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Characterization of Palo Podrido, a Natural Process of Delignification in Wood†

EDUARDO AGOSIN,[1]* ROBERT A. BLANCHETTE,[2]* HERMAN SILVA,[1] CATHERINE LAPIERRE,[3] KORY R. CEASE,[2] REBECCA E. IBACH,[4] ANDRÉ R. ABAD,[2] AND PEDRO MUGA[5]

Laboratorio de Biotecnologia, INTA, Universidad de Chile, Casilla 15138, Santiago 11, Chile[1]; Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota 55108[2]; Centre de Biotechnologie Agro-industrielle, Institut National Agronomique Paris-Grignon, 78850 Thiverval-Grignon, France[3]; Forest Products Laboratory, U.S. Department of Agriculture, Madison, Wisconsin 53705-2398[4]; and Compania Manufacturera de Papeles y Cartones, Santiago, Chile[5]

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Chemical and morphological changes of incipient to advanced stages of palo podrido, an extensively delignified wood, and other types of white rot decay found in the temperate forests of southern Chile were investigated. Palo podrido is a general term for white rot decay that is either selective or nonselective for the removal of lignin, whereas palo blanco describes the white decayed wood that has advanced stages of delignification. Selective delignification occurs mainly in trunks of *Eucryphia cordifolia* and *Nothofagus dombeyi*, which have the lowest lignin content and whose lignins have the largest amount of β-aryl ether bonds and the highest syringyl/guaiacyl ratio of all the native woods included in this study. A *Ganoderma* species was the main white rot fungus associated with the decay. The structural changes in lignin during the white rot degradation were examined by thioacidolysis, which revealed that the β-aryl ether-linked syringyl units were more specifically degraded than the guaiacyl ones, particularly in the case of selective delignification. Ultrastructural studies showed that the delignification process was diffuse throughout the cell wall. Lignin was first removed from the secondary wall nearest the lumen and then throughout the secondary wall toward the middle lamella. The middle lamella and cell corners were the last areas to be degraded. Black manganese deposits were found in some, but not all, selectively delignified samples. In advanced stages of delignification, almost pure cellulose could be found, although with a reduced degree of polymerization. Cellulolytic enzymes appeared to be responsible for depolymerization. A high brightness and an easy refining capacity were found in an unbleached pulp made from selectively delignified *N. dombeyi* wood. Its low viscosity, however, resulted in poor resistance properties of the pulp. The last stage of degradation (i.e., decomposition of cellulose-rich secondary wall layers) resulted in a gelatinlike substance. Ultrastructural and chemical analyses of this substance showed the matrix to have no microfibrillar structure characteristic of woody cell walls but to still be rich in glucan.

White rot basidiomycetes are important in the forest ecosystem because of their unique capacity to degrade all wood components (i.e., cellulose, hemicellulose, and lignin). Many of these fungi possess the ability to selectively degrade lignin, leaving a significant portion of the cellulose intact (7, 9, 37). Several species, including *Phanerochaete chrysosporium*, are being intensively studied because of their potential for use in industrial processes (18, 19, 26, 27). Cellulose fibers left by these fungi after wood decay could be advantageously used as an animal feed or for biological pretreatment of wood for pulping (27). Biological pretreatment of wood chips before mechanical pulping has resulted in improved strength properties of the paper produced from the pulp while decreasing the refining energy requirements (18, 20, 35). In addition, enzymes from white rot fungi may be used for biological bleaching of crude pulp and for treatment of waste effluents generated in pulp and paper production (19, 26, 27). Other lignocellulosic materials (e.g., sugarcane, bagasse, and straw) can also be advantageously treated with white rot fungi to improve digestibility or produce pulp fiber suitable for paper production (3, 4, 23, 40).

Selective lignin degradation by white rot fungi is not a unique phenomenon in nature. Many different species can preferentially degrade large amounts of lignin from wood (5, 7, 24, 36). Usually, hemicelluloses are also degraded to some extent when lignin is removed (10, 14). Selective lignin degradation within woody substrates is often found in localized areas or pockets (6, 32, 36). A combination of delignified and nonselectively degraded wood (in which all cell wall components are removed) also may occur in wood decayed by some white rot fungi (2, 7, 13). There are reports, however, that extensive delignification is present throughout large woody substrates in the temperate rain forests of southern Chile (16, 21, 41). In this region, logs have been delignified by various species of white rot fungi. The decayed wood, first described in 1893 (39), is called palo podrido and has been commonly used as a forage feed for cattle by farmers in Chile for more than 100 years (28). Previous investigation has demonstrated that white rot fungi, in particular *Ganoderma applanatum*, caused a selective degradation of lignin, resulting in wood that was up to 75% digestible (16, 21, 41). Environmental factors (low temperature, high humidity, and microaerobic conditions in the wood) have been suggested to favor delignification (16). Also, the very low nitrogen content of native woods, as compared with the nitrogen levels found in commercial woods, has been suggested as a major factor in the regulation of selective lignin removal by these fungi.

The objectives of this study were to determine the chemical and morphological changes that occurred at different

* Corresponding authors.

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stages of decay in wood of several tree species in southern Chile. Elemental analysis, chemical content, lignin monomer composition, and ultrastructural studies were used to characterize incipient to advanced stages of palo podrido and other types of white rot decay found in wood in southern Chile. Properties of the cellulose that remained after delignification were also evaluated.

MATERIALS AND METHODS

Sampling. Samples of decayed wood, including extensively delignified palo podrido, were collected from the temperate rain forests of Alerce Andino National Park and Chiloe Island in southern Chile. Samples of sound wood and various stages of decayed wood were obtained from several different tree species. Trunks of trees with incipient to advanced stages of decay also were selected. Collections from the same log were visually selected for incipient to advanced stages and labeled 1 to 5, respectively (see Tables 2 to 4). In addition, samples of a gelatinlike stage of advanced decay were collected. All samples were maintained at 4°C in the laboratory until processed. Sound wood of each tree species was also obtained from freshly cut trees.

Fungal isolates. Sporophores of a *Ganoderma* species were found on logs containing palo podrido. Isolations were performed by removing small segments of decayed wood directly behind sporophores as well as within sporophore tissue and placing them on a semi selective agar for basidiomycetes containing 15 g of malt extract (Difco Laboratories, Detroit, Mich.), 2 g of yeast extract (Difco), 15 g of agar (Difco), 0.06 g of Benlate (Du Pont Co., Wilmington, Del.), which consists of a 50% wettable powder of benomyl [methyl-1-(butylcarbamoyl)2-benzimidazolecarbamate], 0.01 g of streptomycin sulfate, and 3 ml of lactic acid.

