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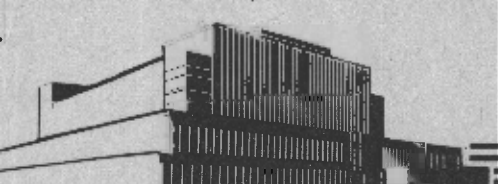

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WOOD-ATTACKING CAPACITIES AND PHYSIOLOGY OF SOFT-ROT FUNGI[1]

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Introduction

Fungal hyphae that grow longitudinally within the walls of wood cells were observed in the latter half of the 19th century by Schacht (24)[3] and others (11, 30), but the fungi responsible for this type of decay remained unknown for many years. The Basidiomycete fungi recognized as the causal agents of decay in wood by Hartig (14) in 1874 were not characterized by their growth within the cell wall. Rather, they penetrated the wall transversely, through pits or by the formation of bore holes, and proliferated in the lumen of the cells. A few of the larger Ascomycetes have also been found to decay wood in a manner similar to that of the Basidiomycetes.

Bailey (3) in 1913 again called attention to fungi that form cavities within the thick, secondary walls of pine. Subsequently, he and Vestal (4) frequently observed these cavities in tracheary cells and fibers during their anatomical comparisons of a wide range of woods from diverse environments. Their observations, published in 1937, characterized these fungi by their ability to grow within the secondary wall, where they

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[3] Underlined numbers in parentheses refer to the literature cited at the end of this report.

enzymatically dissolved the wall substance to form cavities that were oriented either helically around or parallel to the long axis of the cell. The arrangement of the cavities suggested that hydrolysis proceeded along planes determined by the structural orientation of the cellulose. D. H. Linder thought that the responsible fungi might be Pyrenomycetes, or imperfect stages of this group, since he had observed a similar type of attack in maple by a Brachysporium species. A few years later, Barghoorn and Linder (5) showed that wood in marine habitats was attacked in a like manner, and that Fungi Imperfecti and Ascomycetes isolated from the wood were capable of attacking cellulose substrates in pure culture.

Findlay and Savory (12) in 1950 began to investigate this type of decay when they found it was invariably present on the surface of wooden slats over which water flows in industrial cooling towers. A few years later, they demonstrated in laboratory tests (13, 20, 21) that some of the Ascomycetes, notably Chaetomium, and certain Fungi Imperfecti (Trichurus, Bispora, Stysanus, Stemphylium, and Coniothyrium species) isolated from cooling tower slats and wood in contact with the soil were capable, under suitable conditions, of causing decay of a hardwood. Since the surface of the wood, especially where it had been wet for a number of years, was typically softened, the term "soft-rot" was applied to the type of decay produced by fungi that grow within the walls of wood.

A chemical analysis of beech wood decayed by Chaetomium globosum, recently made by Savory and Pinion (23), has further helped to characterize soft-rot. They found that the carbohydrates were virtually depleted while the lignin, although it decreased steadily, was not markedly attacked. In this respect, the wood was similar to that attacked by a brown-rot fungus, and unlike white-rotted wood, in which much of the lignin as well as the carbohydrate is utilized. In respect to its alkaline solubility, however, which indicates the accumulation of primary degradation products formed by enzymatic activity in the progress of decay, the soft-rotted wood was more like white-rotted wood. It showed a slow and steady decrease in solubility, rather than the rapid increase characteristic of brown-rotted wood.

It is increasingly recognized that the type of deterioration known as soft-rot may not only be important in wood under special environmental conditions of extreme wetness or frequent dryness, where Basidiomycete fungi cannot survive, but also may be responsible for many failures of treated wood in the ground. Fungi Imperfecti are often found in decaying

wood, but have been considered harmless casual associates of the wood-destroying Basidiomycetes. Since the work in Britain indicated that Chaetomium and some of the Fungi Imperfecti were capable of causing decay, the present study was made to determine the significance of fungus inhabitants of wood other than Basidiomycetes in certain land environments. Included also were physiological studies of the temperature relationships, oxidase production, relative tolerances to some wood-preserving chemicals, and pH requirements of the fungi.

Isolation and Identification of Soft-Rot Fungi

Isolations of bacteria, actinomycetes, yeasts, and fungi were made from redwood from various parts of cooling towers, and from other woods, mostly pine, in service under miscellaneous environmental conditions. Among the items other than cooling towers from which isolations were made were stakes treated with various preservatives, flooring of railroad cars, piling, boats, and telephone poles. Particular attention was given to wood in which the decay did not appear to be caused by a Basidiomycete. Studies in this report are confined to the fungi, and do not include the actinomycetes, yeasts, or bacteria.

Various types of media and purification techniques were used in making the isolations. Water-agar, nutrient agars based on different carbohydrate and protein sources, with variations in pH and incubation temperatures, were employed. Moist chambers, in which sound wood was placed in contact with infected wood, and the van Tiegham cell were used occasionally. Semi-liquid and liquid media were desirable where bacteria were to be isolated. These various techniques supplied optimum conditions for some of the fungi present but not others, and thereby caused differences in growth rate that aided in obtaining pure cultures. Once isolated, all the fungi grew well on malt agar and on a mineral-vitamin agar covered by filter paper.

More than 100 fungi were isolated. The majority were Fungi Imperfecti, but their taxonomic place within this group was difficult to determine since vegetative stages only were available in most cases.

Certain of the organisma probably represent new species. It appears that most of the Fungi Imperfecti that produced soft-rot in wood under laboratory conditions are in the order Moniliales. These have tentatively been placed in such genera as Acremonium, Alternaria, Bisporomyces, Cephalosporium, Chalaropsis, Cylindrocarpon, Diplococcium, Haplochalara, Helicosporium, Helminthosporium,

Hormiscium, Hymenella, Nematogonium, Phialophora, Pullularia, Sporocybe, Stysanus, Torula, and Trichurus. A few of the Fungi Imperfecti, which were among the most destructive, are in three other orders: Sphaeropsidales, tentatively of the genera Coniothyrium, Cytospora, Cytosporella, and Phoma; Melanconiales, represented by Pestalozzia; and Mycelia Sterilia, represented by Sclerotium. Fusarium, Penicillum, and Trichoderma of the order Moniliales frequently occurred in all isolation attempts, but these isolates have not been found as yet to attack the secondary walls of wood and, therefore, could not be classified as soft-rotters.

Among the isolates that were capable of causing soft-rot, an occasional Ascomycete also was found. There were three species of Chaetomium (C. cochliodes, C. funicolum, and C. globosum), and one species each of Xylaria and Orbicula.

Table 15 summarizes such data for all soft-rot isolates as code designation, tentative identification, wood and wood product from which isolated, and geographic source of host wood. It should be understood that practically all identifications are tentative and subject to change with further study.

Determinations of Soft-Rotting Capacities of Isolates

General Procedure

The isolation work demonstrated that the majority of fungi were capable of growing on a mineral-vitamin agar with filter paper as their carbon source. Any true assessment of the destructive capacity of the fungi from a practical standpoint was therefore considered to be the ability of the fungus to attack cellulose as it occurs with lignin in wood. This ability was ascertained by means of eight tests in which various techniques were used. The test variables were incorporated in an attempt to provide some environment for each fungus that would bring out its rot-producing potential. Moreover, the variables were intended to provide some information about certain special nutritional requirements or aversions to wood extractives that the soft-rot fungi as a group seemed to have. The principal features of the different tests are shown in table 16. This outline description of the tests permits a quick review of the information about any particular test.

The test specimens were cut from either the sapwood of sweetgum (Liquidambar styraciflua), American beech (Fagus grandiflora), ponderosa pine (Pinus ponderosa), and southern pine (any or all of four species: shortleaf (P. echinata), longleaf (P. palustris), loblolly (P. taeda) or slash (P. elliotii), or the heartwood of redwood (Sequoia sempervirens). The specimens were of two forms: blocks 0.75 to 1 inch wide, 0.25 to 0.375 inch thick, and 1 to 2 inches long in the grain direction, and rotary-cut veneer strips 0.0625 inch thick, 0.5 inch wide, and 3 inches long.

The specimens either were placed on the fungus to be appraised without any preliminary treatment, or were leached first in running tap water or in an alkaline, acid, or chlorinated distilled-water solution. Also, some specimens were impregnated with a mineral-vitamin solution. The test substrate was either soil (potting or garden loam to which compost had been added), or a mineral-vitamin agar or solution. The use of soil as a test substrate has been described in ASTM D-1413, 1956 (1). The technique used in this study, however, was sometimes considerably modified as to type of soil and its moisture content.

The inoculum was prepared by covering an agar-slant culture with sterile distilled water, and scraping the mycelium loose. The liquid containing the mycelium was then transferred to a sterile tissue grinder, where the mycelium was broken into small fragments and further diluted by placing it into 10 to 50 milliliters (depending on the amount of mycelium) of sterile distilled water. The mycelial fragments, and the spores if present, were then streaked with a loop or pipette over the surface of filter paper that covered the agar or soil in the test chamber. With this type of inoculation, growth generally was much more rapid and uniform than when the inoculum was placed at one point.

A few of the same fungi were involved in all the tests; however, as it became apparent that an isolate was capable of causing wood deterioration, new ones usually were substituted.

Certain Basidiomycete fungi occasionally were included in the study, so that the effects of the different test variables on them and the soft-rot isolates could be directly compared. Four of these were: the white-rot, Polyporus versicolor (697), and the brown-rots Lentinus lepideus (534), Lenzites trabea (617), and Poria monticola (698), which are economically important Basidiomycete wood-destroyers but not associated

with cooling towers. Ten additional Basidiomycetes, isolated from redwood in cooling towers, included were the white-rots Poria nigrescens (4856, 4963) and Peniophora mollis (ML 21, ML 22 ML 26); and the brown rots Poria oleraceae (4907, ML 27), and unidentified species (ML 19, ML 23, ML 29).

It is apparent that the high moisture content of redwood is an excluding factor to the general presence of Basidiomycete attack in cooling towers. Such attack is generally limited to the heavier structural members, which usually are not wet so continuously in service as are slats from which most of the soft-rot fungi were isolated. The white-rot fungi have occasionally been isolated from fill slats, however, thereby exhibiting a capacity somewhat like that of the soft-rot fungi to tolerate high moistures. In contrast to the soft-rot type of decay, white-rot or brown-rot in cooling towers is interior, and leaves the surface of the redwood relatively sound. A brown, almost charred, crumbly appearance differentiates brown-rot from the somewhat bleached, fibrous to pocket-type white-rot. Figure 1 illustrates samples of cooling tower wood attacked by these Basidiomycete fungi.

Criteria of Attack on Wood

The destructive capacity of the fungi on wood was appraised in four ways: loss in weight, loss of bending tolerance, effect on microscopical structure, and macroscopic evidence of deterioration of the wood.

Loss in weight of inoculated wood is the conventional measure of attack by Basidiomycete fungi. Where weight losses are of sufficient magnitude to be measured reliably, they furnish the simplest means of observing the influence of environmental factors on the activity of a fungus. The weight loss was based on the weight of the oven-dry test specimen before and after it was tested. Controls in uninoculated bottles indicated any loss in weight not attributable to decay. When the weight loss was less then 5 percent, the presence of decay was verified microscopically.

The destructive capacity indicated by loss in bending tolerance was determined with inoculated veneer strips on a step-type series of mandrels. These mandrels, constructed of wood, were designed at the Forest Products Laboratory for determining the relationship of radius of curvature at breakage to slope of grain through sheets of veneer (26).

The specimens of veneer were bent around mandrels of decreasing radius until definite evidence of fracture was obtained. The radius of curvature that preceded the one that caused failure was reported as the breaking radius. This radius was usually determined for two portions of each of three specimens, and the results were averaged. The specimens were bent with the fiber direction at an angle of 90 degrees to the axis of curvature, and with the "tight" side of the veneer toward the mandrel. To insure a reasonably accurate evaluation of the breaking radius, the veneer was pressed as firmly against the mandrel as possible over the entire length of the band, which usually was not greater than about 1.5 inches. Each step, moreover, was started on a radius of curvature several steps larger than the breaking radius. This was done so that all the specimens would be subjected to about the same amount of flexing before they fractured.

When the test was made to determine the correlation of the bending radius to weight loss, every 3 inoculated specimens were represented by one uninoculated specimen. All 4 specimens were cut from the same 1.5 by 6 inch area of the veneer sheet. Any increase in the bending radius of the inoculated specimens over that of the uninoculated meant a decrease in bending capacity.

Microscopical examination could not be used to assess closely the amount of decay in wood, but usually disclosed whether the cell walls had been attacked in the manner described by Bailey (4) and later by Findlay and Savory (13). In this type of deterioration, known as soft-rot, the hyphae ramify within the cell wall, and make tunnels that run longitudinally and follow the cellulose fibrils (fig. 2). In polarized light (2), the hyphae can be seen to lie within cavities with pointed ends. These cavities generally are confined to the less lignified secondary walls of tracheary cells and fibers, and are more conspicuous in the summerwood than in the springwood, especially in softwoods. As seen in longitudinal sections, the hyphae appear as spirals in softwoods, or lie parallel to the long axis of the vessels in hardwoods. In cross sections, the cavities appear as holes that equal or exceed the diameter of the hyphae (fig. 3). As the microscopical examinations were made, the possibility was not overlooked that some of the fungi might penetrate the cell wall by means of bore holes, proliferate in the cell lumen, and cause a general thinning of the wall, in a manner similar to that of the Basidiomycetes. An interesting variation in microscopic characteristics was the similarity to soft-rot attack of a Basidiomycete tentatively identified as Poria nigrescens (fig. 4).

The macroscopic features of infected wood were considered only as possible supplementary evidences of attack by the soft-rot fungi. In nature, the surfaces of wood submerged in water or subjected to excessively wet conditions for a number of years become darkened and softened if attacked by soft-rot. When still wet, the softened surface can easily be scraped away to reveal relatively sound wood. When the wood is dried, small cracks develop across the grain in the darkened surfaces, and give an appearance similar to that of lightly charred wood. In the present tests, unleached wood attacked by the soft-rot fungi usually retained its shape, and the only outward evidence of decay was discoloration and brashness. Figure 5 illustrates different degrees of soft-rot on wood removed from cooling towers. Figures 6 and 7 show soft-rot development on various types of products to illustrate its wide occurrence.

Results of Tests of Soft-Rotting Capacity

The results of the respective tests are reported by test number in order to identify them with the descriptions of procedure given in table 16. The variation in techniques among the tests represented progressive attempts to find more rapid or definitive means of appraising the fungi. Thus the findings have significance for the methodology of growing the fungi as well as for ascertaining their wood-attacking potentials.

Test 1. --Test 1 was based on the standard soil-block technique (ASTM D-1413, 1956 (1)), modified by the use of a thinner test block placed directly on the soil. The modification employed caused the block to absorb more moisture than when a feeder piece of wood is placed between the block and the soil. Also, the incubation period was increased from 12 to 24 weeks. Twenty-eight isolates, including two species of Chaetomium and three Fungi Imperfecti commonly isolated from cooling towers, were used in the tests.

The results (table 1) indicated that the testing technique was unsuccessful in producing attack of the test woods by soft-rot fungi, even where the wood was leached with 0.2 percent sodium carbonate. Although leaching of redwood, especially with sodium carbonate solution, greatly facilitated attack by the white-rot Basidiomycete fungi Poria nigrescens, Peniophora mollis, and Polyporus versicolor, it had no apparent effect on the wood-attacking ability of the brown-rot fungi, Poria oleraceae, P. monticola, and an unidentified species.

Test 2. --In test 2, four further modifications of the standard soil-block technique were made in an attempt to increase the moisture content of the test specimen: (1) a thinner test specimen was used, (2) the soil moisture was increased from 130 to 175 percent of the water-holding capacity of the soil, (3) the bottle was used horizontally rather than in an upright position, so as to allow more of the wood specimen to come in contact with the soil, and (4) wood impregnated with a mineral solution was used.

The results (table 2) indicate that, with the soil-block technique as modified here, all four non-Basidiomycete fungi caused substantial deterioration in sweetgum, and generally in pine also. Decay of redwood was not clearly evident, however, except where the wood was leached in sodium hypochlorite. The addition of minerals to the wood practically always increased the amount of decay. Fungus hyphae were present in the secondary walls of tracheids and vessels when the weight loss was approximately 2 percent or greater. When the weight loss was less, hyphae were present in the cell lumen but not in the secondary walls.

Test 3. --The procedure in test 3 was in most respects like that of test 2, except that 2 percent of peptone was incorporated in the soil water, and the incubation period was shortened by 4 weeks. The aim was to determine (1) whether the modified soil-block technique that proved promising for a few fungi in test 2 would be successful with nearly 60 other isolates, and (2) the extent to which the minimum bending radius was correlated with weight loss as a criterion of attack by the fungi.

The addition of peptone to the soil tended to increase the amount of attack, just as the addition of minerals to the test specimen did in test 2. This was true at least for the three fungi used in both tests. The results (fig. 8 and table 3) indicate that two-thirds of the isolates caused varying amounts of both weight loss and reduction in bending radius in the sweetgum sapwood veneer strips in 8 weeks.

The detailed data in table 3 show the high degree of reproducibility for replicates and the close correlation for the weight loss and bending radius results that were obtained. Weight loss and bending radius were particularly well correlated in the range of radii between about 1.5 and 3.25 inches, which corresponds to weight losses between 0 and about 20 percent. Thus it appears that either measure could be used to appraise

relative amounts of attack by soft-rot fungi. Use of the bending radius has the advantage of not requiring weight determinations, but demands particular care that the initial bending radius of test strips is uniform.

Test 4. --Test 4 was designed to ascertain whether an agar substrate fortified with minerals and vitamins (footnote 1, table 16) would increase the attack in sweetgum, beech, and pine veneer strips over that obtainable on soil. A direct comparison was made with the soil substrate used in test 2 but enriched with manure. Twenty-five isolates were tested (2 Ascomycetes and 23 Fungi Imperfecti), all but 4 of which had been isolated from redwood in cooling towers.

The results (table 4) indicate that, in general, (1) more decay almost always occurred in veneer strips on the agar substrate than on the soil, in many cases considerably more, and (2) decay in gum and beech was similar for the majority of fungi. Leaching the pine in sodium hypochlorite before it was tested resulted in small to moderate increases in the ability of the fungi to attack this wood.

The greater decay on mineral-vitamin agar than on soil in spite of its enrichment is not readily explainable without more knowledge about the nutritional requirements of the fungi. Since the wood strips were more uniformly wet on agar than on soil, it is possible that differences in moisture content as well as nutrients might have been a contributing factor. Observations in nature indicate that the greatest amount of soft-rot occurs in very wet wood.

