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Root rot diseases of Hevea brasiliensis

II. Some host reactions[1]

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Abstract

Hevea brasiliensis (rubber tree) differentiates defense reactions against root rot diseases caused by *Rigidoporus lignosus* and *Phellinus noxius*. These reactions, described at molecular, cellular and histological levels, rarely enable the tree to resist the fungal attack. Trees which initiate new tissues only survive to the infection.

1 Introduction

The severity of attack on the root system of ligneous varieties caused by root rot fungi are sufficient justification for the actions undertaken to combat this disease. The complexity of these diseases and the medium in which the parasite lives are unfavorable for elaborating a combat strategy which is capable of effectively eradicating these infections. Thus, the tree is often left to defend itself and the reactions it develops, not only at the molecular level (Shain 1967, 1971; Hoque 1982), but also at the cell and tissue levels (Shigo and Marx 1977), occasionally enable it to survive.

Hevea brasiliensis, grown in humid tropical climates for rubber production, is one of the examples illustrating the above consideration. In West Africa, especially in the Ivory Coast, certain rubber plantations are ravaged by *Rigidoporus lignosus* and *Phellinus noxius*, two Basidiomycetes responsible for white root rots (Martin and Duplessix 1965; Nandris et al. 1981, 1983 b). A less severe form is the collar canker caused by an Ascomycete, *Sphaerostilbe repens*. To our knowledge, no *H. brasiliensis* clone transferred to Africa has been recognized to be resistant to these diseases. At the individual level, on the

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other hand, some trees tolerate the presence of the fungi in their roots for longer periods of time than others. This is explained either by the variability of pathogenicity which exists in *R. lignosus* and *P. noxius* populations (NANDRIS et al. 1985; NICOLE et al. 1985) or by reactivity capacities which vary from one individual to another. In the latter case, the trees differentiate new roots to compensate for the loss of their tap root (NICOLE et al. 1981, 1982 a). This loss results primarily from the degradation of cell structures (NICOLE et al. 1982 a, b; GEIGER et al. 1983 a) caused by fungal enzymes excreted into the host tissues (GEIGER et al. 1976, 1983 b, 1984; GEIGER and HUGUENIN 1981). Throughout, during the infection, from penetration up to an advanced stage of the disease, rubber trees exhibit defense reactions.

This publication will describe the most typical host reactions elaborated for defense and define their role in the pathogenic process.

2 Materials and methods

2.1 Pathogen material and inoculum

Isolates of *R. lignosus* (strain 1) and of *P. noxius* (strain 4) were collected in 1978 on *H. brasiliensis*, naturally infected in plantation.

Sticks of rubber tree wood (8 cm long by 2 cm diam.) were sterilized in Roux flasks (1l), then seeded with 8 mycelial implants issued from a 2 % malt-agar culture of each fungus. Because of the different degradative abilities of the two parasites, the incubation before plant inoculation lasted 5 months for *P. noxius* and 11 months for *R. lignosus*. Such infected segments constituted the inoculum.

2.2 Artificial infection

The followed methodology was developed under greenhouse conditions by NANDRIS et al. (1983 a). Seeds of *H. brasiliensis* (clône GT1) were collected in IRCA plantation. After germination in sandy tubs, young seedlings were pricked in tubs ($1 \times 1 \times 1$ m) filled up with forest soil of which high humidity level was monitored by watering to saturation. The control of this level was realized with a neutronic moisture gauge (Solo 20).

For each one-month-old plant, 5 inoculum segments were applied against the tap root, 20 cm deep in the soil. Diseased plants were then collected at different stages of infection for light and electron microscopy preparation.

2.3 Microscopy observations

2.3.1 Light microscopy

Segments of root were fixed in an aceton-alcohol-formol solution (1/1/3 vol.), dehydrated in a gradual ethanol series and embedded in paraffin (60 °C). A rotary microtome was used for sectioning the blocks. After removing paraffin, sections were stained either in picroaniline blue, or in phloroglucinol for lignin visualisation. An aqueous solution of aniline blue (1 %) was used for observation by fluorescence microscopy.

2.3.2 Electron microscopy

Classical technics of plant preparation for electron microscopy (HALL 1978) were modified and adapted to rubber root tissues as followed.

At first, all the infected plants were immediately fixed "*in toto*" in a solution of glutaraldehyde (3 %), 2 h at 4 °C, buffered with 0.1 M sodium cacodylate (pH 7.2). The

root was then cut in small segments and fixed in the same solution, 4 h at 4°C. After several washing in buffer (4 × 15 mn and one night), the samples were postfixed (2 h at 4°C) in 1% osmium tetroxid. Following a further rinse in cacodylate sodium, the segments were dehydrated in a gradual ethanol series from 5 to 100 %, infiltrated and embedded in Spurr resin (SPURR 1969) or in Epon 812 preceded by an epoxy propane change (2 × 1 h duration) (LUFT 1961). Because of the woody nature of roots, the pieces were infiltrated in resin at least 5 days. Polymerization was carried out 3 days at 70°C. Sections of 50–60 nm were cut with glass or diamond knives on a LKB ultramicrotome, and mounted on 200 mesh grids. After staining with a satured alcohol solution of uranyle acetate (5 mn), followed by lead citrate (REYNOLDS 1963), the sections were examined on a Siemens Elmiskop 102 electron microscope, operating at 80 kv.

2.4 Enzyme assay

2.4.1 Extraction of soluble peroxidases

The peroxidases are extracted from healthy or infected tissues both from artificially infected seedlings and from adult rubber tree tap roots naturally infected in plantation. For this purpose the tissues are grinded in a wood grinder (Gondar) into sawdust which will macerate (12 hours at 4°C) in a sodium phosphate buffer (0,0125 M pH 6; 5 ml/g fresh weight sawdust). This macerate is then filtrated (glass filter funnel porosity 2) and the filtrate centrifuged 15 minutes at 20000 g (Sorval refrigerated centrifuge RC 2B).

2.4.2 Peroxidase assay

The peroxidase activity is determined using guaiacol and hydrogen peroxide as substrate according to GEIGER et al. (1976). The activity is expressed as Units/ml enzymatic solution. One Unit is the volume of solution (or the amount of proteins) that causes an increase of 0.001 O.D. 420 nm/mn.

2.5 Electrofocusing experiments

The isoperoxidases are fractionated on a LKB preparative electrofocusing column (total volume 110 ml) using a mixture of two types of Ampholine carrior ampholites ranging respectively pH 2.5 to 4.0 and 3.5 to 10.0 in order to produce a pH gradient ranging 2.5 to 10.0. It is stabilised by being incorporated in a 0 to 40 % sucrose density gradient. The enzyme sample [0.5 to 1 ml of a concentrated (Amicon PM10 ultrafiltrer) extract in 20 % sucrose] is applied as a narrow zone at the 20 % sucrose level of the gradient. The pH gradient equilibrium is reached after 3 days under a voltage of 400 V. About 110 fractions of 1 ml are then collected. The pH and peroxidase activity of each second fraction is determined.

3 Results

Microscopic observations

3.1 Early reactions

Beginning with contamination of the plants and the initial stages of parasite penetration, rubber trees initiate a number of reactions which can be observed under the microscope.

