the heartwood surfaces of the sections occurred after five months incubation. At this time free hand sketches were made of each section denoting areas of contrasting mycelial coloration, density of mycelial mat, lines of demarcation where present (compare, Figures 9 and 10), areas of varying degrees of decay, and other features of the heartwood. The limits of the sapwood and heartwood were included in each sketch. These sketches served as a guide during subsequent culturing from each section. There was no apparent difference in surface mycelial growth between the atomized and non-atomized faces of any section. Spraying of one surface apparently left sufficient moisture in the plastic bag to prevent radial cracking and to support fungal growth during incubation of the sections.

Numerous isolations were obtained from the heartwood of each section after incubation. Pairings among these revealed the total number of compatibility genotypes and enabled reconstruction of their lineal development in the bole of each tree. Sampling locations chosen for each section, were based on the previously described

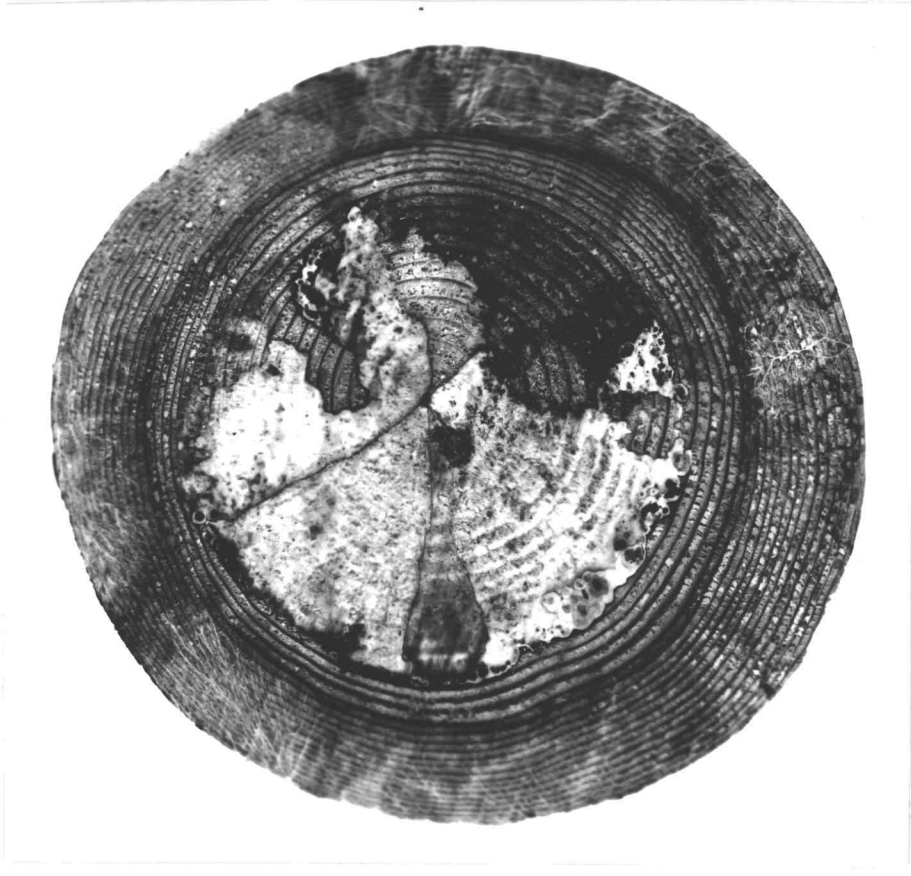

Figure 9. Distinct lines of demarcation formed after five months incubation, in the mycelia covering the surface of the heartwood. Seven individual dikaryotic mycelia were recovered from this section on the basis of the formation of a line of demarcation in paired culture.

