MICROSCOPIC CHARACTERIZATION OF NONMICROBIAL GRAY SAPSTAIN IN SOUTHERN HARDWOOD LUMBER[1]

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ABSTRACT

Southern red oak, ash, and hackberry sapwood containing nonmicrobial discolorations was examined by both light and scanning electron microscopy to determine the causes of these discolorations. Ray parenchyma cells in discolored sapwood of all three species contained globose to amorphous pigmented globules of starch. Ray parenchyma cells in nondiscolored sapwood occasionally contained a few globules. Results indicate that the formation of pigmented starch compounds occurs during normal air-drying operations and is intensified by slow-drying conditions. This results in the macroscopic sapwood discoloration commonly called gray stain.

Keywords: Hardwoods, sapstain, microscopy.

INTRODUCTION

Most sapstains in lumber are caused by fungal colonization. Pigmented spores of mold fungi cause surface discolorations. The colored hyphae of sapstain fungi discolor the entire sapwood portions of many species. These stains can be effectively controlled in hardwoods by dipping freshly cut lumber in a suitable biocide, maintaining a well-drained air-drying site free of weeds, and stacking the lumber to facilitate air movement within and between stacks (McMillen and Wengert 1978), or by kiln-drying shortly after sawing.

Other types of sapstains are chemical rather than fungal. These stains are not associated with any known microbial activity. Chemical brown stain, which occurs mainly in western softwood species, has been studied extensively (Anderson et al. 1960; Barton and Gardner 1966; Cech 1966; Evans and Halvorson 1962; Hulme and Thomas 1975, 1983; Miller et al. 1983; Millett 1952; Shields et al. 1973; Zabel 1953). It is caused by the migration of water-soluble extractives to the surface of lumber during drying and the subsequent enzyme-mediated oxi-

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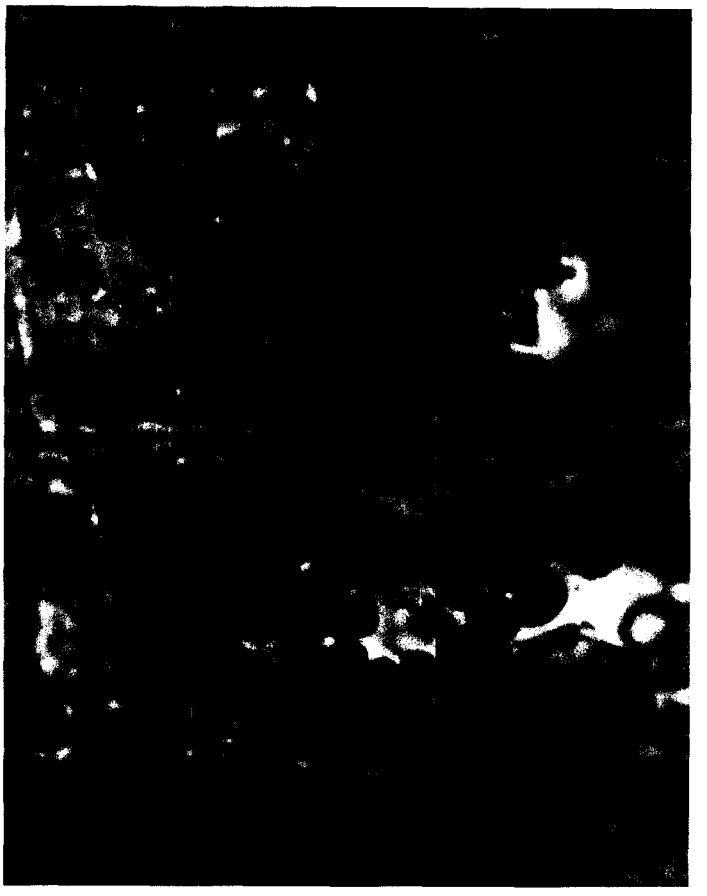

FIG. 1. Light microscope view of amorphous globules in the ray parenchyma cells of gray-stained southern red oak sapwood (100×).

dation of these compounds to a brown polymerized pigment. This discoloration is usually confined to the surface, but it may penetrate throughout affected pieces.