3.1.1 Cellular hyperplasia and hypertrophy

This reaction is encountered in the majority of plants infested by the parasites. There is initially a hyperplasia phenomenon, marked by cell anticlinic and periclinic barriers

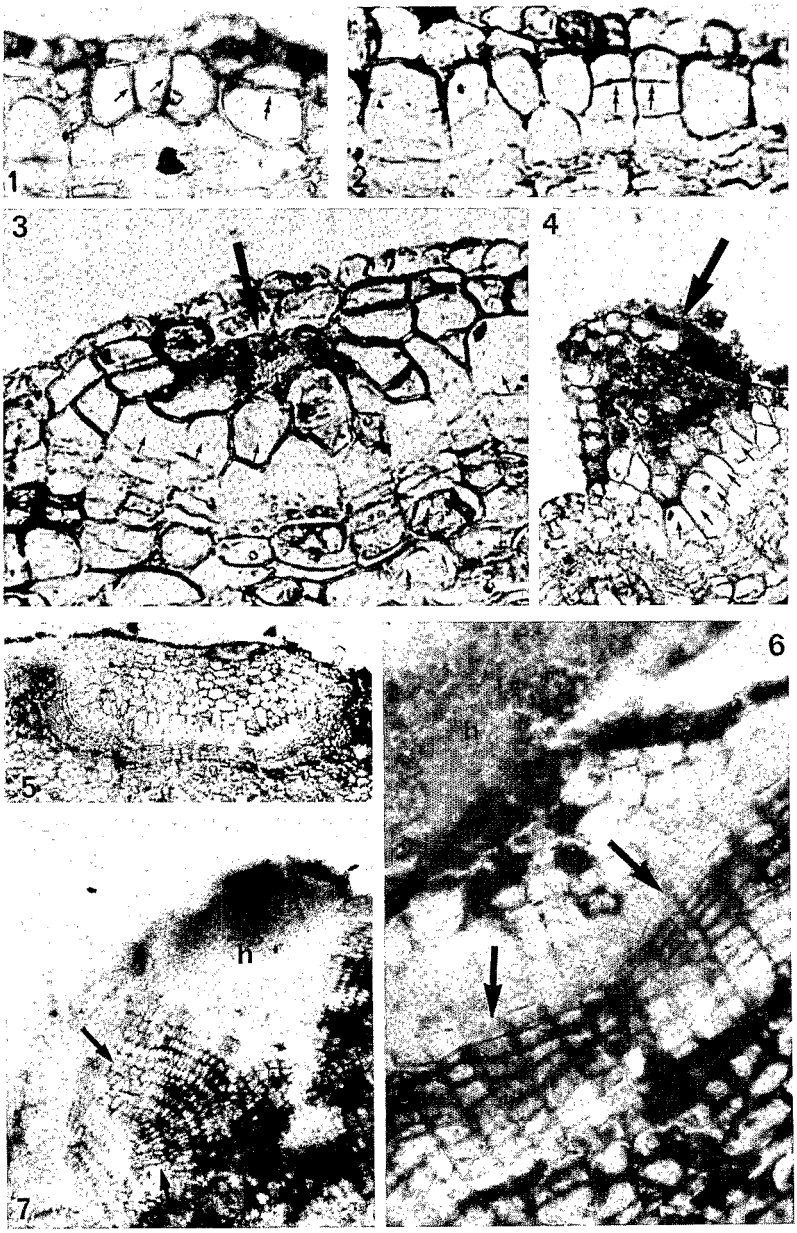


Fig. 1 and 2. Cellular hyperplasia: anticlinic (arrows) and periclinic (double arrows) divisions of some cells occur during the penetration of the first cell layers: (× 250). – *Fig. 3 and 4.* Cellular hypertrophy: after division, some cells increase their volume (small arrows). Cellular hyperplasia and hypertrophy often surround one or several necrotic cells (large arrows); (Fig. 3: × 250; Fig. 4: × 150). – *Fig. 5 and 6.* The cellular modifications of the first cell layers often lead to nodule formation easily perceptible on the root. They exert a pressure (arrows) on the neighbor cells; (Fig. 5: × 40; Fig. 6: × 100; h: hyphae). – *Fig. 7.* The increase in the number of cell layers under the penetration sites results from the stimulation of the phellogen activity (arrows); (h: hyphae; × 150)

(Fig. 1, 2). Subsequently there occurs a cellular hypertrophy process in combination: several cells increase their volume (Fig. 3, 4) and lead to the formation of cell clumps with varying degrees of organization (Fig. 5). These clumps are visible under low magnification. Figure 6 also shows the pressure exerted by the hypertrophied cells on their underlying neighbors. This phenomenon is very often crystallized around one or several necrotic cells and attempts to isolate the first hyphae (Fig. 4).

3.1.2 Stimulation of the phenolic pool and peroxidase activities

Using cytochemical tests, it has been shown that the phenolic pool and peroxidase activities are stimulated, especially under the points of fungal penetration. The xylem also manifests a positive reaction to the two tests, especially in the parenchymatous rays. No quantitative value, however, can be attributed to this result because of the high intensity of the reactions and their constancy during the infection.

3.2 Post-initial reactions

These reactions occur during the penetration and invasion of the first cell layers by the parasites.

3.2.1 At the histological level

One of the most consistent reactions of *Hevea* to attack by *R. lignosus* and *P. noxius* is the increase in the number of cell layers under the points of penetration (Fig. 7). This results from increased activity of the cork-phelloderm-generating layer, either towards the cork or towards the phelloderm. In both cases, the number of cell layers varies from 1 to 4 and may reach the ratio of 1 to 8. The ensemble of these cell layers often forms "nodules", visible to the naked eye, generating swellings on the root surface.

3.2.2 At the cellular level

In the apical region of the root of very young plants, where the cork cambium is not yet active, the walls of some cells undergo considerable thickening. The reaction is most spectacular in the primary cortical parenchyma (Fig. 8). Although the thickness of these walls varies, it may reach 3 μm. The deposits observed in this figure apparently result from the cell lumens and involve only the walls oriented towards the parasite. Attempts to identify the nature of these thickenings have shown that there is a positive reaction to the test for phloroglucinol.

This change in wall architecture has also been observed in the oldest tissues in the supernumerary cork layers. In this case, there is a positive test to Soudan III B; that of lignins, however, is not conclusive.

3.3 Reactions occurring during tissues colonization

The means employed at this stage by the plant to slow the progress of the parasite are observed primarily at the cellular level, principally in the walls. All the root tissues are involved in these reactions.

