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The distribution of plasmodesmata in the phloem of *Hevea brasiliensis* in relation to laticifer loading

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Summary. Cell to cell connections, including plasmodesmata and perforations, were examined in the non-conducting secondary phloem of *Hevea brasiliensis*. Samples were taken from trunks of numerous trees, from several clones, and prepared for thin sectioning and transmission or scanning electron microscopy and as optical sections for fluorescence microscopy. Numerous plasmodesmata were found clustered in primary pit-fields between the ray and axial parenchyma cells. Between the laticifers and adjacent parenchyma sheath cells, structures corresponding to functional plasmodesmata were not observed. But some unusual structural features were occasionally seen in these walls. These observations are discussed in relation to the possible function of the cell types, and to the loss of latex on the tapping of *Hevea*. It is suggested that the loading of the laticifer might first require a symplastic pathway for the transport of metabolites, at the end of which the assimilates must enter the apoplast. A transmembrane active transport system then transfers the metabolites in the laticifer. The presumable role of parenchyma cells in the loading of laticifers is emphasized.

Keywords: Apoplast; *Hevea brasiliensis*; Laticifer loading; Plasmodesmata; Symplast.

Introduction

Although several investigations have been devoted to the anatomy of bark in *Hevea brasiliensis*, especially in relation to its productivity (Bobillioff 1923, Ferrand 1944, Gomez et al. 1972, Narayanan et al. 1973, Gomez 1982, Henon 1984), little attention has been paid to structural features relating to transport of assimilates and the loading of laticifers. In previous papers (Hebant and de Faÿ 1979, 1980; Hebant et al. 1981), it has been shown that the loading of laticifers involves a complex interrelationship between long-distance and short-distance translocation systems. Vertical transport of assimilates from the photosynthesizing leaves, and possibly from the root system (storage starch), occurs via the narrow band of conducting phloem. Short-distance translocation involves parenchyma in both the secondary xylem and phloem, as demonstrated by enhanced enzymatic (acid phosphatase, ATPase) and respiratory activities in the vascular rays. These short-distance transport systems play an important role in the loading of laticifers, especially via ray cells and axial parenchyma associated with the laticifer rings. However, the translocation pathway for nutrients from the parenchyma cells to the laticifers has not been determined. The distribution of plasmodesmata (protoplasmic connections) in cell walls of a tissue might be taken to indicate the existence of a route for symplastic (confined to the cytoplasm) transport (Ziegler 1974, Robards 1975); an absence of plasmodesmata or the presence of degenerate structures might imply that only an apoplastic (occurring in the cell walls and the intercellular spaces) pathway for transport is possible.

In this paper we report the results of a histological study of the distribution of plasmodesmata in the non-conducting secondary phloem of *Hevea*. This portion of the phloem lies outside the band of functional sieve tubes and contains the majority of laticifer rings that are tapped when latex is bled from the plant. The other living cells of the non-conducting phloem are the par-

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† Deceased 1982. This work was initiated by Charles Hébant and is dedicated to his memory.

enchyma cells of the sheaths around the laticifer rings and the phloem rays. We have investigated the wall structure and the connections between neighbouring ray and axial parenchyma cells, between laticifers and ray and axial parenchyma, and between laticifers themselves—including the perforations that join the individual cells of the laticifer system.

Materials and methods

Bark samples were obtained from mature trees of various clones (GT 1, PR 107, RRIM 623) growing in the plantation of the Institut de Recherche sur le Caoutchouc, Ivory Coast. Large portions of bark were removed from the tapping panel with a knife and chisel and immediately immersed in 4% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 1 hour at 0 °C. They were then cut into smaller cubes and fixed in buffered glutaraldehyde for a further 1 or 2 hours. After rinsing and post-fixation in 1% osmium tetroxide, pH 7.2, samples were dehydrated and embedded in Araldite and cut with a Porter-Blum MT 1 ultramicrotome. Thin sections were post-stained with either $KMnO_4$ or according to the Thiéry method (1967), and examined in a Jeol 200 B transmission electron microscope. Thick sections of 1 µm were treated to extract the resin following the method of Sutherland and McCully (1976), using a saturated solution of sodium methoxide, followed by two rinses in methanol. These sections were first viewed by bright field microscopy, and after treatment with aniline blue by fluorescence microscopy. In addition, a few samples were prepared for scanning electron microscopy. After fixation and dehydration in ethanol, these samples were prepared by CO_2 critical-point drying and coated with gold-palladium. The microscope used was a Jeol JSM 35.

