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Penetration and degradation of suberized cells of *Hevea brasiliensis* infected with root rot fungi

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Electron microscopic studies of roots of young *Hevea brasiliensis* plants artificially infected with *Rigidoporus lignosus* and *Phellinus noxius* are described. *Rigidoporus lignosus* differentiates specialized structures which penetrate the suberized tissue. For both fungi, cell colonization is characterized by the degradation of suberized walls. The observations indicate that cell wall alteration is predominantly due to enzymatic activity. The significance of these observations in relation to the pathogenicity of both parasites is discussed.

INTRODUCTION

Rigidoporus lignosus and *Phellinus noxius* are two root-infecting fungi which can cause severe damage to *Hevea brasiliensis* [12]. A study of the infection process by these parasites on young artificially inoculated plants, has shown that root penetration by hyphae occurs during the first 10 days after inoculation [14]. Light microscope observations confirmed that both fungi penetrated through the periderm and indicated that penetration resulted in changes in the suberized cell walls of this tissue [13].

Little work has been done on the degradation of phellem cells and, in particular, of their suberized cell walls [7], by wood rotting fungi, although it is likely that this is important. Suberin-degrading enzymes, which are able to release aliphatic monomers from suberin preparations, have been characterized in *Armillaria mellea* cultures [21]. However, cellular aspects of bark degradation remain poorly documented [16, 19].

This paper describes an ultrastructural study of bark penetration and suberized cell wall degradation of rubber tree roots by *R. lignosus* and *P. noxius*, which was carried out in order to investigate the first steps of the infection process by these two parasites.

MATERIALS AND METHODS

Artificial infections

The method used was developed under greenhouse conditions [11]. Seeds of *H. brasiliensis* (clone GT1) were collected from IRCA plantations. After germination in sand, young seedlings were pricked out into tubs (1 × 1 × 1 m) filled with forest soil. The water content of the soil was brought to field capacity and then maintained at this level using a neutronic moisture gauge (Solo 20).

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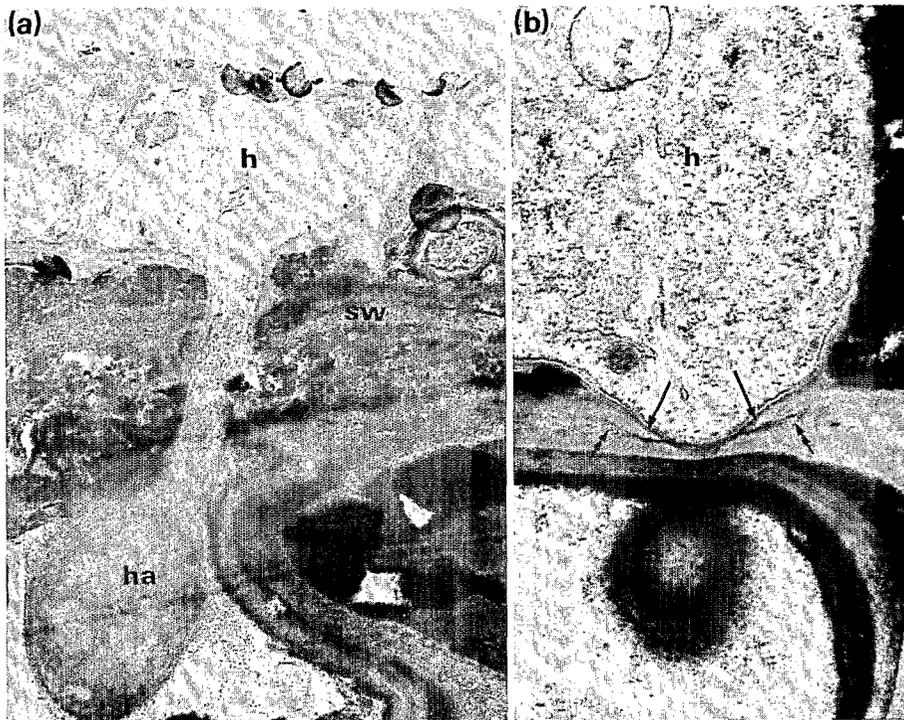


FIG. 1. *Hevea brasiliensis* cells infected with *Rigidoporus lignosus* root rot fungus.

(a) A haustorium-like structure (ha), differentiated by *R. lignosus* from a hypha (h) spreading along the root has perforated the suberized wall (sw) of a cell in the outer periderm of the root. $\times 5400$.

