PRESENCE OF WIDESPREAD BACTERIAL ATTACKS IN PRESERVATIVE-TREATED COOLING TOWER TIMBERS

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ABSTRACT

Microscopic examinations of CCA-treated *Pinus radiata* D.Don timbers in an industrial cooling tower in New Zealand showed bacteria and soft-rot fungi to be primarily responsible for the decay of these timbers. Of these micro-organisms, erosion bacteria appeared to be most widespread, attacking wood cell walls independently as well as in combination with tunnelling bacteria and soft-rot fungi. Tunnelling bacteria attacked wood often with soft-rot fungi, and less commonly with erosion bacteria.

Wood samples were taken from various locations in the cooling tower, including spray line support, panelling above the spray drift eliminators, and from spray drift elimination slats. Examination of these samples indicated that, among the micro-organisms which attacked the wood, erosion bacteria were most tolerant of oxygen-limiting conditions as bacterial erosion was the only type of decay present in wood constantly saturated with water. The evidence provided of the presence of widespread bacterial attacks in an industrial cooling tower timbers is the basis for recognising that bacteria may play an important role in the deterioration of cooling tower timbers.

Keywords: cooling tower timbers; CCA-treated timbers; wood degradation; erosion bacteria; tunnelling bacteria; soft-rot fungi; *Pinus radiata*.

INTRODUCTION

The microbial decay of cooling tower timbers has long been recognised. Savory (1954) described the superficial damage to cooling tower timbers as soft-rot decay caused by members of Ascomycetes and fungi imperfecti. Greaves (1968, 1969) was one of the first to report the presence of bacteria in cooling tower timbers. However, the confirmation in recent years that bacteria can degrade lignified components of wood, and a better understanding of the various types of wood decay caused by bacteria (Blanchette *et al.* 1990; Singh & Butcher 1991) have rekindled interest among wood preservation and biodeterioration researchers in determining the role of bacteria in wood decay in a wide range of situations, including cooling towers (Singh *et al.* 1992; Eaton 1994; Singh, Wakeling & Page 1994b). Bacterial degradation of preservative-treated wood from cooling towers has been confirmed in two recent studies (Singh *et al.* 1992; Eaton 1994).

This paper describes the presence of widespread bacterial attacks in decayed wood examined from an industrial cooling tower. The results reported here were presented at a meeting of the International Research Group on Wood Preservation (IRG) (Singh & Wakeling 1995).

MATERIALS AND METHODS

The timbers examined from the cooling tower in this study had been treated with CCA (copper-chrome-arsenate) preservative prior to being placed in service about 13 years previously. For microscopic examination, decayed wood samples of various moisture content were taken from three regions defined by their proximity to the water spray line. Wood taken from the spray line supports was completely saturated, whereas wood taken from panelling above the spray drift eliminators remained in a moist condition from contact with rising moisture laden air. Wood of an intermediate moisture content was taken from the spray drift eliminator slats.

Hand sections taken from areas showing different degrees of decay were examined under a light microscope using bright field illumination and polarised light. Selected areas from these sections were subsequently processed for observations with a transmission electron microscope (TEM). Samples were either fixed in 3% glutaraldehyde (0.05M sodium cacodylate buffer, pH7) for 5 hours at room temperature and subsequently in 2% osmium tetroxide (in 0.05M sodium cacodylate buffer) overnight at 4°C, or in 1% aqueous potassium permanganate ($KMnO_4$) for 1 hour at room temperature. All samples were dehydrated in acetone and embedded in Spurr's low viscosity resin. Ultrathin sections of approximately 70 nm in thickness were cut with a diamond knife on an LKB ultramicrotome, stained with 1% $KMnO_4$ (in 1% citrate buffer), and examined in a Philips 300 TEM.

RESULTS AND DISCUSSION

The tracheid walls (Fig. 1 and 2) were attacked exclusively by erosion bacteria, which were present in close proximity to sound areas of the secondary wall. The degraded wall shown in Fig. 1 had been transformed into a mass of amorphous material, which appeared partly granular at higher magnification (Fig. 2). In some areas (Fig. 1) the secondary wall had been completely eroded to expose the underlying middle lamella, which had not been attacked. Also resistant to erosion bacteria were initial pit borders and pit tori, as shown in Fig. 3 where it can be seen that the entire secondary wall of pit borders is degraded but the initial pit border areas appear intact.