At least 21 samples, obtained from a minimum of 5 different inclusions with 4–5 sections of 0.3 mm^2 average surface from each sample, were systematically investigated by transmission electron microscopy in order to demonstrate the presence or the absence of plasmodesmata in the walls of the non-conducting phloem cells. The explored surface of phloem was at least equal to 25.10^6 μm^2. More than 5000 cross-sections of laticifers and still more parenchyma cells could be examined and the laticifer walls were closely inspected.

Results

Perforations between individual cells in laticifers of *Hevea* were first observed by Scott (1885). They are conspicuous as large circular openings in the common cross and lateral walls (Fig. 1) of adjacent cells. The perforations distinguish the laticifers as articulated. As the perforations exist both vertically and laterally the laticifer rings of *Hevea* form cylindrical networks around the trunk of the tree.

Numerous primary pit-fields were found in the walls between parenchyma cells of the phloem. Axial parenchyma formed a sheath around the laticifer rings (Hebant and de Faÿ 1979) and exhibited numerous primary pit-fields in their common walls. These are seen in surface view in Fig. 1, occupying 30% of the anticlinical wall and in section in Fig. 2. The wall was locally thin at the primary pit-field (Fig. 2). The ray parenchyma cells also contained numerous primary pit-fields in their walls. Staining with aniline blue induced fluorescence on both sides of the primary pit-fields (Fig. 3), indicating the presence of callose (Currier and Strugger 1956, Currier 1957, Smith and McCully 1978, Hughes and Gunning 1980). Some parenchyma cells also showed shallow depressions in the wall, narrower than typical primary pit-fields (compare Fig. 2 with Fig. 4), and these were transversed by a few plasmodesmata. At primary pit-fields plasmodesmata were more numerous (Fig. 5). Although we did not specifically investigate the ultrastructure of plasmodesmata in the phloem of *Hevea*, they appeared to have the same basic structure as reported by Esau (1977). In the non-conducting secondary phloem of *Hevea*, there were numerous plasmodesmatal connections between the various combinations of axial and ray parenchyma cells,

Abbreviations: ER endoplasmic reticulum; LAT laticifer; M mitochondrion; N nucleus; PAR parenchyma cell; PF primary pit-field

