5

Department of Phytopathology, Institut Français pour la Recherche en Coopération (O.R.S.T.O.M.), Abidjan, Côte d'Ivoire

Biodegradation of Hevea brasiliensis wood by Rigidoporus lignosus and Phellinus noxius[1]

By J.-P. Geiger, B. Rio, M. Nicole and D. Nandris

Abstract

In vitro wood slats degradation assays reveal that both the white root rot fungus *R. lignosus* and the brown root rot fungus *P. noxius* cause a white rot of wood. *In vivo* (infected tap roots) they cause the same type of decay. Nevertheless lignin determination show the rubber-tree ability to react against the parasite aggression by increased lignification of tissues.

1 Introduction

Rigidoporus lignosus (Kl.) Imaz. and *Phellinus noxius* (Corner) G. H. Cunn. are two Polyporaceae which are the major cause of tree loss in rubber plantation by inducing on rubbertrees, respectively, white and brown root rots. Attack by these parasites causes the lignified root tissues of the host to decay.

[1] Paper presented as a poster at the 27th Congress of the French Phytopathological Society, Paris, November 1984.

R. lignosus has been known for a long time as a lignin-consuming (or lignolytic) fungus whereas *P. noxius* was classified among the brown rot agents (ROGER 1954; PICHEL 1956), which degrade the polysaccharide fraction of wood but not lignin (COWLING 1961; KIRK and KELMAN 1965; KIRK 1971; KIRK and HIGHLEY 1973; CRAWFORD and CRAWFORD 1976).

The results of our research involving the nature of enzymes secreted *in vivo* and *in vitro* by both fungi are in disagreement with the classification of *P. noxius*. This fungus in fact does not only secrete enzymes participating in the decomposition of wood polysaccharides (cellulase, pectinase, xylanase, etc.), but also an enzyme identified as a laccase (p-diphenol oxidase), considered to be synthesized only by lignin degrading fungi (KIRK and KELMAN 1965; SCHUBERT 1965) and shown to play a major role in lignin degradation (KIRK et al. 1968 a and b; ISHIHARA and MIYAZAKI 1972; ANDER and ERIKSSON 1976; ISHIHARA 1980; GEIGER et al. 1983 a; KERN 1983; GEIGER et al. 1986 a).

The present research was undertaken in order to verify if *P. noxius* is truly a lignin digesting fungus. This was done by comparing its *in vitro* behavior with that of *R. lignosus* and with that of *Antrodia* sp., a known brown rot fungus of sawed lumber.

Finally, we also investigated the action of *R. lignosus* and *P. noxius* as pathogenic fungi by showing their effect on lignified tap root tissues of adult rubber trees naturally attacked in plantation. These results were compared with those obtained, *in vitro*, on wood slats.

2 Materials and methods

2.1 Plant material

2.1.1 The parasites

The strains of *R. lignosus* and *P. noxius* used were isolated from naturally parasitized *Hevea* taproots in a plantation. They were grown at 28 °C in darkness in 2 % malt 2 % agar medium in Petri dishes.

The *Antrodia* is a strain isolated from sawed lumber.

2.1.2 In vitro experimentation

The tests of wood and lignin degradation by the different fungi were carried out with small slats (5 cm long, 1,5 cm wide and 3 mm thick) cut from the taproots of healthy rubber trees. Three slats were placed in a 2 cm diameter Roux tube. Moisture was assured by adding enough water to fill the lower reservoir of the tube and cover about ¼ of the height of the slats. Tubes and contents were autoclaved for 45 min at 110 °C. After cooling, the slats were inoculated with a mycelial implant taken from a fungal preculture on malt (2 %) agar (2 %).

The effect of a nutrient medium on the capacity of the fungi to degrade the wood was examined. Slats identical to the above were placed in Erlenmeyer flasks containing either 1 cm diameter glass beads and 30 ml of water (in such a way as the slats did not float on the water), or 50 ml of malt (2 %) agar (2 %). In the latter case, the slats were autoclaved separately and deposited on the medium after solidification.

2.1.3 In vivo experiments

The various analyses (lignin assay and monomer composition) were carried out on tissues sampled from the taproots of naturally infected plantation adults. For reasons previously cited (GEIGER et al. 1986 b), experiments were carried out on partially colonized taproots. For each taproot, the sample of healthy tissues (H) was used as the control and basis of

comparison for the other tissue types: HF, healthy tissues close to the front of parasite progression; IF: infected tissues sampled at this front; and I: infected tissues far from the same front. Tissue samples were taken as previously described (GEIGER et al. 1986 b).

2.2 Analytical methods

2.2.1 Estimation of wood weight losses

Fungal capacity to degrade wood was estimated by the dry weight loss of the slats as a function of the culture time. After removing of the fungal mycelium, the slats were dried at 45 °C for 48 hours before weighing. This method was preferred to heating at 105 °C in order to eliminate the possibility of chemical modification of parietal polymers by excessively long and intense heating, since the slats were subsequently used for lignin assays. After such a drying the residual moisture in the slats was less than 5.5 %. The results are expressed as per cent weight loss in comparison to the initial dry weight (48 hours at 45 °C) of the slats, with a correction due to the loss resulting from autoclaving (estimation performed with a group of control slats).

