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Comparative histochemistry and cell morphology of sapwood and heartwood of *Gliricidia sepium* (Fabaceae)

Alejandra Quintanar[1], Laura Rivera[1], Alfonso Torre-Blanco[2] and Silvia Rebollar[1]
[1]Universidad Autónoma Metropolitana-Iztapalapa, Av. Michoacán y Purísima, Col. Vicentina, Iztapalapa, 09340, México, D.F. aqi @ xanum.uam.mx.
[2]Facultad de Ciencias, UNAM, México, D.F.

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Abstract: *Gliricidia sepium* (Jacq.) Steud. (Fabaceae) shows a remarkably high ratio of heartwood to sapwood. Heartwood fibres and vessels have very thick cell walls and the heartwood vessel elements contain many sclerotic and common tyloses, both with gums and crystals in their cavities. Histochemical analysis of these cells and tyloses showed that the cell walls of fibres, vessels and sclerotic tyloses have great quantities of lignin and sterified pectins as matrix components, as well as condensed tannins or phenolic substances in their lumen. Crystals are present in common tyloses and axial parenchyma. Most vessel elements in the sapwood contain tyloses that completely fill their lumen. the majority of these structures are of the common type, whereas only a few of the sclerotic type are present near the heartwood region. Our observations suggest a fast transformation of sapwood into heartwood. We think that the presence of very thick cell walls of fibres, vessels and tyloses, as well as their chemical composition and the inclusion of crystals, influence the high natural resistance to decay of this wood.

Key words: Histochemistry, tyloses, cell morphology, wood, *Gliricidia sepium*, Quintana Roo, Mexico.

Wood of *Gliricidia sepium* (Jacq.) Steud. has been used in tropical America for building purposes because "it never decays and is the strongest wood known" as remarked by Standley (1961). Because of its resistance to termites and fungi it is used for railroads sleepers and poles in Quintana Roo, Mexico (Cabrera *et al.* 1982). The sapwood of *G. sepium* is yellow, the heartwood dark brown. The ratio of heartwood to sapwood is very high. These trees usually reach a considerable height, up to 12 m, and are abundant in tropical regions. for these reasons the specie is appreciated.

Many useful properties of the bark and wood of this tree should be related to chemical components present in the cell walls and lumina. the amount and type of those chemicals are associated with sapwood to heartwood transformation (Datta and Kumar 1987). In addition to the chemical composition of walls, the tyloses should also be of great significance to the properties and uses of this wood, particularly to the heartwood. Details about their morphology and function have been described elsewhere (Metcalfe and Chalk 1950, Foster 1964, Korán and Coté 1965, Sano and Fukazawa 1991, Babos 1993) as well as some evolutionary aspects (Bonsen and Kucera 1990). Carlquist (1988) and Schmitt and Liese (1994) remarked that the occurrence of tyloses in the vessels of spring wood and sapwood of certain species of angiosperms can be the answer to vascular infection, or chemical stimulation. However, when tyloses are normally present, their presence is directly related to heartwood formation. These structures can eventually develop secondary layers, and indeed, completly occlud vessels (Dimond 1970). once occluded, tyloses help to avoid colonization and development of many kinds of fungi which can infect the tree.

This paper deals with the histochemistry of cellular components and the morphology of tyloses in heartwood and sapwood of a tree of *G. sepium*. The relationship between the types of chemical components and some properties of the wood of this tree is also discussed.

MATERIALS AND METHODS

A healthy individual tree of *G. sepium*, 10 m height, with a straight stem 25 cm in diameter at a height of 1.3 m, was collected in a tropical rain forest at the Municipio de Benito Juárez in Puerto Morelos, Quintana Roo, México. The botanical voucher is registered with number 26106 in the Herbario Metropolitano of the Universidad Autónoma Metropolitana Iztapalapa (UAMIZ) and wood samples as UAMIZ-M39 in the Herbario Metropolitano's wood collection.