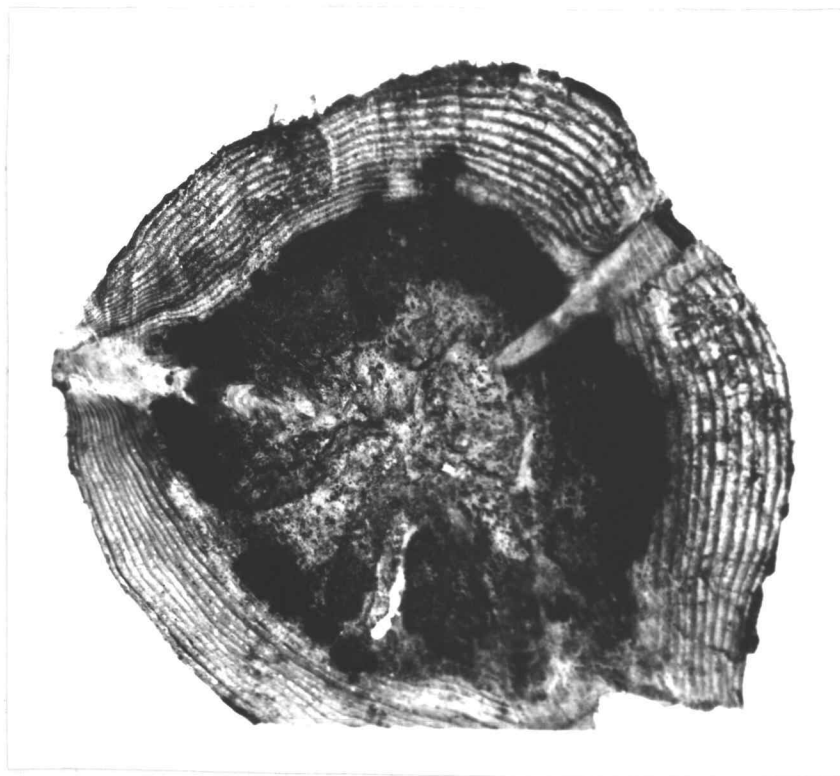

Figure 10. Mycelial growth after five months incubation showing a lack of line of demarcation formation. Seven individual dikaryons were recovered from this section on the basis of the formation of a line demarcation in paired culture. There is little macroscopic distinction between individual dikaryons on this section. Compare with Figure 9. These sections represent two different trees.

characteristics appearing in the sketches.

Samples consisting of wood chips containing mycelia, were taken from beneath the exposed surface of the heartwood and individually cultured on malt extract slants. F. cajanderi was easily recognizable in these cultures by its pinkish mycelium and certain distinctive growth characteristics. Positive isolations were subcultured onto fresh medium and were maintained at 4° C.

Successful isolations from each tree appear in Table 6. Isolations were not obtained from sections 7, 8, 9, 10 and 14 of Tree I due to contamination of the heartwood by fast growing, saprophytic fungi.

Table 6. Number of isolates recovered from all sections of each tree.

Tree Number	Number of sections sampled from each tree	Number of isolates
I	18	60
II	8	31
III	15	121
IV	10	41

Pairings to determine compatibility relationships among the isolates were made on malt-yeast extract medium and incubated at room temperature. In these pairings, three ramets were placed on the medium three-fourths of an inch apart in a triangular pattern.

Pairings were first made among all isolations of each section. After the relations of the dikaryons from each section became clear, pairings were made of isolations from consecutive sections to clarify the vertical relationships among the dikaryons from each tree.

Results

The relationships among the fungi occupying the bole of each of the four trees, as based on the formation of lines of demarcation in paired cultures, are shown in Figure 11.

On the basis of the lack of formation of a line of demarcation in any pairing of isolates from Tree I, this tree is apparently colonized by a single dikaryon as represented in Figure 11. Even so, macroscopic cultural comparisons of the 60 isolates grown as single colonies in Petri dishes, shows some variation in cultural appearance. These variations include differences in coloration of the hyphal mat from white to various shades of pink and to browns, the presence or absence of mycelial exudations, growth rate, and density of aerial mycelium.

