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Reprint from

Springer Series in Wood Science

Editor: T. E. Timell

R. A. Blanchette and A. R. Biggs:
Defense Mechanisms of Woody Plants Against Fungi

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Chapter 10

Defense of Angiosperm Roots Against Fungal Invasion

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10.1 Introduction

The major root disorders currently described in forest pathology mainly concern root rot diseases and vascular diseases. Although different tree-fungus interaction studies have been performed in such pathosystems (Nicole et al. 1982, 1985, 1986a,b, Balanchette 1984, Rishbeth 1985, Geiger et al. 1986a,b, Nandris et al. 1987a, Blanchette and Abad 1988, Blanchette et al. 1989), precise observations regarding defense of roots in angiosperm trees triggered by fungal invasion have rarely been made. Thus, little is known about how the root system of these trees defends itself against fungal infection. However, Thomas (1934) and Sharples (1936) published information about anatomical responses of *Prunus* sp., *Juglans regia*, and *Hevea brasiliensis* infected with *Armillaria mellea* and *Ganoderma* sp. Ito (1949). Sakurai (1952, cited by Akai 1959) and Struckmeyer et al. (1954) also described histological reactions of apple trees, mulberry trees, and *Quercus ellipsoidales* to *Helicobasidium* sp., *Roselinia* sp., and *Endoconidiophora* sp. Rishbeth (1972), probably providing the first review of resistance to fungal pathogens of tree roots but with few references to angiosperms (*Quercus robur*, *Fagus sylvatica*, *Acer pseudoplatanus*, *Populus* sp., some genera of the *Oleaceae* familly, and certain tropical species). Today, research on this subject is still poorly documented in comparison with studies on gymnosperm root defense (Chap. 9).

Our objectives in this review are (1) to describe the main active reactions of root angiosperm tissues; (2) to present the responses of lignified and unlignified cells; (3) to relate previous microscopical observations to biochemical modifications of the tissues; and (4) to discuss the concept of defense mechanisms in roots of angiosperm trees. The major studies dealt with in this chapter are concerned with rubber tree root rot diseases at cellular (Nicole et al. 1983, 1986c) and molecular (Geiger et al. 1986c, 1989) levels both on young artificially infected seedlings (Nandris et al. 1983) and on adult trees naturally infected with *Rigidoporus lignosus* and *Phellinus noxius* (Geiger et al. 1976, 1977).

10.2 Histological Reactions of Roots

Phytopathological surveys of tree plantations showed that among several diseased trees, some seemed to survive infection (Nandris et al. 1988). In rubber tree plantations, careful examination of root systems infected with *Rigidoporus*

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lignosus and *Phellinus noxius* sometimes revealed modifications of their anatomy, characterized by root witches' broom or by hypertrophied lateral roots (Fig. 10.1). In 10-year-old plantations, a survey showed that among taproots of 223 dead trees, only 3.6% had developed visible root reactions. Similar observations were also reported for trees inoculated with *Armillaria* sp. and with *Ganoderma pseudoferreum* (Sharples 1936, Rishbeth 1985). Such tissue modifications strongly suggest great pertubations in root histogenesis with respect to primary and lignified roots.

10.2.1 Increasing Phellogen Activity and Formation of a Secondary Periderm

The formation of protective layers in bark arises from the cork cambium (the phellogen) and can provide strong mechanical, and perhaps biochemical resistance to infection or wounding (Chaps. 2 and 3). The increase in the phellogen activity also occurs in roots and the formation of a secondary bark have been observed in some fruit tree species and specifically in *Juglans nigra* attacked by *Armillaria* sp. (Thomas 1934) and in *Pyrus malus* infected with *Helicobasodium mompa* (Akai 1959). Mathre et al. (1966) observed that penetration of older cotton roots by *Thielaviopsis basicola* was walled off by the development of heavy cork cambium. The pathogen *Armillaria* also stimulated the cork cambium in *Populus tremuloides* and *Fagus grandiflora*, the roots of which were dissected to examine the spatial limit of this reaction (Shigo and Tippett 1981b). In roots of avocado (*Isopogon ceratophyllus*) the necrophylactic periderm (as defined by Mullick and Jensen 1973) was induced by *Phytophthora cinnamomi* to wall off infected tissues (Phillips et al. 1987). In rubber tree root rot diseases, *Hevea brasiliensis* initated such reactions in roots specially contaminated by *Phellinus noxius* and *Rigidoporus lignosus* (Nicole et al. 1986c), as shown in Fig. 10.2. The stimulation of the phellogen activity, common in nonlignified roots, was also described in adult roots of the rubber tree infected with *Rigidoporus lignosus* or *Ganoderma pseudoferreum* (Sharples 1936). In Fig. 10.3, reaction tissues of *Hevea brasiliensis* were differentiated against *Sphaerostilbe repens*, another tropical root parasite decaying cortical tissues only. In some cases, small nodes originating from the phellogen confer an abnormal configuration to the young phellem.

10.2.2 New Vascular Cambium and Meristematic Activity

Barrier zones are differentiated by living trees in response to different stresses (Chap. 5) in both the phloem and xylem. The formation of a new vascular cambium alters the wood anatomy and seems to be initiated from cells adjacent to the former dead cambium (Rishbeth 1972). In rubber trees, such a reaction was observed rarely and only on roots of young seedlings (Nicole et al. 1986c). The newly differentiated cambium (Figs. 10.4 and 10.5) formed the phloem, which contained laticifers, tannin cells, and sieve tubes, and xylem tissue mainly composed of numerous punctated cells (Fig. 10.6). The resulting barrier on

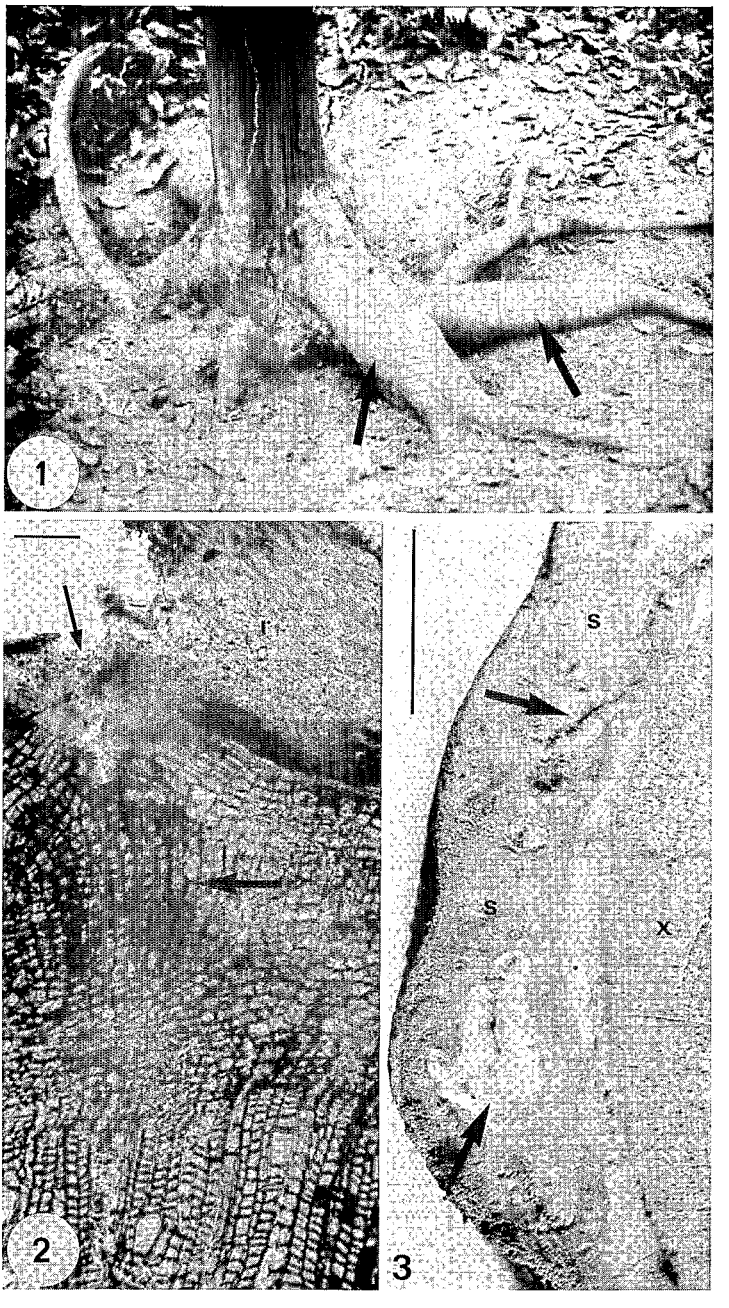


Fig. 10.1. The root system of a *Rigidoporus lignosus*-infected *Hevea brasiliensis* showing hypertrophied lateral roots (*arrows*) enabling the plant to survive root rot diseases

