# Brown Sapwood Stain of Ponderosa Pine Caused by *Cytospora* sp.: Cultural and Histological Aspects

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ABSTRACT

Cultural characteristics of a *Cytospora* species causing brown sapwood stain of ponderosa pine are described in detail. The fungus primarily decays ray parenchyma and produces a diffusing pigment that stains longitudinal tracheids and associated wood elements. The *Cytospora* sp. is similar or identical to one discussed by earlier investigators.

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*Additional key words:* pycnidium, conidiophore, conidium, *Pinus ponderosa*.

Early in 1975 we were queried by quality control personnel of a north-central Washington lumber company about a brown sapwood stain of ponderosa pine *(Pinus ponderosa* Laws.) lumber. The stained lumber had been sawn from stained logs that were decked six to twelve months prior to sawing. This lengthy storage of logs is not usual, but resulted from a depressed market for lumber. In any case, brown-stained lumber was rejected by buyers who were suspicious that it was unsound as well as unsightly.

Hubert (4) reported a brown stain to be common in pines, particularly hard pines, of the Lake States and western North America. Fritz (2) isolated and partially characterized a *Cytospora* causing a brown stain of *Pinus resinosa* Ait. and made a detailed study of its relationship to wood and its effects on strength properties of wood. Fritz believed the *Cytospora* isolated by her to be responsible for the brown stain described by Hubert (4). We isolated a *Cytospora* Ehrenb. ex Fr. from brown-stained lumber and believe it to be similar or identical to Fritz's fungus (2). We were unable to obtain isolates for comparison, however. Because of the prevalence and importance of brown stain in pine logs stored for long periods, we herein describe the fungus isolated by us and supplement previously published observations on stained wood.

MATERIAL AND METHODS.—*Cytospora* sp. was isolated by taking increment cores, with a sterilized increment hammer, from brown-stained areas of wood and plating them on 2% water agar. Hyphae emerging from the cores were excised and plated on 2% potato dextrose agar fortified with 5 g/liter yeast extract (PDYA). Cultures have been placed in American Type Culture Collection as ATCC 28772.

In preparation for sectioning, sapwood was boiled repeatedly, then placed in a mixture of equal parts of 95% ethanol and glycerin for several weeks. Blocks were trimmed to expose radial or tangential faces, then sectioned unembedded at 20 $\mu$m on a sliding microtome. Some sections were stained with picro aniline blue-safranin, using the schedule of Wilcox (7), and examined by bright-field microscopy. Other sections were mounted in 0.2% (w/v) aqueous solution of Calcofluor White PMW (a fluorescence brightener, product of American Cyanamid Co.) or 0.05% (w/v) aqueous solution of acridine orange and examined with a Zeiss microscope equipped with a dark-field condenser and Osram HBO 200 W mercury lamp. Exciter filter GB12 and barrier filter 47 were used in tandem.

Conidia and hyphae from culture were examined unstained or mounted in 0.1% acid fuchsin in saturated aqueous chloral hydrate. Sections of pycnidia were stained with Giemsa in pH 6.5 phosphate buffer. Light photomicrographs were taken on 35-mm Kodak High-Contrast Copy Film 5069 and developed in Kodak Microdol-X.

Sections cut with the sliding microtome were dehydrated through a graded ethanol series followed by an ethanol-Freon TF series. After dehydration, material was subjected to a critical-point apparatus. Processed material was vacuum-coated with gold and examined by an ETEC Autoscan scanning electron microscope.

RESULTS.—Cultural characters of *Cytospora* sp. causing brown stain in sapwood of ponderosa pine is described, as follows.

Colonies on PDYA covering 87-mm diameter petri plate in 3 weeks. Hyphae at advancing edge of colony white to yellowish, villose, becoming tawny to cinnamon and appressed. Reverse at first yellowish, rapidly becoming dark-brown to blackish. Pycnidia produced on colony in one month. Pycnidia subglobose, 0.5-1 (+) mm in diameter, slightly beaked, with one ostiole, blackish, usually covered by tawny hyphae. Pycnidium interior 1-6(+) loculate (Fig. 1), the locules lined with hyaline, complexly branched conidiophores (Fig. 3). Locules releasing conidia through the single ostiole. Conidiophore conidiogenous tips apparently phialides, but this is uncertain owing to minute size. Conidia allantoid, elliptical, or oblong (Fig. 2) 4-6 $\mu$m $\times$ 1-2 $\mu$m, individually hyaline, extruded from pycnidium in a yellowish horn or drop.