The tree was cut at 30 cm from the ground level and two stem sections, 1 m long each, were obtained. From the upper face of the first section, that is at 1.3 m height, a 2 cm section was cut from this, 1x1 cm cubes were obtained from the sapwood and heartwood. The cubes were boiled in water during six hours. Next, they were treated in a microwave oven in 50% glicerol, during 8 minutes at medium power. After this procedure, tangential, radial and cross sections were obtained at thicknesses ranging from 8 to 15 µm. Starch and lipids were revealed by the method of Johansen (1940). The other histochemical methods used in this study to stain heartwood and sapwood sections are described in detail by Krishnamurty (1988). Lugol and Sudan III were employed for detection of starch and lipids. The Toluidine Blue O method was used to stain differentially cellulose (blue) and lignin (green-blue). The zinc-chlorine-iodine method was employed to contrast cellulose (blue to violet) from lignin (yellow to orange). The tannic acid-ferric chloride method was used to reveal the presence of calcium pectate (black to blue-black). The presence of sterified pectins was tested by the alkaline hydroxylamine hydrochloride method. The phloroglucinol method was used to specifically stain lignin (red to violet). Phenolic compounds were specifically stained by the vainillin method. cathechines and leucoanthocianidines are seen as red deposits, whereas their oligomers are seen as dark deposits. The stained sections were mounted in glicerol or synthetic resins. Photographs were taken from fresh and mounted slides.

The thickness of the cell walls of fibres and vessels and their diameters were meassured with the help of a micrometric ocular piece. Pits of vessels and vessel-ray parenchyma were meassured following the specifications of IAWA (1989). Morphological description of tyloses and crystals were made according to IAWA (1989), and Saitoh *et al.*(1993). At least 50 individual measurements were made for every character described.

RESULTS

Fibres. The cell walls of sapwood fibres were thick (10 ± 0.5 µm) and, besides cellulose and lignin, only a light reaction for sterified pectins was detected, whereas heartwood fibres were thicker (12 ± 0.9 µm) and contained a higher amount of lignin and sterified pectins (Fig. 1F). Dark deposits corresponding to condensed tannins were identified inside the lumina of heartwood fibres (Table 1).

TABLE 1

Comparative chemical composition in cells of sapwood and heartwood of *Gliricidia sepium*

Reactive	TB1	TB2	ZCI	TA-FCh	AH	Ph	V1	V2	Lugol	Sudan
Sapwood cells										
Fibres	++++	+			++	+++++				
Vessels	++++				++	++++			+	+++
Tylose 1	++++					+++				
Tylose 2		+++	+++						+	+++
Axial p		+++	++++			+	++	++	+++	+++
Rays		+++	++++			+	++	++	+	+++
ml				+++						
Heartwood cells										
Fibres	+++++		+	+	++++	+++++	++	+++		
Vessels	+++		+	+	++++	+++				
Tylose 1	+++++		+	+	++++	+++				
Tylose 2		+++++	+++++	+++				++++		
Tylose 3		+++++	+++++	+++						
Axial p		+++++	+++++	++				++++		+++
Rays		+++++	+++++	++				++++		+++
ml vessel				+++			+++++			
ml fibres				+++			+++++			
ml tyloses				+++			+++			
ml paren.				+++++			+			

TB1. Toluidine blue for lignins. TB2. Toluidine blue for cellulose. ZCI. Zinc-chlor-iodine for cellulose. TA-FCh. Tannic acid-ferric chloride for calcium pectates. AH. Alkaline hidroxylamine for sterified pectins. Ph. phloroglucinol. V1. Vainillin for dark deposits. V2. Vainillin for red deposits in the lumina. Tylose 1 = sclerotic tylose. Tylose 2 = common tylose. Tylose 3 = common tylose with crystals included. Plus symbols mean: + very light, ++ light, +++ medium, ++++ strong, and +++++ very strong reactions.

Table 2.

