

MORPHOLOGICAL AND CHEMICAL CHARACTERISTICS OF THE WARTY LAYER IN RED PINE (*PINUS RESINOSA* AIT.)¹

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ABSTRACT

Red pine (*Pinus resinosa* Ait.) specimens were successively treated in the following manner: boiled in water for 2 hours, extracted with alcohol-benzene for 4 hours, extracted with 5% NaOH aqueous solution for 1 hour, and delignified with acidified sodium chlorite for 1 hour. The ultrastructure of the warty layer was studied after each sequence of treatment. It was found that the lumen surface of red pine tracheids was relatively free of encrustation. The microfibrils in the surface lamella of the S3 layer were deposited in a bundle form. Two types of warts were found in red pine tracheids. The first type of warts was localized thickenings of the S3 microfibrillar bundles, and the second type of warts was those situated at the end of microfibrillar bundles. Results of studying the effect of chemical treatments suggested that the encrustant lining the lumen wall was hemicellulose in nature and that the warts were made up of a combination of lignin and hemicelluloses. A possible mechanism of the formation of the last lamella of the S3 layer and the associated warts is also discussed.

Keywords: *Pinus resinosa*, warts, electron microscopy, chemical treatment, delignification, S3 layer, microfibrillar orientation, wart formation.

INTRODUCTION

A great amount of information has been accumulated on the development, origin, and chemical nature of the warty layer in wood cells. It is not the main purpose of this report to provide a complete review of the literature on these subjects, but such a treatment can be found in Baird et al. (1974a, b) and Ohtani et al. (1984). However, a summary on these subjects can be made as follows. It has generally been accepted that formation of the warty layer is a final cell-wall differentiation process before cell autolysis. Warts are localized cell-wall thickenings on the lumen surface overlaid by a layer of lignaceous material. Warts and the accompanying isotropic lining on the lumen surface are very resistant to chemical treatments. Evidences have indicated that the warty layer consists of a more condensed form of lignin than the lignin in other parts of the cell wall. The basal area of the warts contains amorphous carbohydrates.

The appearance of warty layer is variable, depending upon the number and size of the warts and the presence or absence of the additional isotropic layer accompanying the warts (Liese 1963; Côté and Day 1969). Baird et al. (1974a) described the warty layer of balsam fir mature tracheids as a layer of encrustant that completely covered the S3 layer, with the warts protruded into the lumen. Côté and Day (1969) found that the warty layer of earlywood tracheids in many southern pines lacked the isotropic layer so that the S3 layer was clearly visible. They also found that the lumen surface of heartwood latewood tracheids of the same species

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was usually heavily encrusted. This type of heavy encrustation is in part due to deposition of extractives. Therefore, the appearance of the warty layer may also be affected by extractive deposition.

During preparation of scanning electron micrographs of domestic hardwoods and softwoods, we observed some unique characteristics of the warty layer in red pine tracheids that have not been reported previously. Therefore, the objectives of this study were to confirm these characteristics of the warty layer in a number of red pine samples and to observe the effects of various chemical treatments on the warty layer.

MATERIALS AND METHODS

Red pine wood blocks were softened by boiling in water for 2 hours. This softening process also served as the hot-water extraction of samples. A radial surface on each block was prepared by removing thin radial sections with a sliding microtome. Then a radial slice about 200 μm thick containing the microtomed surface was cut from each block. These thick slices were successively treated in the following manner: extracted with alcohol-benzene in a Soxhlet extractor for 4 hours, reflexed in a 5% NaOH aqueous solution for 1 hour, and finally, delignified with acidified sodium chlorite solution at 70 C for 1 hour. After each step of treatment including the hot-water extraction, some specimens were randomly selected for microscopic observation. These specimens were immediately washed by three changes of boiling water to remove any trace of chemicals, especially sodium hydroxide and sodium chlorite.

For scanning electron microscope (SEM) observations, specimens were sputter-coated with approximately 250 Å of gold-palladium and then examined with a JEOL-JSM-35 SEM. The direct carbon replica technique of Côté et al. (1964) was used to prepare specimens for the transmission electron microscopy (TEM). Carbon replicas were examined with a Hitachi HU-11E-1 TEM.

