Manganese Accumulation in Wood Decayed by White Rot Fungi

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ABSTRACT

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Black regions and flecks in wood decayed by several white rot fungi (*Cerrena unicolor, Dichomitus squalens, Ganoderma applanatum, G. tsugae, Heterobasidion annosum, Ischnoderma resinosum*, and *Perenniporia medulla-panis*) contained large concentrations of manganese. Two types of decay patterns occurred in wood degraded by these fungi: a selective delignification resulting in the removal of lignin and hemicellulose and a typical white rot causing simultaneous removal of all cell wall components. Atomic emission spectrometry and X-ray microanalyses detected manganese and determined its spatial relationship within selectively delignified wood. Black regions in eastern hemlock wood decayed by *G. tsugae* showed over a 100-fold increase of manganese when compared with sound wood. When black regions were compared with surrounding delignified wood and adjacent white-rotted wood, the increases of manganese were 24-fold and 51-fold, respectively. In contrast to the other decays examined, manganese deposits were found in white-rotted wood attacked by *Fomes fomentarius*. Micromorphological characteristics of decayed wood and manganese deposits were observed with scanning electron microscopy. Leucoberbelin blue reagent confirmed the presence of manganese oxides within the black regions.

Black spots and flecks within decayed wood are characteristically produced by several white rot fungi, including *Dichomitus squalens* (Karst.) D. Reid (*Polyporus anceps* Pk.), *Ganoderma lucidum* (Fr.) Karst., *G. oregonense* Murr., *G. tsugae* Murr., *Heterobasidion annosum* (Fr.) Bref. (*Fomes annosus* (Fr.) Cke.), *Perenniporia subacida* (Pk.) Donk (*Poria subacida* (Pk.) Sacc.), and *Resinicium bicolor* (Fr.) Parm. (*Odontia bicolor* (Fr.) Bres.) (5,16). Although these features were described and illustrated as early as 1878 (15) and have been used macroscopically to identify certain types of decayed wood (5,8,16,26), the factors responsible for causing black areas are not known.

Two distinct types of decay can be found associated with many white rot fungi: a simultaneous removal of all cell wall components and a selective removal of lignin and hemicellulose (2,3,23). In a recent investigation of eastern hemlock wood (*Tsuga canadensis* (L.) Carr.) decayed by *G. tsugae* (2), black spots were always associated with selectively delignified wood. Delignified tissues contained primarily cellulose and were white, contrasting sharply with the black regions within the delignified wood. These black substances also appeared to be located in pockets of white decayed wood (3,5,8). The black spots and flecks are not interaction zone lines or pseudosclerotial plates (2).

This study was initiated to determine the chemical constituents and micromorphological characteristics of black zones associated with decay caused by *Cerrena unicolor* (Fr.) Murr. (*Daedalea unicolor* (Bull.) Fr.), *D. squalens, Fomes fomentarius* (L. ex Fr.) Gill., *G. applanatum* (Pers. ex Wall.) Pat., *G. tsugae, H. annosum, Ischnoderma resinosum* (Fr.) Karst., and *Perenniporia medulla-panis* (Peck) Donk (*Poria medulla-panis* (Pers.) Bres.).

MATERIALS AND METHODS

Wood decayed by *C. unicolor, F. fomentarius, G. applanatum, I. resinosum*, and *P. medulla-panis* was obtained from fallen paper birch (*Betula papyrifera* Marsh.) in Cloquet, MN, Itasca State Park, MN, and the Chequamegon National Forest, WI. Wood decayed by *H. annosum* was obtained from dead Engelmann spruce (*Picea engelmannii* Parry), red pine (*Pinus resinosa* Ait.), and Norway spruce (*Picea abies* (L.) Karst.) from Wasatch National Forest, UT, East Lansing, MI, and Saxonwald, near Hamburg, West Germany, respectively. Wood decayed by *G. tsugae* was taken from dead eastern hemlock in Chequamegon National Forest, and wood decayed by *D. squalens* was obtained from red pine in Cloquet and Ramsey County, MN. All samples were removed from wood directly behind sporophores.

Sections from all samples were cultured as previously described (4) to confirm the presence of each fungus. Radial and tangential sections from each collection were trimmed with a cryostat microtome at −20 C. Sections were mounted on aluminum specimen stubs, dried in a desiccator, and placed in a vacuum evaporator for coating with a fine layer of evaporated spectrally pure carbon. Mounted specimens were examined in a Philips 500× scanning electron microscope fitted with an energy-dispersive X-ray microanalysis unit (EDAX System F) containing a 30 mm^2 Li-drifted, Si-crystal detector. After X-ray microanalyses, specimens were coated with 40% gold and 60% palladium and observed in the scanning electron microscope.