Fig. 10.2. Light photomicrograph of a cross-section through a young root infected with *Rigidoporus lignosus* rhizomorph (*r*) showing additional cell layers produced by phellogen stimulation (*large arrow*) under the penetration site (*small arrow*); *bar* = 12 μm

Fig. 10.3. Cross-section through an mature root infected with *Sphaerostilbe repens*. In the suberized reaction tissue (*s*), tertiar cambium activity (*arrows*) produced woody nodes to prevent fungal invasion of the healthy xylem (*x*); *bar* = 1 cm

Fig. 10.4. Light photomicrograph of a cross-section through a young root infected with *Rigidoporus lignosus*. Meristematic tissue (*5*) is initiated from parenchymatous rays (*6*) of the decayed xylem (*2*). A new vascular cambium (*nc*), formed from the old one (*arrow*) which is probably killed, differentiated new xylem (*8*) and phloem (*7*); *bar* = 35 μm

small roots can be a complete ring, isolating the diseased xylem. Shigo and Tippett (1981a) also described a barrier zone formed by the vascular cambium in xylem of American elm root tissues infected with *Ceratocystis ulmi*. On the other hand, compartmentalization of pathogen in the secondary root phloem seems to be uncommon, although it was reported by Tippett et al. (1983, cited by Shigo 1984) on *Eucalyptus* sp. infected with *Phytophthora cinnamomi* or *Armillaria luteobubalina*.

Meristematic activity appears to be more frequent in trees, and Rishbeth (1985) has suggested that such activity occurs only when a parasite invades a root rather slowly. Our work agreed with this observation; the meristematic activity was considered indeed as a late reaction on rubber trees. On young seedlings this activity completely modified the anatomical organization of the

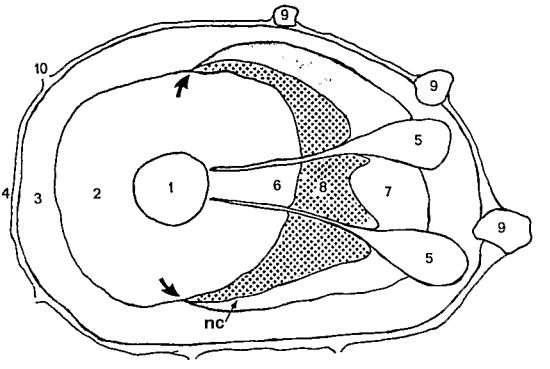


Fig. 10.5. Scheme showing anatomical reactions on a cross-section through a young root infected with *Rigidoporus lignosus*. *1* Medulla parenchyma; *2* decayed xylem; *3* infected phloem; *4* periderm; *5* meristematic tissue; *6* parenchymatous rays; *7* new phloem; *8* new xylem; *9* rhizomorphs; *10* lenticells. The new vascular cambium (*nc*) is formed from the old one (*arrows*)

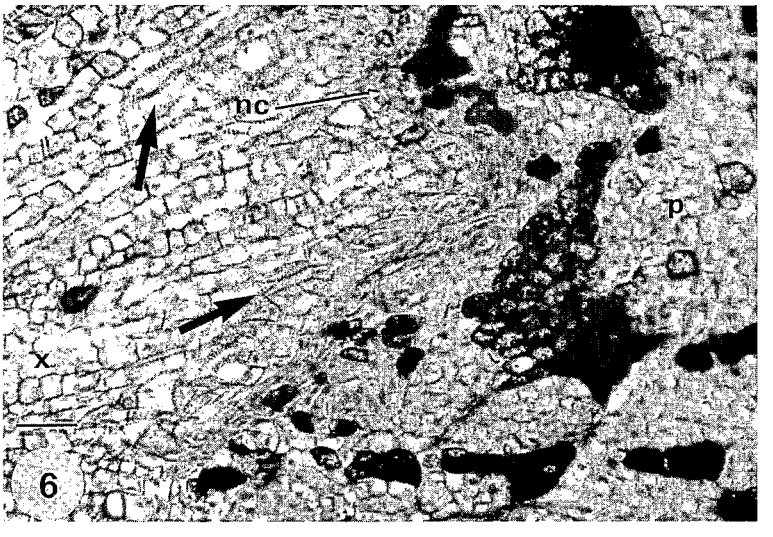
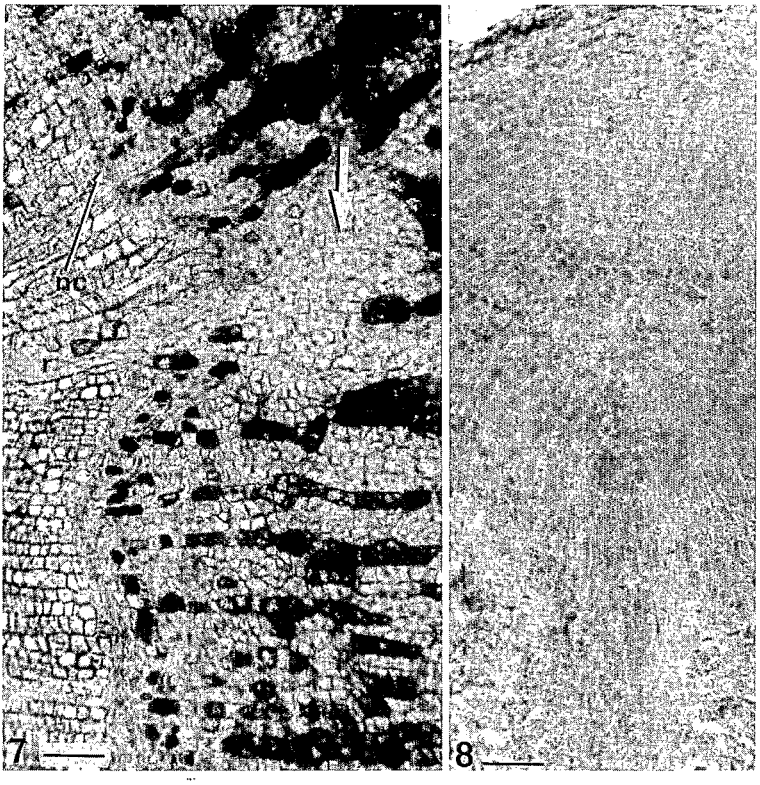


Fig. 10.6. Formation of new xylem (*x*) and new phloem (*p*) by the neo-cambium (*nc*). The new xylem is composed of numerous punctated cells (*large arrows*), typical of healing xylem; cross-section light photomicrograph through a young root infected with *Rigidoporus lignosus*; *bar* = 20 μm

root. The meristematic tissue arose from the parenchyma rays of the former xylem (Figs. 10.4 and 10.5) by dedifferentiation of cells into cellular masses initially composed of several cells (Fig. 10.7), and subsequently forming dense foci, whose organization is not unlike that of a meristem (Fig. 10.8). Such cells have a dense cytoplasm, a large nucleus, and a high nuclear/cytoplasmic ratio



Figs. 10.7 and **10.8.** Dedifferentation of parenchymatous ray cells (*r*) results in the production of unorganized cellular masses (*arrow*) subsequently forming a meristematic tissue composed of cells with a dense cytoplasm and a large nucleus (*Fig. 10.8*); *nc* new cambium. Cross-section light photomicrographs through a young root infected with *Rigidoporus lignosus*; *bar* = 24 μm for both figures

and become generally impregnated with lignin and suberin as mentioned by Aist (1983).

