Reprinted from PHYTOPATHOLOGY, Vol. 60, No. 2, 232-237, February 1970
Printed in U. S. A.

Mapping Columns of Discolored and Decayed Tissues in Sugar Maple, Acer saccharum

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The authors gratefully acknowledge the help of David R. Houston, Walter C. Shortle, Ellis B. Cowling, and William Merrill.

Accepted for publication 2 September 1969.

ABSTRACT

Discolored and decayed wood associated with 8-year-old inoculations with *Fomes fomentarius* and *F. connatus* in trunks of 10 *Acer saccharum* trees showed a basic pattern of physical changes and microbial succession. Moisture, pH, and ash increased as tissues died, discolored, and decayed. Bacteria and nonhymenomycetous fungi were found in distal portions of the columns, consistently in advance of the Hymenomycetes. In decay tests, *Fomes igniarius, Polyporus glomeratus,* and *Pholiota* sp. reduced the wt of only those wood samples that were already decayed in the living tree. *Polyporus versicolor* decayed most of the samples to some extent. Phytopathology 60:232-237.

Discoloration and decay of wood in living trees result from dynamic processes initiated by wounds. Abiotic factors and interactions of microorganisms among themselves and with the host are involved, and all are affected by environment over a long period of time (1, 4, 8, 11, 12, 13, 15).

This paper reports results of a study designed to demonstrate some of the changes that occur in wood following wounding as tissues discolor and decay, and to give additional information on the succession of microorganisms in these tissues.

MATERIALS AND METHODS.—Columns of discolored and decayed tissues associated with 8-year-old inoculation wounds, and with contiguous clear wood (Fig. 1) (13) in 10 sugar maple trees (*Acer saccharum* Marsh.) were sampled and mapped systematically for sp gr, moisture, pH, ash content, microorganisms, and degree of susceptibility, after heat sterilization, to decay by the heart-rot fungi *Fomes igniarius* (L. ex Fr.) Kickx., *Polyporus glomeratus* Pk., and *Pholiota* sp., and by the sap-rot fungus *Polyporus versicolor* (L.) Fr.

The trees (located in the Hubbard Brook Experimental Forest, West Thornton, New Hampshire) ranged in size from 15 to 30 cm diam at 1.5 m. Six trees had been inoculated with *Fomes fomentarius* (L. ex Fr.) Kickx., and four with *F. connatus* (Weinm.) Gill. Felling began in June and ended in September 1967. After each tree was felled, three cuts were made: through the inoculation point 2.4 m above the base and at 1.2 m above and below this point. The ends of the 1.2-m bolts were wrapped immediately with plastic and overlapped with several layers of heavy brown paper. Within 2 hr, the bolts were in the laboratory. Trees 9 and 10 were not cut through the inoculation point in the field, but were brought to the laboratory in 2.4-m bolts. Samples from trees similar to these had been used in preliminary experiments to determine methods.

The bolts were cut into 5-cm discs, and a template was used to mark the position of the samples (Fig. 1). A three-fourth inch plug cutter mounted in an electric drill press was used to extract two sample dowels (A and B) from the column of discoloration and decay and two (C and D) from contiguous clear uninfected wood in the same growth rings (Fig. 1). The dowels were trimmed to 4 cc on a table saw. The 4-cc dowels were weighed approximately 10 min after they were cut from the discs. Although variations in volume were very slight, calipers were used to measure lengths at four positions to determine the precise volume for each dowel. This volume was used in all calculations. Approximately 1,000 dowels were used in the study. The dowels were oven-dried at 105°C for 24 hr, and sp gr and moisture wt were calculated. A hole 1 mm in diam was drilled through each dowel. Each tree was processed to this point before the next was cut.

The ends trimmed off the 5-cm dowels in reducing them to 4 cc were ground in a Wiley mill to pass a 20-mesh screen, and the ground wood was used for ash analysis. One g of oven-dried ground wood was ashed at 550°C for 24 hr with intermittent stirring. Weight of ash for 4 cc of wood was calculated.