Chemical analyses. Water content, water-soluble and organic extractable substances, Klason and acid-soluble lignins, wood neutral sugars, and the multielemental composition of sound and decayed wood were determined by previously described techniques (3, 17, 34, 38; TAPPI method UM250). Lignin monomer composition of sound and decayed woods was followed by lignin thioacidolysis, as described by Lapierre et al. (30, 31). This chemical degradation procedure allowed us to specifically characterize the lignin phenylpropane (C_6C_3) units which are linked essentially by β-aryl ether bonds. The guaiacyl (G) or syringyl (S) C_6C_3 degradation products, liberated by the ether cleavage reaction occurring during thioacidolysis, were quantified by a previously described procedure (31). The total amount (S+G) and the ratio (S/G) of these S and G degradation products are a close reflection of the content and monomer composition of the β-aryl ether-linked units in lignin, respectively. The carboxyl content of the holocellulose of sound and decayed wood was estimated by the procedure described in TAPPI T237. Holocellulose was prepared by the chlorite-acetic acid procedure. Cotton cellulose and oxycellulose (containing 11% of carboxylic-substituted residues) (Sigma Chemical Co., St. Louis, Mo.) were used as standards.

Electron microscopy. Small segments of sound and decayed wood were cut with a freezing microtome or sectioned with a razor blade for scanning or transmission electron microscopy. Samples used for scanning electron microscopy were prepared by the methods of Blanchette et al. (11) and examined with a Philips 500X or Hitachi 450 scanning electron microscope. Small sections of wood were also prepared for transmission electron microscopy. Samples were fixed in 1% aqueous $KMnO_4$, dehydrated, and embedded with Quetol 651 (1). Thin sections were cut with a diamond knife placed on noncoated copper grids and examined with a Hitachi 600 transmission electron microscope. Manganese deposits in decayed wood were determined by using energy-dispersive X-ray microanalysis fitted to a scanning electron microscope (8) and a reagent, Leucoberbelin blue, that reacts with manganese deposits to produce a bright greenish-blue pigmentation within wood (8, 29).

Characterization of pulp form palo podrido. The physical and chemical properties of the paper pulp obtained from an unbleached wood sample of palo podrido showing extensive delignification were determined by TAPPI standard method T248. For all pulps, 60-g/m^2 handsheets were made at each freeness value by TAPPI standard T205. Brightness, viscosity, burst, tear, and tensile indexes were determined by TAPPI standards T525, T254 and T220. A control refiner pulp was made by standard methods with a mixture of woods from southern Chile (80% *Nothofagus* and 20% *Laurelia*, *Drymis*, and other genera).

RESULTS

Sampling. Collections of decayed wood from southern Chile contained many different forms of white rot degradation. Simultaneous decay (i.e., nonselective degradation) and selective delignification were found, as well as a combination of both forms of white rot. The amount of delignified wood varied among the different samples. Usually, the outer portions of the logs lying on the ground were not degraded and consisted of numerous pseudosclerotial plates. Within the interior portions of logs, some greater than 1 m in diameter, a white mass of decayed wood was found. Often, the entire log, except for a few centimeters at the outer edges, was delignified and appeared white. Many logs left on the ground had been split open by farmers and the insides had been completely removed by foraging cattle, leaving only the outer portion of nondecayed wood. The term palo podrido refers to various forms of white-rotted wood, but sometimes palo blanco is more descriptive of the extensively delignified wood and is used to refer to completely white delignified wood. Therefore, we propose to use this term to refer specifically to advanced stages of selective delignification.

Extensive sampling was made from a log of *Nothofagus dombeyi* that contained incipient (stage 1) to advanced (stage 5) stages of delignification and from a log of *Laurelia phillipiana* with incipient (stage 1) to advanced (stage 5) stages of nonselective decay (see Tables 2 to 4). Sporophores of a *Ganoderma* species were found fruiting on both logs. The identity of this fungus could not be confirmed as *Ganoderma applantum* and most probably represents a different species (J. E. Adaskaveg et al., unpublished data). Other collections of delignified wood were obtained from logs of *Eucryphia cordifolia*, *Drymis winterii*, and *Weimannia trichosperma*. In recently felled trees, white-pocket rot was evident. Samples were also collected from zones of extremely advanced decay where the white, delignified wood had become a gelatinous mass of grayish-white pulp. The gelatinlike degraded cellulose was obtained from several different tree species and appeared as an aqueous substance or had a waxy texture.

Chemical characterization of sound wood. Results of lignin and wood sugar analyses of various native Chilean woods are presented in Table 1. Although these woods are all angiosperms, *D. winterii* and *L. phillipiana* have high per-

TABLE 1. Chemical composition of native woods

Component	Composition (% dry wt) of following wood:				
	N. dombeyi	*E. cordifolia*	*W. trichosperma*	*D. winteri*	*L. phillipiana*
Extractives					
Organic soluble substances	9.0	5.0	13.1	5.8	9.8
Water-soluble substances	11.9	10.5	7.1	7.8	9.1
Lignin					
Klason lignin	21.7	22.2	27.3	29.1	30.7
Acid-soluble lignin	5.0	3.1	2.7	2.8	1.6
Guaiacyl (G) content[a]	72	157	119	150	291
Syringyl (S) content[a]	450	435	292	374	220
S+G[b]	2,355	2,640	1,750	2,120	1,860
S/G	6.3	2.7	2.4	2.5	0.8
Polysaccharides					
Glucose	54.2	47.4	43.7	47.8	42.8
Xylose	20.1	16.0	17.8	19.6	13.7
Galactose	2.4	1.4	1.4	1.5	1.7
Arabinose	1.2	0.6	0.5	0.8	1.4
Mannose	0.5	2.7	1.9	3.2	5.1

[a] Values are expressed in micromoles of thioacidolysis products recovered per gram of cell wall.
[b] Values are expressed in micromoles of thioacidolysis products recovered per gram of lignin.

centages of lignin and mannose, characteristic of gymnosperms. The recovery of the S and G thioacidolysis degradation products from these lignins varied, which shows that the relative importance of β-aryl ether bonds in hardwood lignins depends on the origin of the sample. The lowest S/G ratio, 0.8, was observed for *L. phillipiana*, together with a high Klason lignin content. In contrast, the highest S/G ratio, 6.3, was observed for *N. dombeyi*, together with a low Klason lignin content. To our knowledge, this is the first report on the chemical composition of these woods.