3.3.1 Cork

Light microscopy of young cork contaminated by *R. lignosus* shows that wall morphology undergoes extensive modification (Fig. 9). Some are highly scalloped, giving them a notched appearance. This observation is confirmed by fluorescence microscopy of the same tissues impregnated with aniline blue for detecting callose (Fig. 10). These fluores-

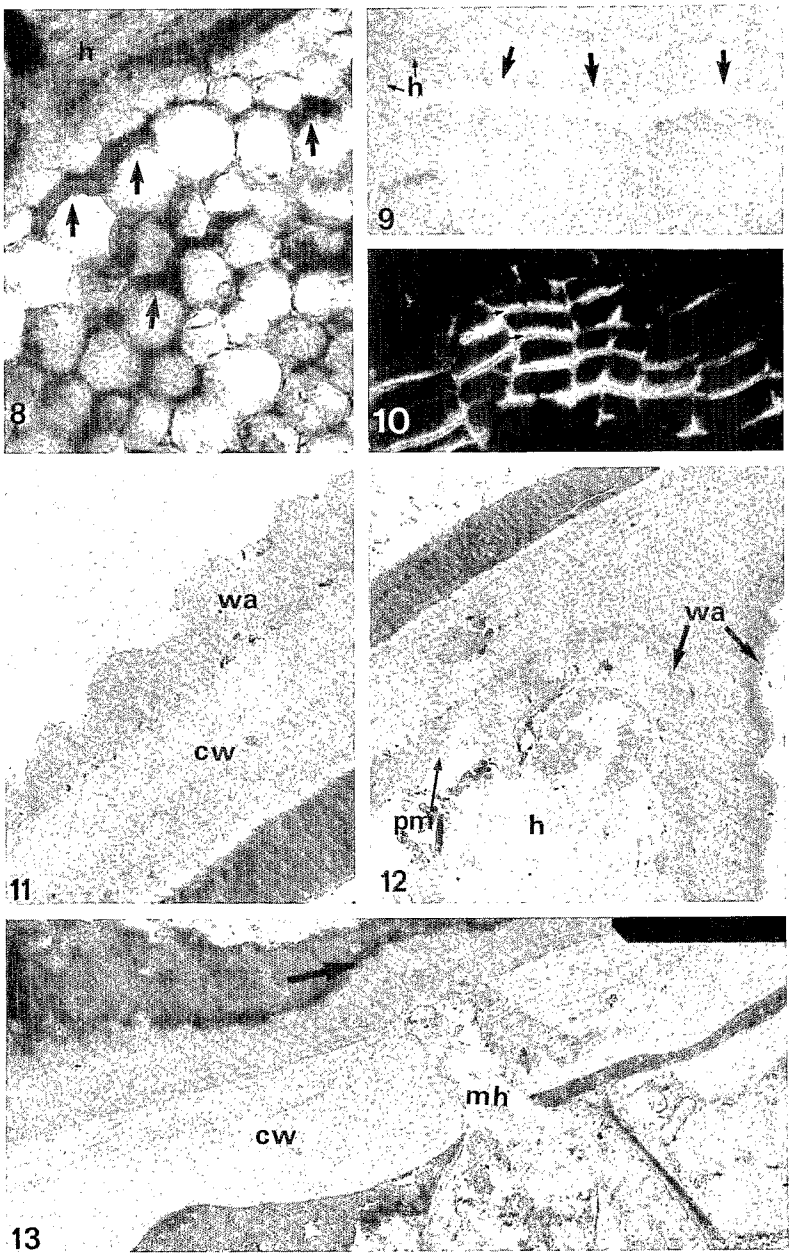


Fig. 8. The thickening of cell walls (arrows) in a young root can develop in the primary parenchyme. In most cases, this modification takes place near the penetration sites and corresponds to a lignification of the cellulosic wall; (h: hyphae: × 250). – *Fig. 9 and 10.* Light microscopy observations show cell walls transformation of the young bark (arrows) (Fig. 9) which fluorescence, under UV light (Fig. 10), differs from cell wall fluorescence of a non infected bark; (h: hyphae; Fig. 9: × 1000; Fig. 10: × 250). – *Fig. 11.* These wall transformations correspond to wall appositions (wa) which have a granular texture; (cw: cell wall; × 20000). – *Fig. 12.* Localization of wall appositions (wa) in the periplasmic space; (pm: plasmamembrane; h: degenerated hyphae; × 11000). – *Fig. 13.* A slight excrescence (arrow) is observed in front of a mycelial filament (mh) which degrades the cell wall (cw); (× 16000)

cent structures, whose emitted light is much more intense than that emitted by cork, is deposited in the course of the infection; healthy tissues are lacking.

Electron microscopy shows these papillae to be electron dense; their organization is granular rather than fibrillar (Fig. 11). Moreover, they are localized in the periplasmic space between the plasma membrane and the wall (Fig. 12). Careful examination of sections suggests that these neoformations are induced by the hyphae. A slight excrescence is in fact observed in front of the mycelial filaments (Fig. 13). This swelling can subsequently thicken to a considerable extent, but these papillae do not resist the parasite, which is capable of partially or totally degrading them (Fig. 14, 15).

3.3.2 Phloem

The only reaction observed in this tissue is the blocking of the sieve tubes. The natural perforations of the sieve tubes can be blocked by slightly osmiophilic masses composed of a matrix which is generally granular (Fig. 16, 17). It appears to be continuous with the fibres of the preexisting wall. Fluorescence light microscopy of the phloem shows that some of the tubes emit green fluorescence, identical to that emitted by callose.

3.3.3 Xylem

Two wall reactions have been noted:

- the tyloses have a swollen appearance and form on fraction S_3 of the secondary wall (Fig. 18);
- a new parietal layer: light microscopy shows that numerous parasitized xylem vessels present deposits on the walls which react positively to tests for lignin (Fig. 19). Electron microscopy shows these formations clearly as a layer superimposed on the secondary wall (Fig. 20). This "wall" is either continuous with irregular contours or else discontinuous. Its granular texture appears different from the other walls and the middle lamella, except where in contact with the S_3 fraction, where fibrils can be distinguished (Fig. 21).

3.4 Late reactions

Some roots very rarely undergo histological changes which totally modify their anatomical organization. *Hevea* can in fact elaborate new tissues, ligneous or not, which the parasites do not contaminate. Figures 22 and 23 represent a transversal section of just such a root, where two types of neoformed tissues can be distinguished, those of a meristematic nature and wood-and-phloem tissues.

3.4.1 Elaboration of cell foci of meristematic nature
(Fig. 22, 23; annotation 5 and 6)

These tissues arise from the parenchymatous rays of the xylem (annotation 6) (Fig. 24), which differentiate and form cellular masses (annotation 5). They are initially composed of several cells and subsequently form dense foci (annotation 5) whose organization is not unlike that of a meristem. The center of these foci in fact contains a focus of cell division around which the newly formed cells are organized (Fig. 25). The latter have a dense cytoplasma, a large nucleus and a high nuclear/cytoplasmic ratio, suggesting an intense metabolic activity.

