

## Some Aspects on Resistance of Acetylated Wood against Biodeterioration

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**Abstract**—Resistance of acetylated wood against biodeterioration was investigated at different levels of acetylation. Spruce (*Picea jezoensis*) wood veneers were treated with uncatalyzed acetic anhydride for different lengths of time and exposed to each two species of wood-rotting fungi and subterranean termites. The protective effect by acetylation was clearly observed for all test organisms but varying with decay type or species used. For a brown-rot fungus *Tyromyces palustris*, the weight loss reached nill at 18 WPG (weight percent gain) of acetylation after the rapid decrease from 10 to 15 WPG. For a white-rot fungus *Coriolus versicolor*, it decreased at higher rate with degree of acetylation and was not counted at just 6 WPG. The attack by termite *Reticulitermes speratus* was almost nill at the highest acetylation of 18 WPG with its rapid decline from 13 WPG. Perfect inhibition was unsuccessful even at 18 WPG for more virulent species *Coptotermes formosanus* but the similar decline of attack was confirmed, too. A large number of bore-holes were produced by fungal attack in the cell walls of acetylated wood below the threshold value for biological resistance. Existence of sporadic erosion in the cell walls was characterized as the limited attack of acetylated wood by fungi.

### 1. Introduction

Dimensional stability and resistance to biological attack of wood have been greatly improved through chemical modification such as acetylation. Goldstein *et al.*<sup>1)</sup> have pointed out that 17 percent gain (WPG) due to acetylation was sufficient to protect the southern yellow pine from attack by the six species of brown-rot fungi used. Stamm and Baechler<sup>2)</sup> also stated that decay was practically eliminated when the reduction in swelling exceeded 70 percent, and the value was attained at about 30 WPG of acetylation. Peterson and Thomas<sup>2)</sup> noted that the lower levels of acetylation was nearly as efficient as the higher acetylated treatment for the protection of wood against white rot. Termite resistance, which is related to the breakdown of lignocellulose in ingested wood by symbiotic organisms, has also been enhanced through wood acetylation<sup>1)</sup>.

Biodeterioration of wood is considered to be the concerted action of the individual enzyme systems responsible for cellulose, hemicellulose, and lignin decomposition<sup>4)</sup>. Therefore protective effect of chemical modification may be

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attributed to its blocking effect of enzymatically-reactive sites in these components. According to Rowell<sup>5)</sup>, who compared the degree of substitution of the components through modification processes, the hydroxyl bond of lignin was always much more substituted than that of the carbohydrates. If so, there should be a clearer difference in effects of chemical modification on biological resistance among the fungal decay types or termite species, because the distinct degrading-systems are recognized in these organisms.

This paper deals with the biological resistance of acetylated wood at various levels of treatment, including SEM observations of the cell-wall structure of acetylated wood exposed to fungal attack.

## 2. Experimental

### 2.1 Acetylation procedure

3.5 mm-thick rotary veneers of spruce (*Picea jezoensis* Carr.) were acetylated with acetic anhydride alone without cosolvent or catalyst. Square-cut veneers (55×55 mm) were oven-dried for 24 hours at 105°C. The oven-dried veneers were placed in glass flask. A prevacuum was applied to the flask (15 mmHg, 30 min.), and then acetic anhydride was impregnated into the specimens under the reduced pressure at 15 mmHg for 30 min. After they were soaked in acetic anhydride for 2 hours at atmospheric pressure, each veneer was transferred to 500 ml round-bottomed flask containing acetic anhydride previously heated at 120°C. The solution was kept at 120°C under reflux and samples were reacted for times ranging from 5 minutes to 3 hours. Then reacted veneers were rinsed in water for 24 hours to remove unreacted acetic anhydride and the by-product acetic acid. The acetylated veneers were oven-dried at 105°C for 24 hours. Acetyl content calculated on the basis of the increase in the oven-dry weight ranged from 1.5 to 18.3 percent. The specimens of veneers were cut and trimmed into samples for decay and termite tests.

### 2.2 Decay test methods

Decay tests were run according to JWPA (Japan Wood Preserving Association) Standard No. 3-1979. Square samples (25 mm wide) of acetylated veneers were sterilized and placed in glass-jars containing a brown-rot fungus *Tyromyces palustris* (Berk. et Curt.) Murr. or a white-rot fungus *Coriolus versicolor* (L. ex Fr.) Qué. Specimens were removed after 8 weeks or 12 weeks, and extent of fungal attack was determined based on weight loss. The moisture content of each specimen at the end of decay test was also estimated.