RESULTS AND DISCUSSION

Figure 1 is an SEM micrograph that reveals several unique characteristics of the lumen surface of a red pine tracheid. First, the lumen surface is relatively free of encrustation so that the microfibrillar orientation of the S3 layer is clearly visible. In addition, the distribution pattern of warts closely follows the microfibrillar orientation of the S3 layer, and warts appear to be localized thickenings of the S3 microfibrillar bundles. Finally, many warts seem to be situated at the end of the S3 microfibrillar bundles.

In the large number of samples studied, we found that the lumen surface of both sapwood and heartwood earlywood tracheids was relatively free of encrustation. This characteristic simplified the ultrastructural study of the warty layer in this species. It was also found that the deposition of warts followed a rather regular pattern, in that many warts were seen aligned in the direction of the S3 microfibrillar orientation (Figs. 2A and 3A). Such an arrangement can also be seen on the bordered pit chamber wall (Fig. 3D) where warts distributed in a circular pattern corresponding to the typical circular microfibrillar orientation in this region. This close relationship between wart distribution and microfibrillar orientation can also be seen in many micrographs published by Côté and Day (1969, especially figs. 33, 62, and 65).



FIG. 1. An SEM micrograph of the warty layer of a water-extracted red pine heartwood tracheid (long arrow = fiber axis).

In some SEM observations, especially when the tracheid lumen surface was viewed directly from the top as shown in Fig. 2A, microfibrils in the surface lamella of the S3 layer appear to be deposited in a bundle form. The diameter of microfibrillar bundles ranges from 0.12 to 0.25 μm , whereas warts have a slightly greater diameter than that of microfibrillar bundles ranging from 0.15 to 0.30 μm . The lumen surface is rather porous because of narrow voids formed between paralleling microfibrillar bundles. These narrow voids might have formed as a result of shrinkage of microfibrillar bundles during the drying of specimens. In addition, Fig. 2A also shows that many warts are seen on a single microfibrillar bundle and that warts seem to be localized thickenings of the microfibrillar bundles. This observation partly agrees with that of Wardrop and Davies (1962) in which they described warts as localized cell-wall thickenings overlain by residual components of the cytoplasm.

In several occasions, the SEM observation of water-extracted specimens reveals that many microfibrillar bundles of the S3 layer appear to terminate at warts