3.4.2 Newly formed wood-and-phloem tissues

Concomitantly with the formation of the above foci, the root differentiates a vascular neocambium which is recognized as several layers of flattened and stacked cells. It appears

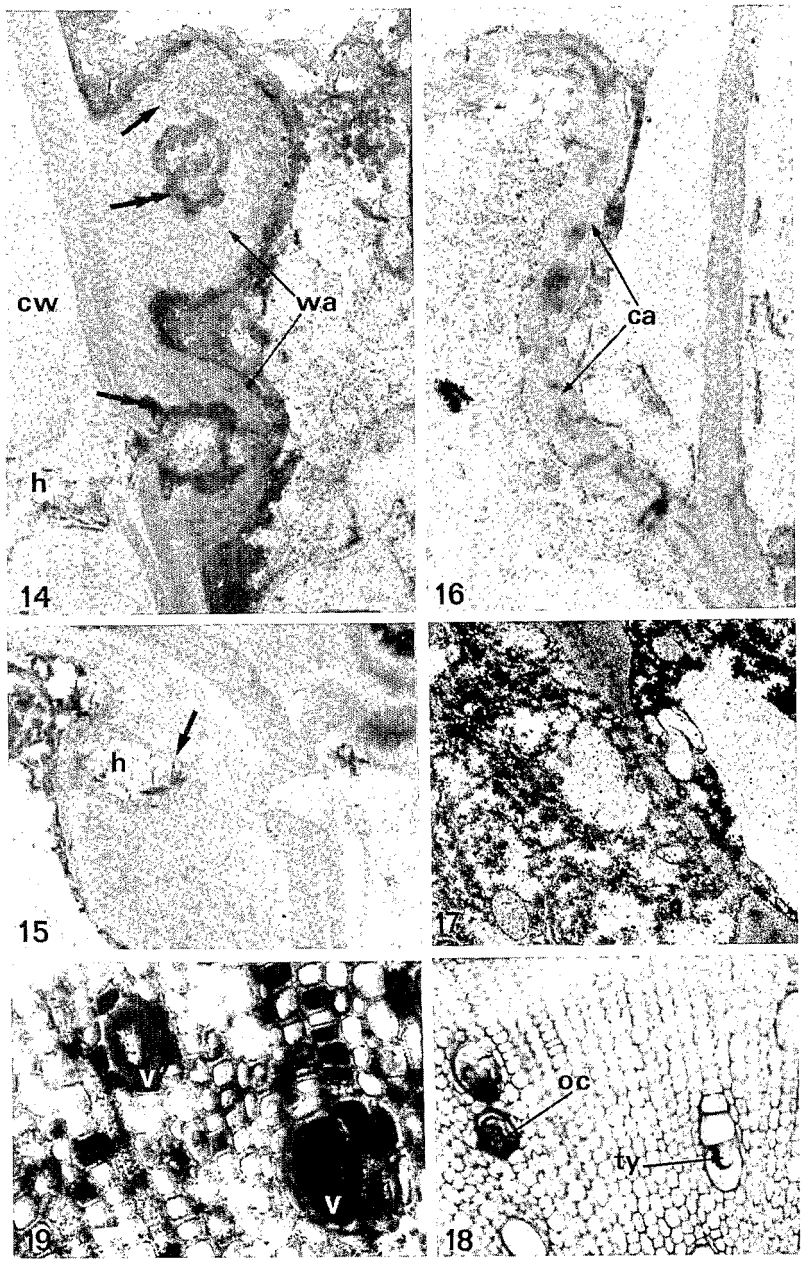


Fig. 14. The parasite can degrade these wall-like depositions which appear then more osmiophilic (double arrows) and eroded (arrows); (cw: host cell wall; h: hyphae; wa: wall apposition; × 16000). – *Fig. 15.* Degradation of wall appositions is observed in contact with the fungus (h) (arrows); (× 13000). – *Fig. 16.* Callose-like depositions (ca) occluding pores of a phloem sieve tube (× 15000). – *Fig. 17.* Section on healthy phloem; no deposition is observed on the wall sieve tube (× 10000). – *Fig. 18.* Development of tyloses (ty) in vessels of the infected xylem; (oc: occluded vessel; × 100). – *Fig. 19.* Observations of necrosed xylem show opaque material deposited along the wall of some vessels (v); (× 250; phloroglucinol coloration for lignin visualisation)

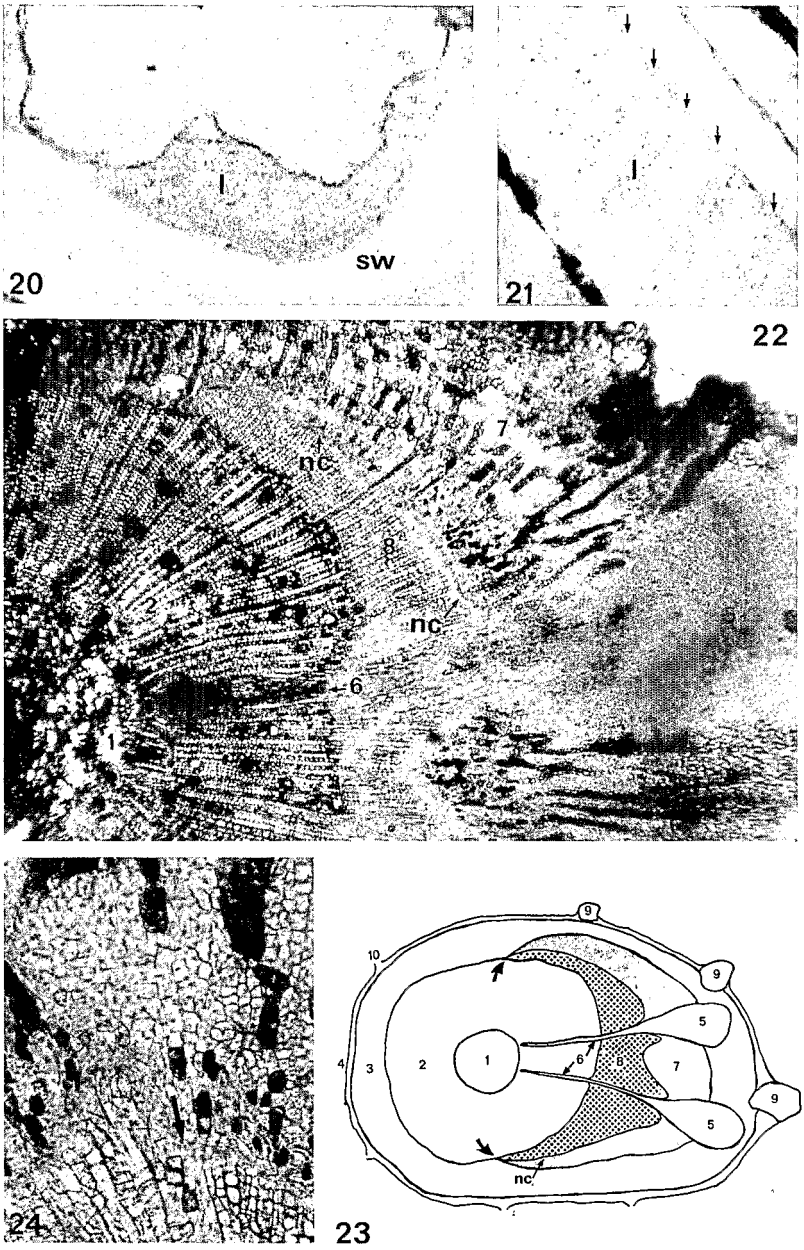


Fig. 20. This wall-like deposition appears as a layer (l) formed on the secondary cell wall (sw); (× 12500). – *Fig. 21.* The contact of this layer with the secondary wall shows fibrils orientated perpendicularly to the S_3 fraction (arrows); (× 25000). – *Fig. 22 and 23.* Tissular neogenesis in a root of a young rubber tree artificially infected with *R. lignosus*. Meristematic tissues (5) are initiated from parenchymatous rays (6) of the necrosed xylem (2). A new vascular cambium (nc) is formed from the old one (arrows), which is probably killed. New tissues, reacting as a barrier to decay, are then differentiated (7 and 8)

