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The Brown Bast Syndrome of Hevea *Part II. Histological Observations*

J.B. GOMEZ*, SAMSIDAR HAMZAH*, H. GHANDIMATHI* AND L.H. HO*

Histological observations on trees suffering from brown bast were made on bark samples from partially dry trees and dry trees which showed external symptoms of dryness or malformations typical of brown bast. The histological behaviour of tissue is microscopically examined to reveal peculiarities evident in the brown bast syndrome.

Formation of tyloses in latex vessels, their ultimate lignification and the significance of these are considered in the paper.

Although histological changes are evident in the laticiferous system and associated tissue, it is shown that the earliest symptoms are evident in the laticifer and hence, the causative mechanism should be sought in the physiology of the laticiferous system.

The brown bast syndrome is a well known physiological disease of *Hevea* which has presumably existed since the inception of the rubber industry. There are schools of thought which favour a physiological explanation for the disease and an older school of thought which considered it to be of pathological origin[1]. More recently, opinion has shifted in favour of the physiological cause and many researchers have put their thoughts on paper.

This paper attempts to bring to focus the various histological symptoms associated with diseased trees. It is expected that such a consideration would highlight the basic nature of the problem — that the tissue is reacting to a problem and tries to cope with the danger it faces by various techniques of defence known in the plant world. From the nature of the reaction in the tissue, some light can also be thrown on the causative mechanism.

Work on the histology of brown bast affected trees is not new. Rands[2,3], Sanderson and Sutcliffe[4] and Rhodes[5] are some of the researchers who have worked on the problem in detail. These authors have described the anatomy of *Hevea* bark affected by the disease. Gomez[6] has briefly reviewed the earlier findings on histological changes occurring in the affected tissue.

Many cells become meristematic, especially those in the vicinity of latex vessels. This meristematic activity partially contributes to the displacement and disorganisation of latex vessels in affected zones in the inner bark of *Hevea*. The latex in such vessels is often coagulated (there is no flow of latex when bark samples are taken from the affected tissue except from yielding vessels). Wound gum formation in the discoloured tissues has been reported[3,4,5]. Paranjothy *et al.*[7] have investigated the general nature of the spread of brown bast and concluded that it is a disease of the laticiferous system. More recently, the histological, biochemical and physiological phenomena[8–11] associated with the brown bast syndrome, have been further investigated.

This paper examines some of these histological features in greater detail with illustrations from many cases of dry trees or partially dry trees examined in our laboratories. A recent paper on the subject by Gomez and Ghandimathi[12] describes the morphological observations.

*Rubber Research Institute of Malaysia, P.O. Box 10150, 50908 Kuala Lumpur, Malaysia

MATERIALS AND METHODS

Tissue samples were secured from trees which had a history of brown bast whenever tumerous abnormalities were investigated. In cases of incipient dryness or early stages of dryness, tissue samples were secured from partially dry zones of the bark indicated by the lack of latex flow along certain regions of the otherwise normal tapping cut. Field experience shows that such trees may continue yielding from the unaffected parts of the bark. A spiral orientation of the abnormal bark arising from excessive meristematic activity along the tissue orientation path is a well-known phenomenon among field workers.

Samples were taken with a bark punch and dropped into formalin-acetic-alcohol fixative[13] and fixed for several days before sectioning. Free-hand sections using a bench microtome were prepared for routine examination of the tissue. Sledge microtome was used to tackle larger pieces of tissue excised from the tree whenever a gross feature was investigated. Finer sections were made by the paraffin wax embedding procedures[13].

The staining technique employed depended on the nature of the sample and investigation required. When rubber was required for *in situ* observations on latex vessels, rubber solvents were avoided in the preparation procedures. Subsequently, rubber stains were employed for investigations[14]. Paraffin wax embedding procedures usually removed rubber from latex vessels and in some instances provided greater clarity for photographic purposes. Thick sections were often first examined by the Sudan III staining method and later by the safranin/fast green method after dissolving the rubber.

For conventional electron microscopy, the procedures used were those normally employed in our laboratories. Fixation of small segments of tissue was carried out in 2% osmium tetroxide solution in phosphate buffer at pH 7.0 and containing 0.35*M* sucrose for tonicity control. Such tissue was dehydrated and embedded in styrene methacrylate for preparation of ultra-thin sections. Sections were stained in uranyl acetate and lead citrate before examination in a Philips EM300.

In addition to conventional electron microscopy, some unconventional electron microscopy was also done to observe particular features such as tyloses quickly and effectively. Tylose-containing latex vessels were first recognised in 50 μm thick sections prepared for light microscopy and selected areas of sections were then re-fixed in osmium tetroxide in the manner earlier described for the electron microscopic examination of such features. Such sections did not provide useful preservation of cellular elements as in conventional electron microscopy but served the purpose of preparing tyloses for electron microscopy.

