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The histology of Hevea brasiliensis phloem necrosis

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Abstract

Phloem necrosis of rubber tree (*Hevea brasiliensis*) is characterized by cell wall degradation, alteration of the middle lamella, the vesiculation of endomembranes, the formation of tylosoids and the internal coagulation of rubber.

1 Introduction

Phloem necrosis of the rubber tree (*Hevea brasiliensis*), a disease of which the aetiology remains unknown, was discovered in the Ivory Coast a few years ago. The continuing increase in numbers of infected trees in plantations is now considered serious.

This disease is mainly characterized by the occurrence of small grey circular patches and thin cracks on the surface of the bark where wood-borer holes also cause latex to flow. Beneath the phellem cells, in the secondary phloem where latex is produced, the necrosis first appears as brown sheets which sometimes reach the cambium and is followed by rotting and the disorganization of the tissue. Eventually, the trunk becomes completely deformed through cracking of the bark between the collar and the tapping area (Nandris et al. 1984). The purpose of this paper is to describe the major histological modifications that occur in the phloem at the brown sheet stage, with the aim of gathering together useful information to help identify the causes of this disease.

2 Material and methods

Samples of bark, comprising suber and phloem tissues, were stamped out from rubber tree trunk and roots (Table 1) on three different occasions, corresponding to the wet season (July), to the period just before defoliation (December) and to the dry season just

Table 1. **Origin of selected trees in plantations for microscope observations**

	Clone	Plot	Quantity	Age (years)
Diseased trees	GT 1	Heke 25	5	11
		Singhe 78	2	7
	PB 235	Baco 30	10	8
		Total	17	
Healthy trees	GT 1	Heke 25	1	11
		Singhe 78	1	7
		Kaco 90	2	7
	PB 235	Baco 30	3	8
		Dole 16	2	4
	PB 217	Baco 17	2	4
	Polyclone	Heke 29	2	4
		Total	13	

after fresh leaf growth (March–April). Samples for TEM examinations were treated as followed: glutaraldehyde (3 % aqueous solution) was injected into the phloem at the point of uptake. After a few minutes, the prefixed phloem, including the cambium and some cells of the xylem, was removed from the tree, cut into small pieces in cold glutaraldehyde (3 %, 4 °C) and fixed for 2 hours in the same solution. After several washings in a sodium cacodylate buffer (0.1 M, pH 7.2 at 4 °C), the fragments of the xylem were removed and the samples were then postfixed (2 h, 4 °C) in 1 % osmium tetroxide. Following a further rinse in sodium cacodylate, the segments were dehydrated in a gradual ethanol series from 5 to 100 % and embedded in Epon 812 resin, as described previously by NICOLE et al. (1987). Ultrathin sections were stained with a saturated alcohol solution of uranyl acetate, followed by lead citrate, before being examined under a TEM Siemens Elmiskop 102 (GERME, Abidjan) or an Elmiskop 101 (University of Dakar). Thin sections were stained with toluidine blue and observed under a light microscope (Leitz Orthoplan).

3 Results

3.1 Some observations on histology of rubber tree phloem

The secondary phloem of *Hevea brasiliensis* is a complex tissue composed of several types of cell; these include

- articulated and anastomosed laticiferous vessels arranged in concentric rings;
- tannin cells and parenchymatous cells closely associated with laticifers;
- sieve tubes;
- stone cells with sclerified walls;
- cells specialized for crystal production;
- parenchymatous rays connected with wood rays.

The organization of the phloem reveals two distinct zones (Fig. 1):

- the soft inner phloem which lies close to the cambium, containing active sive tubes and functional laticifers which are tapped for latex (Fig. 2);
- the hard outer phloem which contains clustered stone cells and the older mantles of laticifers (for more details see DE FAY and JACOB 1989a).

3.2 Ultrastructure of necrosed phloem

TEM examination of diseased tissues, taken from brown sheets, revealed numerous necrosis-linked modifications, mainly concerning cell walls, the middle lamella and the cell membranes.

3.2.1 Cell wall degradation

Cell wall degradation occurred in all necrosed phloem and affected all types of cell. However, the cell wall aspect varies according to the stage of the disease. At the brown sheet stage, it was common to observe a limited erosion of the wall near the periplasmic area, characterized by the disorganization of the cellulose fibres (Fig. 3). Such an effect also occurred in older sieve tubes where observations revealed greater alteration of the wall, with large parts completely digested (Fig. 4).