### 2.3 Termite test methods

Specimens used for termite resistance tests were 25 mm square veneers. For

evaluation of termite resistance, a forced feeding test was employed where untreated or acetylated wood was the only source of nutrient for the termites. The test method was run according to JWPA Standard (No. 11, 1981), using two termite species, *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* Kolbe. The individual test specimens together with 150 sound workers and 15 soldiers were put in a cylindrical, clear plastic container having the bottom sealed with hard plaster of Paris. The plastic containers were placed in a large covered case which had moist cotton wool at the bottom to keep the tests sets in high humidity condition. After a 21 days test duration, the number of dead termites was recorded to determine termite mortality. The test specimens were then removed, cleaned down surface debris and adherent materials by washing, and subsequently oven-dried (60°C) and weighed. The percentage weight loss of each specimen was calculated.

#### 2.4 SEM observations

Scanning electron microscopy was employed for observations of the cell wall characteristics in acetylated wood when attacked by decay fungi.

Preparations of radial-longitudinal surfaces were made by splitting each sample with a knife and microtoming the exposed surfaces. These samples were mounted on specimen stubs, and then coated with gold for examination in Hitachi S-500 SEM.

### 3. Results and Discussion

#### 3.1 Decay resistance

The percent weight loss in the individual test piece after exposure to the two types of decay fungi is plotted against its original weight gain during acetylation (Fig. 1). As shown in the smoothed regression curves, the distinct effect of acetylation was recognized in the decay type. The weight loss was counted until 18 WPG of acetylation for *T. palustris* (brown-rot)-exposed wood, but not for *C. versicolor* (white-rot) even at 8 WPG.

A rapid decrease of weight loss was clearly observed during 10 to 15 WPG for *T. palustris*. This suggests that acetylation over 15 WPG may be sufficient to protect wood from the decay by this fungus. However, a large variance of protective effect was existing below 15 WPG. The failure of decay inhibition may be attributed to the inevitable uneven or localized distribution of acetyl bond in the treated wood during such a medium acetylation.

Rowell<sup>5)</sup> stated that introduction of acetyl bond into wood initiated in lignin and higher lignin substitution did not contribute significantly to the overall protection of wood from brown-rot which has a strong preference to the carbohydrate breakdown. However, the low level of acetylation such as 6 WPG provided

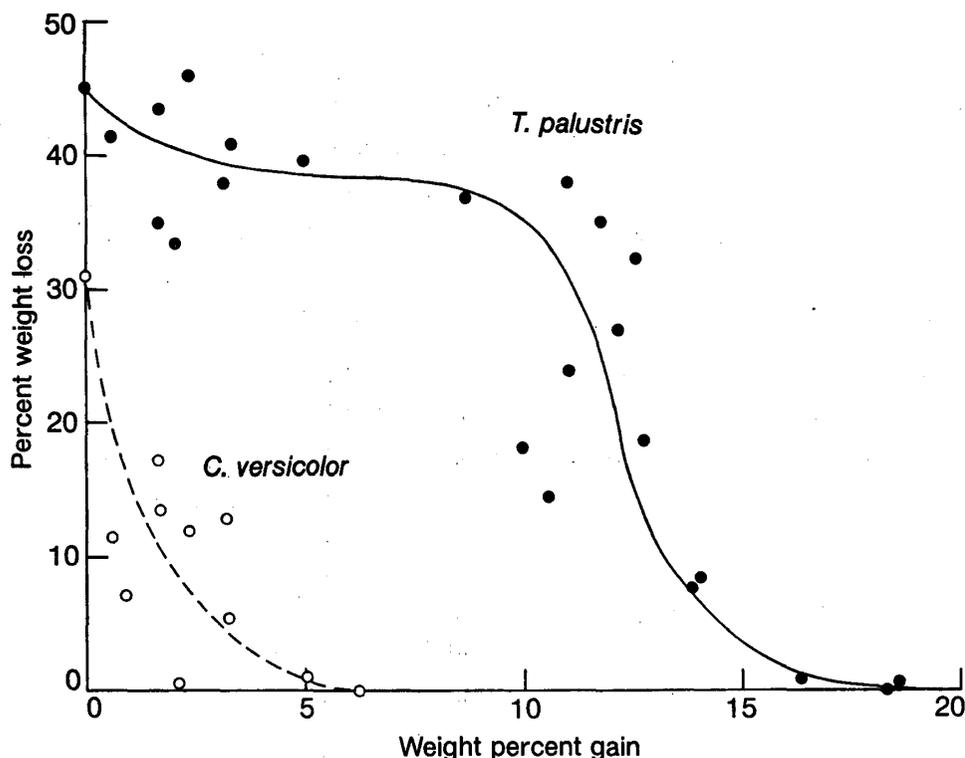


Fig. 1. Relationship between weight percent gain, due to acetylation, and weight loss, due to attack by *T. palustris* or *C. versicolor* for 12 weeks.

a complete protection against a white-rot fungus, *C. versicolor* (Fig. 1). Rowell<sup>5)</sup>, who assumed the ratio of lignin/holocellulose DS was 6 to 7, up to 10 WPG, indicated that the higher lignin substitution in acetylation process was unavoidable in the lower WPG levels. In the case of white-rot fungi, lignin is decomposed simultaneously with polysaccharides<sup>6)</sup> and its decomposition is always essential for their decay activity. Therefore even the lower level of acetylation below 6 WPG might be sufficient to protect wood from the white-rotter.

