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Palo Podrido: Model for Extensive Delignification of Wood by *Ganoderma applanatum*

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Chemical and micromorphological analysis revealed that South Chilean "palo podrido" results from a white-rot fungus that causes highly selective and extensive delignification. Palo podrido samples from 10 different hardwood trunks (*Eucryphia cordifolia*, *Drimys winteri*, and *Nothofagus dombeyi*) decayed by *Ganoderma applanatum* were analyzed. Of 14 samples, 11 had extremely low Klason lignin values, ranging from 6.1 to 0.4% (dry weight). The most remarkable and unusual feature was that delignification and defibration were not restricted to small pockets but extended throughout large areas in the interior of trunks subjected to undisturbed rotting over long periods of time. Comparative analysis of water content, swelling capacity, and lignin content led to the conclusion that besides lignin degradation, suppression of the cellulolytic activity of the rotting organisms plays a decisive role. Among various nutrients added to a palo podrido sample (3% residual Klason lignin), the nitrogen source was the only one leading to almost complete cellulose degradation. We suggest that the extremely low nitrogen content (0.037 to 0.073% [dry weight]) of the investigated wood species was the primary cause for the extensive delignification as well as the concomitant suppression of cellulose breakdown. The low temperatures, high humidity, and microaerobic conditions maintained within the decaying trunks are discussed as additional ecological factors favoring delignification in South Chilean rain forests.

White-rot fungi have developed two distinct ways to attack wood: (i) the simultaneous degradation of lignin, hemicelluloses, and cellulose and (ii) the selective degradation of lignin and hemicelluloses (2, 4, 10). The extensive investigations of Blanchette (2, 3) and Otjen and Blanchette (15) clearly demonstrated that both types of decay can occur side by side in the same substrate.

With a few exceptions, selective lignin degradation could not be obtained under laboratory conditions. Henningsson et al. (8) observed delignification and defibration of asparagine-impregnated birch wood infected with *Phanerochaete chrysosporium*, but at a weight loss of 15% (dry weight) the decay changed towards the simultaneous type. Recently, Otjen and Blanchette (16) succeeded in selectively delignifying wood blocks (aspen wood) with *Ischnoderma resinosum*, *Poria medulla-panis*, and *Xylobolus frustulatus*. However, the authors observed either that only the outer surfaces of the wood blocks were selectively delignified or that the selective type of decay was restricted to small pockets.

In contrast to these experiences, the investigations reported here clearly show that selective delignification can occur in large areas of wood under the specific field conditions prevailing within the evergreen rain forests of South Chile. Philippi in 1893 (17) described a white spongy material found in decayed trunks in the forests of Valdivia (South Chile). This decayed wood, later shown to have a high in vitro rumen digestibility (23), was eaten by the Indians and used as forage for cattle. Knoche et al. (12) described some characteristics as well as the occurrence of this material, called "palo podrido" (meaning rotten wood). González (A. E. González, M.S. thesis, University of Valdivia, Valdivia, Chile, 1980) and González et al. (7) demonstrated that typical palo podrido originates from a white-rot decay with selective degradation of lignin produced by *Ganoderma applanatum*. *G. applanatum* is known to cause either a simultaneous or a selective type of white-rot decay (2), but the mechanisms regulating the type of decay are still poorly understood.

There is no doubt that a better understanding of the ecological principles of wood biodegradation is of fundamental interest. Moreover, exploitation of fungal activities for biotechnological purposes, e.g., for biological pulping processes, livestock feed, or specific wastewater treatments, substantially depends on this basic knowledge.

Despite the remarkable advances made in the field of lignin degradation during the last few years, e.g., the discovery of the important role of nitrogen concentration for lignin degradation (9, 11, 19) and the isolation of ligninolytic enzymes (21), there still are many gaps in our knowledge concerning the background of selective delignification. Therefore, a detailed study of palo podrido as an ecologically well-established system causing extensive delignification may be of particular value.

The purpose of our investigation was to study the chemical composition of palo podrido as well as the microorganisms and environmental factors contributing to its formation (I. Dill, Ph.D. thesis, Technical University of Berlin, Berlin, Federal Republic of Germany, 1985). Some of the results obtained are presented here.

