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Functional Organization of the Bark of *Hevea brasiliensis* (Rubber Tree): A Structural and Histoenzymological Study

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With 16 figures

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Summary

The secondary phloem of *Hevea brasiliensis* presents a repetitive organization in which alternate bands of laticifers, parenchyma cells and sieve tubes occur. The majority of the laticiferous vessel rings are located outside the conducting phloem zone. Radial transport through phloem rays thus appears to be essential in providing the laticifers with those metabolites needed for the biosynthesis of rubber. Enhanced respiratory and phosphatase activities are frequently detectable within ray cells.

Key words: Hevea brasiliensis, secondary phloem, laticifers, vascular rays, radial transport.

Introduction

Very little information is available on the cambium and its derivatives in tropical trees, as compared with the bulk of data accumulated on species of temperate regions (PHILIPSON et al., 1971; ZIMMERMANN and BROWN, 1971). Although several authors have stressed the need for extended investigations on tropical species (ALVIM, 1964; TOMLINSON and GILL, 1973), only a few contributions in the field have appeared within recent years (notably LAWTON and CANNY, 1970; LAWTON and LAWTON, 1971; GHOUSE and HASHMI, 1976).

Arborescent species of economic importance are particularly numerous in the tropics (MANGENOT, 1955), and many of them remain largely underinvestigated. In this paper, we present a new interpretation of the functional organization of the bark of *Hevea brasiliensis*, based on correlated structural and histoenzymological studies. The general anatomy of this plant in relation to its productivity has been described by many authors (cf. e.g. RICHES and GOODING, 1952; BOUYCHOU, 1962; GOMEZ et al., 1972).

Material and Methods

Material

All the specimens of *Hevea brasiliensis* used in this study were grown at the IRCA experimental station at Bimbresso, Ivory Coast. Mature trees, 12 to 21 years old, were selected; such trees are regularly tapped. In addition, some young (2–4 years) and a few older but untapped trees were studied. More than 90 different trees, belonging to the clones GT 1 and PR 107 were sampled.

Methods

Bark samples were taken from the selected trees at various levels. A 4 mm punch was routinely used, although some larger portions of bark were occasionally cut with a knife and chisel. The samples were immediately immersed in fixatives.

The methods used were essentially the same as those described in a recent paper (HÉBANT et al., 1978). In addition, three further cytochemical tests were applied: the IKI reaction for starch (SASS, 1958); a modified WACHSTEIN-MEISEL procedure for the demonstration of ATPase activity (*with* Ca^{++} *and* Mg^{++} *added to the incubation medium*) (BERLYN and MIKSCHE, 1976); a nitroblue-tetrazolium method for the demonstration of NAD-dependent isocitrate dehydrogenase (SAUTER, 1972).

Results

Structural variability is observed in the bark of *Hevea brasiliensis*. This depends upon numerous factors, both internal and external: age of the trees, clones, rhythms and seasons, tapping, state of the bark (virgin, renewed), diseases, etc. In this paper, we only consider the bark of mature, healthy and regularly tapped trees.

Key to labels on figures:

AP, axial parenchyma; C, cambium; CP, conducting phloem; CR, crystal; DC, «definitive» callose; L, laticifer; NCP, non-conducting phloem; PL, parenchyma cells associated to laticifer ring; R, vascular ray (phloem and/or xylem ray); S, sieve tube; SCL, sclereid; T, tannin cell; V, vessel; X, secondary xylem.

