TYLOSES STRUCTURE

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ABSTRACT

A tylosis is an outgrowth from vertical or ray parenchyma cells through bordered pits into the lumen of a xylem vessel element. This study reports additional information on the ontogeny of tyloses, formation of the developing tylosis wall, and chemical composition of the tylosis wall. The development and structure of tyloses in several species of oak (*Quercus*) were studied with the transmitting and scanning electron microscopes. The tylosis wall was layered with a complement of wall layers found in xylem elements. Cellulose and lignin were constituents of the tylosis wall.

INTRODUCTION

Tyloses are saclike intrusions into the lumen of the vessel of wood or ray parenchyma of Dicotyledons. The phenomenon of tylosis formation is common knowledge for it has been a major focus of investigation for the naturalist for a long time. More than three centuries have passed since Marcello Malphigi, the Italian physician, microscopist, anatomist, botanist, and embryologist, first made a drawing of tyloses in chestnut wood (Gerry 1914). Over the next several centuries, naturalists were interested in describing tylosis structure.

The early 20th-century researchers put this information together. From this emerged a picture of tylosis structure with some information on the course of its formation and effect in wood.

With the advent of the electron microscope, Necessany (1955), Koran and Côté (1964, 1965), Meyer (1967, 1968), Kato and Kishima (1965), Kishima (1966), Ishida and Ohtani (1968), and Foster (1964, 1967) provided information on the ultrastructure of tyloses.

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While much of the more recent work on tyloses has been concentrated on the ultrastructure of the tylosis wall, there have been no published data on the direct chemical analysis of the tylosis wall. This investigation is intended to present information on the chemical composition of the tylosis cell wall. In addition, the authors wish to investigate the structure of the tyloses as seen in red oak and compare it to the findings of other investigations on tyloses in different species of trees.

MATERIALS AND METHODS

For direct chemical analysis, samples of *Quercus alba*, white oak, were cut in the tangential plane to expose the tyloses in the vessels. Tyloses were then removed from the vessels by running a blunt glass probe down the length of the vessels and popping tyloses, intact or fragmented, onto the surface of the sample. The tyloses or their fragments were collected with a vacuum siphon at 1×10^{-1} torr and analyzed for lignin, sugars, and extractive content.

The remainder of the study was carried out on *Quercus rubra*, red oak, that had been infected with the fungus *Ceratocystis fagacearum* (Bretz) Hunt, commonly known as oak wilt. The mechanism by which the disease triggers tylosis formation is not yet known; however, the material did provide numerous bud tyloses to observe at one time, which under normal circumstances are not seen. Samples were obtained by boring a 5-mm-diameter cylinder from the trunk of the tree. These cylinders were long enough to include both sapwood and

heartwood, but only the sapwood tyloses were studied. After the samples were removed from the tree, they were placed in 70% ethyl alcohol until they were prepared for viewing with the microscope. Prior to mounting for microscopic observation, the samples were removed from the alcohol and allowed to air dry. When dry they were trimmed so that the vessels were exposed, and then they were cut into pieces about 1 cm long. Aluminum stubs 1.3 cm in diameter were coated with silver conducting paint, and the specimens were glued to them with "Duco cement." The specimens were then coated with a layer of gold 100 to 200 Å thick to prevent primary electron reflection, and examined with a Cambridge Stereoscan Mark II scanning electron microscope at 20 kv.

For examination of the tylosis wall with the transmitting electron microscope, sapwood chips ca. $1 \times 2 \times 2.5$ mm were collected from several heights in the trunk of red oak and fixed for 2 hr in 4% gluteraldehyde. The material was then postfixed for 1 hr in 2% buffered OsO_4. After being washed in distilled water, the specimens were dehydrated gradually in an alcohol series, then in propylene oxide, and finally embedded in epoxy resin. Sections of the embedded material were cut with a diamond knife on a Porter-Blum I ultramicrotome. The sections were stained with lead tartrate according to the method described by Millonigs (1961), followed by uranyl acetate as described by Watson (1958). After staining, the specimens were examined in an RCA-EMU3D electron microscope at 50 kv.

