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Warts in the Evolution of Angiosperm Wood

By R. A. PARHAM and W. M. BAIRD

The Institute of Paper Chemistry, Appleton, Wisconsin

Summary

The occurrence and distribution of the warty layer in twelve species of hardwoods have been investigated by transmission and/or scanning electron microscopy. Samples were selected on the common feature of reportedly having both primitive vessel types with scalariform perforation plates and more evolutionarily advanced vessel types with simple plates. Among the six angiosperm families represented, warts were generally found in the more primitive-type vessel elements. The more advanced vessel types rarely displayed a warty layer. Warts were also sparse or absent in other specialized cells, the fiber tracheids and libriform fibers. From the evidence presented here and in the literature, the variable presence and morphology of the warty layer in hardwoods (or for wood in general) appears to be attributable to a phylogenetic trend. According to this trend, conifer tracheids and primitive hardwood cells are nearly always warted, but as the cell type becomes more advanced or specialized, it becomes increasingly wart-free.

Introduction

Warts

The warty layer is an amorphous, membrane-like layer of material which lines the lumen side of the secondary wall in many wood species [Liese 1965]. Surface and cross-sectional views of the layer in its well-developed state show it to consist of encrusted globules which protrude from the cell wall, giving the appearance of warts. Recent evidence [Cronshaw 1965] concerning the ontogenetic development of this structure has revealed that warts are elaborated onto the cell wall by the cell cytoplasm in its final role before it degenerates and is lost from the cell. This fact has also been confirmed by Kutscha [1968]. Thus, warts may be covered by the cell membrane, tonoplast, and perhaps some cytoplasmic debris. However, warts do not gain their origin solely from cytoplasmic debris, and they are distinct from the microfibrillar portion of the cell wall against which they are deposited.

Since the discovery of the warty layer in wood in 1951 by Kobayashi and Utsumi [1951] and Liese [1951], other investigators have confirmed its presence and distribution in numerous plants. Detailed reviews regarding its occurrence and variability can be found in Wardrop [1964], Liese [1957, 1965], Harada [1965], Scurfield et al. [1970], and Ohtani and Fujikawa [1971]. Liese [1965] suggested that the variable appearance of warts in different species could possibly be due to a different process of protoplasmic disintegration, the frequency and enzymatic lysis of cell organelles, and/or different regulation of lignification, which may contribute to a fixation of components. No further conjecture or evidence to the contrary has since been proposed to elucidate any cause for the wart variability in softwoods or hardwoods.

The resemblance between warts and pit vestures in hardwoods has also been investigated [Côté, Day 1962; Schmid, Machado 1964], and although these two structures are similar in appearance and chemical composition, they are not readily placed in a sensible evolutionary scheme [Scurfield, Silva 1970; Scurfield et al. 1970].

Xylem evolution and warts

It is well established that gymnosperms (conifers or softwoods) are a very ancient type of tree, and that they are the forerunners of the angiosperms (hardwoods), which, in a phylogenetic sense, have arisen comparatively recently [Esau 1965; Fuller, Tippo 1954]. Hardwood vessel elements, fiber tracheids, and fibers have therefore evolved from the conifer tracheid. And, among the vessel elements, those with simple perforations are considered more advanced than the scalariformly perforated vessels.

The presence of warts has been substantiated in essentially all conifers investigated [Liese 1965; Ohtani, Fujikawa 1971], and there is little or no conclusive evidence for any phylogenetic modifications in the warty layer, except perhaps in the two subgenera of *Pinus* [see Frey-Wyssling et al. 1955, 1956; Liese 1965; Côté, Day 1969]. *Gnetum*, perhaps an evolutionary link between gymnosperms and angiosperms [see Scurfield et al. 1970], is also warted. It is only when the more advanced angiosperms are examined for warts that extreme variability is encountered. Some families show a characteristic warty layer while in others it is totally absent [see Wardrop 1964; Liese 1965; Scurfield et al. 1970]. Furthermore, in species that are supposedly warty, not all fibers, tracheids, or vessels contain warts [Jayme, Azzola 1964a, 1964b; Harada 1965].