1: medullar parenchyme; 2: infected xylem; 3: infected phloem; 4: periderm; 5: meristematic tissues; 6: parenchymatous rays; 7: new phloem; 8: new xylem; 9: *R. lignosus* rhizomorphs; 10: lenticells; (Fig. 22: × 40)

Fig. 24. Small unorganized cellular masses are initiated from medullary rays (arrow) (× 100)

to be initiated from the former cambium (Fig. 22) and invaginates in proximity to the meristematic layers. On both sides it produces (Fig. 26):

- xylem, whose often contorted cells have thick walls, occasionally with many punctate formations (Fig. 28). The parenchymatous rays are continuous with those of the secondary xylem (Fig. 27). The cells, as those of the cellular masses, have a very dense cytoplasm, reflecting an unmistakable metabolic activity;
- phloem in which tannin cells, several latex cells, tubes and other cells are distributed.

This process of tissue neogenesis thus leads to the installation of new tissues which isolate the diseased tissues from the rest of the root (Fig. 29). It is often observed that new lateral roots arise from these tissues (Fig. 29, 30). Adult trees naturally infected in plantations also initiate such tissues (Fig. 31). The scarring excrescences they differentiate tend to cover the tissues infected by both *R. lignosus* and *S. repens* (Fig. 32, 33).

The mechanisms described in young artificially infected plants and in older naturally infected trees are similar. Neither of the new tissues formed are contaminated by either parasite.

3.5 Biochemical analysis

Metabolic disturbances, different from those occurring during tissue degradation, accompany both the infectious process and the reaction tissue neogenesis described above. The most important of these changes involves the isoperoxidase spectrum.

There are two levels for viewing this phenomenon:

– quantitatively, there exists a varying degree of increased peroxidase activity (PA) in infected or newly formed tissues in comparison to healthy tissue. In the tap root of an adult infected with *R. lignosus*, this activity can reach or even exceed a factor of 10 in tissues removed from the front of parasite progression. Only tissues colonized for longer periods exhibit merely low PA;

– qualitatively, the soluble isoperoxidase spectrum undergoes a total change. Figure 34 shows a considerable increase in one enzyme species (called P_2) in both parasitized and reaction tissues. In some cases, preexisting activities in the tissues are absent.

Calculations show that P_2 activity increases 190-fold in the best case. These calculations are based on one hand on the overall PA increase in a tissue parasitized by *R. lignosus* in comparison to that of healthy tissues before the front of parasite progression, and on the other hand on the ratio of isoenzyme P_2 to all the other isoenzymes. This increase occurs in tissues attacked by *R. lignosus* or *P. noxius*, as well as in the reaction tissues induced by *R. lignosus* or *S. repens*. This host reaction is thus aspecific.

Isoenzyme P_2 is synthesized by the host and not the parasite. The enzyme species extracted from parasitized tissues is identical to that extracted from reaction tissues, in terms of both its amino acid composition and trypsin fingerprints (GEIGER and HUGUENIN 1981). We were also able to show that the increase in P_2A resulted from new protein synthesis, although this synthesis is more restricted in tissues infected with *P. noxius*. This is probably due to the rapid colonization of *Hevea* tissues and so the plant has relatively little time to react.

The increased PA in parasitized tissues of young artificially infected plants involves not only P_2, but also other preexisting isoperoxidases as well. In addition, the results obtained are highly variable and this variation is probably to be ascribed to a more active metabolism in young tissues, explaining why they respond more rapidly to external stimuli.

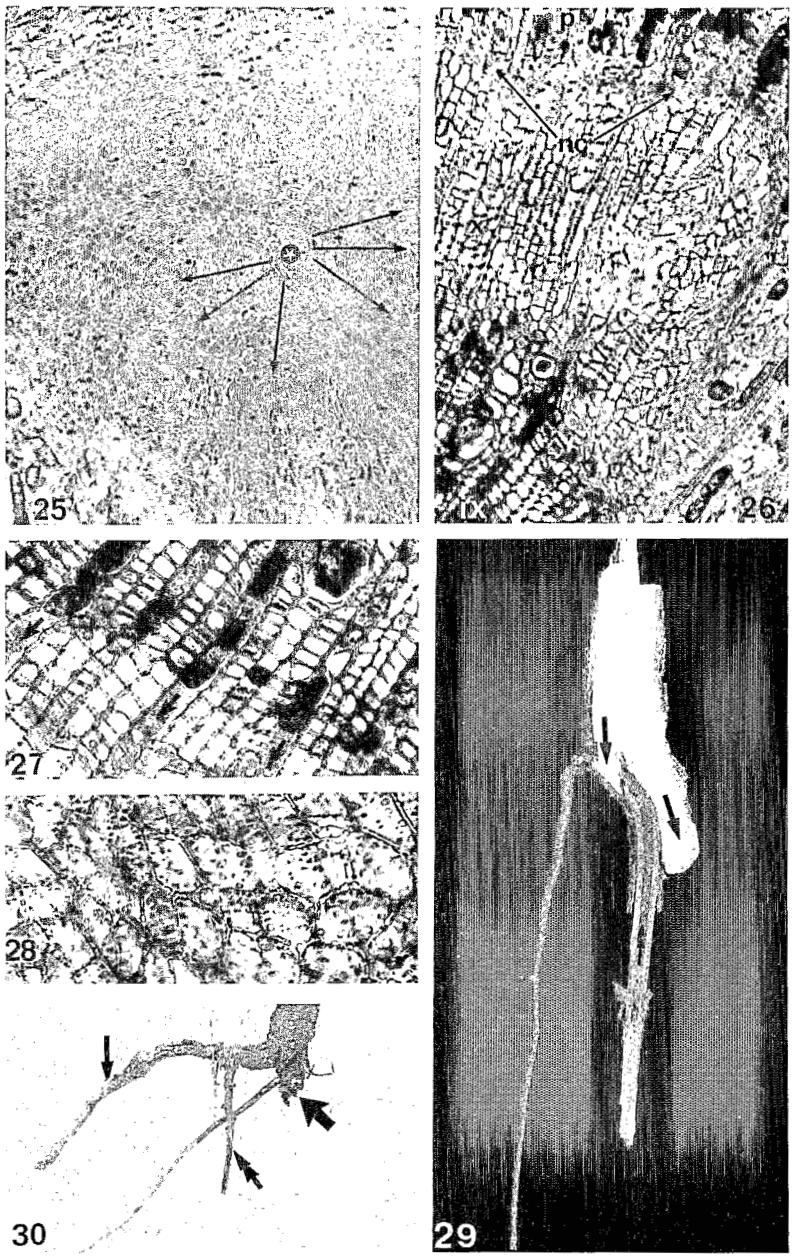
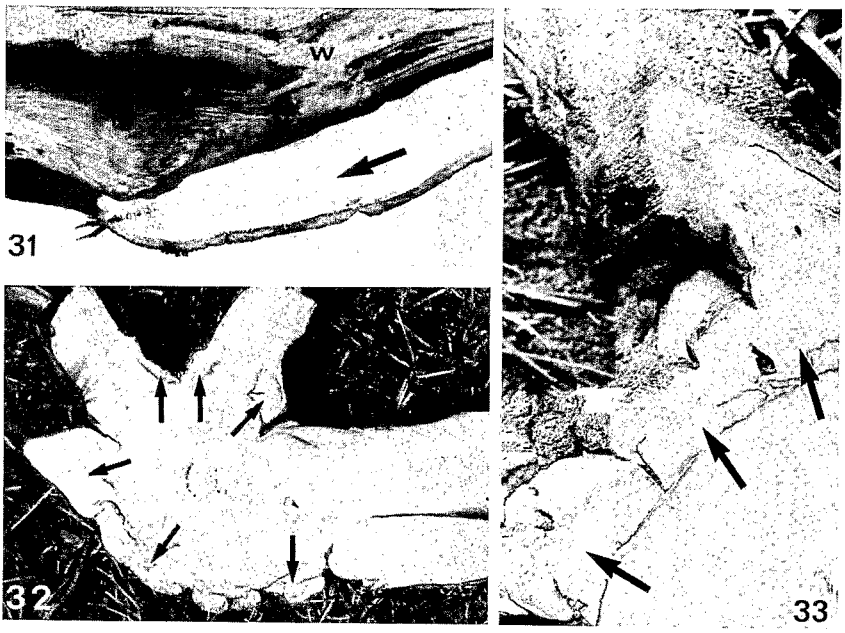