(b) Suberized walls of young periderm cells in the roots of a 6-week-old rubber tree seedling. Both mechanical forces, as indicated by fibre compression (arrows), and enzyme degradation (double arrows) appear to contribute to cell wall penetration by *R. lignosus*. $\times 18\ 000$.

When 1 month old, each plant was inoculated by applying five pieces of rubber tree wood (8 cm length, 2 cm diameter), pre-infected with *R. lignosus* or *P. noxius*, against the tap root, 20 cm deep in the soil.

Microscope observations

Infected and non-infected plants were fixed *in toto* for 2 h at 4 °C, immediately after sampling, in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.2). The roots were then cut into small segments (1 mm³) and fixed again for 2 h at 4 °C. After washing four times in the same buffer, (15 min each wash, with a final wash overnight at 4 °C), the samples were postfixed for 2 h in 1% osmium tetroxide at 4 °C. Following a final rinse in sodium cacodylate, the segments were dehydrated in an ethanol series ranging from 5 to 100% and embedded in Spurr's resin [20] or in Epon 812 [10] preceded by an epoxy propane change (2 \times 1 h duration). The pieces were infiltrated in the resin for at least 5 days. The roots were sectioned, mounted on 200 mesh grids and stained with uranyl acetate and lead citrate [18], and then examined on a Siemens Elmiskop 102 electron microscope operating at 80 kV.

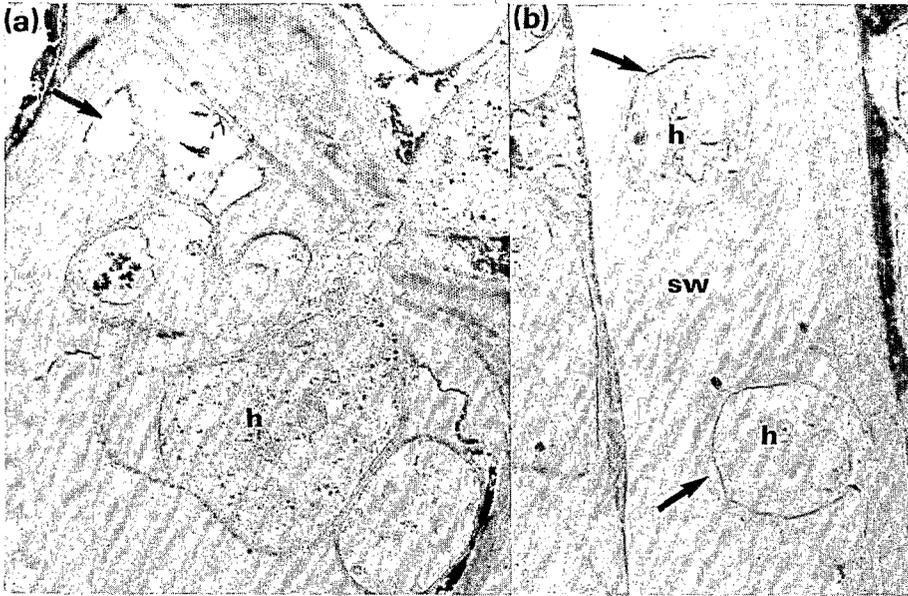


FIG. 2. Hyphae of *Rigidoporus lignosus* in *Hevea brasiliensis* suberized cells.

(a) The hypha (h) of *R. lignosus* is constricted during passage through the wall but resumes a normal dimension on emergence into the lumen of the cell. The formation of holes (arrow) in the wall adjacent to the hypha characterizes one form of cell wall degradation by both fungi. $\times 4500$.

(b) Hyphae (h) of *R. lignosus* growing in mature suberized cell walls (sw). Note that digestion of the wall is limited to a narrow region around the fungal hyphae (arrows). $\times 11\ 250$.

RESULTS

Observations of root penetration by *R. lignosus* show that the penetrating hyphae form haustorium-like structures which perforate the suberized periderm cell wall. Such structures [Fig. 1(a)] are not as well organized as those differentiated by other fungi, and have only been seen on hyphae spreading on the root surface. They seem to be specialized for root penetration. These structures were not observed on roots infected with *P. noxius*. However, the external mycelial sleeve produced on the roots by this fungus is impregnated with stones and sand, and so clear observations were not possible.

Both fungi colonized cork cells, the suberized cell walls being penetrated by a combination of mechanical and enzymatic action [Fig. 1(b)]. The hyphae within the wall were often constricted [Fig. 2(a)] and cell wall breakdown seemed to be limited to regions closely adjacent to the hypha. Different patterns of degradation were observed. First, digestion of the cell walls immediately in contact with the fungal filaments was particularly evident in relation to hyphae growing in the cell wall and during penetration through the wall [Fig. 2(b)]. Second, modification of the fibrillar structure of the walls of young cells, in which suberin deposition on cellulose fibres is actively in process was evident [Fig. 3(a)]. Third, but less frequently, the development of small cavities in the wall adjacent to the penetrating hypha could be seen [Fig. 2(a)].