Elemental analyses of sound wood (Table 2) showed a variety of different concentrations among the different species. Concentrations of K, Ca, Mg, and Na were higher than those of P, Al, Fe, Mn, and Zn.

Chemical characterization of decayed wood. The Klason lignin content of wood of *N. dambeyi* showed an extensive decrease at progressive stages of delignification (Table 3). In the most advanced stage, only 0.6% lignin remained. The yields of the thioacidolysis products, expressed in micromoles per gram of lignin (Table 3, values in parentheses), were not greatly reduced (from 2,354 to 1,830), which reveals that delignification is not very specifically aimed at the lignin β-aryl ether bonds. The thioacidolysis S/G ratio changed drastically from 6.3 (sound wood) to 2.2 (stage 5) (Table 3), which shows that the lignin degraded during the first stages is an S-rich lignin. In contrast, the behavior of *L. phillipiana* during fungal attack was very different. The various stages of nonselective wood decay showed a relatively slight reduction in lignin. The yields of the thioacidolysis degradation products, expressed in micromoles per gram of lignin, were reduced by a factor of greater than 2 (from 1,860 to 739) as the stage of decay became more advanced. This result shows that the β-aryl ether bonds in lignin were preferentially attacked and that the residual lignin in the last stages of decay was therefore richer in carbon-carbon bonds. The thioacidolysis S/G ratio was not substantially changed (it changed from 0.8 to 0.5).

TABLE 2. Comparison of elemental concentrations in sound and decayed wood from southern Chile

Wood sample	Element concn (ppm)								
	P	K	Ca	Mg	Al	Fe	Na	Mn	Zn
Sound wood									
N. dombeyi	22	29	364	306	7	5	82	23	4
E. cordifolia	33	1,046	435	201	5	4	295	13	2
W. trichosperma	40	429	684	288	11	6	274	21	2
D. winterii	142	2,102	294	127	19	15	458	20	4
L. phillipiana	166	3,282	1,785	528	14	32	147	32	6
Decayed wood									
N. dombeyi									
Stage 1	124	459	882	188	6	11	119	15	3
Stage 2	37	280	1,513	378	6	16	197	22	7
Stage 3	16	72	2,459	490	24	14	51	107	11
Stage 4	28	101	3,352	640	16	4	58	51	12
Stage 5	12	129	4,888	895	24	4	132	40	16
L. phillipiana									
Stage 1	6	486	2,146	130	9	7	144	13	2
Stage 2	13	2,712	3,953	413	8	2	442	16	3
Stage 3	61	2,144	9,291	881	10	2	355	84	24
Stage 4	28	3,026	5,731	917	18	3	366	78	18
Stage 5	166	787	9,835	1,340	14	15	350	98	18
E. cordifolia									
Sample 1	90	1,093	2,777	2,690	25	0	371	515	43
Sample 2	14	112	793	91	2	3	79	24	3

Water-soluble substances increased during the decay of *N. dombeyi* and *L. phillipiana* (Table 4), but no differences were found in ethanol-toluene extraction (data not shown). The individual wood sugar data, representing cellulose and hemicellulose content, showed an increase in the glucose content in *N. dombeyi*. As decay progressed, the resulting wood was made up of 88.9 to 92.9% cellulose in stages 4 and 5. The cellulose in the very late stages of decay had

TABLE 3. Lignin content and lignin monomer composition of wood decayed in the field by *Ganoderma* sp.

Wood and decay stage[a]	Klason lignin (% [dry wt])	Amt of thioacidolysis monomers recovered from lignin (μmol/g [dry wt])[b]:			S/G ratio
		G	S	S+G	
L. phillipiana[c]					
Sound	30.7	290	220	510 (1,860)	0.8
Stage 1	31.8	297	151	448 (1,463)	0.5
Stage 2	31.4	294	180	474 (1,642)	0.6
Stage 3	27.2	232	129	361 (1,335)	0.6
Stage 4	25.8	146	64	210 (812)	0.4
Stage 5	22.5	109	57	166 (739)	0.5
N. dombeyi[d]					
Sound	21.7	72	450	522 (2,354)	6.3
Stage 1	20.2	64	355	419 (2,171)	5.6
Stage 2	15.3	57	288	345 (2,316)	5.0
Stage 3	13.4	50	224	274 (2,162)	4.5
Stage 4	3.3	19	47	66 (1,987)	2.5
Stage 5	0.6	3	7	10 (1,830)	2.2

[a] All decay stages reported were found within a single trunk of the corresponding tree species. Stage 1, incipient decay; stage 5, advanced decay.
[b] G, Guaiacyl; S, syringl. Values in parentheses are expressed in micromoles per gram of lignin.
[c] Nonselective delignification.
[d] Selective delignification.

TABLE 4. Water-soluble substances and structural polysaccharides of wood decayed in the field by *Ganoderma* sp.

Wood and decay stage[a]	Water content (%)	Water-soluble substances (%)	Glucose (%)	Xylose (%)	Mannose (%)	DP
L. phillipiana[b]						
Stage 1	ND[c]	2.6	43.0	14.2	4.1	1,070
Stage 2	30.2	7.5	42.1	13.6	3.1	1,030
Stage 3	45.4	13.2	48.4	13.9	2.2	1,000
Stage 4	45.1	17.7	43.2	12.2	2.1	780
Stage 5	50.4	29.3	47.2	10.5	5.1	600
N. dombeyi[d]						
Stage 1	55.1	5.7	53.7	19.6	0	1,150
Stage 2	54.2	7.7	63.9	17.8	0	1,040
Stage 3	63.9	7.8	67.2	17.2	0	1,030
Stage 4	73.2	14.7	88.9	9.0	0.2	450
Stage 5	74.9	12.1	92.9	8.5	0.2	400

[a] All stages of decay reported were found within a single trunk of the corresponding tree species. Stage 1, incipient decay; stage 5, advanced decay.
[b] Nonselective delignification.
[c] ND, Not determined.
[d] Selective delignification.

apparently been altered, since an appreciable decrease in the degree of polymerization (DP) was observed (Table 4). Cellulose depolymerization was most severe after stage 3. The percent xylose decreased from 19.6 in stage 1 to 8.5 in stage 5. In general, the glucose, xylose, and mannose contents remained relatively unchanged in the samples of *L. phillipiana* representing different stages of decay. However, the DP of cellulose was reduced, with the greatest changes observed in the most advanced stages of decay (Table 4).