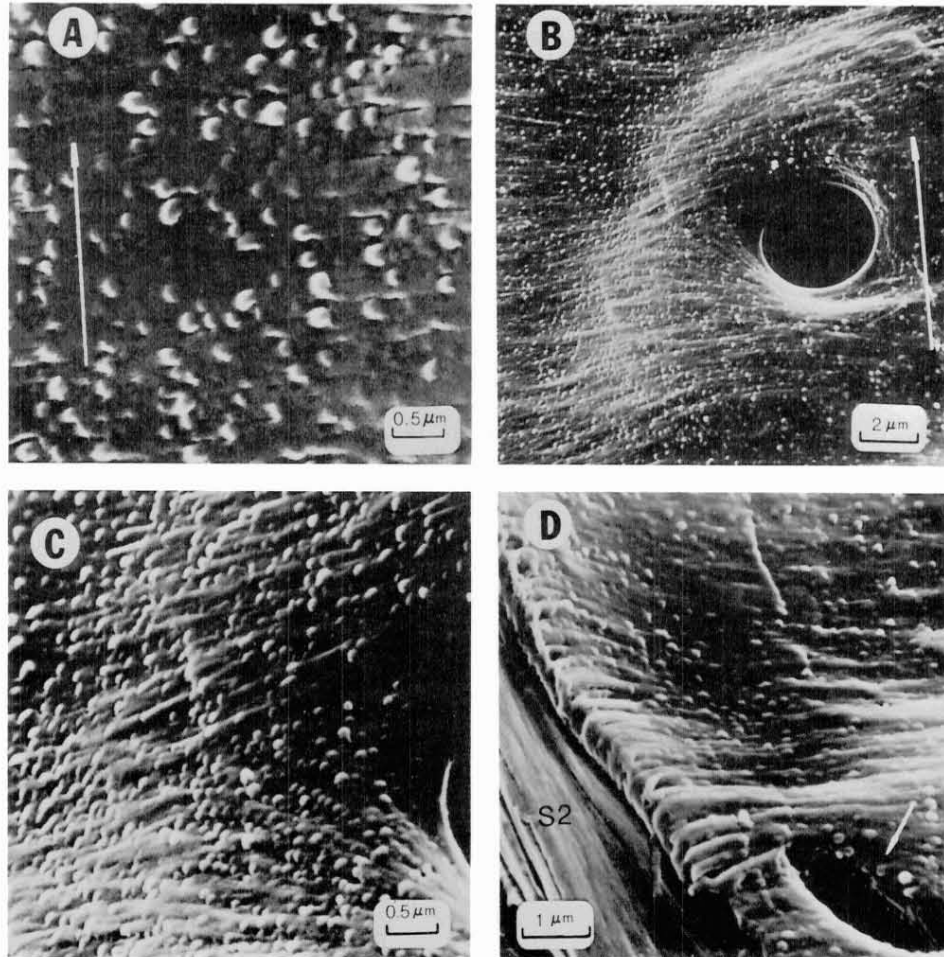


FIG. 2. SEM micrographs of the warty layer in water-extracted heartwood tracheids (long arrows = fiber axes). A. The top view of the lumen surface, showing separation between paralleling microfibrillar bundles. B. Structure of the lumen surface in and around the dome area of a bordered pit. C. An enlarged view of B. D. The cord-like appearance of microfibrillar bundles (lower center) and termination of a microfibrillar bundle at the rim of a pit aperture (arrow) with a wart at the end.

(Figs. 1 and 2B, C). This structure is more evident in or around the dome area of bordered pits probably because the contour in this region provides a better angle to reveal such a structure. Just as the drying of specimens may cause lateral separation of microfibrillar bundles as shown in Fig. 2A, the drying process may also cause vertical separation of microfibrillar bundles that facilitates the observation of the ends of microfibrillar bundles. Figure 2D shows that several microfibrillar bundles are seen terminated at the rim of a pit aperture, and each bundle has a wart at its end. However, the observed structure may simply be an optical illusion caused by the insufficient resolution power of the SEM or an artifact of unknown origin. Future work is definitely needed to confirm this type of structure.

All the above SEM observations were made in the water-extracted specimens. Scanning electron microscopy examinations were also made for specimens sub-

