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Ultrastructure of Laticifers Modifications in *Hevea brasiliensis* Infected with Root Rot Fungi

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With 14 figures

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Abstract

This paper presents some aspects of latex coagulation inside latice vessels of roots of *Hevea brasiliensis* infected by two fungi: *Rigidoporus lignosus* and *Phellinus noxius*. Three stages are described in latex coagulation: the phase of latex destabilisation characterized by the bursting of vacuoles and lysosomes membranes; the phase of latex coagulation characterized by the fusion of rubber particles and the disorganization of the cytoplasm; the formation of shots of rubber clumps indicating the final stage of coagulation.

Zusammenfassung

Ultrastruktur der Modifikationen der Milchröhren von *Hevea brasiliensis*, infiziert mit Wurzelfäulepilzen

Diese Arbeit beschreibt einige Formen der Milchsaftkoagulation im Inneren der Milchröhren der Wurzeln von *Hevea brasiliensis*, die mit 2 Pilzen infiziert wurden: *Rigidoporus lignosus* und *Phellinus noxius*. Es wurden drei Stadien der Milchgerinnung beschrieben: die Phase der Destabilisierung der Latex charakterisiert durch das Aufbrechen der Vakuolen und Lysosomenmembranen; die Phase der Milchgerinnung charakterisiert durch die Fusion von Gummipartikeln und die Desorganisation des Cytoplasmas; die Bildung von Gummiklümpchen, die die letzte Stufe der Gerinnung anzeigt.

Hevea brasiliensis belongs to the Euphorbiaceae, a well-known laticifer family in plant world. It is widely planted in tropical countries for rubber production and constitutes for them a source of important currencies. Variable

losses are caused by parasites in rubber tree plantations. It is the case in the Ivory Coast where *Rigidoporus lignosus* and *Phellinus noxius*, two soil-living fungi, are responsible of root rot diseases (NANDRIS *et al.* 1983 a, 1985). Recent studies have demonstrated different aspects of *H. brasiliensis*-parasite interactions at the level of both host aggression (PERIES *et al.* 1973, GEIGER *et al.* 1976, NICOLE *et al.* 1982 a, GEIGER *et al.* 1983, NICOLE *et al.* 1983) and reaction of trees against these pathogens (NICOLE *et al.* 1986 a). If cellular and molecular aspects of rubber tree tissues degradation are for now well understood (GEIGER *et al.* 1986, NICOLE *et al.* 1986 b), very little data have been reported, on the other hand, on the ultrastructure of latex coagulation which occurs inside laticifers during root decay (NICOLE *et al.* 1982). Such a coagulation has been previously reported on trunk of rubber trees stressed by the brown bast syndrome (DE FAY and HEBANT 1980).

The present paper provides ultrastructural informations on *in situ* latex coagulation in *H. brasiliensis* root system infected with these two fungi and attempts to define the biological signification of this event in pathogenesis of both parasites.

Materials and Methods

Artificial infections: The following methodology was developed under greenhouse conditions by NANDRIS *et al.* (1983 b). Seeds of *H. brasiliensis* (clone GT1) were collected in IRCA plantations. After germination in sandy tubs, young seedlings were pricked in tubs (1 × 1 × 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, constituted of *R. lignosus* or *P. noxius* pre-infected rubber tree wood sticks, were applied against the tap root, 20 cm deep in the soil. Diseased plants were then collected at different stages of infection as for light and electron microscopic preparation.

Microscopic observations: classical techniques of plant preparation for electron microscopy (HALL 1978) were modified and adapted to rubber root tissues as described by NICOLE *et al.* (1986 a). The sections, mounted on 200 mesh grids, were stained with uranyl acetate and lead citrate (REYNOLDS 1963), and then examined on a Siemens Elmiskop 102 electron microscope operating at 80 kV.