The dowels, after oven-drying, weighing, and oven-drying again, were placed in 25 ml of sterile, distilled, deionized water in sterile 50-ml beakers. The beakers were placed in desiccators, and three times a vacuum equal to 25 ml of mercury was drawn for 5 min. After release, all dowels sank. Dowels with sp gr 0.6 contained approximately 85% moisture based on oven-dried wt. The pH of the water in the beaker after the dowels were removed was recorded. The pH was not altered when the dowels were under vacuum for longer periods. This method for determining pH is similar to that given by Stamm (14).

The water-soaked dowels were hung on nickel-chrome wires inserted through small holes made in lids of 8-oz French square bottles (Fig. 4). The holes were sealed with epoxy plastic. The bottles, containing the dowels and 50 ml of sterile, distilled, deionized water (Fig. 4), were placed in an oven at 70°C for 24 hr. This treatment reduced the moisture in the unaffected wood samples, sp gr 0.6, approximately 10%. The dowels were taken out of the bottles and were dipped for 1 min into a slurry of mycelium prepared by either growing *F. igniarius, P. glomeratus, Pholiota* sp., or *P. versicolor* in 25 ml of a medium consisting of 10 g malt extract and 2 g yeast extract/liter in 250-ml Erlenmeyer

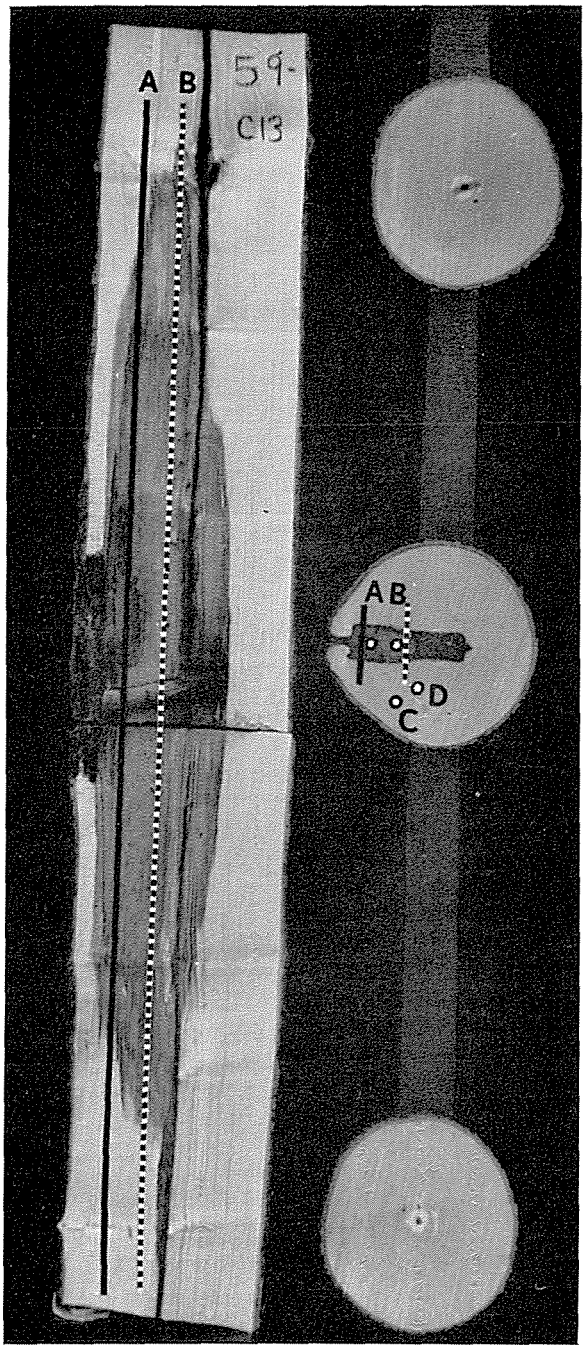

Fig. 1. A typical column of discoloration and decay associated with an inoculation wound in a 1.4-m section of a sugar maple. The 5-cm disc cut from below the inoculation point shows the position of the samples A, B, C, and D. The lines to left of A and to right of B on the disc show where the splits were made for isolations. Two straight rows of 6 chips were extracted from each of both faces of each split, a total of 48 chips/disc. The first chip was always extracted from the clear wood.