Test 5. --The aim of test 5 was to determine the ability of the fungi to decay small blocks of wood in contrast to the thin veneer tested previously by the modified soil-block technique. The test woods, sweetgum and southern pine sapwood and redwood heartwood, were subjected to variables of leaching, with and without sodium hypochlorite in the leach water. A portion of each group of blocks was impregnated with mineral vitamin solution (footnote 1, table 16).

Ninety-nine isolates, representing all those collected at the time, were appraised. The large number of isolates tested, coupled with overall good development of the fungi, made this test particularly significant in comparing the wood-attacking capacity of individual isolates and for estimating the general prevalence and diversity of forms of these fungi that are capable of causing soft-rot.

The results (table 5) indicate that all of the fungi were able to attack the sweetgum blocks. Weight losses varied between 2 and 58 percent. At least three-fourths of the isolates caused weight losses in excess of 10 percent. The presence of minerals and vitamins in the wood tended to promote decay in the majority of cases.

Only about one-half of the isolates attacked the pine sapwood, and all of these attacked blocks showed less decay than did gum sapwood. A certain amount of hyphal penetration of wood cells occurred, however, even though no weight losses were obtained. This was determined by microscopical examination. Leaching in water alone or the addition of minerals and vitamins generally did not greatly increase the amount of attack. However, approximately 40 percent of the fungi that could not attack the normal pine caused small weight losses after the wood was leached in running water.

None of the fungi was able to decay redwood heartwood unless it had been leached in sodium hypochlorite solution. Again, however, there was some hyphal penetration into the redwood. Attack by all of the fungi took place on the hypochlorite-leached redwood; weight losses varied from 1 to 7 percent. A loss of only 1 percent was microscopically verified as due to attack of secondary walls of tracheids in the summerwood, which is typical of soft-rot. The absence of attack on blocks that had been leached in water was considered surprising, since the leaching period was long enough (30 days) to extract significant amounts of fungus-inhibiting components from the wood.

In regard to the Basidiomycetes, comparisons between the results of test 5 and test 1 are of interest, since some of the same isolates were used. The essential differences in the conditions provided by this test were the increased soil moisture, which provided a wetter test block, and a longer test period (36 rather than 24 weeks). Despite the longer test period, decay by the brown-rot fungi (P. oleraceae and unidentified species) was considerably less in gum, pine, and redwood; decay by the white-rots (P. nigrescens isolates), however, was more. Also, many of the soft-rot isolates in test 5 produced as much decay in gum as did the brown-rot fungi.

Since the conditions were so unfavorable for decay by the brown-rot isolates, the addition of minerals or the leaching of pine and redwood generally showed little effect; in fact, the presence of minerals actually seemed to decrease decay. On the other hand, minerals tended to increase decay by the white rotters, as did the leaching of pine and redwood. The presence of chlorine in the leach water further increased the attack of redwood by the white-rot fungi.

Test 6. --Previous tests indicated an apparent inability of the isolates to attack redwood heartwood in the laboratory unless it had been leached in chlorinated water. Chlorine leaching obviously is not necessary for soft-rot fungi in nature, however, since it is now known that soft-rot occurs after various periods of time in many softwoods not exposed to chlorine.

Since soft-rot seems to be especially prevalent in woods exposed to wet soil, test 6 was established to determine the rotting potentials of the isolates when brought into contact with wood covered on all surfaces by a soil medium. This was done by burying unleached redwood blocks in soil made up as described for the soil-block exposures of test 2. Six blocks in each case were exposed for 24 months in soil inoculated with 24 different isolates.

The results (table 6) indicate that a small amount of decay, up to 6 percent, occurred in all cases. This general level of decay is similar to that obtained by the soil-block exposure of redwood blocks that were leached in chlorinated water (test 5). Since the incubation period for the present test was twice as long, the two results are not strictly comparable. There is strong evidence, nevertheless, that attack of redwood by soft-rot isolates had been promoted by long-time contact with wet soil.

Test 7. --Limited tests were made to determine whether a richer soil than that used in test 6 for soil burial might have led to even greater attack by soft-rot fungi. Veneer strips of sweetgum, beech, and pine were partially buried in a garden loam soil and in a potting soil with added compost. Fourteen isolates from redwood and pine sources were tested in these soils during an incubation period of 8 weeks.

The results (table 7) indicate that slightly greater attack of the wood occurred in the composted potting soil than in the garden loam. In the case of one fungus (P 11), the decay was substantially greater in the richer potting soil.

Test 8. --This final test was made to determine whether a shake-culture technique was suitable for producing fairly rapid attack by the fungi on gum sapwood. Twenty isolates, including two prominent wood-destroying Basidiomycetes, were compared during incubation periods of 2 and 4 weeks. In establishing the test, a 1- by 3- by 0.0625-inch strip of sweetgum veneer was placed in an Erlenmeyer flask containing a mineral-vitamin solution. The strip was placed on end so that one-half its length

was exposed above the solution. As the flask was shaken, however, the mineral solution washed over the uncovered portion.

Growth of the fungi was rapid, and the veneer strips were covered with a hyphal slime in three days. The weight losses (table 8) indicate that substantial decay by the soft-rot fungi occurred in 2 weeks. No weight loss was indicated for the two Basidiomycetes, however, which is in line with the apparent special ability of soft-rot fungi to grow in unusually damp situations.

Trials also were made on unleached pine and redwood, but no attack of these woods occurred. Surface growth of the fungi was just as rapid initially as in the case of the sweetgum, but it ceased after a few days.

It appears that the shake-culture technique has promise for rapid appraisals of soft-rot fungi on a susceptible hardwood such as sweetgum sapwood, but not on coniferous woods.

Conclusions on Soft-Rotting Capacities of Isolates

The foregoing tests indicated that a large percentage of the Ascomycetes and Fungi Imperfecti isolated from typically soft-rotted wood were capable of causing substantial decay in sweetgum sapwood under laboratory conditions. Except when the wood was buried in soil, only about 50 percent of the isolates were able to attack normal pine, and none decayed normal redwood heartwood. All of the isolates attacked leached pine and redwood buried in wet soil for a long time. Figure 9 illustrates degrees of attack on different test blocks.

Several factors contributed to an increase in soft-rot attack:

(1) Minerals and vitamins, applied either to the wood or in the agar substrate, or additional organic matter in the soil, generally increased soft-rot attack.

(2) Leaching of the wood in water alone led to a somewhat greater attack of pine sapwood but not of redwood. The presence of chlorine in the leach water, however, increased the susceptibility of both pine and redwood to soft-rot attack. On the basis of these results, it is apparent that the presence of chlorine in the water of redwood cooling towers would accelerate attack of the wood by the soft-rot fungi.

Possible explanations for such accelerated attack are that chlorine contributes to the removal of toxic extractives or changes the lignocellulose complex in wood to make it more susceptible. Whether absence of chlorine would largely or entirely eliminate the soft-rot problem in cooling towers is not known. The slow attack of redwood buried in wet soil in these tests indicates that at least gradual wood attack by soft-rot organisms might occur in the absence of chlorine.

(3) Very moist wood obtained by placing a large portion of the surface of test blocks directly on or in wet soil, or on a vitamin-mineral agar, was subject to more rapid decay.

(4) A mineral-vitamin agar provided a more favorable condition for soft-rotting than even wet woil, probably because it provided both better moisture conditions in the test block and ingredients essential to cellulytic activity.

The soil-block culture technique used so successfully with the Basidiomycete fungi was not a satisfactory procedure for testing soft-rot isolates. This failure was attributed to the drier soil and the use of a "feeder" strip of wood between the test wood and the soil, both of which reduced the wetness of the sample to be tested.

The shake-culture technique appeared to have promise for the rapid appraisal of soft-rot potentials of fungi on a susceptible hardwood, but not on coniferous woods.

Either loss in weight or decreased bending tolerance (determined on mandrels) of infected wood appeared to be suitable as a measure of the relative amount of attack by the soft-rot fungi. Since the use of bending radii necessitates particular care in the selection of test material, however, weight determinations generally seemed preferable.

In appraising a potential soft-rotter, microscopical examination of the wood proved to be a necessary supplement to the other observations, especially in cases where there was little weight loss.

Based on tentative identifications (table 15) and the results of eight tests, fungi of the following types were especially able to attack both hardwood and softwood: Sporocybe, Acremonium, Phialophora, Cytosporella, Bisporomyces, Chalaropsis, Cephalosporium, and Pestalozzia. Most of these were among the most frequently isolated fungi from cooling tower wood. Chaetomium species were only occasionally isolated from cooling

towers, but were obtained frequently from wood in contact with the soil. While Chaetomium species caused losses in weight in these tests, such losses often were less than those caused by most of the fungi just mentioned.

Physiological Characteristics of the Isolates

Several preliminary studies were made to determine the general nature of the soft-rot isolates with respect to commonly investigated physiological characteristics. Also, information was wanted on whether physiological differences exist between selected Basidiomycete wood destroyers and fungi believed to represent fairly well the morphological range of the isolates that cause soft-rot. These are mostly of the Fungi Imperfecti type. It was hoped that the information obtained could be applied immediately in improving techniques to induce soft rot in the laboratory, and perhaps later in providing a basis for broader physiological and control studies. The four preliminary studies were directed at (1) temperature relations, (2) tolerances to toxic materials, (3) pH relations, and (4) oxidase activities of the isolates.

Temperature Relations

General. --Temperature is one of the external factors that influences almost every function involved in the growth of fungi. For each fungus there is a minimum and a maximum temperature, below and above which, respectively, no growth occurs. A characteristic growth curve increases linearly with increasing temperature to the optimum, which may be narrow or broad, and then descends with further increases in temperature. A compilation of data on the optimum temperatures of wood-destroying Basidiomycetes has been made by Humphrey and Siggers (15), Wolf and Wolf (31), and Cochrane (9). These indicate that the optimum for the majority of fungi lies between 25° and 30° C., but a number of fungi, sometimes in ecological groups, have an optimum that is substantially lower or higher than the average.

The growth rate varies among different species within a genus and also among strains and geographical isolates of the same species. Moreover, growth rate is often influenced by a number of factors such as the amount of carbon, nitrogen, and growth substances, and pH of the media. The relative rate may also differ according to whether linear growth or

weight of mycelium has been used in the determination. All metabolic processes do not necessarily respond to temperature differences in the same way as growth; for example, the optimum for growth may not necessarily be the optimum for decay. It is believed, however, that for the Basidiomycetes the temperature most favorable for mycelial growth is likewise conducive to rapid decay (7, 16, 27).

Gross differences found in a temperature study can provide in addition to a physiological basis for comparing fungi, a useful basis for ecological studies and for selecting suitable temperatures for handling the fungi in the laboratory. In the present study, it was important to know whether the incubation temperatures used were near the optimum for the fungi. Also, it was of special interest to know whether those fungi commonly isolated from cooling towers might not comprise an ecological group with a rather high optimum, and those from the soil a significantly lower optimum.

Methods. -- The temperature relations of the isolates were determined by the linear rates of growth of the isolates on malt agar in modified test tubes (25). The tubes were 20 centimeters long and 2 centimeters in diameter at the mouth. They had been modified with a deep indentation of the wall on one side near the mouth. With the indentation kept on the lower side of the tube to prevent the escape of liquid, the sterilized malt agar was cooled and solidified with the tube in a horizontal position. This resulted in a uniform, narrow strip of substrate along one side of the tube, approximately 15 centimeters long, 2 centimeters wide, and about 1 centimeter deep down the middle.

The malt-agar medium contained 2 percent each of Difco malt and agar in distilled water, with the pH adjusted to 6 before sterilization. Fifteen cubic centimeters of the melted malt agar were put into each test tube, which was then plugged firmly with cotton and autoclaved at 15 pounds pressure (121° C.) for 15 minutes.

The inoculum (approximately a 3-millimeter cube) was cut with a two-pronged blade from the growing margin of a 2-week-old petri-dish culture. It was placed on the surface of the agar at the forward end of the strip so that the ensuing mycelial mat extended linearly to the closed end of the tube.

The inoculated tubes were placed in incubators on racks that held them vertically, with the rounded ends uppermost. The agar strip remained firmly in place if it was allowed to harden in the horizontal tube for 24

hours without being disturbed. The incubator temperatures were 10°, 16°, 22°, 28°, 32°, 38°, 42°, and 46°, ±1° C. The constancy of these temperatures in all parts of an incubator was confirmed by thermocouple readings and by the agreement between measurements on replicate isolates in various positions.

Growth of the fungi was measured to 1 millimeter by viewing, with good illumination, the margin of mycelium through the substrate. The position of the advancing margin was marked each time on a narrow ground-glass strip made on the same side of the tube as the indentation. The growth was thus recorded every 2 days, and 6 such measurements were made. The testing was done in duplicate, so that 12 measurements generally were available for each isolate. The average daily growth rate was computed from these measurements. Fewer than 12 readings were used if there was any indication that growth had definitely slowed during the recording period.

Among the 40 fungi studied were eight Basidiomycetes: Lenzites trabea (617), Poria monticola (698), and Polyporus versicolor (697), (all common test fungi, with the previously determined temperature optimum of the first found to be above, and that of the other two similar to, the majority of Basidiomycetes), Poria nigrescens (4856), P. oleraceae (4907), Peniophora mollis (ML 26), and unknown brown rotters (ML 23 and 29), all isolated from redwood cooling towers. The isolates L 2 and R 49A were Chaetomium species, and the remaining were Fungi Imperfecti. Twenty-two of the soft-rot isolates were from redwood cooling towers and 10 were from wood in contact with soil.

Results. --The linear daily growth rates of the fungi at the different temperatures are shown in table 9. Growth curves plotted from the daily growth rates of four isolates with different optima are shown in figure 10.

Twelve percent of the soft-rot isolates showed a temperature optimum at 22° C.; 41 percent at 28° C.; 41 percent at 34° C.; and 6 percent at 38° C. From these data and those gathered by Humphrey and Siggers (15) for 64 Basidiomycete fungi, it is apparent that the temperature optima tend to be considerably higher among the soft-rot fungi than among the wood-destroying Basidiomycetes. Only 12 percent of the soft-rotters, as compared with nearly twice this number among the Basidiomycetes, had optima of 26° C. or below, while 47 percent of the soft-rotters and only 16 percent of the Basidiomycetes had optima of 34° and above. The soft-rot fungi that produced most decay in these tests had high optima.

The temperature study also indicated a relationship between the optimum and maximum temperature, and between the optimum and the temperature range for growth above the optimum (table 10 and 11). For the majority of fungi, the higher the optimum temperature the higher the maximum and also the shorter the range of temperatures that permit growth above the optimum. A comparable relation between optimum and maximum, but not for the range between optimum and maximum points, is indicated for the Basidiomycetes (15).

Four of the five Basidiomycetes isolated from redwood in cooling towers in the present study had an optimum of 34° C. or above, and thus higher than that of the majority of Basidiomycetes. Of particular interest is Peniophora mollis (ML 26), with an optimum of 38° C. This Basidiomycete showed a high daily growth rate at 22° through 42° C., and its maximum temperature for growth, although not determined, was indicated to be more than 8° C. above its optimum.

Among the soft-rot isolates, there also were many more from cooling towers than from soil that had relatively high temperature tolerances. Chaetomium species, isolated more from soil than cooling towers, had the lowest temperature optima. Because of the low optima for Chaetomium, it is probable that their ability to produce decay was not fully evaluated in the decay tests reported earlier in which incubation temperatures, higher than the optimum for Chaetomium species, were used.

The temperature data indicate, therefore, that a great many of the fungi found in cooling towers (Basidiomycete as well as soft-rot isolates) represent an ecological group with a rather high temperature optimum (34° C. or more) for growth. This may be significant in view of the higher temperatures of water that passes through cooling towers. For maximum decay by this high-temperature group, therefore, the incubation temperature commonly used in decay studies with Basidiomycetes (28° C.) may be too low.

Oxidase Production

General. --Bavendamm (6) observed in 1928 that Basidiomycetes known to cause white-rots produced a brown diffusion zone in media that contain gallic or tannic acids. This brown zone was attributed to the oxidation of the acids. Basidiomycetes associated with brown-rots did not produce this reaction. This oxidase test, sometimes known as the Bavendamm

test, when combined with numerous macroscopic and microscopic characters, has been a valuable cultural tool in fungus identification, as shown especially by Davidson and coworkers (10). It permits the separation of the Basidiomycetes, except for a few that give inconsistent reactions, into two distinct groups. Recently, Nobles (17) has shown that 90 percent of the Basidiomycetes that exhibit the oxidase reaction also turn blue when a solution of gum guiac is applied to the mycelium.

Since the identification of Fungi Imperfecti and Ascomycetes by cultural means alone often presents great difficulty, it would be helpful in classifying members if they were known to react differently to the oxidase test. Moreover, a knowledge of the oxidase-producing capacities of the fungi would be expected to give some indication of differences in their rot-producing potential. The oxidase reactions were therefore determined by both methods of test for 32 of the soft-rot isolates.

Methods. --The method of preparing the tannic or gallic acid in malt agar was essentially that described by Davidson and coworkers (10). Five grams of tannic or gallic acid dissolved in 150 cubic centimeters of sterile distilled water were added to 15 grams of Difco malt and 20 grams of agar in 850 cubic centimeters of water after sterilization of the malt-agar solution at 15 pounds pressure for 20 minutes and cooling to approximately 50° C. The two solutions were thoroughly mixed, after which 30 cubic centimeters of the mixture were poured into petri dishes and cooled rapidly.

The inocula (5 millimeters square) were cut from a 2-week-old petri-dish culture and slightly pressed, mycelium down, on the surface of each agar plate. Incubation was at 28° C. and 70 percent relative humidity for 2 weeks.

The blue-coloration test consisted of putting two or three drops of a filtered gum guiac solution (0.5 gram gum guiac in 30 cubic centimeters of 95 percent alcohol) on mycelium growing on malt agar either in a tube slant or petri dish. Since the majority of soft-rot isolates form darkly pigmented colonies, color changes were obscured except occasionally at colony edges, where the younger, less colored, hyphae were present. All color changes, however, could be detected by pressing filter paper that was saturated with gum guiac solution on the mycelium.

Results. --The results (table 12) show that 13 of the isolates gave a positive reaction with both the oxidase and gum guiac tests, while 9 gave a negative reaction. Ten of the isolates were inconsistent in that 5 were

positive only in the oxidase test and 5 only in the gum guiac test. Fungi commonly isolated from cooling towers and shown in earlier tests to cause appreciable decay, gave positive and negative reactions.

The oxidase test should be applied to a much larger number of Fungi Imperfecti isolates, and the reproducibility of the results should again be checked. Present indications are that the oxidase test can be used as a cultural tool in distinguishing between some of the soft-rot species. It remains to be determined whether any relationships can be developed between the oxidase-producing capacity of the fungi and the visible characteristics of the rot produced by them.