Examination of sections of brown-stained wood

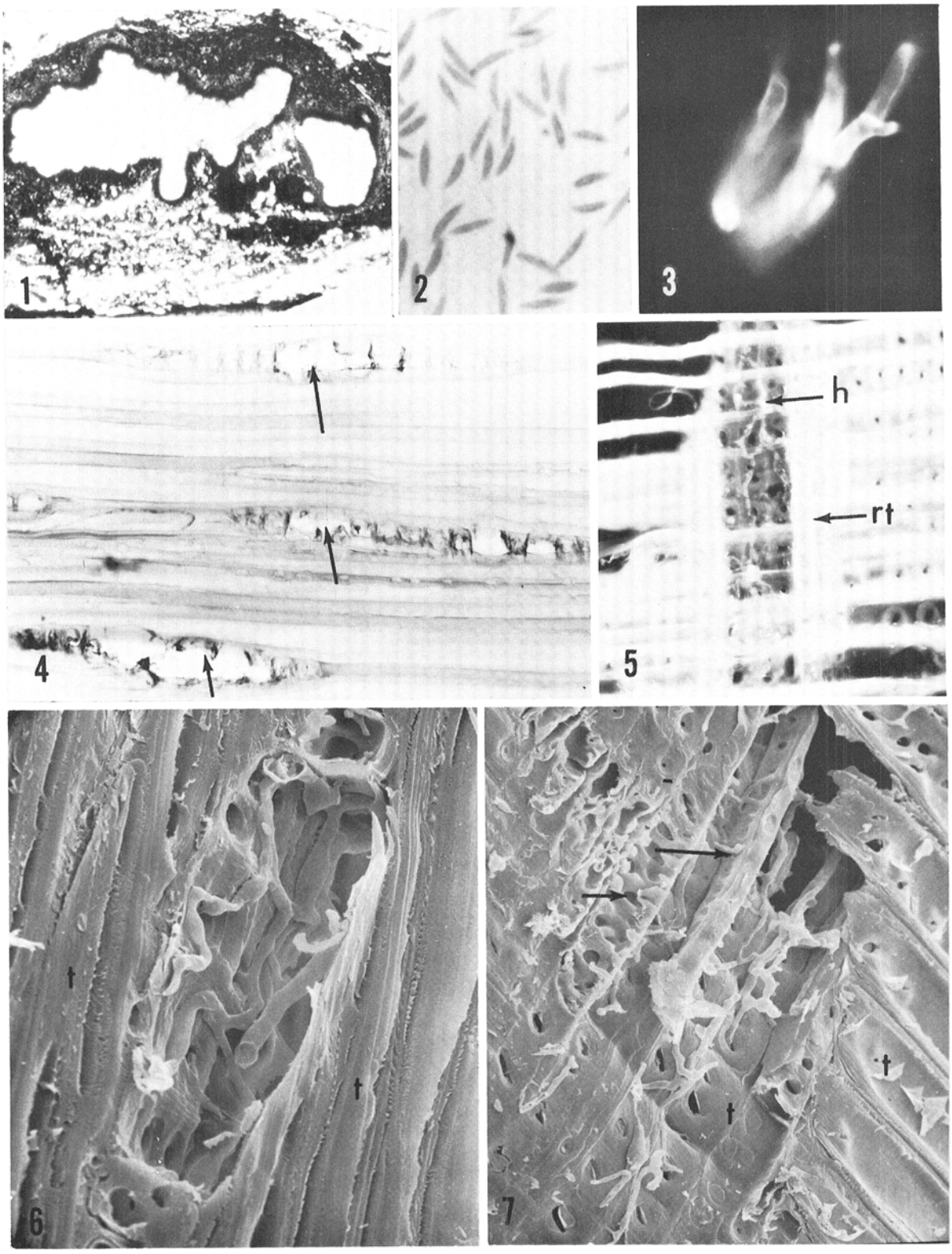


**Fig. 1-7.** *Cytospora* sp. and the histology of its association with ponderosa pine wood. **1)** *Cytospora* conidial locules from freezing microtome section of single pycnidium (stained with Giesma; bright field microscopy) (× 54). **2)** *Cytospora* conidia (stained with acid fuchsin, bright field microscopy) (× 2500). **3)** *Cytospora* conidiophores (mounted in brightener, dark field fluorescence) (× 1800). **4)** Tangential section of brown-stained wood. Arrows denote areas in rays where parenchyma has been decayed (picro aniline blue-safranin, bright field microscopy) (× 165). **5)** Radial section of brown-stained wood. Hypha (h) in area formerly occupied by ray parenchyma, with pits of underlying longitudinal tracheids showing. Ray tracheids (rt) (acridine orange, dark field fluorescence) (× 170). **6)** Tangential section of brown-stained wood. Former ray packed with hyphae. Surrounding longitudinal tracheids (t) (scanning electron microscopy) (× 840). **7)** Radial section of brown-stained wood. Hyphae accumulated in void created by lysis of ray parenchyma and entering pits of underlying and overlying longitudinal tracheids (t). Dentate ray tracheids (arrows) invaded, but intact (scanning electron microscopy) (× 420).

showed that the fungus is confined primarily to rays and to longitudinal wood elements at ray crossings (Fig. 4, 5, 6, 7). Parenchymatous ray elements, including resin duct epithelial cells, are completely lysed by the fungus (Fig. 5, 7). Low-power light microscopy and scanning electron microscopy of tangential sections showed partial voids in areas formerly occupied by rays (Fig. 4, 6). Dentate ray tracheids and longitudinal tracheids of the wood are entered via pits (Fig. 7). Tracheid walls are stained brown, but bore holes or thinning of walls were not observed.

Steam-sterilized pieces of ponderosa pine wood (approximately 6 cm × 3 cm × 0.5 cm) placed in cultures with the fungus became deteriorated and stained within 6 weeks in the same manner as naturally affected wood.

