Concentrations of Manganese and Microorganisms in Discolored and Decayed Wood in Sugar Maple[1]

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Columns of discolored and decayed wood associated with 8-year-old wounds and contiguous clear wood in five sugar maple trees, *Acer saccharum* Marsh., were mapped systematically for microorganisms and concentrations of manganese. Numbers of wood chips yielding microorganisms decreased as the vertical distance above and below the wounds increased. Bacteria and nonhymenomycetous fungi were isolated from the discolored wood at the distal margins of the columns, Hymenomycetes were not. In columns of discolored wood alone, without decayed wood, the concentrations of manganese were the same as in the contiguous clear wood. In columns of discolored and decayed wood, but where few Hymenomycetes were isolated from the decayed wood, the concentrations of manganese were the same as in the contiguous clear wood. The concentrations of manganese were higher in those tissues at the interface between discolored wood, and decayed wood that yielded Hymenomycetes frequently in culture, than contiguous clear tissues. Changes in ash, moisture, and pH may be more related to the tree response to wounding while manganese concentrations may be more related to the concentration and species of microorganisms in the tissues.

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Des colonnes de bois décoloré et carié associé à des blessures vieilles de 8 ans et au bois clair contigü, dans cinq arbres d'érable à sucre, *Acer saccharum* Marsh, ont servi à une étude systématique de la distribution des microorganismes et des concentrations en manganèse. Le nombre de copeaux de bois renfermant des microorganismes diminuait avec l'augmentation de la distance verticale en haut et en bas des blessures. Des bactéries et des champignons non-hyménomycètes ont été isolés du bois décoloré à la périphérie distale des colonnes, alors que les hyménomycètes étaient absents. Dans les colonnes de bois décoloré seulement, sans que le bois soit carié, les concentrations en manganèse étaient les mêmes que dans le bois clair contigü. Les concentrations en manganèse étaient plus élevées dans ces tissus, à l'interface entre bois décoloré et bois carié fournissant fréquemment des hyménomycètes en culture, qu'elles ne l'étaient dans les tissus clairs contigüs. Les changements du contenu en cendres et en humidité, ainsi que le pH peuvent être davantage reliés à la réponse de l'arbre à une blessure, alors que les concentrations en manganèse peuvent être plutôt reliées à l'abondance et à l'espèce de microorganismes dans les tissus.
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Introduction

Discolored wood in sugar maple, *Acer saccharum* Marsh., and some other deciduous hardwoods contains higher concentrations of minerals than clear wood of the same species (Scheffer 1939). The concentration of minerals, the pH, and the moisture content increase as tissues die, discolor, and decay (Shigo and Sharon 1970).

The major mineral constituents studied by Good *et al.* (1955) in discolored wood of sugar maple were calcium, potassium, iron, magnesium, and manganese. They showed that iron concentrations varied little in the samples —2 samples of clear wood and 18 samples of discolored wood; designated as slight, medium, dark, and very dark stain. The concentrations of manganese in these samples varied greatly from 19 p.p.m. in sound wood to 1250 p.p.m. in a sample of very darkly stained wood. Such

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large differences in the concentration of a microelement as manganese could have an effect on microorganisms and the decay that follows discoloration.

Decayed wood of grand fir, *Abies grandis* (Dougl.) Lindl. contained higher concentrations of manganese than sound wood (Ellis 1959). Bark around cankers on red oak, *Quercus rubra* L., contained higher concentration of manganese than bark not near cankers (Ross 1961). *Phialophora melinii* (Nannfeldt) Conant, one of the most common fungi associated with discolored wood in sugar maple, produced a dark green-black pigment in liquid media amended with manganese (Shigo 1965). Manganese is the most abundant microelement in wood of deciduous trees in northeastern United States (Young and Carpenter 1967), and the concentration of manganese in trees appears to vary with site (Riou and Delorme 1939).

Our purpose was to investigate further the relationship of manganese to discoloration and decay in sugar maple.

Materials and Methods

Columns of discolored and decayed tissues associated with 8-year-old inoculation wounds, and contiguous clear wood (Fig. 1) in five sugar maple trees (numbers 1, 2, 3, 4, and 9 from the study of Shigo and Sharon (1970)) were mapped systematically for microorganisms and concentrations of manganese.