Purpose of the investigation

The failure of the warty layer to persist in all hardwood families or in all cells of hardwoods that are reportedly warty raises the question: Can the warty layer be considered a feature, the appearance and/or presence of which is attributable to an evolutionary trend?

The majority of hardwood trees can be categorized into those that possess vessel elements with scalariform perforations and those with simply perforated vessels. However, only a limited number of species exhibit both vessel types [Panshin, de Zeeuw 1970; Dept. Sci. Indust. Res. 1960; Metcalfe, Chalk 1957]. It was felt by the authors that perhaps by examining wood species of the latter type a phylogenetic trend, if present, correlating xylem cell morphology and wart structure could possibly be detected. The most comprehensive study would entail the examination of *all* hardwood species for vessel type, general cell advancement, and presence or absence of warts. Such an undertaking would be monumental but is really unnecessary, since a good deal of this information is extractable from the literature. Unfortunately, such data do not indicate there is any clear relationship of this sort. Warted and unwarted vessels are found to contain simple or scalariform perforations with no decipherable trend. Perhaps this situation only serves to illustrate the fact that in different plant families, even if all species are evolving convergently, some trends can be accelerated while others may be retarded [Esau 1965].

It can be argued that the above condition also holds within an individual species or even a single tree, since xylem evolution is influenced by environment, plant age, and habit of the species [Carlquist 1965]. However, if one or more species from different plant families are seen to exhibit simultaneously along with an already established phylogenetic trend a second trend that is consistent in its direction, it seems reasonable to conclude these trends to be parallel. The present investigation represents an attempt to correlate such trends in hardwood xylem, particularly with reference to the vessel elements.

Materials and methods

Metcalfe and Chalk [1957] indicated that in a survey of 1801 hardwoods the following distribution of vessel perforations was found: wholly scalariform, 206; scalariform and simple, 91; wholly simple, 1504. Only about 5% of all the species studied possessed vessels with both types of perforations. Twelve of these woods were examined in the present work (Table 1), including all of the North American hardwoods of this type listed by Panshin and de Zeeuw [1970]. All species were surveyed with the scanning electron microscope for the presence of warts in the various cell types. Two species, *Fagus grandifolia* (American beech) and *Platanus occidentalis* (sycamore), were studied in greater detail using transmission electron microscopy.

All samples were cut from the sapwood portion of dry wood blocks from a collection at The Institute of Paper Chemistry, Appleton. After resaturation in water, blocks were sectioned radially at 150 μm with a sliding microtome. Sections were then dried between glass slides at 60° C.