Inductively coupled plasma atomic emission spectrometry (24) was used for multielement analyses of sound eastern hemlock, *G. tsugae* sporophores, delignified wood, black regions in delignified wood, white-rotted (simultaneous rot) wood, and zone lines or pseudosclerotial plates. Decayed wood and black areas were separated with fine-pointed forceps. Five separate samples, each from a different collection, were analyzed for each determination.

To test for deposition of manganese oxides in wood, an acidified solution (pH 3–4) of 0.2% leucoberbelin blue (obtained from H. J. Altmann, Gehlberge Str. 9, D-1000 Berlin 20, West Germany) was applied to the cut surface of decayed wood. The colorless leucoberbelin blue reagent reacts with manganese oxides (20) to produce a bright greenish blue within the wood. Phloroglucinol-HCl and zinc-chlor-iodide (17) were also applied to the cut surfaces of decayed wood to test for the presence of lignin and cellulose, respectively.

RESULTS

Black regions within decayed wood were intermittently dispersed throughout the white, delignified wood. Large, irregular patches were observed in wood decayed by *G. tsugae* and small black flecks in wood decayed by *D. squalens* (Fig. 1). Two types of decayed wood were associated with all fungi examined except *F.*

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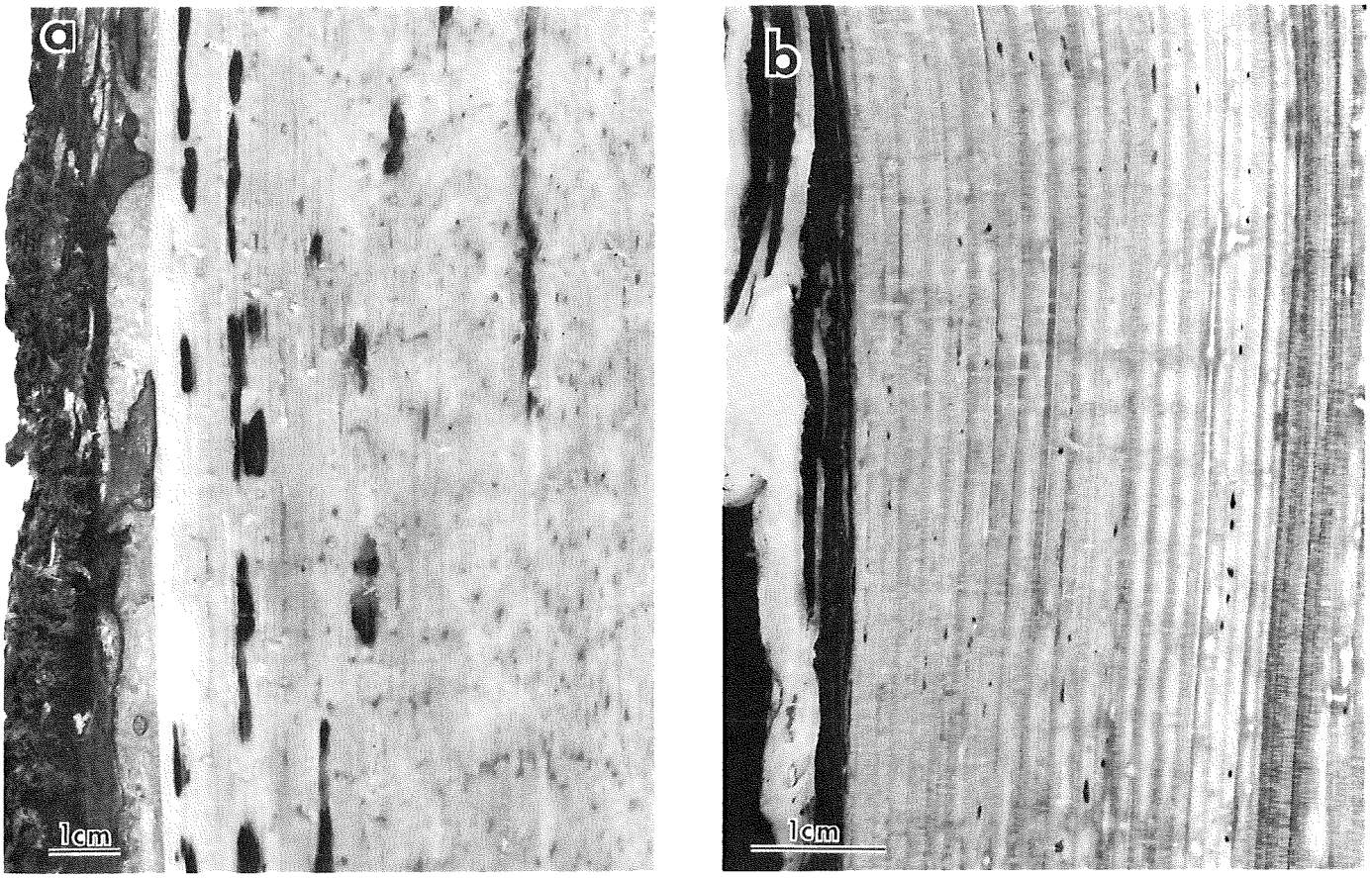

Fig. 1. A, Eastern hemlock wood decayed by *Ganoderma tsugae* showing large, irregular patches of black pigmentation within white, delignified wood. **B,** Red pine wood decayed by *Dichomitus squalens* showing small black flecks within white pockets.