Relative Tolerances to Some Wood-Preserving Chemicals

General. --The tolerances of different wood-destroying Basidiomycetes to the same chemical may vary greatly. It is apparent, therefore, that the toxicity of given chemicals to any new group of fungi found capable of attacking wood cannot be predicted but must be ascertained directly. With this in mind, Price (18) tested 6 soft-rot fungi and found them to react fairly uniformly to the toxicity of 5 different chemicals.

In laboratory tests by Savory (22), the amount of pentachlorophenol needed to reach the toxic limit for Chaetomium globosum was considerably higher than that for Polyporus versicolor, one of the most phenol-tolerant Basidiomycetes in hardwoods. Also, Scholles (29) found that the toxic limits for C. globosum were greater than those for other standard test fungi in trials with compounds containing copper, zinc, and mercury.

Observations of treated woods exposed in the soil suggested that the soft-rot fungi probably have a higher group tolerance for preservatives than do the Basidiomycetes. For instance, soft-rot occurs at times on the surfaces of poles, piling, and other wood installations in the soil that have been treated to sufficiently large retentions of creosote, pentachlorophenol, or various water-borne compounds to prevent attack by Basidiomycetes. Also, microscopical evidence of soft-rot attack was found in more than half of 50 variously treated test stakes that had been removed from one exposure plot at the first indication of softness. Characteristic decay by Basidiomycetes was absent, moreover, and only Fungi Imperfecti could be isolated from the stakes.

The aim of the present tests was to determine the preservative tolerances among the soft-rot fungi. The toxicities of nine fundamentally different preservatives to 32 representative isolates were determined. To have a familiar point of reference, concurrent studies were conducted with 9 Basidiomycetes, 4 of which were known to be especially tolerant of one or more of the chemicals.

Methods. --To obtain an initial estimate of the relative tolerances, toxicity tests were made to determine the minimum concentrations of preservative in malt agar that would inhibit growth of the fungi. The method described by Schmitz (28) was used with certain modifications.

Difco malt and agar sufficient to provide a 1.5 percent concentration of each in the final solution were made up with distilled water in a 500 milliliter flask, which was then sterilized and cooled to 60° C. The liquid lost during sterilization, determined by weight, was then replaced with hot, sterile distilled water. The preservative solution was added next and mixed with the malt agar by rotating the flask. By inverting the flask, the medium--in 15 cubic centimeter amounts--was dispensed aseptically into test tubes by means of a stopcock on the delivery tube. The tubes were slanted so that the surface of the liquid was approximately 3.5 inches long, and the medium was then allowed to harden.

Inoculum (5 millimeters square and 2 millimeters thick) was cut from the margin of a 2-week-old petri-dish culture and placed approximately 2 inches from the forward edge of the agar slant. The inoculated tubes were then incubated at 80° F. and 70 percent relative humidity for 14 days and examined for evidence of growth. The inhibition point was considered to lie between the highest concentration that permitted growth from the inoculum onto the agar surface and the next highest concentration. Killing points were not determined.

Results. --The results, summarized in tables 13 and 14 show that (1) there was a considerable range of tolerances among the soft-rot isolates for most of the preservatives, (2) at least one-fourth, and generally considerably more, of the soft-rot isolates were more tolerant of sodium fluoride, sodium arsenate, sodium chromate, zinc chloride, and possibly creosote than were the nine Basidiomycetes, and (3) none to only a few isolates were more tolerant of mercuric chloride, copper sulfate, or sodium pentachlorophenate, than the most tolerant Basidiomycete. Relative tolerances to sodium borate are not clear because the lowest concentration used (0.5 percent) inhibited most fungi.

Fungi showing particularly high tolerance to most of the chemicals tested included R2 (Cytosporella), R34 (unnamed), and P36 (Sporocybe, Acremonium). Interesting examples of tolerance to specific chemicals were R34 (unnamed) to sodium pentachlorophenate, P12E (Alternaria) to sodium borate, and P13 (Cephalosporium) to copper sulfate.

pH Preferences

General. --An appraisal of the pH preferences of soft-rot isolates was expected to serve as a partial guide to the choice of substrate for culturing soft-rots, and also to indicate whether these fungi represent an ecological type with respect to pH relationships.

There was some evidence that many soft-rot fungi might be able to grow in a more alkaline environment than the wood-destroying Basidiomycetes. A malt-agar substrate with an initial pH of 5 to 6, which is satisfactory for the growth of Basidiomycetes (although not necessarily optimum), was likewise satisfactory for the growth of all soft-rot isolates. Most of the latter were capable of good growth at a pH of 7.4 in the vitamin-mineral agar, however, whereas the several Basidiomycetes grew poorly or not at all on this medium.

There also is evidence, in their natural environment, that some of the soft-rot fungi have a capacity to develop in an alkaline situation. Fungi that grow in redwood cooling towers generally are subjected to slightly alkaline water. Although the alkalinity of cooling-tower wood is not necessarily optimum for the growth of these fungi, neither does it prevent their growth nor the deterioration of wood in the tower. Similarly, Barghoorn and Linder (5) have shown that certain marine fungi capable of decaying wood, and which belong to the same broad taxonomic groups as the soft-rot fungi, have an alkaline tolerance well above that for the majority of Basidiomycetes.

Methods. --To obtain suitably circumscribed pH data on the many isolates of this study, preliminary observations were made on the ability of 32 isolates to develop at prescribed levels of alkalinity and acidity. In addition, the average daily linear growth at various pH's was further determined for 8 of the fungi, 4 of which were isolated from cooling towers and 4 from soil-contact installations.

The sterilized substrate, which consisted of 1 percent malt and 2 percent agar, was adjusted with hydrochloric acid and sodium hydroxide to initial pH's from 3 to 9. The medium was buffered with sodium orthophosphate and citric acid (McIlvaine's standard (8)) to maintain the intial pH. Since the buffering action was not totally effective above pH 8, 0.3 to 0.4 gram of calcium carbonate was added to the alkaline media. Little change of pH occurred during the experiments, since initial and final pH's were within 0.2.

Fifteen cubic centimeters of the medium were dispensed aseptically into test tubes and hardened with a slanted surface, or, when linear growth was measured, the medium was carried in growth tubes similar to those used in the temperature studies. A 3-millimeter cube of inoculum cut from the margin of a 2-week-old petri-dish culture was placed 1 inch from the base of the agar slant or at the forward end of the growth-rate tube. Incubation was at 28° C. and 70 percent relative humidity. Growth in the test tubes was observed only after 12 days, but, as before, six measurements were recorded for growth in growth-rate tubes.

Results.--The results indicated that 13 (about 40 percent) of the isolates failed to grow at pH 3. All 32 grew at pH 4 and 5, however, and had apparently reached their maximum rate of growth at pH 6. About half of the isolates maintained the maximum rate of growth at pH 7, and one-fourth at pH 8. A pH of 9 had a retarding effect on all the isolates, but did not prevent their growth. An evident decrease in growth on the alkaline medium was often accompanied by a change in appearance of the mycelial mat, which became thickened.

Similar results are illustrated by the linear growth rates of eight isolates, at different pH levels, in figure 11.

When these results are compared with what is known about the pH relations of Basidiomycetes, it can be said that both the soft-rot isolates and the Basidiomycete wood-destroyers commonly have a growth optimum at or near pH 6, but only among the soft-rot fungi does the optimum commonly extend to a much higher pH. Most of the Basidiomycete wood-destroyers seem to be inhibited by a mildly alkaline substrate (between 7 and 8 (32)). These data indicate also that the faster growing isolates at pH 7 generally were substantially more tolerant than the slower growing isolates at pH 9.

Summary

This research has indicated that a large percentage of the wood-inhabiting Ascomycetes and Fungi Imperfecti may be capable of causing a type of decay known as soft-rot. Their attack of wood usually is slower than that by Basidiomycetes except where the wood has a high moisture content, a condition that retards decay by typical brown- or white-rot Basidiomycetes. Soft-rot developed much more rapidly in such hardwoods as sweetgum and beech than in such softwoods as pine and redwood. The presence of minerals and vitamins, however, leaching the wood (especially in chlorinated water), or long exposure of the wood to wet soil tended to accelerate attack of the softwood species.

Certain physiological characteristics of the soft-rot and wood-attacking Basidiomycete fungi were also compared:

Temperature optima tended to be considerably higher among the soft-rot fungi than among the Basidiomycetes.

Tolerances among the soft-rot fungi to common preservative chemicals varied widely. At least one-fourth and generally more of the soft-rot isolates were more tolerant of sodium fluoride, sodium chromate, sodium arsenate, and zinc chloride than were the Basidiomycetes tested.

Both Basidiomycete and soft rot rungi had growth optima at or near pH 6. but only the soft-rot fungi were capable of growth at pH 8 or 9.

Since some soft-rot fungi exhibited a positive and others a negative oxidase reaction, this test may also help to differentiate members of the soft-rot group as well as Basidiomycetes.

Tentative identifications for some of the soft-rot fungi, along with isolation data for over 100 organisms, are summarized. Identifications in all cases require further study.

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Table 1.--Weight losses produced in 24 weeks by selected soft-rot isolates in wood blocks. Test No. 1[1]

Isolate		Redwood heartwood			Southern pine sapwood		Sweetgum sapwood	
		Normal	Leached in water	Leached in sodium carbonate	Normal	Leached in water	Normal	Leached in water
		Percent	Percent	Percent	Percent	Percent	Percent	Percent
Fungi Imperfecti								
26 isolates[2]		0	0	0	0	0	0	0
Ascomycetes								
Chaetomium globosum	1669	0	0	0	0	0	0	0
Chaetomium cochliodes	2 SF	0	0	0	0	0	0	0
Basidiomycetes (Brown rots):								
Poria oleraceae[3]	4907	49	48	46	36	48	49	53
Unidentified brown rot[3]	ML19	56	61	63	68	68	74	75
Unidentified brown rot[3]	ML23	56	59	62				
Poria monticola[4]	698	46	49	54	63	65	67	65
Basidiomycetes (White rots):								
Poria nigrescens[3]	4856	25	33	71	47	31	52	51
Poria nigrescens[3]	4963	18	45	56	54	39	60	57
Peniophora mollis[3]	ML21	0	0	28	26	16		
Peniophora mollis[3]	ML22	0	0	15				
Polyporus versicolor[4]	697	0	32	43	43	40	68	63

[1] Each figure is an average for three test blocks. Considerable drying of cultures was evident, and active decay may have ceased before the end of the 24-week test period.

[2] In this test, in which the moisture content of the soil was initially 130 percent of its water-holding capacity, none of the 26 isolates tested were able to cause weight loss. These isolates included R 8, R 9, and R 18, which represented the Fungi Imperfecti in additional tests.

[3] Isolated from redwood structural members in cooling towers where the wood is not so wet as in slats.

[4] Test fungi used in ASTM D1413 (1956).

Table 2.--Weight losses[1] produced in 12 weeks by four isolates in variously leached and unleached veneer strips, with and without addition of supplementary minerals to the wood. Test No. 2

Isolate[2]	Chemicals in leach water[3]	Soil substrate					
		Redwood heartwood		Ponderosa pine sapwood		Sweetgum sapwood	
		Without minerals	With minerals[4]	Without minerals	With minerals[4]	Without minerals	With minerals[4]
		Percent	Percent	Percent	Percent	Percent	Percent
FPRL S 121	None (Distilled water)	0	0	2	4	17	26
	Sodium hypochlorite	5	8	10	13	20	35
	Sodium carbonate	0	0	2	3	12	32
	Acetic acid plus hydrogen peroxide	0	0	0	3	19	23
R 2	None (Distilled water)	0	0	5	8	8	12
	Sodium hypochlorite	3	2	8	15	13	17
	Sodium carbonate	0	0	2	6	13	14
	Acetic acid plus hydrogen peroxide	0	0	2	8	7	12
R 5	None (Distilled water)	0	0	8	13	9	30
	Sodium hypochlorite	2	2	6	16	14	26
	Sodium carbonate	0	0	3	13	12	23
	Acetic acid plus hydrogen peroxide	0	0	4	10	7	22
R 7	None (Distilled water)	0	0	0	2	6	25
	Sodium hypochlorite	6	9	9	13	20	23
	Sodium carbonate	0	0	1	2	6	24
	Acetic acid plus hydrogen peroxide	0	0	0	1	6	24

[1] Each weight loss is an average for four test specimens.

[2] FPRL S 121 was from Chilean hardwood timber (supplied by Forest Products Research Laboratory, England). R 2, R 5, and R 7 were Fungi Imperfecti from redwood cooling towers.

[3] See Appendix 2, test 2, for leaching details.

[4] Mineral composition of the impregnating solution (grams per 1,000 cubic centimeters of distilled water): ammonium nitrate, 3.0; potassium phosphate, 2.0; potassium dihydrogen phosphate, 2.5; magnesium sulfate, 2.0.

Table 3.--Breaking radius and weight loss of inoculated and uninoculated sweetgum specimens in Test No. 3

Fungus Isolate No.[1]	Breaking radius					Weight loss				
	Inoculated specimens				Matched uninoculated specimens	Inoculated specimens				Matched uninoculated specimens
	1	2	3	Average	4	1	2	3	Average	4
R 1	2.50	2.50	2.00	2.3	1.25	12	10	6	9.3	0
2	2.50	2.25	1.75	2.2	1.25	10	9	5	8.0	0
2B	1.25	1.25	1.50	1.3	1.00	0	0	0	0	0
2C	1.50	1.00	.75	1.1	.75	0	0	0	0	0
3	1.00	1.25	1.25	1.2	1.00	0	0	0	0	0
4	1.00	1.00	1.25	1.1	1.00	0	0	0	0	0
5	2.00	2.25	2.00	2.1	1.25	6	5	7	6.0	0
6	2.25	2.00	2.25	2.2	1.50	7	7	7	7.0	0
7	2.50	2.50	2.50	2.5	1.25	12	10	14	12.0	0
7B	1.00	.75	1.00	.92	1.00	0	0	0	0	0
8	3.50	3.25	3.00	3.3	1.00	20	18	15	17.7	0
9	3.25	2.50	3.25	3.0	1.00	20	16	17	17.7	0
9B	1.25	1.00	1.00	1.1	1.00	0	0	0	0	0
10	3.00	2.75	2.75	2.8	.75	17	14	15	15.3	0
11	3.50	3.25	3.50	3.4	1.25	28	20	25	24.3	0
12	3.50	3.50	3.50	3.5	1.00	30	27	26	27.7	0
13	2.25	2.00	2.00	2.1	1.25	10	9	10	9.7	0
14	3.00	2.50	3.25	2.9	1.25	19	15	20	18.0	0
15	2.50	2.25	2.75	2.5	1.00	15	13	12	13.3	0
16	.75	1.00	1.00	.92	1.00	0	0	0	0	0
17	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0
18	3.25	3.50	3.25	3.3	.75	27	24	29	26.7	0
19	.75	1.00	1.00	.92	1.00	0	0	0	0	0
20	1.25	1.25	1.25	1.3	1.50	0	0	0	0	0
20B	1.00	1.00	1.00	1.0	1.25	0	0	0	0	0
20C	.75	1.00	1.00	.92	1.25	0	0	0	0	0
21	3.50	3.25	3.25	3.3	1.00	30	26	24	26.7	0
22	2.25	2.50	2.75	2.5	1.00	12	11	12	11.7	0
23	3.50	3.00	3.25	3.3	.75	25	21	23	23.0	0
24	3.50	3.50	3.50	3.5	1.00	37	35	31	34.3	0
25	1.25	1.00	1.00	1.1	1.00	0	0	0	0	0
26	2.50	2.25	2.75	2.5	.75	15	10	12	12.3	0
27	2.00	1.75	1.75	1.8	1.00	2	1	0	1.0	0
28	3.25	3.25	3.00	3.2	1.00	23	19	18	20.0	0

Table 3.--Breaking radius and weight loss of inoculated and uninoculated sweetgum specimens in Test No. 3--Continued

Fungus Isolate No.[1]	Breaking radius					Weight loss				
	Inoculated specimens				Matched uninoculated specimens	Inoculated specimens				Matched uninoculated specimens
	1	2	3	Average	4	1	2	3	Average	4
R 29	2.75	2.50	2.50	2.6	1.00	14	12	15	13.7	0
30	2.75	2.50	2.75	2.7	1.00	15	11	16	14.0	0
31	3.00	2.75	2.75	2.8	1.25	18	12	15	15.0	0
32	3.25	2.50	3.25	3.0	1.25	20	14	19	17.7	0
33	1.25	1.25	1.00	1.2	1.00	0	0	0	0	0
34	2.00	1.75	2.00	1.9	1.25	10	6	7	7.7	0
35	2.75	2.75	2.50	2.7	1.00	16	14	13	14.3	0
37A	2.75	2.25	2.00	2.3	1.25	12	9	8	9.7	0
37B	3.25	2.50	2.75	2.8	1.25	19	15	17	17.0	0
37C	1.00	.75	1.00	.92	1.00	0	0	0	0	0
38	3.25	2.50	2.50	2.8	1.00	17	14	12	14.3	0
39	2.25	2.50	2.25	2.3	1.00	9	12	10	10.3	0
40	2.00	2.00	1.75	1.9	.75	6	4	3	4.3	0
41	2.50	2.25	2.00	2.3	1.00	10	7	6	7.7	0
42A	2.75	2.50	2.50	2.6	1.00	12	14	12	12.7	0
43	1.00	1.00	1.25	1.1	1.00	0	0	0	0	0
45	1.75	1.50	1.50	1.6	1.25	2	1	1	1.3	0
46	1.25	1.25	1.25	1.3	1.50	0	0	0	0	0
47A	2.25	2.25	2.00	2.2	1.00	13	9	10	10.7	0
47B	1.00	.75	.75	.83	1.00	0	0	0	0	0
47C	3.25	2.25	2.75	2.8	1.00	17	14	15	15.3	0
47D	.75	1.00	.75	.83	1.00	0	0	0	0	0
47E	1.25	1.50	1.25	1.3	.75	0	0	0	0	0
47F	3.50	3.25	3.00	3.3	1.00	22	19	17	19.3	0
58B	1.50	1.75	1.50	1.6	1.00	2	2	2	2.0	0
60	3.00	2.75	3.25	3.0	1.00	17	13	19	16.3	0

[1] All organisms were Fungi Imperfecti isolated from redwood in cooling towers.