2.2.2 Elimination of extractable substances from wood

After drying, the slats were ground (Willey blade grinder) to obtain 60 mesh sawdust. "Extractable" material was removed from the sawdust with hot (95 °C) water for 12 hours, ammonium oxalate (0.5 %, 85 °C, 24 h) and finally sodium carbonate (2 %, 85 °C, 8 hours). Each extraction involved 50 ml of extracting liquid per gram of dry sawdust. After each extraction, the sawdust was rinsed with hot water until neutrality. After carbonate extraction it was rinsed and dried at 45 °C. It was then successively extracted in a Soxhlet apparatus with alcohol/benzene (1/2, v/v) and alcohol, each for 8 hours. After drying, lignin assays and monomer composition were performed on the residue which is an extractive-free wood.

2.2.3 Lignin assay

Lignin was assayed by quantitative extraction with thioglycolic acid (from 250 mg of 60 mesh extractives free sawdust) in the following conditions: incubation with a mixture of 0,4 ml of thioglycolic acid and 5 ml of 2 N HCl for 5 hours at 90 °C. The suspension was filtered through sintered glass (porosity 5) and the residue was washed with water (until pH 5). It was then resuspended in 5 ml of 2 % NaOH for 24 hours at ambient temperature. The suspension was again filtered through sintered glass (porosity 5) and washed twice with 5 ml aliquots of water. The filtrate and washes were pooled in a tared centrifuge tube and brought to pH 1.5 with 5 N HCl, which caused the flocculation of thioglycolic lignin.

After centrifuging for 10 min at 15000 g the flocculate was washed twice with 5 ml of water. The pellet from the last centrifugation was dried at 80 °C for 48 h and then weighed. The results are expressed as per cent lignin (as the thioglycolic form) in comparison to the initial dry weight of the extractive-free material (105 °C dry weight estimated with a sample previously dried at 45 °C for 48 hours, then heated at 105 °C for 48 hours).

2.2.4 Monomer composition of lignin

Monomers were analyzed after oxidizing the extractive-free sawdust with alkaline nitrobenzene in the following conditions: 250 mg of sawdust were suspended in a mixture of 0.5 ml of nitrobenzene and 5 ml of 2 N NaOH. Oxidation was carried out at 160 °C for 3 h in a sealed stainless steel tube with intermittent agitation. After oxidation, the suspen-

sion was filtered through sintered glass porosity 5 and the residue was washed three times with 5 ml of water and then twice with 20 ml of ether. The filtrate and washes were transferred to a separatory funnel and extracted twice with 40 ml of ether in order to eliminate the residual nitrobenzene. The aqueous phase was brought to pH 2.5 with 5 N HCl, saturated with NaCl and then extracted twice with 60 ml of ether. The ether phase was recovered and filtered over anhydrous Na_2SO_4. The solvent was evaporated to dryness and the residue taken up with 3×2 ml of methanol.

This methanol solution was used for the analysis of monomers. The aldehydes of the benzoic series, resulting from the nitrobenzene oxidation of lignin, were separated with HPLC (Waters apparatus, column "C 18") in the following conditions: 5 to 45 % methanol gradient in 1 % acetic acid, flow-rate 1 ml/min, runing time 15 min, internal standard T7 (3,4,5-tri-methoxybenzaldehyde at 0.5 mg per ml of solution injected), volume injected: 5 to 10 μl.

2.3 Electron microscopy observation

Samples of roots infected plants (*in vivo*) and slats of *in vitro* degraded wood were fixed with 3 % glutaraldehyde (2 h, 4 °C) buffered with 0.1 M sodium cacodylate (pH 7.2). After several washes, samples were postfixed (2 h, 4 °C) with 1 % osmium tetroxyd. After further rinses, segments were dehydrated in a gradual ethanol series (5 to 100 %) and embedded in Epon 812 preceded of an epoxy propane change. Sections of 60–50 nm were stained with lead citrate (REYNOLDS 1963) and uranyl acetate and examined on a Siemens Elmiskop 102 electron microscop, operating at 80 kv.

3 Results and discussion

3.1 In vitro degradation of Hevea slats; lignolytic nature of P. noxius

3.1.1 Weight loss and lignin degradation

Figure 1 a shows the capacity of the fungi to degrade the wood of *Hevea*. In our experimental conditions, it is shown that *Antrodia* is much more active than the *P. noxius* strain
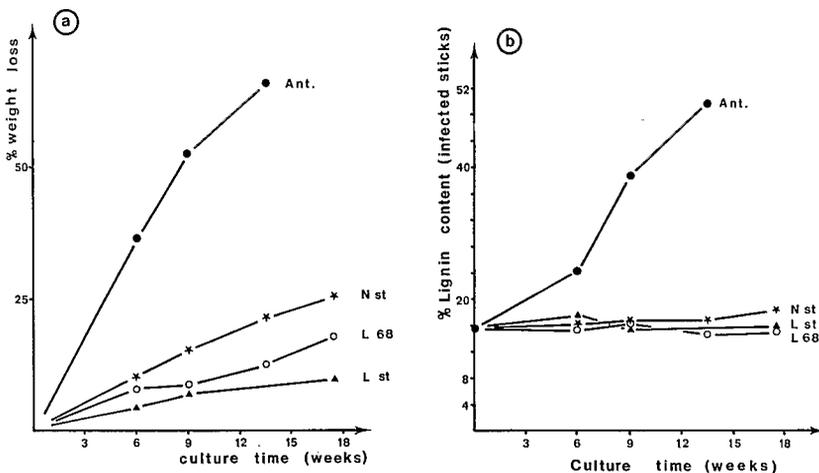