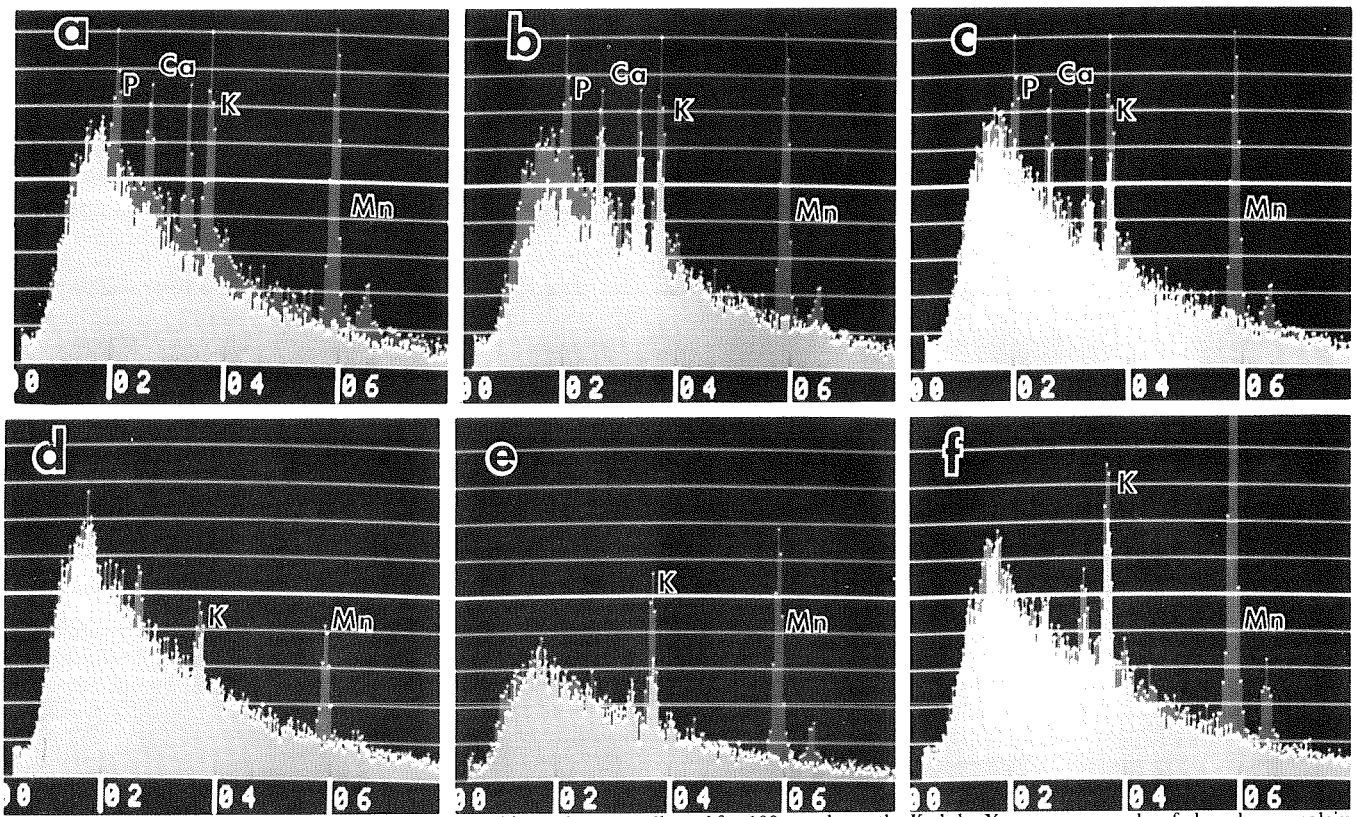

Fig. 2. Each graph of two superimposed X-ray spectra (white and gray), collected for 100 sec, shows the K alpha X-ray energy peaks of phosphorus, calcium, potassium, manganese, and other elements, by use of a stationary beam probe. **A–C,** Black regions from wood decayed by *Ganoderma tsugae* (gray spectra) compared with **A,** sound eastern hemlock (white spectra), **B,** surrounding delignified wood (white spectra), and **C,** adjacent white-rotted wood (white spectra). Black regions contained higher concentrations of manganese than did sound or decayed wood (gray spectra). **D–F,** X-ray microanalyses of black regions in wood decayed by **D,** *Dichomitus squalens*, **E,** *Fomes fomentarius*, and **F,** *Heterobasidion annosum* (gray spectra) compared with surrounding delignified wood (white spectra) showed large amounts of manganese.