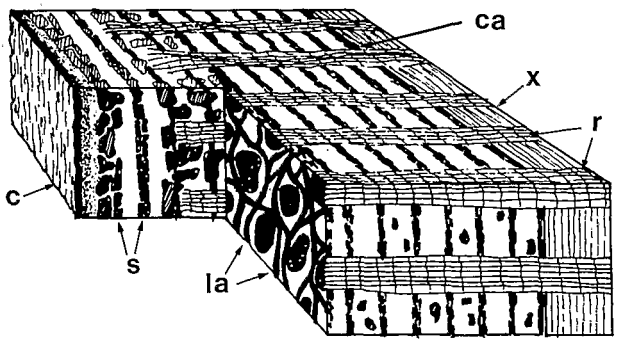


Fig. 1. Histological organization of *Hevea brasiliensis* phloem: laticifers are organized in concentric mantles (la) which alternate with parenchymatous rays (r) and sieve tubes. (c: cork; ca: cambium; s: sclerids; x: xylem)

Results

The secondary phloem of *H. brasiliensis* presents a sequential histological organization (BOBILIOFF 1923, HEBANT and DE FAY 1980, i.a.) (Figs. 1 and 2). The concentric mantles of laticifers alternate with the parenchymatous cells and sieve tubes. They are constituted of longitudinal articulated vessels, parallel to the main axis of the tree; anastomoses are frequent within a ring. The youngest vessels are confined to the cambium, in the conducting phloem which contains the main active sieve tubes. These vessels are tapped for latex production, avoiding cambium damages in order to ensure bark renewal. Close contacts have also been established between tannin cells and laticifers (TRANCARD 1979). In older phloem, this organization is perturbated by sclerids differentiation (Fig. 2).

In a rubber tree, latex is defined as the fluid cytoplasm of laticifers (ARCHER *et al.* 1963). It is a colloidal suspension, or a hydrosol from the physico-chemical aspect, with organelles, rubber and non-rubber particles (Fig. 3). Electron microscope observations have revealed:

— typical rubber particles, constituted of isopren polymer of 500 nm diam. for the larger (Fig. 4);

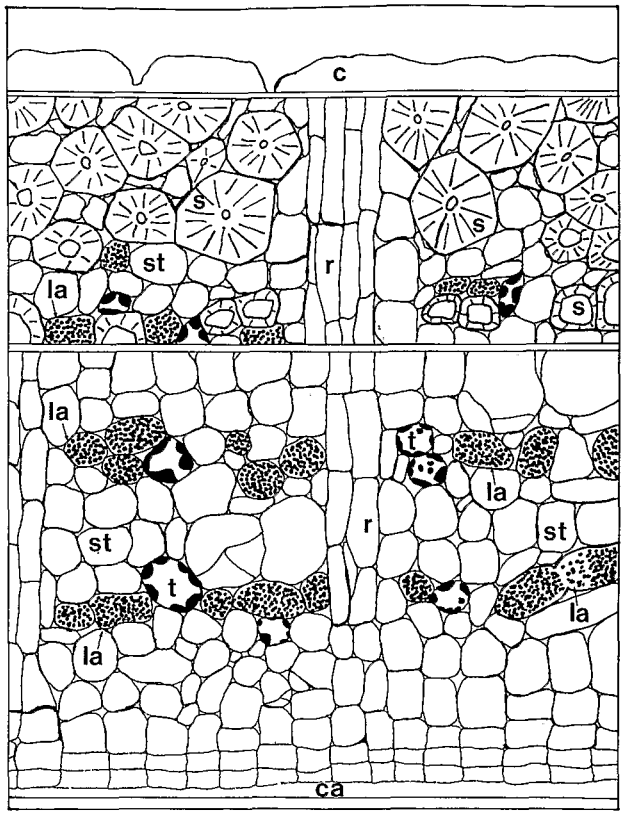
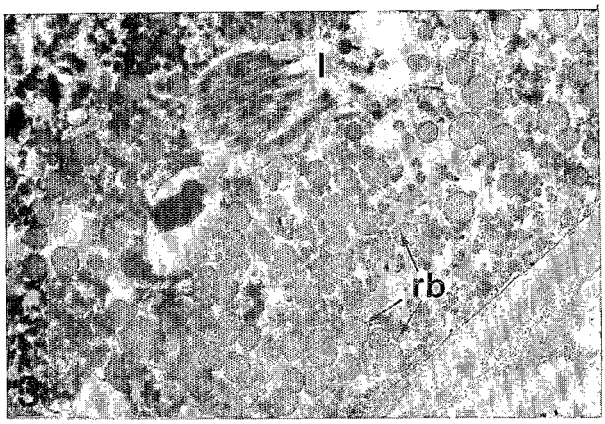