RESULTS

Tyloses originate in the parenchyma cells that line the vessels of angiosperms (Fig. 1). In the initial stage of growth, the tylosis advances from the parenchyma cell through the pit aperture of the adjoining vessel wall as a fingerlike probe that balloons or enlarges upon reaching the vessel lumen (Fig. 2). Vessels of angiosperms are the most prominent elements of the xylem tissue. In these more or less tubular structures, pits normally occur in all parts of the vessel wall and are contiguous with ray vessels and vertical parenchyma cells. Tyloses in the vessel lumen of red oak first become visible at the junction where the xylem ray cell pitting is in contact with the vessel wall (Fig. 3). Later they arise from many of the pits in the vessel wall (Fig. 4).

Young budding tyloses appear to have a wrinkled surface when they first protrude into the vessel lumen (Fig. 5). As the tyloses mature and balloon, the wrinkles disappear and their surfaces appear smooth (Figs. 6 and 7). On the surfaces of some of the immature tyloses (a mature tylosis fills the entire vessel cavity), we observed numerous projections (Fig. 8). These prominences or blisters are of distinct shape and fairly uniform size. They average 1 μ in diameter and height.

Other observations show that a single tylosis that meets no obstacle except the opposite wall of the vessel will grow to an enormous size. Conversely, when several tyloses come into contact with each other, their size is reduced. In addition, usually where the tyloses butt against each other, intertylosic pitting occurs (Fig. 9). These pits appear circular and are without a membrane or torus. The pits are of a simple type and resemble enlarged pores. The pits measure from 0.5 to 1 μ in diameter.

Ultrastructural studies on tyloses walls of red oak show a laminated structure similar to that found in tyloses of other species of woods. The layers of tyloses that butt against each other resemble the middle lamella in electron density (Fig. 10). In addition, very fine randomly oriented microfibrils were apparent in the tylosis wall (Fig. 11). The microfibrils measured from

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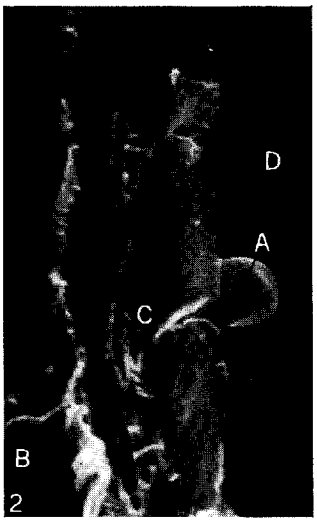
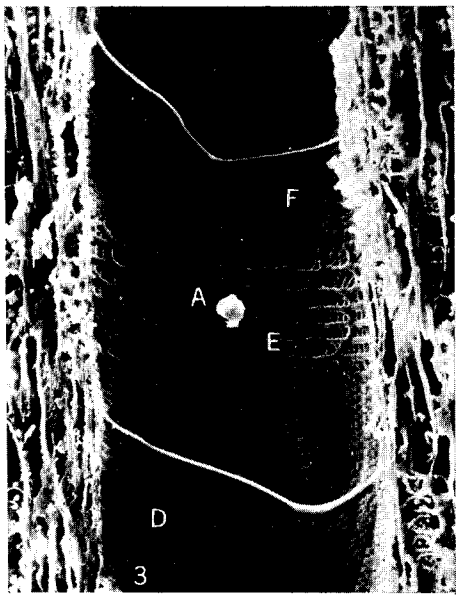
Legend: Tylosis bud—A
(A_1, smooth; A_2, wrinkled)
Vertical parenchyma cell—B
Pit membrane—C
Vessel—D
Ray parenchyma pitting—E
Vertical parenchyma pitting—F
Intertylosic pitting—G
Microfibrils—H
Electron Dense Lamella—I