Fig. 1. In vitro biodegradation of wood. a. results expressed as weight loss of wood sticks (slats) after different culture times and infected by different fungi (means for 5 cultures). (Ant.: *Antrodia* sp., N St: *P. noxius*; L St and L 68: two different isolates of *R. lignosus*). b. evolution of lignin content in residue of the wood sticks [as in a)]
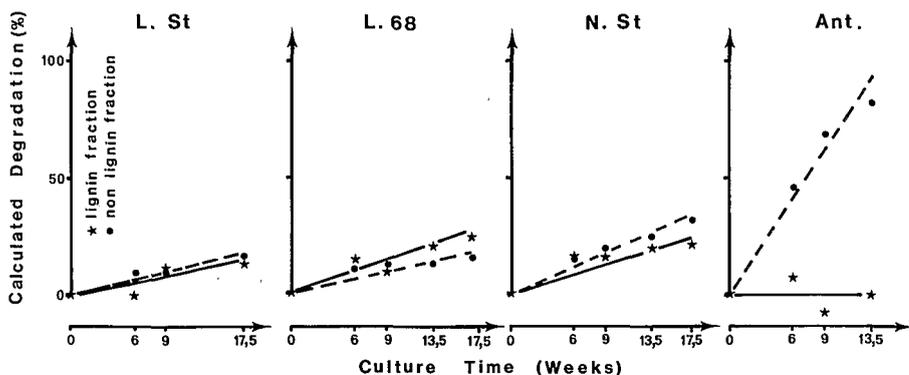
Fig. 2. Percentage of lignin and non lignin fractions degradation calculated from results presented in Fig. 1 a and b

(N St), which in turn has a higher degradation capacity than that of the two *R. lignosus* strains (L st and L 68). These two strains differ from each other by their aggressiveness, measured in conditions described elsewhere (NANDRIS et al. 1983). This first approximation of the capacity to degrade the wood was extended by estimating the quantities of lignin present in the slats after different times of colonization by the fungi (Fig. 1 b). These results are highly demonstrative. The quantity of lignin in the wood invaded by *R. lignosus* and *P. noxius* does not vary to any great extent, while in the case of *Antrodia*, there is a considerable increase in the lignin content of the slats residues. We may conclude that the first two organisms degrade both the lignin and polysaccharide fractions of the wood in a nearly "equilibrated" manner. *Antrodia*, on the other hand degrades only the polysaccharides, leading to an enrichment of the lignin fraction in the wood residue.

In order to better appreciate these findings, we used the preceding data to calculate the quantity of lignin effectively consumed in the course of colonization and deduced the rate of consumption of the "non-lignin" fraction (containing a majority of – but not exclusively – polysaccharides). Figure 2 confirms the following points:

a. *R. lignosus* and *P. noxius* both degrade lignin. *P. noxius* should thus be classified as a white rot agent, just as *R. lignosus*.

b. *Antrodia* sp., on the other hand, is a brown rot agent, since it does not depolymerize the lignin fraction.

c. *P. noxius* exhibits a tendency for preferential degradation of polysaccharides, while *R. lignosus* degrades lignin and polysaccharides in a relatively balanced manner, although with a slight preference for lignin. These data agree with the respective capacities of the two fungi to excrete polyosidases and laccase, respectively involved in the degradation of polysaccharides and lignin. The former are excreted in very large quantities by *P. noxius*, whereas *R. lignosus* releases predominantly laccases (GEIGER et al. 1986 b).

3.1.2 *Effect of an exogenous nutrient source on the capacity of R. lignosus and P. noxius to degrade wood*

This experiment was undertaken in light of observations of fungi such as *Phanerochaete chrysosporium* and *Coriolus versicolor*, showing that lignin is not degraded in the absence of a nutritive substrate such as glucose, or even cellulose (KIRK et al. 1976). These fungi cannot develop in the presence of lignin as sole carbon source. ANDER and ERIKSSON (1975, 1977) also showed that some white rot agents degraded the lignin fraction of wood slats more rapidly in the presence of exogenous nutrients, such as malt extract, than in their absence.
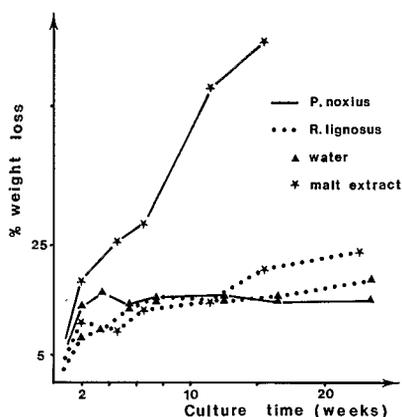
Fig. 3. Effect of malt extract on the wood degradation capacities of *R. lignosus* (St. strain) and *P. noxius* (St. strain) (mean for 5 to 10 inoculated slats)

Our results show that the addition of malt extract has only a slight effect on the rate of wood degradation by *R. lignosus* (Fig. 3). The efficiency of *P. noxius* for wood degradation, however, increases considerably in these conditions. It is not possible to determine if this increased wood degradation results from an increased total mycelial mass invading the wood or from a quantitative increase in the secretion of the enzymes responsible for degrading the different polymers of the wood.

The increased capacity for wood degradation applies to all types of polymers. Table 1 shows that after 4 months of growth on slats in the presence of malt extract, *P. noxius* degrades 62 % of the wood, corresponding to 61.9 % of the lignin fraction and 62.1 % of the non-lignin fraction. After 6 months of culture on slats alone (with water) the total degradation of the wood and its components is much lower, 14.4, 18 and 13.7 % respectively.