Fig. 2. Histological organization of *Hevea brasiliensis* phloem: the conducting phloem lies near the cambium (ca) and contains the functional sieve tubes (st) and laticifers (la), tapped for latex production. Tannin cells (t) are associated with laticifers mantles. The differenciation of sclerids (s) in the older phloem modifies its organization, thus preventing latex production. (c: cork; r: parenchymous rays)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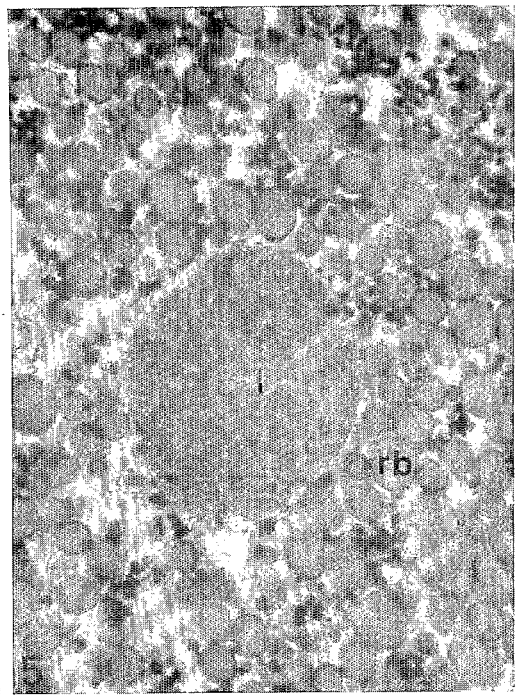
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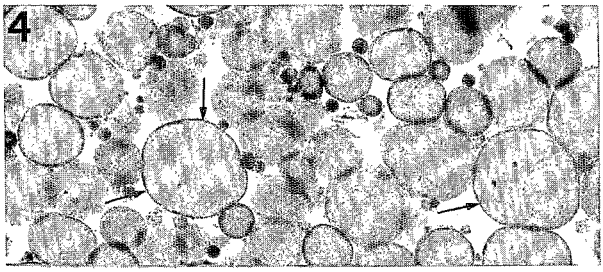
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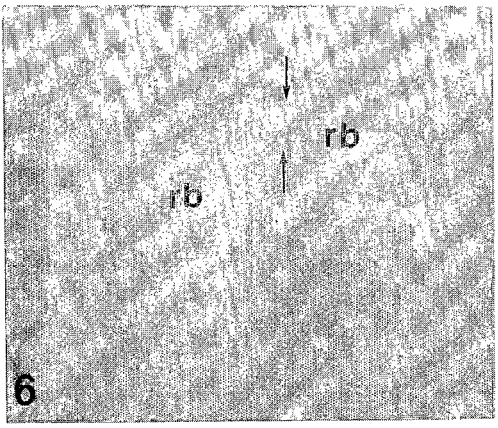
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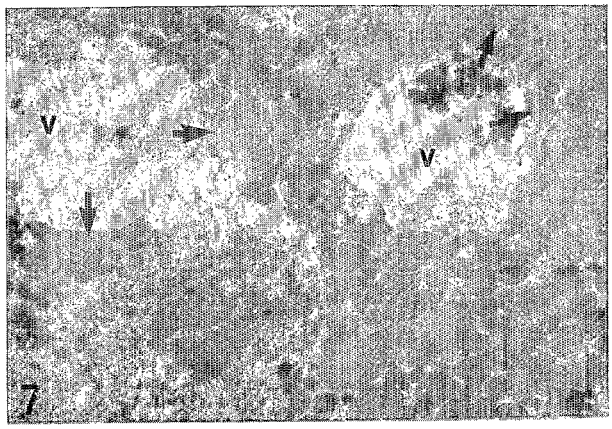
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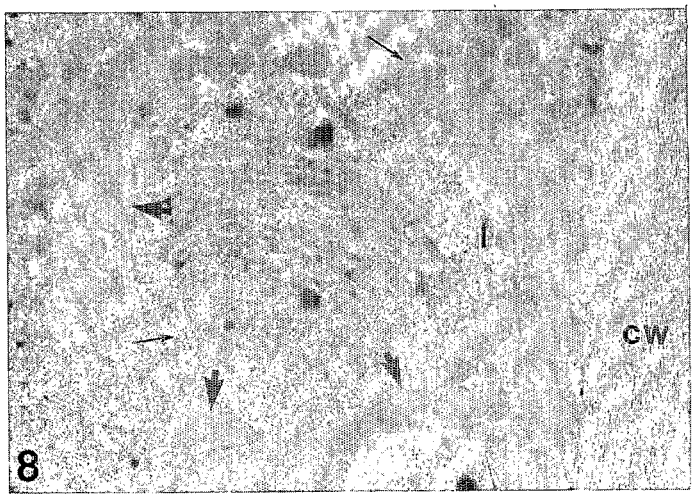
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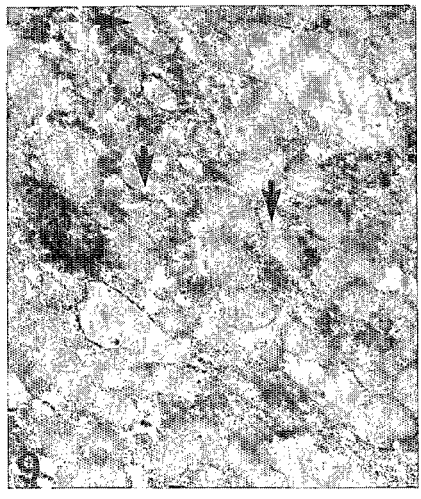
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— Frey-Wissling particles bounded with a double phospholipids membrane; this organelle is rare in latex (DICKENSON 1969) and very little is known about its role in the latex metabolism;
— lutoids are specialized single-membrane forms of polydispersed vacuom with lysosomal characters (PUJARNISCLE 1968, RIBAILLER *et al.* 1972, D'AUZAC *et al.* 1982). They show a relationship with the ER and can incorporate rubber particles (HEBANT 1981). In immature latex, lutoids possess inclusions consisting of oriented protein microfibrills (Fig. 5). In laticifer metabolism they control the cytosolic homeostasis by acting as a detoxicating trap (CHRESTIN *et al.* 1984a), favouring a high rubber production.