Table 4.--Weight losses[1] produced in veneer strips in 8 weeks by various isolates grown on mineral-vitamin agar or potting soil. Test No. 4

Isolate[2]	Mineral-vitamin agar				Potting soil			
	Beech	Gum	Ponderosa pine		Beech	Gum	Ponderosa pine	
			Not leached	Leached in chlorine			Not leached	Leached in chlorine
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
R 2	14	14	0	5	10	12	2	5
6	8	15	9	10	7	13	5	7
7	24	29	0	6	15	18	0	2
11	55	54	21	28	41	43	4	8
15	19	28	3	7	12	21	2	6
18	54	55	11	10	41	46	5	6
21	35	54	12	17	45	50	6	11
24	31	57	26	28	30	52	12	18
30	18	20	0	4	9	11	0	2
34	14	24	1	3	16	20	0	2
38	45	54	1	4	40	48	0	2
39	15	38	10	14	40	18	0	4
40	30	21	1	5	6	13	7	12
47C	25	29	11	17	15	19	6	15
47F	55	56	19	19	45	46	9	11
48B	48	43	15	19	40	43	12	15
49A	52	53	1	5	41	45	0	2
53B	51	54	13	14	41	44	6	10
58G	64	65	6	11	51	55	3	5
60	17	15	3	4	7	8	0	1
Re 1B	18	4	1	3	16	14	0	1
P 6B	33	41	6	8	26	37	2	4
12E	3	1	3	4	2	2	0	0
13	25	28	4	11	20	25	0	2
16	38	28	1	5	18	29	1	3

[1] Each figure is an average for three test specimens.

[2] R - Isolates from redwood in cooling towers
Re- Isolate from redwood in soil
P - Isolates from pine in soil
R 49A is Chaetomium and R 58G is Xylaria; the others are all Fungi Imperfecti.

Table 5.--Weight losses[1] produced by 99 soft-rot and 6 Basidiomycete isolates in 9 months in variously treated wood blocks. Test No. 5

Isolate[3]	Wood species and preliminary treatment of test block[2]											
	Sweetgum sapwood		Southern pine sapwood				Redwood heartwood					
	A	AA	A	AA	B	BB	A	AA	C	CC	D	DD
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
R 1	18	38	4	6	2	2	0	0	0	0	6	6
2	16	20	6	4	6	2	0	0	0	0	3	4
5	6	4	0	0	0	0	0	0	0	0	3	4
6	16	18	4	8	4	2	0	0	0	0	6	4
7	12	26	0	0	0	0	0	0	0	0	3	3
8	16	28	0	0	0	0	0	0	0	0	4	5
9	22	24	4	6	4	2	0	0	0	0	5	3
10	22	20	6	4	4	4	0	0	0	0	6	4
11	6	28	6	8	14	16	0	0	0	0	4	5
12	44	28	6	4	4	4	0	0	0	0	4	6
13	6	6	6	4	0	0	0	0	0	0	6	4
14	22	28	6	4	4	4	0	0	0	0	3	4
15	28	32	6	8	6	8	0	0	0	0	4	3
18	32	40	10	14	14	14	0	0	0	0	4	7
21	34	42	12	18	16	26	0	0	0	0	5	3
22	16	22	6	4	4	4	0	0	0	0	2	3
23	....	30	0	0	4	2	0	0	0	0	3	3
24	32	44	8	8	20	20	0	0	0	0	2	3
26	22	22	0	0	0	0	0	0	0	0	3	3
27	4	5	6	4	0	0	0	0	0	0	5	4
28	20	34	0	0	4	4	0	0	0	0	3	3
29	20	24	0	0	4	4	0	0	0	0	2	2
30	14	42	6	4	0	0	0	0	0	0	4	5
31	16	20	0	0	0	0	0	0	0	0	3	4
32	18	30	4	8	4	4	0	0	0	0	3	3
34	20	24	0	0	0	0	0	0	0	0	3	4
35	10	....	....	2	....	....	....	....	....	....	....	....
37A	24	28	12	12	12	18	0	0	0	0	3	3
37B	16	30	0	0	0	0	0	0	0	0	4	4
38	34	56	0	0	4	4	0	0	0	0	3	2
39	12	16	0	0	4	8	0	0	0	0	2	1
40	22	30	0	0	0	0	0	0	0	0	4	3
41	20	24	0	0	0	0	0	0	0	0	3	3
42A	14	20	0	0	0	0	0	0	0	0	2	2

Table 5.--Weight losses[1] produced by 99 soft-rot and 6 Basidiomycete isolates in 9 months in variously treated wood blocks. Test No. 5--Continued

Isolate[3]	Wood species and preliminary treatment of test block[2]											
	Sweetgum sapwood		Southern pine sapwood				Redwood heartwood					
	A	AA	A	AA	B	BB	A	AA	C	CC	D	DD
	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
R 42B	26	26	0	0	0	0	0	0	0	0	2	2
44	4	2	0	0	0	0	0	0	0	0	2	2
45	4	4	0	0	0	0	0	0	0	0	0	0
46B	4	4	0	0	0	0	0	0	0	0	3	3
47A	18	26	0	0	4	4	0	0	0	0	4	4
47C	34	64	10	14	4	14	0	0	0	0	4	4
47F	34	38	10	10	16	24	0	0	0	0	7	7
48A	14	26	0	0	4	2	0	0	0	0	3	2
48B	58	36	0	0	10	20	0	0	0	0	2	3
49A	34	36	0	0	0	0	0	0	0	0	3	4
49B	4	4	0	0	0	0	0	0	0	0	2	2
49C	16	36	0	0	6	2	0	0	0	0	4	4
50A	24	30	6	4	6	8	0	0	0	0	4	3
51A	18	26	8	4	6	4	0	0	0	0	4	2
51B	2	26	0	0	0	0	0	0	0	0	2	2
51C	24	30	4	6	0	0	0	0	0	0	3	3
52B	20	26	0	0	0	0	0	0	0	0	2	3
53B	36	40	12	12	16	20	0	0	0	0	2	2
54A	....	28	0	0	0	0	0	0	0	0	....	3
54B	14	18	0	0	4	2	0	0	0	0	2	2
55B	20	42	0	0	0	0	0	0	0	0	4	4
55F	20	24	6	6	12	10	0	0	0	0	4	4
56A	36	40	8	12	6	12	0	0	0	0	5	5
56B	....	28	0	0	0	0	0	0	0	0	3	3
57B	16	56	0	0	2	6	0	0	0	0	2	3
57D	6	4	0	0	0	0	0	0	0	0	4	4
58A	6	4	0	0	0	0	0	0	0	0	5	3
58B	8	4	0	0	0	0	0	0	0	0	4	4
58C	30	38	16	12	14	12	0	0	0	0	4	3
58E	28	42	0	0	0	0	0	0	0	0	1	1
58F	12	18	0	0	0	0	0	0	0	0	3	2
58G	50	58	12	18	12	14	0	0	0	0	4	4
59A	22	38	0	0	4	4	0	0	0	0	3	4

Table 5.--Weight losses[1] produced by 99 soft-rot and 6 Basidiomycete isolates in 9 months in variously treated wood blocks. Test No. 5--Continued

Isolate[3]	Wood species and preliminary treatment of test block[2]											
	Sweetgum sapwood		Southern pine sapwood				Redwood heartwood					
	A	AA	A	AA	B	BB	A	AA	C	CC	D	DD
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
R 59B	4	6	0	0	4	2	0	0	0	0	5	4
60	18	28	0	0	0	0	0	0	0	0	4	4
62	6	....	2	....	....	....	....	....	....	....	....	....
63B	4	....	....	....	....	....	....	....	....	....	....	....
63D	4	....	1	....	....	....	....	....	....	....	....	....
63F	2	....	1	....	....	....	....	....	....	....	....	....
Re 1A	12	14	0	0	0	0	0	0	0	0	3	2
1B	10	28	0	0	4	2	0	0	0	0	4	5
1C	24	....	0	0	0	0	0	0	0	0	3	2
G 1A	28	56	8	10	4	14	0	0	0	0	5	3
2	4	4	4	4	0	0	0	0	0	0	2	3
3	(4)	....	(4)	....	....	....	....	....	....	....	....	....
4	(4)	....	(4)	....	....	....	....	....	....	....	....	....
O 2	15	....	2	....	....	....	....	....	....	....	....	....
P 1A	18	28	0	0	0	0	0	0	0	0	2	3
2B	10	24	0	0	4	8	0	0	0	0	3	2
6B	30	....	3	....	6	....	....	....	....	....	....	....
10A	34	36	0	0	0	4	0	0	0	0	2	3
11	18	....	2	....	....	....	....	....	....	....	....	....
12B	26	36	0	0	0	0	0	0	0	0	4	4
12C	18	22	0	0	0	0	0	0	0	0	2	2
12E	....	2	....	....	....	3	....	....	....	....	2	2
12F	30	32	0	0	4	2	0	0	0	0	2	2
12G	6	8	0	0	2	4	....	....	....	....	3	2
13	30	38	10	10	8	6	....	....	....	....	2	4
14	16	....	....	....	....	....	....	....	....	....	....	....
16	26	....	....	2	....	....	....	....	....	....	3	....
17	12	....	2	....	....	....	....	....	....	....	....	....
26B	18	....	....	....	....	....	....	....	....	....	....	....

Table 5.--Weight losses[1] produced by 99 soft-rot and 6 Basidiomycete isolates in 9 months in variously treated wood blocks. Test No. 5--Continued

Isolate[3]	Wood species and preliminary treatment of test block[2]											
	Sweetgum sapwood		Southern pine sapwood				Redwood heartwood					
	A	AA	A	AA	B	BB	A	AA	C	CC	D	DD
	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
P 27	(4)		(4)									
35	(4)		(4)									
36	(4)		(4)									
Basidiomycetes (Brown rots)[5]												
4907	22	20	21	22	21	18	18	21	16	13	25	22
ML 27	24	18	28	21	18	24	16	16	16	16	21	20
ML 19	29	16	22	15	25	17	11	10	15	10	18	9
ML 23	43	29	42	29	35	41	13	13	18	17	22	21
Basidiomycetes (White rots)[6]												
4856	69	65	53	60	56	66	30	33	51	54	55	60
4963	83	85	51	59	67	69	17	28	43	43	47	59

[1] Each weight loss is an average for two test specimens.

[2] A = Normal, no preliminary treatment; AA = Same as A but impregnated with mineral-vitamin solution; B = Leached daily for 8 hours in running water, 16 hours in standing water for 1 month; BB = Same as B but impregnated with mineral-vitamin solution; C = Leached in 110° F. distilled water with daily change for 3 months; CC = Same as C but impregnated with mineral-vitamin solution; D = Leached in 110° F. distilled water containing 2 p.p.m. available chlorine. Daily change of chlorinated water for 6 weeks; DD = Same as D but impregnated with mineral-vitamin solution.

[3] All soft-rot isolates are Fungi Imperfecti except R 49A, P 12B - Chaetomium funicolum, and R 58G - Xylaria sp.

[4] Weight losses not determined but substantial rot observed microscopically.

[5] Brown-rots isolated from cooling towers: Poria oleraceae (4907, ML 27); unidentified species (ML 19, ML 23).

[6] White-rots isolated from cooling towers: Poria nigrescens (4856, 4963).

Table 6.--Weight loss produced by isolates in redwood blocks without prior treatment, buried in inoculated loam soil for 24 months. Test No. 6

Isolate[1]	Weight loss[2]	Isolate[1]	Weight loss[2]
	Percent		Percent
R 2	2	R 39	0
6	4	40	4
7	4	47C	5
11	6	47F	1
15	6	48B	1
18	6	49A	1
21	3	53B	1
24	4	58G	2
30	5	60	1
34	4	Re 1B	3
35	5	G 1A	6
38	5	P 13	1

[1] R 58G - Xylaria, R 49A - Chaetomium funicolum; the remainder are Fungi Imperfecti.

[2] Each weight loss is an average for three test specimens.

Table 7.--Weight losses produced in 8 weeks by additional Fungi Imperfecti in veneer strips partially buried in inoculated soil of two kinds. Test No. 7

Isolate	Weight loss in strips of wood, partially buried in silt loam garden soil[1]			Weight loss in strips of wood, partially buried in a loam potting soil[1]		
	Beech	Sweetgum	Ponderosa pine	Beech	Sweetgum	Ponderosa pine
			Not leached / Leached in chlorine			Not leached / Leached in chlorine
	Percent	Percent	Percent / Percent	Percent	Percent	Percent / Percent
R 2	7	10	1 / 4	8	12	2 / 5
7	.....	12	0 /	.....	17	2 / .....
40	.....	12	0 /	.....	13	7 / .....
56B	.....	14	1 /	.....	14	2 / .....
62	.....	2	2 /	.....	5	5 / .....
63B	.....	0	0 /	.....	9	0 / .....
63D	.....	4	1 /	.....	1	3 / .....
63F	.....	2	3 /	.....	4	3 / .....
O 2	11	17	2 / 5	13	22	3 / 7
P 11	9	20	2 / 5	42	48	9 / 14
14	.....	19	..... /	.....	19	..... / .....
16	.....	18	2 /	.....	18	3 / .....
17	.....	14	1 /	.....	17	2 / .....
26B	.....	17	..... /	.....	18	..... / .....

[1] Each weight loss is an average for four test pieces.

Table 8.--Weight loss in sweetgum veneer strips in shake culture. Test No. 8

Isolate[1]	Weight loss[2] after exposure		Isolate[1]	Weight loss[2] after exposure	
	2 weeks	4 weeks		2 weeks	4 weeks
	Percent	Percent		Percent	Percent
R 2	5	7	R 39	10	12
6	7	9	40	7	10
7	8	10	47C	5	7
11	7	12	47F	6	9
15	6	10	49A	10	12
18	3	7	Re 1B	5	8
21	8	12	P 6B	7	12
24	7	15	16	5	9
34	6	9	697	0	0
38	10	15	698	0	0

[1]Isolate 49A was Chaetomium funicolum; 697 and 698 were Polyporus versicolor and Poria monticola, respectively; the remainder are Fungi Imperfecti.

[2]Each weight loss is an average for five test specimens.

Table 9.--Average daily rates of linear growth by soft-rot fungi and Basidiomycetes on malt agar at different temperatures[1]

Isolate[2]	Temperature of malt-agar medium						
	10° C.	16° C.	22° C.	28° C.	34° C.	38° C.	42° C.	46° C.
	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.
OPTIMUM TEMPERATURE NEAR 22° C.								
R 20	1.1	1.8	2.5	2.3	0	0	0	.....
49A	.7	2.6	4.7	3.4	0.3	0	0	.....
G 3	.5	1.5	1.5	1.0	0	0	0	.....
L 2	.7	2.3	2.3	.....	1.5	0	0	.....
OPTIMUM TEMPERATURE NEAR 28° C.								
R 7	.3	1.1	1.8	2.4	2.0	0.3	0	.....
21	0	1.0	2.3	5.7	4.0	2.9	0	.....
34	.3	.9	1.5	1.9	1.2	0	0	.....
40	.3	.9	1.4	1.7	1.5	0	0	.....
47C	.1	.7	1.2	1.8	1.6	.6	0	.....
58G	.2	.9	1.9	2.9	2.0	0	0	.....
60	.6	1.2	1.7	2.5	2.0	0	0	.....
Re 1B	2.0	5.0	6.0	6.9	.5	0	0	.....
G 1A	.2	.8	1.3	1.7	1.1	0	0	.....
P 6B	.1	.5	.9	1.6	.3	0	0	.....
12E	2.1	4.1	6.0	8.2	1.5	0	0	.....
13	.7	1.5	2.0	2.3	1.2	0	0	.....
35	1.3	2.9	4.7	6.0	4.2	0	0	.....
697	2.7	5.4	7.3	10.0	7.8	2.4	0	.....
4856	.2	4.1	6.8	8.9	5.2	0	0	.....
ML 29	.5	2.7	3.7	4.8	3.4	0	0	.....
698	1.8	4.4	6.0	7.0	4.1	0	0	.....
OPTIMUM TEMPERATURE NEAR 34° C.								
R 2	.3	.7	1.1	1.5	1.8	.6	0	0
6	0	1.1	2.4	3.3	4.2	2.5	0	0
11	0	1.0	1.8	2.7	3.5	2.7	0	0
15	.5	1.3	1.8	2.2	2.3	1.0	0	0
18	0	1.1	2.5	3.6	4.0	3.0	0	0
24	0	1.0	1.9	2.9	3.5	2.5	0	0
30	.3	1.1	2.0	2.3	2.5	.6	0	0
39	.1	1.5	2.9	4.0	4.3	2.2	0	0

Table 9.--Average daily rates of linear growth by soft-rot fungi and Basidiomycetes on malt agar at different temperatures[1]--Continued

Isolate[2]	Temperature of malt-agar medium							
	10° C.	16° C.	22° C.	28° C.	34° C.	38° C.	42° C.	46° C.
	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.
R 47F	0	0.7	1.7	2.5	3.1	2.6	0	0
48B	0	.5	1.8	3.2	4.5	2.2	0	0
53B	0	1.1	2.0	3.0	4.0	3.1	0	0
P 16	1.6	4.2	6.0	6.9	7.4	0	0	0
36	0	1.5	2.3	3.3	4.0	2.1	0	0
617	1.1	3.3	5.2	6.9	8.2	5.3	1.0	0
4907	.4	1.9	2.9	4.4	5.2	1.5	0	0
ML 23	2.3	4.0	5.8	7.4	8.9	2.5	0	0
OPTIMUM TEMPERATURE NEAR 38° C.								
R 35	0	1.0	2.9	4.9	6.6	7.3	4.9	1.0
38	.1	.4	1.4	2.3	3.3	3.6	0	0
ML 26	0	4.2	12.5	22.5	31.0	37.0	34.0	8.0

[1] Each figure is an average of 12 measurements.

[2] Basidiomycetes: Nos. 697, 4856, ML 29, 698, 617, 4907, ML 23, ML 26
Ascomycetes: R 49A, L 2, R 58G
Remainder are Fungi Imperfecti.