These features are consistent with previous electron microscopic views of the attack of wood cell walls by erosion bacteria (Singh *et al.* 1990; Singh, Wakeling & Drysdale 1994), which confirms the pattern of degradation shown in Fig. 1 and 3 as bacterial erosion. Initial pit borders were shown to be resistant to erosion bacteria in another recent study (Singh *et al.* 1995). The resistance of both middle lamella and initial pit borders to erosion bacteria is probably related to their high lignin content. In *P. radiata* wood, the lignin content of middle lamella is about 80% (Donaldson 1985). Although no information is available on the lignin content of initial pit borders in *P. radiata*, judging from their high contrast after $KMnO_4$ staining (Fig. 3) these areas of pit borders are also likely to be rich in lignin. According to Murmanis & Sachs (1969), initial pit borders are primary structures, which would suggest

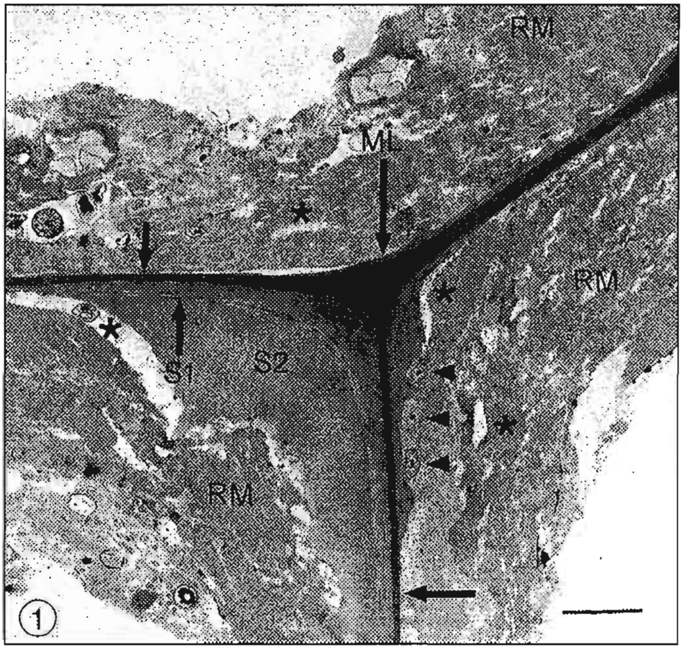
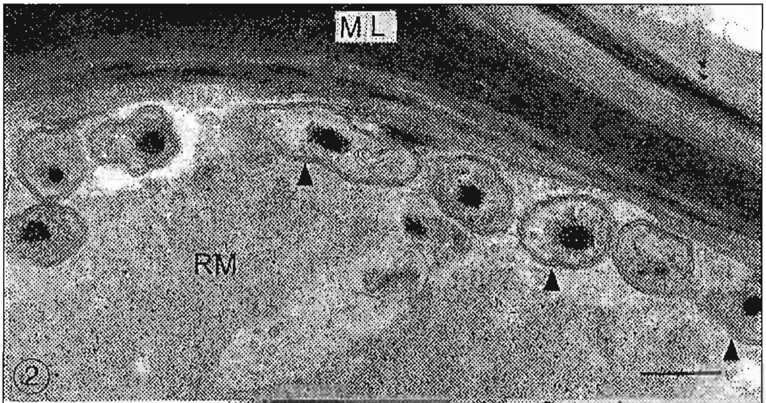


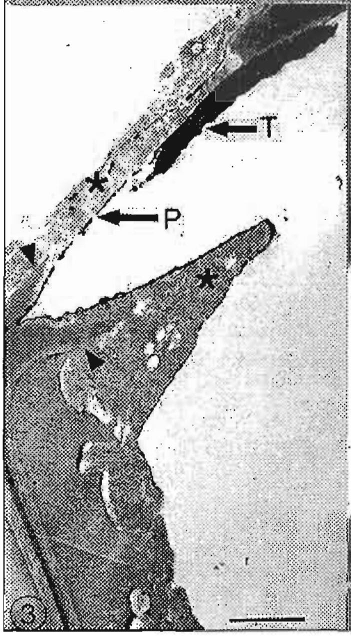
FIG. 1—Transverse section through a corner region shared by three adjoining tracheids. Tracheid walls are attacked exclusively by erosion bacteria (arrowheads), and the residual wall material (RM), after the secondary walls have been degraded, appears as an amorphous mass at this magnification. The transparent areas (asterisks) within the residual material are probably shrinkage artefacts formed during sample preparation for TEM. The unlabelled arrows indicate those areas where the secondary wall has been eroded up to the middle lamella. ML = middle lamella; S1, S2 = secondary wall layers. TEM: Bar = 2 μm.

that the lignin content of initial pit borders is comparable to that of the primary wall areas of the compound middle lamella. The results of the work of Parham & Côté (1971) and Timell (1973) with conifer wood, which involved removal of polysaccharides from wood cell walls by hydrofluoric acid, support this view.