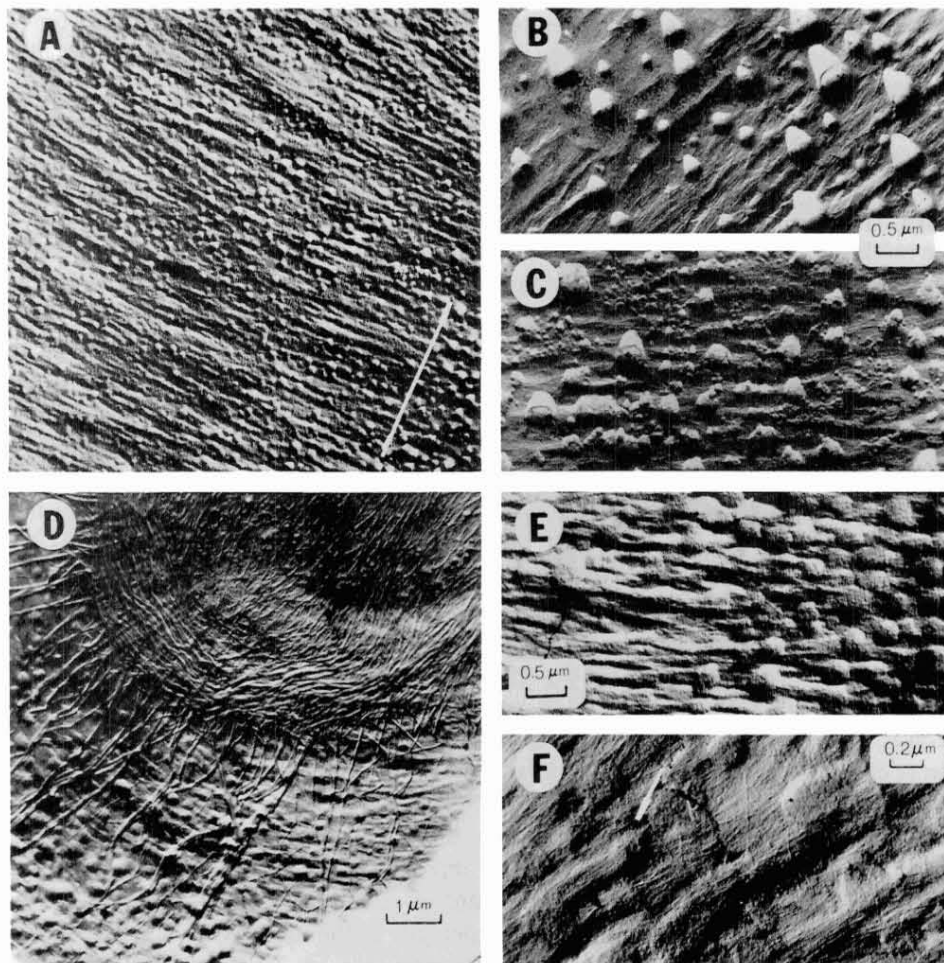


FIG. 3. Carbon replicas of red pine warty layer. A. The lumen surface of a water-extracted sapwood tracheid (long arrow = fiber axis). B and C. Heartwood specimens extracted with water and alcohol-benzene, showing two types of lumen wall encrustation. D. Sapwood bordered pit extracted with water, alcohol-benzene, and 5% NaOH. Removal of the encrustant reveals a circular wart distribution pattern on the pit chamber wall. E. Heartwood, same treatment as in D, showing eroded warts in a cell corner. F. Same as E, showing ribbon-like microfibrillar bundles and flattened warts. Also note the outline of the end of a microfibrillar bundle (arrow) covered with collapsed warts.

jected to further treatments, but very little detail of the warty layer was resolved. This result was somewhat anticipated for NaOH-treated and delignified specimens because these two treatments would erode the warty layer and thus reduce its resolution by the SEM. However, it was unexpected to find that after alcohol-benzene extraction, the microfibrillar orientation on the lumen wall could no longer be recognized and warts were only poorly resolved.

A TEM examination of carbon replicas of water-extracted specimens also shows that there was very little encrustation on the lumen wall of sapwood earlywood tracheids (Fig. 3A). The lumen surface of heartwood tracheids was found lined with slightly more encrustant than that in sapwood tracheids, but the lining was

usually not heavy enough to embed the S3 microfibrillar orientation. Two types of heartwood lumen wall encrustation were observed; one type that lines the lumen wall as a thin film (Fig. 3B), and the other type appears as patches of loosely packed granules (Fig. 3C). Both these two types of encrustation were resistant to the alcohol-benzene extraction but could be removed by a 5% NaOH treatment (Figs. 3D, E). Pit chamber walls in sapwood tracheids were usually lined with a thin layer of encrustant that resisted the alcohol-benzene extraction but that could be readily removed by the NaOH treatment (Fig. 3D). Removal of the encrustant on pit chamber walls reveals the circular pattern of microfibrillar orientation and a similar pattern of wart distribution.

In addition to removing the lumen surface encrustant, the 5% NaOH treatment also eroded the warts. Warts in the cell corner where they are usually more numerous, larger in size, and protrude higher into the lumen were reduced to low mounds (Fig. 3E). In some cases, warts were flattened or collapsed as a result of the NaOH treatment (Fig. 3F). Baird et al. (1974b) reported that the basal area of balsam fir warts contained an amorphous carbohydrate. It is possible that the carbohydrate in the basal area of red pine warts was removed by the NaOH treatment and the subsequent collapse of the remaining shell of lignaceous substance resulted in a flattened appearance as shown in Fig. 3F. Furthermore, after the NaOH treatment, microfibrillar bundles transformed from a cord-like (Fig. 2D) to a ribbon-like (Figs. 3E, F) appearance. This observation suggests that a considerable amount of material is also removed from microfibrillar bundles by NaOH. When the NaOH-treated specimens were subsequently delignified, warts were eroded further, leaving a relatively smooth appearance of the S3 layer surface without recognizable wart structure (Fig. 4A).