Holocelluloses prepared from selective and nonselective decayed woods contained similar and even less carboxylic residues than the respective sound woods did. The higher carboxyl content of holocellulose of sound woods, as compared with that of cotton cellulose, is probably related to the presence of uronic acids in the hemicelluloses of these woods. Furthermore, the delignification process does not have any influence on the carboxyl content of the delignified samples (Table 5).

Analysis of other collected samples of decayed wood showed variable amounts of lignin and wood sugars (Table 6). Samples with a combination of selective and nonselective lignin degradation had a lignin content of 6 to 13%, and the glucose content was 67 to 84%. Another sample of delignified wood contained 0.9% lignin and 97.6% glucose. However, the cellulose in this sample was highly depolymerized (cellulose DP = 195). Samples of gelatinlike substances from the most advanced stages of delignification had 4 to 9% lignin, 54 to 80% glucose, and 1 to 5% xylose (Table 5).

TABLE 5. Carboxyl content of holocellulose of wood decayed in the field by *Ganoderma* sp.

Delignified sample and decay stage	Carboxyl content (meq/100 g)
N. dombeyi	
Sound	24.1 (1.1%)
Stage 5	15.5 (0.7%)
L. phillipiana	
Sound	52.1 (2.3%)
Stage 5	51.2 (2.2%)
Oxycellulose[a]	228.0 (10.3%)
Cotton[a]	0.3 (0.01%)

[a] Standards.

Elemental analyses of decayed wood showed substantial increases in the Ca, Mg, Mn, and Zn content. In sample 1 of *E. cordifolia*, there was a 25-fold increase in Mn content over that in sound wood (Table 2).

Physical and chemical properties of pulp from palo podrido. The unbleached pulp obtained after refining stage 4 and 5 *N. dombeyi* had a high brightness, indicating that only a mild bleaching would be necessary to reach brightness values similar to those found for the bleached control pulp (Table 7). As expected from the DP determinations (Table 4), the viscosity of the biopulp was very low (Table 7). The fiber length of the control sample was approximately twice that of pulp made from decayed wood. The decayed wood was easy to refine, as shown by the small number of revolutions necessary to obtain handsheets. Freeness values were higher for the decayed wood than for the control. Tensile strength, burst index, and tear index were low when compared with handsheets made from the control pulp (Table 8).

Morphological aspects of wood decay. Advanced stages of palo blanco showed extensively delignified fibers and vessels (Fig. 1A). No middle lamella was present within the cell walls, and cells detached easily from one another. Secondary wall layers were still intact in the cells that remained (Fig. 1B). In decayed wood of *L. phillipiana*, cells had a nonselective type of degradation. All parts of the cell wall were removed, and eroded cell wall layers were evident (Fig. 1C).

Ultrastructural changes that occurred in the cell walls of delignified *N. dombeyi* wood are presented in Fig. 2B to D. Cell wall layers of sound wood show the distribution of lignin (electron-dense areas) throughout the fiber and vessel walls (Fig. 2A). Stages 1 and 2 typically had parts of the secondary wall delignified (Fig. 2B). Lignin was removed from the lumen toward the middle lamella. In stage 3, secondary walls of most cells were delignified. The middle lamella between cells and cell corner regions were also removed (Fig. 2C). In

TABLE 6. Lignin and wood sugar analyses of field-collected wood with advanced stages of decay

Sample	Decay type	% Lignin		% Wood sugars		
		Klason	Acid soluble	Glucose	Xylose	Mannose
1	Delignified	6.4	2.9	83.9	5.3	0.7
2	Delignified	0.9	1.1	97.6	1.0	0
3	Delignified and simultaneous	12.9	3.5	67.7	12.6	4.3
4	Delignified and simultaneous	10.3	3.1	73.0	1.4	1.5
5	Gelatin	9.6	5.0	54.7	4.7	1.8
6	Gelatin	5.4	2.0	79.7	2.8	5.1
7	Gelatin	4.4	3.0	79.6	1.2	1.4

TABLE 7. Chemical properties of an unbleached pulp from palo blanco

Wood sample	% Cellulose	% Hemicellulose	% Lignin	Brightness (%)	Viscosity (cP)	% of specks >0.2 mm	Fiber length (mm)
Control[a]	50.8	22.9	22.2	89.7	106.0	ND[b]	0.82
Palo blanco	92	8.8	0.9	77.5	11.0	22.5	0.42

[a] The control was bleached pulp from a mixture of Chilean native woods (see Materials and Methods).
[b] ND, Not determined.

TABLE 8. Physical properties of 60-g/m^2 handsheets of an unbleached pulp from palo blanco

Wood sample	No. of beating revolutions	Freeness (SR)	Specific gravity (g/cm^3)	Tensile index (Nm/g)	Burst index (kPa · mq^2/g)	Tear index (nM · m^2/g)
Control[a]	—	20	—	31.2	1.6	6.3
	—	32	—	56.9	3.6	8.9
	—	41	—	7.6	4.8	8.8
	—	53	—	83.3	5.9	8.5
Palo blanco	0	28	0.62	48.6	1.6	2.5
	200	39	0.67	51.3	2.0	2.7
	500	53	0.72	56.2	2.3	2.4

[a] The control was bleached pulp from a mixture of Chilean native woods (see Materials and Methods.)

some cells, delignification was apparent in the secondary wall, but the middle lamella had not been degraded. Decayed wood from stages 4 and 5 had cells that appeared completely delignified, and all parts of the middle lamella were removed (Fig. 2D). The S_1 and S_2 layers were intact and were not eroded. In some areas a few cell corners were found between the delignified cells. Although the secondary walls were not eroded, some separation within the secondary wall was apparent (Fig. 2D).