Fig. 25. The organization of this tissue is like that of a meristem; cells are small, with a large nucleus and a dense cytoplasm; a focus of cellular division is also perceptible (arrows); (× 500). – *Fig. 26.* Xylem (x) and phloem-like (p) tissues are the differentiated tissues from the new vascular cambium (nc); (ix: infected xylem; × 100). – *Fig. 27.* Medullary rays newly differentiated are in continuity with those of the decayed xylem (arrows); (× 250). – *Fig. 28.* The numerous pits of cells of the new xylem are well perceptible on this photography; (× 150). – *Fig. 29.* Macrophotograph of a longitudinal section in a tap root of a young rubber tree artificially infected with *R. lignosus*. New tissues (arrows) surrounding the decayed tap root (double arrow) are not contamined by the fungus. – *Fig. 30.* Lateral root (arrow) and new tap root (double arrow) are formed from the differentiated xylem (large arrow: initially decayed tap root)



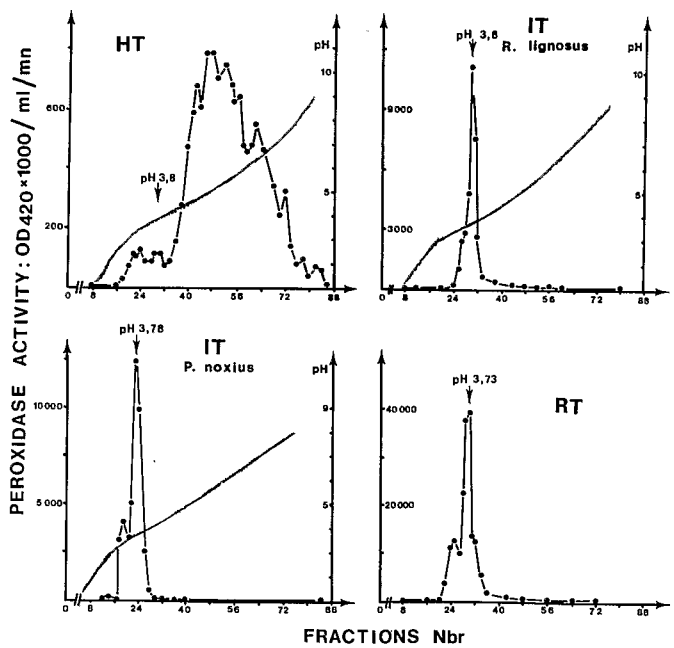


Fig. 31. Longitudinal section in a mature rubber tree infected with *R. lignosus*. Newly differentiated tissues (arrow) surrounded the decayed wood (w). – *Fig. 32 and 33.* Cross section in a mature tree tap root infected with *S. repens*. The tissue neogenesis process induces new bark and xylem to prevent fungal invasion (arrows). – *Fig. 34.* Electrofocusing diagrams of isoperoxidases respectively extracted from healthy (HT) reaction (RT) and infected (IT) tissues (adult rubber tree tap roots). The latter ones are colonized either by *P. noxius* or *R. lignosus*. The diagrams show the qualitative and aspecific changes occurring after infection by *P. noxius* and *R. lignosus* and host reaction tissues. They show especially the predominance of one major isoperoxydase (called P_2) characterized by a pH value of nearly 3,75

Discussion

Cellular hyperplasia and hypertrophy are reactions which are frequently encountered in plants (AKAI 1959) and result from the activation of growth mechanisms (multiplication and division) of preexisting histological structures. In the roots, however, this type of reaction is of a special nature in that it concerns a tissue (cork) which is *a priori* only slightly capable of undergoing metabolic modifications of this rapidity. Thus, they appear exclusively in young organs whose cell walls contain only little or no suberin. The presence of several necrotic cells within the cell masses thus formed implies a hypersensitivity reaction which in certain plants is a symptom of resistance to fungal attack (KIRALY 1980). The presence of phenols at this level is superimposed on an intense natural brown pigmentation of the tissues, whose function would be to protect living tissues against the external medium (MULLICK and JENSEN 1973).

The increased suberization of certain cork cell walls has also been described. The process of suberisation in plants is intimately related to the healing process (AKAI 1959); suberin is often deposited within cortical barriers, as is the case for those induced by *R. lignosus* and *P. noxius*. This point has an analogy with the suberin-containing barriers created by oak trees (*Quercus robur*) to limit the spread of rot caused by *Stereum* sp. (PEARCE and RUTHERFORD 1981).

The newly formed papillae in the young cork cells appear to be composed of callose, as suggested by ultraviolet fluorescence observations. Callose is electronlucent in electron microscopy, however, and so does not appear on the micrographs. It is thus probably mixed with other components. The papillae induced in *Solanum tuberosum* by *Phytophthora infestans*, for example are composed of callose and cellulose (HOHL et al. 1980). This heterogeneity in the composition of the papillae, gives them varying electron densities (AIST 1976). The same problem obtains to the deposits obstructing the pores in the phloem tubes. Even though their morphology (electronlucent) is identical to that of the deposits encountered in the phloem necrosis disease of elm trees (BRAUN and SINCLAIR 1976), however, the composition of these obstructions is nevertheless heterogeneous.