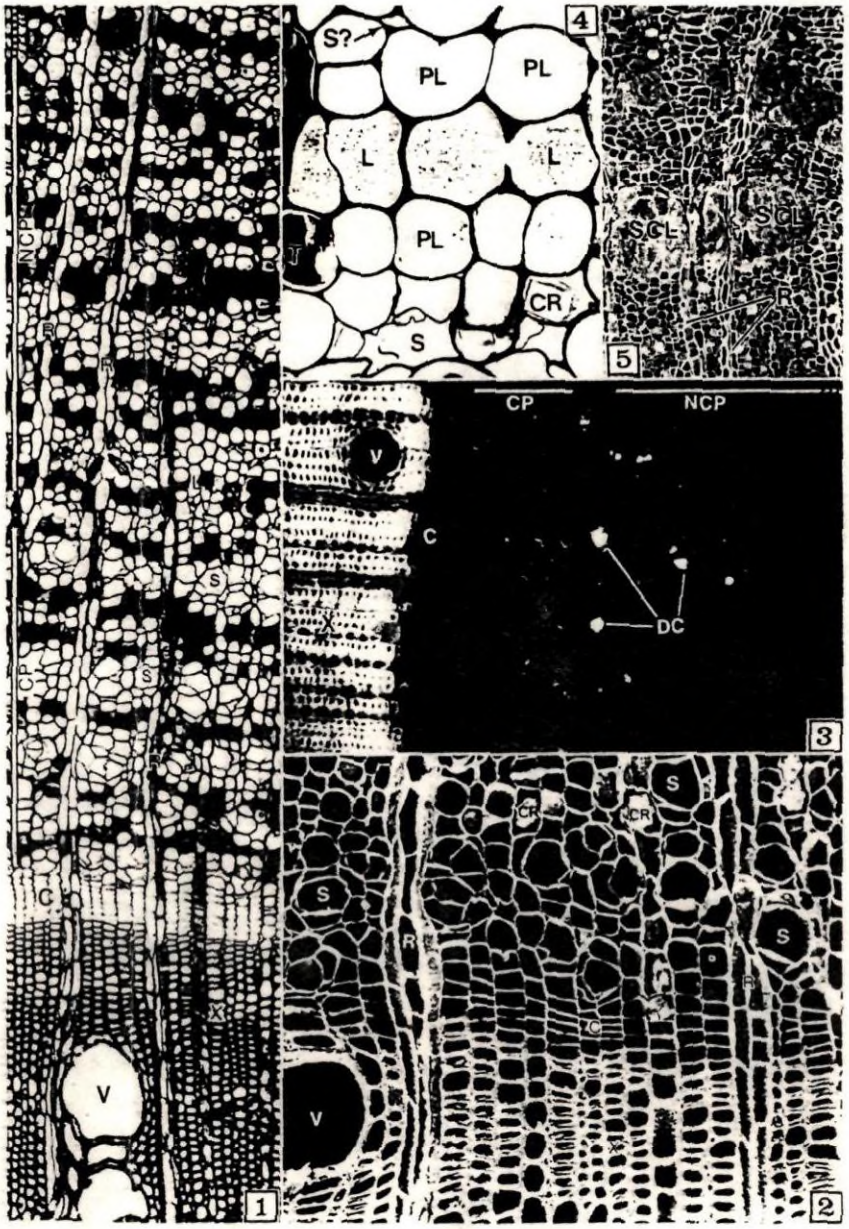
Fig. 1: Secondary phloem and xylem from stem of *Hevea brasiliensis* in transverse section. Alternate bands of laticifers (with associated parenchyma cells), and of sieve tubes (the latter crushed in the non-conducting phloem), are identifiable within the phloem. The depth of the conducting phloem zone (CP) in the specimen shown here is ca. 0.6 mm. Section of araldite-embedded material stained with Paragon. ×80.

Fig. 2: Transverse view of cambium and derivatives (scanning electron micrograph). Note that more or less differentiated ray elements establish the link between phloem and xylem rays, through the cambium – which appears here to be non-functional. × 160.

Fig. 3: Transverse section of phloem of *Hevea brasiliensis* treated with buffered aniline blue and viewed under the fluorescence microscope. Moderate amounts of callose characterize the sieve tubes of the conducting phloem. Outside this zone, sieve plates are occluded by large amounts of «definitive» callose (DC), which eventually disappears. Note also primary fluorescence of xylem. ×70.

Fig. 4: Detail of laticifers and associated parenchyma cells in transverse section. Degenerated sieve tubes also seen. Section of araldite-embedded material stained with Paragon. ×400.

Fig. 5: Transverse view of aged portion of bark showing development of sclereids and alteration of rays. Scanning electron micrograph. ×160.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The vascular cambium of *Hevea* undergoes rhythmic activity, and there is no synchronization between one tree and another within a given population.

Wood is of the diffuse-porous type, with multiseriate rays and axial parenchyma (paratracheal and banded apotracheal).

Within the secondary phloem, a narrow layer of conducting phloem is seen adjacent to the cambium (fig. 1). Its width was found to vary from 0.2 to 0.8 mm for a total bark thickness of ca. 10 mm in samples taken from just below the tapping cuts.