3.2.2 Middle lamella degradation

The middle lamella was also affected by the necrosis; it may be attacked at the same time as the cell wall which suggests that chemical mechanisms are probably involved in pectin dissolution (Fig. 5 and 6).

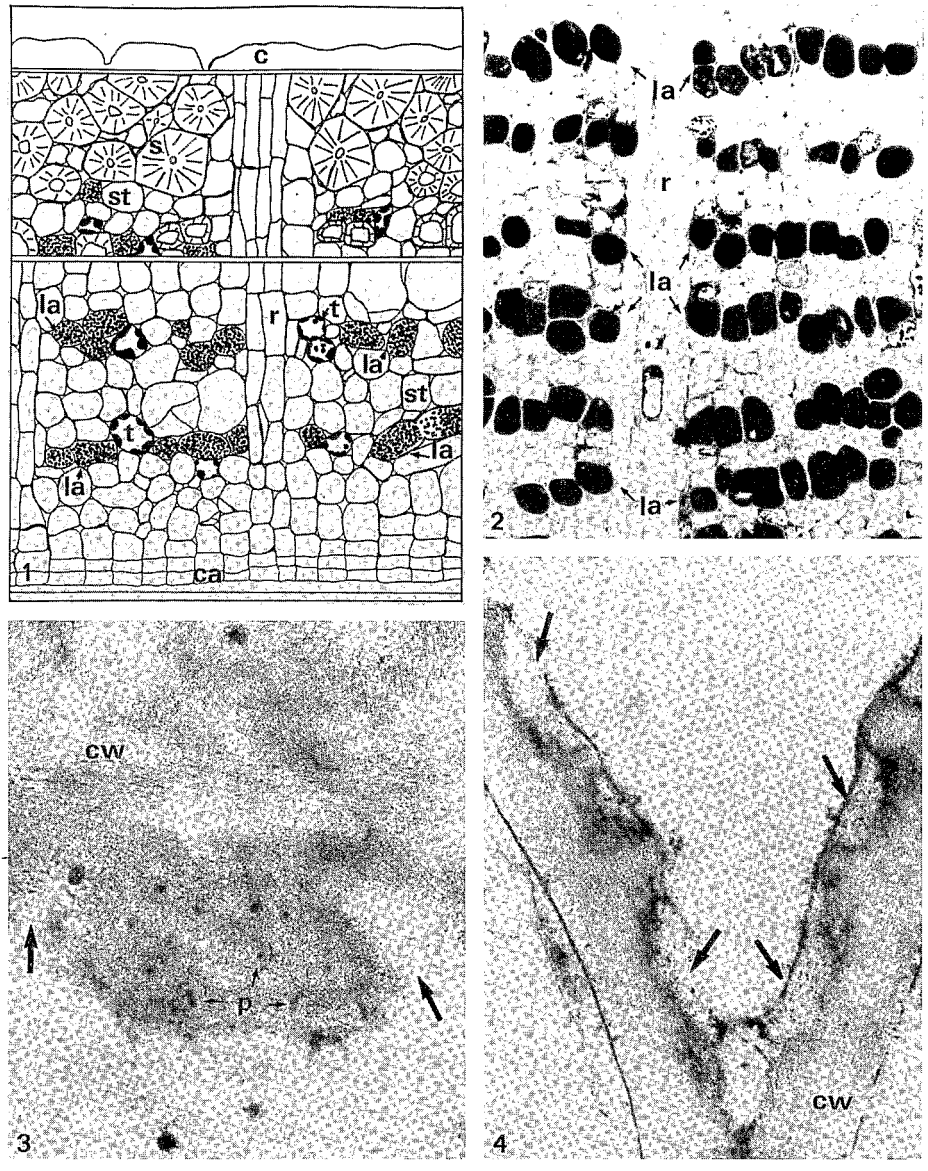

Fig. 1. Histological organization of phloem of *Hevea brasiliensis*. The soft inner phloem, where functional sieve tubes (st) and laticifers (la) are localized, lies near the cambium (ca). Tannin cells (t) are close to the latice vessels. The differentiation of stone cells (st) in the hard outer phloem modifies the organization of the rubber liber, thus preventing latex production; c: cork; r: parenchymatous rays – *Fig. 2.* Half-thin cross-section stained with toluidine blue in the soft secondary phloem showing laticifer rings (la); r: parenchyma rays, (×660) – *Fig. 3.* Degradation (large arrows) of the sieve tube cell wall (cw) close to a zone rich in plasmodesmata (p), (×48 000) – *Fig. 4.* Strong digestion (arrows) of a cellulose cell wall (cw) of parenchyma cells, (×48 000)