flasks in the dark for 4 weeks. The mycelium of each species was washed thoroughly in sterilized, distilled, deionized water and chopped for 5 sec in a blender. The isolates of *F. igniarius* and *P. glomeratus* came from decayed wood in sugar maple. The isolate of *Pholiota* sp. was isolated earlier from decayed wood in tree 10, and *P. versicolor* was a stock culture obtained from *Acer saccharum*.

Dowels A, B, C, and D from the 10 trees were inoculated with each of the test fungi and incubated in the dark at approximately 25°C for 6, 8, and 10 weeks. Noninoculated dowels dipped in sterile water for 1 min were included. After incubation, the mycelium was scraped from the dowels. The percentages of moisture and wood decayed were determined after oven-drying at 105°C for 24 hr.

Microorganism maps were made from the results of isolations. Isolation chips were extracted from the discs after the sample dowels had been extracted. The discs were split at right angles to the columns immediately in front of dowel A and in back of dowel B (Fig. 1). Chips of wood approximately 3×10 mm were extracted with a gouge and placed in an upright position in the agar, making certain that they touched the bottom of the petri dish without cracking the agar. The agar medium contained 10 g malt extract and 2 g yeast extract/liter of distilled water. The chips were extracted six in a row from the columns of discoloration and decay and from contiguous clear wood (Fig. 1). The chips were examined after two time periods, depending on growth rate. The bottoms of the chips were examined for bacteria under the stereoscope at ×30. Approximately 7,000 chips were cultured.

Detailed maps on all trees can be obtained from the authors.

RESULTS.—*Patterns in columns of discolored and decayed tissues.*—As the wound was approached longitudinally from above or below the distal margins of the columns, increases occurred generally in moisture, pH, and ash. These increases continued even after sp gr began to decrease (Fig. 2, 3). Moisture and ash content at the distal margins of the columns were slightly lower than the clear wood. Moisture and ash contents increased as the inoculation point was approached. The drier tissues at the distal margins contained vessels plugged with a granular-type material as described by Good et al. (5). When these tissues surrounded the discolored columns, a bleached zone was evident on the freshly cut surfaces. Specific gravity was slightly higher at the distal margins, and then began to decrease as the inoculation point was approached. The tissues that were slightly higher in sp gr than the unaffected wood contained cells plugged with masses of dark, gumlike substances. It is possible that some of these slight changes were missed in some columns because of the 5-cm distance between sample dowels.

Analysis of clear wood samples contiguous to columns of discolored and decayed tissues.—The differences between the samples of clear wood from the C and D lines were very slight. The only recognizable gradation in the clear wood samples was a progressively lower