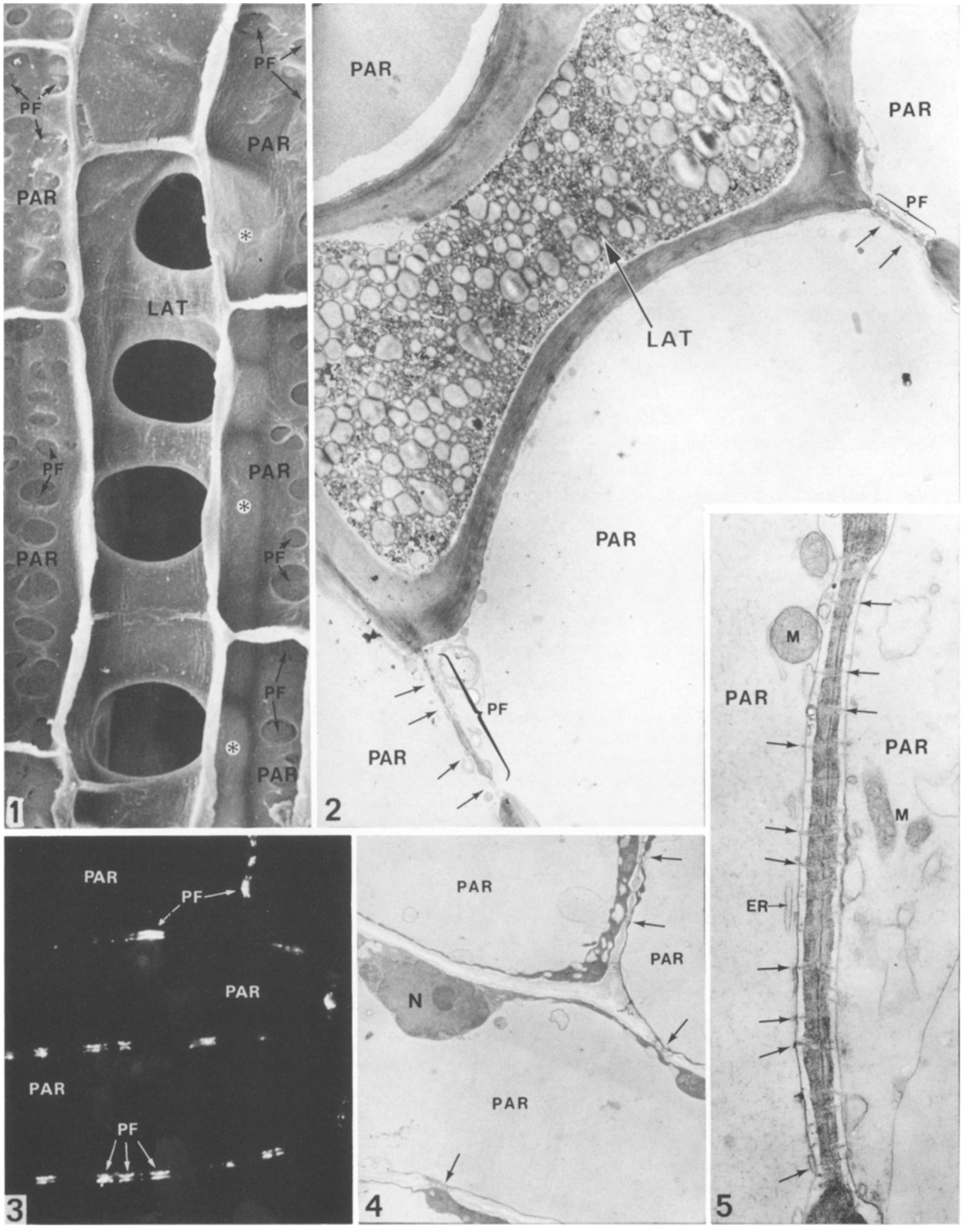
Fig. 1. Radial longitudinal view of a laticifer and its adjacent axial parenchyma sheath cells. Four large circular perforations connect this laticifer with other adjacent laticifer cells. Numerous primary pit-fields occur on the walls between adjacent parenchyma cells. Pit-fields are absent in the common wall between the laticifer and its axial parenchyma cells. Scanning electron micrograph. × 1,100

Fig. 2. A laticifer and its adjacent axial parenchyma cells in cross-section, treated by the Thiéry method for the detection of polysaccharides. Two primary pit-fields are present between the adjacent parenchyma cells. Unlabeled arrows indicate sites of plasmodesmata. Transmission electron micrograph. × 4,500

Fig. 3. Radial longitudinal section of phloem parenchyma cells stained with aniline blue and viewed under the fluorescence microscope. Fluorescent areas, which seem to be double, are numerous in the walls between the parenchyma cells. They correspond to deposits of callose at sites of plasmodesmata in the primary pit-fields. × 520

Fig. 4. Radial longitudinal section showing portion of four phloem ray cells post-stained in $KMnO_4$. Some plasmodesmata occur in their walls, in local depressions (arrows). × 3,400

Fig. 5. Detail of a pit-membrane between two ray parenchyma cells. Plasmodesmata are visible and numerous penetrating the wall (arrows), but at this magnification structural details are not resolved. Some profiles of endoplasmic reticulum lie near the plasmodesmata. Radial longitudinal section contrasted by $KMnO_4$. × 18,000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especially in the anticlinical walls of the parenchyma sheathing the laticifers and in both the anticlinal and periclinal walls of the rays (Fig. 6).

The parenchyma cells that formed a sheath around the laticifers lacked primary pit-fields in walls adjacent to the laticifers (Fig. 1). There was no fluorescence here after aniline blue staining (Fig. 6). The laticifers had very thick electron dense walls without pits or plasmodesmata (Figs. 2 and 8). None of the numerous sections sampled of phloem from trunks of several trees, belonging to various clones, showed functional plasmodesmata in walls between laticifers and parenchyma cells. Nevertheless, some unusual structures were noted in the walls. Eight were counted in the cross-sections in 107 walls between laticifer and parenchyma. These were mostly small recesses on the parenchyma cell side of the wall. A minute deposit of callose was detected at the recess, as shown by the aniline blue induced fluorescence (Figs. 7A and B). Higher magnifications of these depressions often revealed small swellings in the wall, but only on the parenchyma cell side (Fig. 9). An electron translucent zone appeared just below each of these wall protuberances. A peculiar fibrillar arrangement, in the form of a transverse "groove", was sometimes visible within the wall of the laticifer (Fig. 10). However, an inner layer of wall material separated this structure from the protoplast of the laticifer (Fig. 10). Occasionally, the inner layer of the wall formed a swelling (Fig. 11), which seemed to plug the groove. An electron transparent zone was observed once in the wall, appearing as a hole opening into the laticifer protoplasm (Fig. 12).