RESULTS

Tyloses and their Influence on Latex Vessels

Figure 1 shows a widespread occurrence of tyloses in laticifers in a tissue taken from partially dry bark. It is important to retain the rubber in the tissues to recognise these structures readily when tissue samples are routinely scanned for the presence of tyloses. Artefacts related to latex coagulation have to be eliminated by micro-chemical tests if necessary.

If the phloroglucinol test[13] is applied, the contrast between the red coloured lignifying elements and the remaining laticifers is quite clear *(Figure 2)*.

Figure 3 shows lignified tyloses embedded in the rubber in laticifers. *Figure 4* shows a laticifer, at the upper part of the photograph, there is a latex vessel with many compartments. Whether this vessel has been invaded by tyloses or whether a process of de-differentiation is occurring is still doubtful. Coagulated rubber is seen in the periphery of the vessel; calcium oxalate crystals are plentiful in the section and lignified tyloses are present in latex vessels, shown in the lower left section of the same photograph.

Figure 5 shows a fine section of a latex vessel which includes a group of tyloses. Three or four

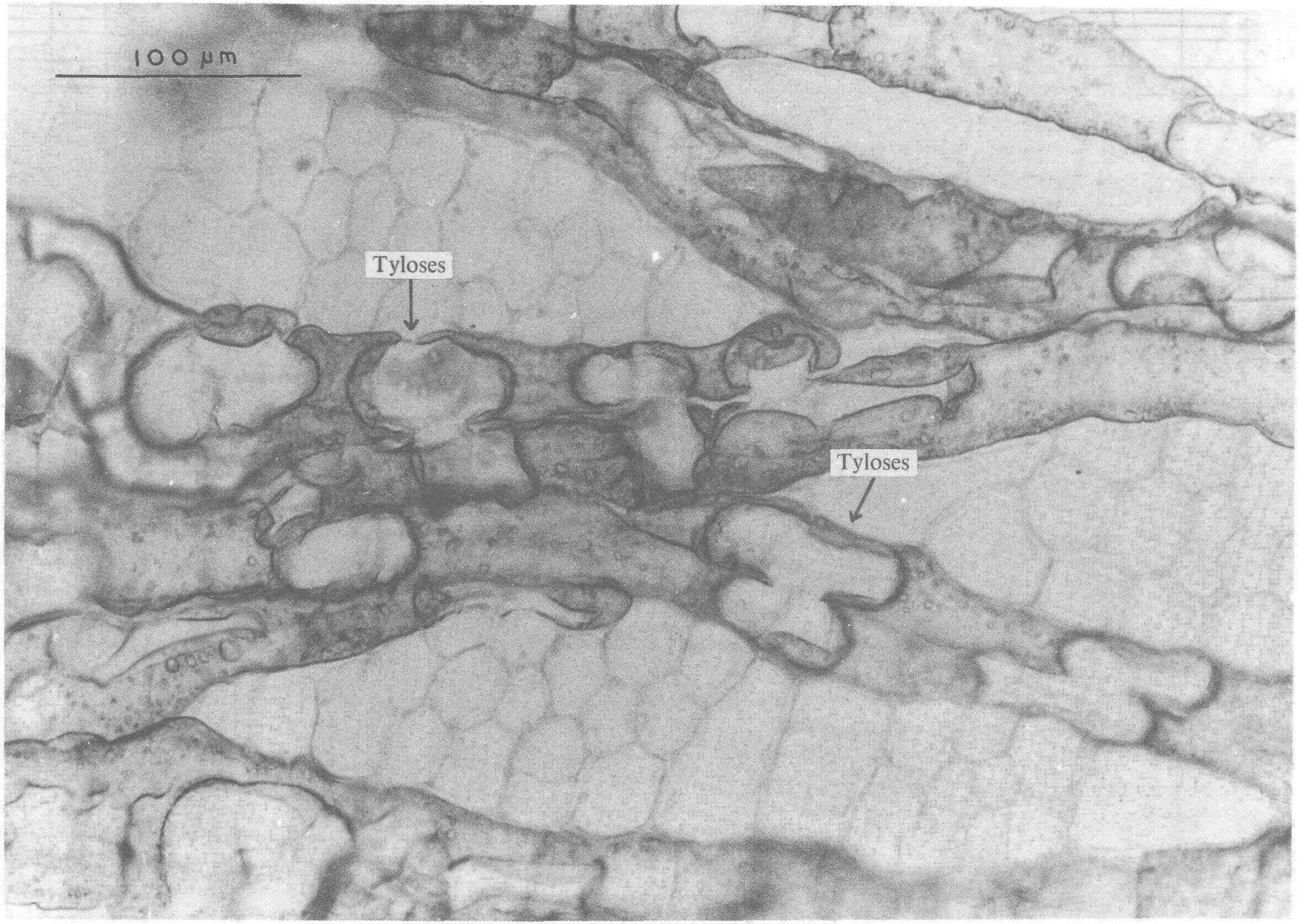

Figure 1. Section through dry bark showing profuse occurrence of tyloses. Magnification 385 ×.