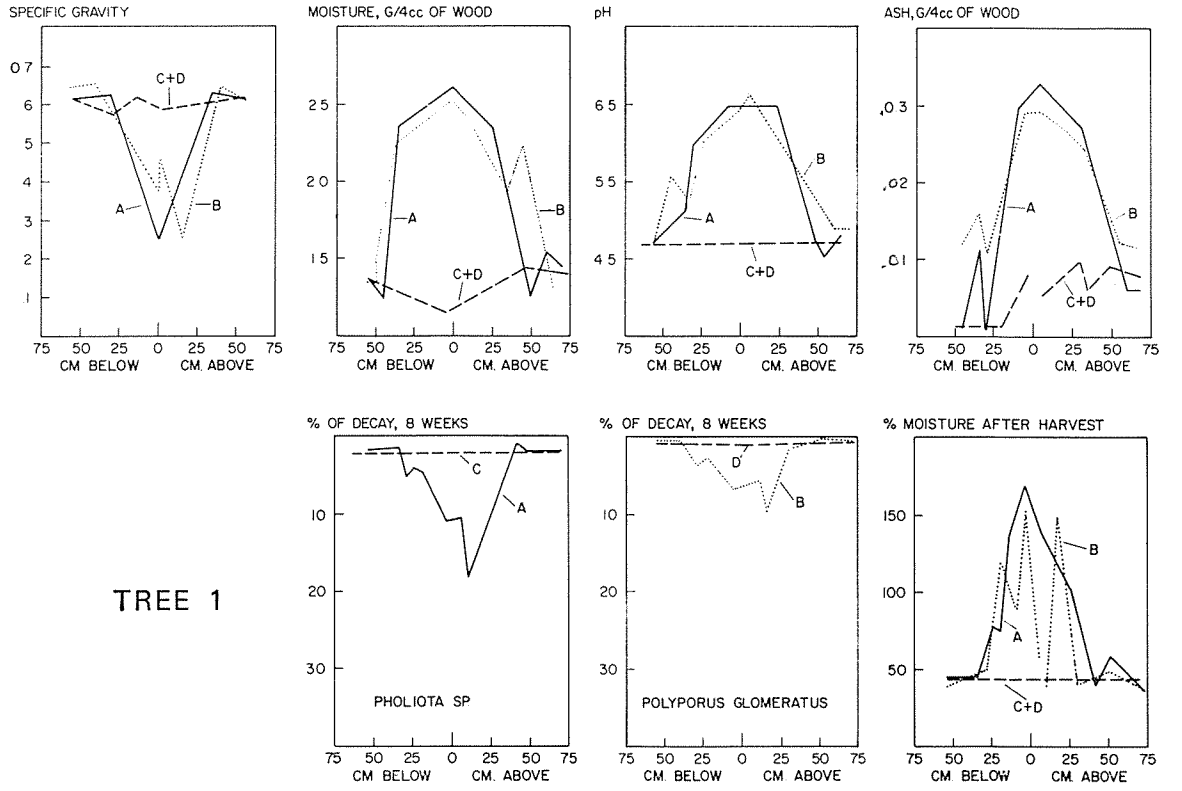

Fig. 2. Data on sp gr, moisture, pH, ash, and decay susceptibility of 4-cc wood samples extracted at 5-cm intervals above and below the 8-year-old inoculation wound in tree 1, *Acer saccharum*. The inoculum, *Fomes fomentarius,* was not reisolated. Samples A and B were taken in a straight line from discolored and decayed tissues above and below the inoculation point, 0. Samples A were proximal to the bark, and samples B were proximal to the pith. Samples C and D were from clear wood contiguous to the discolored and decayed tissues. For more details as to the positions of A, B, C, and D, see Fig. 1.

moisture content as the wound area was approached. This trend was evident in trees 9 and 10 also, which were not cut at the inoculation point until immediately before the samples were processed in the laboratory. Also, the lack of a corresponding gradation in the A and B samples at these areas indicated that if drying of the bolts at the cut ends occurred, it was not sufficient to cause the observed decrease in moisture of the C and D samples. In some trees, ash content was slightly and progressively greater as the inoculation point was approached. Ash content of the clear wood samples was slightly variable, pH was fairly constant at 4.7, and sp gr was approximately 0.6.