Sieve tubes in the active phloem are 7–16 µm in diameter. Small amounts of callose are frequently identifiable on their sieve plates by the aniline-blue fluorescence method (fig. 3). Outside this zone, aging sieve tubes are encountered, the sieve areas of which may be occluded by large amounts of callose («definitive» callose) (fig. 3). In older regions of secondary phloem, crushing of the sieve elements is observed (figs. 1 and 4). No callose is usually detectable in them (fig. 3).

Laticiferous vessels are rhythmically produced within cambium derivatives, forming concentric hollow cylinders in the bark (figs. 1 and 6). Each latex vessel ring is embedded within a sheath of parenchyma cells (fig. 4).

Alternate bands of laticifers, parenchyma cells and sieve tubes (intermixed with other cell types) thus constitute a characteristic pattern in the secondary phloem (fig. 1). However this alternation is not absolutely regular, for instance sieve tubes may be lacking between two successive vessel rings.

Only a limited number (2 to 6) of laticiferous vessel rings are found within the conducting phloem zone, whereas many more rings (e.g. 25–35) occur in the outer non-conducting phloem.

Fig. 6: Tangential view of laticiferous vessel ring. Thick freezing microtome section of osmium tetroxide treated material. ×82.

Fig. 7: Disorganized laticifers in aged bark. Tangential longitudinal section of osmium tetroxide treated material. ×72.

The multiseriate rays of the phloem are continuous with those of the xylem. When the cambium is non-functional, more or less differentiated ray elements establish the link between xylem and phloem ray cells (fig. 2). These elements dedifferentiate at the onset of reactivation of the vascular cambium.

In aged portions of the secondary phloem, sclereids differentiate, the progressive development of which eventually disorganizes the fundamental pattern described above : the rays are seriously altered (fig. 5) whereas the laticiferous vessel rings progressively disappear (fig. 7).

One important structural fact retains the attention: the various parenchyma cells in the recently formed wood and secondary phloem constitute a continuous three-dimensional network. The following are thus spatially connected with each other: the cells of axial parenchyma in the xylem; the ray cells, both of xylem and phloem; the parenchyma cells associated with the laticifers in each ring.

Cytochemical results

Starch

Starch frequently occurs in ray and axial parenchyma cells of the wood (fig. 8). Small starch grains are occasionaly encountered in certain parenchyma cells of the secondary phloem, but they remain quite scanty.

Enzyme activities

Enhanced respiratory and phosphatase activities are frequently observed in the parenchyma cells of the threedimensional network described above. Lack of staining is occasionaly noted, but it is not yet known whether this is due to preparation hazards, or to variations in the physiological state of the tissues studied.