All these particles and the classical organelles composing the laticifer cytoplasm generated repulsive electrostatic forces from the negative charges present on their membranes (CHRESTIN *et al.* 1984b), remaining the stability of the colloidal suspension. Perturbations of this organization cause a rupture of the electrostatic balance and induce latex coagulation.

Such a coagulation occurs in laticifers of rubber tree roots infected with *R. lignosus* and *P. noxius*. Examination of diseased roots showed well the absence of latex flow in healthy tissues, suggesting the inside coagulation in front of the fungal progression line. Electron microscope observations of infected root samples, at different stages of the infective process, revealed several modifications of laticifer organization.

a) the phase of latex destabilisation: rubber particles merge after bridging establishment to form microcoagula (Fig. 6) which accumulate around the vacuolar system (Fig. 7), and especially adhere to lutoids whose membrane begins to burst (Fig. 8). During this phase, microvesicles appear in latex cytoplasm (Fig. 9).
b) the phase of latex coagulation is characterized by the fusion of clusters of vesicles (Fig. 10) favouring the fusion of individual microcoagula, thus eliminating large volume of cytoplasm (Fig. 11). The lysis of classical vacuoles, of lutoids and of Frey-Wissling particles (not observed under

Fig. 3. The latex cytoplasm is a stable colloidal suspension composed of classical organelles, lutoids (l), Frey-Wissling particles and rubber particles (rb); (healthy tissue: control; ×9000)