Table 10.--Relation between optimum and maximum temperatures for growth

Optimum temperature	Number of isolates with indicated optimum temperature	Number of isolates with indicated maximum temperature			
		34° C.	38° C.	42° C.	46° C. or greater
°C.					
22	4	2	2		
28	13		10	3	
34	13		1	12	
38	2			1	1

Table 11.--Relation between optimum temperature and the temperature range for growth above the optimum

Optimum temperature	Number of isolates with indicated optimum temperature	Number of isolates with indicated differences between optimum and maximum temperatures					
		4° C.	8° C.	10° C.	12° C.	14° C.	16° C.
°C.							
22	4				2		2
28	13			10		3	
34	13	1	12				
38	2	1		1			

Table 12.--Oxidase reaction and growth of representative soft-rot isolates and two Basidiomycetes on gallic and tannic acid media, and reaction with gum guaic

Isolate[1]	Gallic acid medium		Tannic acid medium		Gum guaic
	Growth	Reaction[2]	Growth	Reaction	Reaction[3]
	Mm. per day		Mm. per day		
R 2	0	++++	0	++++	+
6	0	-	0	-	+
7	10	++	12	+++	-
11	10	-	0	++++	-
15	10	-	0	++++	+
18	0	-	0	-	+
20	2	-	0	++++	-
21	0	-	0	-	-
24	0	-	0	-	-
30	10	-	6	++++	+
34	1-2	+++	10-12	+++	+
35	0	++++	0	++++	+
38	0	-	0	-	-
39	0	-	0	-	-
40	5	++++	5	++++	+
47C	8-10	+++++	5-6	+++++	+
47F	0	-	0	-	-
48B	0	-	0	-	-
49A	0	+++	0	+++	+
53B	0	-	0	-	+
58G	0	+++++	4	++++	+
60	10	++	7	++	-
Re 1B	6	-	0	-	+
G 1A	4-5	++++	4-5	++++	+
3	0	-	0	-	-
P 6B	8	+++	0	+++	+
12E	6-8	+++	15	+++	+
13	12	-	5	++++	-
16	25	++++	40	++++	+
35	0	-	0	-	+
36	6-8	-	0	-	-
S 70B	0	-	0	-	-
4907	20	-	30	-	-
4856	0	++++	0	++++	+

[1] R 49A is Chaetomium funicolum; S 70B, C. globosum (obtained from Forest Products Laboratory, England); R 58G, Xylaria sp.; 4907, Poria oleraceae; and 4856, P. nigrescens. All others are Fungi Imperfecti isolates.

[2] Intensity of reaction on gallic and tannic acid media:

- No brown discoloration of agar
- ++ Brown diffusion zone formed only under inoculum or never extending beyond margin of growth
- +++ Brown diffusion zone extending beyond margin of growth about 1 to 2 centimeters
- ++++ Brown diffusion zone extending beyond margin of growth about 2 to 3 centimeters
- +++++ Brown diffusion zone extending beyond margin of growth to edge of petri dish.

[3] Blueing on gum guaic indicated by +, no blueing by -.

Table 13.--Concentrations of chemical compound bracketing the concentration which might be expected to inhibit the growth of 32 soft-rot fungi and 9 Basidiomycetes

Isolate[1]		Percentages of indicated compounds tested in malt agar bracketing the concentration preventing growth[2]								
		Sodium arsenate (Na_2HAsO_4)	Sodium borate ($Na_2B_4O_7$)	Sodium chromate (Na_2CrO_4)	Sodium fluoride (NaF)	Sodium pentachlorophenate (commercial)	Copper sulfate ($CuSO_4$)	Mercuric chloride ($HgCl_2$)	Zinc chloride ($ZnCl_2$)	Coal-tar creosote (low residue)
R	2	2.5+	0.5-1.0	0.2-0.4	0.3-0.5	0.006-0.008	0.05-0.10	0.02-0.04	0.7+	0.05-
	6	1.0-1.5	0.5-	1.2+	.5- .7	0.002-	.01- .03	.02- .04	0.3-0.5	.05-
	7	0.1-	0.5-	.2- .4	.3- .5	.002- .004	.01- .03	.02- .04	.05-	.05+
	11	2.5+	0.5-	1.2+	.5- .7	0.002-	.01- .03	.01- .02	.3- .5	.05-
	15	2.5+	0.5-	.2- .4	.3- .5	.002- .004	.03- .05	.02- .04	.05-	.05-
	18	1.5-2.0	0.5-	1.2+	.5- .7	0.002-	.03- .05	.01- .02	.3- .5	.05+
	20	0.1-	0.5-	0.2-	.1- .3	0.002-	.03- .05	.02- .04	.1- .3	.05-
	21	2.5+	0.5-	1.2+	.1- .3	0.002-	.03- .05	.02- .04	No test	.05-
	24	1.5-2.0	0.5-	1.2+	.5- .7	0.002-	.01- .03	.01- .02	.1- .3	.05-
	30	.5-1.0	0.5-	.4- .6	.1- .3	.002- .004	.05- .10	.02- .04	.05-	.05+
	34	2.5+	.5-1.0	0.2-	.1- .3	.01 - .04	.03- .05	.02- .04	0.7+	.05+
	35	1.0-1.5	0.5-	0.2-	.3- .5	0.002-	.03- .05	.02- .04	.05-.1	.05-
	38	.5-1.0	0.5-	1.2+	0.7+	0.002-	.01- .03	.01- .02	.5- .7	.05-
	39	2.0-2.5	0.5-	1.2+	0.7+	0.002-	.01- .03	.02- .04	0.7+	.05-
	40	2.5+	0.5-	0.2-	.3- .5	.006- .008	.05- .1	.02- .04	0.7+	.05-
	47C	.1- .5	0.5-	0.2-	.3- .5	.004- .006	.05- .1	.02- .04	.1- .3	.05+
	47F	2.5+	0.5-	1.2+	.3- .5	0.002-	.01- .03	.01- .02	No test	.05+
	48B	1.0-1.5	0.5-	1.2+	.3- .5	0.002-	.01- .03	.02- .04	.1- .3	.05-
	49A	0.1-	0.5-	.4- .6	.1- .3	0.002-	.01- .03	.02- .04	.05-.1	.05-
	53B	2.0-2.5	0.5-	1.2+	.5- .7	0.002-	.01- .03	.02- .04	No test	.05-
	58G	0.1-	0.5-	0.2-	.1- .3	0.002-	.03- .05	.01- .02	.05-	.05-
	60	.1- .5	0.5-	.6- .8	.1- .3	.002- .004	.03- .05	.01- .02	.05-	.05+
Re	1B	0.1-	0.5-	0.2-	.1- .3	0.002-	.03- .05	.01- .02	0.7+	.05-
G	1A	0.1-	0.5-	0.2-	.5- .7	.002- .004	.03- .05	.02- .04	.05-.1	.05+
	3	.5-1.0	0.5-	0.2-	.3- .5	0.002-	.01- .03	.02- .04	.05-.1	.05-

Table 13.---Concentrations of chemical compound bracketing the concentration which might be expected to inhibit the growth of 32 soft-rot fungi and 9 Basidiomycetes--Continued

Isolate[1]	Percentages of indicated compounds tested in malt agar bracketing the concentration preventing growth[2]								
	Sodium arsenate (Na_2HAsO_4)	Sodium borate ($Na_2B_4O_7$)	Sodium chromate (Na_2CrO_4)	Sodium fluoride (NaF)	Sodium pentachlorophenate (commercial)	Copper sulfate ($CuSO_4$)	Mercuric chloride ($HgCl_2$)	Zinc chloride ($ZnCl_2$)	Coal-tar creosote (low residue)
P 6B	0.1-	0.5-	1.2+	0.1-0.3	0.002-	0.05-0.1	0.01-0.02	0.3-0.5	0.05-
12E	0.1-0.5	1.0-2.0	0.2-	0.7+	0.002-0.004	.05- .1	.01- .02	.5- .7	.05-
13	2.5+	0.5-	0.2-	.3- .5	0.002-	0.5+	.01- .02	0.7+	.05-
16	0.1-	0.5-	0.2-	.3- .5	0.002-	.1 - .3	.02- .04	.05-	.05-
35	.1- .5	0.5-	0.2-	.1- .3	0.002-	.01- .03	.01- .02	.05-.1	.05-
36	2.5+	0.5-	1.2+	0.7+	0.002-	.03- .05	.01- .02	0.7+	.05-
S 70B	.5-1.0	0.5-	0.2-	.1- .3	0.002-	.01- .03	.02- .04	.05-.1	.05-
ML 23	0.1-	0.5-	0.2-	.1- .3	0.002-	.03- .05	.01- .02	.05-	.05-
4907	0.1-	0.5-	0.2-	.1- .3	0.002-	.05- .10	.02- .04	.05-	.05-
ML 26	.5-1.0	0.5-	0.2-	.1- .3	.002- .004	.03- .05	.05- .1	.05-.1	.05-
29	.5-1.0	0.5-	.8-1.0	.05-	0.002-	.01-	0.006-0.008	.05-	.05-
4856	1.0-1.5	0.5-	0.2-	.1- .3	0.002-	.03- .05	.02- .04	.1- .3	.05-
697	0.1-	0.5-	0.2-	No test	.008- .01	.01- .03	.02- .04	.1- .3	.05-
534	No test	0.5-	0.2-	...do...	0.002-	.01- .03	.01- .02	.05-	.05-
617	.5-1.0	0.5-	0.2-	...do...	0.002-	.03- .05	.02- .04	.05-.1	.05-
698	0.1-	0.5-	0.2-	...do...	0.002-	.05- .1	.02- .04	.05-.1	.05-

[1] R 49A (Chaetomium funicolum), R 53G (Xylaria sp.), and S 70B (Chaetomium globosum) were Ascomycetes. ML 23 (unknown brown rotter), 4907 (Poria oleracea), ML 26 (Peniophora mollis), ML 29 (unknown), 4856 (Poria nigrescens), 697 (Polyporus versicolor), 534 (Lentinus lepideus), 617 (Lenzites trabea), and 698 (Poria monticola) were Basidiomycetes. All others were Fungi Imperfecti.

[2] Minus sign indicates no growth occurred at lowest concentration tested; plus sign indicates growth at highest concentration tested.

Table 14.--Summary of results of malt-agar toxicity tests

Chemical	Indicated inhibition point	Number of isolates[1] with indicated inhibition point		Relative tolerance indicated for soft-rot fungi as a group, compared with tested Basidiomycetes
		Fungi Imperfecti and Ascomycetes	Basidiomycetes	
	Percent			
Sodium arsenate (Na_2HAsO_4)	Less than 0.1	8	4	Considerably higher
	0.1 - 0.5	4		
	.5 - 1.0	4	3	
	1.0 - 1.5	3	1	
	1.5 - 2.0	2		
	2.0 - 2.5	2		
	More than 2.5	9		
Sodium borate ($Na_2B_4O_7$)	Less than 0.5	29	9	Not clearly evident
	0.5 - 1.0	2		
	1.0 - 2.0	1		
Sodium chromate (Na_2CrO_4)	Less than 0.2	14	8	Considerably higher
	0.2 - 0.4	3		
	.4 - .6	2		
	.6 - .8	1		
	.8 - 1.0		1	
	More than 1.2	12		
Sodium fluoride (NaF)	Less than 0.05	0	1	Considerably higher
	0.1 - 0.3	11	4	
	.3 - .5	11		
	.5 - .7	6		
	More than 0.7	4		
Sodium pentachlorophenate (commercial)	Less than 0.002	22	7	Similar
	0.002 - 0.004	6	1	
	.004 - .006	1		
	.006 - .008	2		
	.008 - .01		1	
	.01 - .04	1		

Table 14.--Summary of results of malt-agar toxicity tests--continued

Chemical	Indicated inhibition point	Number of isolates[1] with indicated inhibition point		Relative tolerance indicated for soft-rot fungi as a group, compared with tested Basidiomycetes
		Fungi		
		Imperfecti Ascomycetes	Basidiomycetes	
	Percent			
Copper sulfate ($CuSO_4$)	Less than 0.01	0	1	Similar
	0.01 - 0.03	13	2	
	.03 - .05	11	4	
	.05 - .1	6	2	
	.1 - .3	1		
	More than 0.5	1		
Mercuric chloride ($HgCl_2$)	0.006 - 0.008		1	Similar
	.01 - .02	13	2	
	.02 - .04	19	5	
	.05 - .1		1	
Zinc chloride ($ZnCl_2$)	Less than 0.05	6	4	Considerably higher
	0.05 - 0.1	6	3	
	.1 - .3	4	2	
	.3 - .5	4		
	.5 - .7	2		
	More than 0.7	7		
Coal tar creosote (Low residue)	Less than 0.05	24	9	Moderately higher
	More than 0.05	8		

[1] Total number of soft-rot fungi used with all chemicals was 32, except only 29 were tested against zinc chloride. Total number of Basidiomycetes used was 9, except only 8 were tested against sodium arsenate and 5 against sodium fluoride.

Table 15.--Soft-rot isolates: their code designation, tentative identification, wood and product from which isolated and geographic source[1]

Designation	Tentative identification[2]		Source of isolate		
	Group	Genus	Wood	Product[3]	Locality
R 1	Fungi Imperfecti	Phialophora richardsiae (Nannf.) Conant	Redwood	Cooling tower	Philadelphia, Pa.
R 2	do	Cytosporella	do	do	Lima, Ohio
R 5	do	Alternaria	do	do	Meadville, Pa.
R 6	do	Coniothyrium; Sporocybe; Acremonium	do	do	Philadelphia, Pa.
R 7	do	Phialophora richardsiae (Nannf.) Conant	do	do	Front Royal, Va.
R 8	do	Phialophora sp.	do	do	Lawtonia, Ky.
R 9	do	Cytosporella	do	do	San Angelo, Tex.
R 10	do	Cytosporella, Phoma	do	do	Lawrenceville, Ill.
R 11	do	Sporocybe, Acremonium	do	do	Do.
R 12	do	Phialophora sp.	do	do	Baton Rouge, La.
R 13	do	Hormiscium	do	do	Forest City, Ark.
R 14	do	Cytosporella	do	do	San Angelo, Tex.
R 15	do	Chalaropsis, Pullularia	do	do	Jackson, Miss.
R 18	do	Sporocybe, Acremonium	do	do	Arizona
R 20	do		do	do	Philadelphia, Pa.
R 21	do	Sporocybe, Acremonium	do	do	Whiting, Ind.
R 22	do	Cytosporella	do	do	Do.
R 23	do	do	do	do	Etiwanda, Calif.
R 24	do	Sporocybe	do	do	Handly, Tex.
R 26	do		do	do	Albuquerque, N. Mex.
R 27	do		do	do	Do.
R 28	do	Cytosporella	do	do	Do.
R 29	do	Phoma, Cytosporella	do	do	Do.
R 30	do	Phialophora richardsiae (Nannf.) Conant	do	do	Do.
R 31	do	Phialophora sp.	do	do	Do.
R 32	do	Phoma, Cytosporella	do	do	St.Catherines' Ont.
R 34	do		do	do	Jonesboro, Ark.
R 35	do	Cephalosporium	do	do	Oakridge, Tenn.
R 37A	do	Nematogonium	Douglas-fir	do	Amarillo, Tex.
R 37B	do	Phialophora richardsiae (Nannf.) Conant	do	do	Do.
R 38	do	Sporocybe, Haplochalara	Redwood	do	Whittier, Calif.
R 39	do	Sporocybe, Acremonium	do	do	Lima, Ohio
R 40	do	Cytosporella	do	do	Jackson, Miss.
R 41	do		do	do	Do.
R 42A	do		do	do	Do.
R 42B	do	Phialophora sp.	do	do	Do.

Table 15.--Soft-rot isolates: their code designation, tentative identification, wood and product from which isolated and geographic source[1] -- continued

Designation	Tentative identification[2]		Source of isolate		
	Group	Genus	Wood	Product[3]	Locality
R 44	Fungi Imperfecti		Redwood	Cooling tower	Enterprize, Miss.
R 45	do		do	do	Pittsburg, Pa.
R 46B	do		do	do	Glendale, Calif.
R 47A	do		do	do	Whiting, Ind.
R 47C	do	Cephalosporium	do	do	Do.
R 47F	do	Sporocybe, Acremonium	do	do	Do.
R 48A	do		do	do	California
R 48B	do	Sporocybe, Acremonium	do	do	Do.
R 49A	Ascomycetes	Chaetomium funicolum Cooke	do	do	Tuscon, Ariz.
R 49B	Fungi Imperfecti		do	do	Do.
R 49C	do	Phialophora richardsiae (Nannf.) Conant	do	do	Do.
R 50A	do		do	do	Riverside, Calif.
R 51A	do		do	do	Etiwanda, Calif.
R 51B	do		do	do	Do.
R 51C	do		do	do	Do.
R 52B	do	Phialophora richardsiae (Nannf.) Conant	do	do	Lordsburg, N. Mex.
R 53B	do	Sporocybe, Acremonium	do	do	Amarillo, Tex.
R 54A	do	Phialophora richardsiae (Nannf.) Conant	do	do	Jackson, Miss.
R 54B	do		do	do	Do.
R 55B	do	Phialophora richardsiae (Nannf.) Conant	do	do	Sugar Creek, Mo.
R 55F	do		do	do	Do.
R 56A	do		do	do	Baton Rouge, La.
R 56B	do	Phialophora richardsiae (Nannf.) Conant	do	do	Do.
R 57B	do		do	do	Los Angeles, Calif.
R 57D	do		do	do	Do.
R 58A	do		do	do	Savannah River, Ga.
R 58B	do		do	do	Do.
R 58C	do		do	do	Camden, S. C.
R 58E	do		do	do	Chattanooga, Tenn.
R 58F	do		do	do	Kinston, N. C.
R 58G	Ascomycetes	Xylaria sp.	do	do	Rochester, N. Y.
R 59A	Fungi Imperfecti		do	do	Do.
R 59B	do		do	do	Do.
R 60	do	Phialophora richardsiae (Nannf.) Conant	do	do	Do.
R 61	do		do	do	Philadelphia, Pa.
R 62	do		do	do	St. Louis, Mo.

Table 15.--Soft-rot isolates: their code designation, tentative identification, wood and product from which isolated and geographic source[1] -- continued

Designation	Tentative identification[2]		Source of isolate		
	Group	Genus	Wood	Product[3]	Locality
R 63A	Fungi Imperfecti		Redwood	Cooling tower	Kinston, N. C.
R 63B	do		do	do	Do.
R 63C	do		do	do	Do.
R 63D	do		do	do	Do.
R 63E	do		do	do	Do.
R 63F	do		do	do	Do.
R 63G	do		do	do	Do.
R 64	Ascomycetes	Chaetomium cochliodes Palliser	do	do	
R 66	do	Chaetomium globosum Kunze	do	do	Milwaukee, Wis.
R 67	Fungi Imperfecti		do	do	Savannah River, Ga.
R 68	do		do	do	Do.
R 69	do		do	do	Do.
R 70	do	Phialophora sp.	do	do	Do.
R 71	do	do	do	do	Do.
R 72	Ascomycetes	Chaetomium funicolum Cooke	do	do	Los Angeles, Calif.
R 73	do	do	do	do	Paducah, Ky.
R 74	Fungi Imperfecti		do	do	Los Angeles, Calif.
R 75	do	Cephalosporium	do	do	Port Arthur, Tex.
P 1A	do	Phialophora sp.	Pine	Railroad car (Wolman salts)	Ohio
P 2B	do		do	do	Do.
P 5A	do		do	Boat	Madison, Wis.
P 6B	do	Bisporomyces, Haplochalara	do	Ammunition box	Panama Canal Zone
P 7A	Ascomycetes	Chaetomium cochliodes Palliser	do	Telephone pole (Pentachlorophenol)	Cincinnati, Ohio
P 10A	do	Chaetomium globosum Kunze	do	Telephone pole (creosote)	Do.
P 11	Fungi Imperfecti		do	Telephone pole (zinc-meta-arsenite)	Illinois
P 12A	do		do	Test stake (rosinamine D, pentachlorophenate)	Madison, Wis.
P 12B	Ascomycetes	Chaetomium funicolum Cooke	do	Test stake (pentachlorophenol)	Do.