Table 1

Effect of an exogenous nutrient on wood degradation

Culture conditions	Culture time (months)	Weight loss (1)	% lignin		% of non lignin fraction degraded (2)	S/V (1)
			in sticks residue (1)	degraded (2)		
Control (sterilized sticks)	—	0	23,2	0	0	1,72
R. lignosus/ sticks + water	0,5	8,3	23,2	8,3	8,3	1,69
	6	19,0	21,0	26,6	17,7	1,60
R. lignosus/ sticks + malt extract	0,5	12,1	22,7	14,1	11,4	—
	6	23,2	22,9	24,8	23,5	1,57
P. noxius/ sticks + water	0,5	14,1	22,8	16,3	13,6	1,61
	6	14,7	22,3	18,1	13,7	1,31
P. noxius/ sticks + malt extract	0,5	18,1	22,7	20,1	17,6	—
	4	62,1	23,3	61,9	62,1	1,32
(1): experimental values (2): calculated values.						

3.1.3 Monomer composition of lignin before and after colonization of the slats

The fungal attack may result either in a "recurrent" depolymerization of lignin without modifying the monomer composition of the residual polymer, or in a progressive modification of the polymer in terms of both the degree of polymerization and the monomer composition. The results Table 1 show that infection leads to a considerable change in syringaldehye/vanillin (S/V) ratio of the residual lignin.

Only the S/V ratio was calculated since the p-coumaryl units [analysed as p-hydroxybenzaldehyde (B) after nitrobenzene oxidation of the sawdust] is encountered in very low quantities: 0.6 to 1 % of total V + S + B units.

This modification of lignin is truly progressive. After two weeks of culture of *R. lignosus*, the decreased S/V ratio is barely perceptible (1.72 initial down to 1.69), while it is more evident after 6 months (1.60). *P. noxius* exerts a more intense action, since the initial

S/V ratio of 1.72 decreases to 1.61 after two weeks and reaches 1.31 after 4 months of culture. In addition we can note that an exogenous nutrient supply has only a negligible effect on the changes of the S/V ratio in comparison to that in the slats cultured in the absence of the substrate (1.57 vs. 1.60 for *R. lignosus* and 1.32 vs. 1.31 for *P. noxius*).

The decreased S/V ratio implies either a preferential elimination of the sinapinic residue from the original macromolecule, or a chemical transformation of the residue into a different compound. It is to be noted in this context that the laccase of *Coriolus versicolor* can demethylate vanillic acid and alcohol, as well as the lignin from ground maple wood (ISHIHARA and MIYAZAKI 1974; ISHIHARA 1980). This observation can also be correlated with the fact that *R. lignosus* and *P. noxius* laccases have a higher affinity for sinapinic acid than for the other lignin basal units (GEIGER, unpublished data).

An alternative interpretation is that the fungi preferentially degrade syringic-type lignin-rich parietal structures, which would result in the reduction of the quantity of this residue in the infested wood in comparison to the original levels in healthy wood.

3.2 Effect of R. lignosus and P. noxius infestation of Hevea taproots on the lignin content of different tissues

This analysis was performed on 5 taproots infested by *R. lignosus* and 6 taproots infested by *P. noxius*. In each case, the adult taproots (4 to 6 years old) were partially invaded, enabling the four types of tissues (H, HF, IF and I described above) to be sampled. The results are expressed either as the percent of lignin content in extractive-free sawdust (Fig. 4a, b) or as the relative percentages of lignin calculated for each taproot in comparison to the quantity of lignin present in H tissues (base 100) (Fig. 4c, d). The latter means of presenting the results leads to a better visualization of the changes in lignin contents as a function of the type of tissue analyzed (especially for the taproots attacked by *P. noxius*).
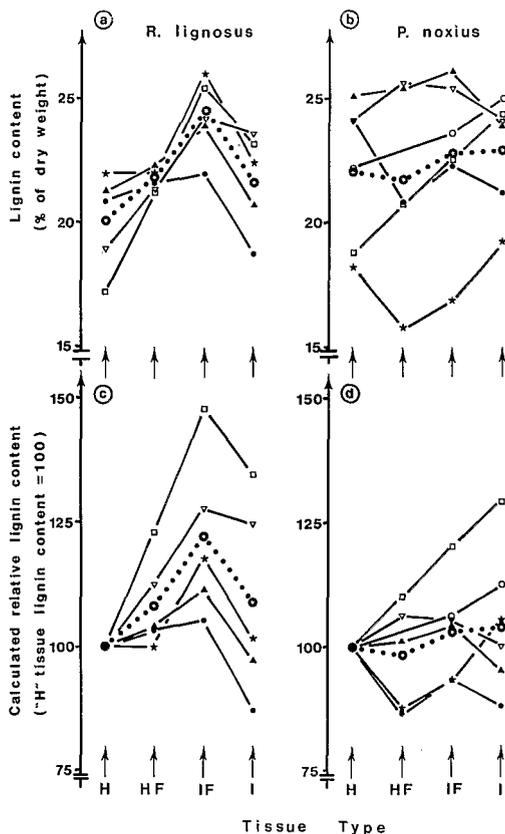

Fig. 4. Lignin content recorded for different tissue-types of taproots (each line joins the values recorded for tissues belonging to the same taproot; dotted lines join mean values). a and b: values expressed as a percentage of dry weight of tissue types respectively in case of taproots partially colonized by *R. lignosus* and *P. noxius*. c and d same analysis; for each taproot results registrated for HF, IF and I tissues are expressed as a percentage of corresponding H tissue lignin content (H and I: respectively healthy and infected tissues located far from the front of intratissular parasite progression; HF and IF: healthy and infected tissues removed respectively near – and at – the front of parasite progression)

The first comment concerns the variability of lignin contents in H tissues from one taproot to another, which may be attributed to genetic differences among trees (the taproots sampled in plantations arose from plants of different origin) or to variations in the assay technique. In order to eliminate the latter possibility, the four types of tissues from the same taproot were systematically treated in the same experimental serie (exhaustion of extractives, lignin assay, monomer composition). In addition, the experiment performed on a sawdust sample divided into 4 batches undergoing the treatments in standard conditions showed uniformity of the results of lignin assays: differences in extreme values are lower than 3 % in comparison to the mean value. Thus, for each taproot, the variations of lignin content recorded for different tissue types (H, HF, IF and I) are significant.