MATERIALS AND METHODS

Sampling. Palo podrido samples were collected in the rain forests of the Isle of Chiloé from the following locations: Pauldeo, Coipomó, Puntra, and Gamboa. The samples were withdrawn from the interior of decaying hardwood trunks of *Eucryphia cordifolia*, *Drimys winteri*, and *Nothofagus dombeyi*. All trunks bore fruiting bodies which were identified as *G. applanatum* by macromorphological characteristics.

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Chemical analysis. Decayed wood was dried at 105°C, pretreated in a Waring blender, and then ground to pass through a 60-mesh screen. For all gravimetric analyses, the samples were weighed to the nearest 0.01 mg.

Water content. Decayed wood (3 to 12 g) was dried at 105°C for about 24 to 48 h, cooled in a desiccator, and weighed.

Swelling capacity. Dry wood meal (0.5 g) was quantitatively transferred into a graduated cylinder (10 ml). After the addition of 7 ml of distilled water, the sample was stirred until no more visible air bubbles appeared. With another 3 ml of water, attached wood meal was rinsed from the spatula and the glass wall into the cylinder. After 24 h, air bubbles were removed again by stirring. Changes in the height of the "wood column" as a measure of swelling capacity were read daily until constant values were obtained. The final results were expressed as the deviation in percent from the values for the corresponding sound wood.

pH of samples. The pH was determined potentiometrically after suspension of the samples in distilled water for about 30 to 45 min. Analysis of the decayed wood was made with a few grams of fresh material, and that of the corresponding sound wood was made with 1 g of dry wood meal.

Ash content. About 2 g of dry wood meal was placed in a porcelain crucible and ashed at 600°C for 4 h. After being cooled in a desiccator, the crucible was weighed.

Solubility in ethanol-benzene. About 1.5 g of dry wood meal was extracted with ethanol-benzene (1:2 [vol/vol]) for 4 h in a Soxhlet extractor, keeping the liquid boiling briskly. Each extracted sample was washed with 50 to 100 ml of ethanol and dried at 105°C. After evaporation of the solvent, each extract was dried at 105°C for 24 h, cooled in a desiccator, and weighed.

Solubility in hot water. About 1 g of dry wood meal was placed in a 200-ml Erlenmeyer flask. After the addition of 100 ml of distilled water, the mixture was slowly stirred at 80°C for 3 h. The sample was then transferred to a tared fritted-glass crucible, washed with hot water, dried at 105°C for about 24 h, cooled in a desiccator, and weighed.

Nitrogen content. N was determined by the Kjeldahl method. About 2 g of dry wood meal was placed in a 250-ml Kjeldahl flask. The common weighing boats were not used because they contain traces of nitrogen. Digestion was done with 7.5 g of a selenium reaction mixture (E. Merck AG, Darmstadt Federal Republic of Germany) and 20 ml of concentrated H_2SO_4. The ammonia was distilled into a boric acid solution (2%). Titration was performed with 0.01 N H_2SO_4, and the pH was additionally controlled potentiometrically.

Acid-insoluble lignin. Acid-insoluble (Klason) lignin was determined by a modification of TAPPI (Technical Association of the Pulp and Paper Industry, Atlanta, Ga.) standard method T 222 os-74. Flasks containing 1 g of ethanol-benzene-extracted wood meal and 20 ml of H_2SO_4 (72%) were gently shaken in a water bath at 30°C for 1 h. The acid was then diluted with H_2O to 4% (wt/vol), and the samples were autoclaved at 121°C for 30 min. The lignin that settled overnight was quantitatively collected by filtration through a crucible, washed free of acid with hot water, and dried. The lignin content was calculated as a percentage of oven-dried, nonextracted wood meal.

Acid-soluble lignin. Acid-soluble lignin was determined by a modification of TAPPI method UM 250. The first 50-ml supernatant solution obtained after settling of the acid-insoluble (Klason) lignin was used for the spectrophotometric determination of acid-soluble lignin. The A_{205} of the solution was measured in a Beckman spectrophotometer (model 35) in a cuvette with a 10-mm light path. H_2SO_4 (4%) was used as a reference solution, and at A_{205}s higher than 0.5 the samples were diluted with 4% H_2SO_4. In accordance with TAPPI, the lignin content was calculated as follows: percent lignin = $[(A_{205} \times D)/110] \times$ [total volume × 110]/[oven-dried weight (grams) × 1,000], where E is the extinction coefficient and D is the dilution factor.