The trees, located in the Hubbard Brook Experimental Forest, West Thornton, New Hampshire, ranged in size from 15 to 30 cm diameter at 1.5 m. The 1.2 m bolts that contained the inoculation wound and the column of discoloration and decay were cut into 5-cm discs, and a template was used to mark the position of the samples relative to the center of the discs (Fig. 1). A plug cutter 19 mm in diameter mounted in an electric drill press was used to remove two sample dowels, A from the outer edge and B from the inner portion of the column of discolored and decayed wood, and two dowels, C and D, from contiguous clear uninfected wood in the same growth rings (Fig. 1). The dowels were trimmed to 4 cm^3 on a table saw as described by Shigo and Sharon (1970) for other analyses. The wood remaining after the dowels were trimmed was used for ash and manganese determinations. In a few cases, there was not enough wood remaining for ash and manganese determinations, and blanks appear in the data.

Microorganism maps were made from the results of isolations. Isolation chips were cut from the discs after the sample dowels had been removed. The

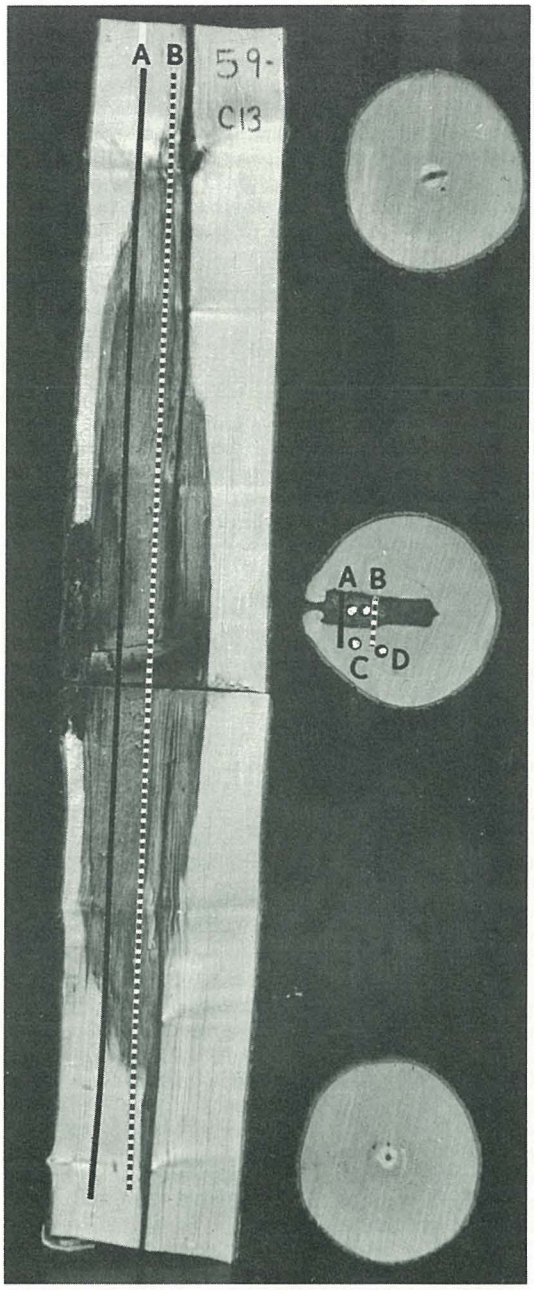

FIG. 1. A typical column of discoloration and decay associated with an inoculation wound in a 1.4-m section of a sugar maple. The 5-cm disc cut from below the inoculation point shows the position of the samples A, B, C, and D. The lines to left of A and to right of B on the disc show where the splits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

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. The agar medium contained 10 g malt extract and 2 g yeast extract per liter of distilled water and it was approximately pH 6 after autoclaving. The chips were cut 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 3000 chips were cultured from the five trees. Additional details on materials and methods are given by Shigo and Sharon (1970).

All ash samples used for manganese determinations were derived from 1-g samples of oven-dried ground wood that was ashed to constant weight in tared porcelain crucibles in a muffle furnace at 550 °C. The ash was dissolved in 10 ml of 5% (v/v) HCl. The acid solution was heated to boiling, cooled, filtered through Whatman No. 40 filter paper, rinsed, and made up to a volume of 50 ml with deionized and distilled water. An appropriate aliquot of this solution was evaporated to dryness. The manganese content was then determined coloremetrically at 525 mμ by the Hunter–Coleman procedure, using a para-sodium periodate solution (Hunter and Coleman 1960).

Soluble ash and soluble manganese were determined on paired unextracted and extractive-free wood samples equivalent to 1-g of oven-dried tissue. Extractive free wood was obtained by using A.S.T.M. Standard D 1105 (American Society for Testing and Materials 1969). Soluble ash and soluble manganese were calculated as the difference between determinations on unextracted and extracted samples. All samples were corrected using specific gravity to show concentrations of manganese per 4 cm^3 of wood.