10.2.3 Differentiation of New Roots

The formation of a lignified barrier, and of new lateral roots or taproots to prevent fungal invasion is the consequence of both new vascular cambium and meristematic activity. *Hevea brasiliensis* initiated these responses on young and adult trees infected with *Rigidoporus lignosus* and *Phellinus noxius* (Nicole et al. 1983, 1986c) or attacked by *Armillaria* sp. (Fox cited by Rishbeth 1972). Infected tissues were surrounded by the wall 4 barrier zone (Figs. 10.9, 10.10), as described by Pearce and Woodward (1986) on the butt of *Acer saccharinum* infected with *Ganoderma adspermum*. They were then replaced and compartmentalized by other roots (Fig. 10.11) to restrict the spread of decay. Differen-

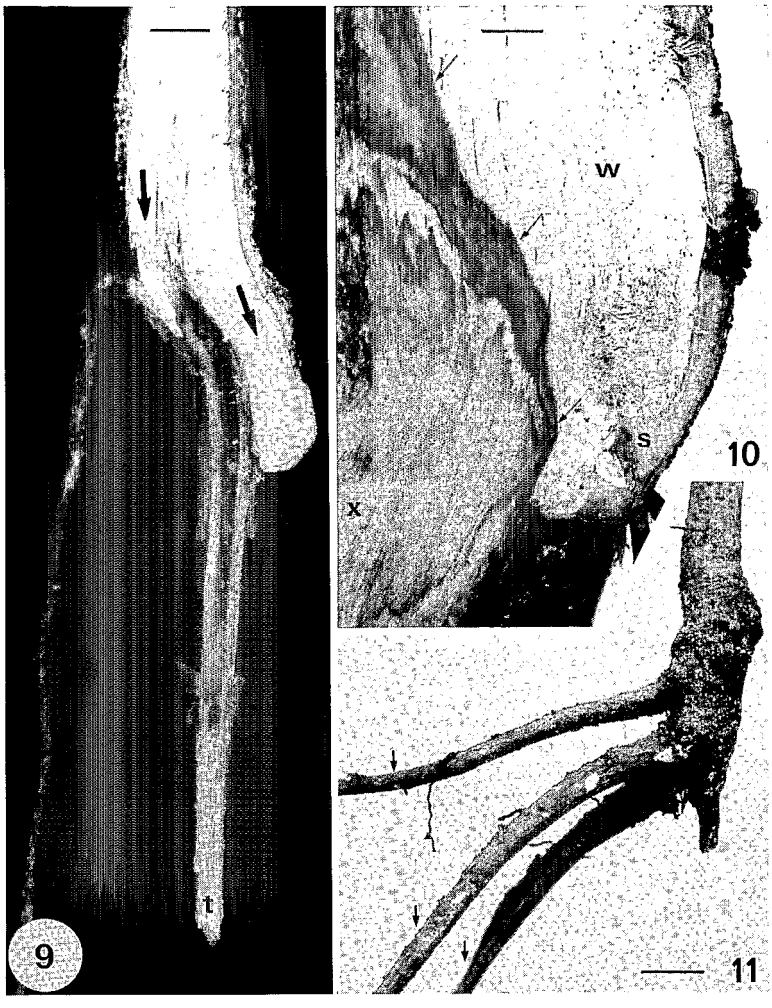


Fig. 10.9. Macrophotograph of a 2-month-old Hevea infected with *Rigidoporus lignosus*; longitudinal section through the root. The former taproot (*t*) is decayed by the fungus and compartmentalized by new tissues (*arrows*) differentiated from a new cambium; *bar* = 1.5 cm

Fig. 10.10. Macrophotograph of an adult Hevea taproot decayed by *Rigidoporus lignosus*; longitudinal section. The degraded xylem (*x*) and the reaction wood (*w*) are separated by a barrier zone (*arrows*) which prevent fungal spread into healthy tissues; *s* suberized tissue; *bar* = 1.5 cm

Fig. 10.11. Macrophotograph of a 2-month-old Hevea infected with *Rigidoporus lignosus* showing the complete decay of the former taproot and the differentiation of lateral roots (*arrows*) allowing the tree to survive; *bar* = 1 cm

tiation of new lateral roots often occurred after the former taproot had decayed, probably involving plant growth substances (see Sect. 10.4.3). Although there is no direct evidence, lignification and suberization in the barrier zone generally confer resistance to degradation of sapwood by fungi (Pearce and Rutherford 1981).

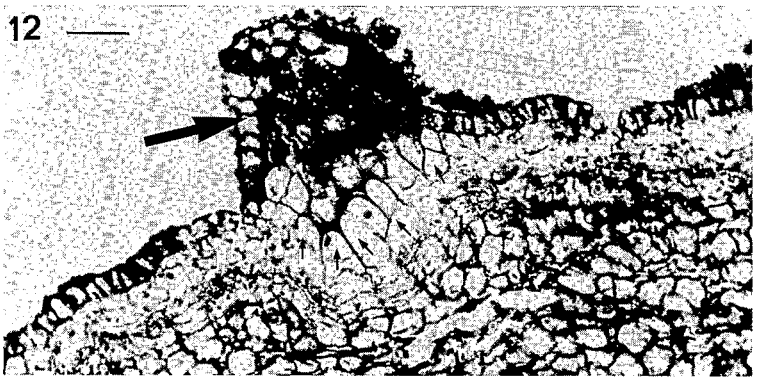


Fig. 10.12. Light photomicrograph of a cross-section through a root infected with *Phellinus noxius*. Hypertrophy of young phellem cells (*small arrows*) near cells colonized by fungal hyphae (*large arrow*); *bar* = 24 μm

10.3 Cellular Defenses

10.3.1 Cell Hyperplasia and Hypertrophy: The Hypersensitive-Like Response

A common response of *Hevea brasiliensis* to infection with *Phellinus noxius* concerns the first cell layers of the young root periderm. Hypertrophy and hyperplasia of the cells were observed during the penetration of hyphae into the root. The volume of some cells greatly increased near the penetration sites or around infected cells (Fig. 10.12), often in association with hyperplasia. Periclinal cell divisions also occurred in roots of avocado infected with *Phytophthora* sp., especially in the primary parenchyma, which acquired the appearance of an exophylactic periderm (Phillips et al. 1987).

The hypersensitive response, well known in foliar diseases, entails a reaction with an early tissue necrosis (Kiraly 1980). It merely constitutes a physical barrier, reducing the fungal penetration by surrounding the dead cells (Fig. 10.13) as specifically induced by *Phellinus noxius* on rubber tree roots. The hypersensitive necrosis probably results from recognition mechanisms, as yet unknown with root rot diseases, and might suggest the implication of fungal elicitors. Albersheim (1989) showed that oligosaccharide fragments of host cell walls, released by pathogen exo-enzymes, can kill plant cells. Enzymes secreted by *Phellinus noxius*, which assist hyphal penetration in roots (Geiger et al. 1986b), produce such components, which are able to elicit the hypersensitive-like response described in *Hevea* root.

10.3.2 Vascular Occluding Reactions

Gel, gum plugs, and tyloses are of widespread occurrence as root reactions in fungal wilt diseases (Beckman and Talboys 1981). Vascular gelation was

Fig. 10.13. Light photomicrograph of hypersensitive-like response in *Phellinus noxius*-infected root. Hypertrophied parenchyma cells develop around necrotic cells (*arrow*); *bar* = 8 μm

reported by VanderMolen et al. (1977) within vessel lumina in *Fusarium*-infected *Citrus* and *Verticillium*-infected cotton. These authors also described the pectic nature of this material. Tyloses, originating from the bulbing of adjacent parenchyma cells, are the more usual response in xylem vessels, for example in *Juglans regia* (Thomas 1934) and in *Quercus* sp. (Struckmeyer et al. 1954). However, formation of tyloses has rarely been described in root rot diseases, although they probably contribute to impede water transport, as observed on young rubber trees artificially infected with tropical root rot fungi. When gel, gums, and tyloses solidify they may act as physical barriers (Chap. 13).