3.2.1 Taproots infested by R. lignosus

There is a systematic increase occasionally up to 50 %, of the lignin content in IF tissues. (Fig. 4 a, c). This increase may be correlated with the considerable increase of the peroxidase activity observed in the same tissue type (IF), corresponding to a host reaction (GEIGER et al. 1976, 1986 b). As this peroxidase is able to polymerize phenylpropanoid alcohols (GEIGER and HUGUENIN 1981) it is tempting to attribute the increased lignin content to a reaction lignification of tissue in response to parasite aggression. In fact such reaction could be histologically observed in artificial infected young plants (GEIGER et al. 1983 b; NICOLE et al. 1986). The reaction lignification is ultimately ineffective, since the intratissue progression of the fungus in most cases continues, and the lignin which accumulates in the IF tissues is progressively degraded, leading to the decreased lignin contents observed in I tissues.

3.2.2 Taproots infested by P. noxius

These results are more difficult to interpret because of the heterogeneity of response to the parasite attack. Any attempt at interpretation must take into account the fact that the anatomical reactions of *Hevea* are rare. Similarly, the stimulation of peroxidase activity in IF tissues is much lower than in the case of aggression by *R. lignosus*. These observations are consistent with the possibility that *P. noxius* rapidly kills the tissues it attacks. Nevertheless, histological reactions, e. g. hyperplasia, supernumerary cells, and even lignification reactions are often observed in the cell layers situated under the points of fungal penetration in artificially infected young plants (NICOLE et al. 1986).

Can these facts be extrapolated to adult taproots and explain only low increase of the lignin content of HF tissues in some taproots? How may then be explained the decreased lignin content, in the same tissue types, in the case of two of the taproots analyzed?

The same difficulties of interpretation arise when we consider the stages IF and I. In three of the taproots, the lignin content increased, which could be attributed to the preferential degradation of the polysaccharide fraction of the tissues. For other taproots, on the other hand, we note decreased quantities of this polymer, leading to the supposition of a preferential lignolytic activity. Finally, in the case of the last taproot, there is a balanced degradation of the different tissue fractions.

Considering that IF and I type tissues are dead and thus the only parameter capable of variation is the "pathogenic agent", we may explain this heterogeneity by the fact that taproots are attacked by strains of *P. noxius* which differ by their physiological capacities to degrade the different polymers of the wood. This hypothesis is supported by the fact that populations of *P. noxius* (and *R. lignosus*) are heterogeneous at the level of their pathogenicity (NICOLE et al. 1986). Similarly, recent *in vitro* experiments have shown that these same isolates differ in their potentials for enzymatic secretion (GEIGER, unpublished data).

3.3 Effect of infection on the monomer composition of residual lignin

The monomers of residual lignin were analyzed in samples of extractive-free sawdust from different taproot tissues, of which a part was used to determine total lignin content. In general and regardless of the nature of the infesting pathogen, the S/V ratio decreases, preferentially in I tissues, i. e. those colonized for the longest times (Fig. 5 a, b). This result agrees with *in vitro* observations in the case of infested *Hevea* slats. It would seem that the process leading to modification of lignin, prior to (or accompanying) the degradation of the polymer, is comparable, whether fungal attack is on sterilized slats or on naturally infested *Hevea* taproots. This is probably explained by the fact that the fungi act as saprophytes in both cases, taproot type I tissues being dead, just as are the sterilized slats.

A last comment concerns the very low p-coumaric residues (lower than 1 % of total monomers) in the lignin analyzed, regardless of the tissue source.
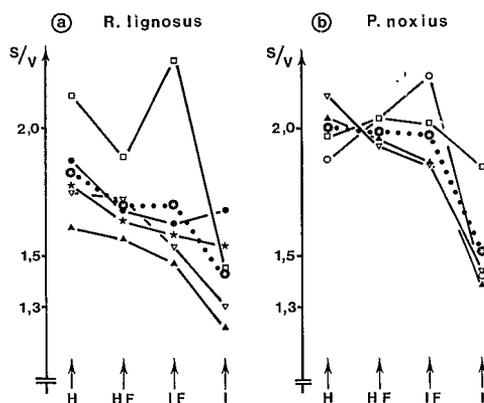

Fig. 5. Lignin monomer composition expressed as the ratio of syringyl/vanilin groups in different tissue types (H, HF, IF and I: for signification, see Fig. 4) of taproots respectively attacked by a. *R. lignosus*, b. *P. noxius*

4 Conclusions

The present investigation involves two aspects: a. the determination of the degradation of wood, *in vitro*, by two root parasites of *Hevea* and the verification of their rot types, brown or white; b. the determination of the *in vivo* effect of the same fungi colonizing the taproots of adults in a plantation and thus, acting as parasites.