Additional samples of delignified wood from the field displayed masses of black deposits (sample 1 of *E. cordifolia*). X-ray microanalyses showed that these deposits had high concentrations of Mn. Leucoberbelin blue applied directly to the surface of the decayed wood reacted with the black deposits and produced a greenish-blue coloration. Other samples from advanced stages of decay showed delignified cells that were in various stages of degradation (Fig. 3A). The S_1 and S_2 layers of the secondary walls were expanded and separated. Separations also were evident along the microfibrils within the secondary wall (Fig. 3A). Stages in the degradation of the cellulose within delignified wood were also found. Secondary wall layers appeared to become extremely disorganized (Fig. 3B). In some areas microfibrils were visible, but others assumed an amorphous appearance. Macroscopically, areas of delignified wood had changed into a gelatinous substance. Gelatinous samples 5 to 7 (Table 6) did not have cell wall layers that were discernible. The degraded cellulose-rich secondary walls apparently combined, forming a unique morphological matrix (Fig. 3C and D). The lumina of many cells and degraded secondary walls also appear to coalesce, forming the characteristics of the gelatinous matrix (Fig. 3C and D).

The decay pattern observed for the *L. phillipiana* wood (stages 1 to 5) was a nonselective type of degradation (Fig. 4A to D). Cell walls of wood from stages 1 and 2 were eroded in localized areas, causing a typical simultaneous decay of the cells (Fig. 4A). In more advanced stages (stages 3 and 4), the cell walls were eroded severely. A thinning of the cell walls also was evident, and degradation around the entire circumference of the cell occurred (Fig. 4B and C). Degradation had apparently progressed from the lumen to the middle lamella. In stage 5 decayed wood, extensive degradation was apparent (Fig. 4D). Remnants of cell walls and portions of middle lamellae remained. Masses of hyphae were also present within the degraded cells. The cell corner regions appeared to be more resistant to attack.

DISCUSSION

Palo podrido and palo blanco are two terms used to describe white-rotted wood in forests of southern Chile. Wood that is pure white and represents a high degree of delignification is often referred to as palo blanco, whereas other stages of decay and forms of white rot may be called palo podrido. Previous investigators have shown that a wide range of different types of decayed wood may be considered to be palo podrido (21, 41). Nonselective white rot degradation and combinations of selective and nonselective decay also occur in wood found in this region. The presence of white-pocket rot in standing trees suggests that the *Gano-*

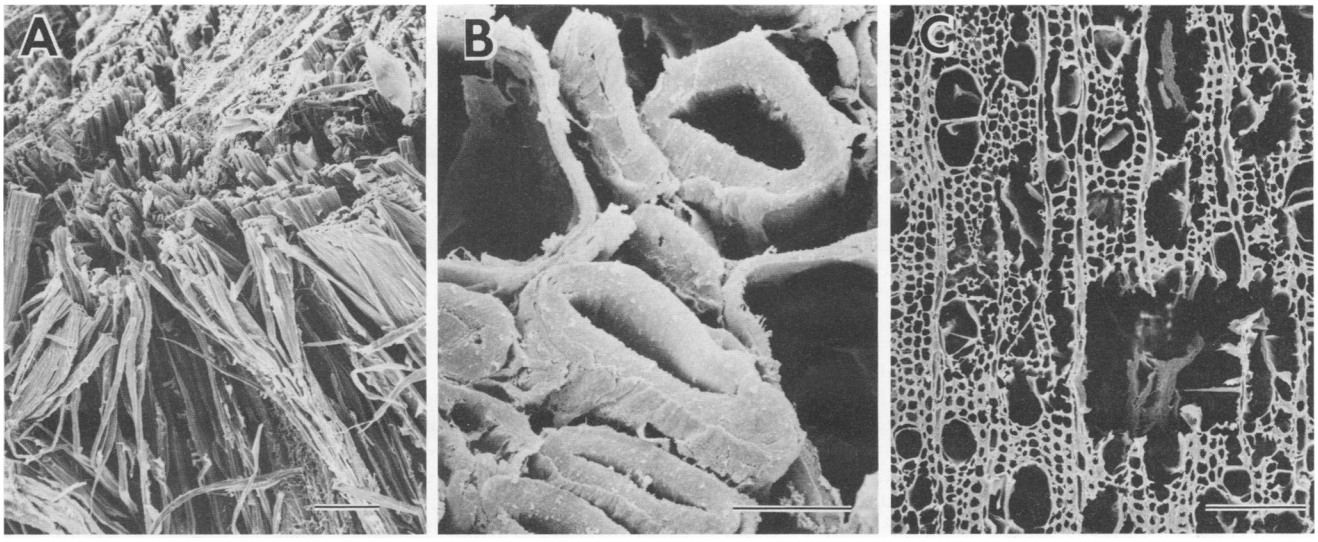

FIG. 1. Scanning electron micrographs of delignified wood from palo blanco stage 5 (A and B) and an intermediate stage of nonselective degradation (C). (A) Delignified wood showing cells that are detached from each other. Individual fibers and vessel elements can be seen. Bar, 100 μm. (B) Cells from an area of decay as shown in Fig. 2A, with only secondary wall layers evident. The middle lamella between cells has been completely removed. Bar, 10 μm. (C) Nonselective white rot causing an attack of all cell wall layers. Cells are eroded, and many voids can be seen in the wood where cells have been removed. Bar, 100 μm.