DISCUSSION.—Results of our study indicate that the *Cytospora* sp. described here, and its activities in wood, are identical to those described by Fritz (2). Fritz concluded that the effect of the fungus on major mechanical properties of pine wood is negligible. We did not perform mechanical tests, but, based on histological observations, assume that mechanical properties are not adversely affected if wood is used in ordinary situations with common safety allowances.

It would be premature to assign a species name to this *Cytospora* sp. There are at least 320 *Cytospora* descriptions, one-third of which possibly are "good" species (1). Most of them have been described, in part, on the basis of host. Little cross-inoculation or cultural work has been done. Most, if not all, *Cytospora* species are imperfect states of *Valsa* Fr. or closely related genera. *Valsa pini* (Alb. and Schw.) Fr. and *V. abietis* Fr., both of which are reported to have *Cytospora* imperfect states, are reported on ponderosa pine in the Pacific Northwest (5, 6). *Cytospora abietis* Sacc., reported to be the imperfect state of *V. abietis* (6), was shown by cross-inoculation studies to cause cankers of white fir [*Abies concolor* (Gord. and Glend.) Lindl.] and California red fir (*A. magnifica* A. Murr.) in California and Nevada (8). The *Cytospora* sp. described herein may be the imperfect state of one of these *Valsa* spp.

In a widely circulated (but not officially published) report, Funk (3) described a *Cytospora* from Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] stem cankers that, in culture, has resemblances to the fungus described herein. Unfortunately, cultures of Funk's fungus are no longer available (A. Funk *private correspondence*).

*Cytospora* brown stain appears to develop, at least noticeably, in logs that have been stored for 6 months or longer. Material examined also showed blue stain which can develop much more rapidly. Indeed, the lumber company which supplied the brown-stained wood regularly saws blue-stained logs. It seems probable that *Cytospora* brown stain will be a problem primarily of logs stored for long periods and can be controlled by more rapid rotation of inventories. If it should become a problem under other circumstances, controls based upon more detailed studies of the fungus are indicated.

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