TABLE 1. Comparison of elemental concentrations in sound wood, *Ganoderma tsugae* sporophores, and associated decay[a]

Elements	Sound hemlock	Sporophores	Zone lines	White rot	Delignified wood	Black zones in delignified wood
Al	7.7 ± 5.6	15.2 ± 8.8	34.1 ± 5.5	36.9 ± 10.7	53.9 ± 13.9	56.5 ± 6.9
B	3.5 ± 0.4	0.9 ± 0.5	2.8 ± 1.1	5.6 ± 2.0	6.9 ± 3.3	8.7 ± 5.3
Ca	1,107.5 ± 142.8	751.1 ± 268.6	5,038.6 ± 1,939.7	3,498.6 ± 1,194.0	5,350.0 ± 1,913.1	3,336.8 ± 572.4
Cu	3.4 ± 6.5	14.0 ± 2.7	3.7 ± 2.2	6.8 ± 2.9	9.4 ± 2.4	10.9 ± 2.5
Fe	7.4 ± 7.3	18.3 ± 11.6	22.0 ± 15.8	14.7 ± 3.6	31.2 ± 14.5	49.0 ± 8.3
K	685.6 ± 34.9	7,849.7 ± 1,772.4	278.7 ± 42.1	1,566.6 ± 1,989.8	1,622.5 ± 693.0	1,174.8 ± 762.8
Mg	184.0 ± 6.9	842.3 ± 169.9	185.4 ± 30.6	508.5 ± 239.6	926.9 ± 359.1	520.3 ± 101.8
Mn	75.5 ± 13.6	49.6 ± 28.7	53.2 ± 9.3	168.3 ± 68.0	353.9 ± 92.2	8,543.0 ± 2,696.2
Na	23.7 ± 16.0	5.1 ± 3.3	111.8 ± 63.9	259.4 ± 132.5	658.8 ± 388.8	267.6 ± 160.0
P	187.1 ± 27.4	5,620.8 ± 768.5	178.7 ± 30.7	313.3 ± 134.3	596.1 ± 337.8	902.8 ± 91.8
Zn	4.5 ± 7.2	38.6 ± 8.3	14.9 ± 5.9	25.6 ± 13.3	31.7 ± 13.1	38.5 ± 33.6

[a] Values are means of five replicate determinations from different trees; parts × 10^{-6} (± one standard error).

