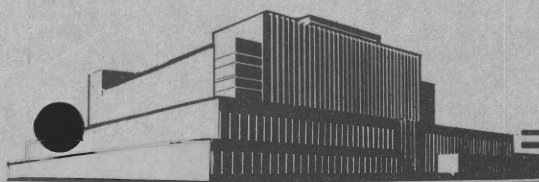
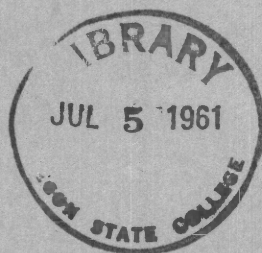


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In Cooperation with the University of Wisconsin

COPPER TOLERANCE OF SOME WOOD-ROTTING FUNGI

By

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Summary

The generally high tolerance of acid-forming brown-rot fungi and the low tolerance of white-rot fungi to copper sulfate was confirmed for 16 species, including Poria cocos Wolf., a fungus associated with the failure of copper-napthenate treated fence posts in Florida, and four other brown-rot species not previously reported as copper tolerant. The tolerance of the brown-rot species was strikingly increased by lowering the pH of the substratum from pH 6 to pH 2 with sulfuric acid. Several mechanisms for fungus tolerance to copper have been suggested; all require a decrease in the pH of the medium.

Introduction

Samples of pine fence posts containing brown cubical decay at or near the ground level were received by this office for examination. The posts came from a farm near Gainesville, Florida, and had been pressure-treated with copper naphthenate by the solvent-recovery process for a retention of one-half pound of the preservative per cubic foot of wood.

Sections cut from the posts through the decayed wood were placed in a moist chamber. In a few days mycelium grew over the surface of these sections and in about two weeks fungus fruiting bodies (sporophores) began to develop. Pure culture isolations were made from the surface mycelium, the context of the sporophores, spores from the sporophores, and the brown cubical rotted wood. In all, 25 isolations from these sources yielded the same fungus, identified by its cultural characteristics as Poria cocos Wolf., the tuckahoe fungus. This identification was confirmed by Dr. J. L. Lowe after examination of the sporophore and the spore prints. Culture No. 104264-Sp, listed in Table 1, is a polysporous isolate from one of the sporophores.

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The fungus was obviously attacking wood that had been penetrated by copper naphthenate, indicating that the preservative treatment was ineffective in preventing decay by it. On that basis it was considered advisable to determine the copper tolerance of Poria cocos and to include in the test a number of other decay fungi that have been used in the past in this Laboratory and at the Forest Products Laboratory, U. S. Forest Service, Madison, Wisconsin.

Materials and Method

The toxicant used was the hydrated sulfate of copper, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.² Sorted, perfectly blue crystals were used. The medium was the standard substratum formerly used in this Laboratory: 2 percent Fleischmann's diamalt, and 2 percent Difco agar in distilled water. The media and the toxicant solutions were autoclaved separately for 20 minutes at 15 lbs. pressure, mixed while hot and, with a pipette, 30 ml. lots were poured into Petri dishes.

The concentrations of copper sulfate used were in geometric progression with a common ratio of 1.75, i. e., each concentration was 1.75 times that of the next below and ranged from 0.1 percent to 2.87 percent. In terms of copper, the concentrations are one-fourth of those shown for the copper sulfate.

In sterilizing the copper sulfate solutions by autoclaving, it was found that a precipitate separated from the solutions. This precipitate was very heavy, particularly at the higher concentrations. It was dissolved by adding concentrated sulfuric acid (66Be; sp. Gr. 1.8355). The heaviest concentration required 12 drops (by eye-dropper) of the acid per liter of agar for clearing. The same amount of acid was added to each of the other concentrations, including the controls which contained no added copper, in order to establish uniform conditions of growth.

The species of fungi used in this test are listed in Table 1. Inoculations were made from fresh Petri dish cultures of each fungus. Uniform discs of inoculum were cut from an arc equidistant from the center of each culture to insure that each inoculum was approximately of the same age. The plates were incubated at room temperature maintained at approximately 25° C.