Based on the presence of a line of demarcation forming in paired culture, tree II contains three compatibility genotypes. Eight compatibility genotypes are found in Tree III, and four in Tree IV (Figure 11).

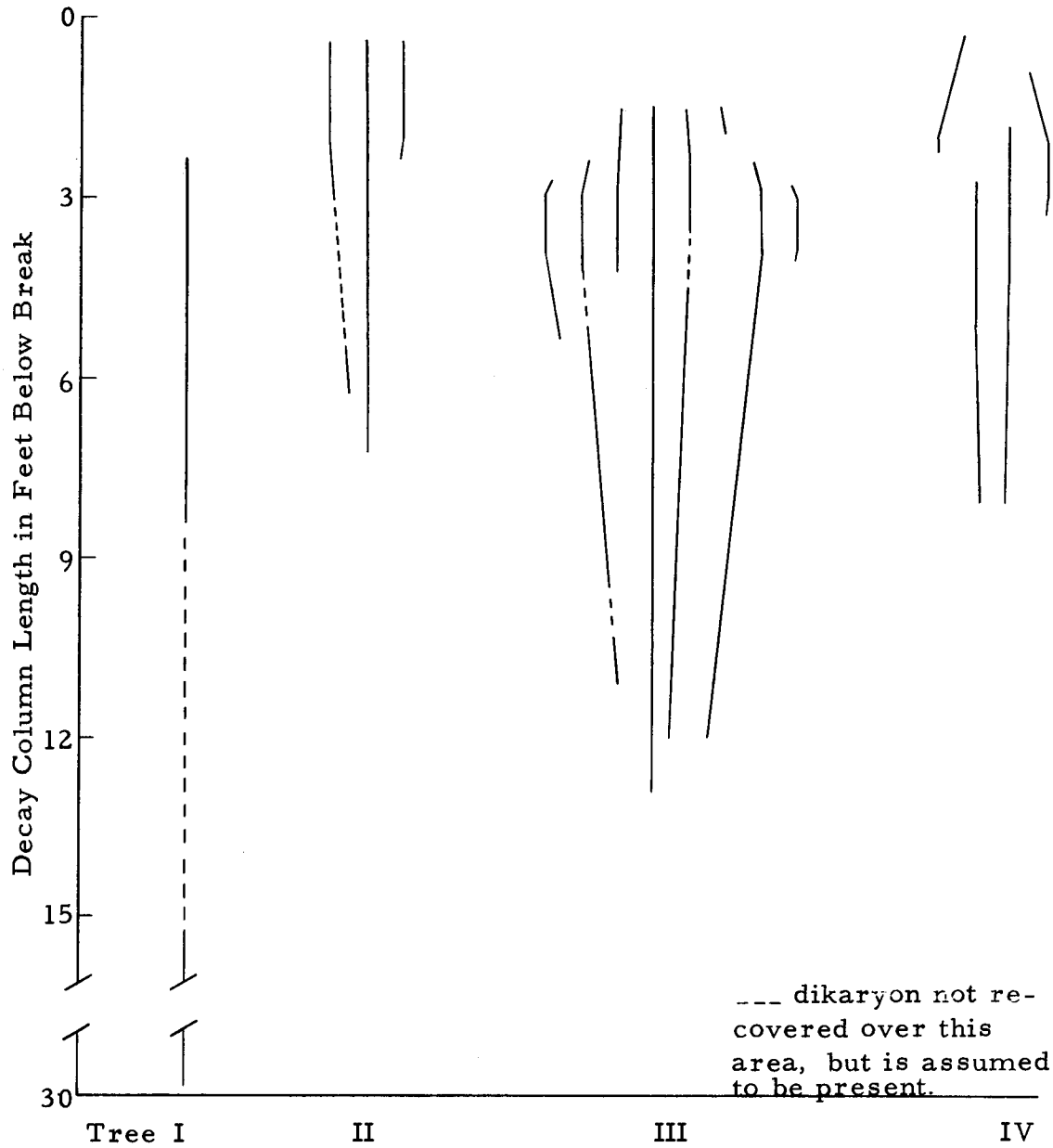

Figure 11. Lineal development of compatibility genotypes of F. cajanderi in four glaze-damaged Douglas firs.