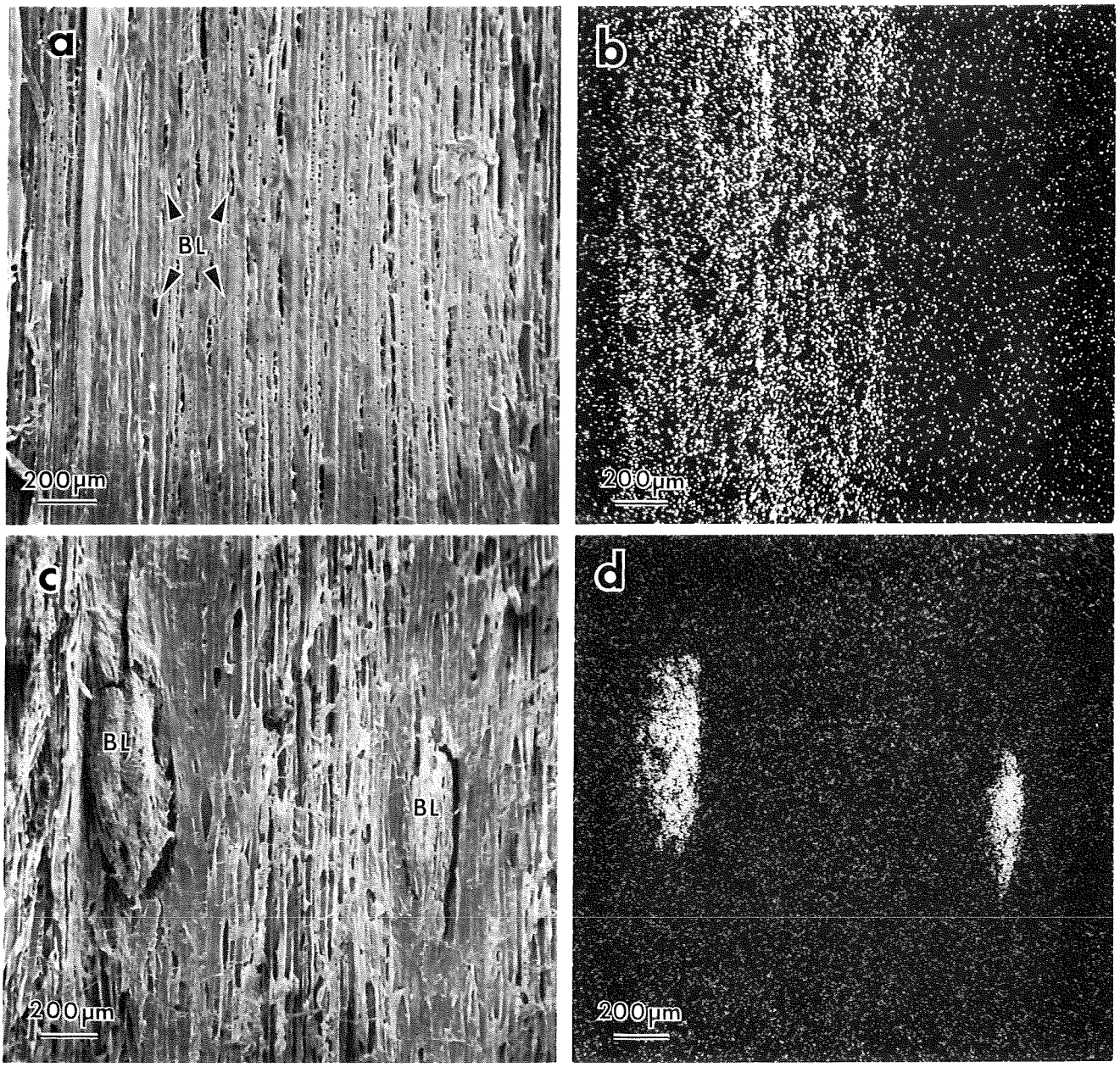

Fig. 3. A, Scanning electron micrograph of wood delignified by *Ganoderma tsugae* showing location of the black area (BL). **B,** X-ray microanalysis dot mapping for manganese in the same area shown in **A. C,** Wood decayed by *Heterobasidion annosum* with two black spots (BL) surrounded by delignified wood. **D,** X-ray microanalysis dot mapping for manganese in the same area shown in **C.**

fomentarius. White wood containing the black regions did not stain with phloroglucinol-HCl but did stain blue with zinc-chlor-iodide, indicating lack of lignin and presence of cellulose, respectively. Tan wood adjacent to the white areas stained bright carmine when phloroglucinol-HCl was applied, indicating a positive reaction for lignin. Large voids within the tan wood were filled with mycelium. Isolations confirmed the presence of each basidiomycete in both the white and the tan areas.

X-ray microanalyses of black regions from various decay types revealed high concentrations of manganese. When black regions in wood decayed by *G. tsugae* were compared with sound eastern hemlock, small peaks of phosphorus, potassium, and calcium were observed in addition to an exceedingly large peak of manganese (Fig. 2A). A comparison of the X-ray spectra for black spots with the spectra for surrounding white wood (Fig. 2B) and adjacent tan wood (Fig. 2C) demonstrated the high concentration of manganese in black pigmented areas. X-ray analyses of wood decayed by *D. squalens, F. fomentarius*, and *H. annosum* (Fig. 2 D–F), as well as those of wood decayed by *C. unicolor, G. applanatum, I. resinosum*, and *P. medulla-panis* (*data not presented*), also showed larger amounts of manganese in black spots than in adjacent decayed wood. Net peak comparisons of spectral data confirmed high levels of manganese in the black regions and absence of significant amounts of manganese in surrounding wood.

Scanning electron microscopy, for all wood except that decayed by *F. fomentarius*, indicated the white delignified wood containing the black regions consisted of tracheids that lacked middle lamellae. The secondary wall also appeared to be