10.3.3 Cell Wall Thickening

Cell wall thickening occurs very early in the infection process at the point of hyphal contact. In the apical region of the root of young infected rubber trees, the cells of the primary parenchyma undergo a spectacular thickening of the walls, which is orientated toward the parasite (Fig. 10.14) (Nicole et al. 1986c). Responding cells thus show enlarged walls, which can reach more than 2 μm in thickness. Positive tests enabled the identification of lignin in this new wall. In the cork barrier induced by *Phellinus noxius*, cell wall thickening becomes heavily suberized. Although suberization was mainly described in relation to wound-associated changes and postinfection (Pearce and Rutherford 1981), both lignification and suberization often take place in the barrier zone (Enebak and Blanchette 1989), the cell walls of which also contain unknown components (Ride 1983).

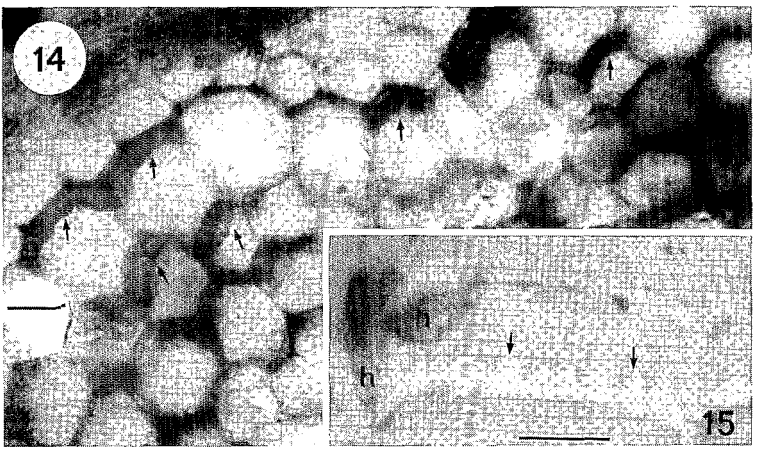


Fig. 10.14. Wall thickening of primary parenchyma cells (*arrows*) of a young root infected with *Rigidoporus lignosus*; histochemical tests (phloroglucinol/HCl; $KMnO_4$ oxidation) demonstrated the lignin nature of these walls; *r* rhizomorph. Light photomicrograph; *bar* = 8 μm

Fig. 10.15. Light photomicrograph of a cross-section through a root infected with *Rigidoporus lignosus*. Wall modification (*arrows*) of phellem cells colonized by fungal hyphae (*h*); UV illumination of sections after aniline blue staining reveals callose deposition along the wall; *bar* = 3 μm

10.3.4 Deposition of New Wall-Like Material

Wall appositions represent a general response of plants to infection by forming molecular barriers such as lignin, callose, and hydroxyproline-rich glycoprotein (Mazau and Esquerré-Tugayé 1986). In diseased woody plants, deposition of wall-like material was described in lignified and unlignified roots. Light microscopic observations of *Rigidoporus lignosus*-infected *Hevea brasiliensis* revealed that young, suberizing cell walls present a notched appearance (Fig. 10.15) with a fluorescent aspect when observed under UV light. Ultrastructural examination showed deposition of material along the cell wall between the plasmalemma and the wall itself, suggesting that these wall appositions could be induced by fungal progression inside the wall (Fig. 10.16). Electron microscopy also revealed that they are electron-dense, indicating the presence of other components than the callose shown by histochemical tests. The efficiency of these induced papillae in young phellem cells is probably low because *Rigidoporus lignosus* produces the enzymes (β-glucanases) able to degrade them (Nicole et al. 1986a). Coating material in vessels of the root xylem has also been described in infected *Hevea brasiliensis* (Nicole et al. 1986c). It appeared as a new, irregular, and lignified layer, located on the secondary cell wall of vessels, and was never observed in healthy roots. In trees, such a "protective layer" was also investigated in paravascular and ray parenchyma cells of elm infected with *Ceratocystis ulmi* (Ouellette 1981).

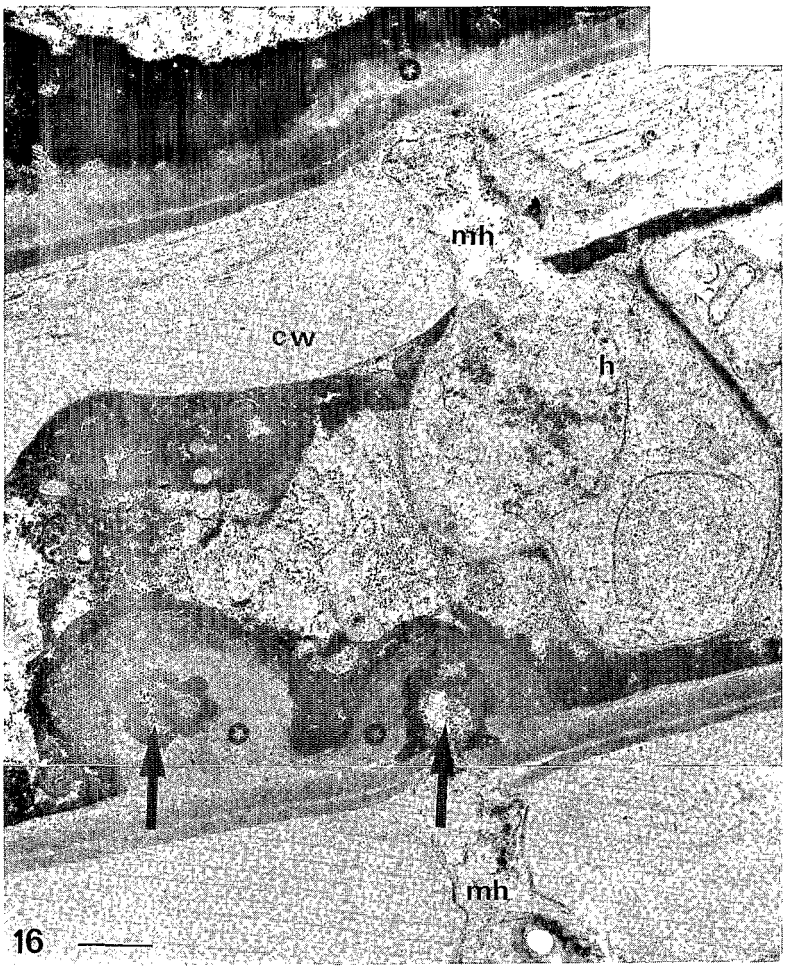


Fig. 10.16. Transmission electron micrograph of a cross-section through a *Rigidoporus lignosus*-infected root. Papillae (★) deposited along the cell wall in contact with fungal hyphae (*h*). The pathogen can degrade these wall appositions which become more electron-dense (*arrows*). *mh* Microhyphae; *cw* cell wall; glutaraldehyde/osmium tetroxide fixatives; *bar* = 0.5 μm

10.4 Biochemical Modifications of the Xylem

10.4.1 Lignification

Histological and cytological reactions of wood include various metabolic disturbances in response to stresses (Chaps. 5, 7, and 8). Among them, stimulation of the lignin biosynthesis pathway is of importance in resistance (Vance et al.

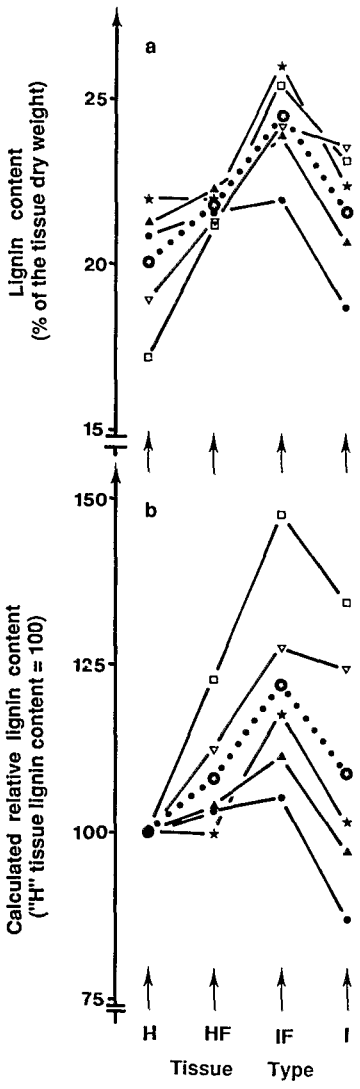


Fig. 10.17a,b. Lignin content recorded for different tissue types of rubber tree taproots. **a,b** Values expressed as a percentage of dry weight of tissue types in case of taproots partially colonized by *Rigidoporus lignosus*; for each taproot, results registered for *HF*, *IF*, and *I* tissues are expressed as a percentage of corresponding *H* tissue lignin content. Healthy tissue *H* and *I* infected tissue are located far from the fungal progression line; *HF* and *IF* are, respectively, healthy and infected tissues located close to the fungal progression line