CLARIFICATION OF THE RELATIONSHIP BETWEEN COMPATIBLE, YET CULTURALLY DISSIMILAR APPEARING ISOLATES FROM THE SAME TREE

The literature suggests the concept that cultures of similar appearing mycelia isolated from the same tree, are of the same thallus, while those mycelia of differing appearance are of different thalli. Compatible dikaryotic isolations of F. cajanderi isolated from different locations in the same tree are generally quite similar in their cultural appearance. Occasionally, however, compatible isolates are found to differ in color, growth appearance or other macroscopic characteristics. Compatible cultures numbers 184 and 187, referred to in the first part of the thesis, were isolated from different locations in the same Douglas-fir in the summer of 1963. Their mycelial mats differ in amount and color of the aerial mycelium, and the presence of mycelial exudations from one isolate and not the other. The question arises as to whether or not these isolations are of the same thallus or of two different, but by chance, compatible thalli.

To clarify the relationship existing between the culturally dissimilar yet compatible isolates 184 and 187, ten monokaryons derived from each were cross-paired in culture, and also were paired with ten monokaryons from culture 188, which is noncompatible with either 184 and 187. The results of these 300 pairings, representing

all possible pairing combinations, are given in Figures 12 and 13.

These results show cultures 184 and 187 to be the same, even though their colonies differ in appearance. Pairing of the monokaryons from culture 184 with those of 187 results in the typical bipolar mating pattern of F. cajanderi (Figure 12) as would be expected in pairings between monokaryons originating from the same mycelium. When monokaryons of cultures 184 and 187 were paired with monokaryons of culture 188, the formation of clamp connections in every pairing (Figure 13) indicated the presence of different alleles at the incompatibility locus, and therefore the presence of two compatibility genotypes. There appears therefore to be limitations on the use of cultural appearance as a criterion for distinguishing isolates of the same species.

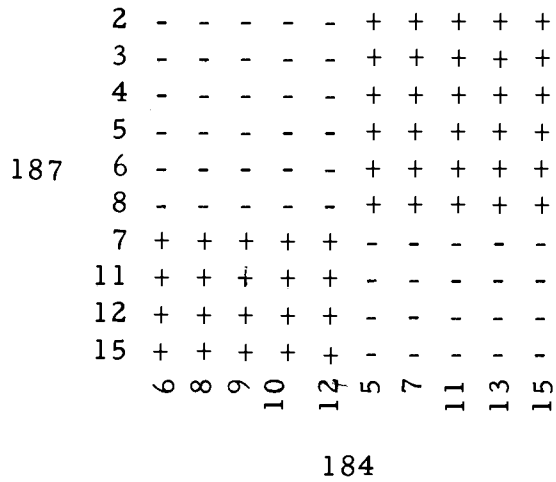

Figure 12. Pairing of monokaryons from cultures 184 and 187 to determine their genetic relationship on the basis of clamp connection formation in paired culture. A typical bipolar mating pattern is found in this pairing.