After 10 days of incubation, 8 of the 17 fungi used in this test had failed to make growth even in the control plates. The pH of the medium, it was realized,

²-Copper sulfate was used because this compound is most generally used in tests of copper toxicity, and to avoid any delay in procuring the naphthenate.

must have been at the critical level when sulfuric acid was added to clear the precipitates brought about by autoclaving. The acidity of the medium was pH 2 as shown by the Alkacid Test Ribbon.

A second series of tests was made, similar in every respect to the above except that the copper sulfate solutions were sterilized by steaming in the Arnold steamer to avoid the formation of the precipitate and the necessity to add sulfuric acid for clearing. The initial acidity of the medium of this second series was pH 6.

Results

The fungi that failed to grow at the pH 2 level included all four of the white-rot species and four of the brown rots (table 1). All of these, except Poria oleracea Dav. and Lomb. and P. carbonica Overh., were also extremely sensitive to copper, the lowest concentration of 0.1 percent copper sulfate being sufficient to check their growth entirely.

Of the fungi that did make growth at the pH 2 level, all made substantial growth and in much higher concentrations of copper than they did at the pH 6 level (fig. 1). Coniophora puteana (Fr.) Karst. was outstanding in this respect. Whereas a concentration of only 0.18 percent of the toxicant was sufficient to stop its growth on the pH 6 media, at the pH 2 level only the highest concentration of the series (2.87 percent) checked its growth. Merulius americanus Burt was only moderately tolerant to copper on the standard medium (0.31 percent), but a three-fold concentration (0.94 percent) was required to check its growth at pH 2. This three-fold advantage at the pH 2 level is also exhibited by Poria monticola Murr. and Daedalea quercina Fr. Poria radiculosa (Peck.) Sacc. showed the highest tolerance to copper of the species tested, the fungicidal as well as the fungistatic concentration at the pH 6 level was 2.87 percent. At the pH 2 level it made growth, although very slow, even in the highest concentration of the series. Measurable growth in this highest concentration did not begin until the 22nd day of incubation and on the 38th day the diameter of the colony was 35 mm. The two isolates of Poria cocos Wolf. also were very tolerant to copper, especially in the low pH range. In this test P. monticola which has heretofore been regarded as perhaps the most tolerant (1, 5).³

The "lag period" to which Horsfall (8) calls attention and frequently observed in fungi grown on toxic substrata, is well shown in this work (fig. 1). P. monticola is the only exception. This species grew as well or better in the more acid medium and in all concentrations of copper, even in the controls, attesting to its acidophilous character as well.

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

For species that failed to grow in the more acid agar the killing point concentrations were usually higher than the inhibiting concentrations. For the acid-tolerant species, however, the inhibiting and killing concentrations appeared equal.

Discussion

The present work at two pH levels and with copper sulfate as the toxicant, indicates that for a number of brown-rot fungi, tolerance to copper sulfate is conditioned by the acidity of the medium. Worthy of note in this respect is that the four white-rot species used in this test are all in the lowest tolerance group. Also in the lowest tolerance group are the brown-rot fungi, Lenzites saepiaria (Fr.) Fr. and L. trabea (Fr.) Fr. It should be mentioned here, however, that while these last two species cause brown-cubical decay in wood, on gallic and tannic acid agar media they sometimes give weak oxidase reactions (2), and thus in this respect tend to react like white rotters.

The difference between the fungi in this test are in general agreement with those of other tests involving copper sulfate in agar (10, 13); copper naphthenate in agar (7); and copper naphthenate in wood (1, 3, 4, 5, 14). In addition to the 10 brown-rot fungi that were moderately to highly tolerant of copper in the present study, the following 7 have been previously reported tolerant to one or more copper compounds in agar or wood: Fomes subroseus (Weir.) Overh.; Lentinus lepideus Fr. (1); Merulius lacrymans Fr. (1); Polyporus sulfureus Fr. (10); Poria vaillantii (Fr.) Cke.; P. vaporaria (Fr.) Cke. (10); and a Ptychogaster sp. (10). For the white-rot species, in addition to the four in the present study, intolerance to copper compounds has been reported for Fomes pini (Fr.) Karst. (1); Pleurotus ostreatus Fr. (10); Polyporus abietinum Fr. (1); Poly. adustus Fr. (1); Poly. hirsutus Fr. (1); Schizophyllum commune Fr. (10); and Stereum purpureum (Fr.) Fr. (10). In all of these reports, copper tolerance is generally associated with the brown-rot fungi and intolerance with the white. Only in Cowling's (1) results is there any material departure from this.