1980). However, the difficulty to quantify induced lignin-like material in lignified tissues has strongly restricted research on the subject, as mentioned by Ride (1983). Nevertheless, interesting results were published by Geiger et al. (1986c) concerning the evolution of the lignin content of *Hevea brasiliensis* taproots during root rot diseases. These authors showed an increase of up to 50% of the lignin content in *Rigidoporus lignosus*-infected lignified tissues close to the fungal progression line (Fig. 10.17). On the other hand, accumulation of lignin in xylem infected with *Phellinus noxius* is rather heterogeneous. It would be interesting to test the monomer composition of the newly formed defense lignin of barrier zones to see whether it is different from the native xylem as demon-

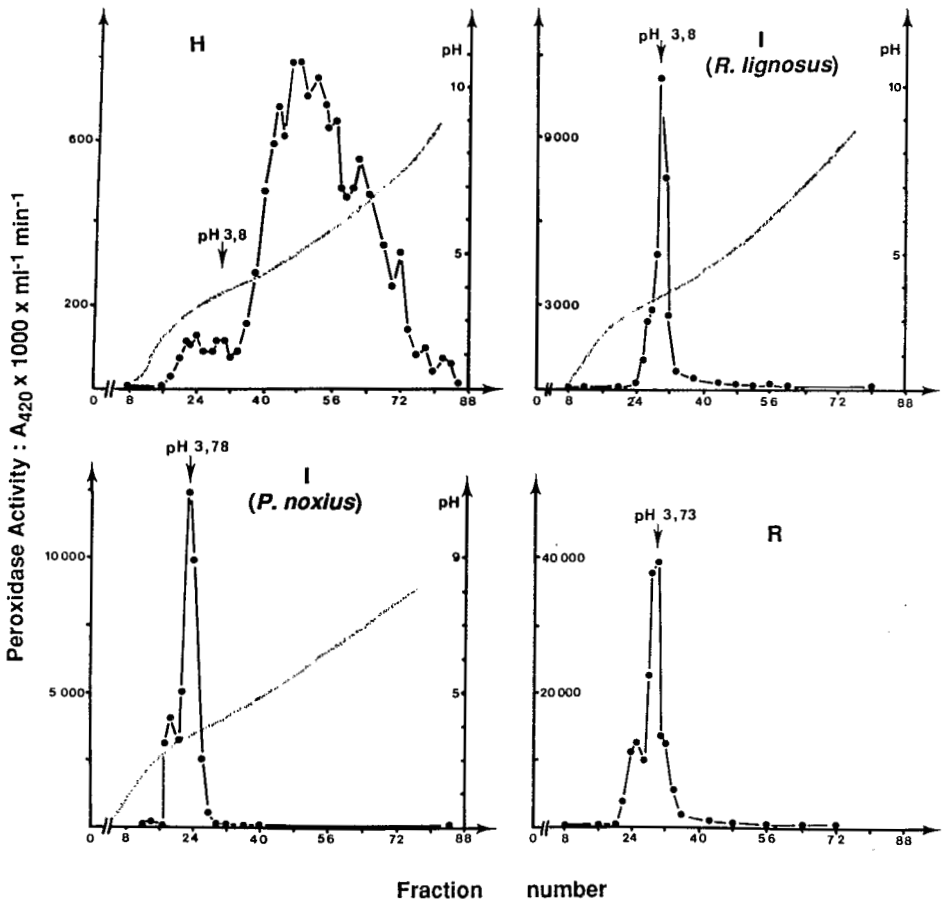


Fig. 10.18. Electrofocusing diagrams of isoperoxidases extracted from healthy (*H*), reaction (*R*), and infected (*I*) tissues of adult rubber tree taproots, respectively. This figure shows the qualitative and specific changes occurring after infection by *Rigidoporus lignosus* and *Phellinus noxius* and in host reaction tissues. The predominance of a major isoperoxidase (pH = 3.8) is indicated. The *light curves* in H, I (*R. lignosus*), and I (*P. noxius*) indicate the graded series of pH

strated by Asada and Matsumoto (1972) and Hammerschmidt et al. (1985) on herbaceous angiosperms.

10.4.2 The Peroxidase Reaction

Peroxidase activity has been the most studied plant reaction to infection, except in highly lignified tissues. Research carried out with rubber trees showed that healthy and infected tissues, both localized close to the fungal progression line, had a high peroxidase activity (Geiger et al. 1976). Figure 10.18 reports

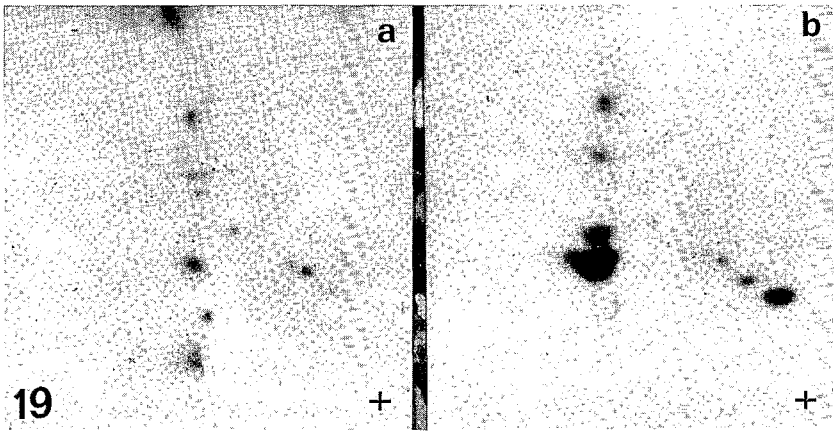


Fig. 10.19a,b. Trypsic map (fingerprint) of the peroxidase (P2a) purified from infected tissues of **a** *Rigidoporus lignosus*-infected taproots and **b** reaction zone tissues produced in *Sphaerostilbe repens*-infected taproots. This result with respect to other physicochemical characteristics reveals that the isoperoxidases produced in infected roots and in the reaction zone are identical

electrofocusing results, showing a considerable increase in one enzyme species in both parasitized and reaction tissues. The identified isoperoxidase, called P2a, was responsible for the major enzyme activity, which may reach a factor as great as 170 by comparison with healthy tissues. Polyacrylamide gel electrophoresis, amino acid composition, and trypsic fingerprints (Fig. 10.19) demonstrated that the physicochemical characteristics of the purified peroxidases were similar in *Rigidoporus lignosus*-infected taproots and in the uninfected reaction zone tissues induced by *Sphaerostilbe repens*. Thus, the increase of the P2a isoperoxidase activity was considered as a host reaction to infection (Geiger et al. 1989). This activity probably results in the stimulation of a de novo synthesis of the enzyme or in the activation of a preexisting apoenzyme inactivated in healthy tissue because the heme of the peroxidase is lacking.

Close correlations between peroxidase activity and lignification enhancement and host resistance (Vance et al. 1980) have often been established. In the rubber tree, P2a isoperoxidase can carry out the terminal step in lignin biosynthesis, i.e., the polymerization of p-coumaryl and coniferyl alcohols to form condensed products analogous to lignin (Geiger and Huguenin 1981, cited by Nicole et al. 1986c). This indicates that P2a activity enhancement may induce specific functions during the biosynthesis of lignin, like other peroxidases during lignification of trees (Harkin and Obst 1973). Although the peroxidase reaction is widespread in herbaceous plants, this research revealed that the root system of trees is able to initiate similar responses, specific in terms of its expression and the intensity of its stimulation. However, the induced peroxidase activity may also be involved with other biochemical processes occurring during root resistance, such as those involved in growth substance metabolism, synthesis of hydroxyproline-rich glycoprotein, and/or suberization.