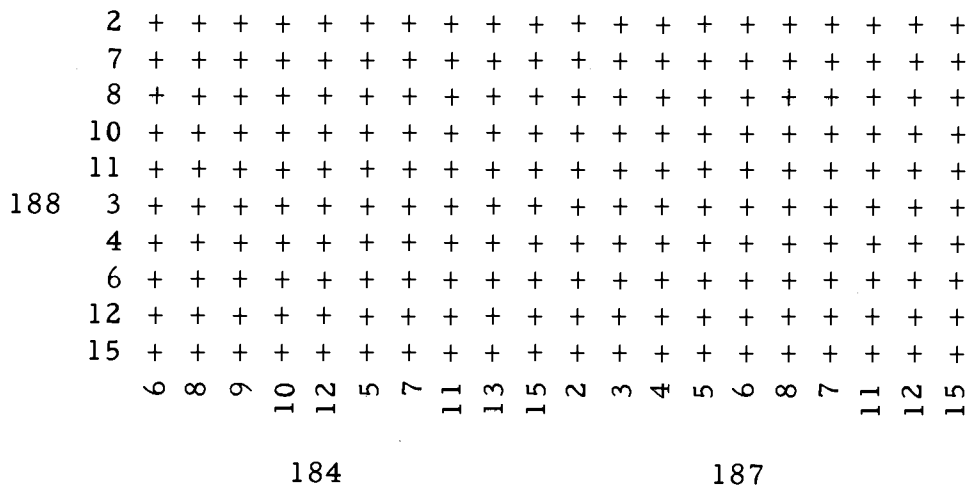

Figure 13. Pairing of monokaryons from cultures 184 and 187 with monokaryons from 188 to determine their genetic relationships on the basis of clamp connection formation in paired culture. Clamp connections were found in all pairings.

INCIDENTAL LABORATORY OBSERVATIONS

During the course of this study several observations were made incidental to the main objectives. These are presented in this section.

Diploidization of a Monokaryon Through Nuclear Migration

The two mating loci (A and B) of tetrapolar basidiomycetes have different functions in dikaryon formation (16, p. 197-198). The A locus controls clamp connection formation, while the B locus controls nuclear migration. Bipolar basidiomycetes are considered to lack nuclear migration during dikaryon formation, and regularly form clamp connections as the dikaryon. In this way, the mating type locus of the bipolar species is considered to be homologous with the A locus of the tetrapolar species due to the presence of clamp connections and lack of nuclear migration during dikaryon formation.

Therefore, the bipolar species having no locus comparable to the B locus of the tetrapolar species are thought to form the dikaryon only in the contact zone between two compatible monokaryons. However, occasionally monokaryons of F. cajanderi in paired culture appeared to become diploidized through nuclear migration.

Clamp connections could be found throughout the mycelium of the "monokaryon" in paired culture, while the stock culture remained without clamp connections during this time. A "dikaryotic sector"

(Figure 6) was present in these pairings as in the more usual situation in which the constituent monokaryons were not diplodized.

The Significance of Zone Line Formation

Zone lines are not common in wood decayed by brown-rot fungi, such as F. cajanderi, while being quite common in wood decayed by white-rot fungi. Zone lines in decayed wood result from the interaction of mycelia of the same or different species in host tissue (20, 40) and show the limits of each colony. Even though zone lines were not plainly visible in the decayed heartwood of the Douglas-fir sections used in this study, the interaction effects between compatibility genotypes were present as shown by the localized development of mycelia on the surfaces of the sections. This growth localization denotes macroscopically the delimitation that each dikaryon had undergone in the stem. Zone line formation by itself, i. e., the formation of a discolored zone along the contact margin of two mycelia in host tissue, appears not to be responsible for the non-intermingling of F. cajanderi dikaryons in Douglas-fir. The competetive mycelia are restricted in their intermingling in the absence of any discoloration of host tissue.

Resistivity of the Inner Sapwood

It can be hypothesized, lacking direct proof, that the presence of the fungus in the dead heartwood stimulates the living sapwood to

produce materials which are fungistatic toward F. cajanderi. The materials probably are not naturally occurring products of the sapwood, because the inhibitory zone is found only on the innermost region of the sapwood immediately adjacent to the heartwood. However, there is the possibility that during the conversion of sapwood to heartwood some organic changes may occur which could result in the production of substances unfavorable to fungal growth. Even so, any restrictive effect on fungal growth that may be present, becomes lost upon formation of heartwood. The actual cause of the inhibition needs further clarification.