The effect of large concentrations of manganese on the growth of *Phialophora melinii*, which is commonly associated with discolored tissue in sugar maple, was tested using a basal medium containing 10 g/l D-glucose, 2 g/l asparagine, salts, buffer, trace elements, and vitamins as described by Lilly and Barnett (1951). The basal medium containing trace amounts of manganese (0.1 p.p.m.) was amended with a stock solution of $MnSO_4 \cdot H_2O$ to treatment concentrations of 0, 10, 25, 50, 75, 100, 150, 200, 300, 500, and 1000 p.p.m. Mn^{++}. Initial pH was 5.0. The fungi were grown in 25 ml of medium in 250-ml Erlenmeyer flasks and replicated five times per treatment. Sterilization was done by autoclaving at 15 p.s.i. and 121 °C for 15 min. The sterilized media were inoculated with washed mycelium from 18-day-old cultures of *P. melinii* chopped 5 s in a blender.

After incubation at 20 °C for 18 days, the mycelium was harvested and oven-dried at 105 °C for 24 h in tared 10 ml beakers. Growth was recorded as oven-dried weight of mycelium.

The ability of *P. melinii* to accumulate manganese was tested on the same basal medium at three levels of manganese concentration—20, 60, and 200 μg Mn^{++}/2000 ml solution. The fungus was grown in 200 ml of medium in 1000-ml Erlenmeyer flasks replicated twice. The mycelium was harvested at 21 days and rinsed several times with deionized distilled H_2O and dilute HCl solution. The mycelium was then oven-dried at 105 °C for 24 h and weighed to determine the oven-dried weight of mycelium. The mycelium was then ashed in a muffle furnace and manganese determinations made as previously described. The concentration of manganese was recorded as micrograms of manganese per milligram of mycelium.

Tables giving detailed data from each tree can be obtained from the authors.

Results

Microorganisms and Manganese in Discolored and Decayed Wood

Four of the five sample trees (numbers 1, 2, 3, and 4) had large columns of decayed wood; two of these columns (trees 1 and 3) yielded many cultures of Hymenomycetes, the other 2 (trees 2 and 4) yielded relatively few Hymenomycetes. Tree 9 had little decayed wood.

Discolored wood associated with large columns of decayed wood that yielded many cultures of Hymenomycetes (trees 1 and 3) contained higher concentrations of manganese than the contiguous clear wood (Table 1). The highest concentration of manganese in these columns was at the interface between discolored and decayed wood. The concentration of manganese in the decayed wood was approximately the same as that in the contiguous clear wood. In tree 1, the nonhymenomycetous fungi isolated most frequently from the discolored tissues were *Phialophora melinii*, *Phialophora* sp., and *Fusarium* sp. Only a few other nonhymenomycetous fungi were isolated. Although *Fomes fomentarius* (Fr.) Kickx. was inoculated into this tree, it was not recovered. *Corticium vellereum* Ellis and Dragin and an unidentified Hymenomycete were isolated from the decayed wood. In tree 3, the nonhymenomycetous fungi isolated most frequently from the discolored wood were *Phialophora melinii*, *P. lignicola* (Nannf.) Goidanich, *Ascocoryne* sp., and *Trichicladium canadense* Hughes. *Fomes fomentarius* was not recovered. The Hymenomycete isolated frequently was not identified.

The columns of discolored and decayed wood

TABLE 1. Microorganisms and manganese in columns of discoloration and decay above 8-year-old wounds, and manganese in contiguous clear wood in sugar maple, trees 1 and 2[1]

Centimeters above wound	Tree 1					Tree 2				
	Microorganisms in sample line B			Manganese µg/4 cm³		Microorganisms in sample line B			Manganese µg/4 cm³	
	b	n	h²	B	D⁴	b	n	h²	B	D⁴
65	10	0	0[3]	214	96					
55	23	1	0	286	313	2	1	0[3]	157	150
45	15	1	9	314	106	4	4	0	163	160
35	17	1	13	$\underline{197_5}$	109	17	16	1	148	163
25	17	0	11	174	112	20	14	0	286	170
15	16	0	16	142	123	22	15	1	$\underline{191_5}$	160
5	8	0	19	192	99	22	22	1	584	205
Wound										

[1]Data given represent only a very small portion of total data on record. Data given here show two typical patterns of microorganisms and manganese.
[2]b = bacteria, n = nonhymenomycetous fungi, and h = hymenomycetous fungi.
[3]Number of chips from 24 at each locus that yielded microorganisms.
[4]Only sample lines B and D shown here; B represents a vertical line through a column of discoloration and decay and D is a line through contiguous clear wood as shown in Fig. 1.
[5]Horizontal line indicates interface between discolored wood–above–and decayed wood–below—based on specific gravity measurements.

in trees 2 and 4 in which few Hymenomycetes were isolated from the decay presented another pattern (Table 1). There was little difference between the concentrations of manganese in the discolored and decayed tissues and the contiguous clear wood. This was true even though the column of decay in tree 4 was extensive. In tree 4 the manganese concentration of the decayed wood was lower than the contiguous clear wood.