Rabanus (10) and Shimazono and Takubo (12) have suggested that the high tolerance of the brown rots is due to their habit of producing oxalic acid which, it was supposed, precipitates the copper into the insoluble form of the oxalate thus rendering the copper metabolite inert. However, if precipitation of the metal into the inert oxalate form were the principal factor in copper tolerance, the brown rots should show an even greater superiority over the white rots with respect to zinc tolerance. Zinc is a more potent poison than copper and zinc oxalate more insoluble and more inert than copper oxalate. In Richards' (11) study, however, the white rots actually outranked the brown rots in zinc tolerance.

This study indicates that the low pH reaction generally produced by the brown-rot fungi in their metabolism, irrespective of the acid produced, may be more important as a factor in their copper tolerance. In this study sulfuric acid was used and, in the case of Coniophora puteana (Fr.) Karst., its tolerance to copper was increased more than nine-fold at the pH 2 level. Lin (9) found that hydrochloric acid antidoted copper sulfate for spores of Sclerotinia fructicola Wint. in the pH range of 2 to 6 covered by this study. Horsfall (8) attributes the greater tolerance in acid substrata as due to the protection of amino acids against replacement of their hydrogen by chelating the copper. The copper tolerance of the oxalic-acid-producing brown-rot species, he continues, may thus be due not to the low solubility of copper oxalate but mainly to the lowering of the pH of the substratum.

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Table 1.--Tolerance of some wood-rotting fungi to copper sulfate in agar

Fungus Species	Isolate number:	Type:	Lowest inhibiting		Lowest killing	
			concentration	Percent	concentration	Percent
			pH 6	pH 2	pH 6	pH 2
Poria radiculosa (Peck) Sacc.	Mad. 5096-29	Brown:	2.87	2.87+	2.87	2.87+
P. cocos (Schw.) Wolf	Mad. 104-R	"	1.64	2.87+	1.64	2.87+
P. cocos (Schw.) Wolf	FP 104264-Sp	"	1.64	2.87	1.64	2.87
P. oleracea Davidson & Lombard	Mad. 4907	"	1.64	----	1.64	no data
P. monticola Murr.	Mad. 698	"	.94	2.87	1.64	2.87+
P. xantha (Fries) Cooke	Mad. 5096-35	"	.94	1.64	1.64	1.64
P. incrassata (Berk. & Curt.) Burt	Mad. 563	"	.94	1.64	.94	2.87
Daedalea quercina L. ex Fries	FP 59058-R	"	.54	1.64	.54	1.64
Merulius americanus Burt	FP 97439-Sp	"	.31	.94	.31	.94
Poria carbonica Overh.	FP 94160-R	"	.31	----	.31	no data
Coniophora puteana (Fries) Karst.	Mad. 515	"	.18	2.87	.18	2.87
Lenzites trabea Pers. ex Fries	Mad. 617	"	.1-	----	.31	.31
L. saepiaria (Fries) Fries	Mad. 604-S	"	.1-	----	no data	.18
Stereum frustulosum (Pers. ex Fries) Fckl.	FP 56461-R	White:	.1-	----	.18	no data
Polyporus versicolor L. ex Fries	Mad. 697	"	.1-	----	.18	no data
Poria ambigua Bres.	FP 104029-Sp	"	.1-	----	.18	.18
Odontia bicolor (Fries) Bres.	Flo 126-A	"	.1-	----	.1	.1

a = At pH 2 no test for copper because fungus failed to grow even in the controls.

+ = Greater than this concentration.