The resistance of torus in *P. radiata* pits to erosion bacteria (Fig. 3) is likely to be related to its high extractive content. The torus depicted in Fig. 3 appears extremely dark, which would suggest that it has been impregnated largely by phenolic-type extractives as phenolics are known to react strongly with osmium tetroxide (OsO_4) as well as $KMnO_4$, the reagents used in this study to prepare samples.

The degradation of tracheid walls was also a result of combined attacks by erosion bacteria and soft-rot fungi (Fig. 4), tunnelling bacteria and soft-rot fungi (Fig. 5), and by erosion bacteria, tunnelling bacteria, and soft-rot fungi (not illustrated). Soft-rot cavities were present in the S2 layer of a tracheid wall (Fig. 4), which was also attacked by erosion bacteria. Although only a few bacteria were present in the part of the tracheid shown in Fig. 4, the pattern of wall degradation was identifiable as bacterial erosion as the presence of residual wall material in degraded areas of wood cell walls, as shown here, is a characteristic





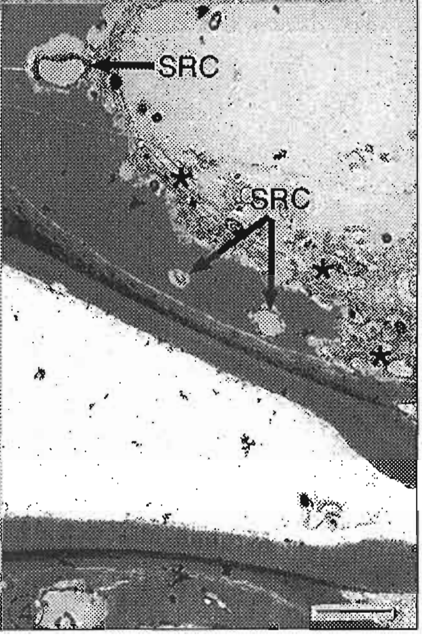


FIG.2–High magnification view of erosion bacteria (arrowheads) present in proximity to sound areas of the secondary wall in a tracheid. The residual wall material (RM) appears partly granular at this magnification. ML = middle lamella. TEM: Bar = 0.5 μm.

FIG.3–Transverse section through an inter-tracheid pit region. The entire secondary wall of pit borders is degraded by erosion bacteria (asterisks). However, initial pit borders (arrowheads) appear to be resistant. The highly dense torus (T) is also resistant. P = preservative layer. TEM: Bar = 2 μm.

FIG.4- Transverse section through an area of the tracheid wall which has been attacked by soft-rot fungi and erosion bacteria. Soft-rot cavities (SRC) are present in the S2 layer; the inner face of this wall has been eroded and the residual wall material is present in eroded areas (asterisks). TEM: Bar = 2 μm.

feature of bacterial erosion of wood cell walls (Singh *et al.* 1990; Singh, Wakeling & Drysdale 1994).

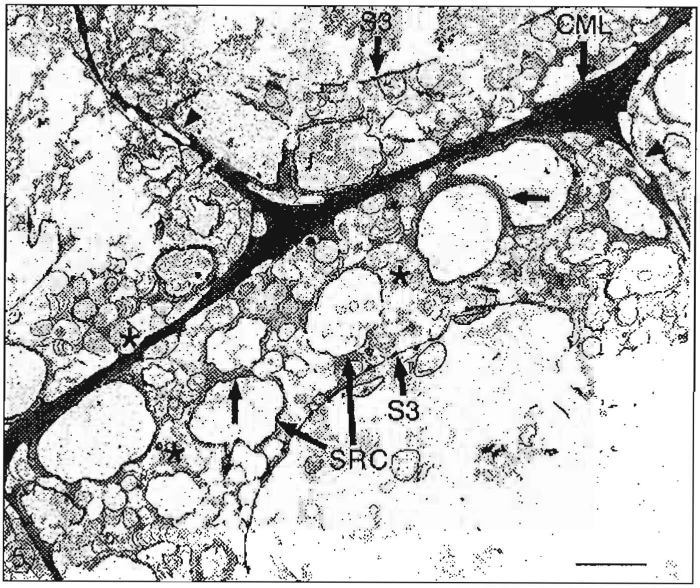


FIG.5–Transverse section through tracheids heavily attacked by soft-rot fungi and tunnelling bacteria. Soft-rot cavities (SRC) and bacterial tunnels (asterisks) are present throughout secondary walls, which are heavily degraded. Soft-rot cavities are excluded from the middle lamella, but this wall is penetrated by bacterial tunnels and is consequently thinned in some areas (arrowheads). The unlabelled arrows indicate those areas where adjacent soft-rot cavities are situated very close to one another; bacterial tunnels are excluded from the S2 wall in these areas. CML = corner middle lamella; S3 = secondary wall layer. TEM: Bar = 2 μm.