Mycelial Bridges

At a particular level in the tree decay of the bole appears to fully occupy the heartwood in the natural state. The heartwood here being distinguished from the sapwood by its dark compared to light color of the sapwood. Direct hyphal penetration of the inner sapwood from mycelia in the heartwood, even after eight months' incubation of the sections, does not appear to occur. The outer sapwood, however, apparently has no special resistance and is attacked on the sections through the formation of "mycelial bridges" over the inner sapwood (Figure 14). The mycelial bridges are firmly attached to the inner sapwood, but do not penetrate deeply.

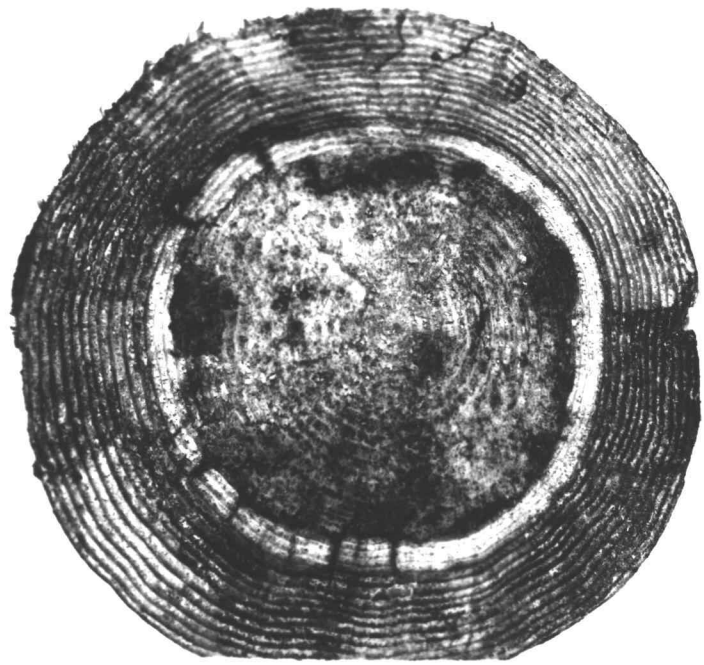

Figure 14. Mycelial bridge formation by F. cajanderi. Mycelial bridges have been formed across a narrow band of apparently decay resistant sapwood adjacent to the heartwood during incubation of the sections. Various secondary organisms inhabiting the outer sapwood are also unable to attack the inner sapwood.

The Effect of Catechol on Fungus Infected Heartwood

An effort was made to find some means of quickly detecting the location of F. cajanderi in infected Douglas-fir wood. A one percent catechol solution sprayed on the surface of the section indicated the location of the fungus (Figure 15). The infected region of the heartwood became darkened, while the uninfected heartwood remained unchanged in color. The reaction is complete in 24 hours at 4^{o} C.