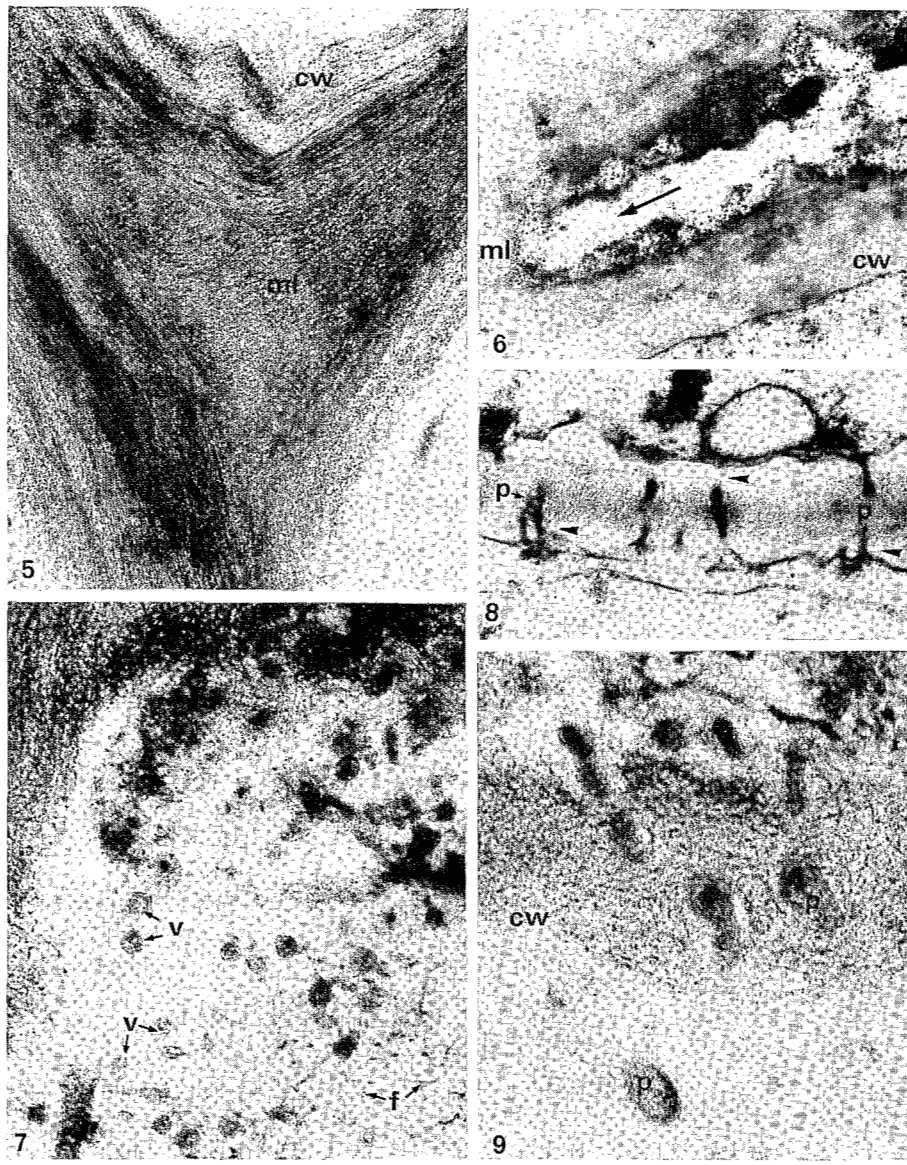

Fig. 5. Middle lamella (ml) between associated parenchyma cells of healthy phloem; cw: cell wall, (×40 000) – *Fig. 6.* Digestion (arrowed) of a middle lamella between two sieve tubes; cw: cell wall, (×21 800) – *Fig. 7.* Vesicles (v) associated with cellulose fibres (f) resulting from the degradation of the plasmalemma, (×75 800) – *Fig. 8.* The cellulose cell wall is eroded (arrowheads) close to the plasmodesmata (p) which seem to be an important target of the necrosis process, (×40 000) – *Fig. 9.* Extrusion of plasmodesmata (p) through the cell wall (cw) after cellulose fibre degradation, (×120 000)

3.2.3 Endomembrane degradation

An examination of phloem cells affected by brown sheets also showed disturbances of the organelle membranes and of the plasmalemma. In the sieve tubes and in the parenchymatous cells of rays, the plasmalemma was broken up into small vesicles localized near the cell