Status of columns of discolored and decayed tissues. —The vertical extensions of discolored tissues were fairly consistent at approximately 55 cm above and below the inoculation points, while decayed tissues varied considerably from 0 to 47 cm (Table 1). There was no consistent relationship between the vertical distal margin of the discolored tissues and the vertical distal margin of the decayed tissues. In cross section, the columns were the shape of the drill bit near the inoculation point, and they sloped toward the pith as the vertical distance increased away from the inoculation point (Fig. 1). There was more decay in the portion of the column proximal to the bark. The A lines in the graphs show a lower sp gr than the B lines, which were deeper in the column. Because the samples were extracted along straight lines, samples along the A line encountered clear wood before those in the B line when going from the inoculation point distally. The vertical distance from decayed to discolored to clear tissues was shorter in the A lines than in the B lines (Fig. 2, 3).

TABLE 1. Extension of discoloration and decay in *Acer saccharum* above and below 8-year-old inoculation wounds

Tree	Discoloration		Decay	
	Above inoculation wound	Below inoculation wound	Above inoculation wound	Below inoculation wound
	cm	*cm*	*cm*	*cm*
1	63	52	35	32
2	63	47	32	27
3	57	62	22	32
4	62	72	42	47
5	67	57	27	17
6	62	57	0	12
7	57	57	32	32
8	47	40	17	15
9	32	27	22	12
10	80	52	22	17