The induction of papillae is undoubtedly of a pathologic origin, but the causative agent is still a subject of controversy, according to HEATH (1980). Thus, some authors consider the parasite the primary inducing agent, while others believe that wall degradation products are the causative factor. The former hypothesis seems tenable in *Hevea* because the papillae are very often observed in contact with or in close proximity to fungal filaments.

At the cellular level, the production of tyloses, invoked in this particular case by PERIES and IRUGALBANDARA (1973), is associated with the formation of a new parietal layer in the vessels or fibers. OULETTE (1981) has investigated this aspect on Dutch elm disease caused by *Ceratocystis ulmi*. He observed that this fraction may replace S_3, indicated the stratified appearance of this layer, and stressed its ligneous nature. There is thus apparent concordance between the modifications of certain xylem walls, the ligneous nature of this layer can nevertheless be advanced as a result of the positive colorimetric test.

The significance of the stimulation of lignification in both cortical tissues and in xylem is undoubtedly identical. It would nonetheless be interesting to determine the composition of these reaction lignins since – as reported by ASADA (1978) and VANCE et al. (1980) – they probably differ from that of natural lignins. This mechanism is generally encountered in plants and its role in resistance has been examined in detail (TOUZE and ROSSIGNOL 1977; RIDE 1978; HAMMERSCHMIDT and KUC 1982). RIDE (1975), among others, reported the rapidity of lignin synthesis in response to a fungal attack. At a different scale, the lignification observed in the root parenchyma of *Hevea* is also rapid. In this context, the rapidity and efficacy of the stimulation of lignin biosynthetic pathways and its precur-

sors is essential. In terms of peroxidase stimulation, the situation is highly variable at the level of the interval preceding the increased activity of these enzymes. In some cases it appears late (LEGRAND et al. 1976), while in others it is early (HISLOP and STAHMANN 1971). Similarly, the degree of augmentation varies between 50 and 300 %. Finally, stimulation involves all – or at least a large majority – of the isoenzymes preexisting in healthy tissues (HISLOP and STAHMANN 1971; SEEVERS et al. 1971; BIRECKA et al. 1975 a, b; OHGUCHI et al. 1974; RETIG 1974; ASADA et al. 1975; OHGUCHI and ASADA 1975). Moreover, these enzymes are considered either as markers of senescence (MAZAU and ESQUERRE-TUGAYE 1976) or as markers of rejuvenation (BIRECKA et al. 1975 a, b).

In comparison with the "peroxidase" reaction developed by the various hosts cited in the references, that of adult *Hevea* presents two exceptional characteristics on the level
- of the intensity of the stimulation. The overall increase of activity in parasitized tissues may reach or exceed a factor of 10 in comparison to PA extracted from healthy tissues;
- of the specificity of this stimulation, since it appears that only one isoenzyme is induced and in considerable proportions in the course of attack by *R. lignosus*.

What significance can be attributed to this phenomenon? Can it be taken as a resistance mechanism? A relationship between resistance and peroxidase stimulation has in fact been suggested by some authors (JOHNSON and CUNNINGHAM 1972; RETIG 1974; VEGETTI et al. 1975) while others were unable to establish this correlation (GRZELINSKA 1970; JOHANSSON and THEANDER 1974).

In the context of the hypothesis of a positive effect of PA on plant resistance, we can imagine its participation in a mechanism of reaction lignification, whose efficacy has been demonstrated by others (HIJWEGEN 1963; ASADA and MATSUMOTO 1969, 1972; RIDE 1975, 1978). A role of isoperoxidases in the neosynthesis of a lignin specific to parasitized tissues has been proposed in interactions between *Raphanus sativus* and *Peronospora parasitica* (OHGUCHI et al. 1974; OHGUCHI and ASADA 1975; ASADA 1978).

In the case of *H. brasiliensis*, isoperoxidase P_2 can carry out the terminal step in the biosynthesis of lignin, i. e. the polymerization of p-coumaryl and coniferyl alcohols to form condensation products whose structure is analogous to that of lignine (DHP) (GEIGER and HUGUENIN 1981). It is not yet possible to prove, however, that this enzyme is responsible for the increased quantities of lignin in certain tissues sampled from tap roots infested by *R. lignosus* (GEIGER et al. 1984). Regardless of the actual situation, this lignification is generally ineffective in *Hevea*, since the pathogen eventually degrades all the tissues, except in the case of tissue neogenesis. The process may nevertheless delay the fatal outcome by conferring a partial resistance to the tissues.

It thus seems that there is a positive relation between increased PA and that of the reaction lignification of the tissues. Although this reaction plays a positive role as a defense mechanism in many plants, it is only slightly effective in the case of infestation by a lignin-digesting parasite.

At the level of histogenesis, the cork-phelloderm cambium is systematically stimulated in all plants examined. It is not specific, however, since this is a classical response to bark injury (AKAI 1959; AKAI and FUKUTOMI 1980; TIPPETT et al. 1982) and to other fungal attacks (SAKURAI 1952; BIGGS et al. 1983). The results do not, however, lead to an unambiguous establishment of a relationship between the number of newly formed cell layers and the extent of penetration. But, the parasite exhibits difficulties in penetrating this barrier independently of the cell volume in question.

In older rubber trees, the reaction tissues differentiated by a new vascular cambium lead to the formation of a ligneous healing callus (NICOLE et al. 1981). Only individuals with superior physiological capacities could reach this ultimate degree of defense mechanisms. For other subjects, the mobilization of the defense system at the expense of basal metabolism would considerably exhaust the plants, favoring their middle-term vul-

nerability. Thus, according to McLaughlin and Shriner (1980), the possibility of having sufficient energy reserves available constitutes an important advantage for the survival of the plant. In this context, Sharples and Gunnery (1933) described the same histogenesis in *Hevea*, stressing the trophic role of these neformations and their importance in the resumption of growth by the tree.

Recent works on the anatomical reactions of ligneous trees has shown that the elaboration of these barriers from the xylem favors the compartmentalization of the rot (Shigo and Marx 1977). The diseases caused by *Fomes annosus* (Shigo 1975; Tippett and Shigo 1980) and by *Armillaria mellea* (Shigo and Tippett 1981 a; Tippett and Shigo 1981) are subjected to identical constraints, just as the elm parasitized by *Ceratocystis ulmi* (Shigo and Tippett 1981 b; Shigo 1982 a).

Published data show that the differentiation of anatomical barriers in the xylem unambiguously results from the combined action of the wood and cambium, according to Blanchette (1982 a, b), however, appears to be intimately related to the partial destruction of native cambium. The death of a fraction of the cambial cells would in this case be considered as an inducing element.

Role of histological reactions in pathogenesis: The most diverse type of reactions occur in the course of penetration. Supernumerary cortical layers and cellular nodules locally slow the progression of fungal hyphae, but this is not the case for reinforcement of walls, whether they be woody or corky. The parasites in fact possess the corresponding degradative enzymes (Geiger et al. 1983 a, b). The increased volume of certain cortical cells, as well as their being partitioned, also delays tissue invasion, but only temporarily. The consequences of these phenomena on the longterm course of the infection are on the whole negligible. In certain varieties of cultivated plants, however, e. g. citrus (de Vallavieille 1980) and peanuts (Harris and Beute 1982), these reactions are a mechanism of resistance. Parietal responses of host only slightly modify the decay progression. The tyloses oblige the mycelial filaments to circumvent them. The papillae offer no obstacle to the hyphae which can probably destroy them with its extracellular glucanases (Geiger et al. 1984).