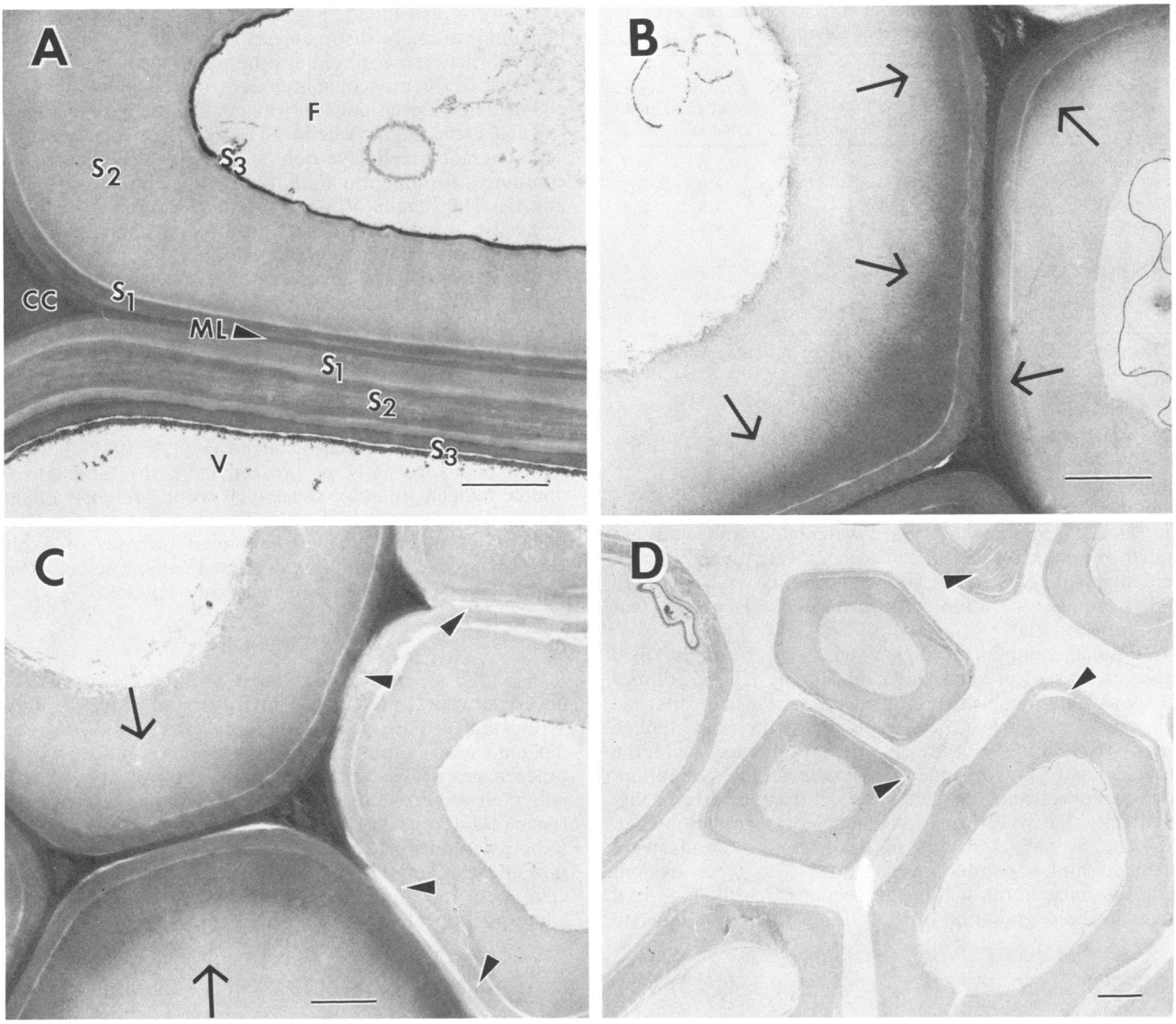

FIG. 2. Transmission electron micrographs of transverse sections from *N. dombeyi* with various stages of delignification caused by *Ganoderma* spp. Wood was fixed with $KMnO_4$ to stain lignin within cell walls. (A) Sound wood in areas adjacent to incipient decay showing the electron-dense cell walls of a fiber (F) and vessel (V). The secondary wall layers (S_1, S_2, and S_3) and middle lamella (ML) are evident. The cell corner region of the middle lamella (CC) was most electron dense. (B) Incipient stage of decay (stage 1 from Table 2), showing a progressive loss of electron density from the lumen toward the middle lamella (arrows). The area where no stain is apparent represents lignin loss. (C) More advanced stages of decay (typical of stage 2 or 3 from Table 2) show some cells with delignified secondary walls and degraded middle lamella (arrowheads). Other cells show loss of lignin from the secondary wall (arrows), but delignification had not reached the middle lamella. (D) Cells from stage 4 (Table 2) with delignified secondary walls and no middle lamella. At this stage of decay, even the cell corner regions of the middle lamella have been removed. Some degradation of the remaining secondary wall is apparent. Separations are evident at the S_1 and S_2 boundaries, and separations within the S_2 layer are also beginning (arrowheads). Bar, 2 μm.

derma species responsible for the formation of palo podrido and palo blanco may have originated in the heartwood of the living tree. As selective delignification extends within the wood, this could result in the formation of large selectively delignified areas, probably arising from the pockets of delignification already present, either in the standing tree or after the corresponding infected tree has fallen.

The unique environmental factors present in southern Chile apparently are ideal for fungal growth and rapid decay. Dill and Kraepelin (16) have suggested that low temperatures, high humidity, and microaerobic conditions in decaying trunks may favor delignification. In addition, favorable conditions for decay are maintained in the substrates by the formation of pseudosclerotial plates. These zones, formed by the fungus around the perimeter of the wood, protect the substrate from invasion by other microorganisms and are apparently responsible for preserving high concentrations of cellulose within the interior of the decayed logs for long periods. The ability of other *Ganoderma* species to produce pseudosclerotial plates or zone lines to maintain a proper environment and to protect selectively delignified wood from cellulolytic microorganisms has been previously demonstrated (7). In other regions the pseudosclerotia may be acting to reduce desiccation (33), whereas in the evergreen