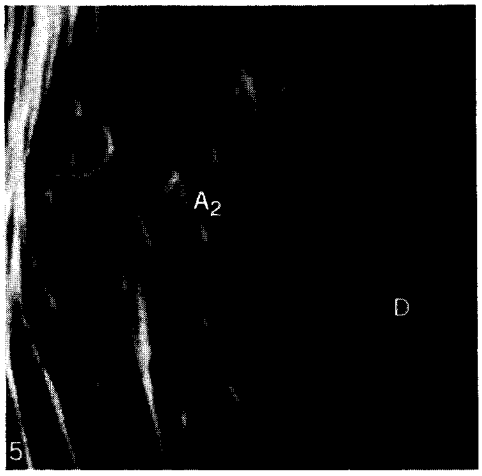


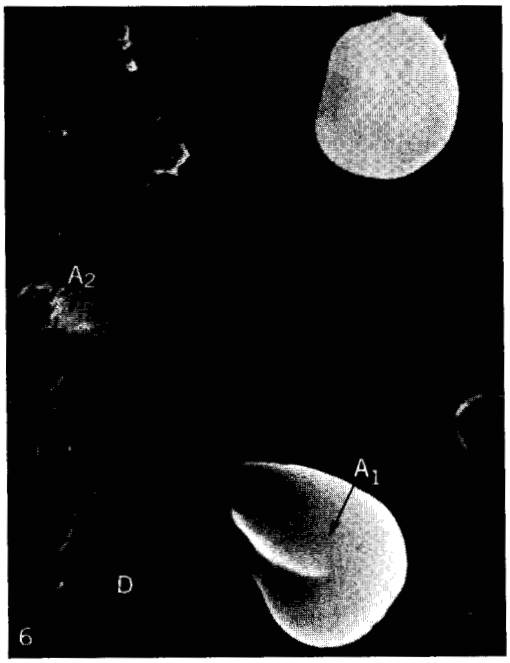
Fig. 1. Tylosis bud (A) arising from a vertical parenchyma cell (B). 6500×.

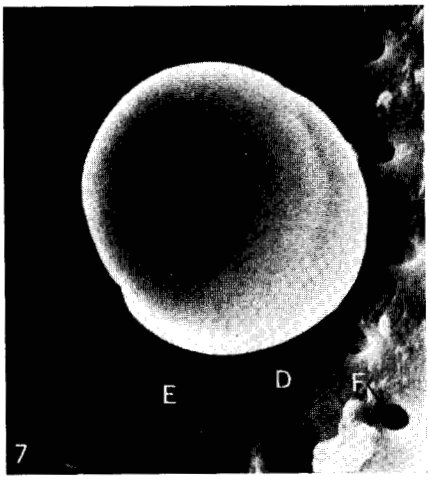
Fig. 2. Tylosis bud (A) advancing from the parenchyma cell (B) through the pit aperture of the adjoining vessel wall. 6050×.

Fig. 3. Tylosis arising at junction where ray pitting (E) is in contact with the vessel wall. Vertical pitting (F) lies on either side of the ray pits. 240×.

Fig. 4. Tyloses arising from adjacent vertical parenchyma pitting in the vessel wall. 1440×.

Fig. 5. Very young tyloses with wrinkled surfaces (A_2). 2625×.





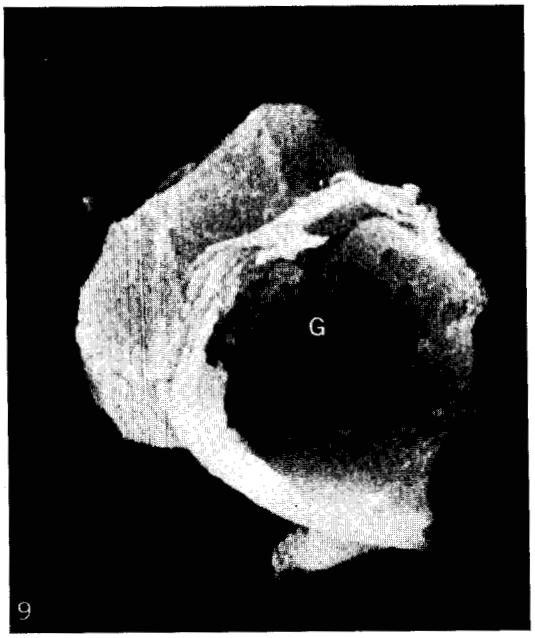


Fig. 6. Ballooning tyloses (A_1) with smooth surfaces. Contrast this with the very young wrinkled tylosis (A_2). 8250×.

Fig. 7. Smooth surface tylosis. 3225×.

Fig. 8. Tylosis bud with projections on the surface. 3225×.

Fig. 9. Intertylosic pitting (G). 2500×.

an electron micrograph under a Macroscope are ca. 50 Å or less in diameter. Many form bundles of fibrils 100 to 200 Å in diameter or larger. The randomly oriented microfibrils of the tylosis wall appear arranged in a fashion similar to that of the primary wall of woody plant cells. In addition, the tylosis wall appears much like the lignocellulosic walls of woody plants when viewed with an electron microscope. Stratification of the tylosis wall is clear (Fig. 12).