Table 15.--Soft-rot isolates: their code designation, tentative identification, wood and product from which isolated and geographic source[1] -- continued

Designation	Tentative identification[2]		Source of isolate		
	Group	Genus	Wood	Product[3]	Locality
P 12C	Fungi Imperfecti		Pine	Test stake (pentachloro-phenol)	Madison, Wis.
P 12D	do.		do.	Test stake (rosinamine D penta-chlorophenate)	Do.
P 12E	do.	Alternaria	do.	Test stake (drop liquor concentrate)	Do.
P 12F	do.		do.	Test stake (CCZC)	Do.
P 12G	do.	Torula	do.	Test stake (pyrosote)	Do.
P 12I	do.		do.	Test stake (CZC)	Do.
P 13	do.	Cephalosporium	do.	Telephone pole (zinc-meta-arsenite	Illinois
P 14	do.		do.	Test stake (copper naphthenate)	Madison, Wis.
P 15	do.		do.	Test stake (Urea "Bl" glue)	Do.
P 16	do.	Pestalozzia sp.	do.	Test stake (creosote-petroleum)	Texas
P 17	do.		do.	Test stake	Madison, Wis.
P 18	do.		do.	Telephone pole (creo-sote)	
P 19	do.		do.	do.	Denver, Colo.
P 21	do.		do.	Test stake (Minilith)	Saucier, Miss.
P 22	do.		do.	Test stake	Do.
P 23	do.		do.	Test stake (zinc chloride)	Do.
P 24	do.		do.	Test stake (Tanalith)	Do.
P 25	do.		do.	Test stake (Boliden salts)	Do.

Table 15.--Soft-rot isolates: their code designation, tentative identification, wood and product from which isolated and geographic source[1] -- continued

Designation	Tentative identification[2]		Source of isolate		
	Group	Genus	Wood	Products[3]	Locality
P 26A	Fungi Imperfecti	Alternaria sp.	Pine	Test stake (copper naphthenate)	Saucier, Miss.
P 26B	do.	Dendryphium; Diplococcium	do.	do.	Do.
P 27	do.	Helicosporium aureum (Corda) Linder.	do.	Top of test stake	Madison, Wis.
P 28	do.		do.	Test stake	Corvallis, Ore.
P 29	do.		do.	Test stake (Boliden salts)	Do.
P 30	do.		do.	Test stake (rosin oil)	Do.
P 31	do.		do.	Test stake (fuel oil)	Do.
P 32	do.		do.	Test stake (pentachloro-phenol)	Do.
P 33	do.		do.	do.	Do.
P 34	do.		do.	Test stake (oleo-resin)	Do.
P 35	Ascomycetes	Orbicula	do.	Test stake (Tanalith)	Saucier, Miss.
P 36	Fungi Imperfecti	Coniothyrium; Acremonium; Sporocybe	do.	Telephone pole (creosote)	Oregon
P 37	do.		do.	Pier (copper formate)	Florida
P 38	do.		do.	Test stake (pyrosote)	Corvallis, Ore.
P 39	Ascomycetes	Ceratocystis pilifera (Fries) C. Moreau	do.	Lumber	Medford, Ore.
P 40	do.	Pestalozzia sp.	do.	Test stake (copper naphthenate)	Saucier, Miss.
P 41	do.	do.	do.	Test stake (creosote)	Orange Park, Fla.
Re 1A	do.		Redwood	Test stake	Madison, Wis.
Re 1B	do.	Pestalozzia	do.	do.	Do.
Re 1C	do.		do.	do.	Do.
G1A	do.	Haplochalara, Cephalosporium	Sweetgum	Lumber	Charleston, S. C.
G 2	do.		do.	Plywood (treated)	Saucier, Miss.

Table 15.--Soft-rot isolates: their code designation, tentative identification, wood and product from which isolated and geographic source[1] -- continued

Designation	Tentative identification[2]		Source of isolate		
	Group	Genus	Wood	Product[3]	Locality
G 3	Fungi Imperfecti	Stysanus sp.	Sweet-gum	Plywood	Madison, Wis.
G 4	...do...	Sclerotium sp.	...do...	Test blocks	Do.
G 5	Ascomycetes	Chaetomium cochliodes Palliser	...do...	Plywood	Do.
O 2	Fungi Imperfecti		Scarlet oak	Test post (treated)	Norris, Tenn.
YP 1	...do...	Bonordeniella sp.	Yellow poplar	Plywood	Madison, Wis.
L 1	...do...		Locust	Insulator pin on telephone pole	Johnston, Pa.
L 2	Ascomycetes	Chaetomium globosum Kunze	...do...	...do...	Do.
C 1	Fungi Imperfecti		Cedar	Post	Ann Arbor, Mich.
C 1B	...do...		...do...	...do...	Do.
1669	Ascomycetes	Chaetomium globosum Kunze			
E 1	...do...	...do...	Elm	Mushroom house	England
CBS	Fungi Imperfecti	Bispora effusa Pk.			Centraalbureau voor Schimmelcultures, Holland
FPRL S 132	...do...	Bispora pusilla Sacc.	Scots pine	Cooling tower	Forest Products Laboratory, Princes Risborough England
FPRL S 70B	Ascomycetes	Chaetomium globosum Kunze	Picea	...do...	Do.
FPRL S 121	...do...	Chaetomium funicolum Cooke	Laurelia aromatica (Chile)	Lumber	Do.
FPRL S 109C	...do...	Chaetomium cochliodes Palliser	...do...	...do...	Do.
FPRL S 603	Fungi Imperfecti	Coniothyrium sp.	Eucalyptus saligna (S. Africa)		Do.

Table 15.--Soft-rot isolates: their code designation, tentative identification, wood and product from which isolated and geographic source[1] -- continued

Designation	Tentative identification[2]		Source of isolate		
	Group	Genus	Wood	Product[3]	Locality
FPRL S 102	Ascomycetes	*Orbicula porietina* (Schrad. ex. Fr.) Hughes	Scots pine	Cooling tower	Forest Products Laboratory, Princes Risborough, England
FPRL S 91	Fungi Imperfecti	Stysanus sp.	Hard-wood	Plywood	Do.
FPRL S 605	do.	do.	Scots pine	Test blocks	Do.
FPRL D 128	do.	*Trichurus terrophilus* Swift and Povah	*Eucalyptus saligna* (S. Africa)	Fence post	Do.

[1] During course of this study all isolates listed caused soft-rot as determined by loss in weight, reduction in bending tolerance, or microscopical attack of secondary wall in wood. Most frequently isolated fungi have been placed tentatively in the genera: Phialophora, Cytosporella, Phoma, Coniothyrium, Acremonium, and Pestalozzia. No names have been suggested for some of those frequently isolated.

[2] All identifications are tentative and subject to change on further study. When two or more names have been suggested for an isolate all are given.

[3] Where product contained treated wood, preservative present is indicated.

Table 16.--Summary of incubation methods used for testing the capacity of the various isolates to attack wood

Test No.	Incubation substrate	Fungi tested	Test specimen			Incubation period
			Kind and size	Species	Preliminary treatment	
1	Silt loam soil (100 grams oven-dry basis), containing moisture equal to 130 percent of its water-holding capacity, placed in an 8-ounce bottle in upright position. Essentially the same as ASTM D 1413 (1956) except the test specimen was placed directly on the soil, i.e., without an intervening feeder block. Sterilized at 15 pounds pressure for 30 minutes.	Basidiomycetes: 9 Ascomycetes: 2 Fungi Imperfecti: 26 Many were later shown to be mixed cultures.	Block: 1 by 1 by 0.25 inch (along grain)	Sweetgum sapwood	(a) None (b) Leached in running tap water for 2 weeks.	24 weeks
				Southern pine sapwood	(a) None (b) Leached in running tap water for 2 weeks.	
				Redwood heartwood	(a) None (b) Leached in distilled water for 1 week with heat (100°C.) for 1 hour each day, followed by change of water (c) Leached in 0.2 percent sodium carbonate solution for five 1-hour periods (100°C.) with change of solution after each steaming.	
2	Silt loam soil (100 grams oven-dry basis), containing moisture equal to 175 percent of its water-holding capacity, placed in an 8-ounce bottle in horizontal position.	Ascomycete: 1 Fungi Imperfecti: 3	Veneer strip: 0.0625 inch thick, cut to 0.5 by 3 inches (along the grain)		Half of all specimens impregnated with mineral solution (minus vitamins)[1] after treatment.	12 weeks
				Sweetgum sapwood	(a) One-fourth specimens leached by heating in water (100°C.) for 1 hour, transferred to fresh water for 23 hours. Process repeated three times.	

Table 16.--Summary of incubation methods used for testing the capacity of the various isolates to attack wood--Continued

Test No.	Incubation substrate	Fungi tested	Test specimen			Incubation period
			Kind and size	Species	Preliminary treatment	
2	(Cont.)			Ponderosa pine sapwood	(b) Same as (a) followed by steaming for 1 hour in 2.62 percent sodium hypochlorite solution.	
				Redwood heartwood	(c) Same as (a) followed by leaching in 0.2 percent sodium carbonate solution for five 1-hour periods (100°C.) with change of solution after each steaming.	
					(d) Same as (a) followed by leaching at 60°C. in equal parts of glacial acetic acid and hydrogen peroxide (3 percent H_2O_2) for 2 days.	
3	Soil substrate used same as in Test 2, with the addition of 2 percent peptone in the soil water.	Fungi Imperfecti: 60	Veneer strip: 0.0625 inch thick, cut to 0.5 by 3 inches (along the grain)	Sweetgum sapwood	None	8 weeks

Table 16.--Summary of incubation methods used for testing the capacity of the various isolates to attack wood--Continued

Test No.	Incubation substrate	Fungi tested	Test specimen			Incubation period
			Kind and size	Species	Preliminary treatment	
4	(a) Soil substrate; as in Test 2 except enriched with manure compost. (b) 25 cc. mineral-vitamin agar[1] placed in 80 cc.-capacity test tube, sterilized at 15 pounds pressure for 15 minutes and allowed to harden with a 4-inch surface slant. Sterile 1 by 3 inch filter paper put on agar surface.	Ascomycetes: 2 Fungi Imperfecti: 23	Veneer strip: 0.0625 inch thick, cut to 0.5 by 1 inch (along the grain)	Sweetgum sapwood Beech sapwood Ponderosa pine sapwood	 Leached in sodium hypochlorite solution (2 p.p.m. available chlorine), changed daily for 3 weeks.	8 weeks
5	Same as Test 2.	Ascomycetes: 3 Fungi Imperfecti: 95 Basidiomycetes: 6	Block: 0.75 by 0.375 inch (thick) by 2.25 inches (along the grain)	Sweetgum sapwood Southern pine sapwood Redwood heartwood	None (a) None (b) Leached alternately in running water for 8 hours and standing in water for 16 hours for 30 days. (a) Leached in distilled water (43°C.) with daily change for 30 days. (b) Leached in sodium hypochlorite solution (2 p.p.m. available chlorine) kept at 43°C. changed daily for 6 weeks Half of all blocks impregnated with mineral-vitamin solution[1]	36 weeks

Table 16.--Summary of incubation methods used for testing the capacity of the various isolates to attack wood--Continued

Test No.	Incubation substrate	Fungi tested	Test specimen			Incubation period
			Kind and size	Species	Preliminary treatment	
6	Silt loam soil with moisture content equal to 175 percent of its water-holding capacity, placed in an 8-ounce bottle standing upright. Sterilized at 15 pounds pressure for 30 minutes. Test block buried 1.75 inches deep in soil.	Ascomycetes: 2 Fungi Imperfecti: 22	Block: 0.75 by 0.375 inch (thick) by 2.25 inches (along the grain)	Redwood heartwood	None	104 weeks
7	(a) Silt loam soil with moisture content equal to 130 percent of its water-holding capacity, placed in an 8-ounce bottle standing upright. (b) Potting soil with added compost and moisture content equal to 130 percent of its water-holding capacity. Test specimen pushed vertically 2 inches into soil.	Fungi Imperfecti: 14	Veneer strip: 0.0625 inch thick, cut to 0.5 by 3 inches (along the grain)	Sweetgum sapwood Beech sapwood Ponderosa pine sapwood	(a) None (a) None (a) None (b) Leached in sodium hypochlorite solution (1 p.p.m. available chlorine) for 2 weeks.	8 weeks
8	Mineral-vitamin solution[1] (50 cc.) placed in 250 cc. flask and sterilized 15 pounds pressure for 20 minutes. Test specimen inserted so that one end touched bottom and the other rested against the side of the flask, above the solution. Inoculated by shake-culture technique.	Basidiomycetes: 2 Ascomycetes: 1 Fungi Imperfecti: 17	Veneer strip: 0.0625 inch thick cut to 0.5 by 3 inches (along the grain)	Sweetgum sapwood Southern pine sapwood Redwood heartwood	None None None	2 and 4 weeks

[1] The mineral-vitamin solution, used as a solution solidified with agar, or for impregnation of test specimens, contained 4 basic minerals, 8 trace elements, and 4 vitamins. The amounts, as grams per liter of distilled water were: ammonium nitrate (NH_4NO_3), 3.0; potassium phosphate (K_2HPO_4), 2.0; potassium dihydrogen phosphate (KH_2PO_4), 2.5; and magnesium sulfate ($MgSO_4 \cdot 7H_2O$), 2.0; manganese sulfate ($MnSO_4$), 0.0044; ferric sulfate ($Fe_2(SO_4)_3$), 0.0025; zinc sulfate ($ZnSO_4$), 0.0015; boric acid (H_3BO_3), 0.0016; calcium chloride ($CaCl_2$), 0.01; sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$), 0.001; cobalt chloride ($CoCl_2$), 0.0006; and copper sulfate ($CuSO_4$), 0.005.
The vitamins added, as milligrams per liter, were: nicotinic acid, 0.0005; thiamine hydrochloride, 0.0001; pyridoxine hydrochloride, 0.0005; and calcium pantothenate, 0.0001.

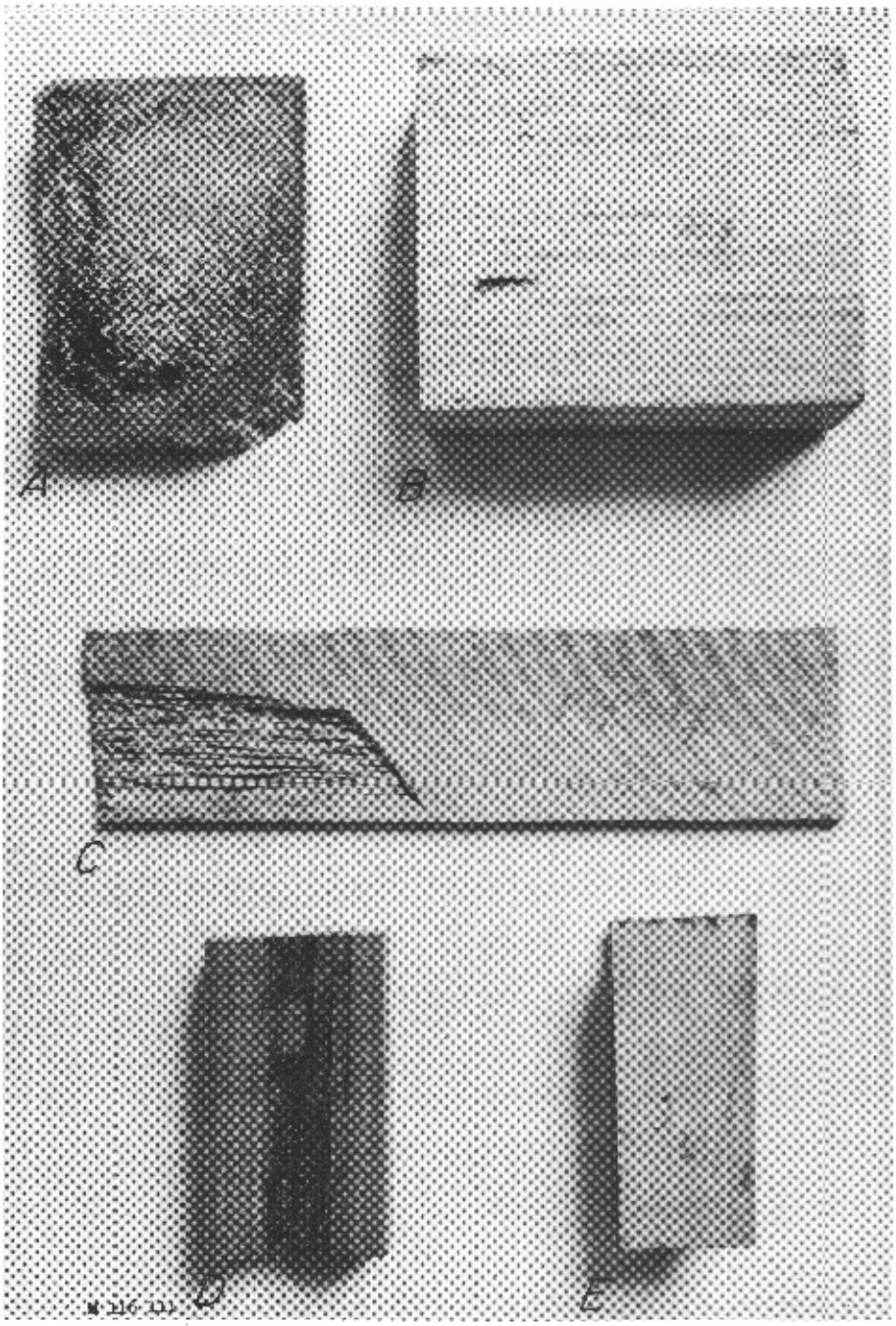

Figure 1. --Interior decay caused by fungi of Basidiomycete group in redwood of cooling towers. A, B, and C: fibrous to pocket-type white rot caused by Poria nigrescens - A, interior and B, exterior of structural timber; C, slat. D and E: cubical, brown rot in a structural timber, caused by Poria oleraceae - D, interior, showing the charred appearance of the decay, and E, exterior of the same timber.