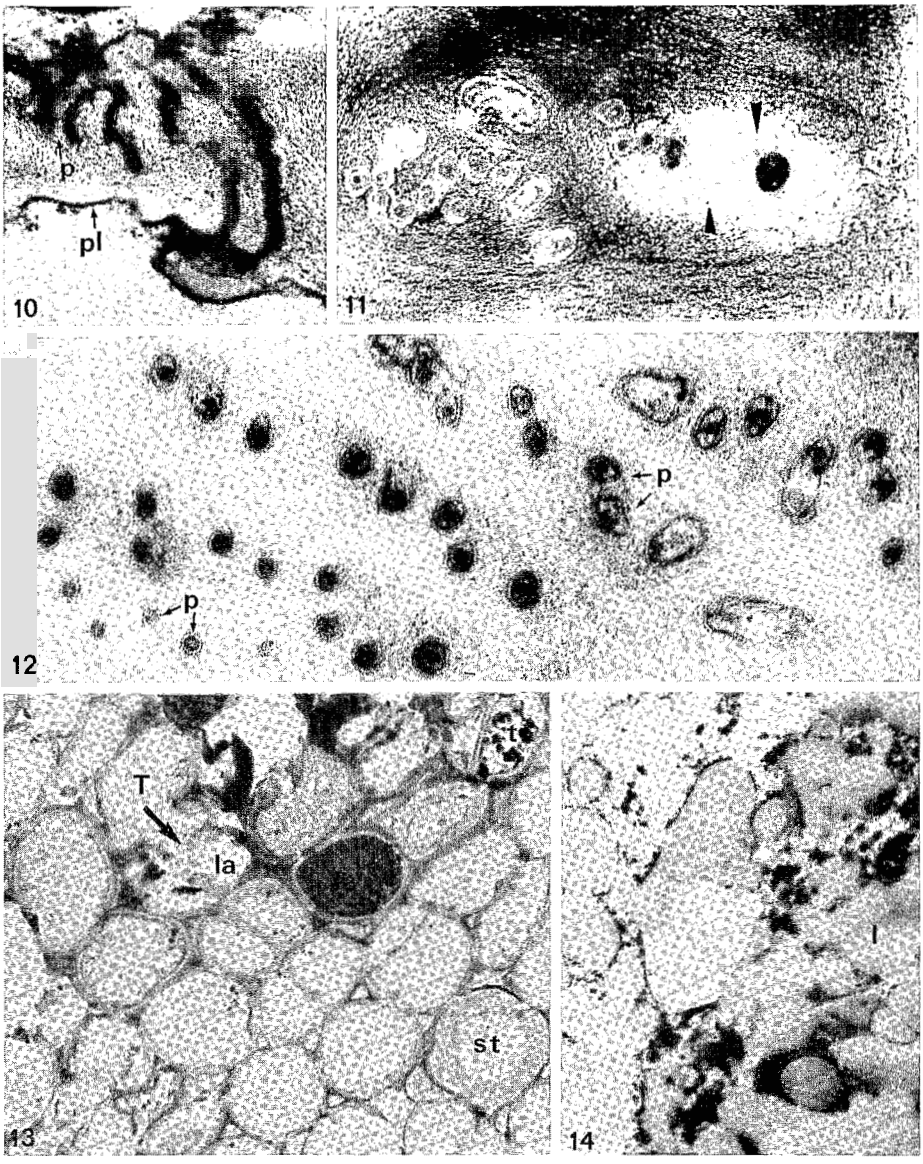

Fig. 10. The degradation of the cellulose fibres causes a loss of rigidity to the cell wall (cw), thus giving the plasmodesmata (p) a distorted aspect, (×70 000) – *Fig. 11.* Degradation of the cell wall close to the plasmodesmata which seem to float in an empty space (arrows), (×54 400) – *Fig. 12.* Plasmodesmata (p) in the cell wall of a parenchyma cell of healthy phloem, (×100 000) – *Fig. 13.* Half-thin cross section stained with toluidine blue. Formation of a tylosoid (T) from an associated parenchyma cell inside a latice vessel (la); t: tannin cell; st: stone cell; (×825) – *Fig. 14.* Internal latex coagulation. Latex particles have merged to form a clump of rubber (l), (×23 000)

wall. These vesicles may be dispersed often associated with cell wall fibres (Fig. 7), or more or less aggregated and associated with other broken pieces of membrane.