Phellinus noxius appears to degrade cell structures more rapidly and more completely than *R. lignosus*. However, with both fungi the lumen of suberized phellem cells can become totally filled with hyphae [Fig. 3(b)].

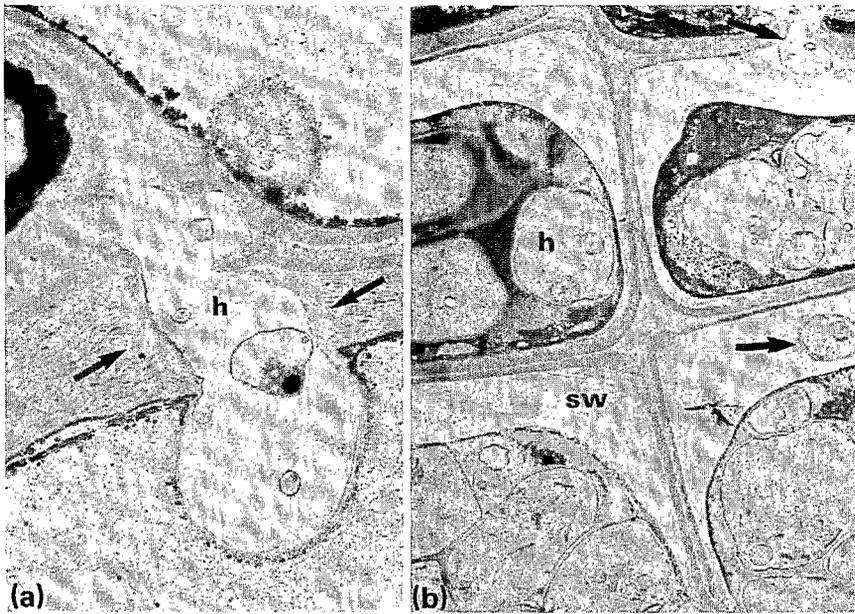


FIG. 3. Suberized cells of *Rigidoporus lignosus*-infected *Hevea brasiliensis*.
 (a) Modification of the wall of a young suberized cell, in contact with a penetrating *R. lignosus* hypha (arrows). The hypha (h) has degenerated. $\times 9000$.
 (b) Total colonization of the lumen of suberized phellem cells by *R. lignosus*. $\times 4500$.

DISCUSSION

The differentiation of haustorium-like structures by *R. lignosus* indicates that active mechanisms are involved in the penetration of rubber tree roots by this fungus. These structures have not been noted before on this species and are probably specialized for periderm cell wall perforation. Indeed, this pathogen normally develops two types of mycelium which are different from each other morphologically and metabolically [1] including their enzymatic secretions [3]. One type occurs in the rhizomorph and is probably specialized for superficial dissemination, while the other for infection.

For both fungi, ultrastructural observations indicate that hyphal growth within the corky layers of the bark involves enzymatic degradation of suberized cell walls. These studies show that cell wall digestion is localized around hyphae, suggesting that enzyme diffusion is limited. Enzymatic degradation of plant polyesters has been well described for cutin [6, 8, 9] but more rarely for suberin [2, 21]. Suberin is a complex polymer consisting of an aromatic moiety, similar to lignin, attached to aliphatic fatty acids and associated with alcohols and waxes. Its degradation probably involves a range of enzymes including oxidases and esterases [6, 7, 17]. Laccases, which contribute to the degradation of lignin-like polymers [4], have been shown to be produced by both *P. noxius* and *R. lignosus* in the outer bark tissues [13]. Enzymes capable of degrading fatty acid esters (C6 to C18) have been identified in culture filtrates when these fungi have been grown on sterilized rubber tree bark [5]. However, no experimental work has been done *in vivo* to determine the implication of these enzymes in suberin degradation.

At the present time the only evidence for some degradation of this substrate in *H. brasiliensis* comes from microscopic observations.

Nevertheless, our observations strongly implicate some fungal activity in the degradation of the suberized walls of phellem cells. Such enzyme activity is probably also involved in the degradation of the suberized walls of the primary cortical parenchyma in young roots. Changes in the suberin content of the walls of these cells (characterized by histochemical tests) have been described as reactions in rubber tree roots against these parasites [15].

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