10.4.3 Other Biochemical Responses

Biochemistry of tree defense reactions in the xylem of gymnosperms and angiosperms is described in Chapters 7 and 8, respectively. In roots of angiosperms, some reactions are of interest and concern, especially proteins, the phenolic pool, and hormonal stimulations.

Infection-related enzymes were differentiated in the phloem of roots of *Quercus rubra*, *Q. alba*, and *Acer saccharinum* infected with *Armillaria mellea*. It was demonstrated that chitinase and β-1,3 glucanase hydrolized the hyphal cell walls of this pathogen (Wargo 1975) and may account for the resistance of healthy tissues to fungal invasion.

In cotton (*Gossypium hirsutum*) catechin and gallocatechin, localized in the endodermis of young roots, played a role as chemical barriers to penetration and colonization of the root of vascular pathogens (*Verticillium dahliae*) by inhibiting sporulation and fungal pectinases (Mace and Howell 1974). In the same way, dihydroxyphenols, normally absent from the stele tissue of cotton roots, were synthesized by the xylem ray cells, whereas paravascular cells and the root cortex produced terpenoids (Bell 1981). Production of phytoalexins was also stimulated in root tissues of resistant cultivars of *Gossypium hirsutum*, as for example gossypol, a phenolic antioxidant, which was accumulated in response to infection by *Fusarium oxysporum* f. sp. *vasinfectum* and *Rhizoctonia* infection (Dallakyan et al. 1978, Bell 1981). Lastly, stilbenes were associated with resistance of avocado against *Phytophthora* root rot.

Several kinds of reactions and symptoms concern modifications of hormone metabolism (Pegg 1988). Ethylene is produced, for example, in roots of *Phytophthora*-infected peach trees (Ugalde and Taylor 1988). The role of ethylene was also stressed in hyperplasia and hypertrophy responses. Gibberellic acid and auxins promote the formation of tyloses and the differentiation of xylem (Pegg 1976). Generally, differentiation of vascular tissues is under hormonal control (Aloni 1987, Roberts et al. 1988). In addition, histological and biochemical reactions appear to be regulated by plant growth substances as reported by Hoque (1982) in his review on biochemical aspects of stress physiology of ligneous plants. The scheme outlined below (Fig. 10.20) summarizes the different pathways involved in the defense of the rubber tree that also may be under hormonal control. It reveals how complex the regulation of the root defense can be.

10.5 Defense in Angiosperm Roots Triggered by Fungi

10.5.1 The Time Sequence of Root Defense

Root reactions to fungal invasion depend greatly on plant aging, which changes their ability to defend themselves. Mature trees thus become less susceptible to the pathogens. Although natural physical barriers locally limit fungal colonization, they are often inefficient in stopping the infection with time. Thus, roots

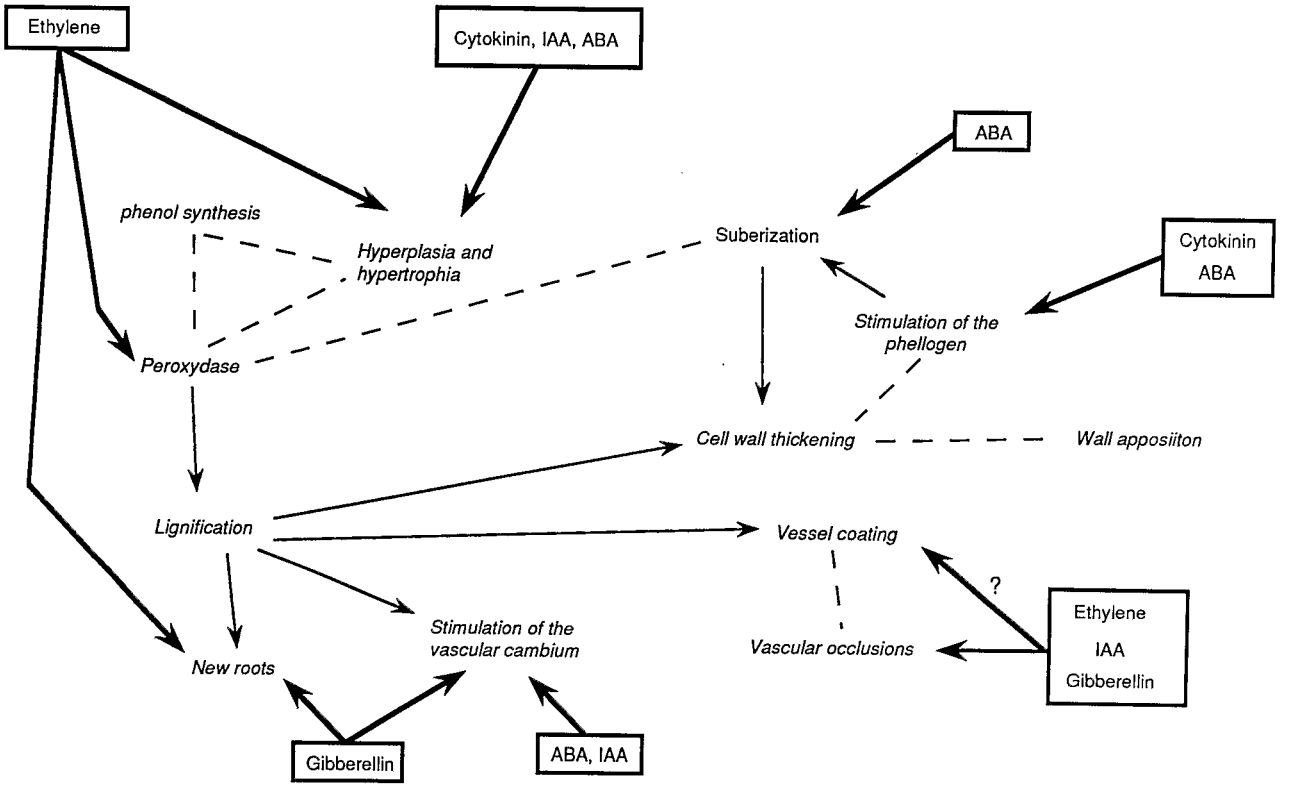


Fig. 10.20. Possible relations between root reactions and hormonal balance in rubber tree infected with root rot fungi (→ hormonal control; → connected responses; .... responses occurring simultaneously)

produce sequential reaction zones whose chemical components, such as lignins, terpenoids, or enzymes, help the plant to resist. Plant defense accordingly increases with maturity; this is likely to be the case with roots which are infected very early. Bell (1980) reported that "defense against root diseases increases rapidly and remains at a high level, even in the mature plant", as described in roots infected with *Fusarium*, *Rhizoctonia*, or *Phytophthora*. Older cotton roots were more resistant to *Thielavopsis* root rot because of cork cambium stimulation (Mathre et al. 1966).

Induced defense by roots of the rubber tree infected with *Rigidoporus lignosus* and *Phellinus noxius* illustrate well the sequence of events in root defense (Fig. 10.21). Among the responses observed within these root rot diseases, most were concerned with roots of yound seedlings (Nicole et al. 1986c). Some are developed as soon as the hyphae penetrate the root periderm (cell hypertrophia and hyperplasia, stimulation of peroxidase activity, and the hypersensitive-like response). Other responses, such as cork-phelloderm cambium stimulation, wall thickening, wall appositions, and cell wall lignification or suberization occur during the colonization of nonlignified tissues. Protective layers, peroxidase activity, plugging material, the stimulation of the vascular cambium, and the lignification of phloem or medulla parenchyma cells are induced when the fungi degrade the vascular tissues. However, the newly differentiated tissues that wall off the decayed roots occurred late and only in a few trees.

Early reactions suggest recognition mechanisms between host and pathogens. In root-fungi interactions, these mechanisms are probably common and nonspecific. In this respect, the rapid cell death of rubber tree periderm only occurs after close contact with *Phellinus noxius*. In foliar diseases, for example, immediate responses of cells appear within seconds or minutes after infection (or wonding), whereas the rapid reactions in root infections, and especially with root-rot diseases, occur over a period of several hours to some days. Rubber tree roots attacked with *Rigidoporus lignosus* initiated the first observable reactions only 5 days after artificial infection, and slow reactions such as root differentiation were produced several weeks later (Nicole et al. 1983). Thus, in infected roots the sequence of defense must not only be considered in time but also in space as a result of a precise situation (i.e., periderm penetration, xylem colonization, or middle lamella degradation).