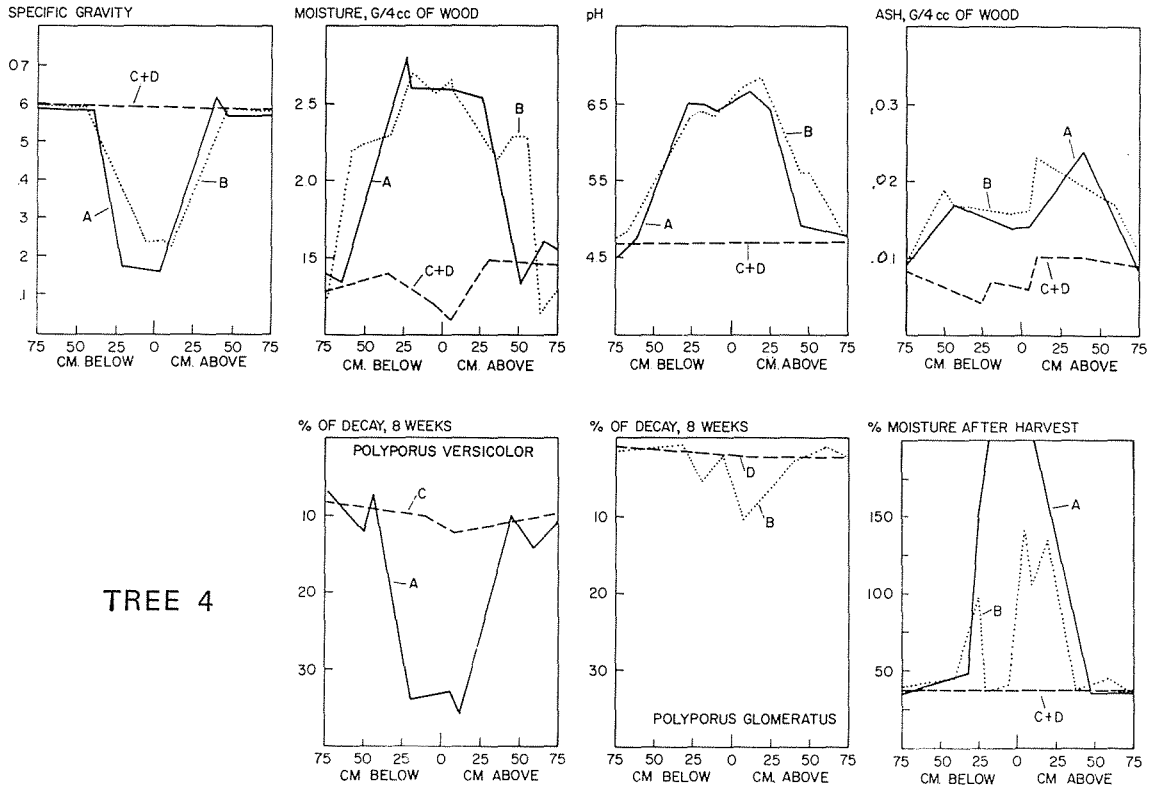

Fig. 3. Data on sp gr, moisture, pH, ash, and decay susceptibility of 4-cc wood samples extracted at 5-cm intervals above and below the 8-year-old inoculation wound in tree 4, *Acer saccharum*. The inoculum, *Fomes fomentarius,* was not reisolated. Samples A and B were taken in a straight line from discolored and decayed tissues above and below the inoculation point, 0. Samples A were proximal to the bark; samples B were proximal to the pith. Samples C and D were from clear wood contiguous to the discolored and decayed tissues. For more details as to the positions of A, B, C, and D, see Fig. 1.

Microorganism patterns.—Bacteria were isolated more frequently than other organisms. They were in the tissues most distal to the inoculation wounds, but the number of chips yielding bacteria decreased as the vertical distal margins of the columns were approached. Bacteria were isolated sometimes from the bleached zones beyond the darkly discolored margins of the columns. Some bacteria grew only on the bottom of the agar medium where the chip touched the glass. Bacteria and nonhymenomycetous fungi were isolated frequently from the same chip, and grew well together in culture.

The principal nonhymenomycetous fungi isolated were *Phialophora* spp., *Ascocoryne* sp., *Trichocladium canadense* Hughes, and *Fusarium* sp. *Trichocladium canadense* and *Ascocoryne* sp. were isolated from sound discolored tissues as well as from tissues in an advanced stage of decay. *Trichocladium canadense* was isolated most frequently from tissues near the pith. The principal fungi had phialophores.

Hymenomycetes were not recovered from all decayed tissues. Where Actinomycetes were isolated frequently from decayed wood, Hymenomycetes were not. Actinomycetes were isolated from five trees. They were isolated most frequently from the old inoculation dowels and nearby tissues. Nematodes were also in some of these areas.

The fungi introduced as inocula 8 years previously, *Fomes fomentarius* and *F. connatus,* were not isolated from the columns. *Pholiota* sp., although not inoculated, was isolated from decayed tissues in trees 9 and 10.

Many species of microorganisms were isolated from tissues near the inoculation point, but the number of species present decreased as distance from this point increased.

Decay tests.—The heart-rot fungi, *P. glomeratus, F. igniarius,* and *Pholiota* sp., grew on all samples, but caused little or no wt loss of the clear wood samples from along the C and D lines (Fig. 4). In contrast, *Pholiota* sp. and *P. glomeratus* incited additional decay in samples from along the A and B lines previously decayed in the living tree (Fig. 4). *Fomes igniarius* induced very slight additional decay in wood that was decayed in the living tree. The shapes of the sp gr and decay graphs were similar, indicating that the amount of further decay in culture was proportionate to the sp gr of the samples. The amount of mycelium on the samples was inversely related to the sp gr and distance from the wound.

Mycelium scraped from the samples inoculated with *F. igniarius* grew on malt-yeast extract agar medium, indicating that the fungus was viable at harvest time. Mycelium of *Pholiota* sp. covered the entire surface of