Discussion

The mature laticifers of *Hevea* bark have no obvious functional plasmodesmata in their walls. However, it is possible that the unusual fibrillar transverse "grooves" observed in their walls are the remnants of aborted plasmodesmata. Similar instances have been reported in the leaf stomata of many species. Mature guard cells lack functional plasmodesmata in their walls, even though they existed earlier in development (Brown and Johnson 1962, Allaway and Setterfield 1972, Srivastava and Singh 1972, Singh and Srivastava 1973, Ziegler et al. 1974, Vela and Lee 1975, Peterson and Hambleton 1978, Rutter and Willmer 1979, Willmer and Sexton 1979, Galatis and Mitrakos 1980, Wille and Lucas 1984). Furthermore, incomplete plasmodesmata are sometimes visible embedded in wall material in mature stomata (Willmer and Sexton 1979,

Abbreviations: CST crushed sieve tube; LAT laticifer; M mitochondrion; N nucleus; P plastid; PAR parenchyma cell; PF primary pit-field; rPAR ray parenchyma cell; sPAR sheath parenchyma cell

Fig. 6. 1 μm thick cross-section of phloem tissue viewed by fluorescence microscopy after staining with aniline blue. Numerous bright fluorescent areas are distributed in walls between the parenchyma cells of the rays and the sheaths around the laticifers, and correspond to deposits of callose at sites of plasmodesmata. Light primary fluorescence is evident in the crushed sieve tubes, not nothing in the walls of the four laticifers. × 600

Fig. 7. A and B. 1 μm thick cross-section of phloem **A** The section is viewed by bright field microscopy and shows a laticifer and the adjacent parenchyma cells. Two characteristic primary pit-fields are seen in the walls between the parenchyma cells. In the wall between the laticifer and one of the lower parenchyma cells, a small depression is detected on the parenchyma cell side (arrowhead) but nothing on the laticifer side. × 1,150. **B** The same section viewed by fluorescence microscopy, after staining with aniline blue. Prominent fluorescence occurs in the two primary pit-fields but only a minute fluorescent spot (arrowhead) is evident in the small wall depression on the side of the axial parenchyma cell. × 1,150

Fig. 8. Cross-section of two laticifers and an adjacent ray parenchyma cell, stained by the Thièry method. Primary pit-fields, depressions and plasmodesmata are absent in the wall between the laticifer and the ray cell. × 14,000

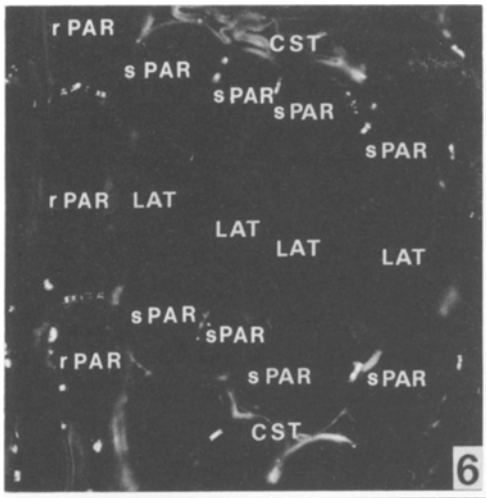
Figs. 9–12. Details of unusual structures in walls between laticifers and surrounding parenchyma sheath cells

Fig. 9. Portion of the wall between a laticifer (*LAT*) and a parenchyma cells (*PAR*) in a recess occupied by protoplasm of the parenchyma cell. Arrowheads indicate three small swellings bordered by plasmalemma. There is a small electron translucent zone in the wall below the protuberances. Cross-section treated by the Thièry method. × 24,000

Fig. 10. The wall shows a fibrillar arrangement in the form of a transverse "groove" (bracket). An innermost layer of the laticifer wall overlies the "groove". Cross-section treated by the Thièry method. × 26,000

Fig. 11. Detail of the inner layer of a laticifer wall where it forms a true swelling. No transverse "groove" is observed in the wall. Cross-section stained with $KMnO_4$. × 40,000