Wall thickness of vessels and tyloses from sapwood and heartwood

Wall thickness $p< 0.05$	Sapwood	Heartwood
<	13 ± 1.1 µm	16 ± 0.9 µm
----Moderately large vessels		
Regular vessels	7.5 ± 0.5 µm	8 ± 4 µm
Sclerotic tyloses	35 ± 2.7 µm	37.5 ± 3 µm
Common tyloses	$5 + 0.6$ µm	8 ± 0.4 µm

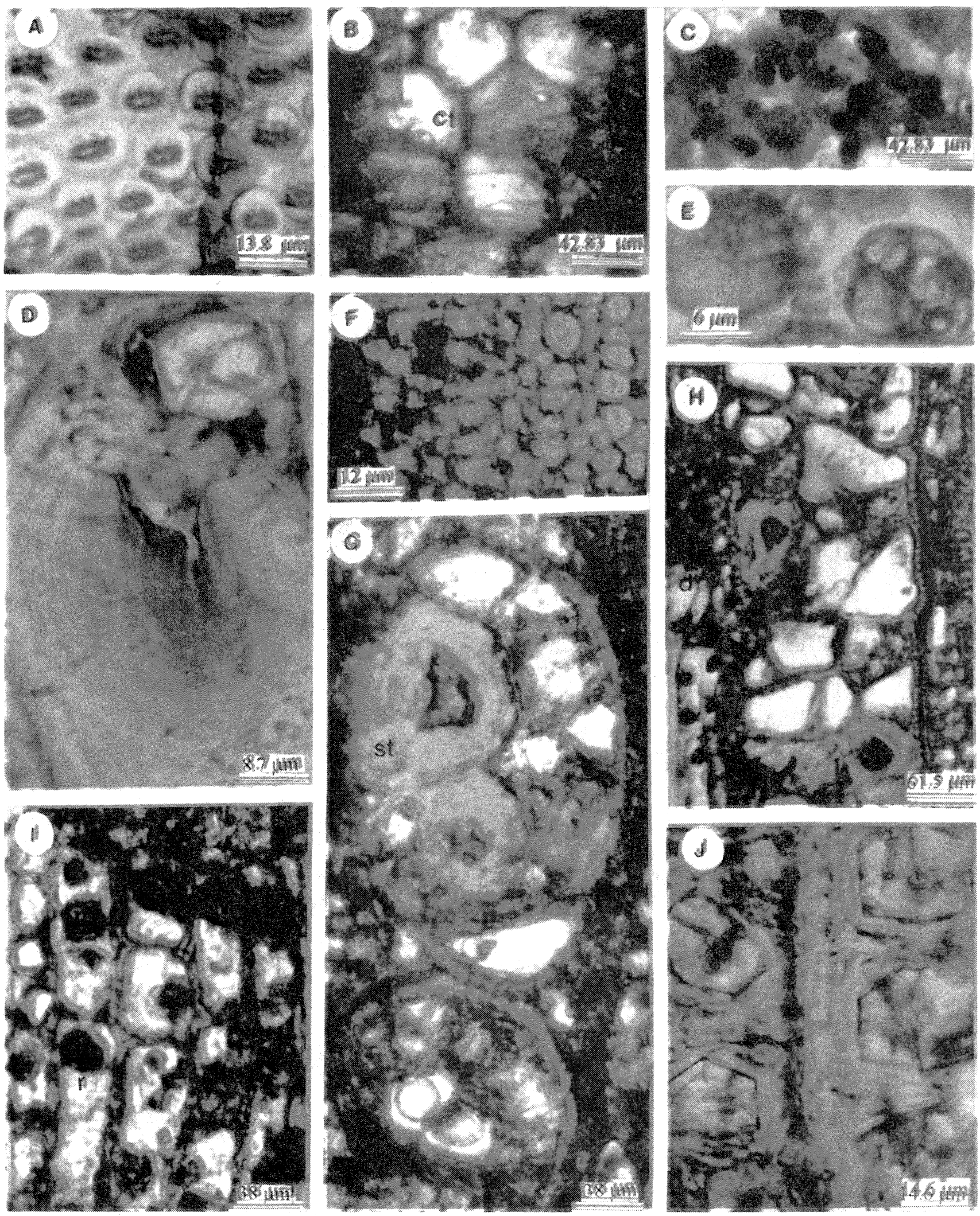

Fig. 1A-D: Sapwood. A. Intervessel pittings. B. Common tyloses (ct). C. Starch granules in axial parenchyma. D. Sclerotic tyloses and crystal (st). Fig. 1E-J: Heartwood. E. Lipid inclusions in radial parenchyma. F. Fibres. G. Vessels of two different sizes showing common and sclerotic tyloses. H. Longitudinal vessel showing tyloses. I. Red (r) and dark (d) deposits in axial parenchyma. J. Crystals in axial parenchyma.

Vessels. According to their tangential diameter the vessel elements could be distributed in two different groups: a group of moderately large vessels measuring 180 ± 20 μm in sapwood and 221±22 μm in heartwood, and a second group of regular vessels measuring 80±8 μm in sapwood and 114±10 μm in heartwood (Fig. 1G). The chemical composition of the cell wall of vessels was very similar to that of fibres (Table 1). The cell walls of heartwood vessels were slightly thicker than those of sapwood (Table 2). The intervessel pits were large in both groups (12.5±0.7 μm) (Fig. 1A). The vessel-ray pits were also large (15±0.5 μm), vertical and simple.

Tyloses. Almost all vessels examined contained tyloses that completely blocked their lumen. Two to four longitudinal series of tyloses were found in the moderately large vessels, whereas only one or two were found in the regular vessels. Common tyloses were most frequently found in sapwood vessels (Fig. 1B) whereas sclerotic tyloses were more frequent in heartwood (Fig. 1G and 1H). Only very few of the sclerotic tyloses, with a layered structure of lignin, were present in sapwood, usually near the heartwood (Fig. 1D). These sclerotic tyloses in sapwood had very thick walls (Table 2) and were