Fig. 2 shows the values of moisture content of exposed wood after 12 weeks. It has been recognized that the exclusion of cell-wall water was one of the most important factors in the mechanism of wood protection from decay by wood modification processes. However, as shown in the results, acetylated wood contained much free water in cell lumina even at the high acetylation levels and some colonization and growth of hyphae occurred.

As increase of WPG, the moisture content was gradually reduced and where WPG exceeded 15, the value reached below 20 percent for *T. palustris* and 30 percent for *C. versicolor*. Above 5 WPG no loss in weight occurred for *C. versicolor*, while moisture content gradually decreased with increase of WPG due to acetylation, and declined rapidly at 12 WPG. The reduction in permeability due to the

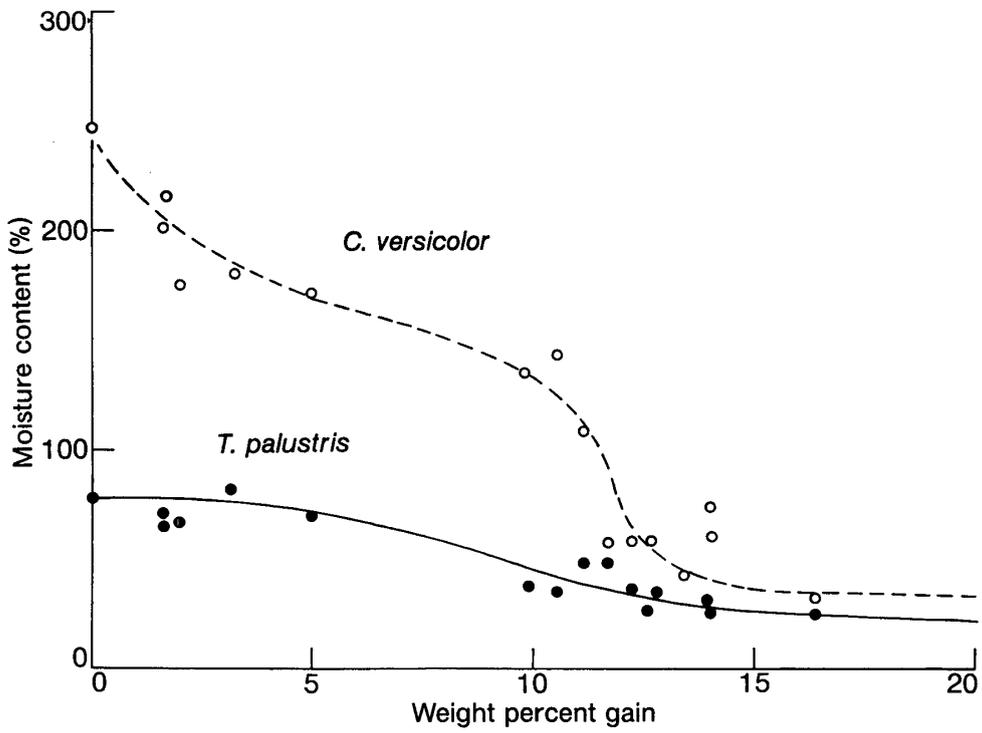


Fig. 2. Moisture content of specimens of different weight gain due to acetylation, after exposure to *T. palustris* or *C. versicolor* for 12 weeks.