One of the consequences of cell wall degradation is the disorganization of the plasmodesmata. Figure 8 shows the beginning of cell wall erosion close to plasmodesmata,

which later causes them to extrude through the cell wall (Fig. 9). This degradation is characterized by a decrease in cell wall rigidity and results in the plasmodesmata becoming distorted (Fig. 10). Moreover, the complete digestion of cellulose fibres surrounding the plasmodesmata produces a space in which these structures seem to float (Fig. 11 and 12). At an advanced stage, large parts of the cell wall, containing numerous plasmodesmata, become separated from the main frame.

More generally, the degradation of the organelle membranes, such as those of the nucleus for example, is coupled to the degeneration of the cytoplasm, characterized by the storage of osmiophilic particles.

3.2.4 Modification of laticiferous vessels

Histological observation showed the existence of tylosoid structures (Fig. 13), defined by De Fay and Hebant (1980) as being outgrowths of parenchyma cells in the latificer, which become progressively lignified. These tylosoids were observed as in the trunk than in roots, whereas they were previously only described in the phloem of the rubber tree trunk. The main alteration of the laticifers, however, consists of *in situ* coagulation, a consequence of the fusion of rubber particles (Fig. 14) which leads to the cessation of latex yield.

3.2.5 Abnormal structures in diseased phloem

Abnormal structures were also observed in the necrosed phloem as compared to healthy tissues. Their monophology consisted of clusters of circular particles localized near the cell wall (Fig. 15) and surrounded by a membrane delimiting an osmiophilic area (Fig. 16). They were never related to other cell organelles.

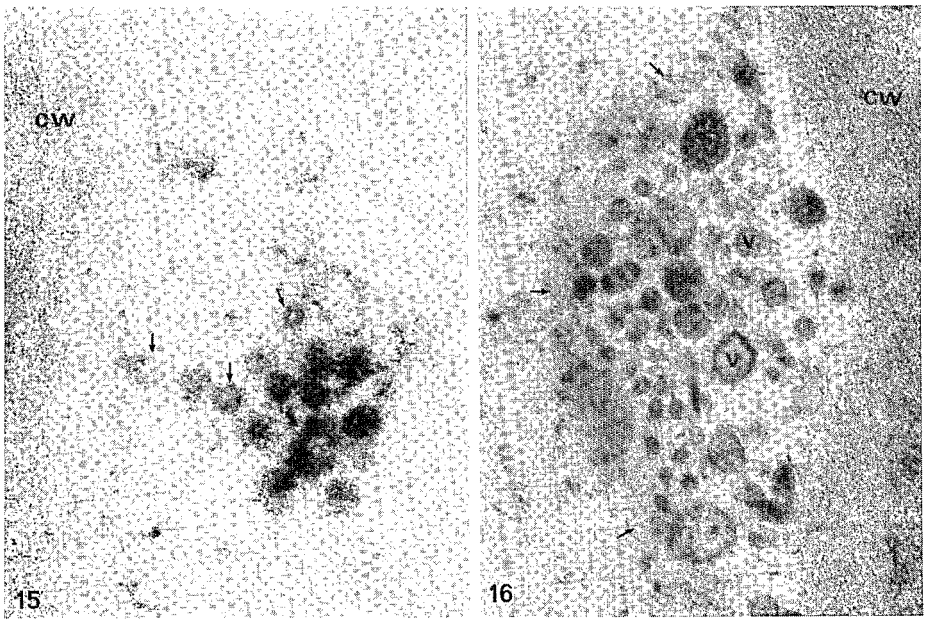

Fig. 15. Vesicles (arrows) of undetermined origin localized near the cell wall (cw), (×80 000) – *Fig. 16.* Vesicles (v) localized in the paramural space between the cell wall of a parenchyma cell (cw) and the highly degraded plasmalemma (arrows), (×60 000)

4 Discussion

Samples taken from roots with brown sheets and examined under a light microscop revealed structures similar to the tylosoids previously described for phloem dryness syndrome (De Fay and Hebant 1980). This shows that tylosoids are also differentiated in the phloem of trees affected with diseases other than brown bast and suggests that tylosoids cannot be considered as typical of bark dryness, as proposed by De Fay and Jacob (1989b) but probably result from perturbations of rubber tree phloem whose laticifer network has been seriously disrupted. This is true of internal latex coagulation, already described at the cellular level (Nicole et al. 1986) and at the molecular level by Chrestin (1989). Thus, tylosoid formation seems to be non-specific and associated with phloem disorders of physiological or pathological origins.