Direct chemical analysis provided further evidence as to the chemical nature of the tylosis wall. Tyloses, intact wood, and vertical parenchyma cells and fibers of white oak were analyzed for alcohol-benzene extractives, lignin, and sugars obtained by acid hydrolysis. Pectin analyses were not completed in time for this publication. Comparisons of each component in the tylosis wall with those of the cell walls of intact wood and cell walls of vertical parenchyma and fibers indicated cellulose was the prominent component. Lignin was determined as the acid insoluble residue after acid hydrolysis, Moore and Johnson (1967). Lignin ranked next to glucose in amount and xylose followed. The arabinose, galactose, and mannose constituted less than 5% of the cell-wall constituents (Fig. 13). The high proportion of xylose and glucose in the tylosis wall is very similar to that of the developing primary wall of xylem tissue.

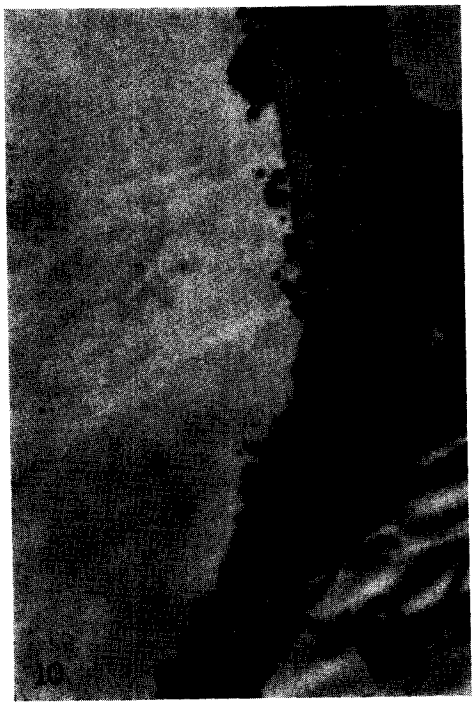
DISCUSSION

Recently observers have made great strides in the revelation of tylosis formation. Koran and Côté (1964) reported that after a tree is felled, the negative pressure in the conducting vessels approaches that of atmospheric pressure. This decrease in pressure perhaps stimulates the action of auxin induced by wounding, along with an intense diffusion of water from the vessel into the parenchyma cell. As the osmotic pressure increases, protoplasmic activity is forced against the pit membrane, which then becomes partially dissolved or completely ruptured. These two researchers further noted that after the pit membrane is ruptured, part of the protoplasm contained in the parenchyma cell is still enclosed by the ectoplasmic membrane and bulges into the vessel lumen.

In the same year, Foster (1964) showed that a layer distinguishable from the pit membrane enlarges to form the tylosis. A layer that closely resembles this was reported by Schmid (1965). This layer is situated within the parenchyma cell adjacent to the vessel-parenchyma pit membrane. In the present study, we have not as yet had a clear view of this layer in our specimens; this may have been the fault of specimen preparation.

The process of wall formation within the tylosis is apparently similar to the process of wall formation in a woody plant cell. The formation of the expanding tylosis wall, which is similar to a primary wall, has been shown to be accompanied by a random arrangement of microfibrils and evidence of secondary wall layering. In one of the early studies on tylosis ultrastructure, Necessany (1955) found the walls of tyloses formed of randomly oriented microfibrils and encrusted with lignin. He found no evidence of secondary wall structure. Brown, Panshin, and Forsaith (1949) found in the vessels of *Quercus emoryi* Torr., emory oak, extremely thick-walled tylosis marked with ramiform pits. Secondary thickening of the tylosis wall was reported by Koran and Côté (1964). They, too, found the tylosis wall encrusted with lignin and reported that the secondary wall structure of tyloses was presumably a maturing phase of normal developing xylem cells. Other studies have confirmed the random microfibrillar orientation of the developing tylosis wall (Foster [1967] and Meyer [1968]). The electron microscope and heavy metal staining of the tylosis wall have helped in the discernment of tylosis wall structure and now pave an avenue for continued research in determining the chemical nature of the tylosis wall as well as the nature of the woody plant cell wall.