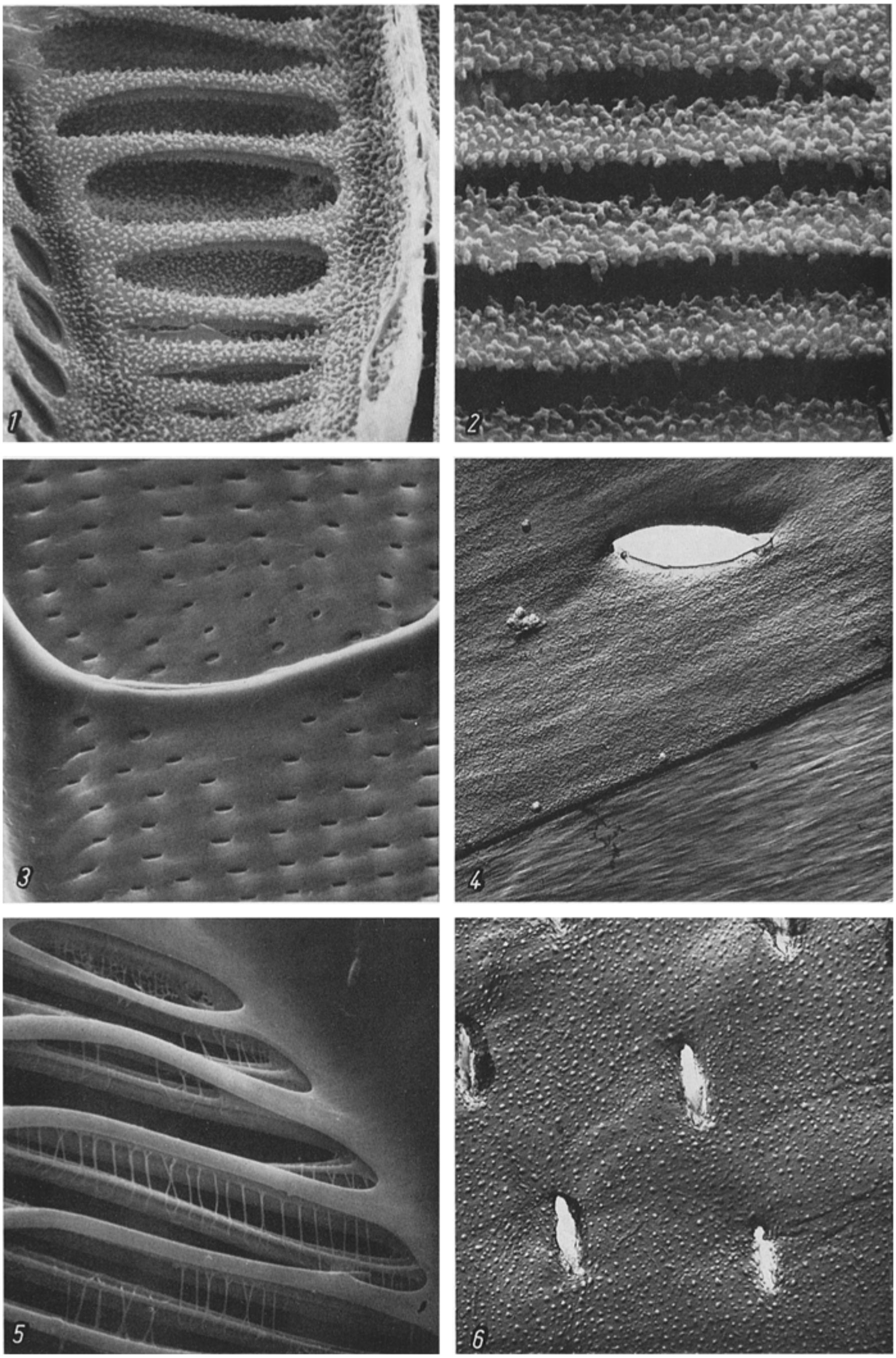
For scanning electron microscopy (SEM), sections were affixed to standard specimen pedestals with transfer adhesive tape. They were then coated with approximately 20 nm of carbon followed by 20 nm of 60 : 40 Au/Pd, all evaporations being carried out during specimen rotation and oscillation. This amount of coating was required to avoid specimen charging over the rough section surface and/or surface blistering at high magnification and accelerating voltage. Specimens were examined using a JSM-U3 microscope at 15 and 25 kV. Micrographs were recorded on Polaroid P/N 55 film.

Surface replicas for transmission electron microscopy (TEM) were prepared by the techniques of Côté et al. [1964] and Dunning [1968]. They were subsequently examined with a RCA EMU-3F microscope using 50 kV.