Degradation of palo podrido. Undried decayed wood (1.0 to 1.2 g) was quantitatively transferred into 100-ml Erlenmeyer flasks under sterile conditions and supplemented with 20 ml of various nutrient solutions. The complete nutrient solution contained, per liter of distilled water, the following: $(NH_4)_2SO_4$, 0.5 g; L-asparagine, 0.5 g; KH_2PO_4, 1.0 g; KCl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; yeast extract, 0.2 g; and trace element solution, 10 ml. The pH of the medium was adjusted to 5.0 before autoclaving. The trace element solution contained per liter of distilled water, the following: EDTA, 5.2 g; $FeCl_2$, 1.5 g; $ZnCl_2$, 70 mg; $MnCl_2 \cdot 4H_2O$, 100 mg; H_3BO_3, 62 mg; $CoCl_2 \cdot 6H_2O$, 190 mg; $CuCl_2 \cdot 2H_2O$, 17 mg; $NiCl_2 \cdot 6H_2O$, 24 mg; and $Na_2MoO_4 \cdot 2H_2O$, 36 mg. The pH was adjusted to 6.5. The added nutrient solution was varied as follows: (i) pH 5.0 or pH 7.2 and (ii) omission of nitrogen, phosphate, nitrogen and phosphate, trace elements, or yeast extract. After 10 months of incubation at 20°C in the dark, the contents of the flasks were collected on filter membranes by vacuum filtration, dried at 105°C, cooled in a desiccator, and weighed. The dry weight was calculated as a percentage of the dry weight of the original sample used.

RESULTS

Chemical analysis and macromorphological characteristics of palo podrido. A total of 28 samples of decayed wood collected from 10 different trunks were analyzed. The following results are for 14 samples from these trunks chosen as characteristic examples.

The decaying trunks were identified as *E. cordifolia*, *D. winteri*, and *N. dombeyi*. Table 1 shows the results of the chemical analysis of the sound wood. Two independent specimens of each wood species were analyzed. The results for 14 samples of decayed wood are summarized in Table 2 (individual trunks are identified by capital letters). Except for the water content, all values were expressed on the basis of dry weight.

The content of H_2SO_4-insoluble lignin (Klason lignin) in all samples of decayed wood was below that in the corresponding samples of sound wood. Most of the samples had an extremely low lignin content, and some were almost completely delignified. For example, samples R, Q, K, Xa, Xb, Sc, Va, Vb, and C merely contained 0.4 to 4.4% Klason lignin. The content of H_2SO_4-soluble lignin appeared to decrease with decreasing Klason lignin, but there was no clear-cut correlation.

Figure 1 shows the macromorphology of wood from a degraded trunk of *E. cordifolia* (sample Q). With a Klason lignin content of 3.6% (Table 2), this sample showed a high degree of delignification. Between white delignified areas there were light-brown patches showing simultaneous white rot. It was apparent that the decay of polysaccharides must have been low because the decayed wood still retained some stability and fibrous structure. An even more extreme sample, with a Klason lignin content of 0.4% (Table 2), from a degraded trunk of *D. winteri* (sample Xb) is shown in Fig. 2. The interior of this large trunk had been transformed into

TABLE 1. Analysis of sound wood from *E. cordifolia*, *D. winteri*, and *N. dombeyi*[a]

Wood species	pH	Chemical composition (% [dry wt])						
		Total lignin	Acid-insoluble (Klason) lignin	Acid-soluble lignin	Ethanol-benzene-soluble substances	Hot-water-soluble substances	N	Ash
E. cordifolia	5.7	32.3	27.9	4.4	1.40	1.9	0.058	0.42
	4.8	23.7	20.3	3.4	4.70	ND[b]	0.037	0.46
D. winteri	5.3	30.0	26.8	3.2	0.81	2.0	0.073	0.33
	5.3	29.4	25.5	3.9	1.06	ND	0.062	0.40
N. dombeyi	4.3	23.8	19.7	4.1	3.61	8.1	0.051	0.17
	4.5	20.4	16.4	4.0	2.29	6.2	0.042	0.18

[a] All data except pHs are the averages of duplicate determinations.
[b] ND, Not determined.

white fibrous material. In contrast, the decayed wood from another *D. winteri* trunk (sample Sc) (Fig. 3) also showed a high degree of delignification (Klason lignin content, 4.1%; Table 2), but the macroscopic appearance indicated that very high amounts of polysaccharides must also have been degraded. Thus, the wood structure was totally destroyed, and the sample had a pulpy consistency.