The overall efficacy of these reactions, on the other hand, is difficult to estimate. In certain individuals, they contribute to a type of "partial resistance" of the plant to the disease. Some *Hevea* individuals from the GT1 clone infected by *R. lignosus* (strain 1) exhibit a phase of tolerance to the parasite during which the infestation process stagnates (Nicole et al. 1983). This equilibrium would result from the combined effects of morphological, histological and biochemical reactions.

The comparison of these results with the C.O.D.I.T. model [Compartmentalization of Decay In Trees, Model defined by Shigo and Marx (1977)] furnishes interesting information. The model is based on four "barriers" which limit the spread of the fungus (parasite or saprophyte) responsible for a wood rot, by compartmentalizing the degraded tissues. In agreement with Pearce (1982) we also note that the C.O.D.I.T. model is applicable not only to the reactions elaborated in the wood, but also to those initiated in non-lignified tissues. The differentiation of supernumerary cortical layers from the cork-phelloderm cambium combined with a certain degree of suberization of cell walls constitutes the wall 4 barrier in the C.O.D.I.T. model. This is also the case for tissue neogenesis, which prevents the decay extension and the contamination of newly formed tissues. Similarly, the C.O.D.I.T. model shows that, in the *Hevea*-root rot fungi couple, these reactions, at least in clone GT1 studied, occur selectively as a function of the type of progression of the disease. Thus, a given histological reaction will be differentiated only in response to a well determined situation in the infectious process. This reflects the constancy of certain reactions e. g. those occurring during penetration as well as the low frequency of other histological manifestations (tissue neogenesis). Finally, this analysis confirms that the C.O.D.I.T. model can be applied to root rots, in agreement with Tippett and Shigo (1980, 1981).

5 Conclusion

The reaction mechanisms described for *H. brasiliensis* (GT1 clone) infected by *R. lignosus* (strain 1) and *P. noxius* (strain 4) are characterized by their aspecific nature. They have in fact all been described for other identical couples. In addition, these defense reactions rarely enable the tree to resist the fungal attack. Moreover, the pathogenicity of these two parasites varies from one strain to another, thereby determining the highly variable reaction levels of the host. Thus, as mentioned by JOHANSSON and UNESTAM (1982) in *Picea abies* infected by *Heterobasidion annosum*, selection by cloning of resistant individuals appears to be haphazard and thus not very reliable. There nevertheless remains a path open which has not been extensively explored in root rot diseases the search for factors which can elicit one or another of these reactions.

A number of histological reactions, especially in ligneous species, are under hormonal control (HOQUE 1982). The stimulation of tissue neogenesis by hormonal treatment for example may now enable certain diseased trees to be saved or even to prevent parasitic attack.

Summary

Reactions of *Hevea brasiliensis* against root rot diseases caused by the fungi *Rigidoporus lignosus* and *Phellinus noxius*, are described at molecular, cellular and histological levels. It has been shown that rubber trees differentiate defense reactions during all stages of the infection. Cellular hypertrophy and hyperplasia, cambium activity stimulation and lignification and suberification of certain walls occur in the course of root penetration. Wall appositions in young suber cells, callose depositions on pores of sieve tubes in phloem, tyloses formation and new cell wall layer differentiation in xylem, are the main reactions observed during tissues colonization. Elaboration of meristematic tissues and induction of a new vascular cambium constitute late reactions.

Biochemical studies have revealed the increase of an isoperoxidase activity in both infected and reaction tissues. This isoenzyme is synthesized by the host and can be detected as early as penetration.

These reactions rarely enable rubber trees to resist the fungal attack. Their actual effectiveness is discussed in comparison with the C.O.D.I.T. model.

Résumé

Les pourridies d'Hevea brasiliensis. II. Quelques réactions de l'hôte

Les réactions d'*Hevea brasiliensis* aux pourritures racinaires, causées par les champignons *Rigidoporus lignosus* et *Phellinus noxius*, sont décrites aux niveaux moléculaire, cellulaire, et histologique. L'Hévéa réagit à tous les stades de l'infection.

L'hypertrophie et l'hyperplasie cellulaires, la stimulation du cambium subéro-phellodermique, la lignification et la subérification des parois ont été observées au cours de la pénétration des hyphes dans la racine. Les dépôts de callose sur les parois des jeunes cellules du liège et au niveau des pores des tubes criblés du phloème, la formation de tyloses et la différenciation d'une nouvelle couche pariétale dans les vaisseaux du xylème constituent les principales réactions qu'oppose l'Hévéa à la colonisation des tissus par ces parasites. Enfin l'élaboration de tissus à caractère méristématique ainsi que l'initiation d'un néocambium libéro-ligneux sont des réactions tardives, peu fréquentes.

Les études biochimiques ont révélé la très forte augmentation de l'activité d'une isoperoxydase tant dans les tissus infectés que dans les tissus réactionnels. Cette isoenzyme est induite par l'hôte dès la pénétration de la racine.

L'efficacité de ces réactions est discutée par comparaison avec le modèle C.O.D.I.T.; mais rarement les arbres présentent une résistance aux attaques fongiques.

Zusammenfassung

Wurzelfäule bei Hevea brasiliensis. II. Einige Reaktionen des Wirtes

Reaktionen von *Hevea brasiliensis* auf die Infektion durch die Wurzelfäuleerreger *Rigidoporus lignosus* und *Phellinus noxius* werden beschrieben. Sie finden auf molekularer, zellulärer und histologischer Ebene statt. *H. brasiliensis* zeigt Abwehrmechanismen während aller Phasen der Infektion.

Zellhypertrophien und Hyperplasien, Anregung der Kambiumaktivität sowie Lignifizierung und Suberinisierung bestimmter Zellwände wurden im Laufe des Eindringens der Pathogene in die Wurzeln beobachtet. Wandauflagerungen in jungen Korkzellen, Kalloseablagerungen auf die Poren der Siebröhren im Phloem, Thyllenbildung und die Ausbildung neuer Zellwandschichten im Xylem sind die wichtigsten während der Gewebebesiedlung beobachteten Vorgänge. Als späte Reaktionen sind die Bildung von meristematischen Geweben und die Induzierung neuer Gefäßkambien anzusehen. Biochemische Untersuchungen haben gezeigt, daß die Aktivität einer Isoperoxidase sowohl in den infizierten Geweben als auch in den Reaktionsgeweben ansteigt. Dieses Isoenzym wird vom Wirt gebildet und kann schon beim Eindringen der Pathogene nachgewiesen werden.

Die aufgezeigten Reaktionen ermöglichen es *H. brasiliensis* jedoch nur selten, Angriffen von Pilzen zu widerstehen. Ihre Wirksamkeit wird im Vergleich mit dem C.O.D.I.T.-Modell diskutiert.

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