Results

Sycamore

According to Panshin and de Zeeuw [1970], sycamore is a diffuse-porous wood having vessels predominantly with simple perforations or occasionally scalariform in the smaller, latewood vessels. Specimens in the present study displayed more than just an occasional vessel of the latter type, and the plates often possessed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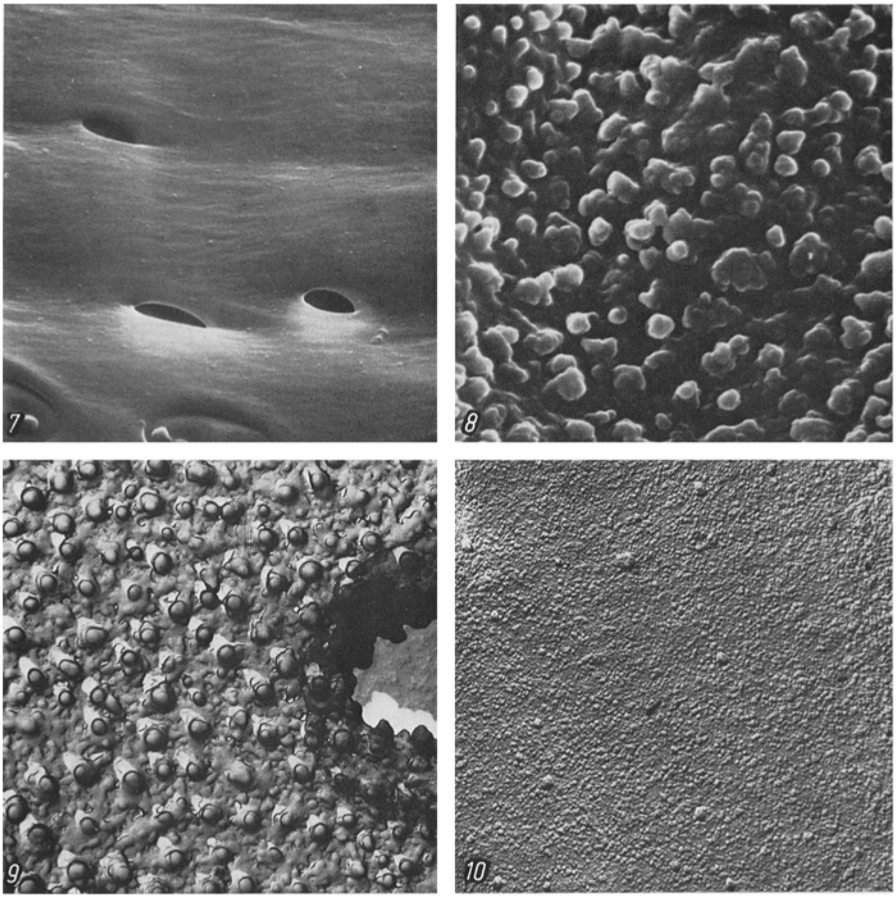

Fig. 1. Scalariform perforation plate in small vessel member of sycamore. Note dense covering of warts. SEM. (SEM: scanning electron micrograph, TEM: transmission electron micrograph) Magn.: 1520 ×

Fig. 2. Wart stacks on perforation bars in sycamore. SEM. Magn.: 3160 ×

Fig. 3. Rim of simple perforation plate at junction of two unwarted vessel elements in sycamore. SEM. Magn.: 890 ×

Fig. 4. Close-up of lumen surface in same vessel type as in Fig. 3. Note the very smooth but finely granular surface structure. TEM. Magn.: 9750 ×

Fig. 5. Unwarted sycamore vessel with scalariform perforations. Note the microfibrillar connections between the perforation bars. SEM. Magn.: 1900 ×

Fig. 6. Warty layer in sycamore fiber tracheid. TEM. Magn.: 2440 ×

Fig. 7. Smooth lumen surface of a simply perforated beech vessel. SEM. Magn.: 2530 ×

Fig. 8. Warts on lumen surface of beech vessel with scalariform perforations. SEM. Magn.: 6330 ×

Fig. 9. Lumen surface of same vessel type as in Fig. 8. TEM. Magn.: 8050 ×

Fig. 10. Granular lumen surface of beech fiber tracheid. TEM. Magn.: 9820 ×

up to 15 or more bars. However, the majority of vessels were simply perforated and larger than the scalariform cells. Fig. 1 illustrates a typical scalariform perforation and the presence of a distinct warty layer. The warts occur singly as well as in short, linear or curved stacks with the terminal wart often tapered (Fig. 2). In other scalariform vessels, warts were distributed in a more isolated fashion. As a rule, all warted vessels in sycamore were small and contained scalariform perforations consisting of many bars.