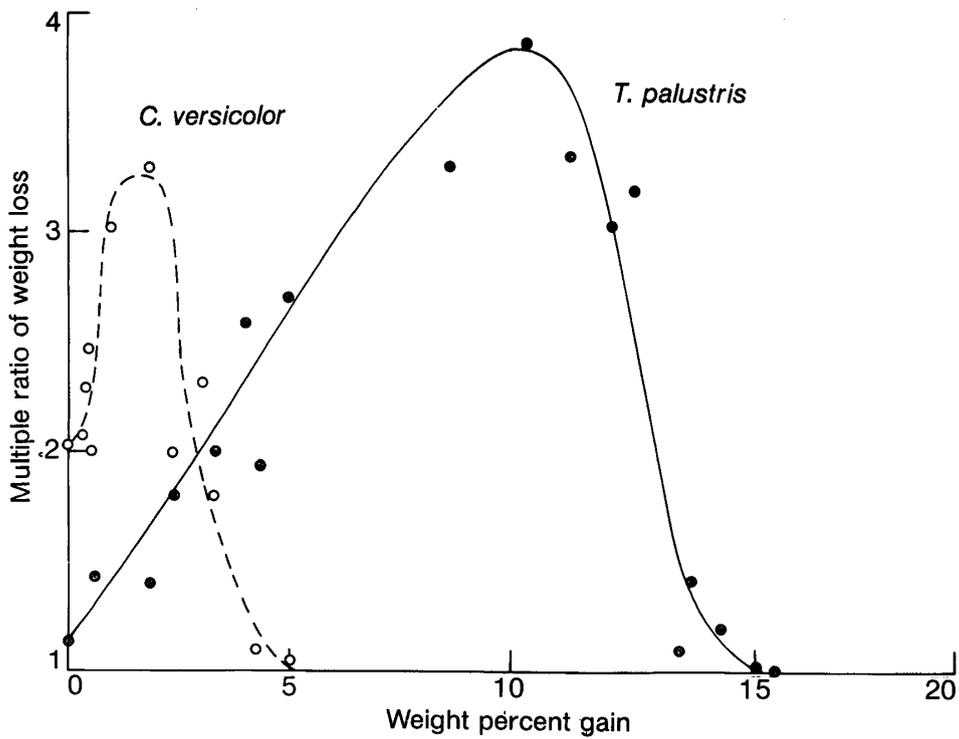


Fig. 3. Effect of weight percent gain due to acetylation on the ratio of wood weight loss after exposure for 8 weeks to that after 12 weeks in decay tests.

bulking chemicals which restricted pore spaces in the cell walls also might cause the reduction of moisture absorption.

Fig. 3 shows the relationship between WPG and the weight loss ratio in 12 weeks/8 weeks. The size of specimens used for decay tests was small and thin which allowed to bring a severe decay of control specimens in a shorter period of this test, so that little difference of weight loss was observed between the two periods for control and the lower acetylated specimens. By rising of acetylation level, the invasion of fungus hyphae into the acetylated wood was delayed and successive degradation of the cell walls was kept at lower extent. Delay of the initiation of fungal colonization and cell-wall lysis is also one of the important factors for the protective treatment of wood. The present results showed that any increase of weight loss with exposure time was not found in the acetylated wood over 5 WPG for *C. versicolor* and 15 WPG for *T. palustris*, respectively. The acetylation treatment may be characterized as its longer effect than other preservative treatments for decay control.

### 3.2 Termite resistance

For termite tests, an exposure method was selected wherein treated wood was the only source of nutrients for the termites because this would reveal whether undigestibility might be an antitermitic property of the acetylated wood.

In the control sets of the forced-feeding tests, the termite survivals after test

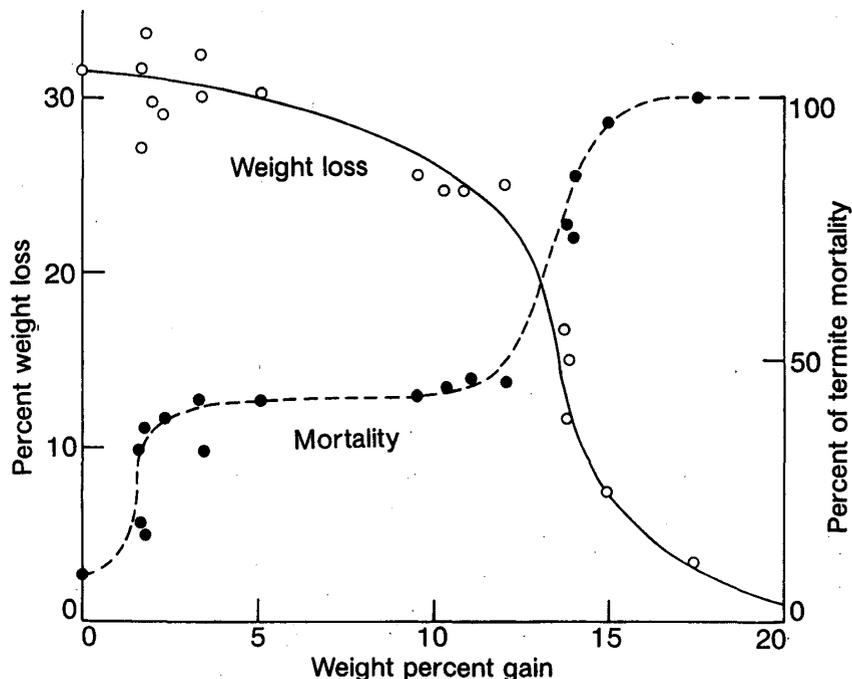


Fig. 4. Effect of acetylation on termite mortality and wood weight loss in forced-feeding tests by *R. speratus* for 3 weeks.

period for 3 weeks were always over 90 percent both for *R. speratus* and *C. formosanus*, while the matching percentages for acetylated specimens above 18 WPG were 0 percent, and below 60 percent, respectively (Figs. 4 and 5). It has been said that acetylation of wood caused greater mortality for *R. speratus* than for *C. formosanus*<sup>7)</sup>. For *R. speratus*, acetylated wood above 12 WPG induced 100 percent mortality within 3 weeks or a few additional weeks, and higher weight gain from acetylation virtually eliminated attack on the wood by this termite. The lower acetylation from 3 to 12 WPG could hardly attain 100 percent mortality within the same test period, even though the values were much more higher than those of controls. For *C. formosanus*, 100 percent mortality could not be attained even in the acetylated wood over 18 WPG. However, the termites should be died if the test period would be prolonged to over 3 weeks, judging from the course of its mortality curve.