A great problem in determining the degree of degradation of field material is the lack of a direct measure for the weight loss caused by decay. Determination of the swelling capacity of wood meal is a very simple physical test which, in our opinion, can be used as an indirect measure for the degree of wood degradation. The swelling capacity should be dependent on the lignin content of wood because lignin impregnates the polysaccharides and inhibits their swelling. On the other hand, we observed that dried wood meal samples, although having the same lignin contents, may substantially differ in their swelling capacities. For example, sample K and sample R were both almost completely delignified (Klason lignin content, 1.8 and 2.2%, respectively; Table 2); however, sample K, with only a +2% swelling capacity, almost corresponded to sound wood (0%), while in sample R, the swelling capacity was strongly increased (+53%). This difference was also demonstrated by a comparison of sample Vb (Klason lignin content, 4.0%; swelling capacity, +36%) with sample C, which had nearly the same lignin content (4.2%) but a strongly decreased swelling capacity (−48%). The high swelling capacity of samples R and Vb apparently reflects a higher content of uninjured polysaccharides. Actually, the physical characteristics of delignified dry wood meal depend on the degree of degradation of the cell elements.

We observed an evident relationship between the swelling capacity of individual wood meal samples and the water content of the original (fresh) samples. Decayed wood samples (U, Sb, Sc, and C) containing 87% or more water (fresh weight) showed the lowest swelling capacity (0 to −48%). We therefore suggest that the water content falling between 53 and 96% (Table 2), in the case of our material, considering the ecological situation in Chile (see Discussion), corresponds to the degree of degradation (weight loss) of the samples. Therefore, in Table 2 we arranged the samples according to their water content. Obviously, the swelling capacity within each series of wood meal samples initially

TABLE 2. Analysis of decayed wood from rotting trunks of *E. cordifolia*, *D. winteri*, and *N. dombeyi*[a]

Wood species	Sample	Water content (% [fresh wt])	pH	Swelling capacity[b]	Chemical composition (% [dry wt])						
					Total lignin	Acid-insoluble (Klason) lignin	Acid-soluble lignin	Ethanol-benzene-soluble substances	Hot-water-soluble substances	N	Ash
E. cordifolia	H	53	5.0	+11	22.4	18.3	4.1	1.79	18.0	0.090	3.54
	G	65	4.1	+29	9.9	7.5	2.4	0.58	10.4	0.046	1.48
	R	66	4.7	+53	4.2	2.2	2.0	2.31	6.0	0.029	0.84
	Q	71	3.9	+146	5.6	3.6	2.0	1.70	6.2	0.043	0.47
	K	80	4.1	+2	4.3	1.8	2.5	4.64	10.1	0.120	1.42
	U	91	4.5	0	8.2	6.1	2.1	1.51	9.9	0.095	1.20
D. winteri	Sa	78	3.6	+2	12.6	11.0	1.6	1.53	4.4	0.047	0.12
	Xa	80	4.1	+66	5.9	4.4	1.5	0.94	7.6	0.064	0.35
	Xb	83	4.0	+34	1.9	0.4	1.5	0.54	6.9	0.057	0.17
	Sb	87	4.2	−27	7.6	5.3	2.3	1.70	7.4	0.051	0.20
	Sc	95	4.2	−39	6.0	4.1	1.9	1.13	8.2	0.142	0.22
N. dombeyi	Va	80	4.2	+54	4.7	3.0	1.7	1.23	4.3	0.037	0.21
	Vb	84	3.8	+36	5.8	4.0	1.8	1.78	6.2	0.045	0.28
	C	96	5.1	−48	6.5	4.2	2.3	6.88	6.5	0.202	ND[c]

[a] All data except pHs are the averages of duplicate determinations.
[b] Deviation of the swelling capacity as a percentage of the swelling capacity of the corresponding sound wood; +, increased swelling capacity; −, decreased swelling capacity.
[c] ND, Not determined.