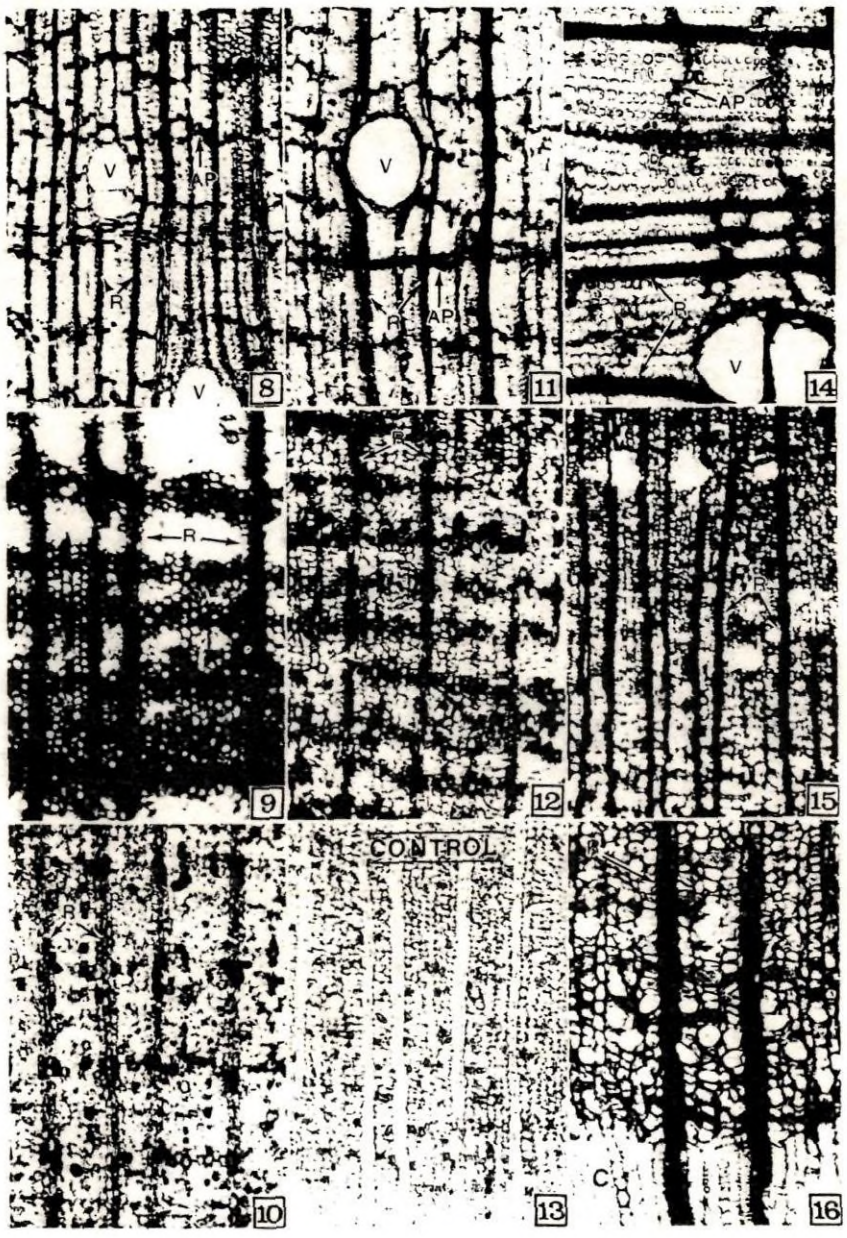
The following activities were revealed:

- Acid phosphatases, by the methods of GOMORI, using β-glycerophosphate as substrate (figs. 11 to 13), and of BURSTONE, using naphtol-AS-MX-phosphate as substrate.
- ATPase (figs. 14 to 16).
- Succinate dehydrogenase, with the nitroblue-tetrazolium procedure of NACHLAS et al. (fig. 10).
- Isocitrate dehydrogenase.
- Cytochrome oxidase, by the method of BURSTONE (fig. 9).

Particularly high levels of activity are evident in the elements of the vascular rays (both xylem and phloem rays). In addition, intense staining of the companion cells in the conducting phloem is observed.

Special mention must be made of the laticiferous vessels which show variable degrees of staining when acid phosphatase activity is tested.

When the GRAHAM-KARNOVSKY D.A.B. procedure for peroxidase is carried out, such an intense reaction of the bark is obtained, even after short incubation periods, that no differential staining can be deciphered in most cases. Some differential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oxidative activity however appears to be revealed, by the BURSTONE method for cytochrome-oxidase, which is known to yield coloured reaction products under the influence of a number of factors, in addition to the cytochrome-oxidase (HARWIG, 1967): positively stained bands are revealed by this method in the secondary phloem (fig. 9). These «bands» correspond to the degenerating sieve-elements and associated cells, the staining in them being mostly located within the cell walls.

Discussion

In all the mature trees studied, a majority of the laticiferous vessel rings in the bark are located outside the narrow band of conducting phloem. Therefore radial transport through the phloem rays appear to be essential in providing such laticifers with those metabolites needed for the biosynthesis of rubber. In addition, laticiferous vessels in each ring are closely associated with each other and, apart from a few parenchyma cells, only the rays interrupt their continuity: these appear to be the only possible pathway for effective radial transport of nutrients.

In this context, the occurrence of enhanced respiratory and phosphatase activities in ray cells of *Hevea brasiliensis* is of significance. A bulk of evidence from various other plant materials suggest that vascular rays may play an important role in radial transport, and that the enhanced enzyme activities demonstrated at this level are related to the transport phenomena (HÖLL, 1975). It is worth noting that normally constituted laticifer rings disappear precisely at the level where rays are collapsed.