By and large, it may be said that the woody plant cell wall is composed of microfibrils that contain a high percentage of



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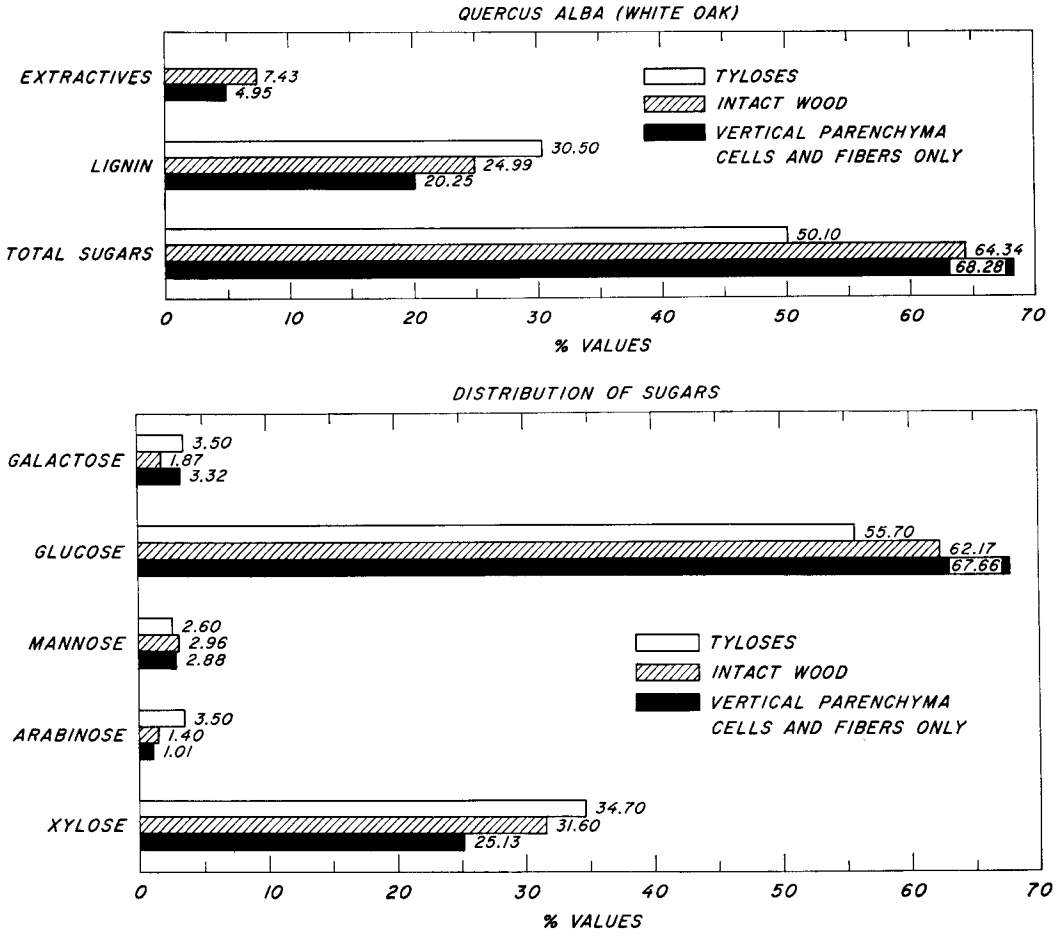

Fig. 13. Chemical analysis of the tyloses, vertical parenchyma and fibers, and intact wood. Total sugars were calculated as glucose. The ratios of the individual sugars were calculated from the sum of the individual sugars.

glucose residues and that the xylose and mannose are more or less confined to the encrusting substances surrounding them. This seems to be true of the tylosis wall as well. Isenberg (1933) first showed that the tylosis wall was composed mainly of lignin and cellulose. Kato and Kishima (1965) verified this fact by staining woody cell walls with various dyes such as zinc-chloride-iodine, phloroglucinol-hydrochloric acid. They also stained sections with sudan IV and ruthenium red to determine the presence of lipid and pectin in the cell wall. In the determination of the chemical constituents of the tylosis wall in this study, essentially all of the component sugars in

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Fig. 10. Transmission electron micrograph of three tyloses (A) in contact with each other. Note electron dense lamella (I) between contacting tyloses. 21,600×.