FIG. 1–3. Macromorphology of different palo podrido samples. Fig. 1. Sample Q (Klason lignin content, 3.6%) from a trunk of *E. cordifolia* showing white fibrous delignified material with some distinct light-brown patches consisting of simultaneously degraded wood. Fig. 2. Extensively delignified white fibrous sample (Xb) from a trunk of *D. winteri* containing only 0.4% Klason lignin. Fig. 3. Advanced stage of decay of *D. winteri* (sample Sc; Klason lignin content, 4.1%). The original wood is totally destroyed and transformed into an amorphous greyish-white pulp. Scales are in millimeters.

increased with the increasing water content of the fresh samples up to a maximum but then decreased with a further increase in the water content and progressive decay. We think that the phase of increasing swelling capacity reflects highly selective lignin degradation, with little degradation of polysaccharides. The maximum swelling capacity should characterize the typical state of palo podrido, while in more advanced stages, polysaccharides remaining in the delignified material become degraded as well. Therefore, the lignin content (dry weight) measured in late stages may be somewhat increased.

On the basis of the water content of individual samples, we calculated the percentage of weight loss for lignin and for polysaccharides. The increase in the water content in decayed wood in comparison with that in the corresponding sound wood (theoretically presumed value, 30% [fresh weight]) was attributed to metabolically produced water (Dill, Ph.D. thesis). Two examples follow. In sample R, about 93% of the lignin but only about 36% of the polysaccharides had been degraded, indicating the high selectivity of lignin degradation. In contrast, in sample U, about 96% of the lignin but also about 83% of the polysaccharides had been degraded. This result agrees with the low swelling capacity as well as the loss of wood structure of sample U and confirms the presumed high degree of polysaccharide degradation.

The nitrogen and ash contents could hardly be used as a measure for the degree of wood degradation. Theoretically, ash and nitrogen contents (dry weight) proportionally increase with increasing weight loss. Actually, this was not the case. Both values could have been reduced through the export of nitrogen and ash constituents by the fruiting bodies of *G. applanatum* which were found on all investigated trunks. Thus, the nitrogen content of sample R, instead of being increased, was significantly lower than that of sound wood. An additional problem was reflected by the values for ash. Despite the basidiocarp formation, we occasionally found abnormally high ash values (up to 9% [dry weight]) (data not shown). Presumably, this was the result of the accumulation of calcium oxalate by the fungus since, in some decayed wood samples, many calcium oxalate crystals were visible. It was not possible, even after raising the temperature of ashing to 1,000°C, to completely eliminate secondarily produced carbonates and to obtain carbonate-free ash.

The range of pH values was between 3.6 and 5.1 (Table 2). Most of the samples had pHs lower than those of the corresponding sound wood samples.

The hot water solubility of all decayed wood samples, except those of *N. dombeyi*, was increased as compared with sound wood, but no relation to other parameters could be detected. This was also the case for solubility in ethanol-benzene, for which some values were increased and others were decreased as compared with controls.

Micromorphological characteristics of palo podrido. In palo podrido samples showing highly selective lignin degradation, the cell elements of the wood were separated from each other because of the degradation of the middle lamellae but otherwise appeared unaffected. Interestingly, in samples with a high water content, although showing defibration, the cell elements had many large erosion troughs.

Figures 4 and 5 characterize the micromorphology of palo podrido samples (R and Xb) showing highly selective lignin degradation.

Figure 4 shows complete defibration in sample R from *E. cordifolia*. Except for the lysis of the middle lamellae, the cells obviously retained their original structure. Some transverse channels of boring hyphae but almost no erosion troughs were visible. Remarkable was the swelling of the cell walls and the fine spiral structure of the cellulose fibrils. Treatment with phoroglucinol hydrochloride did not produce a staining reaction.

Figure 5 shows defibrated tracheids of *D. winteri* (sample Xb), a hardwood species showing some analogies to softwood species in that it lacks wood fibers. In this case, swelling of the cell walls was hardly visible. Treatment with phloroglucinol hydrochloride did not produce a staining reaction. The lack of swelling in these tracheids reflects differences in the two tree species, as verified by a comparison of the chemically macerated sound woods.