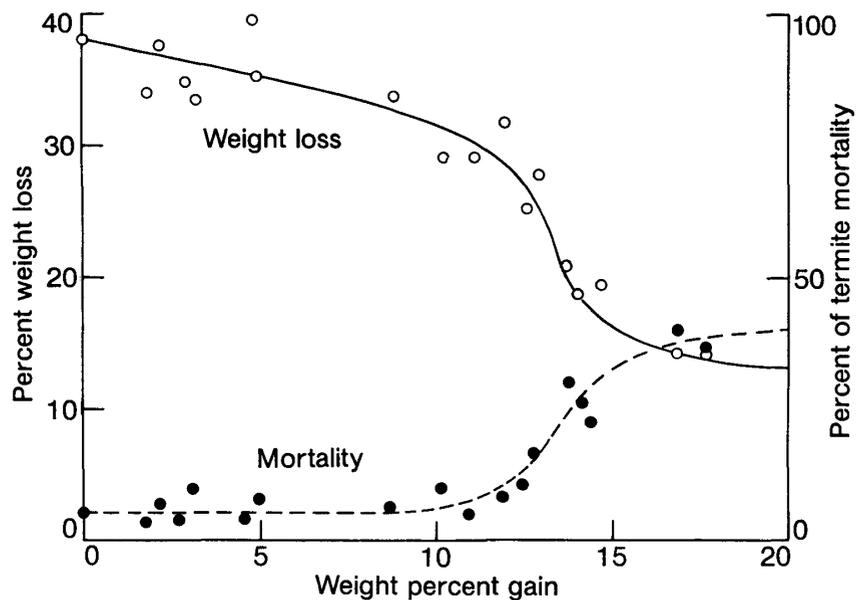


Fig. 5. Effect of acetylation on termite mortality and wood weight loss in forced-feeding tests by *C. formosanus* for 3 weeks.

According to the studies of the authors<sup>7)</sup> and others<sup>8)</sup> on termite resistance of chemically modified wood, greater mortality may be attributable to an enhanced starvation effect. At the higher weight gain due to acetylation the termites obtained little nourishment from the wood and expired due to starvation, and percentages of mortality were always higher than those in the starved sets. This indicated that the energy expended to eat the acetylated wood was not balanced by nutritional energy from the wood. At the lower level of acetylation, the insects were able to get nutrients from the breakdown of the ingested wood by their symbiotic intestinal

organisms and were thus able to remain alive for an additional days.

It has been pointed out that the perfect resistance to the brown-rot fungi was attained above 18 WPG. The termite tests also showed that loss of wood volume and termite mortality had an apparent threshold at a WPG of approximately 18. It is of interest that cell wall decomposition by this fungus and utilization of wood by the symbiotic protozoa in termites were almost inhibited at the same level of acetylation. This suggests that the similar degradation mechanism is existing in these quite different organisms in taxonomic status.

### 3.3 SEM observations

Some relevant information on the mechanism of acetylation on degradation of the wood has been obtained from the present visual approach using SEM techniques. Differences of the molecule's configuration due to wood acetylation may modify the characteristic pattern of cell wall lysis caused by decay fungi.

In the highly acetylated wood, a few cells adjacent to the specimen surfaces were densely filled with fungus hyphae. However, a number of them decreased in the cells locating in the inner part of the specimen (Fig. 6-A). The hyphae were able to invade easily and colonize into the surface cells contact with fungal mat. It seems likely that the supply of nutrient source from the medium would be important in supporting the fungus development during invasion and reinvasion