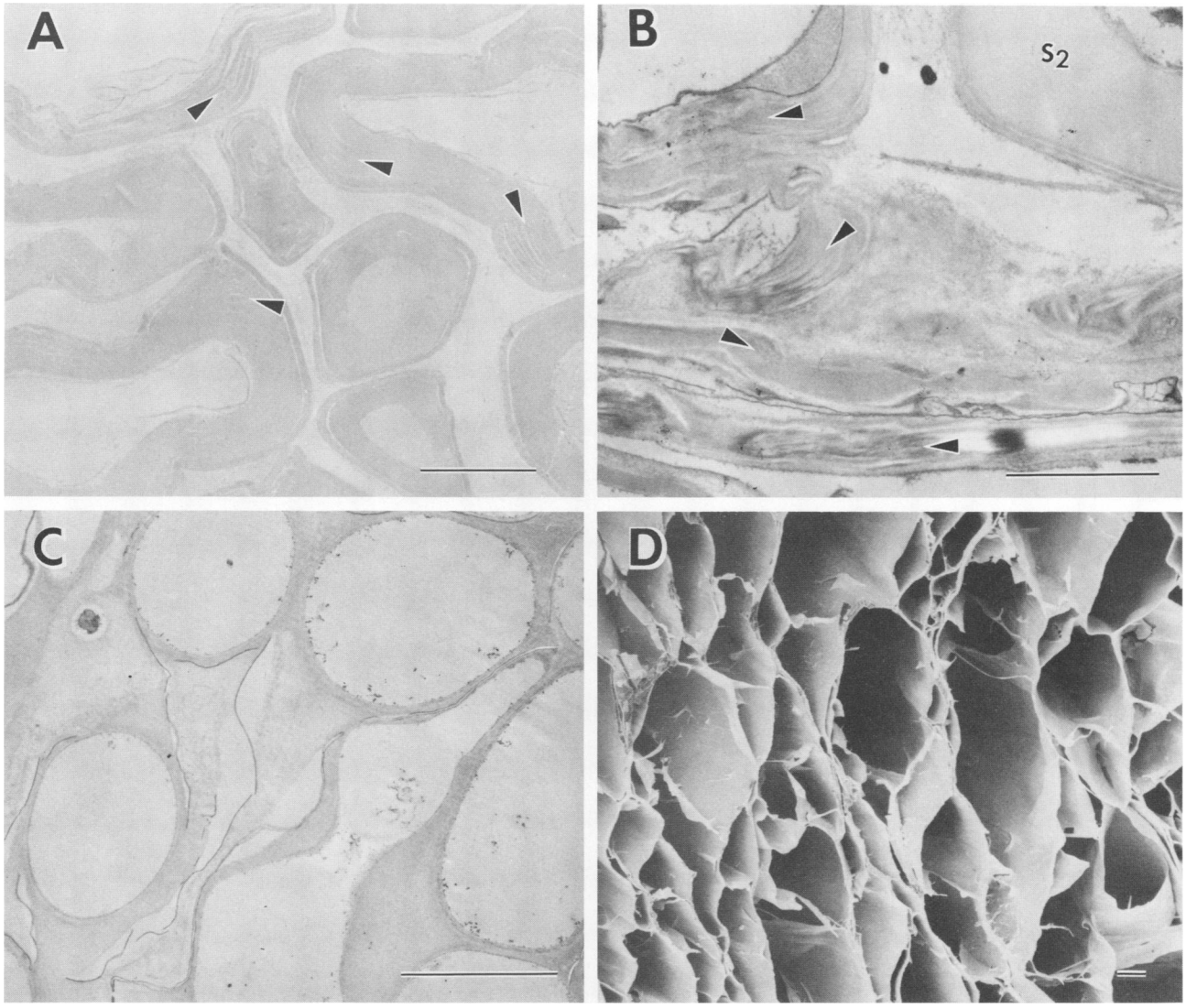

FIG. 3. Transmission electron micrographs (A to C) and scanning electron micrograph (D) of advanced stages of palo blanco (A) and gelatinous decayed wood representing the degradation process of delignified cells (B to D). Samples were fixed with $KMnO_4$. (A) Delignified cells with secondary wall layers that are separating along microfibrils (arrowheads). Separations are also seen at the S_1 and S_2 boundaries. (B) A stage of delignified cell wall decomposition showing a relatively unaltered delignified secondary wall (S_2) and transformation to an amorphous matrix. Within the matrix, a residual microfibrillar structure can be observed in various stages of degradation (arrowheads). (C) Gelatinous decayed wood shows no organized cell wall structure. The degraded secondary cell wall substances coalesce to form the amorphous material. (D) The gelatinlike substance has an unorganized structure with no cell wall layers that are characteristic of wood. Bar, 5 μm.

rain forests of southern Chile the pseudosclerotia are most probably preventing unusually high levels of moisture from accumulating in the wood. When there is a break in the pseudosclerotia from weathering or other factors, the external microflora may enter and degrade the cellulose. These areas are the common locations for the gelatinlike material. Samples of stage 5 *N. dombeyi*, left in the field after the pseudosclerotia and delignified wood had been cut with a saw, showed extensive degradation of the cellulose when the site was revisited several months later. However, a new zone line had already formed at some distance from the cutting edge, which would presumably protect the delignified area from external microbial attack.

The lignin content, and particularly lignin monomer composition, appears to be a major factor in influencing selective delignification. Wood from *N. dombeyi* and *E. cordipholia* have the lowest lignin content, highest S/G ratio, and largest amount of β-aryl ether linkages in lignin (Table 1). In contrast, *L. phillipiana* has a high lignin content, low S/G ratio, and smaller quantity of β-O-4 ether linkages. The β-O-4 ether linkages of lignin were preferentially degraded only in the nonselective type of white rot found in *L. phillipiana*. The type of lignin within cells of deciduous woods has been found to affect wood decomposition (11, 22). In the results presented in our study, it is evident that the high syringyl content could influence not only the rate of degradation but also the type of white rot attack. The most extensively delignified wood has commonly been found in *N. dombeyi* and *E. cordifolia*, but not in *L. phillipiana* (16, 21, 41).