Fig. 12. An electron translucent zone in the wall of a laticifer (arrowhead) that might represent a hole opening into the laticifer. Cross-section stained by $KMnO_4$,. × 18,000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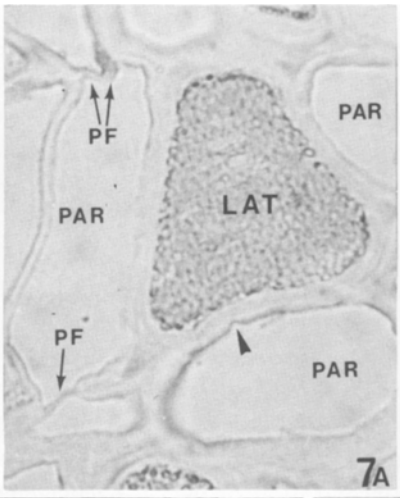
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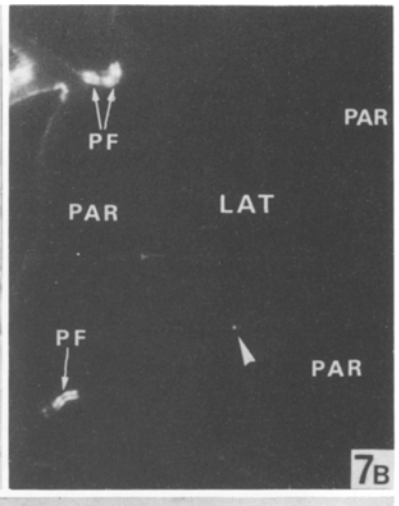
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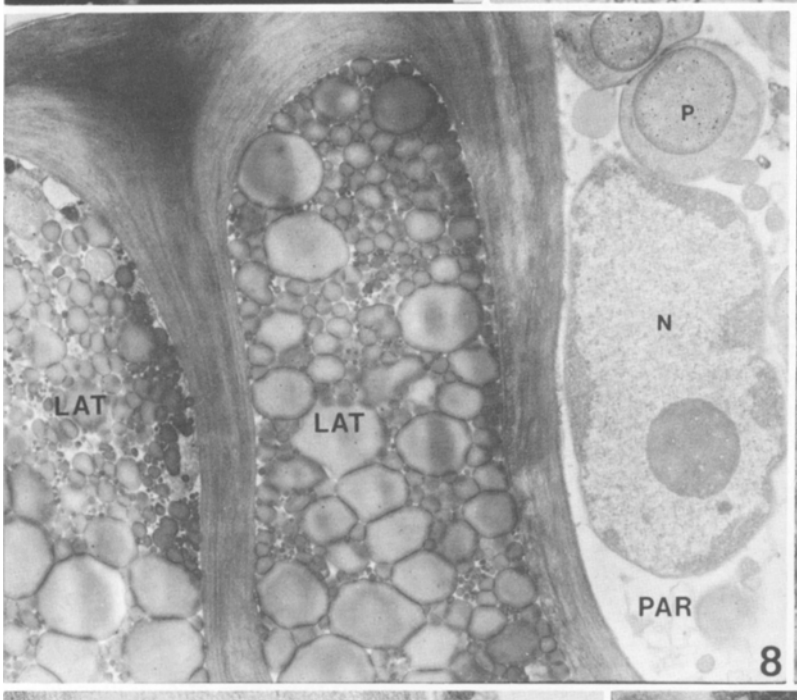