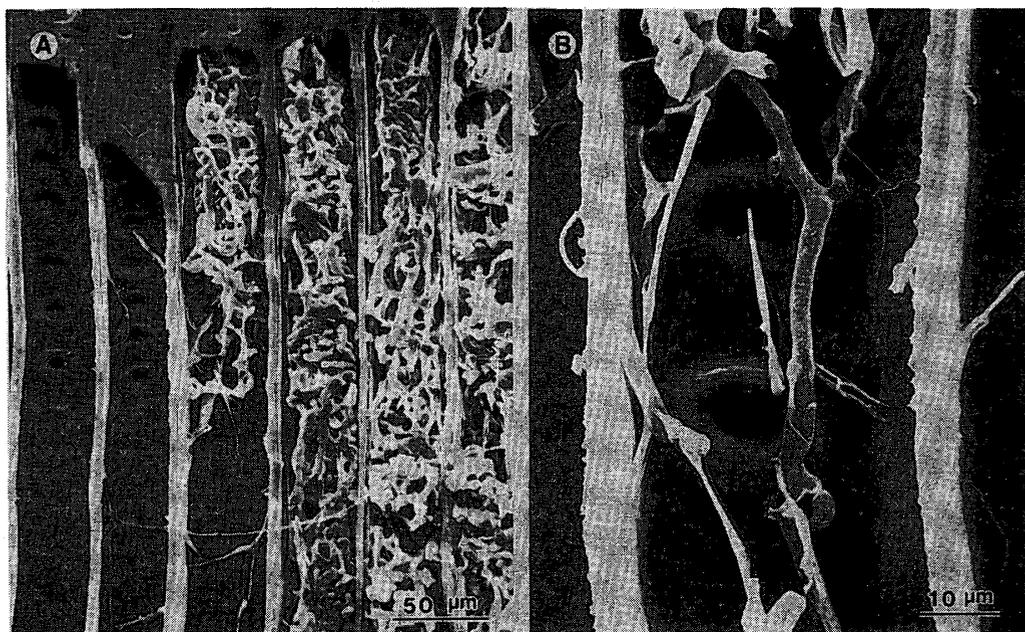


Fig. 6. A, Excessive hyphal development in tracheids adjacent to the specimen surface (in the direction of the right-hand side of the photo). B, Close-up view of hyphae and cell walls without lysis or erosion. Acetylated wood of 15 WPG, exposed to *T. palustris*.

into the acetylated wood. The hyphae observed in the cells of acetylated wood with high WPG was not considered to utilize cell-wall components and they were still depending on the culture substrates (Fig. 6-B). Hyphae were able to penetrate into the wood cells to a certain extent, but the cell walls, which were blocked with stable bond during acetylation, probably retarded the rate of subsequent invasion of hyphae into the cells apart from the specimen surfaces.

Attack by the brown-rot fungus is known to remove cellulose first from the S2 layer while all other cell wall layers remain intact<sup>9</sup>. In the present study, such a characteristic pattern of cell-wall lysis by *T. palustris* were substantially unchanged by the low-level acetylation below 10 WPG, and only the rate of lysis were relatively retarded. Through the development of acetylation during 10 to 15 WPG, different features were recognized between untreated and acetylated specimens. During this stage, small and scattered bore-holes emerged in acetylated wood exposed to *T. palustris* (Fig. 7-A), whereas in untreated wood a limited number of original bore-holes continued to enlarge even in the advanced stages of degradation<sup>10</sup>, accompanying the cracks or breaks in the cell walls. The sporadic erosion of the cell walls occurred severely in the latewood (Fig. 7-B) and the ray contact areas in the earlywood tracheids. The severer lysis of the cell walls in the latewood may coincide with Rowell's observation<sup>11</sup> that a greater amount of the substituted components was found in the earlywood than in the latewood.

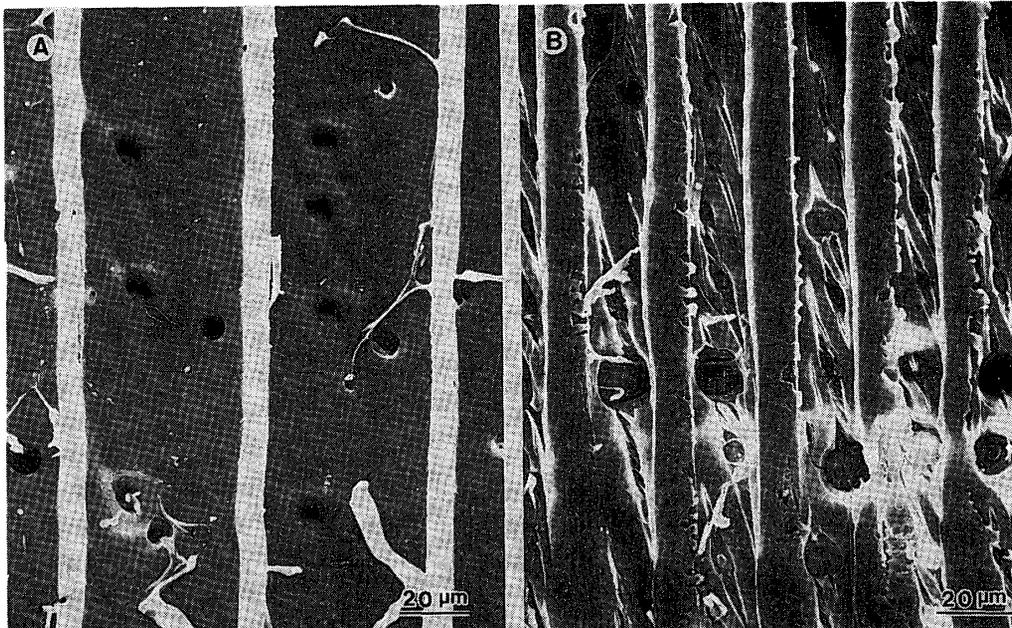


Fig. 7. A, Bore-holes in the earlywood tracheid walls, without lysis around pit openings. B, Erosion in the pit regions of the latewood tracheid walls. Acetylated wood of 12 WPG, exposed to *T. palustris*.