Ultrastructural observation of phloem sections revealed disorganization of cell walls, of the middle lamella and of plasmodesmata and membranes structures.

The alteration of cellulose walls probably results from the actions of cellulases and glycosidases contained in the enzymatic pool of rubber cells and which are actively involved in laticifer anastomosis mechanisms in healthy trees (Sheldrake and Moir 1970). Stimulation of the activity of such enzymes, triggered by the necrotic process, can favour tissue autolysis.

The cause of the digestion of the middle lamella, which is mainly composed of pectic compounds, cannot yet be offered. None of the studies of enzymatic activity of diseased tissue, has demonstrated the existence of pectic hydrolases to which the middle lamella degradation could be attributed. Nevertheless, three hypotheses may be suggested to explain these observations:

- the pectic enzymes responsible for the degradation of the middle lamella are of external origin; the organism which secretes them has yet to be identified.
- one, or several, pectic enzymes exist in rubber tree phloem but remain undetected by the standard techniques. Indeed, such enzymes have been cytochemically characterized in non-articulated laticifers of other plants (Wilson et al. 1976; Allen and Nessler 1984). Hence, under stress, they could contribute, as cellulases, to phloem lysis.
- the disease induces a pectinolytic chemical reaction which causes the alteration of the middle lamella.

The degradation of the membranes could be explained by the liberation of hydrolase and oxidase enzymes, widespread in the rubber tree phloem (Chrestin 1989). Thus, the cytotoxicity of these enzymes could be the cause of the production of plasmodesmic structures and of the rupturing of the plasmalemma, followed by a vesiculation of the resulting fragments. This phenomenon seems to be similar to the plasmalemma endocytosis described by Giordani (1980, 1981) during the differentiation of articulated laticifers in other plants. This vesiculation of the plasmalemma is moreover associated with the activities of various enzymes. The accumulation of vesicles adjacent to the cell wall has often been described as a plant defense reaction to fungal infection (Beckman 1980) associated with the detoxification of diseased cells (Chamberland et al. 1989).

In conclusion, some of these observations suggest that a microorganism could be the cause of the phloem degradation. Experiments seeking to confirm this hypothesis are in progress and involve microscopic observations, the characterization of external nucleic acids, attempts at pathogen transmission to *Hevea* and different chemical treatments of infected and healthy trees to prevent the development of the disease. The results of these investigations will be the subject of a future paper.

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Summary

Microscopic observation of phloem samples taken from rubber trees (*Hevea brasiliensis*), affected with necrosis, revealed major cellular modifications of this tissue. Cell walls and the middle lamella showed different types of alteration which varied according to the severity of the disease. All cell membranes appeared degraded, especially the plasmalemma which was vesiculated and the plasmodesmata which were extruded from the cell wall. Tylosoids in diseased roots and coagulation of rubber inside latice vessels are also described. The origin and the nature of the alteration to the phloem are discussed.

Résumé

L'histologie de la nécrose du phloème d'Hévéa

L'examen microscopique d'échantillons prélevés dans le phloème d'Hévéa (*Hevea brasiliensis*) atteint de nécrose révèle de profondes modifications de l'organisation cellulaire de ce tissu. Les parois ainsi que la lamelle moyenne présentent des figures d'érosion dont l'importance varie selon le niveau de la maladie. L'ensemble des systèmes membranaires paraissent déstabilisés ce qui se traduit entre autre par la vésiculation du plasmalemme et l'extrusion des plasmodesmes des parois. Des tyllosoides ont en outre été observés dans le phloème de racines atteintes de nécrose et le phénomène de coagulation du caoutchouc dans les laticifères est fréquent. La discussion des faits observés s'articule principalement autour de la nature et des causes des altérations subies par le phloème.

Zusammenfassung

Histologie der Phloemnekrose bei Hevea brasiliensis

Mikroskopische Untersuchungen von Phloemproben von mit Phloemnekrose befallenen *Hevea brasiliensis* ergaben starke Zellveränderungen in diesem Gewebe. Zellwände und Mittellamellen zeigten verschiedene Arten von Veränderungen in Abhängigkeit von der Schwere der Krankheit. Alle Zellmembranen schienen zersetzt, besonders das Plasmalemma wies Vesikel auf, und die Plasmodesmen waren aus der Zellwand verdrängt. Thyllenähnliche Strukturen in kranken Wurzeln und die Koagulation von Gummi in Gefäßen werden ebenfalls beschrieben. Ursache und Art der Veränderungen des Phloems werden diskutiert.

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