A similar oxidative stain, commonly called gray stain, occurs in certain hardwood species and has periodically caused significant decreases in lumber value (Amburgey and Forsyth 1987; Clark 1957). As with brown stain, this discoloration is believed to be caused by the enzyme-mediated oxidation of certain wood constituents. It usually develops during warm, humid conditions that retard air-drying. Gray-stained red oak (*Quercus* spp.) lumber often has a nonuniform, mottled gray appearance on the flat-sawn surface. On the cross-section, gray stain uniformly discolors the entire sapwood, unlike fungal stains, which form wedge-shaped areas of discolorations along the rays. Usually, the discoloration first appears at the heartwood-sapwood interface and progresses throughout the sapwood, taking up to two weeks to develop fully. The reason for this progression is not understood at this time. Generally, gray stain is not noticeable until the outer surface is removed by planing or sanding. It was found that either oven-drying or steam-heating specimens at 212 F for 30 minutes before air-drying, a method that will not prevent fungal stains, prevented gray stain development (Anon. 1956;

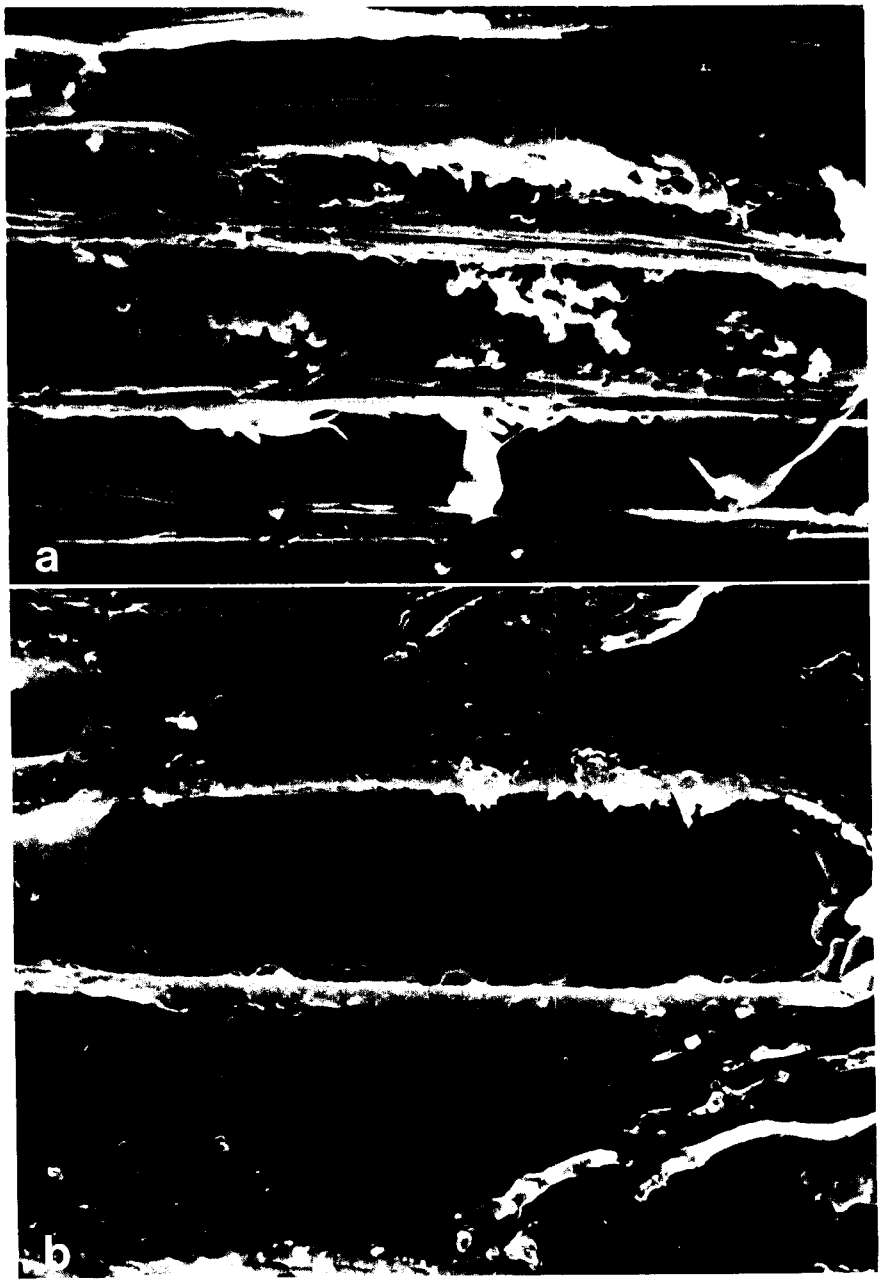

FIG. 2. Globules nearly filled the lumena of ray parenchyma cells of gray-stained southern red oak sapwood (a) (950×) but were either absent or few in number in nondiscolored sapwood (b) (1,500×).