The interpretation of the functional organization of the laticiferous tissue of *Hevea* that we propose here thus implies both vertical (in the conducting phloem) and radial transport of metabolites. One might also expect a participation under certain circumstances of the reserves (starch) of the parenchyma cells in the wood, via the vascular rays and through the cambium, in the loading of the laticifers.

From another point of view, this model might also provide an explanation of the fact that the new method of puncture tapping provides latex with a higher sucrose

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Fig. 8: Demonstration of starch in parenchyma cells of wood by IKI reagent. Transverse section. ×45.

Figs. 9–16: Demonstration of enzyme activities in transverse sections of wood and secondary phloem:

Fig. 9: Cytochrome-oxidase and other oxidase activities in phloem, as revealed by the method of BURSTONE. Darkly stained tangential bands correspond to degenerating sieve tubes and associated parenchyma cells. – Fig. 10: Succinate-dehydrogenase in phloem, by the method of NACHLAS et al. – Figs. 11 to 13: Acid phosphatase activity (GOMORI method) in wood (fig. 11) and secondary phloem (fig. 12). Fig. 13 shows a no-substrate control for phloem. Darkly stained tangential bands in fig. 12 correspond to laticifers and associated parenchyma cells. – Figs. 14 to 16: ATPase activity (after a modified WACHSTEIN-MEISEL procedure) in wood (fig. 14) and phloem (figs. 15 and 16). Fig. 16 shows detail of cambium and newly formed secondary phloem.

Figs. 8 to 13, ×45; fig. 14, ×75; fig. 15, ×27; fig. 16, ×68. – Figs. 9, 10, 12, 13, 15 and 16 are orientated so that cambium is located towards base of plate.

content (PRIMOT and TUPY, 1976): in this procedure, deeper incisions in the bark reach the conducting phloem zone (unattained by conventional procedures), with the result that contamination of the latex by sieve-tube sap occurs.

Irregular staining of the laticifers when tested for acid phosphatases has been recorded. Inconsistent preservation of the structures may be responsible for this. Should the «lutoids» (= polydispersed vacuo-lysosomal system; cf. PUJARNISCLE, 1968; RIBAILLIER et al., 1971) be altered, then an intense coloured reaction occurs, whereas a more discrete particulate staining is observed when they remain intact.

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Myrosinase Activity in Differentiated and Undifferentiated Plants of Brassicaceae

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With 3 figures

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Summary

The occurrence and distribution of myrosinases in different plant organs in several species of Brassicaceae (*Brassica chinensis*, *B. napus* L., *B. oleracea* L., *Iberis amara* L., *Iberis amara* var. *coronaria*, *Lepidium sativum* L., *Raphanus sativus* L. and *Sinapis alba* L.) was studied. Myrosinase solutions were prepared from roots, stems, leaves, callus cultures and protoplasts and after dialysis the enzymatic activity was detected after hydrolytic cleavage of sinigrin. Specific activity was calculated in relation to protein, and it was found to vary within the range of 0.490 to 0.010 μmol · min^{-1} · mg^{-1} in 1 to 2 day old seedlings. The activity was found to decrease throughout the development of the plant but even at the flowering stage myrosinase activity could be detected.

In callus cultures originally isolated from stem segments of wild candytuft and chinese cabbage myrosinase activity was demonstrated 48 and 64 weeks, respectively, after the start of the cultivation period.

Protoplasts from the abovementioned species were also isolated from leaf tissue and their morphological shape examined by light – and scanning electron microscopy. Although myrosinase activity was not detectable in protoplasts of cabbage, cress and rape, the myrosinase extracts from protoplasts of the other species showed higher specific activity than the intact leaf cells.

The distribution of myrosinases has been interpreted and correlated to the development stage of the plant tissue.

Key words: Myrosinases, Brassicaceae, protoplasts, callus cultures.

Introduction

The naturally occurring thioglucosides called glucosinolates are hydrolysed in neutral solutions by myrosinases or thioglucoside glucohydrolases (E.C. 3.2.3.1) to isothiocyanate, glucose and sulphate. Myrosinases seem to be accompanied by one or more glucosinolates. They are present in all Brassicaceae species examined (Etllinger and Kjaer, 1968) but have also been found in the plant families Capparidaceae, Resedaceae, Tropaeolaceae (Snowden and Gaines, 1969), Limnantraceae, Caricaceae