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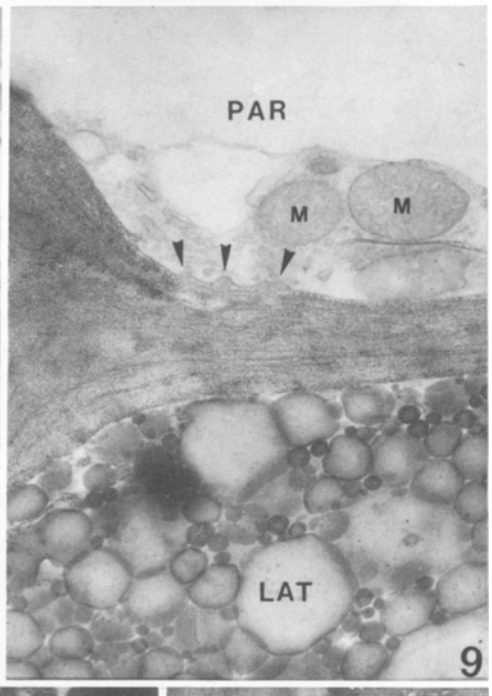
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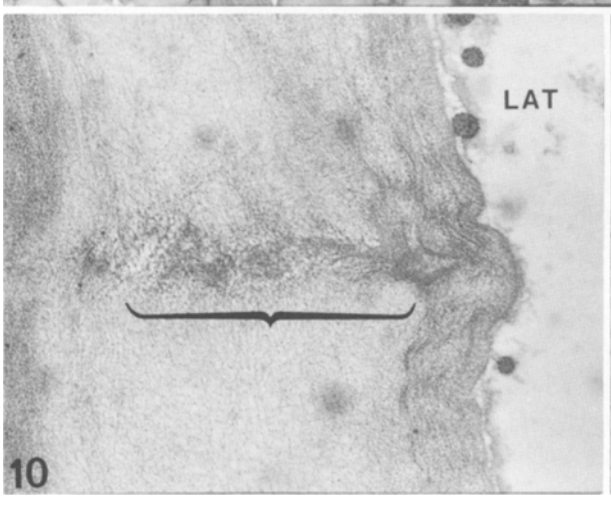
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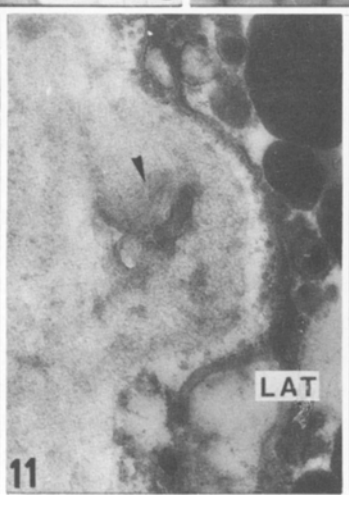
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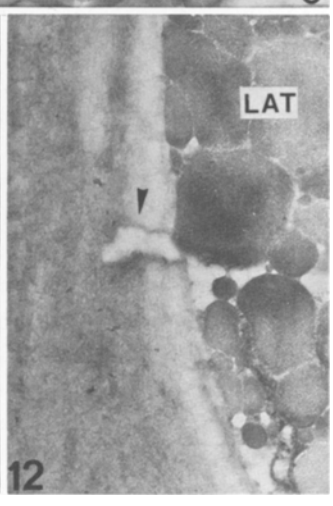
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Wille and Lucas 1984). The guard cell isolation from cell to cell communication appears to be a common phenomenon.