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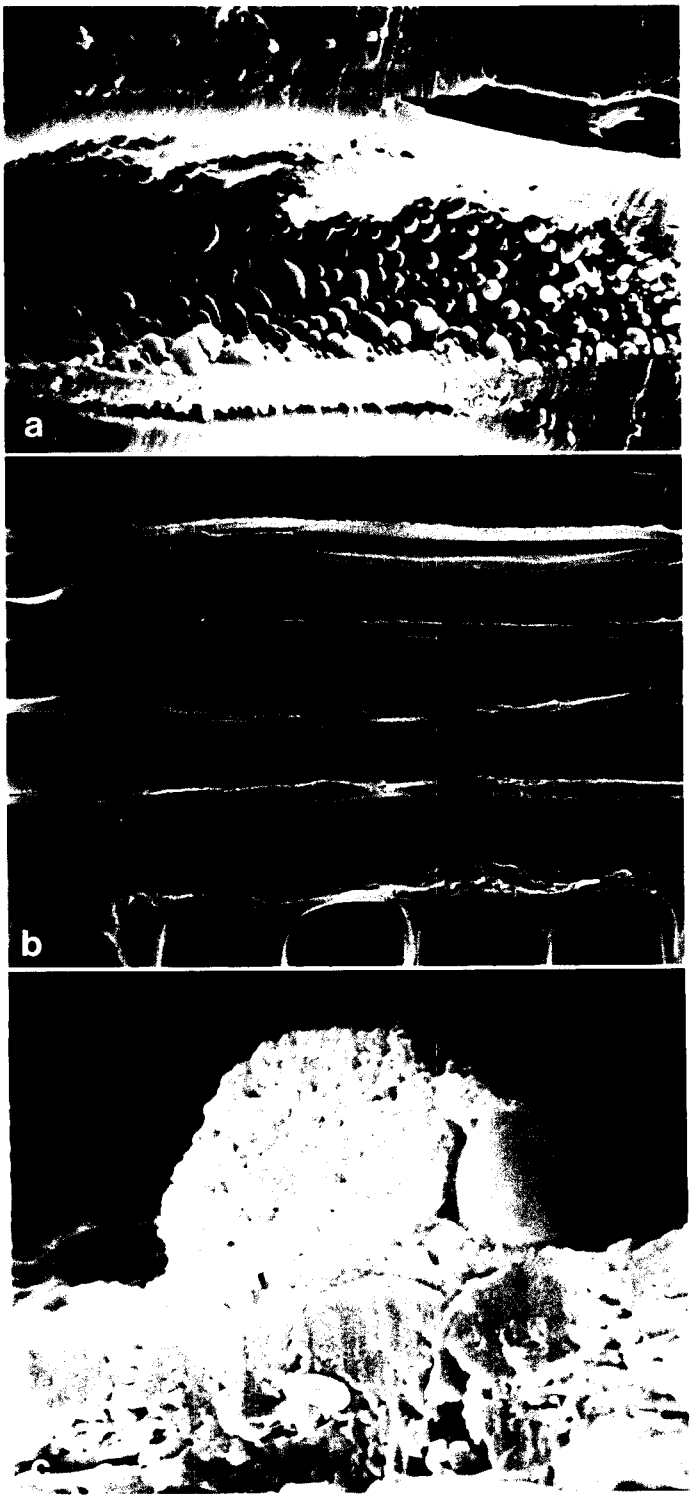
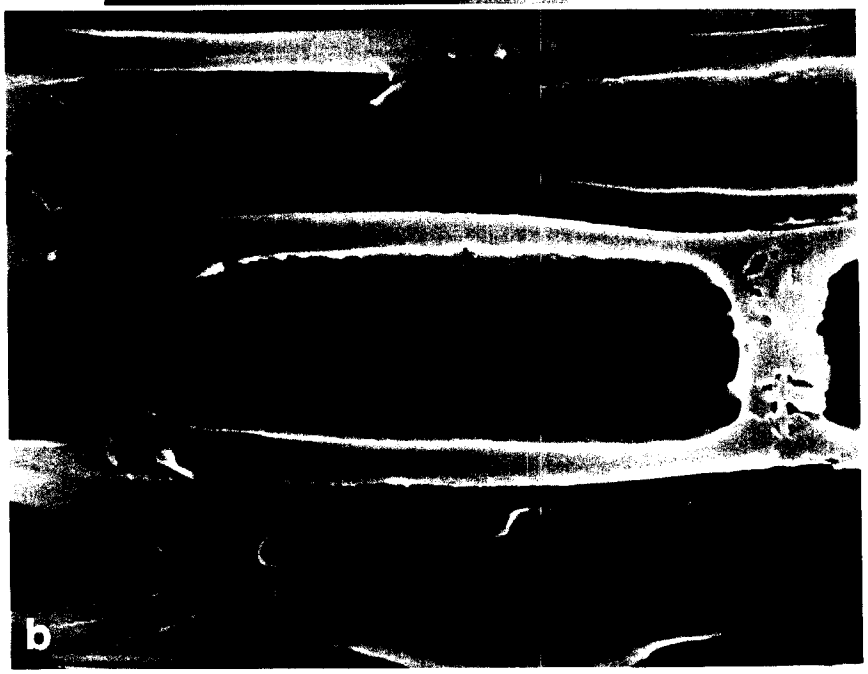
FIG. 3. Globules in the ray parenchyma cells of sticker-stained ash were less amorphous than those found in gray-stained oak sapwood (2,000×) (a). Ray cells in nondiscolored ash usually had no globules (700×) (b). Many globules appeared to be secreted from the lumen side of the cell wall (10,000×) (c).


a
b


a


b

Gill 1957). Laboratory and field tests at Mississippi State University have indicated that dipping freshly cut southern red oaks in a formulation containing sodium bisulfite will control gray stain, but this is not being used commercially (Amburgey and Forsyth 1987; Forsyth and Amburgey in preparation).