The vast majority of vessels in sycamore contained simple perforation plates and were unwarted. Fig. 3 illustrates the end-wall area of two such vessel members. A detailed view of the lumen surface in this vessel type is seen in Fig. 4. Here there is a finely granular deposition on the cell wall but no wart structure.

Only a few of the unwarted vessels contained scalariform perforations. An example is shown in Fig. 5. This micrograph also illustrates the "reticulate" and "orthogonal" microfibrillar connections between the perforation bars referred to by Meyer and Muhammad [1972].

Other evolutionarily advanced cells in sycamore, the fiber tracheids and libriform fibers, generally displayed a warty layer (Fig. 6), but sycamore was one of the few species among the twelve woods that contained such cells. Warts here were much smaller than vessel warts, and a few libriform fibers were completely wart-free.

Beech

The vessel majority in beech was composed of the wider elements that exhibited simple perforation plates exclusively and no warty layer (Fig. 7). At high magnification the lumen surface of this cell type showed only a granular covering, similar to that in the unwarted sycamore vessels.

Small diameter vessels in beech (as in sycamore) were largely restricted to the latewood zone, and in contrast to the larger, unwarted, and simply perforated vessel members, they most often possessed scalariform perforations and distinct warts. These warts presented an interesting morphology which is shown in Fig. 8. Along with single warts the lumen surface is covered with aggregates or wart clusters. Beneath these more obvious protrusions, a second tier of warts seems to emerge. However, these latter warts are masked and appear tightly bound to the cell wall by an amorphous membrane or coating. This masking is seen more clearly in Fig. 9.

Fiber tracheids and libriform fibers in beech tended to intergrade. Fig. 10 shows the lumen surface of a narrow fiber. This cell, as was characteristic of the other fibers observed, exhibited no real wart structure. The lumen surface was obscured by a coarse, granular material similar to that found by Harada [1965, Fig. 5] in fibers of *Fagus crenata*, Japanese beech. In some fibers these incrustations were larger and resembled an incipient warty layer. However, further examination would be required to confirm such a supposition. In any event, the warty layer was not present in American beech fibers in a distinguishable form. This is apparently in contrast to results obtained by Harada [1965] on Japanese beech since he found distinct warts in some fibers as well as in vessel elements.

Other species

Observations on the occurrence of a warty layer in the vessels and fibers of 12 hardwood species including sycamore and beech are summarized in Table 1. A general observation which is not listed is the absence of warts in axial and ray parenchyma of all twelve woods. Also, no pit vestures were found in any of the species.

Discussion

With the exceptions of *Magnolia acuminata, Ostrya virginiana,* and *Castanea dentata,* Table 1 confirms that all woods surveyed contained both simply and scalariformly perforated vessels. However, the rare occurrence in these species of scalariform perforations was expected [Panshin, de Zeeuw 1970].

Of the twelve species examined, only six revealed a warty layer, and in one of these *(Castanopsis)* it was very sparse. Of course, this is not to imply that if both vessel types (scalariform as well as simple) had been found in some of the unwarted species, both would have been without warts.

Warts were found for the most part in only the vessel elements. Fiber tracheids sometimes displayed small warts, as did a few fibers. However, in eleven of the twelve species fibers were completely devoid of warts.

If, for the twelve woods in Table 1, one segregates the vessels and fibers according to their degree of phylogenetic advancement, and at the same time associates with each cell type the presence or absence of a warty layer, a general but striking trend becomes evident. In the vast majority of cases, warts occur in the more primitive cells. Furthermore, considering the even broader phylogenetic relationship between angiosperms and gymnosperms, this same correlation appears valid.

There is no intuitively obvious explanation why some of the primitive-type, scalariform vessels were without warts or why some of the simple vessels contained them. However, from Table 1 one must conclude these two cell types to be a definite minority, except in one species, *Magnolia grandiflora.* The overall situation can only be reconciled by proposing that in some species the phylogenetic association between wart development (or loss) and cell specialization (or advancement) has been either retarded or accelerated. The same can be said for all those hardwoods that possess only simple or scalariform perforations and exhibit warts irrespective of the vessel type.