10.5.2 Efficacy of Root Defense to Fungal Invasion

As postulated by Beckman and Talboys (1981), roots are known to be the most vulnerable plant organs, in particular young roots. Although root defense increases with plant maturity, efficacy of reactions often decrease with the frequency and the successful penetrations of the tissues by the parasites. Root-rotting fungi penetrate the root system in several locations (Nicole et al. 1987), and the ectotrophic infection habit of these fungi suggests that the more rapidly a pathogen progresses into the taproot, the less the plant can react (Garrett 1970).

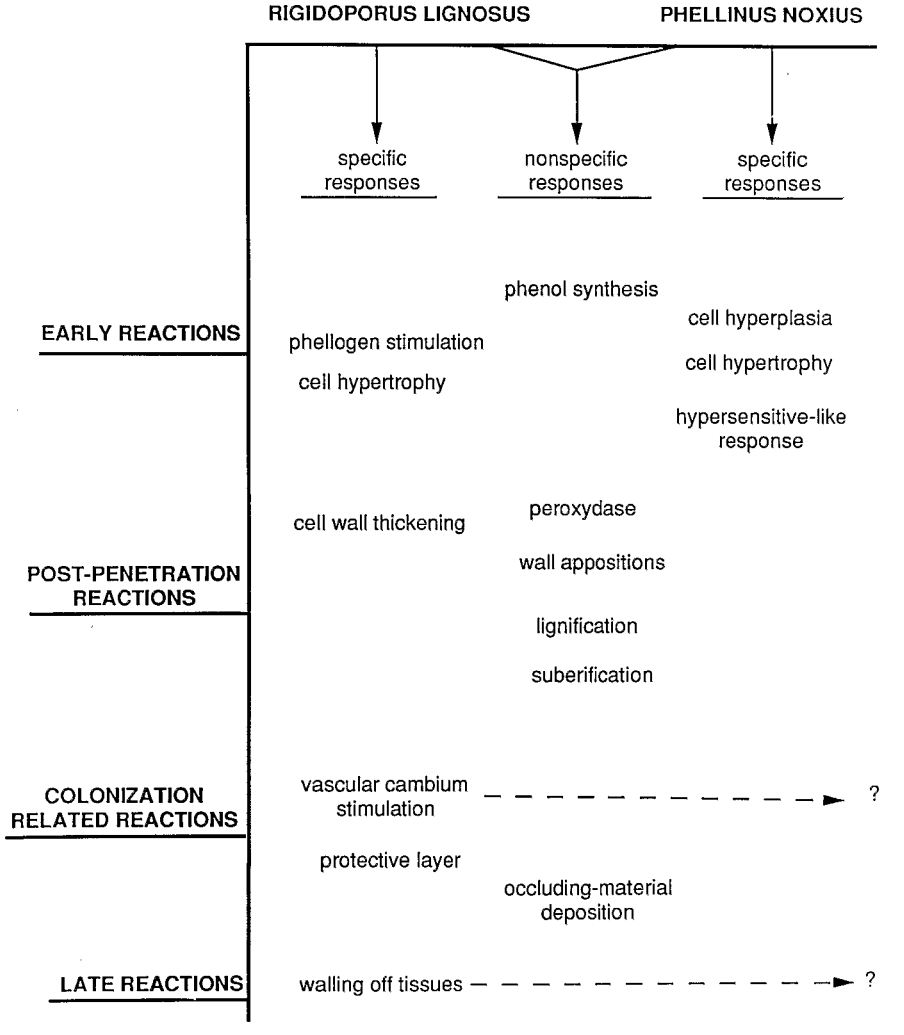


Fig. 10.21. Sequence of histological and biochemical reactions in *Hevea brasiliensis* roots following infections with *Rigidoporus lignosus* and *Phellinus noxius*. Rapid responses occur within a few days, late responses within several weeks after infection

When considered individually, none of the root responses described above are specific; moreover, none of them confers complete resistance to the whole tree; at best a balance is established between fungi and trees (Nicole et al. 1983). As Shigo (1984) stated, if environmental conditions do not favor the tree, the pathogen has an advantage. However, local efficacy was often reported for some of these reactions. Hypersensitivity has been suggested to precede the cessation of fungal growth (Ingram 1982), as observed in rubber tree roots infected with *Phellinus noxius*. The hypersensitive-like response of *Hevea*

brasiliensis is always associated with hypertrophied cells that prevent local colonization of cortical cells. This suggests that cell death or damage occurring in a hypersensitive response can induce host defense responses (Hahn et al. 1989). Similar efficient associations have also been described; xylem hyperplasia and hypertrophy, for example, often accompanied tylosis formation in vascular diseases (Beckman and Talboys 1981). However, to our knowledge, few references have reported resistance of callose to woody root rot fungi. Callose deposition in the young phellem cells of rubber tree roots do not resist fungal hyphae, which possess enzymes able to degrade these wall appositions (Fig. 10.16) (Nicole et al. 1986a,c).

When all reactions are considered together, survival of trees to root infection often depends on the success of root regeneration. Both meristematic and cambial activities contribute to the differentiation of new tissues (including lignification, suberization, and polysaccharide pathway processes), which may play a more positive role in resistance mechanisms than responses at the cellular level. Necrophylactic periderm and barrier zones restrict fungal spread by compartmentalizing decayed tissues in both bark and vascular tissues. No fungal penetration into and beyond the neodifferentiated tissues was observed (Pearce and Woodward 1986). Indeed, microscopic observations and isolation tests performed on the xylem barrier zone of roots of rubber trees infected with *Rigidoporus lignosus*, never allowed identification of the fungus. Although it has not been investigated, it is possible that the monomer composition of this neoxylem was different, thus preventing lignin degradation by the fungus (Geiger et al. 1986a).

The CODIT model elaborated by Shigo and Marx (1977) was applied to angiosperm roots (Shigo and Tippet 1981b, Nicole et al. 1986c). Accordingly, responses such as vessel occlusion, wall apposition, and hyperplasia or hypertrophy correspond to the movable walls 1, 2, and 3, whereas protective tissues correspond to the stationary wall 4. However, defense by compartmentalization is not always successful (Shigo 1984), and the tree often dies when the system does not function. In rubber tree plantations or under greenhouse conditions, tree survival of root rot diseases depends mainly on the efficacy of the barriers decribed by the CODIT model.

10.5.3 Elicitation of Some Defense Reactions in Tree Roots

Attempts to trigger defense reactions of woody plants by fungal elicitors have been made rarely in comparison with elicitation of herbaceous plant defense (De Wit 1986, Mazau et al. 1987, Esquerré-Tugayé et al. 1990). However, some authors reported in young trees, different aspects of elicitation as gossypol stimulation by conidia cell walls of *Verticillium* in *Gossypium barbadense* (Bell 1981). Miller et al. (1986) showed that monoterpene synthesis was induced in stem phloem of lodgepole pine (*Pinus concorta*) after inoculation with *Ceratocystis clavigerum* or treatment with chitosan. Rapid callose synthesis was also elicited in *Pinus elliotii* hypocotyls after injection of a conidia suspension of the pitch canker fungus (*Fusarium subglutinans*) (Valluri and Soltes 1990).