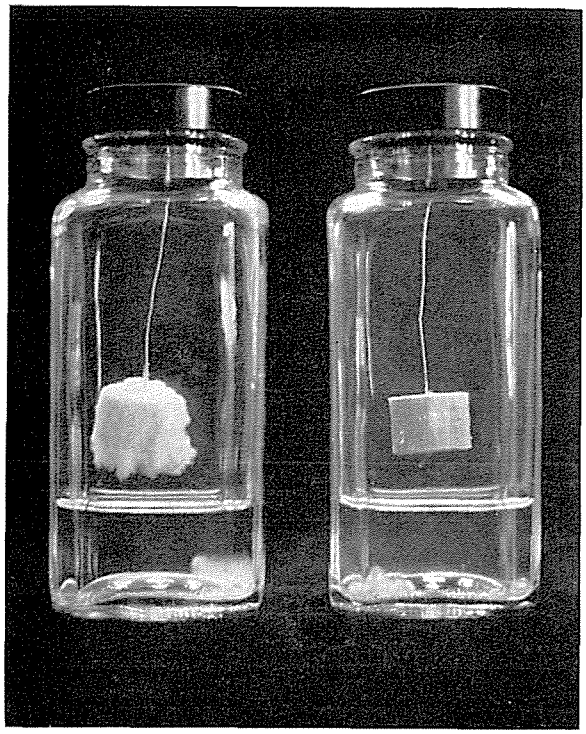

Fig. 4. A sample of clear wood, *Acer saccharum,* (right) and decayed wood (left) inoculated with *Pholiota* sp. and incubated for 6 weeks. The fungus grew on the clear wood sample, and mycelial bits that fell into the water also grew, but the sample was not decayed. The sample of decayed wood was decayed further.

the samples a few days after inoculation, but the fluffy mycelium then collapsed, especially on the clear wood samples.

Polyporus versicolor to some extent decayed most of the samples. As with the other fungi, the amount of decay was inversely related to sp gr (Fig. 3). Least decay with all fungi occurred in the zone where sp gr was slightly higher than the sound wood. This zone contained deposits of dark substances in the cells.

Noninoculated samples lost approximately 0.5% wt during incubation.

The moisture content of the samples was recalculated at the end of the incubation period. All samples, including noninoculated ones, remained above the fiber-saturation point at a minimum of 35%. Often, the decayed samples contained a lower percentage of moisture than the nondecayed sound wood samples. The graphs give these data in percentages as they are usually expressed. In the living tree, wood in the beginning stages of decay had approximately 80% moisture. The clear wood samples had approximately the same percentage of moisture after inoculation.

Discussion.—Data on discolored and decayed tissues and the differences between these tissues and clear wood and heartwood have been reported (3, 4, 5, 6, 7, 11). Data presented in this paper illustrate patterns of changes, and include the affect of microorganisms and the susceptibility of tissues to further decay after heat sterilization. These results show that, although every column of discoloration and decay is different, general patterns of changes exist when time is not a limiting factor.

Because gradations exist, it is clearly evident that all the discolored or decayed tissues in a column are not uniform. Discolored or decayed tissues resulting from early stages of the processes differed greatly from such tissues produced during later stages. As more attention is given to these processes and to the tissue changes that result, greater care must be given to the exact nature of the tissues under study. The stage to which the tissues have been altered will also determine the presence or absence of certain microorganisms.

Isolation techniques are also of extreme importance. If a medium unsuitable for bacteria is used, the importance of these organisms will be overlooked. If the chips do not touch the bottom of the dish, certain bacteria will not grow. This suggests that many of the bacteria are facultative anaerobes. Isolation maps of the microorganisms growing from chips systematically extracted give the best indication of the flora and their position.

The decrease in moisture in the clear wood contiguous to the wound suggests that a gradient may also exist in the white wood along the column of discoloration and decay. These samples were extracted outside the zone of plugged vessels.

The laboratory decay tests were in many ways severe for the wood and the fungi, yet *Polyporus versicolor* did incite decay in most samples. It is possible that incubation was not long enough for the other fungi. But the tissues decayed previously in the living tree were decayed further in most cases. This agrees with results of other studies, where previously decayed wood continued to decay more rapidly than sound wood (2, 3, 9, 10). The fungi grew on all samples, indicating that there was sufficient moisture and inoculum, and the clear wood samples contained approximately the same amount of moisture as did the tissues in the early stages of decay in the living tree. Samples from locations with high sp gr that contained cells impregnated with dark substances were decayed least by all fungi. In the living tree, this was the zone in advance of the decay fungi.