The nonhymenomycetous fungi isolated most frequently from the discolored wood in tree 2 were *Fusarium* sp., *Phialophora melinii*, *Phialophora* spp., *Ascocoryne* sp., and *Trichocladium canadense*. Fourteen chips yielded Actinomycetes. The nonhymenomycetous fungi isolated most frequently from the discolored wood in tree 4 were *Phialophora melinii* and *Phialophora* sp. Actinomycetes were isolated from 72 wood chips. *Fomes fomentarius* was not recovered.

Tree 9 had a very small amount of decay. There was little difference in the manganese concentration between the discolored and decayed wood and the contiguous clear wood. *Fomes connatus* (Weinm.) Gill was inoculated into the tree, but only *Pholiota* sp. was isolated from the chips. Very few nonhymenomycetous fungi were isolated.

Manganese and Phialophora melinii

There was an increase in dry weight of mycelium of *P. melinii* from 70 to 120 mg when 10 p.p.m. manganese was added to the medium. The dry weight remained almost the same at 120 mg in media with concentrations

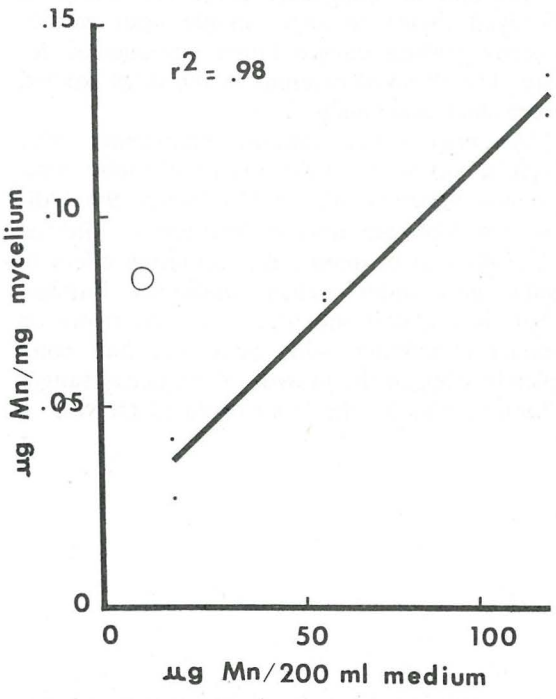

FIG. 2. Relationship between the concentration of manganese in the mycelium of *Phialophora melinii* and the initial substrate concentration of manganese.

of manganese up to 1000 p.p.m. As the amount of manganese in the medium increased, the amount of manganese in the mycelium increased (Fig. 2).

Discussion

Concentrations of manganese in discolored tissues associated with decayed tissues that had a high concentration of Hymenomycetes were higher than in contiguous clear wood. Concentrations of manganese in decayed tissues that had a high concentration of Actinomycetes and low concentrations of Hymenomycetes were the same as those in contiguous clear wood.

The interface between decayed wood and discolored wood had the highest concentration of manganese. This would be the most active site for microorganisms. *Phialophora melinii* accumulated high amounts of manganese in the mycelium. Other microorganisms including the Hymenomycetes may be able to accumulate even higher amounts. Ellis (1959) stated that high concentrations of manganese were in tissues decayed by *Echinodotium tinctorium* (Ellis) E. and E. It is possible that high concentrations of manganese could also occur in decayed tissues in sugar maple after certain microorganisms caused larger amounts of decay. The decayed columns in the trees studied were small and young.

At high concentrations, manganese may have a role in detoxification of phenolic compounds. Shortle *et al.* (1971) showed the addition of high amounts of manganese allowed *P. melinii* to overcome the inhibiting effect of gallic acid under certain conditions. Further, they showed that once *P. melinii* had grown on media containing gallic acid that had completely stopped the growth of the decay fungus *Fomes connatus*, the latter resumed growth.

Ash and moisture content and pH increased as the tissues in sugar maple discolored and decayed (Shigo and Sharon 1970). Manganese did not follow this pattern. Changes in ash, moisture, and pH may be more related to the tree response to wounding while manganese concentrations may be more related to the concentration and species of microorganisms in the tissues.

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