- = The given concentration or less.

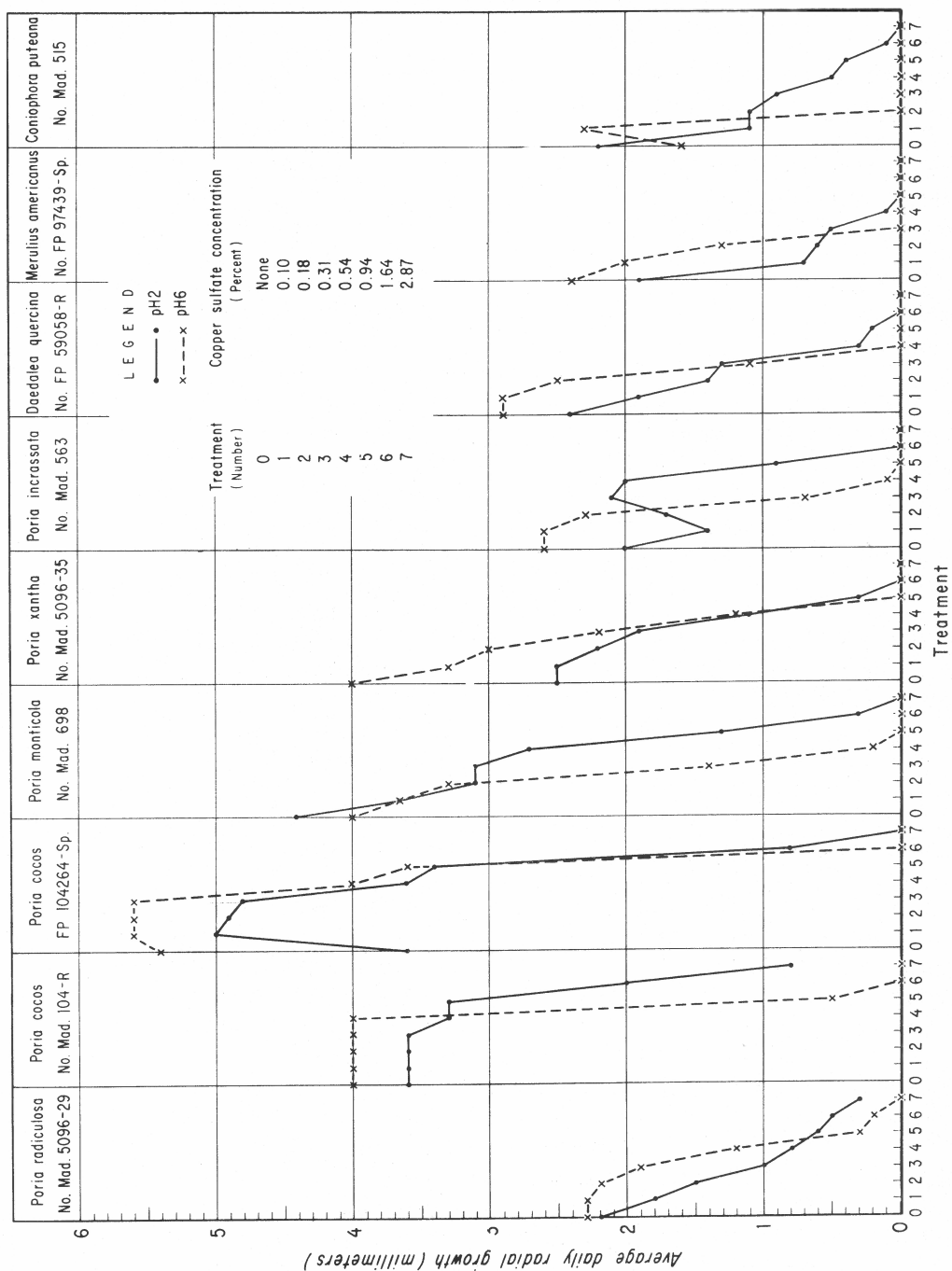


Figure 1. --Effect of pH on tolerance to copper of some wood-rotting fungi.

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