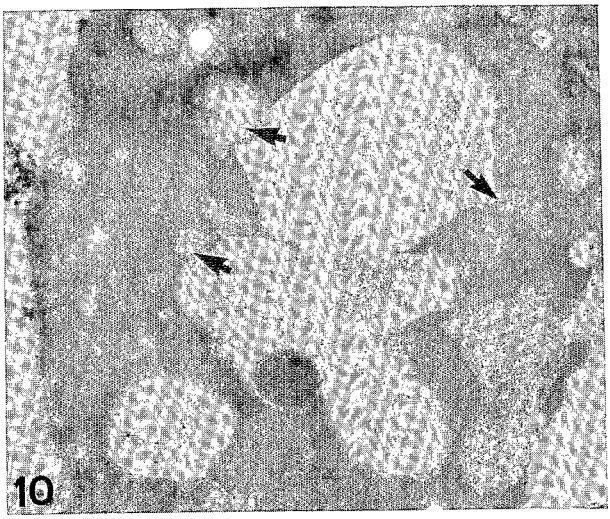
Fig. 4. Rubber particles, of different sizes, are surrounded by a thin film (arrows), more electron-dense than the rest of the particle; (healthy tissue: control; ×18750)

Fig. 5. Lutoids (l) are single-membrane vacuoles with lysosomal characteristics; the young form of lutoids contains fibrillar proteins visible on this microphotograph, in cross and longitudinal sections; (rb: rubber particle). (Healthy tissue: control; ×20000)

Fig. 6. In infected phloem, the first stage of latex coagulation is characterized by the fusion (arrows) of rubber particles (rb) to form microcoagula (×37500)

Figs. 7 and 8. During latex destabilization, microcoagula (thick arrows) accumulate around vacuoles (v) (Fig. 7) and lutoids (Fig. 8) which membrane is burst (thin arrows); (cw: host cell wall) (Fig. 7: ×15000; Fig. 8: ×15000)