Conclusions

In some angiosperm woods which have been shown to contain both simply and scalariformly perforated vessels, the presence or absence of a warty layer follows a trend which can be generally associated with the degree of phylogenetic advancement of the xylem cell. The primitive-type vessels, those with scalariform perforations, are nearly always warted in those species that exhibit warts. On the other hand, in the same woods, simply perforated vessels almost never exhibit warts. In hardwood fibers, another highly specialized but imperforate cell type, warts are also rare and very small when present.

Table 1. Observations on the occurrence of the warty layer in twelve angiosperm woods

Family	Species	Vessels		Fibers
		Scalariform perforations	Simple perforations	
Magnoliaceae	*Magnolia acuminata* L.	Very rare (none observed)	Vessel majority; no warts	No warts
	M. grandiflora L.	Vessel majority; spiral thickenings; no warts	Very rare; no warts	No warts
Betulaceae	*Carpinus caroliniana* Walt.	Very rare; small cells with spiral thickenings; no warts	Vessel majority; spiral thickenings; no warts	No warts
	Ostrya virginiana (Mill.) K. Koch	Very rare (none observed)	Vessel majority; no warts	No warts
Platanaceae	*Platanus occidentalis* L.	Vessel minority; 50% or more with warts, these cells with 10 or more bars per perforation plate	Vessel majority; wide cells; all unwarted	Small warts
Fagaceae	*Fagus grandifolia* Ehrh.	Vessel minority; cells narrow and in last portion of LW; always warted	Vessel majority; cells wide and in EW; warted vessels small, in or near LW; vast majority unwarted	No distinct warts
	F. sylvatica L.	Vessel minority; narrow cells, usually with prominent warts; some with small warts or unwarted	Vessel majority; wide cells; all unwarted	Some small warts in fiber tracheids
	F. orientalis Lipsky	Rare; small cells and in LW zone; all warted	Vessel majority; wide cells, most unwarted; warts rare, in smaller LW vessels	Some small warts in fiber tracheids
	Castanopsis crysophylla (Doug.) A. DC.	Very rare; small and in LW zone; no warts	Vessel majority; most unwarted; some small warts in a few small vessels	Very small warts in most fiber tracheids
	Castanea dentata (Marsh.) Borkh.	Very rare (none observed)	Vast majority; no warts	No warts
Lauraceae	*Sassafras albidum* (Nutt.) Nees	Vessel minority; small cells; all with large warts	Vessel majority; unwarted; few small LW cells with warts	No warts
Vacciniaceae	*Oxydendrum arboreum* (L.) DC.	Rare; small vessels; no warts	Vessel majority; spiral thickening; no warts	No warts

The usefulness of the present study is limited due to the small number of species examined (12 out of possibly 91 or more) and the consequent failure to ascertain the extent of variability within and between species. However, based on the evidence presented, it is the strong contention of the authors that the variable occurrence and morphology of the warty layer in angiosperm wood (or wood in general) parallels xylem evolution. More specifically, as the cell type becomes increasingly specialized and thereby further separated phylogenetically from its ancestor, the primitive and warty gymnosperm tracheid, the wart structure is reduced and eventually lost. Throughout the hardwood species there are exceptions to this proposal, perhaps due to evolutionary retardation or acceleration, but in those species which exhibit both primitive and advanced vessel types, the supposition appears valid.

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A recent publication by Ohtani, J., Ishida, S. 1973. (An observation of the sculptures of the vessel wall of *Fagus crenata* BL. using scanning electron microscopy. Res. Bull. Coll. Exp. For., Hokkaido Univ. **30** (1): 125–144) also cites the fact that "warts on the vessel wall are scarcely present in the early stage and remarkably in the late stage within an annual increment of beechwood studied". These authors contend that it may be reasonable to distinguish roughly between earlywood and latewood vessels of this species on the basis of the degree of wart development.

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Dr. R. A. Parham, Assistant Professor
W. M. Baird, Graduate Student
The Institute of Paper Chemistry
Appleton, Wisconsin 54911 U.S.A.