The results unambiguously show the capacity of both fungi to degrade lignin. The comparison with the behaviour of *Antrodia* sp., a known brown rot agent, leaves no further doubt on this point. *R. lignosus* and *P. noxius* are thus both to be classified among the white rot group of fungi. In addition the lignolytic abilities of the two latter fungi are confirmed by cytological observation (Fig. 6 a and b).

There are some differences in the capacities of these two fungi in terms of the relative rates of degradation of the major fractions of wood, lignin and polysaccharides. *R. lignosus* tends to depolymerize lignin more rapidly than polysaccharides, while *P. noxius* exhibits a preference for polysaccharides. Finally, *P. noxius* "responds" more than *R. lignosus* to exogenous nutrients. In the former case this response involves a considerable increase in the rate of wood degradation, regardless to the type of polymer attacked.

The results obtained *in vivo* furnish matter to discussion, especially in the case of infestation by *P. noxius*. The infective situation varies for one individual (taproot) to another, generating contradictory interpretations, e. g. preferential degradation of lignin in some cases, of polysaccharides in others. These contradictions can be explained by the hypothesis according to which different *P. noxius* strains exist, differing by their capacities
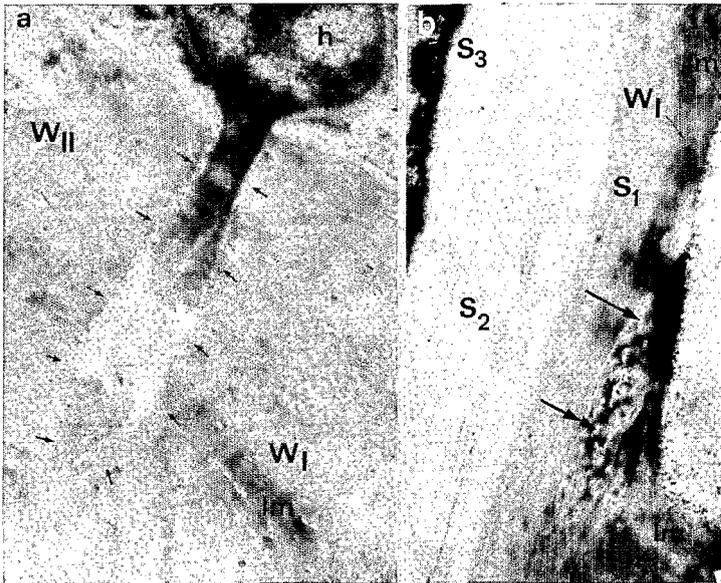
Fig. 6. a. lignified cell wall alteration caused by *R. lignosus* (*in vitro*); hyphae (h) differentiate micro-hyphae which degrade the secondary wall (W_{II}), the primary wall (W_I) and the middle lamella (lm). Dissolution of cell wall components occurs both in contact – and in front of the hyphae (arrows) (× 30000). b. lignified cell wall alteration caused by *P. noxius* (*in vivo*). Degradation of the primary wall (W_I) consist first in a disorganization of the native structure (arrow) and secondly in a wall perforation (double arrow). (lm: middle lamella; S1, S2, S3: secondary wall layers; × 40000)

to degrade the two major fractions of wood. Anyway, attack by *P. noxius* causes the rapid necrosis of infested tissues, thus limiting the intensity of lignification reactions, especially at IF stages.

Attack by *R. lignosus* induces a more uniform response. On the average and in comparison to healthy tissues, there is a 25 to 30 % increase of the lignin content in IF tissues. This reaction lignification of the tissues is a response to parasite attack. It may appear slight in comparison to that observed in the case of radishes attacked by *Peronospora parasitica* (ASADA and MATSUMOTO 1969) and beans infected by tobacco necrosis virus (TNV) (KIMMINS and WUDDAH 1977). We may keep in mind that in the case of *Hevea* aggression, the tissues which will be the site of reaction lignification are already highly lignified, which is not the case for radishes or beans. In addition, it is reasonable to suppose that the reaction potentials of an adult *Hevea* taproot are much lower than those of a leaf. Nevertheless, the possibilities of taproot reactions exist, as shown by the increase in peroxidase activities in IF tissues (GEIGER et al. 1986 b).

If we examine the lignification variations of tissues in the framework of "resistance of plants to infection", we should consider not only the quantitative, but also the qualitative aspect, i. e. changes occurring in the monomer composition of the polymer (RIDE 1978). Thus, the monomer of parasitized radish tissue lignin is quite different in comparison with that of the healthy one (high p-coumaric unit content, absence of sinapinic units; ASADA and MATSUMOTO 1972). In beans infested by TNV, however, changes in the monomer composition are slight (KIMMINS and WUDDAH 1977).

In *Hevea*, there was never a significant increase in the proportion of p-hydroxybenz-aldehyde among the oxidation products of the tissues, regardless of their nature (HF, IF or I). This implies that, on a structural level, the reaction lignin of HF and IF tissues is probably not very different from that of healthy tissues. On the other hand, we note a

considerable decrease in the S/V ratio in I tissues. It is to be noted that we observed comparable decreases in the slats infested by both fungi. This is consistent with the possibility that both fungi adopt a saprophytic behaviour in the dead I type tissues.

The lignification reaction may lead to the edification of one of the barrier as proposed in the C.O.D.I.T. hypothesis (SHIGO and MARX 1977).

In practice, this reaction is ineffective in terms of the course of the pathogenic process, since the pathogens generally invade the entire taproot, causing the death of the tree.