Degradation of palo podrido. It appears paradoxic that under field conditions a material consisting of rather pure cellulose like palo podrido can accumulate at all. Besides some extractives and hemicelluloses, cellulose should be the most easily degraded component of wood. Moreover, typical soft-rot fungi known to degrade cellulose were isolated from all palo podrido samples. Therefore, we were interested to find out what kind of limitations could be responsible for the overall inhibition of cellulolytic activity. For this purpose, we tested whether autochthonous microorganisms in the degraded wood samples were still able to produce additional weight losses. Equal portions of palo podrido sample Va (Klason lignin content, 3.0%) were incubated in Erlenmeyer flasks after the addition of a nutrient solution lacking a carbon source. Besides bacteria, sample Va mainly contained *Scytalidium lignicola*, *Trichoderma polysporum*, and *Sistotrema brinkmannii*, all known to degrade cellulose in pure cultures. After the sample stood for 10 months at 20°C, the weight loss in each flask was determined (Table 3). Even after such a long incubation period, the cultures without an added nitrogen source had only small weight losses, in the range of 12 to 24%. In contrast, all cultures with an added nitrogen source had extremely large weight losses, in the range of 81 to 91%. Taking into account the production of biomass, this means a rather complete degradation of the substrate which was already evident after a few months. Raising the pH of the medium from 5.0 to 7.2 or omitting phosphate, trace elements, or yeast extract had no significant effect on the percent weight loss. This result convincingly demonstrates that nitrogen must play an essential role in palo podrido decay.

DISCUSSION

The ability of the white-rot basidiomycete *G. applanatum* to degrade the lignin of wood either selectively or simultaneously is already known (2). The specific conditions in the South Chilean rain forests obviously force this fungus to increase the extent of selective lignin degradation while inhibiting cellulose-degrading activity. The result is that the interiors of rotting trunks of the three investigated hardwood species become transformed into extensively delignified wood, so-called palo podrido.

Under German field conditions, lignin degradation up to such an extent as that observed in Chile cannot be found. We repeatedly observed a comparable delignification with defibration in birch wood degraded by *Fomes fomentarius*, but this was always restricted to a few small pockets surrounded by the simultaneous decay of typical white rot. As expected, the values for Klason lignin in these mottled samples were somewhat lower than those in sound wood (16 versus 19% [dry weight]). Recently, we analyzed a trunk of