Failure to isolate Hymenomycetes from decayed tissues has also been experienced by others (9, 11). Shain (11) stated that the fungi may die back under certain conditions. The absence of Hymenomycetes in decayed tissues that yielded Actinomycetes suggests that the latter may create conditions unfavorable for Hymenomycetes.

It is certain that Hymenomycetes decay wood in living trees. In this study and others (13), Hymenomycetes were never isolated from the discolored distal margins of the columns, while bacteria and nonhymenomycetous fungi were. Therefore, in *A. saccharum,* Hymenomycetes seldom confront living tissues, but rather tissues that not only are dead, but also are altered as a consequence of host-response to injury and of infection by other organisms.

Apparently death of the sound wood alone is not enough to permit decay by some Hymenomycetes. Tissues that were decayed further by heart-rot fungi differed in many ways from the sound tissues. Wood probably must be altered in very specific ways before the heart-rot fungi can cause decay, and it is also likely that, once Hymenomycetes become established in a living tree, they may be able to elicit conditions that further enhance their growth. The studies reported here deal with the early stages of these processes in *Acer saccharum*.

LITERATURE CITED

1. BASHAM, J. T. 1966. Heart rots of jack pine in Ontario. 1. The occurrence of Basidiomycetes and microfungi in defective and normal heartwood of living jack pine. Can. J. Bot. 44:275-295.
2. FINDLAY, W. P. K. 1940. Studies in the physiology of wood-destroying fungi. III. Progress of decay under natural and under controlled conditions. Ann. Bot. 4:701-712.
3. FINDLAY, W. P. K., & C. B. PETTIFOR. 1941. Dark coloration in western red cedar in relation to certain mechanical properties. Emp. For. J. 20:64-72.
4. GOOD, H. M., J. T. BASHAM, & S. D. KADZIELAWA. 1968. Respiratory activity of fungal associations in zones of heart rot and stain in sugar maple. Can. J. Bot. 46:27-36.
5. GOOD, H. M., P. M. MURRAY, & H. M. DALE. 1955. Studies on heartwood formation and staining in sugar maple, *Acer saccharum* Marsh. Can. J. Bot. 33:31-41.
6. HART, H. H. 1965. Formation of discolored sapwood in three species of hardwoods. Mich. Agr. Exp. Sta. Quart. Bull. 48:101-116.
7. HART, H. H. 1968. Morphological and chemical differences between sapwood, discolored sapwood, and heartwood in black locust and osage orange. For. Sci. 14:334-338.
8. MALOY, O. C., & V. S. ROBINSON. 1968. Microorganisms associated with heart rot in young grand fir. Can. J. Bot. 46:306-309.
9. ROTH, E. R. 1943. Effects of invisible decay on deterioration of untreated oak ties and posts. J. Forestry 41:117-121.
10. ROTH, E. R., & B. SLEETH. 1939. Butt rot in unburned sprout oak stands. USDA Tech. Bull. 684, 34 p.
11. SHAIN, L. 1967. Resistance of sapwood in stems of loblolly pine to infection by *Fomes annosus*. Phytopathology 57:1034-1045.
12. SHIGO, A. L. 1967. Successions of organisms in discoloration and decay of wood. *In* Internat. Rev. Forestry Res. II. Academic Press, N. Y. 237-299.
13. SHIGO, A. L., & E. M. SHARON. 1968. Discoloration and decay in hardwoods following inoculations with Hymenomycetes. Phytopathology 58:1493-1498.
14. STAMM, A. J. 1961. A comparison of three methods for determining the pH of wood and paper. Forest Prod. J. 11:310-312.
15. SUCOFF, E., H. RATSCH, & D. D. HOOK. 1967. Early development of wound-initiated discoloration in *Populus tremuloides* Michx. Can. J. Bot. 45:649-656.