ZM 116 111

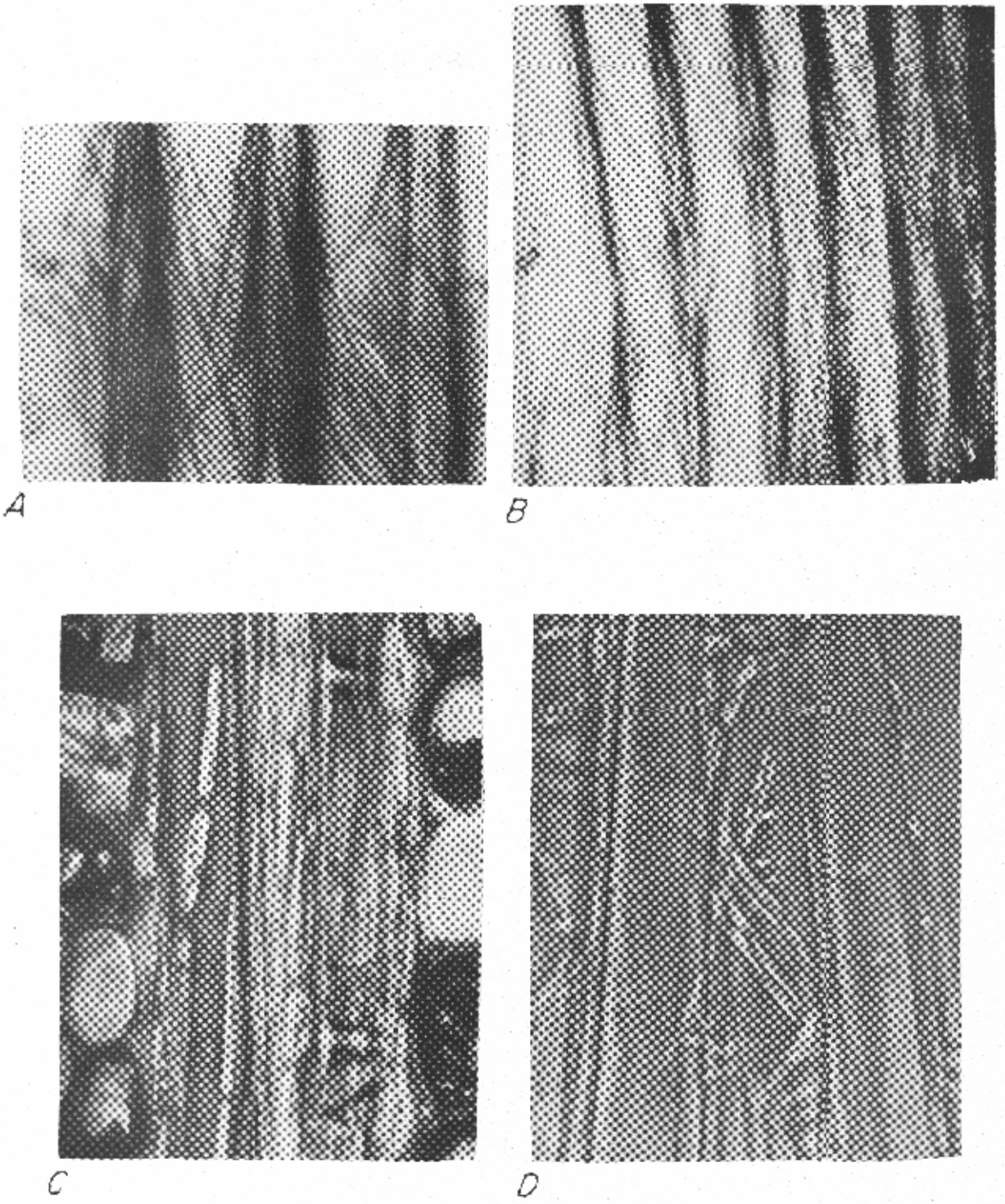

Figure 2. --Longitudinal microscopical sections of soft-rotted wood. A and B: Pine showing the typical spiral course of the hyphae within the secondary wall. Thickening of the hyphal walls may be noted in a portion of B. C and D: Minusops sp. showing the hyphae lying within larger spindle-shaped cavities which they have formed in the secondary wall.

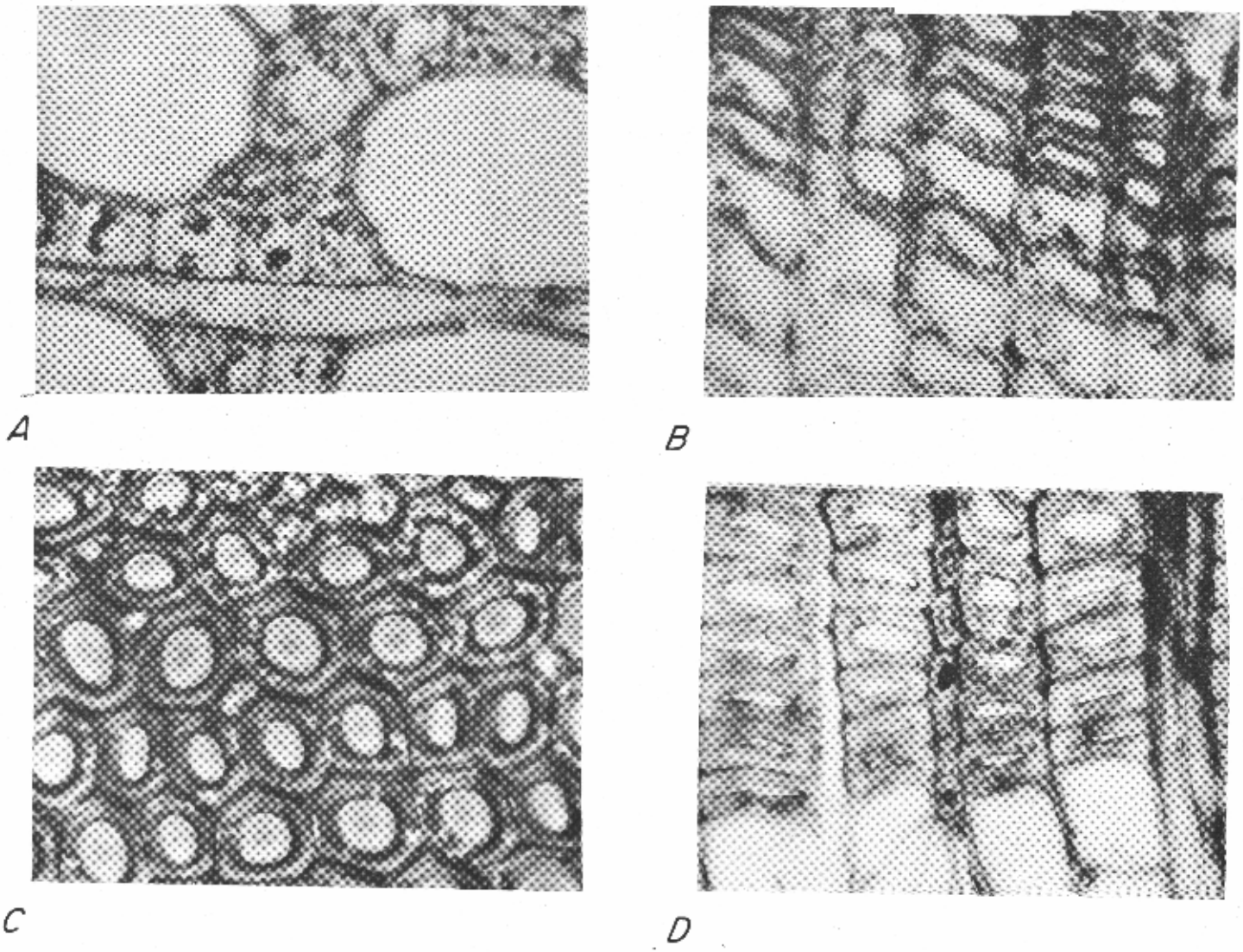

Figure 3.--Transverse microscopical sections of soft-rotted wood. A, sweetgum; B, pine; C, Minusops sp.; and D, redwood. Darker areas within the wall are fungal hyphae cut transversely.

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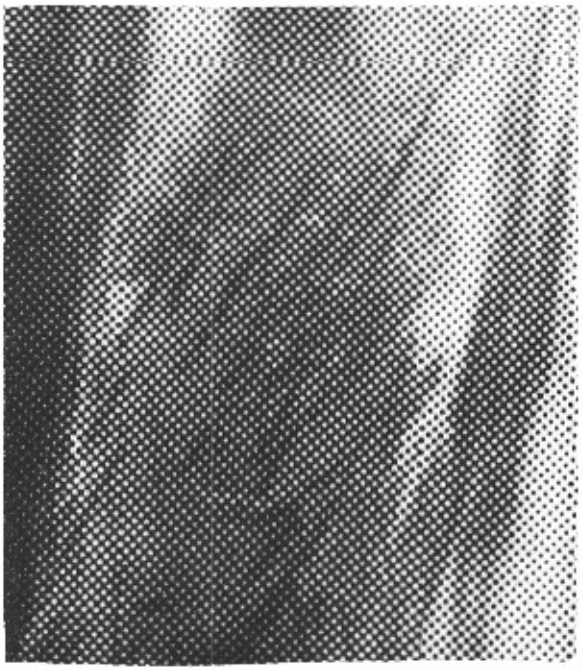
A



B

Figure 4.--A, cross- and B, longitudinal microscopical sections of redwood decayed by the white-rot fungus, Poria nigrescens, showing the similarity of its attack to that by soft-rot fungi. P. nigrescens is the only Basidiomycete decay fungus that has been observed to develop within the secondary wall.

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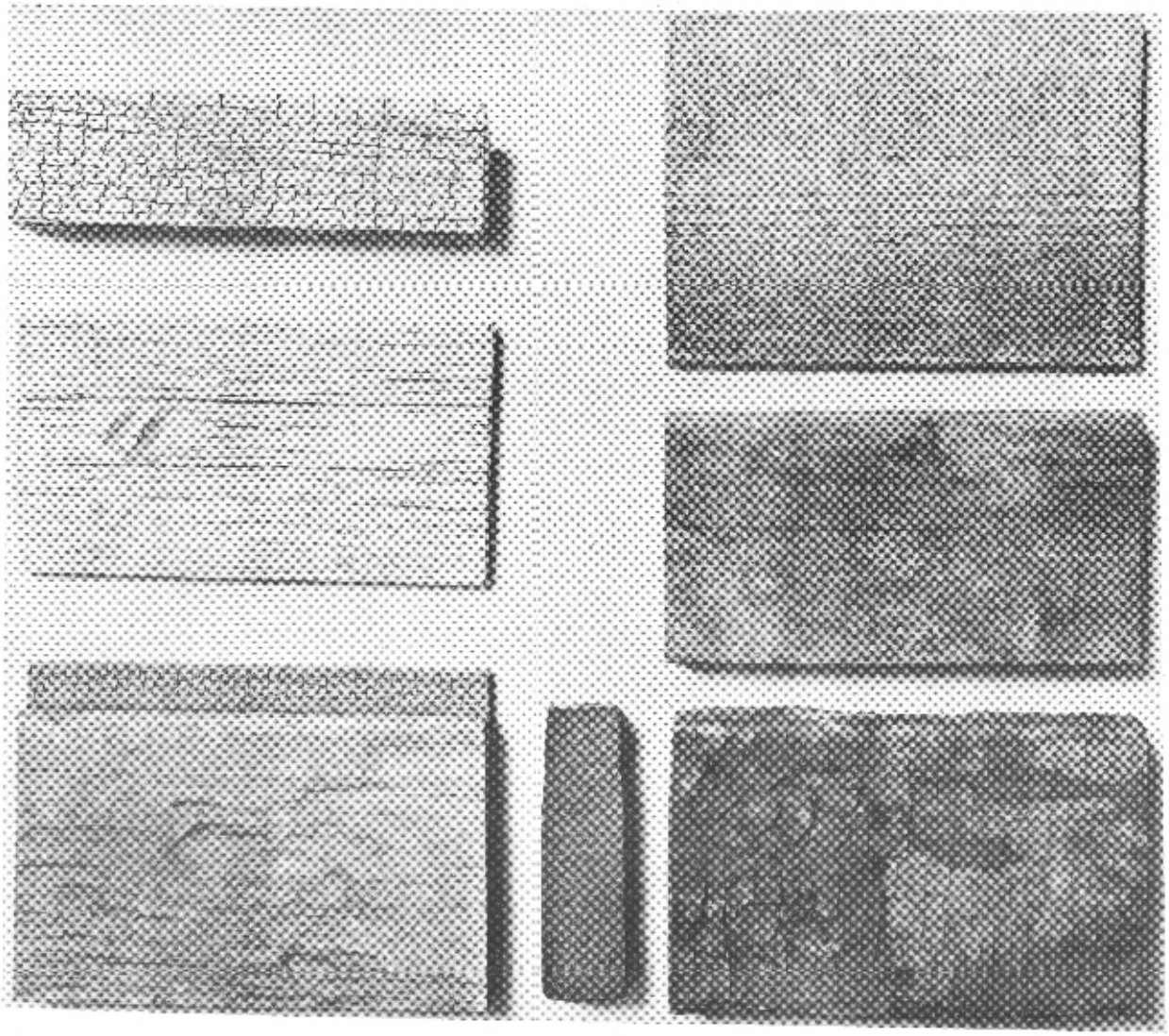

Figure 5. --Characteristic cross-checking of the softened surface of redwood members from cooling towers attacked by soft-rot fungi.

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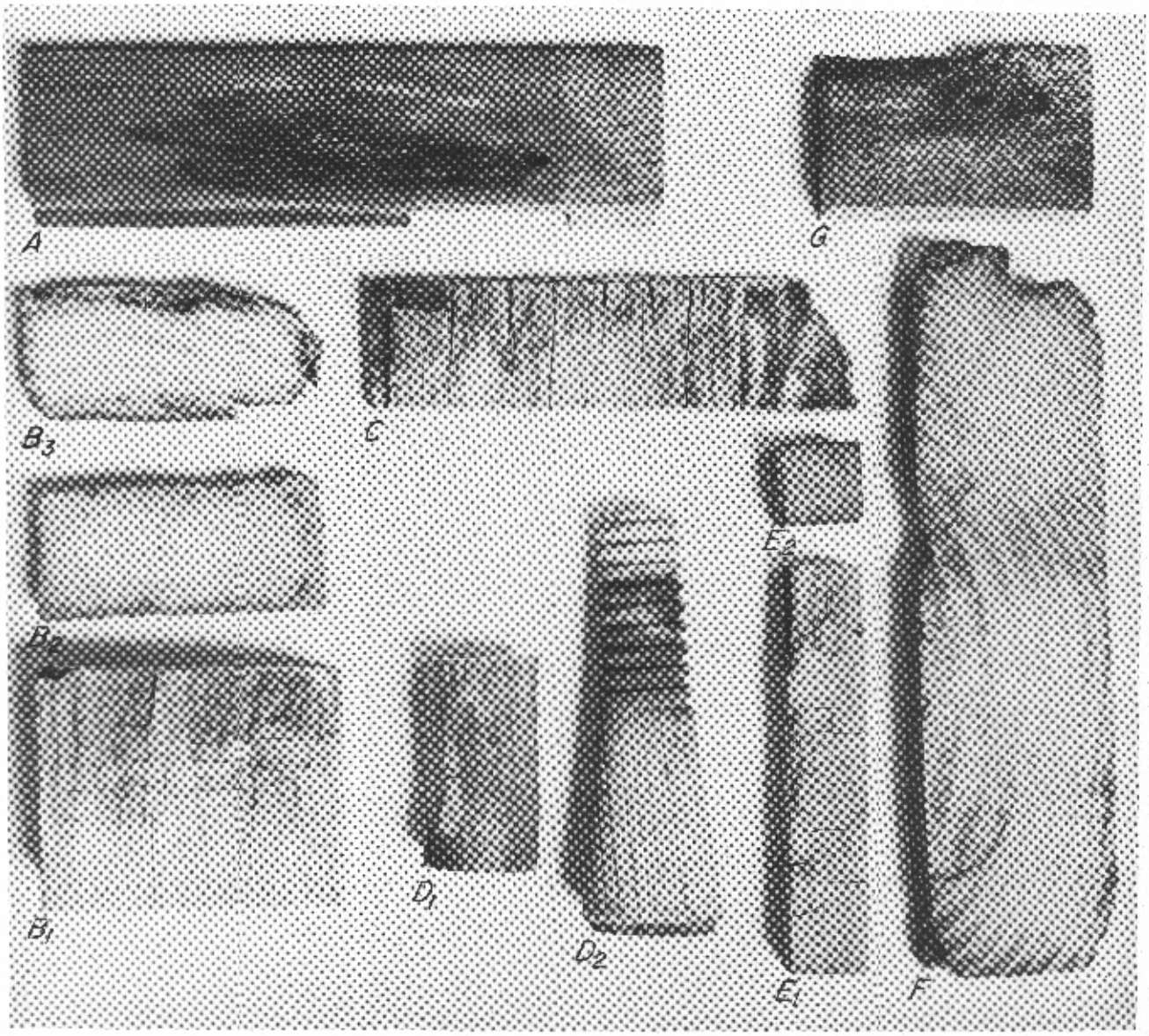

Figure 6. --Soft-rot in various types of products. A: section of a ponderosa pine window frame that had been stored in a warm, damp room. B 1, 2, and 3: cross sections and an exterior view of a preservative treated southern pine stake. C: sweetgum plywood panel exposed above ground. D 1 and 2: locust eliminator pins from a telephone pole. E 1 and 2: cross section and exterior of a redwood stake. F: preservative-treated southern pine from a railroad car. G: Yew fence post.