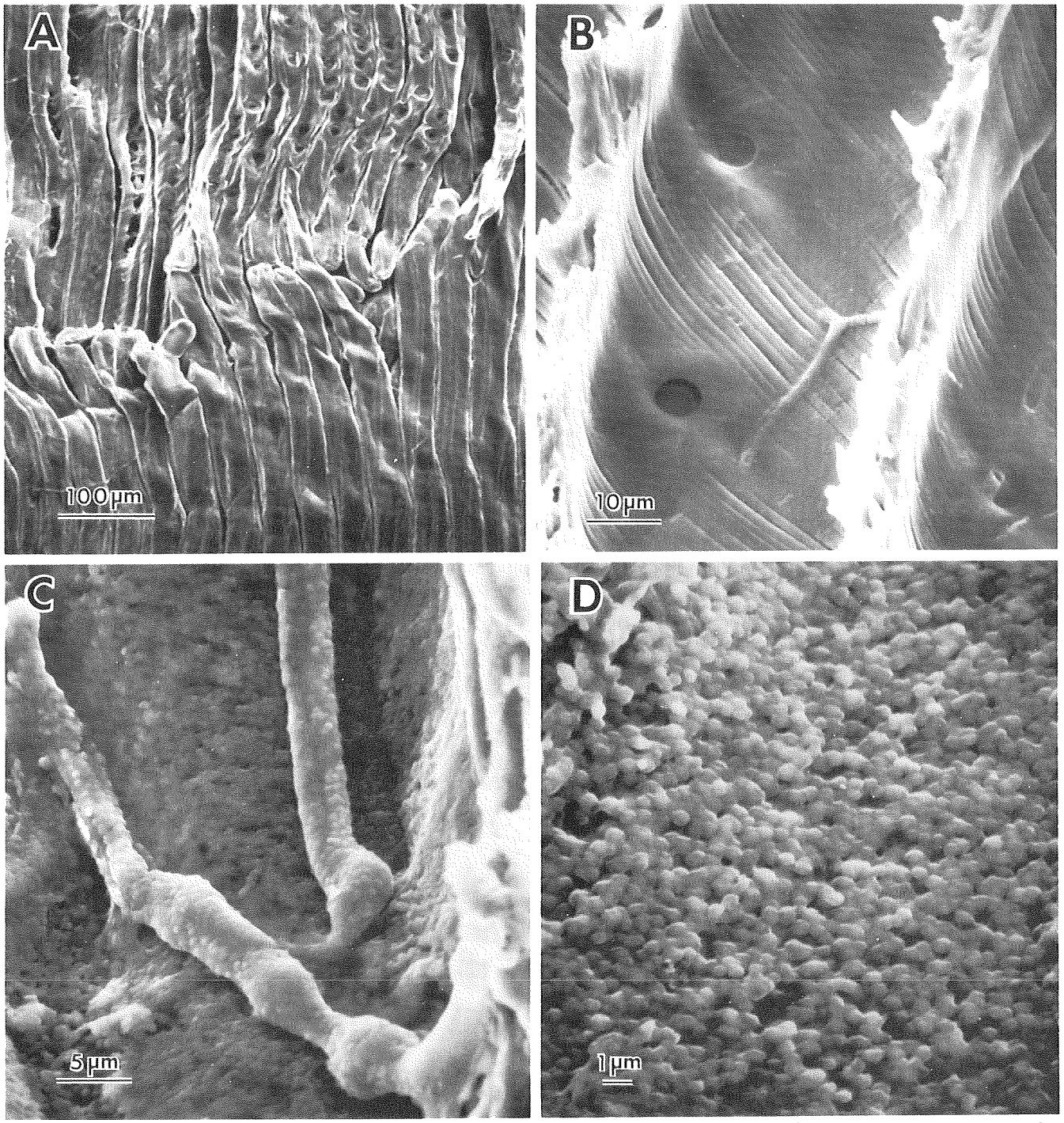

Fig. 4. Scanning electron micrographs of wood delignified by *Heterobasidion annosum*. **A,** Delignified cells lacked middle lamellae, and cells separated from one another. **B,** No deposits were observed within cell lumens. **C,** Black regions within delignified wood showed manganese deposits on and around thick-walled fungal hyphae. **D,** The manganese deposits coated tracheid walls throughout the black region.

delignified. Tan wood was white-rotted with erosion troughs and holes in the cell walls; a selective delignification was not apparent. Masses of mycelium that filled voids were found only in the tan, white-rotted wood. These micromorphological characteristics were similar to those previously described (1–3). X-ray dot mapping revealed the localization of high manganese concentrations in the black regions (Fig. 3A,B) or flecks (Fig. 3C,D).

Multielement analyses with atomic emission spectrometry showed a >100-fold increase of manganese in black zones caused by *G. tsugae* when compared with sound wood (Table 1). The increase in manganese when black areas were compared with delignified and white-rotted wood was 24-fold and 51-fold, respectively. Small increases of phosphorus, potassium, calcium, and other elements were also evident. Sporophores had large amounts of phosphorus and potassium, whereas zone lines contained increased quantities of calcium. Concentrations of manganese were low in sporophores and zone lines.

Black areas of all decayed wood tested reacted positively with leucoberbelin blue reagent, indicating the presence of manganese oxides. No reaction was obtained when the reagent was applied to zone lines, sporophores, or decayed wood. Sections observed with a microscope revealed brown deposits and some thick-walled hyphae in the black regions. Scanning electron microscopy demonstrated a diffuse pattern of deposits that adhered to fungal hyphae and coated the cell walls (Fig. 4).

DISCUSSION

Manganese oxide deposits in wood decayed by wood-destroying fungi vary in size and shape. In all the decays examined, except that caused by *F. fomentarius*, black spots were located in white, delignified wood. Hartig (15) noted the occurrence of these black areas in the middle of white pockets caused by *Trametes radiciperda* (*H. annosum*). From his keen observations, he reported that white tissues lacked middle lamella, allowing single tracheids to be removed. Wood between the decay, however, was attacked differently, with the middle lamella degraded last. Results from scanning electron microscopy reported here and previously (1–3,25) confirmed Hartig's observations and demonstrated that many different species of white rot fungi can cause two distinct types of decay.