Fig. 11. Transmission electron micrograph of a tylosis wall showing microfibrils (H). 38,200×.

Fig. 12. Scanning electron micrograph of three tyloses in contact with each other. Note stratification of tyloses walls and intertylosic pitting. 1600×.

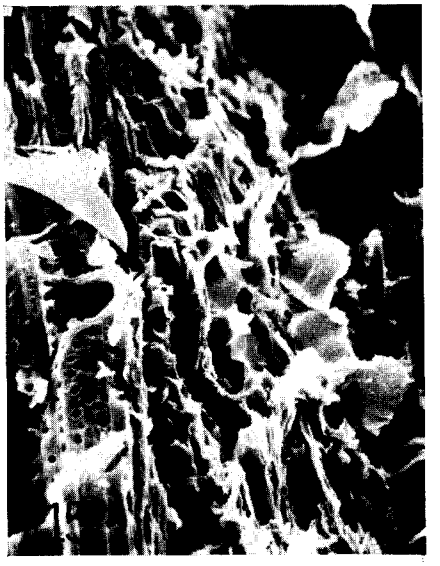

Fig. 14. A longitudinal view of tyloses in a vessel. Balloonlike tyloses form a stopple along the entire length of the vessel element. 260×.

Fig. 15. Densely packed tyloses in a red oak vessel from a tree heavily infected with oak wilt. 500×.

the sample were determined. As the determination of sugar units that compose the polysaccharide is essential to the study of wood components, they are equally important in the study of tylosis wall components. On the basis of this study we see, in the tylosis wall, cellulose units containing sugar residues other than glucose.

The high ratio of xylose in the carbohydrate fraction is about the same order of magnitude as found in ray cells and new formed wood. Perhaps it is necessary in maintaining the desired elasticity of the tylosis balloon.

It was clearly found that smooth and wrinkled tyloses appear in the same specimen. Meyer (1968) reported that in white oak the natural tyloses are spherical, and traumatic tyloses assume a cylindrical or cigar shape. In our red oak specimens taken from trees infected with oak wilt (*Ceratocystis fagacearum*), traumatic tyloses appear elongated-wrinkled as well as smooth-round. These were not found at different growing season levels, but often were seen in the same vessel.

In some instances protrusions were observed on the surface of the rounded tylosis. Ishida and Ohtani (1968) report prominences on the surface of tyloses in Harunire (*Ulmus* sp.) and suggest that such prominences on the tylosis wall may be a characteristic structure for some species. Though the protrusions are very prominent in infected red oak, it is questionable as to whether these are limited to trees with fungal infections, for they have not been observed on tyloses in noninfected white oak or other species.

As the tyloses grow from the parenchyma cells into the lumen of the vessels, these growths continue to enlarge until they fill considerable portions of the vessel cavity and form a stopple along the entire length of the vessel element (Fig. 14). In the heavily infected red oak, the tyloses were smaller in size and densely packed (Fig. 15). This could possibly indicate a natural resistance factor that leads to great wood durability. Behr et al. (1969) in a study of the microscopic examination of pressure-treated wood, found a vast degree of packing of hardwood vessels by tyloses in the same and various species of wood.

The cytological study of the development of tyloses is only now at the beginning. Knowledge of their development and understanding of their formation could lead not only to explanations of tylosis wall thickening but could help expand our present concepts of plant cell-wall origin and thickening.

CONCLUSIONS

(1.) Direct chemical analyses of wall formation by tyloses studied here has confirmed the presence of lignin, cellulose, and hemicelluloses in the wall of tyloses and that the process of wall formation within the tylosis is apparently similar to the developing process of wall formation in the woody plant cell.

(2.) The random orientation of microfibrils as seen in the tylosis wall of red oak apparently occurs as a natural consequence, as reported by other investigators for different species of trees.

(3.) Electron micrographs reveal distinct layers in a double tylosis wall of red oak. These may be compared to the compound middle lamella and the secondary walls.

(4.) In red oak, tyloses may first arise in vessels through the ray parenchyma-vessel pitting and then from the vertical parenchyma-vessel pitting.

(5.) Early tyloses buds of infected red oaks are wrinkled and become smooth-walled as they expand and mature in the vessel lumen.

(6.) Many young tyloses are covered with prominences of unknown function or origin.

(7.) Intertylosic pits occur between tyloses that butt against each other.

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