Many authors (Côté and Day 1962; Liese 1963; Scurfield and Silva 1969; Baird et al. 1974b; Ohtani et al. 1984) have reported that the warty layer of many hardwoods and softwoods is very resistant to chemical treatments. In red pine, however, we found that when present, especially in the heartwood, the encrustant lining the lumen wall could be readily removed by a 5% NaOH extraction. Dunning (1969) and Baird et al. (1974b) suggested that the warts in longleaf pine and balsam fir were made up of a combination of lignin and amorphous carbohydrates. The latter authors also found that the encrustant lining the lumen wall contained an amorphous carbohydrate. Meier (1964) also reported that the S3 layer of spruce and pine tracheids contained a large amount of glucuronoarabinoxylan. Therefore, it is possible that in red pine the substance removed from the lumen surface, microfibrillar bundle, and warts by the NaOH treatment is hemicellulose in nature. The substance that resisted the NaOH extraction is probably lignaceous material since it can be removed by a delignification treatment.

It was found that the surface lamella of the S3 layer tends to be easily peeled off from the cell wall during sample preparation. Figure 4B shows that a portion of the S3 surface lamella was torn apart from, and folded back to, the cell wall revealing its backside. It is evident that the backside of the S3 surface lamella is as warty as the lumen-side surface. This observation strongly suggests that some warts are the thickened portions of microfibrillar bundles.

The SEM observation that many warts are situated at the end of microfibrillar bundles could not be duplicated in the TEM study. When comparison is made between TEM and SEM micrographs of water-extracted specimens, it was found