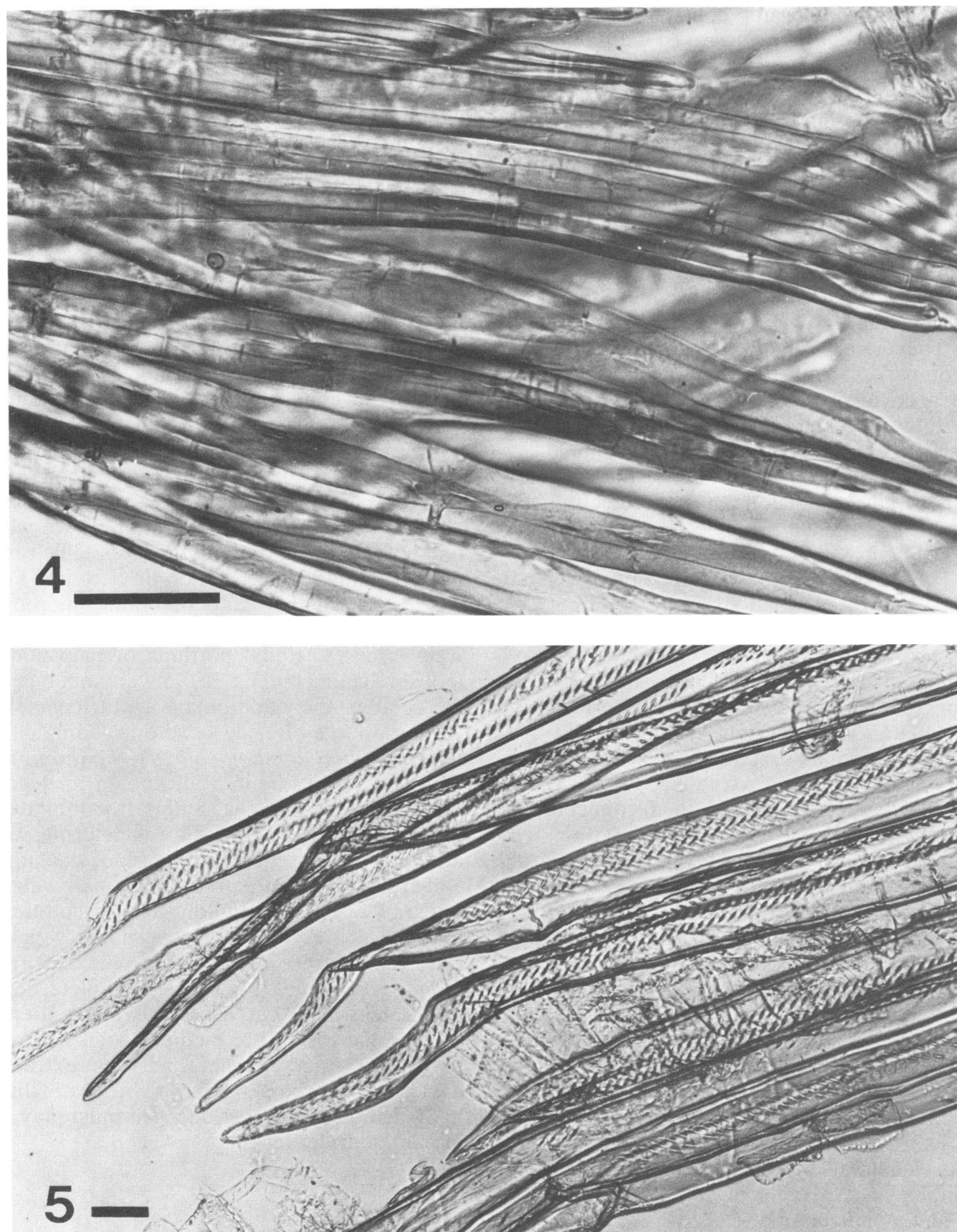

FIG. 4 and 5. Micromorphology of palo podrido samples. Fig. 4. Sample R (Klason lignin content, 2.2%) from *E. cordifolia* showing defibration caused by biodegradation of middle lamellae. Delignified cell walls appear strikingly swollen, sometimes revealing a fibrillar substructure. Bar, 50 μm. Fig. 5. Sample Xb (Klason lignin content, 0.4%) from *D. winteri* showing delignified and defibrated wood tracheids lacking detectable swelling of cell walls (characteristic for this wood species). Bar, 50 μm.

Populus sp. degraded by *G. applanatum* in a forest in Berlin. The micromorphological as well as chemical analyses revealed unusually large areas of selective lignin degradation, with lignin contents as low as 5%, but most of the trunk showed simultaneous decay. Surprisingly, the areas of selective lignin degradation were located within closed compartments (pseudosclerotia) produced by the fungus itself.

Here we propose an explanation for the extensive and highly selective delignification regularly found in Chile.

The microclimate in South Chilean rain forests that are not destroyed by woodcutting is characterized by extremely high humidity as well as constantly low temperatures (8 to 13°C) during the entire year. Therefore, decaying trunks never desiccate and retain the metabolic water produced during decay. This means that the trunks keep their outer integrity over long periods, and in their interiors, decay can proceed undisturbed. Conservation of the microenvironment is increased by the bark and by sediments of humus. Moreover, we observed only minimal activity of insects within such trunks. Consequently, decaying trunks constitute rather closed systems with presumably microaerobic conditions, very low O_2 concentrations, and high CO_2 concentrations.

Many experiments with *P. chrysosporium* have demon-

TABLE 3. Weight loss in palo podrido samples (original sample Va from *N. dombeyi*) after 10 months of incubation

Nutrient solution[a]	pH	Weight loss (%)[b]
Complete	5.0	87, 90, 90
Complete	7.2	83, 89
Without nitrogen	5.0	12, 16, 22
Without phosphate	5.0	81, 87
Without nitrogen and phosphate	5.0	21, 24
Without trace elements	5.0	87, 88
Without yeast extract	5.0	87, 91

[a] See Materials and Methods.
[b] Calculated as a percentage of the dry weight of the original sample; individual values are from individual flasks.

strated that the highest lignin degradation rates are achieved under a 100% O_2 atmosphere (1, 11, 22). On the other hand, Leisola et al. (13) observed that efficient degradation of dioxane-hydrochloride-isolated lignin by *P. chrysosporium* is possible under an atmosphere of 30% CO_2 and merely 10% O_2, and they suggested that pure oxygen may even be toxic. Reid and Seifert (20), using several white-rot fungi, showed that oxygen stimulates the degradation not only of lignin but also of polysaccharides and thus, in turn, does not enhance the selectivity of the process.