Fig. 9. The apparition of small vesicles (arrows) in laticifers cytoplasm initiates the latex coagulation *sensus stricto* (×18750)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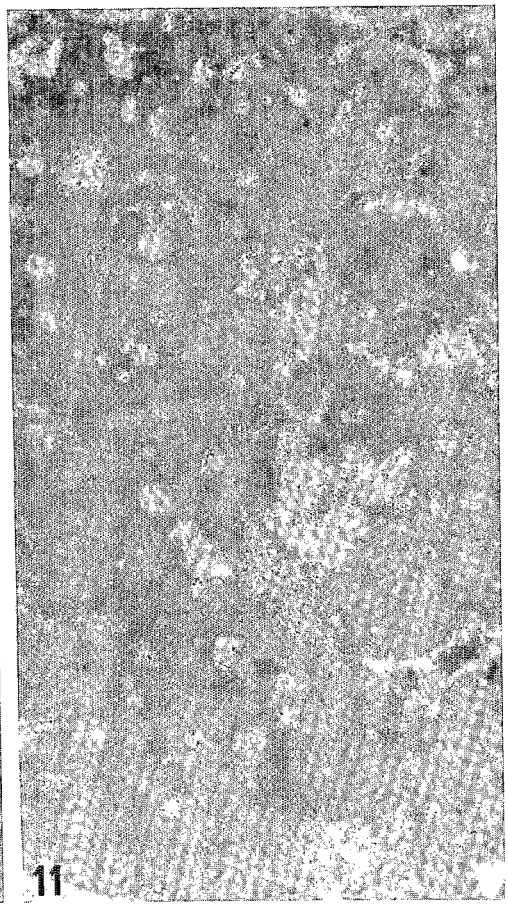
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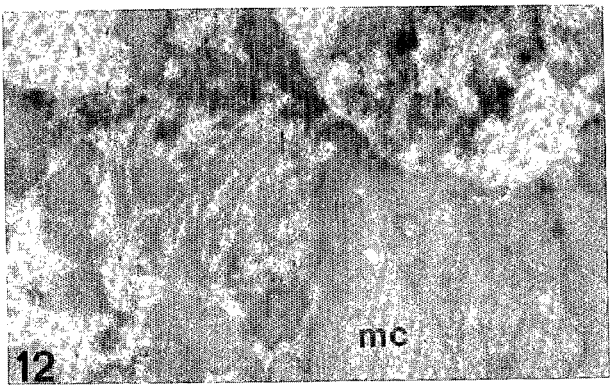
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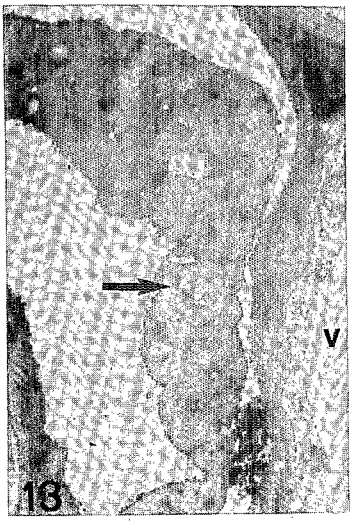
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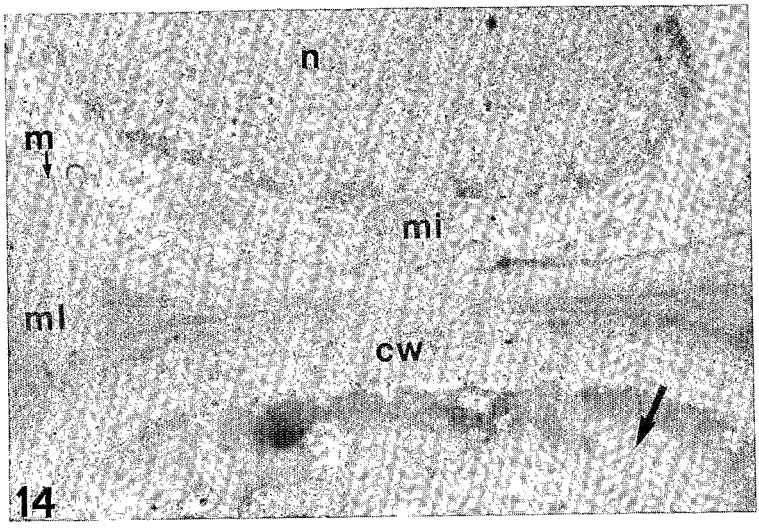
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TEM), resulting in membrane bursting, causes the disorganization of their content (Fig. 12).

c) the final phase of coagulation: the laticifer cytoplasm is completely digested; no membrane structures and organelles can be detected. All the latex is solidified (Fig. 13).

These three phases are characterized by the absence of mycelium inside latice vessels, or in contact with them, as well as in decay tissues than in tissues recently contaminated. Inside latex coagulation can be induced before phloem colonization. In this case, associated parenchymatous cells of laticifers do not present any cellular modifications, the organelles keeping their integrity (Fig. 14).

Discussion

Generally, latex coagulation is conditioned by external stresses as phloem wounds caused by the tapping cut for rubber exploitation. During root infection with *R. lignosus* and *P. noxius*, and the brown bast syndrome, on the other hand, latex coagulation is induced inside laticifers without external injuries. In this last case, from an anatomical point of view, DE FAY and HEBANT (1980) have characterized the inside coagulation first by the presence of protuberances in laticifers, called thyllosoids and originated in neighbouring cells, and secondly by a proliferation of hyperplasic tissues. Such thyllosoids have never been observed in roots of infected rubber trees. However, hyperplasic tissues were also described, but rather as a tree reaction against aggression by the pathogens (NICOLE *et al.* 1986b).

Biochemical studies of latex coagulation, have shown that coagulating factors are compartmentalized in lutoids and Frey-Wissling particles (HANOWER *et al.* 1976, BRZOZOWSKA-HANOWER *et al.* 1979, D'AUZAC *et al.* 1982). Among these factors, some ions such as H^+, Ca^{++}, Mg^{++}, enzymes such as polyphenol-oxidases and proteases are the most active (HANOWER *et al.* 1976, YIP and GOMEZ 1980). Mechanism of "inside coagulation" revealed that lutoids contain an enzymatic activity that generates superoxyde anions ($O^{\cdot}_2$) from NAD(P)H and O_2 (CRETIN and BANGRATZ 1983, CHRESTIN *et al.* 1984b and CHRESTIN 1984). This oxygen form ($O^{\cdot}_2$) is toxic and leads to a peroxidative degradation of the insaturated membrane lipids. Destabilization of organelles, especially of the vacuolar complex, causes the decompartmentalization of coagulating factors which spread through the cytoplasm, initiating latex coagulation. So, as mentioned by D'AUZAC and JACOB (1984), "lutoids play the role of a suicide bag".