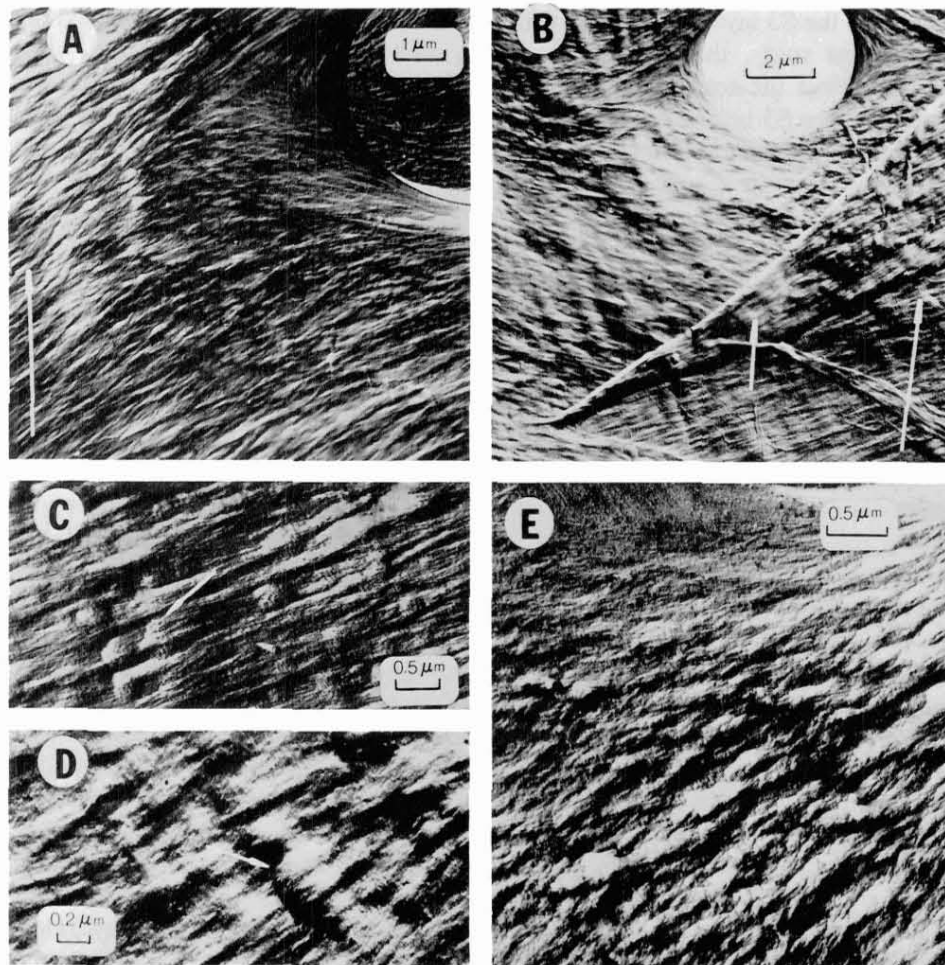


FIG. 4. Carbon replicas of red pine warty layer (long arrows = fiber axes). A. Delignified heartwood, showing warts were completely removed. B. NaOH-extracted heartwood, showing a warty appearance of the backside of the S3 surface lamella (arrow). C and D. NaOH-extracted heartwood, showing the ends of some microfibrillar bundles (arrows). E. Delignified heartwood, showing the bare ends of the S3 microfibrillar bundles in the dome area of a bordered pit.

that the lumen surface became a rather tight structure with the carbon replica technique. As water-extracted samples were further treated, the lumen surface became even tighter and the orderly microfibrillar orientation was somewhat disturbed. It is probable that the combination of the tightening of the lumen surface and the disturbance and flattening of microfibrillar bundles makes the recognition of the ends of microfibrillar bundles very difficult. Despite this difficulty, the structure of microfibrillar bundles ended at warts can be sporadically identified (Figs. 3F and 4C, D). In addition, such a structure was frequently observed in the dome area around pit apertures in the delignified specimens (Figs. 4A, E).

Scurfield and Silva (1969) considered that warts were formed at the time of cell autolysis by depositing less than completely elaborated wall constituents on the

surface of the S3 layer through invaginations of the plasma membrane. Based on the present study, their statement can be elaborated further in the following manner. Near the completion of cell-wall formation, microfibrils in the surface lamella of the S3 layer are deposited in an aggregated form. Occasionally, due to sluggish growth just before cell autolysis, deposition of aggregated microfibrils is accompanied with a large quantity of lignin and hemicellulose, resulting in localized thickening of the microfibrillar bundles. At the time of cell autolysis, the cell stops producing microfibrils and deposits a terminating mixture of lignin and hemicellulose at the end of microfibrillar bundles. This final process of cell-wall formation would be able to account for the unique relationship between warts and the microfibrillar bundles in the surface lamella of the S3 layer observed in this study.

SUMMARY AND CONCLUSIONS

The lumen surface of red pine tracheids was found to be relatively free of encrustation, and a simple hot-water extraction of specimens was sufficient to expose the S3 layer. This characteristic simplified the ultrastructural study of this species. This study also found that the distribution pattern of warts closely followed the microfibrillar orientation of the S3 layer. The microfibrils in the surface lamella of the S3 layer were deposited in an aggregated form, and warts were found to be localized thickenings of the microfibrillar bundles. Many warts were found, especially with the SEM, situated at the ends of microfibrillar bundles.

If present, most of the encrustation lining the lumen surface was resistant to extractions with neutral solvents but could be readily removed by a 5% NaOH treatment. The NaOH treatment also removed a substantial amount of material from microfibrillar bundles and warts. A delignification treatment of specimens completely dissolved the warts. On the basis of these results, it is concluded that, in red pine, the S3 microfibrillar bundles are rich in hemicellulose content and that warts are composed of a combination of lignin and amorphous carbohydrates, and the accompanying encrustant on the lumen surface is mainly carbohydrate.

Because the elaboration of the lumen surface is a final process of cell-wall formation, the ultrastructural study of the surface lamella of the S3 layer and the associated warts would be able to provide crucial information as to how this final process is accomplished. A possible mechanism of this final process is proposed. Further work has been planned to study this possibility.

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