Acknowledgements

Summary

The study of the degradation of slats shows that *P. noxius*, just as *R. lignosus*, is capable of degrading lignin. Both pathogens are thus white rot fungi. In this framework, the comparison of the behaviour of these two fungi with that of *Antropodia sp.* , a known brown rot agent, is particularly illustrative. Lignin assays showed the tendency of *R. lignosus* to preferentially degrade lignin, while *P. noxius* preferentially degrades the polysaccharide fraction. Finally, both fungi also cause considerable changes in the residual lignin by modifying the monomer composition of the polymer.

In vivo and in comparison to healthy tissues, there is a significant increase in the lignin content of infested tissues removed from the front of *R. lignosus* progression. It would appear that *Hevea* reacts by an increased synthesis of lignin. This reaction is ineffective, since the pathogen ultimately degrades the polymer. Such reaction is much less evident in case of attack by *P. noxius*. In terms of the monomer composition of lignin, there are changes in the tissues colonized for the longest times, comparable to that observed *in vitro*.

Résumé

Biodégradation du bois d'Hevea brasiliensis par Rigidoporus lignosus et Phellinus noxius

L'étude de la dégradation de bûchettes d'Hévéa montre que *P. noxius* est capable, tout comme *R. lignosus*, de dégrader la lignine. Les deux agents pathogènes sont donc à classer parmi les agents de pourriture blanche du bois. Dans cette optique, la comparaison entre le comportement de ces deux champignons et celui d'*Antrodia sp.*, agent de pourriture brune, est particulièrement démonstrative. Cependant les dosages de lignine mettent en évidence une tendance de la part de *R. lignosus*, à dégrader préférentiellement la lignine, alors que la tendance inverse, dégradation préférentielle de la fraction polysaccharidique, est observée pour *P. noxius*. Enfin, ces deux champignons provoquent une forte altération de la lignine résiduelle qui se traduit par une modification de la composition monomérique du polymère.

In vivo, et par rapport aux tissus sains, on observe une augmentation significative du taux de lignine dans les tissus infectés prélevés au niveau du front de progression de *R. lignosus*. Il semblerait que l'Hévéa réagisse par une synthèse accrue de lignine. Cette réaction n'est cependant pas efficace, puisque l'agent pathogène finit par dégrader le polymère. Cette manifestation est beaucoup moins nette en cas d'attaque par *P. noxius*. Au plan de la composition monomérique des lignines, on note une altération au niveau des tissus les plus anciennement colonisés. Cette modification est comparable à celle enregistrée *in vitro* et conduit à penser que, dans ces tissus les deux parasites adoptent un comportement purement saprophytique.

Zusammenfassung

Holzabbau bei Hevea brasiliensis durch Rigidoporus lignosus und Phellinus noxius

Die Untersuchung des Abbaus von Holzspänen zeigt, daß *P. noxius* wie *R. lignosus* in der Lage ist, Lignin abzubauen. Beide Pathogene sind demnach Weißfäuleerreger. Unter diesem Gesichtspunkt ist der Vergleich der Eigenschaften beider Pilze mit denen von *Antrodia* sp., einem bekannten Braunfäuleerreger, besonders aussagefähig. Versuche mit Lignin zeigten bei *R. lignosus* die Tendenz, vorzugsweise Lignin abzubauen, während *P. noxius* hauptsächlich die Polysaccharidfraktion

abbaut. Beide Pilze bewirken auch deutliche Veränderungen im Restlignin, indem die Monomeren-zusammensetzung des Polymers verändert wird.

In vivo zeigt sich im Vergleich zu gesundem Gewebe bei infiziertem Gewebe aus dem Bereich des vordringenden *R. lignosus*-Myzels ein deutlicher Anstieg des Ligningehaltes. Es scheint, als ob *Hevea* mit erhöhter Ligninproduktion auf den Angriff reagiert. Diese Reaktion ist allerdings un-wirksam, da das Pathogen letztlich das Polymer abbaut. Solch eine Reaktion ist bei der Besiedlung durch *P. noxius* sehr viel weniger deutlich. Hinsichtlich der Monomerenzusammensetzung des Li-gnins gibt es Veränderungen in den am längsten besiedelten Geweben, die mit den *in vitro* beobach-teten vergleichbar sind.

References

ANDER, P.; ERIKSSON, K. E., 1975: Influence of carbohydrates on lignin degradation by the white-rot fungus *Sporotrichum pulverulentum*. Sven. Papperstidn. **78**, 643–652.

– – 1976: The importance of phenol oxidase activity in lignin degradation. Arch. Microbiol. **109**, 1–8.

– – 1977: Selective degradation of wood components by white-rot fungi. Physiol. Plant. **41**, 239–248.

ASADA, Y.; MATSUMOTO, I., 1969: Formation of lignin-like substance in the root tissues of japanese radish plant infected by downy mildew fungus. Ann. Phytopath. Soc. Japan **35**, 3, 159–167.

– – 1972: The nature of lignin obtained from downy mildew infected japanese radish root. Phytopath. Z. **73**, 208–214.

COWLING, E. B., 1961: Comparative biochemistry of the decay of sweetgum sapwood by white-rot and brown rot fungi. U. S. Department of Agriculture, Technical Bulletin No. 1258, 79p.

CRAWFORD, D. L.; CRAWFORD, R. L. 1976: Microbial degradation of lignocellulose: the lignin com-ponent. Appl. Environ. Microbiol. **31**, 714–717.

GEIGER, J. P.; HUGUENIN, B., 1981: La peroxydase des tissus racinaires d'Hévéa parasités par *R. lignosus*: origine et rôle physiologique potentiel. Proceed. Congrès sur la Protection des Cultu-res Tropicales. Lyon.