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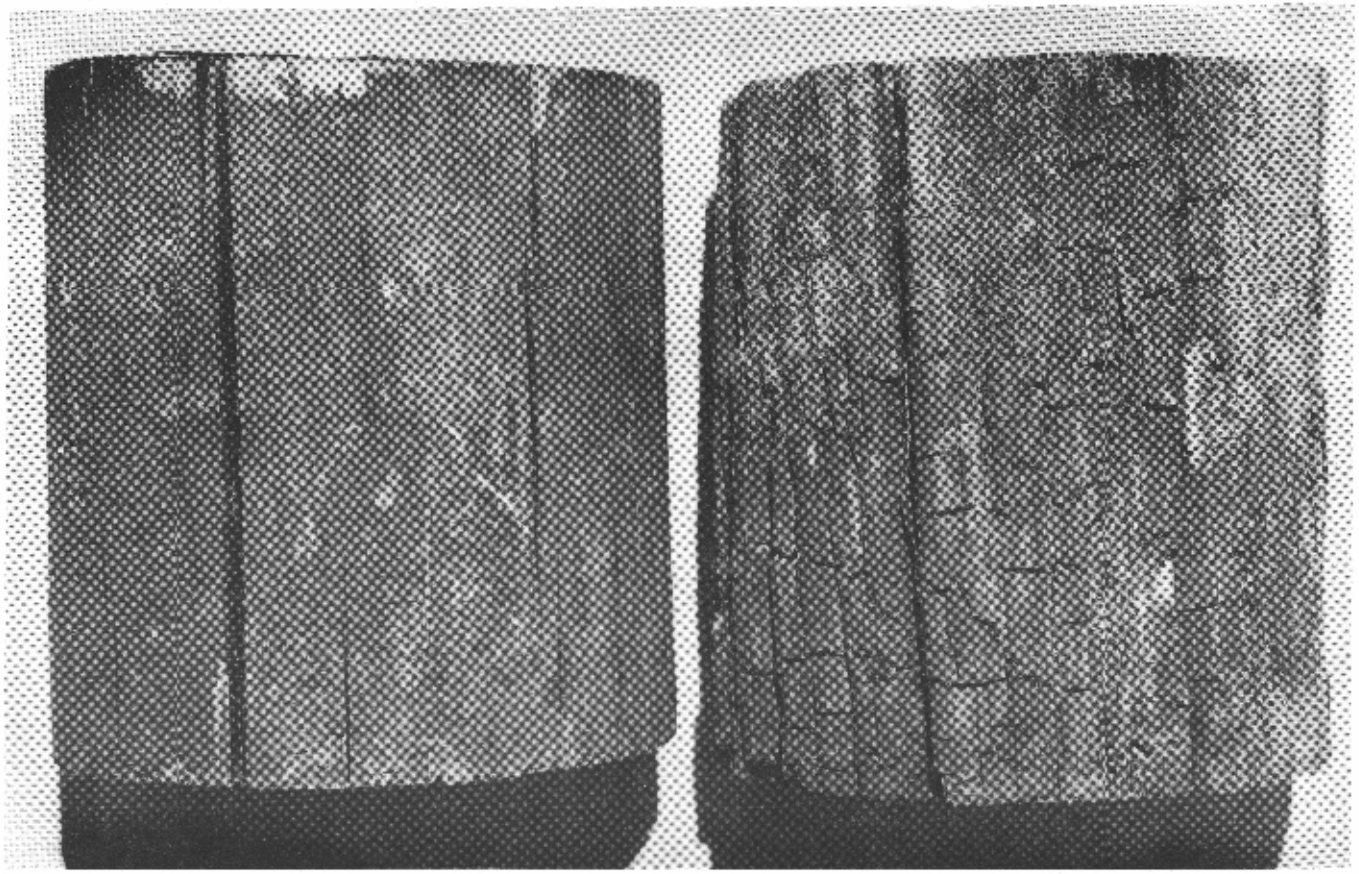

Figure 7. --Soft-rot in above-ground (left) and below-ground (right) segments of a preservative-treated telephone pole.

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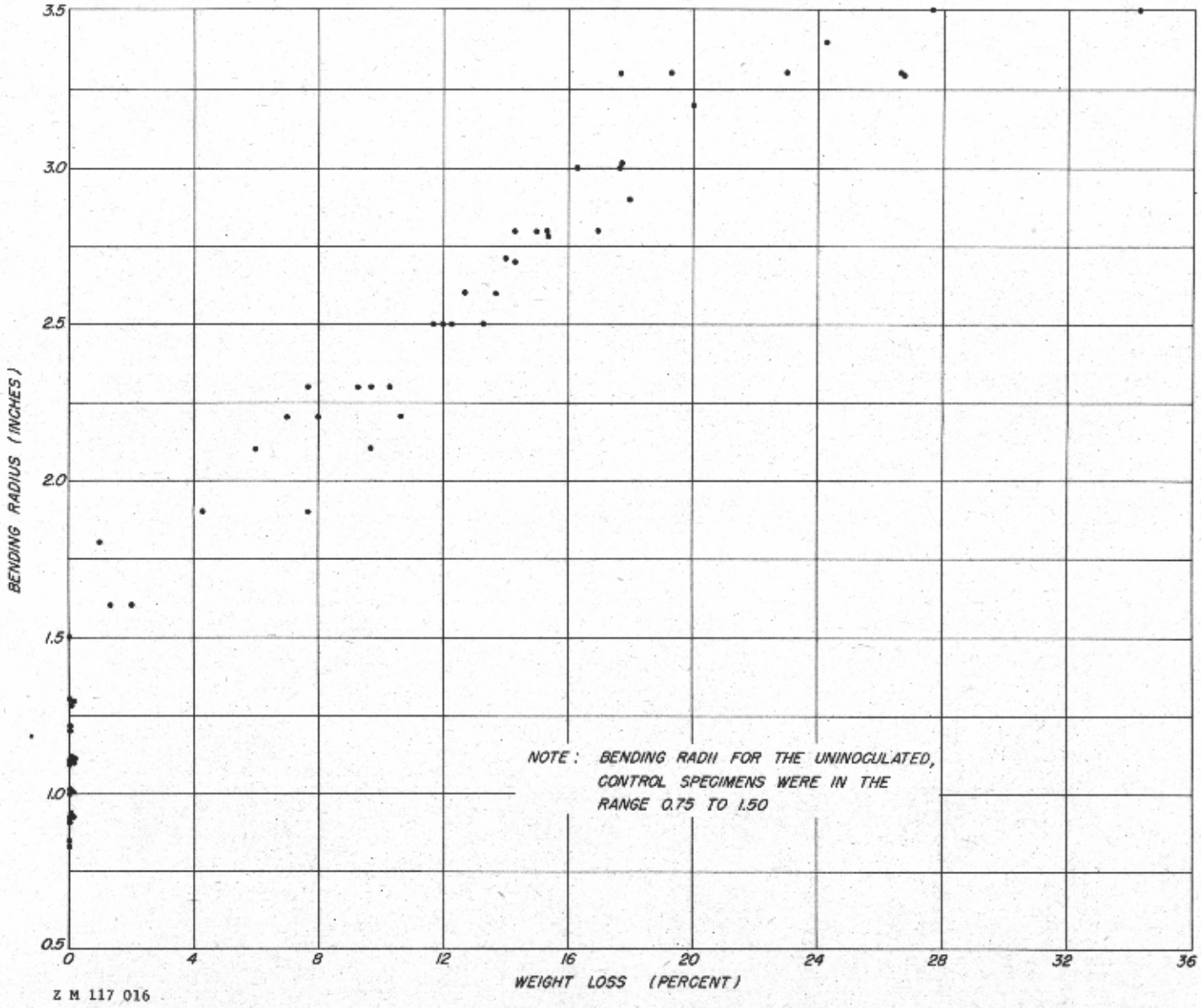

Figure 8. --Minimum bending radius and weight loss of inoculated veneer strips in Test No. 3. Inoculations were made with 60 isolates of Fungi Imperfecti. Each point represents the average radius and weight loss obtained with a different isolate on three different strips.

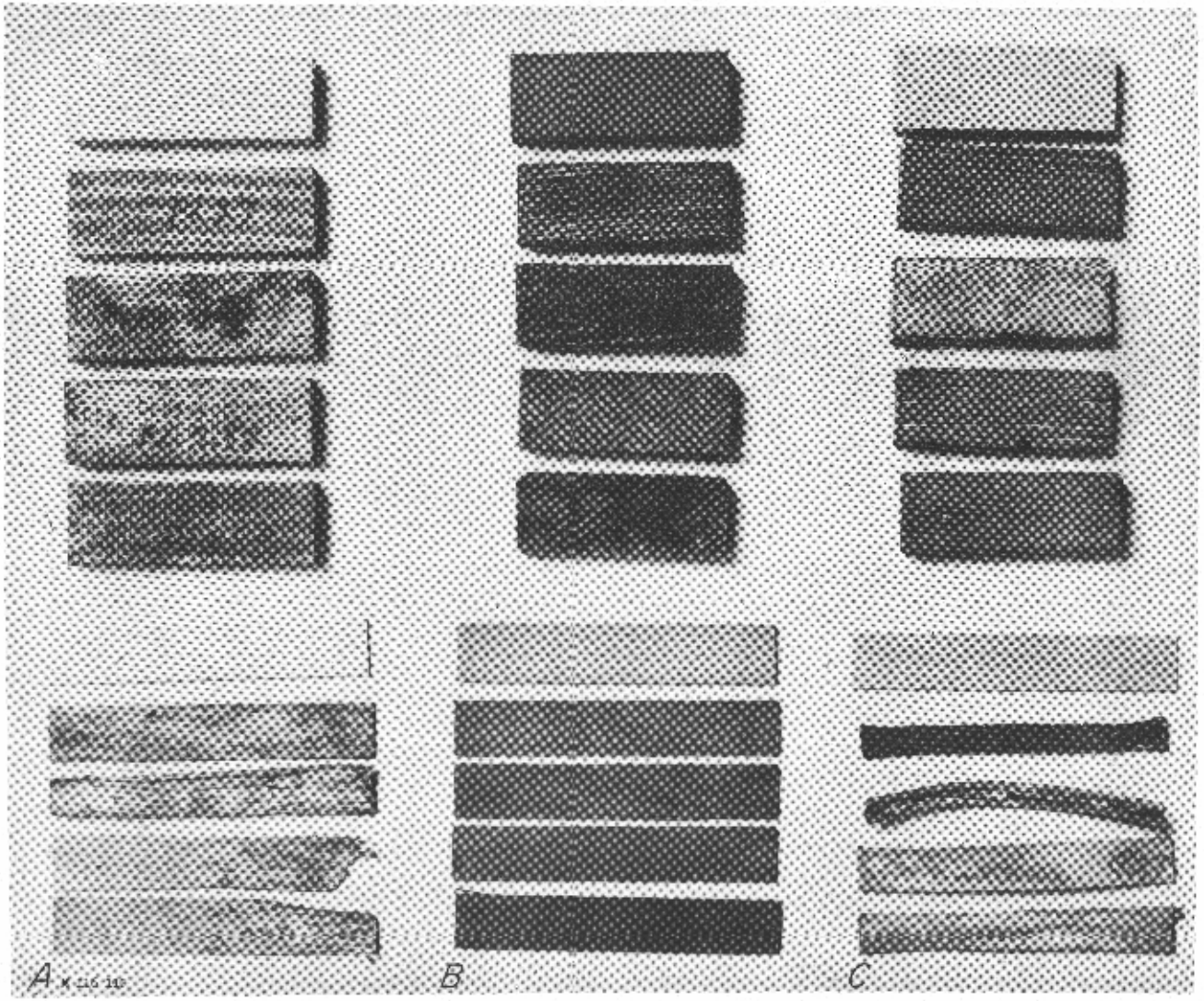

Figure 9. --Various degrees of attack by soft-rot fungi on pine (A), redwood (B), and sweetgum (C) test blocks and veneer strips. The uppermost specimen in each group was not inoculated.

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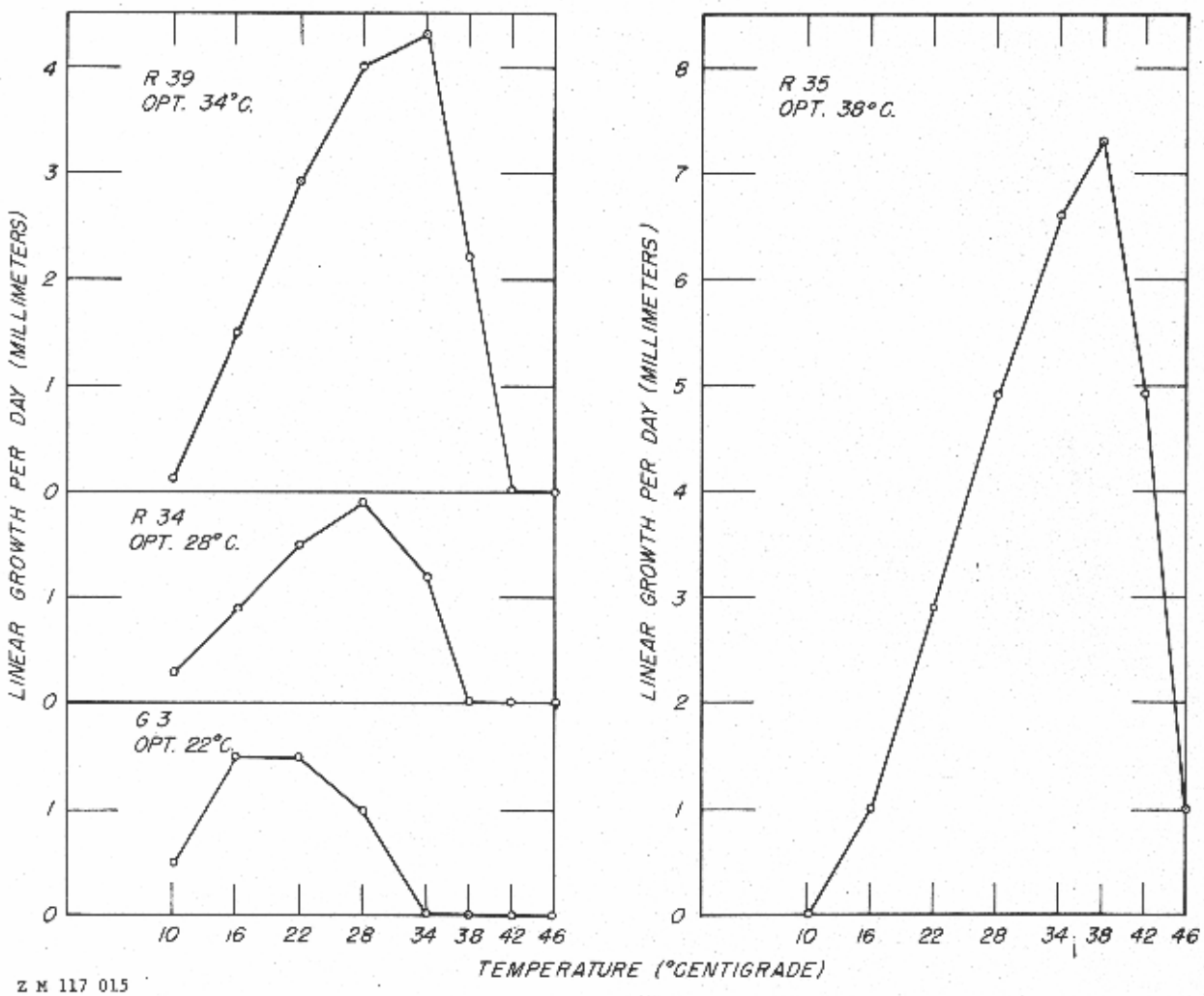

Figure 10. --Growth-temperature curves for four of the soft-rot fungi, representing groups with different optima.

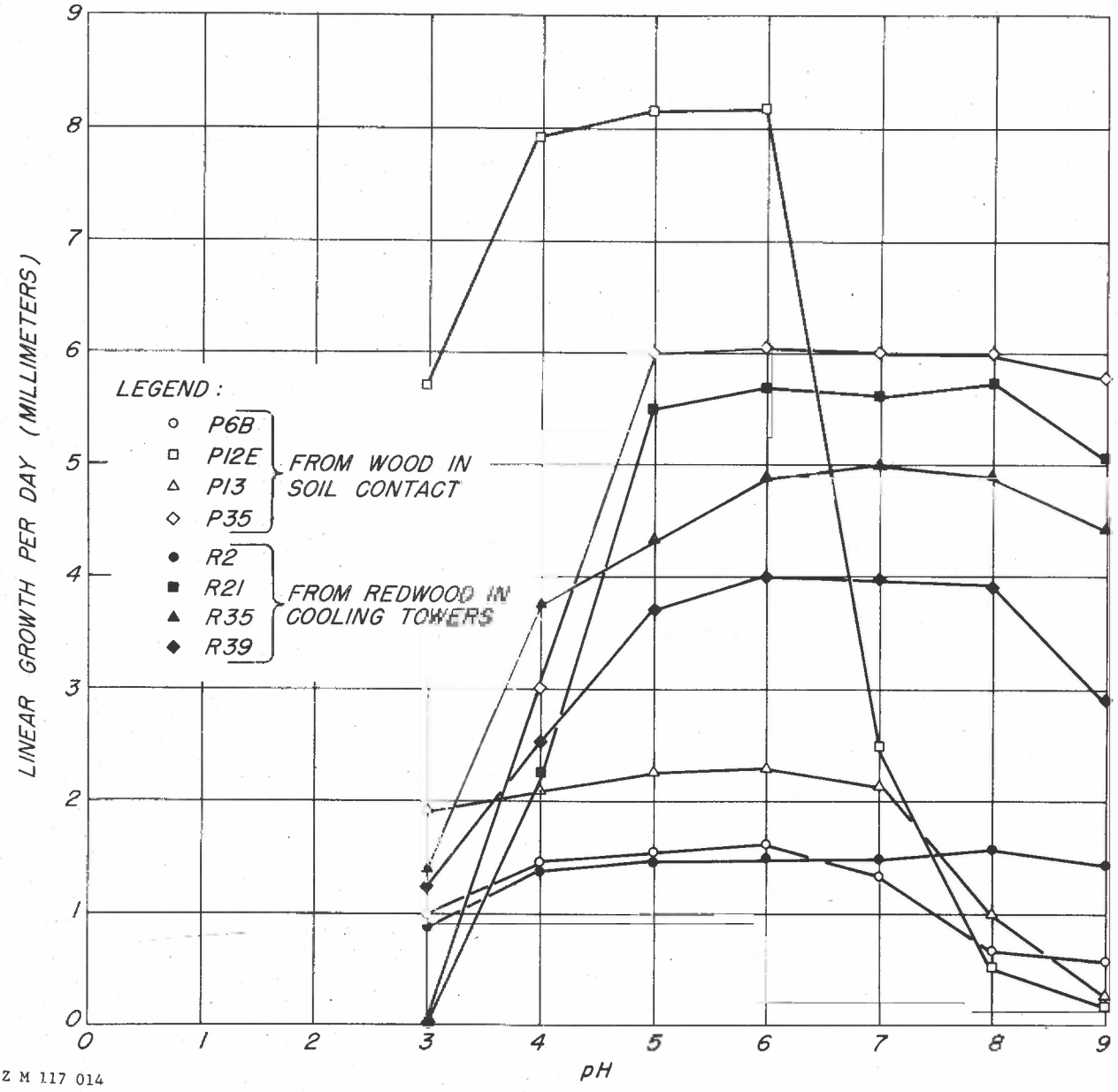

Figure 11. --Growth of eight Fungi Imperfecti on malt-agar at different pH levels.