Attack by the white-rot fungus is characterized to produce a gradual thinning of the wood cell wall from the lumen to outward. The thinning of the secondary walls of untreated wood was proved by complete removal of the wall layers from S3 (Fig. 8-A). However, in the lower acetylated specimens, such as 2 WPG, which showed the same loss of weight with the untreated ones, a random erosion and pocket-like removal of the cell walls was detected (Fig. 8-B).

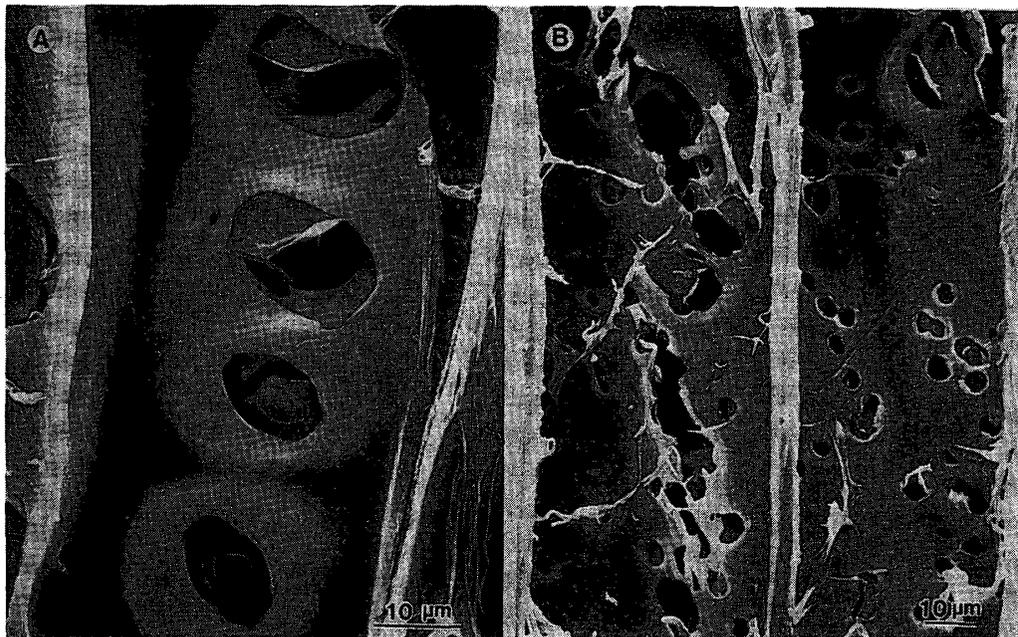


Fig. 8. A, A gradual thinning of tracheid walls from S3 to S2 in untreated wood. B, A random erosion and pocket-like holes in tracheid walls of acetylated wood (2 WPG). Attacked by *C. versicolor*.

In the early stages of fungus colonization in untreated wood, hyphae penetrate into wood more frequently through pit opening than by direct boring through lignified cell walls. Active transverse penetration of the cell walls producing "bore-holes" was observed only in the later stages of decay, but a number of them was limited<sup>9)</sup>. In acetylated wood with low levels at 3 WPG, a large number of bore-holes were present not only in the ray contact areas but also in the unpitted regions of the cell walls especially in *C. versicolor*-attacked wood (Fig. 9-A).

In acetylated wood above 7 WPG, in which no loss of weight occurred, a small number of bore-holes were recognized from the lumen surfaces (Fig. 10-A). Weight loss by formation of bore-holes might be compensated by weight increase due to existing of hyphae in the cell cavities and to the absorption of the nutritional components from the medium. Above 12 WPG, any bore-holes were not observed although a few hyphae were still present in the intact cells of acetylated wood (Fig. 10-B).