The two types of decay caused by white rot fungi may not always be found together. A white rot causing simultaneous removal of all cell wall components often was the only decay type present. In other samples, delignified wood occurred in small areas intermittently dispersed throughout the white-rotted wood or in larger regions such as those produced by *G. tsugae*. Manganese oxide accumulations appeared to be found only in delignified wood and not in white-rotted wood. When white-rotted wood was the only type of decay present, no black spots were found. One exception, however, was the decay caused by *F. fomentarius*. Small black flecks containing manganese oxides were associated with decayed wood that had a bleached white appearance but was not completely delignified. I have examined many other white rots from field collections and herbarium samples that did not contain black spots. In contrast, most white pocket or white mottled rots that contained selectively delignified wood characteristically had black manganese oxide deposits.

White pocket rot fungi that cause a selective delignification in living trees, such as *Phellinus pini* (1), may not have accumulations of manganese in the decayed wood. However, manganese oxides are frequently found in standing dead trees or down wood colonized by *P. pini* (*data not presented*).

Many microorganisms can oxidize manganous compounds in aquatic and soil environments (6,13,21), but the accumulation of large concentrations of manganese in wood decayed by white rot fungi has not been shown previously. Nutrient concentrations in basidiocarps and rhizomorphs have been determined in several investigations for fungi that colonize conifer and decidious litter (10,28,29), but excessively large concentrations of manganese have not been observed. Sporophores of *G. tsugae* analyzed in this study also showed no manganese accumulation despite the large increase of manganese in wood decayed by the fungus. Phosphorus and potassium concentrations were high in sporophores and were similar to concentrations observed in sporophores of other basidiomycetes (10,28,29). Zone lines in decayed wood also had low amounts of manganese but large amounts of calcium. Zone lines or pseudosclerotial plates contain components that are similar to those found in rhizomorphs (7,22). Elemental analyses of rhizomorphs produced by various basidiomycetes also indicated increased amounts of calcium (10).

Research with white rot fungi has shown that the process of lignin degradation is oxidative (19) and enzymatic (27). The lignin polymer is apparently attacked by extracellular, nonspecific oxidizing agents (18). The results presented here indicate that in areas of decayed wood where selective delignification is present, manganese is also oxidized. The precipitation of manganese oxides appears to initiate a gradient that results in additional manganese being transported into the region. Since selectively delignified wood usually has manganese deposits associated with it, manganese may regulate the type of cell wall attack that occurs. Therefore, manganese may be an important component in the degradative processes resulting in the selective removal of lignin by white rot fungi.

Excess or deficient amounts of manganese have important effects in agricultural soils and aquatic systems and have received considerable investigation (11,12,14,21). Relatively little information is available concerning the effects of manganese in forest soils, however. Soil manganese has recently been associated with black stain root disease caused by *Ceratocystis wageneri* Goheen et Cobb (30). Variations in both oxidation reduction potential (Eh) and hydrogen ion activity (pH) influenced manganese accumulation (9) and affected disease severity (30). The effects of manganese on root rot fungi such as *H. annosum* and various other wood-destroying fungi deserve additional attention.

The quantity of manganese within black regions located in delignified substrates far exceeds the amounts present in sound wood or surrounding decayed wood. An external source of manganese appears essential for the concentrations observed. The processes of manganese transport, accumulation, and immobilization in decayed wood and subsequent release to other microorganisms or plants should become an important component of future forest ecosystem studies.

LITERATURE CITED

1. Blanchette, R. A. 1980. Wood decomposition by *Phellinus (Fomes) pini*: A scanning electron microscopy study. Can. J. Bot. 58:1496-1503.
2. Blanchette, R. A. 1984. Selective delignification of eastern hemlock by *Ganoderma tsugae*. Phytopathology 74:153-160.
3. Blanchette, R. A., Otjen, L., Effland, M. J., and Eslyn, W. E. 1984. Changes in structural and chemical components of wood delignified by fungi. Wood Sci. Technol. In press.
4. Blanchette, R. A., and Shaw, C. G. 1978. Associations among bacteria, yeasts, and basidiomycetes during wood decay. Phytopathology 68:631-637.
5. Boyce, J. S. 1961. Forest Pathology. 3rd ed. McGraw-Hill, Inc., New York. 572 pp.
6. Bromfield, S. M., and Skerman, U. B. D. 1950. Biological oxidation of manganese in soils. Soil Sci. 69:337-348.
7. Campbell, A. H. 1934. Zone lines in plant tissues. II The black lines formed by *Armillaria mellea* (Vahl.) Quél. Ann. Appl. Biol. 21:1-22.
8. Cartwright, K. St. G., and Findlay, W. P. K. 1958. Decay of Timber and Its Prevention. Her Majesty's Stationery Office, London. 332 pp.
9. Collins, J. F., and Buol, S. W. 1970. Effects of fluctuations in the Eh-pH environment on iron and/or manganese equilibria. Soil Sci. 110:111-118.
10. Cromack, K., Todd, R. L., and Monk, C. D. 1975. Patterns of basidiomycete nutrient accumulation in conifer and deciduous forest litter. Soil Biol. Biochem. 7:265-268.
11. Ehrlich, H. L. 1981. Geomicrobiology. Marcel Dekker, New York. 393 pp.
12. Gerretsen, F. C. 1937. Manganese deficiency of oats and its relation to soil bacteria. Ann. Bot. 1:207-230.
13. Ghiorse, W. C., and Hirsch, P. 1978. Iron and manganese deposition by budding bacteria. Pages 847-909 in: Environmental Biogeochemistry