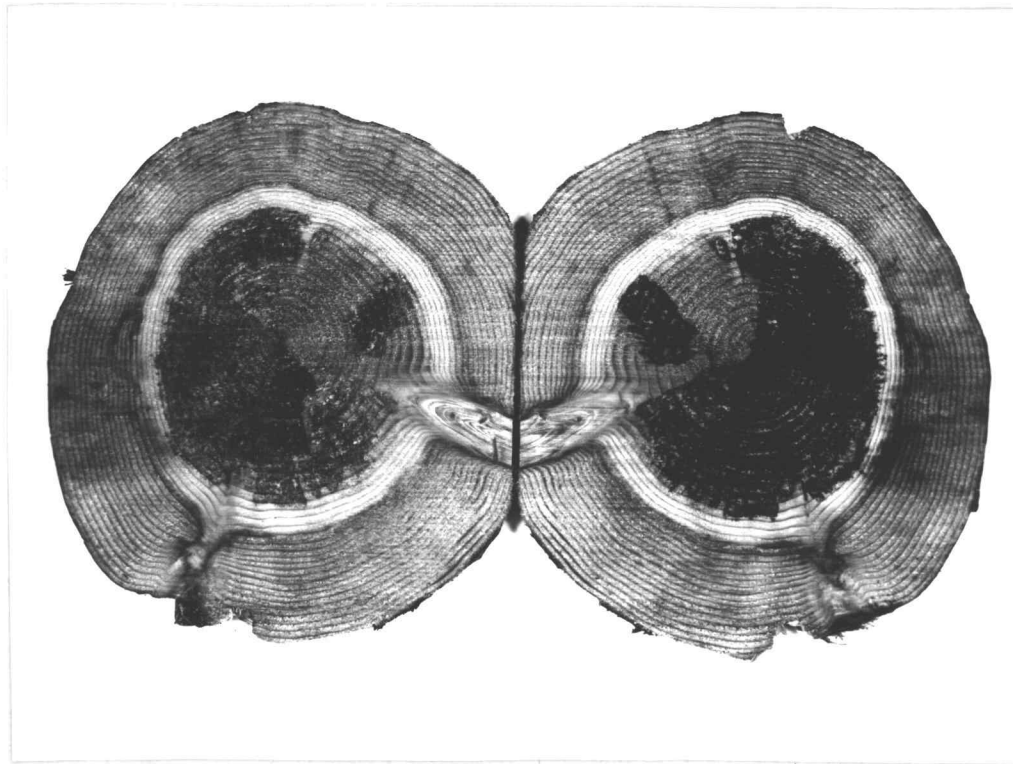

Figure 15. Facing surfaces of a section showing the effect of catechol in distinguishing the heartwood area occupied by F. cajanderi. The surface on the left was sprayed with sterile distilled water and the surface on the right with a one percent solution of catechol in sterile distilled water. The contrasting inner zone (light colored) consists of sapwood adjacent to the heartwood. Discoloration of the outer sapwood results from activity of various secondary fungi during eight months of incubation. The section was opened just prior to the surface treatments.

DISCUSSION

The formation of a line of demarcation in culture has been found to occur to some extent in all the genetic relationships examined, except those in which ramets of the same clone were paired. The degree of discoloration in the contact zone varies with the genetic relationships of the mycelia involved. Comparatively speaking, closely related mycelia show less tendency toward formation of lines of demarcation with less discoloration of the contact zone where lines do form in paired culture than do mycelia of more distant relationship. Dikaryons of F. cajanderi of like genetic origin intermingle freely in culture and supposedly would act similarly in natural substrate.

The formation of the line of demarcation in paired culture was found to be a highly reliable diagnostic character for determining the number and distribution of distinct compatibility genotypes of F. cajanderi inhabiting decay columns of glaze-damaged Douglas-firs. The information presented here support and extend the work of Childs (14, 15), Mounce (23), Verrall (40), and Weir (41), and in so doing indicate the line of demarcation formed in paired culture to have wide application for diagnostic work within the genus Fomes (Fr.) Kickx, and is applicable to one species of Polyporus Mich. ex Fr. and one of Poria Pers. ex S. F. Gray emend. Cke. This is not

to suggest that the formation of a line of demarcation in paired culture of the same species is of universal occurrence even in the genus Fomes. Roth [1/] did not find lines as experienced here in pairings of F. pini (Thore ex Fr.) Karst. from Douglas-fir, but rather he found a mutual aversion of mycelia leaving a clearly defined gap or "barrage" (37) between the ramets. Even though expression of the interaction between colonies may vary the same genetic relationships appear to underlie all.