GEIGER, J. P.; HUGUENIN, B.; NANDRIS, D.; NICOLE, M., 1983 a: Effect of an extracellular laccase of *Rigidoporus lignosus* on *Hevea brasiliensis* lignin. Proceed. lignocellulose biodegradation con-ference, Littlehampton. Summary in Appl. Biochem. Biotechnol. (1984), **9**, 359–360.

GEIGER, J. P.; HUGUENIN, B.; NICOLE, M.; NANDRIS, D., 1986 a: Laccases of *Rigidoporus lignosus* and *Phellinus noxius*. II. Effect of *R. lignosus* laccase L1 on thioglycolic lignin of Hevea. Appl. Biochem. Biotechnol. (in press).

GEIGER, J. P.; NANDRIS, D.; GOUJON, M., 1976: Activité des laccases et des peroxydases au sein des racines d'Hévéa attaquées par le pourridié blanc [*Leptoporus lignosus* (Kl.) Heim]. Physiol. Vég. **14**, 271–282.

GEIGER, J. P.; NANDRIS, D.; NICOLE, M.; HUGUENIN, B., 1983 b: Comparative studies of rubber root rot caused by *Rigidoporus lignosus* and *Phellinus noxius* in Ivory Coast. Proceed. 6th Con-gress of IUFRO, Melbourne. KILE, G. A. (ed.), 407–408.

GEIGER, J. P.; NICOLE, M.; NANDRIS, D.; RIO, B., 1986 b: Root rot diseases of *Hevea brasiliensis*. I. Physiological and biochemical aspects of host aggression. Eur. J. For. Path. **16**, 22–37.

ISHIHARA, T., 1980: The role of laccase in lignin biodegradation. In: Lignin Biodegradation: micro-biology, chemistry and potential applications. Vol. II. KIRK, T. K.; HIGUCHI, T.; CHANG, H. M. (eds.), C. R. C. Press. 17–31.

ISHIHARA, T.; MIYAZAKI, M., 1972: Oxidation of milled wood lignin by fungal laccase. Mokuzaï Gakokaishi **18**, 415.

– – 1974: Demethylation of lignin and lignin models by fungal laccase. Mokuzaï Gakokaishi **20**, 39.

KERN, H., 1983: Action of the white rot fungus *Sporotrichum pulverulentum* on lignosulfonates. Holzforschung **37**, 287–292.

KIMMINS, W. C.; WUDDAH, D., 1977: Hypersensitive resistance: Determination of lignin in leaves with a localized virus infection. Phytopathology **67**, 1012–1016.

KIRK, T. K., 1971: Effects of microorganisms on lignin. Ann. Rev. Phytopathology **9**, 185–210.

KIRK, T. K.; CONNORS, W. T.; ZEIKUS, J. G., 1976: Requirement for a growth substrate during lignin decomposition by two wood-rotting fungi. Appl. Environ. Microbiol. **32**, 192–194.

KIRK, T. K.; HARKIN, T. M.; COWLING, E. B., 1968 a: Oxidation of guaïacyl- and veratrylglycerol-β-guaïacyl ether by *Polyporus versicolor* and *Sternum frustulatum*. Biochim. Biophys. Acta **165**, 134–144.

KIRK, T. K.; HARKIN, J. M.; COWLING, E. B., 1968 b: Degradation of the lignin model compound syringylglycol-β-guaïcyl ether by *Polyporus versicolor* and *Stereum frustulatum*. Biochim. Biophys. Acta **165**, 145–163.

KIRK, T. K.; HIGHLEY, T. L., 1973: Quantitative changes in structural components of conifer woods during decay by white- and brown-rot fungi. Phytopathology **63**, 1338–1342.

KIRK, T. K.; KELMAN, A., 1965: Lignin degradation as related to the phenoloxidases of selected wood-destroying Basidiomycetes. Phytopathology **55**, 739–745.

NANDRIS, D.; NICOLE, M.; GEIGER, J. P., 1983: Infections artificielles de jeunes plantules d'*Hevea brasiliensis* par *Rigidoporus lignosus* (Kl.) Imaz. et *Phellinus noxius* (Corner) G. H. Cunn. Eur. J. For. Path. 13, 65–76.

NICOLE, M.; NANDRIS, D.; GEIGER, J. P.; RIO, B., 1985: Variability among African populations of *Rigidoporus lignosus* and *Phellinus noxius*. Eur. J. For. Path. 15, 293–300.

NICOLE, M.; GEIGER, J. P.; NANDRIS, D., 1986: Root rot disease of *Hevea brasiliensis*. II. Some host reactions. Eur. J. For. Path. 37–55.

PICHEL, R. J., 1956: Les pourridiés de l'Hévéa dans la cuvette congolaise. INEAC, Sér. Techn. 49, 480 pp.

REYNOLDS, E. S., 1963: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. cell. Biol. 7, 208–212.

RIDE, J. P., 1978: The role of cell wall alterations in resistance to fungi. Ann. Appl. Biol. 891, 302–305.

ROGER, L., 1954: Phytopathologie des pays chauds. T. III, LECHEVALIER, P. (ed.). Paris.

SCHUBERT, W. J., 1956: Lignin Biochemistry. New York: Academic Press.

SHIGO, A. L.; MARX, M., 1977: Compartimentalization of decay in trees (CODIT). U. S. Dept. Agriculture. Agric. Inf. Bull. 405, 73 pp.

Authors' address: ORSTOM, Centre d'Adiopodoumé, Laboratoire de Phytopathologie, B.P. V-51, Abidjan, Côte d'Ivoire