From our own investigations of palo podrido, we presume that microaerobic conditions like those established within a rotting trunk may be one factor that increases selective lignin degradation. This presumption is also supported by the observation that areas of selective lignin degradation under field conditions in Germany were always located in the deeper zones of trunks or even within closed pseudosclerotia.

The influence of temperature on wood decay also appears to be important. The results obtained with *P. chrysosporium* (11), a rather thermotolerant fungus, may not be valid for other white-rot basidiomycetes. Drew and Kadam (6) showed that *P. chrysosporium* and *Sporotrichum pulverulentum* are more active in lignin degradation at 28°C than at their growth optimum of 38°C. In this context, we wish to point out that at the low temperatures prevailing in the South Chilean rain forests, the growth rate of *G. applanatum* should be suboptimal. It is known that ligninolytic activity, at least of *P. chrysosporium*, is correlated with secondary metabolism (9). It is possible that this physiological state can be provoked by suboptimal temperatures.

Besides the above-mentioned factors, the extremely low nitrogen content of the investigated Chilean hardwoods is perhaps the most important one promoting selective delignification. The nitrogen concentration in the sound woods ranged from only 0.037 to 0.073% (dry weight). It is a well-known phenomenon that the nitrogen supply plays a crucial regulatory role in wood decay and that high N concentrations inhibit lignin degradation (11). On the other hand, high N concentrations stimulate polysaccharide breakdown (14, 18).

Our degradation experiments clearly demonstrated that the addition of a nitrogen source resulted in a rapid and almost complete decay of the cellulose in the palo padrido samples. We also changed other factors, such as moisture and gas atmosphere, but these were also changed in the flasks without added nitrogen. The addition of nitrogen relieved the limitation for cellulose degradation but, as we showed in further experiments (Dill, Ph.D. thesis), it did not enhance lignin degradation.

In a previous paper (5), we showed that the nitrogen in wood consists of hydroxyproline-rich amino compounds and that about half of it appears in the insoluble lignin fraction. We therefore suggested that the middle lamellae, known to be lignin and glycoprotein rich, contain remarkable portions of nitrogen in the form of lignoprotein complexes. Thus, the stimulation of lignin degradation by limited nitrogen could be the only way for the fungus to gain access to these nitrogen sources. In this context, it would be interesting to find out if the primary determinant for the mottled-rot pattern, a type of decay characterized by alternating areas of selective and simultaneous lignin degradation (15), is a nonhomogenous distribution of nitrogen within wood.

In palo podrido, cellulose degradation is suppressed, but as we observed in samples with an extremely high water content at advanced stages of decay, cellulose is degraded as well. As already mentioned, we think that palo podrido in its typical form refers to that phase of decay in which maximal loss of lignin and minimal loss of cellulose are reached. Our results revealed that the material we collected contained only a few samples that were in this particular phase, e.g., samples R and Q.

In German forests, the environmental conditions are very different from those in South Chile. Periodic desiccation of the decaying trunks together with the influence of frost during the winter months exert serious mechanical stress. The overall integrity of the trunks is usually disrupted, and decay cannot continue undisturbed over longer periods. Moreover, insect activity contributes to further destruction. Therefore, the higher N concentration of the surrounding soil increasingly influences the type of decay. Besides these environmental factors, nitrogen concentrations in sound woods as low as those that we found in Chilean woods are unusual in German woods. For instance, we noted two to three times higher nitrogen concentrations in German birch wood and beech wood. This result agrees with the predominance of simultaneous white rot in German forests.

Many white-rot fungi are known to produce a mottled type of white rot, strikingly proving their ability to selectively delignify. The question is how to specifically stimulate this ability. We emphasize that palo podrido constitutes a model system that works successfully under natural conditions by extending delignification over the whole interior of trunks.

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