Some other hardwoods develop a similar discoloration during drying. Sugar hackberry (*Celtis laevigata* Willd.) sapwood lumber often develops a brownish-gray to greenish-gray discoloration just below the surface and sometimes throughout the cross section of the piece. This discoloration is difficult to control because the discoloration can develop within two to three hours after sawing during warm, humid weather. Steaming the wood prior to air-drying or kiln-drying the wood immediately after sawing will prevent discoloration (Price 1982). Ash (*Fraxinus* spp.) lumber develops a stain similar to that in red oak and hackberry, but the stain occurs only beneath drying sticks. The reason for sticker staining is not clear, but it is believed to be due to slower drying conditions beneath the stickers. Nonmicrobial discolorations in tupelo gum (*Nyssa aquatica* L.), evergreen magnolia (*Magnolia grandiflora* L.), and sweetbay magnolia (*M. virginica* L.) are associated with high wood moisture content and are caused by the conversion of starch in parenchyma cells to brownish gum-like deposits (Scheffer and Lindgren 1940).

The purpose of this study was to observe and characterize oxidative discolorations in southern red oak, ash, and hackberry via light and scanning electron microscopy.

METHODS AND MATERIALS

Light microscopy

Thin sections of gray-stained red oak sliced with a hand-held razor blade were soaked in 5% aqueous potassium hydroxide for several seconds to soften and swell the tissue. Each section was then stained with a saturated aqueous solution of phloxine and mounted on glass slides for transmission light microscopy observation.

Scanning electron microscopy

Small cubes (5 millimeters per side) of gray-stained and clear red oak, ash, and hackberry were vacuum impregnated with water and allowed to soak overnight. Then, a new hand-held razor blade was used to remove a thin layer of the surface to be observed. Cuts were made to assure that some ray tissue was present on the exposed surface. These specimens were air-dried for 24 to 48 hours in a partially covered petri dish to prevent dust accumulation, and then the dried specimens were attached to aluminum stubs with silver paste. After the adhesive was allowed to dry for 24 to 48 hours, the specimens were coated with gold/palladium in a Polaron E5100 sputter coater and observed in a Hitachi HHS-2R scanning electron microscope at 20 kV.

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FIG. 4. Globules in parenchyma cells of discolored hackberry appeared to be flattened or saucer-shaped (2,500×) (a). Parenchyma cells in nondiscolored hackberry usually contained no globules (2,000×) (b).

RESULTS AND DISCUSSION

Gray-stained and unstained specimens appeared similar under the light microscope, with the exception of the ray tissue. The ray parenchyma cells of stained specimens were occluded with tiny amber to brown globules when viewed from the transverse and radial sections (Fig. 1). The electron microscope revealed similar inclusions in stained red oak, ash, and hackberry. The shape of the inclusions ranged from globose to ovoid to nearly completely amorphous incrustations, their shape and texture being unique for each genus. Globules in red oak varied most, but the shapes were usually globose to ovoid with a rough surface (Fig. 2A). The globules in ash had smooth surfaces and were more consistent in appearance (Fig. 3A). Many of the globules appeared to be secreted from the lumen side of the cell wall (Fig. 3C). In hackberry, the ray parenchyma cells, as well as many upright parenchyma cells, were filled with globules that were more flattened or saucer-shaped (Fig. 4A).

In general, globules were absent from the ray cells of unstained specimens of all three species (Figs. 2B, 3B, and 4B). Although a few scattered globules were found in the parenchyma cells of unstained specimens, they were not concentrated enough to cause a macroscopic discoloration. This result indicates that globule formation while lumber is drying is a natural reaction that is intensified during slow drying conditions and results in gray-stained lumber. The globules become blue in color when treated with iodine-potassium iodide solution and viewed microscopically. This is a reaction generally considered to be indicative of the presence of starch (Johansen 1940). It is hypothesized by the present authors that these globules are the end-product of the enzyme-catalyzed reaction believed to occur in gray-stained lumber. Results of this and other studies indicate that nonmicrobial sapstains in several hardwood species are caused by enzyme-catalyzed reactions that covert starch to pigmented deposits (Scheffer and Lindgren 1940; Panshin and DeZeeuw 1980).

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