totally devoid of extractives. The sclerotic tyloses in heartwood had very thick walls (Table 2) and its chemical composition appeared to be similar to that of the cell walls of fibres (Table 1), but showing a layered structure of matrix components (lignin, sterified pectins and polyphenols). Dark (condensed tannins) and red deposits (polyphenols) were observed within their lumen. A smaller group of heartwood sclerotic tyloses had thinner cell walls and contained a rhomboidal crystal 60±4 μm wide that completely blocked their lumina. The common type of tyloses had a much thinner cell wall (Table 2) and its composition showed to be qualitatively different to that of sclerotic tyloses. Common tyloses in sapwood contained only a small amount of lignin in their cell wall and starch grains, some lipids and crystals measuring about 50±2.4 μm whitin their lumen. Common tyloses in heartwood contained also polyphenols and calcium pectates in their cell wall. Some of the heartwood common tyloses contained lipids, gums of the same kind seen in sclerotic tyloses (red deposits) or up to three crystals similar to those present in the sclerotic ones (56 ±2.6 μm wide).

Axial and radial parenchyma: these cells had cell walls similar to those of the common tyloses. Starch grains and lipids were present in sapwood radial parenchyma (Fig. 1C and 1E) or red deposits of gums in heartwood radial cells (Fig. 1I). There is also a specialized type of axial parenchyma cells containing one to three romboidal crystals smaller than those present in the common tyloses (22 ± 1.5 μm) (Fig. 1J).

Middle lamella. In the sapwood this region contained calcium pectates. In the heartwood its chemical composition varied according to its location. The middle lamella found between fibres and vessels showed a higher amount of dark deposits (condensed tannins) than the lamella betwen the tyloses. on the other hand, these dark deposits were nearly absent from the lamella between the parenchyma and ray cells.