Figs. 10, 11 and 12: The fusion of vesicles (Fig. 10; arrows) favours the formation of coagula (c), leading to the disorganization of the laticifer cytoplasm (Fig. 11) and the degradation of membrane structures (Fig. 12); (l: lutoid). (Fig. 10: ×37500; Fig. 11: ×37500; Fig. 12: ×22500)

Fig. 13. Final stage of coagulation indicated by shots of clump of rubber (arrow); no organelles can be detected in laticifers; (v: vacuole) (×6000)

Fig. 14. Fungal filaments have not been seen inside laticifers or in contact with them during phloem degradation; when coagulation occurs before phloem colonization (arrow), the organization of bordering cells is not pertubated; the organelles keep their integrity; (cw: host cell wall; m: cell membrane; mi: mitochondria; ml: middle lamella; n: nucleus) (×15000)

Our TEM observations of infected roots have revealed the bursting of lutoid membrane and the simultaneous latex coagulation. The absence of fungal filaments in (or near) laticifers, and the initiation of latex coagulation in the non yet contaminated phloem, suggest that the determinism of coagulation might take place from a distance of laticifers. The functional organization of *H. brasiliensis* phloem, described by HEBANT and DE FAY (1980), is based upon a three dimensional network assuring connections between axial parenchyme in the xylem, ray cells of both phloem and xylem, and parenchymatous cells associated with the laticifers. This structural organization allows radial and vertical transport of metabolites through ray cells which interrupts the continuity of laticifer mantles. Fungal extracellular enzymes (laccases and hydrolases), or secreted metabolites, and the different degradation products released by the digestion of the host cell walls by the parasites (GEIGER *et al.* 1986), are probably conveyed in the ray cells until laticifers. Among them, several are able to break the electrostatic balance of latex cytosol.

According to ERIKSSON (1981 a) the main white rot fungi secrete enzymes as cellobiose oxidase or glucose oxidase implied in cellulose degradation. Cellobiose oxidase of *Sporotrichum pulverulentum*, for example, is known to generate $O^{\cdot}_2$, which may be involved in the primary attack of cellulose and lignin (ERIKSSON 1981 b). Attempts to detect such fungal enzymes activities in roots infected with *R. lignosus* and *P. noxius* did not succeed (GEIGER *et al.*, unpublished). Nevertheless, measurements of superoxide dismutase (SOD) and catalase activities, two constitutive membrane protective enzymes of the rubber tree latex (FRIDOVICH 1975), would further precise the $O^{\cdot}_2$ enhancement in the infected phloem, and thus indicate if the mechanism of inside coagulation described in the "brown bast syndrome" can be applied to the latex coagulation observed in decayed roots. Such experiment is presently in process.

Observations of healthy rubber trees as control, have revealed that *in vivo* latex coagulation also occurs in roots, but at a very low level. Biological signification of inside coagulation can be explained by the senescence of laticifers. Membrane bursting and cytoplasm disintegration during laticifers maturation have been reported on other latex plants (RACHMILEVITZ and FAHN 1982). Moreover, SHELDRAKE and MOIR (1970) have detected an endogenous cytoplasmic cellulase activity in *H. brasiliensis* laticifers, also found in *Ficus carica* by GIORDANI (1981). This hydrolase occurs in differentiation and maturation of laticifers, and could be stimulated during root decay, thus causing latex destabilization.

So, considering biochemical results and microscopic observations, it is possible that in infected roots, *in situ* latex coagulation square with an activation of laticifers senescence and can be considered as a secondary event in rubber tree root rot diseases.

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