From a physiological point of view, the existence of numerous functional plasmodesmata connecting the cytoplasm of mature guard cells, subsidiary cells and epidermal cells would hinder the increase in concentration of osmoticum in guard cells, and thus the increase in turgor pressure which controls stomatal opening. Like functional guard cells, the laticifers of exploited *Hevea* need to maintain high turgor pressure, because the flow of latex on tapping is linked to the turgor pressure (7–15 bars) prevailing in the laticifers (Buttery and Boatman 1966, Milford et al. 1969) and such a pressure requires a high concentration of osmoticum which is inconsistent with possible leaks through the plasmodesmata.

There is at least another physiological reason why the rings of laticifers should be symplastically isolated from the surrounding parenchyma. The laticiferous vessels of *Hevea* are characterized by an intense isoprenic metabolism (30–50% of rubber in latex), especially when the tree is exploited by tapping. Sucrose, the major precursor of rubber, must enter the laticifers intensively at the level of tapping panel. Thus, the laticifers are the recipient sink cells for metabolites in the trunk of exploited *Hevea* and it seems difficult to believe that substrates, intermediates, enzymes, micro rubber particles can leak through plasmodesmata to non-specialized cells.

The lack of plasmodesmata in mature laticifers implies that metabolites which enter the laticifer must penetrate the laticifer wall apoplastically, and then be actively transported across the plasmalemma of the laticifer. Experimental work corroborates this view. The technique of radioactive labeling and tracing has already been employed on trunks of *Hevea*; a tracer was applied to a small area, where periderm had been scraped, and it was subsequently relocated in the latex (Lustinec and Resing 1965, Lustinec et al. 1966). Inorganic phosphate (^{32}Pi), sucrose (^{14}U—C or ^{3}H) or $^{86}Rb^{+}$ applied in this manner to the apoplast have been detected, after a short interval, in the tapped latex not far-removed from the site of application (Lacrotte et al. 1985). Conversely, mannitol (^{14}C), considered conventionally impermeable to the majority of phospholipid membranes, has not been relocated in the latex (Lacrotte unpubl). Sucrose, inorganic, Rb^{+} are carried through the laticifer plasmalemma, whereas mannitol moving through apoplast cannot cross the membrane. Furthermore, inhibitors of metabolic energy supply (2,4-dinitrophenol, NaF), applied at the same time as the (^{14}U—C) sucrose, partially inhibits its entry into the latex (Lacrotte unpubl). Since sucrose is the major precursor of rubber and inorganic phosphate is a normal component of latex, this tends to support the view of an active loading of the laticifer from the apoplast and across the plasmalemma.

Sieve tubes in leaves are also specialized cells intensively loaded with metabolites (photosynthates). But, the apoplast concept of phloem loading has no universal validity (van Bel 1987). Only the sieve tube-companion cell complexes of beet leaves (Geiger et al. 1973), the thin-walled sieve tube-companion cell complexes of Monocotyledons such as *Zea mays* (Evert et al. 1978), *Commelina benghalensis* (van Bel 1986) have been proven to be symplastically well-isolated from the rest of the leaf. In these cases, sucrose is considered to be actively accumulated from the apoplast by the sieve tube-companion cell complexes with a carrier mediated energy dependant mechanism such as a sucrose/proton cotransport system at the level of plasmalemma (Cronshaw 1981, Giaquinta 1983). In contrast, the sieve tube-companion cell complexes of *Amaranthus retroflexus* (Fisher and Evert 1982), the thick-walled sieve tubes of *Zea mays* (Evert et al. 1978), the two sets of phloem-loading cells of *Coleus blumei* (Fisher 1986) and *Themeda trianda* (Botha and Evert 1988) are symplastically connected with surrounding cells. The symplastic phloem loading is considered as being a potential alternative for or a complement to loading via the apoplast and the mechanism of phloem loading is species specific (van Bel 1987). Without other data on laticifer loading, it is advisable to state that this strictly apoplastic loading of laticifer itself applies only to these mature secretory cells in the trunk of exploited *Hevea brasiliensis*. The knowledge of this laticifer loading mechanism might have implications in the control of the yield of rubber trees.

What are the pathways for the short-distance transport of assimilates up to the laticifers? The short-distance translocation in stems was first studied from a physiological point of view: apoplast seems to be the principal route for radial photosynthate transfer in stems of sugar cane (Glasziou and Gayler 1972), willow (Gardner and Peel 1971), cotton (Hampson et al. 1978), broad bean (Wolswinkel 1984) and common bean (Patrick and Turvey 1981). But a structural approach in the stems of *Phaseolus vulgaris* (Hayes et al. 1985) indicates that plasmodesmata interconnect all contiguous cell types which constitute the stem symplast and that photosynthate transfer from sieve tube-companion cell

complexes could be either direct to the apoplast or follow a symplastic route to the phloem parenchyma which is the principal stem sink for photosynthates. The translocation rates and the frequency of plasmodesmata point to a symplastic transport of assimilates in parenchyma cells of wood rays of poplar (Sauter and Kloth 1986) and the cortex of *Laminaria hyperborea* and *L. saccharina* (Schmitz and Kühn 1982). The role of vascular rays as being the short-distance translocation pathways and the three-dimensional network of the various parenchyma cells are demonstrated in the stem of *Hevea brasiliensis* (Hebant and de Faÿ 1979). The spacially connected parenchyma cells of phloem rays and laticifer sheaths are shown (in this present work) to have numerous plasmodesmata in their common walls. Although the frequency of plasmodesmata between ray cells, sheath cells and between the two sets of cells has not been measured, we think that the parenchyma transport of sucrose might follow a symplastic pathway, at least partially, because of the high sucrose consumption by the laticifers. A part of metabolites and other nutrients might still be translocated in the apoplast. For example, water that rapidly enters the laticifers when the latex is tapped (i.e., dilution reaction of osmotic origin) or which leaves the laticifer during the day because of transpiration (Buttery and Boatman 1966, 1976) is presumably transported in the apoplast.

If the symplast of parenchyma is a route for the short-distance transport of assimilates in *Hevea* trunk, the existence of a transient apoplastic step implies that the parenchyma cells sheathing the laticifers play an important role in loading the apoplast for subsequent uptake by the laticifer protoplasma.

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