DISCUSSION

A remarkable feature of *G. sepium* is its very low proportion of sapwood. The cell morphology of the sapwood, particularly the presence of fibres and vessels with thick cell walls and the widespread occurrence of tyloses in vessel elements could be related to a high rate of transformation into heartwood in this tree. It is noteworthy that the common type of tyloses are present even in vessel elements near the vascular cambium, whereas the more sparse sclerotic type of tyloses are more frequently seen close to the heartwood. Schmitt and Liese (1994) have described a similar ocurrence of tyloses in wounded trees, nevertheless, we think that the characteristic distribution of tyloses in the sapwood of this tree could suggest an intrinsic, regulated mechanism of transformation of sapwood into heartwood, more than an induced one. Moreover, the starch grains in the parenchyma cells and common tyloses are seen only in the sapwood, whereas lipid droplets, in addition to their presence in the sapwood, are also associated to the extractives present in heartwood cells (Fig. 1E).

The heartwood of this tree is rich in chemical components like condensed tannins, cathechines and leucoanthocianidines and also rich in crystals which are present in parenchyma cells and tyloses. This abundance of extractives and crystals suggests a low hygroscopic capacity of cell walls and cavities, since nearly all of the latter are almost completely occluded by these substances. These features, together with the toxic properties of its bark, make it reasonable to predict a high resistance to biological attack. These properties are undoubtedly related to the usages traditionally given to this species. Trees are widely grown locally to serve as hedges, whereas the wood has been used as building material, particularly for external use, as well as for making poles and railroad sleepers.

The amount and type of tyloses in the heartwood are noteworthy. These structures, especially the types studied here, undoubtedly have an important role in the defense strategy of plants. The longitudinal series of tyloses here described very closely agree with the morphological description made by Saitoh *et al.* (1993) for some japanese woods. The tyloses present in the moderately large vessels of *G. sepium* are similar to Saitoh's closely packed tyloses, and those present in the regular vessels correspond to the uniseriate tyloses in which upper and lower walls are in contact. According to Schmitt and Liese (1994), tyloses in wounded wood tissue can divide and form several longitudinal series in large vessels. A similar event can probably occur in the sapwood of *G. sepium*, since these structures were present near the vascular cambium. The presence of large intervessel pits can also be correlated to the high amount of tyloses. according to the same authors, pits larger than 5 mm favour their growth. Moreover, applying the categories proposed by Bonsen and Kucera (1990) to the structure of this wood, it can be clearly assigned to the tyloses-forming group. Notwithstanding, according to the mentioned authors, the tyloses-forming species rarely have gums deposited inside the tyloses or in the middle lamella. We think that the characteristic cell structure and composition of the wood of this treee indicate a fast transformation of sapwood into heartwood. this could give *G. sepium* an advantage in an aggressive habitat of high temperature and humidity with severe competition among other species of trees.

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RESUMEN

Gliricidia sepium (Jacq.) Steud. Fabaceae posee un tronco con una proporción muy alta de duramen en relación a la albura. Las fibras y vasos en el duramen de este taxon poseen paredes muy gruesas. Los vasos en el duramen poseen grandes cantidades de tílides, tanto simples como esclerosadas, conteniendo gomas y cristales en sus cavidades. El análisis histoquímico de las células y tílides en el duramen mostró que las paredes celulares de las fibras, vasos y tílides esclerosadas contienen grandes cantidades de lignina y pectinas esterificadas como componentes matriciales, así como taninos condensados o substancias fenólicas en sus lúmenes, mientras que las células parenquimatosas y las tílides simples contienen una menor cantidad de estos componentes. Los cristales están presentes en el parénquima axial y en las tílides simples. La mayoría de los vasos en la albura contienen tílides que ocluyen completamente sus cavidades. la mayoría de estas tílides son del tipo simple y solamente se encuentran algunas esclerosadas en la vecindad del duramen. Nuestras observaciones sugieren que en esta especie hay una rápida transformación de albura en duramen. La presencia de paredes celulares muy gruesas en fibras, vasos y tílides, así como su composición y la inclusión de cristales, seguramente influyen en la alta resistencia natural de esta madera a los ataques biológicos.

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