Interaction within host tissue, between dikaryons possessing dissimilar compatibility genotypes results in a delineating of mycelial growth during colonization of the living tree. This observation was based on the patterns of colony location found in the bole sections of the trees examined (Figure 9). Presumably, similar interactions in paired culture result in the formation of the line of demarcation. It appears this interaction between mycelia functions in host tissue especially to protect each infection dikaryon and its substrate from invasion by other members of the same species.

The number of dikaryons found in any tree probably depends to a large extent upon the type of break and on the frequency and location of dikaryon formation. The location of haploid pairing to form the dikaryon is not known, but it can be presumed to be on or very near

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[1/] Roth, Lewis F., personal communication, March, 1965.

the surface of the exposed heartwood. Decay studies have shown the rate of decay of wood by monokaryons to be somewhat less than that by dikaryons during a comparable growth period for F. igniarius (38). Dikaryon growth in nature could consequently surpass that of the monokaryon and block out further development of the latter.

From the number of individual dikaryons inhabiting the decay column of tree III (Figure 7), it thus appears that more than one dikaryon can readily become established in the exposed heartwood. As decay progresses away from the area of initiation, in those trees in which more than one compatibility genotype was found, a maximum number of individual dikaryons are competing for the substrate. Beyond this point the number of competitors decreases.

Depth of penetration and persistance of individual dikaryons depends on several factors, among them is the rate of growth of the individual and its position in the stem relative to the other competing dikaryons. The data suggest that eventually the decay column will contain but one, or at most, two compatibility genotypes.

Mycelial development through the stem need not follow a strictly vertical descent. In tree V, the four dikaryons which had the greatest linear growth descended the bole in a long spiral, making approximately one revolution of the stem in 11 feet. This effect is probably related to a spiral growth pattern in the wood and does not represent a lateral growth movement of the fungus.

Degrees of decay resistance vary within an individual tree. Under the usual conditions of decay resistance testing, all wood of Douglas-fir is liable to decay by wood-decaying fungi (31). Tests of this kind have been conducted using wood from "healthy trees", i. e., wood not closely associated with wood-decaying fungi under natural conditions. This is not the situation with the wood used for isolation purposes in this study. All wood removed from the trees included a cross-section of the decay column, as well as living sapwood.

The wood immediately adjacent to the fungus infected heartwood, the inner sapwood as it is called in this study, displays high decay resistant properties. This resistance is quite evident upon examination of Figure 14 in which the inner sapwood remains without fungus infestation even after eight months incubation in the presence of F. cajanderi, as well as various saprophytic fungi inhabiting the outer sapwood since the preparation of the section. Studies with "healthy wood", such as that by Scheffer and Englerth (31) have failed to demonstrate this zone of resistant wood probably because it does not exist in the "healthy" tree. If this is the situation, then it is probable that the presence of F. cajanderi is inciting the host to produce a substance possessing fungistatic properties toward the decay organism. This probability is further substantiated by the presence of the readily decayable outer sapwood (Figure 14), the sapwood not closely

associated with the primary decay organism.

The above observation may help to explain why the presence of F. cajanderi does not appear to affect the vigor of the host tree; it simply is unable to penetrate the living tissue.

SUMMARY

The formation of a line of demarcation in paired culture of Fomes cajanderi is of common occurrence. Paired monokaryons from the same parent segregate to form the typical bipolar mating pattern of this species, with the new dikaryon developing as a "sector" between compatible monokaryons. On the basis of a number of experiments, the formation of the line of demarcation in paired culture was concluded to be valid evidence to allow separation of naturally occurring dikaryotic genotypes. Numerous isolates from the boles of four infected Douglas-firs were examined in paired culture. From the presence or absence of the line of demarcation, these trees were found to contain as many as eight distinct dikaryotic compatibility genotypes of F. cajanderi.

Evidence indicates that variations in cultural appearance of isolates from the same decay column are not necessarily indicative of multiple infections. Paired culture tests must be used to establish the relationships involved.

Indirect evidence indicates the possibility of the production of fungistatic substances in response to infection by F. cajanderi.

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