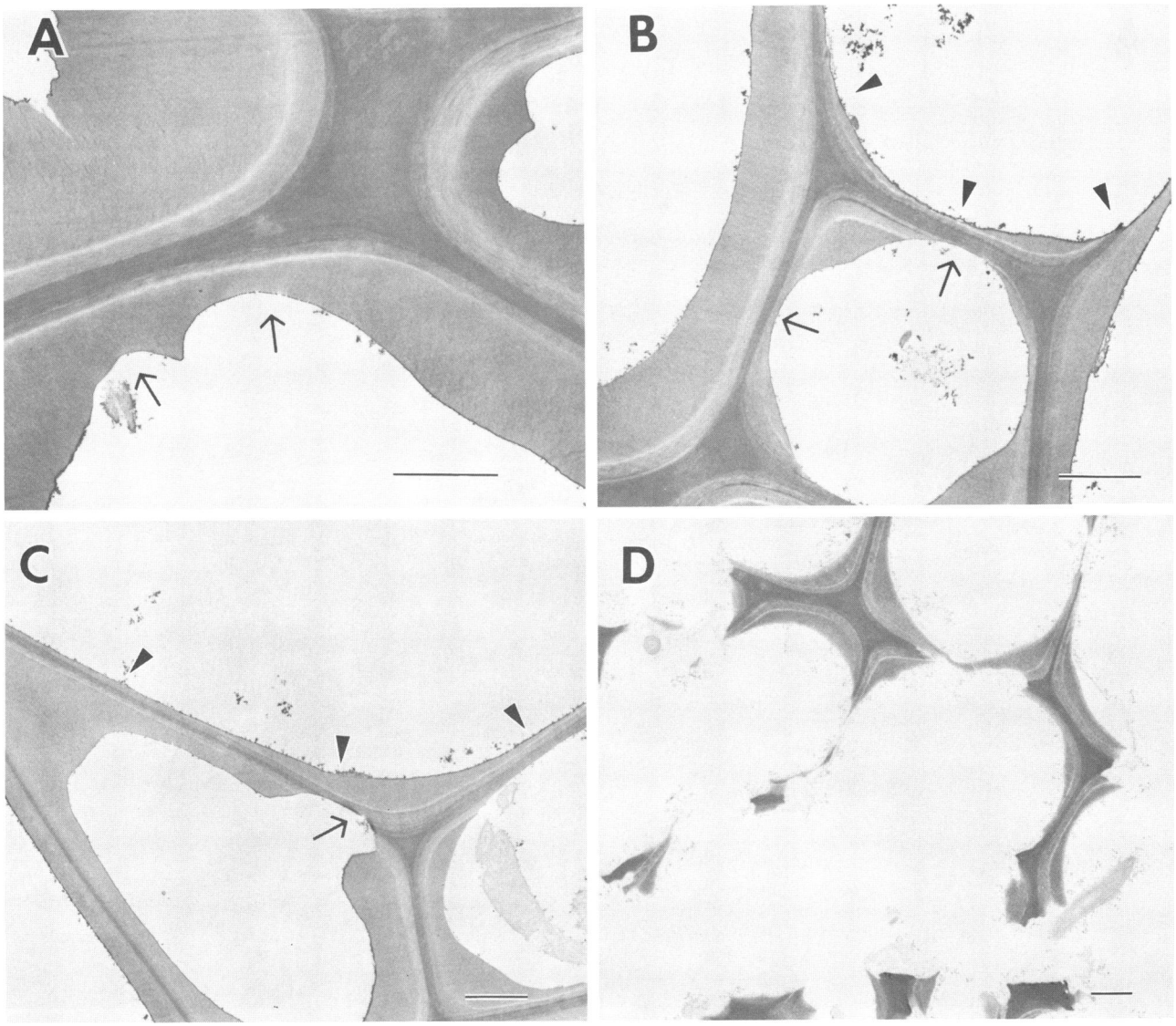

FIG. 4. Transmission electron micrographs of transverse sections of *L. phillipiana* wood with nonselective white rot. (A) Incipient stage of decay, stage 1 (Table 3), showing eroded cell walls (arrows). (B and C) Nonselective decay is apparent in wood from stage 2 (Table 3), showing erosion troughs (arrows) and cell wall thinning throughout the cell surface (arrowheads). All cell wall layers are degraded from the lumen toward the middle lamella. (D) Advanced stages of decay typical of wood from stages 4 and 5 (Table 3), showing remnants of cells that remain. In some cells the entire cell wall has been completely degraded, whereas in other cells parts of the middle lamella and secondary walls remain. Bar, 3 μm.

The process of delignification (Fig. 2A to D) demonstrates the removal of lignin from the secondary walls during early stages of decay. The stained areas in sections fixed with $KMnO_4$ (15) represent the areas where lignin is located in the cell walls, and X-ray microanalysis of brominated wood has confirmed these results for other samples (12). In Fig. 2A to D presented here, the loss of lignin is evident by the lack of stain. Lignin is first removed from the secondary wall near the lumen and progresses inward. Although only one or two fungal hyphae are present within each cell, the delignification process is diffuse throughout the cell wall. In more advanced stages, lignin is removed from the middle lamella between cells and finally from cell corner regions. One remarkable aspect is the extensive removal of lignin from the cells without extensive destruction of the cellulose (Fig. 2B and C). Only after almost complete lignin removal is some alteration of cellulose observed in terms of reduced DP (Table 4). Cellulases and not oxidoreductases seem to be responsible for the decrease in DP, since the carboxyl contents of sound and decayed wood samples are very similar. This suggests that the highly oxidative compounds, such as Mn(III) and activated radical species produced during lignin biodegradation (25, 26), do not react with cellulose.

Manganese accumulates in some woods with selective delignification (8). The fungi apparently require manganese for ligninolytic systems that involve Mn-dependent peroxidases and the generation of different oxidative states of manganese. Some samples of delignified wood from southern Chile had black manganese deposits (Table 6) within the wood, whereas others did not. The presence of manganese in decayed wood is detectable visually only after large quanti-

ties have precipitated, forming MnO_2. Elevated levels of manganese also are present in other samples (Table 2). However, environmental conditions were apparently not always suitable for manganese precipitation, and large concentrations did not accumulate very often. An interesting aspect of the volcanic soils of southern Chile is their low manganese content (42). Even with small amounts of manganese present in the soil and low manganese concentrations in sound wood (Table 2), the white rot fungi are able to obtain the amounts necessary for delignification. Indeed, in some samples, exceedingly large amounts are found as black manganese deposits.

The decrease in cellulose DP that occurred in advanced stages of delignification is the main factor responsible for the poor strength properties observed in the paper sheets. The drop in DP appears to be associated with extremely high levels of lignin loss (Table 4) and with separations that form in the delignified cell walls (Fig. 2D and 3A). Although these changes in cellulose would not affect digestibility and previous study has indicated that palo podrido may be as much as 77% digestible (41), cellulose of this type would not be suitable for paper production. Moderate stages of delignification in which the DP has not been drastically reduced should provide good paper quality. Therefore, fungi from southern Chile, such as *Ganoderma* spp., should be of great value in investigating the rapid degradation of secondary wall lignin early in the decay process. Recently, pretreatment of wood chips with fungi that removed only small amounts of lignin have produced refiner mechanical pulps with increased strength properties and reduced energy requirements (35). Fungi from southern Chile should also be important in ascertaining the mechanisms of ligninolytic activity.

The gelatinlike material that occurs in delignified wood represents a stage of cellulose degradation. The micrographs in Fig. 3A to D show the changes from cellulose-rich secondary walls with no apparent erosion to (i) separations occurring between the S_1 and S_2, (ii) separations within the S_2 layer along cellulose microfibrils, and (iii) gradation from an organized structure (S_2 layer) to an amorphous state. The remaining matrix (with low electron density) apparently absorbs water, readily imparting a gelatinous appearance. Little leaching of water-soluble sugars occurs, however, since the wood sugar analyses, after acid hydrolysis, showed a high glucose concentration. In addition to cellulases that are produced by *Ganoderma* spp., other microorganisms that have breached the pseudosclerotial barriers also undoubtedly contribute to the formation of the gelatinous decayed wood.

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