In Fig. 5 is illustrated a combined attack on tracheid walls by soft-rot fungi and tunnelling bacteria. Typical soft-rot cavities and bacterial tunnels are present in the secondary walls. Both soft-rot cavities and bacterial tunnels are present mostly in the S2 layer, where tunnels penetrate virtually all areas of this wall including those between soft-rot cavities. Tunnels are, however, excluded from those areas of the S2 wall where two or more soft-rot cavities occur in very close proximity to one another, physically restricting penetration of the wall by tunnelling bacteria. Although tunnelling bacteria are known to change their shape dramatically in response to the characteristics of the substrate (Singh & Butcher 1991), passages between some of these closely placed soft-rot cavities appeared to be too narrow even for tunnelling bacteria to negotiate. Tunnelling bacteria also penetrate the S1 and S3 layers, and the lignin-rich middle lamella (Fig. 5). Whereas the highly lignified middle lamella can be degraded by tunnelling bacteria, as indicated by the thinning as well as disruption of the middle lamella, the middle lamella appears to be resistant to soft-rot attack (Fig. 5), except where the fungus penetrates this wall by means of a fine penetration hypha (Singh, Wakeling & Page 1994). Clearly, the effect of a combined attack by tunnelling bacteria and soft-rot fungi is likely to be far more devastating to wood tissues than attack by soft-rot fungi alone.

The observations in this study provide evidence that bacteria have a major role to play in the decay of CCA-treated *P. radiata* wood under conditions of high humidity in a water cooling tower. Of the micro-organisms which degraded cooling tower wood, erosion bacteria were observed to be most widespread, occurring alone as well as together with soft-rot fungi, and sometimes with soft-rot fungi and tunnelling bacteria (Singh *et al.* 1992). Tunnelling bacteria were also common, often co-existing with soft-rot fungi.

Eaton (1994) recently reported that cooling tower wood treated with ACQ13 (ammoniacal copper quaternary ammonium formulation) was more susceptible to bacterial decay than to soft-rot fungi. His study involved a comparison of two different preservative formulations, ACQ13 and CCA, at different target retentions. Our studies have provided evidence that erosion bacteria, tunnelling bacteria, and soft-rot fungi all attacked and degraded *P. radiata* cooling tower wood treated with CCA to a retention of around 13.5 kg/m^3 (Singh *et al.* 1992) and 15.0 kg/m^3 (Singh, Wakeling & Page 1994).

Clearly, the role of bacteria in the decay of timbers exposed to the unique environments of cooling towers is likely to assume greater importance in future, especially as the response of wood-degrading bacteria to different preservatives and different concentrations of the same preservative is becoming better understood, and we are now able to clearly identify various patterns of bacterial decay by light and electron microscopy (Singh & Butcher 1991).

Based particularly on more recent studies (Singh *et al.* 1992; Eaton 1994; Singh, Wakeling & Page 1994) and present observations, we are now able to suggest that the types of microbial decay present in cooling tower timbers are likely to be related to preservative treatments as well as to local environmental conditions (micro-environments) within cooling towers. We already have some information on the effect of moisture content of timbers on the type(s) of microbial decay present. It appears that erosion-bacteria-only attack occurs in timbers which are excessively moist (i.e., highly saturated with water), and soft-rot-only attack occurs in timbers placed in those parts of a cooling tower where moisture is at a low level. A combined attack by tunnelling bacteria and soft-rot fungi, and by erosion bacteria, tunnelling bacteria, and soft-rot fungi is common for timbers which are moderately moist.

It has been suggested that the main reason erosion-bacteria-only attack occurs in wood that is highly saturated with water is that under such conditions oxygen availability is likely to be very limiting (Singh *et al.* 1990). Indeed, there is increasing evidence to suggest that of the known wood-degrading micro-organisms, erosion bacteria may be the most tolerant to near-anaerobic conditions (Singh *et al.* 1990; Blanchette & Hoffmann 1993; Kim & Singh 1994; Singh, Wakeling & Drysdale 1994).

CONCLUSIONS

The evidence provided shows that bacteria alone or together with soft-rot fungi are the main factors responsible for the deterioration of CCA-treated cooling tower timbers. In order to seek ways to prolong the service life of cooling tower timbers in New Zealand and abroad, further work is needed to resolve the interaction among these micro-organisms and with the micro-environmental conditions in various parts of the same as well as different cooling towers, and also with the preservatives used to treat these timbers.

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