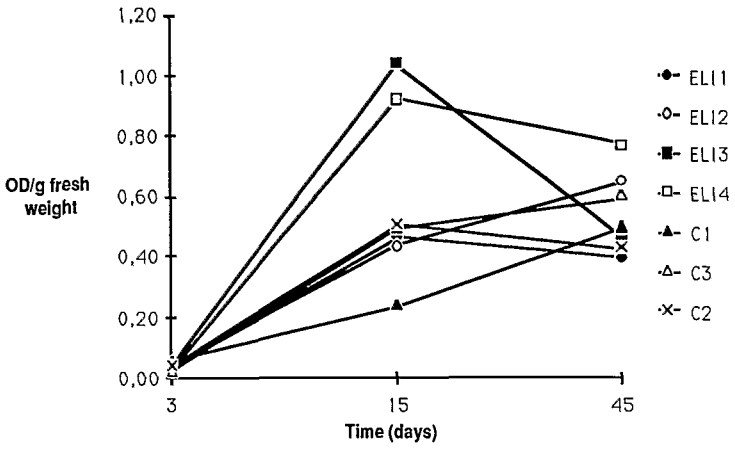


Fig. 10.22. Elicitation of the cinnamyl-alcohol dehydrogenase (CAD) of rubber tree roots. The CAD activity is expressed in OD/g fresh weight; *ELII*, *2*, *3*, and *4* different *Rigidoporus lignosus* elicitors; *C1* healthy plants; *C2* plants wounded with a needle; *C3* plants injected with water

Recents works on root rot diseases of the rubber tree revealed that elicitation of some reactions also succeed on roots of lignified plants (Nicole et al. 1991). Cambium activity and callose depositions in parenchyma cells were induced by *Rigidoporus lignosus* elicitors. Lignification was also triggered by fungal extracts according to microscopic observation and by assaying cinnamy-lalcohol dehydrogenase (CAD), a specific enzyme for lignin synthesis (Fig. 10.22). Elicitation of these defense responses by fungal cell wall fragments contributes to the strengthening of the structural barriers of rubber tree roots in order to prevent the extension of decay.

10.5.4 Lignification in Wood: a Model in Root Defense

Many papers point out the role of lignification in plant defense (Vance et al. 1980, Touzé and Esquerré-Tuguayé 1982). Lignification is known to restrict fungal progress in numerous ways, but in trees little attention has been devoted to the importance of lignification in root defense. An analysis of data collected on *Hevea brasiliensis* infected with *Rigidoporus lignosus* and *Phellinus noxius* suggest that lignification is strongly involved in wood defense and contributes to the resistance of some trees to decay (Fig. 10.23). Even though no close relations were established between the different rubber tree root responses, cell wall thickening, xylem neosynthesis, enhancement of peroxidase activity, involvement of this peroxidase in lignin monomer polymerization, increase in the lignin content of infected tissues, and the induction of the CAD with fungal elicitors, there is evidence for the contribution of lignin synthesis to tree defense (Nicole et al. 1986c). Survival of trees attacked by soil fungi depends mainly on the

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impossible. However, in tropical tree plantations (i.e., the rubber tree), the genetic origin of the "clones" is almost unknown. Each population of a root rot fungus is considered as a mosaic of clones with different pathogenic potential (Redfern 1975, Nicole et al. 1985). In addition, fungi such as *Rigidoporus lignosus* are known to infect more than 100 species of trees. Thus, selection of resistant or tolerant trees should increase genetic diversity in order to maintain a low level of disease.

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efficacy of the wall 4 barrier of the CODIT model, often concerned with intense lignification. It is therefore likely that specific genes may be involved in the biosynthesis of defense lignin (Walter 1990).

10.6 Root Defense: Tolerance or Resistance to Fungal Invasion?

Tolerance and resistance are concepts that have been used to describe the host-parasite interaction. However, the definitions given by several authors are sometimes confusing and their use in tree infections, such as root rot diseases, might be debatable. Clarke (1986) suggested that we should establish tolerance scale ranging from highly tolerant to acutely intolerant plants; a similar continuum has been described for resistance. He proposed different forms of tolerance related to the parasite (tolerance to the parasite), to the disease (tolerance to disease), or to both (overall tolerance). Tolerance can be shown not only by the whole plant, but also at the cellular and/or field level (Mussell 1980), assuming that the physiological mechanisms of their reactions are known.

Identification of tolerance or resistance among trees infected with root-rotting fungi, if foliar symptoms do not occur, requires the uprooting of the plants to provide visual evidence of infection. The tolerance of the rubber tree to root-rotting fungi seems to be mainly determined by the severity of the disease. A given strain of *Rigidoporus lignosus* or *Phellinus noxius* causes damage which varies from tree to tree. In a population of artificially infected seedlings of *Hevea brasiliensis* clone GT1, only 5% survived the disease. Although the level of infection was identical with that in dead trees, infection was severely restricted in the surviving plants by host reactions, decayed roots being replaced by newly formed tissues.

More generally, in tree root diseases, it would be interesting:

1. To understand the role of tree vigor in regrowth of damaged root tissues. As stressed by Rishbeth (1972), survival of a tree often depends on the balance between regeneration and killing of roots. The level of infection-induced disturbances of the tree metabolism interferes strongly with the plant's ability to allocate resources to defend itself against root fungal infection (McLaughlin and Shriner 1980), thus determining the level of tolerance.
2. To know whether root tolerance varies with the variable aggressiveness of strains. Such a variation of pathogenicity is well known in root rot fungi (Raabe 1967, Prillinger and Molitoris 1979, Nandris et al. 1987b) as well as in vascular pathogens. Evidence of pathotypes of *Rigidoporus lignosus* and *Phellinus noxius* was demonstrated in isolates originating from various countries and even from the same plantation (Nicole et al. 1985).
3. To find out if tolerance, or resistance, of tree roots is of genetic origin. In tree breeding projects, resistance or tolerance to diseases is now being taken into account. This implies, first, the identification of resistance mechanisms and, second, the determination of the possible level of specificity. According to Carson and Carson (1989), without specificity, genetic control seems

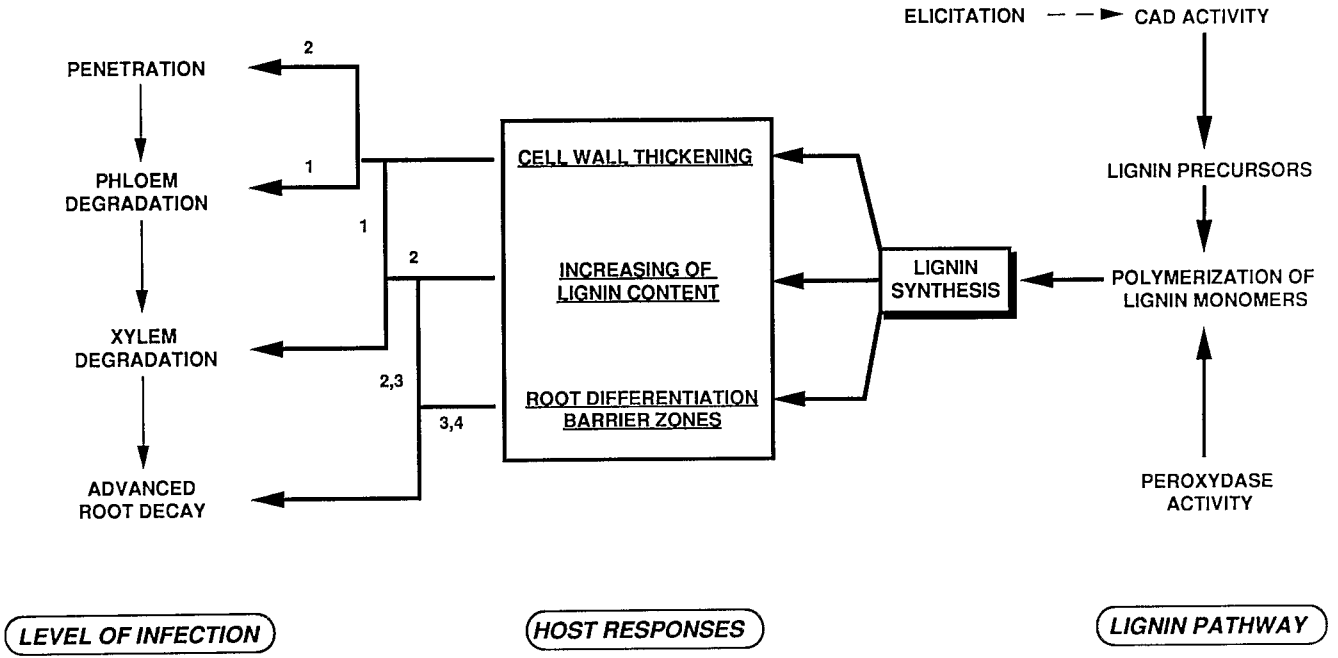

Fig. 10.23. Lignification as a defense reaction in roots of rubber trees infected with root rot fungi. *1* Possible local efficacy; *2* stop fungal spread; *3* stop decay extension; *4* induced tolerance or resistance of trees

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