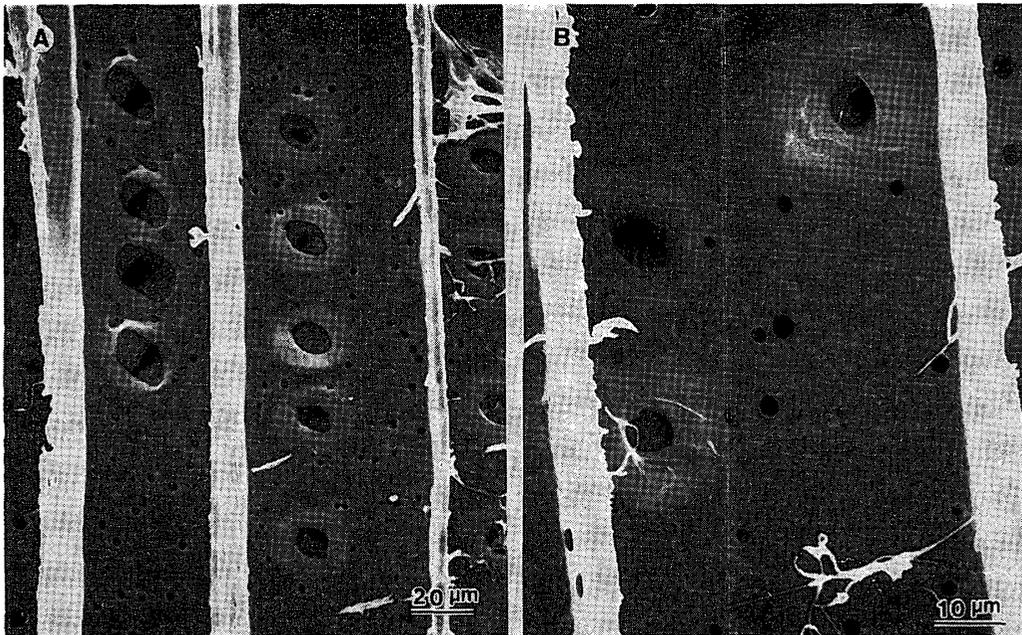


Fig. 9. A, Bore-holes numerous distributed in tracheid walls of acetylated wood (3 WPG). B, Erosion troughs and bore-holes in tracheid walls of acetylated wood (5 WPG), without evenly lysis of the S3 layer. Attacked by *C. versicolor*.

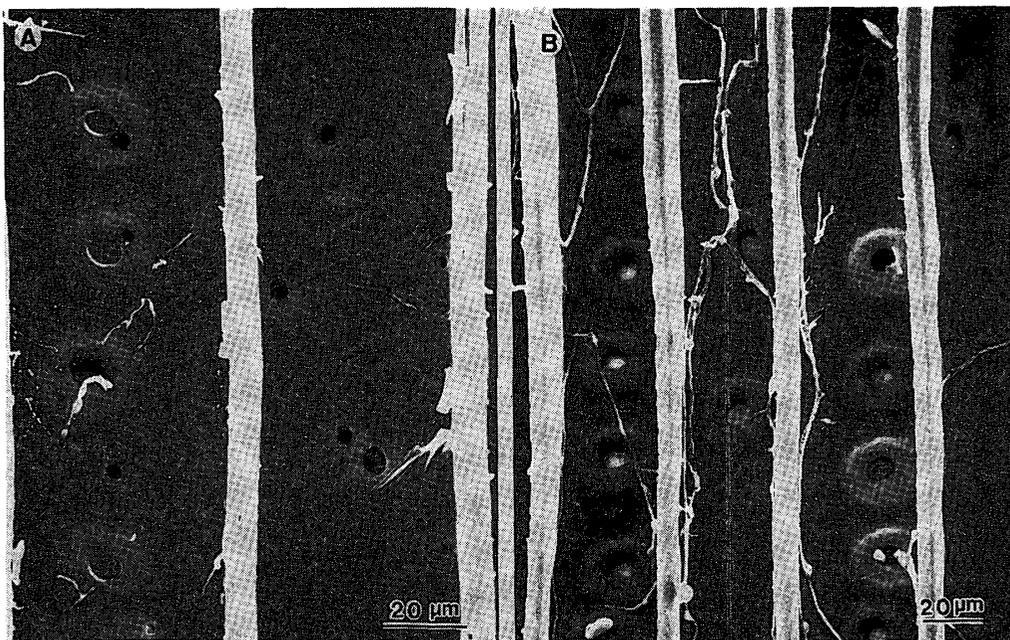


Fig. 10. A, A small number of bore-holes in tracheid walls of acetylated wood (7 WPG). The specimen showed no sign of weight loss due to fungus attack. B, Sound tracheid walls with fungus hyphae in the cell cavities of the highly acetylated specimen above 12 WPG. Attacked by *C. versicolor*.

#### 4. Conclusions

Resistance to biological degradation has been greatly improved through acetylation of wood. The decay resistance depended on adequate distribution of acetyl bond in water-accessible regions of the cell walls. The complete protection was attained at 18 WPG level of acetylation for brown-rot fungi, but it was available at the lower levels of acetylation, 6 WPG, for white-rot fungi.

The weight losses of acetylated wood decreased at rapid rate during 10-15 WPG for *T. palustris* and 4-5 WPG for *C. versicolor*, which meant that ability of these fungi to decompose the treated wood was influenced by the different levels of substitution of accessible hydroxyls by stable chemical bond.

The termite tests also showed that loss of wood volume and termite mortality had the same threshold value of 18 WPG to that in the attack by the brown-rot fungus.

For the white-rot fungus, attack was inhibited at the lower levels of acetylation than those reported before. However, a large number of bore-holes were observed in the cell walls of acetylated specimens. The formation of bore-holes is not assumed to cause a significant influences on mechanical properties of wood because it occurred rather sporadically.

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