and Geomicrobiology. Vol. 3. W. E. Krumbein, ed. Ann Arbor Science, Ann Arbor, MI.
14. Ghiorse, W. C., and Hirsch, P. 1982. Isolation and properties of ferromanganese-depositing budding bacteria from Baltic Sea ferromanganese concentrations. Appl. Environ. Microbiol. 43:1464-1472.
15. Hartig, R. 1878. Die Zersetzungserscheinungen des Holzes der Nadelholzebaume und der Eiche in Forstlicher Botanischer und Chemischer Richtung. Springer-Verlag, Berlin. 151 pp.
16. Hubert, E. E. 1931. An Outline of Forest Pathology. John Wiley & Sons, Inc., New York. 543 pp.
17. Jensen, W. A. 1962. Botanical Histochemistry. W. H. Freeman, San Francisco. 408 pp.
18. Kirk, T. K. 1981. Toward elucidating the mechanism of action of the ligninolytic system in basidiomycetes. Pages 131-141 in: Trends in the Biology of Fermentations. A. Hollaender, R. Rabson, P. Rogers, A. San Pietro, R. Valentine, and R. Wolfe, eds. Plenum Press, New York.
19. Kirk, T. K. 1983. Degradation and conversion of lignocelluloses. Pages 266-295 in: Filamentous Fungi. Vol. 4. J. Smith, D. Berry, and B. Kristiansen, eds. Edward Arnold, Ltd., London. 401 pp.
20. Krumbein, W. E., and Altmann, H. J. 1973. A new method for the detection and enumeration of manganese oxidizing and reducing microorganisms. Helgol. Wiss. Meeresunters. 25:347-356.
21. Leeper, G. W., and Swaby, R. J. 1940. The oxidation of manganous compounds by microorganisms in the soil. Soil Sci. 49:163-169.
22. Lopez-Real, J. M. 1975. Formation of pseudosclerotia (zone lines) in wood decayed by *Armillaria mellea* and *Stereum hirsutum*. Trans. Br. Mycol. Soc. 64:465-471.
23. Meek, B. D., Mackenzie, A. J., and Grass, L. B. 1968. Effects of organic matter, flooding time, and temperature on the dissolution of iron and manganese from soil in situ. Soil Sci. Soc. Am. Proc. 32:634-638.
24. Munter, R. C., and Grande, R. A. 1981. Plant tissue and soil extract analysis by ICP-atomic plasma spectochemical analyses. Pages 653-673 in: Developments in Atomic Plasma Spectochemical Analyses. R. M. Barnes, ed. Heyden and Son, Philadelphia. 751 pp.
25. Otjen, L., and Blanchette, R. A. 1982. Patterns of decay caused by *Inonotus dryophilus* (Aphyllophorales: Hymenochaetaceae), a white-pocket rot fungus of oaks. Can. J. Bot. 60:2270-2279.
26. Partridge, A. D., and Miller, D. L. 1973. Major wood decays in the inland northwest. Idaho Res. Found. Nat. Resour. Ser. 3. 125 pp.
27. Tien, M., and Kirk, T. K. 1983. Lignin-degrading enzyme from the hymenomycete *Phanerochaete chyrsosporium* Burds. Science 221:661-663.
28. Vogt, K. A., and Edmonds, R. L. 1980. Patterns of nutrient concentration in basidiocarps in western Washington. Can. J. Bot. 58:694-698.
29. Vogt, K. A., Edmonds, R. L., and Grier, C. C. 1981. Biomass and nutrient concentrations of sporocarps produced by mycorrhizal and decomposer fungi in *Abies amabilis*. Oecologia 50:170-175.
30. Wilks, D. S., Gersper, P. L., and Cobb, F. W., Jr. 1983. Relation of soil redox potential to infection of ponderosa pine by *Ceratocystis wageneri*. Phytopathology 73:1120-1125.