Figure 2. Application of phloroglucinol test to dry bark. Note that the latex vessels are brownish whereas the lignified tyloses are reddish. Magnification 960×.

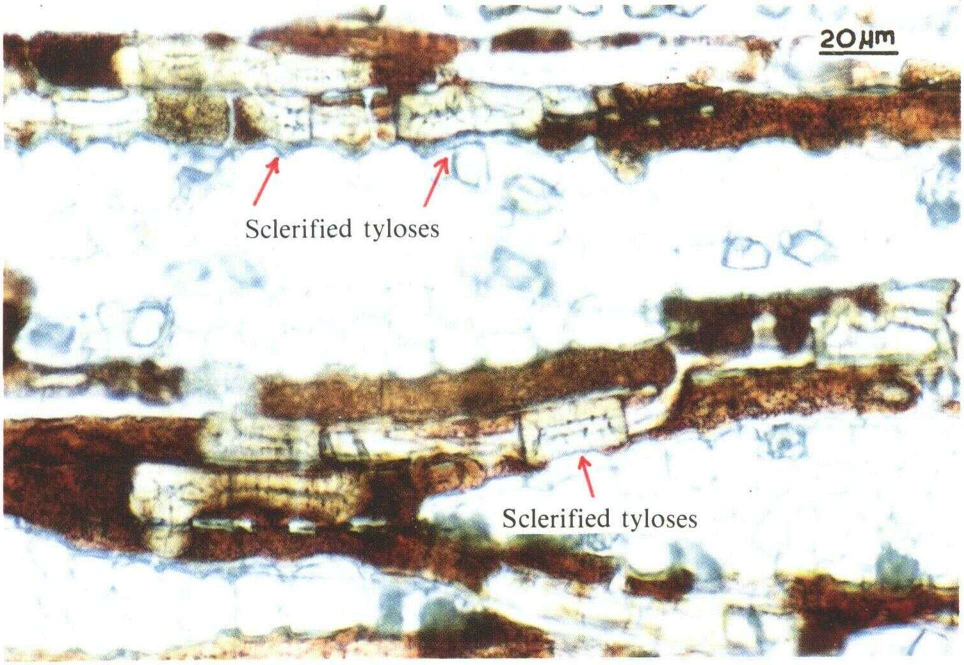

Figure 3. Section through tylose-bearing laticifer zone stained in Sudan III and fast green. Magnification 500×.

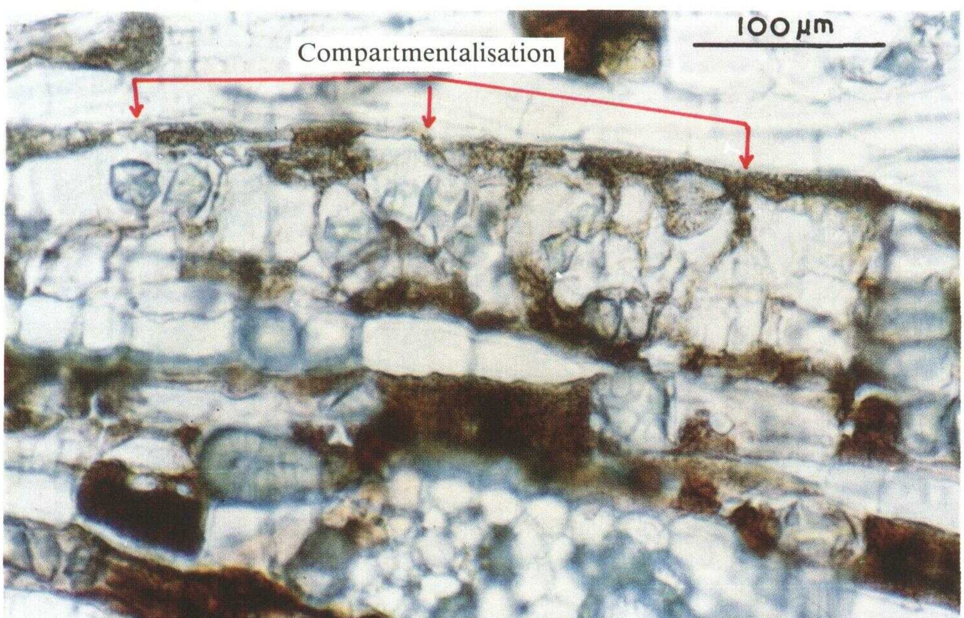

Figure 4. Section through tylose-bearing laticifers showing one vessel with compartmentalisation reflecting de-differentiation. Note the occurrence of brownish material (rubber) in the periphery of the laticifer. Stained in Sudan III and fast green. Magnification 256×.

units are clearly visible and the tylose wall is only beginning to thicken. Nuclei are seen in two units. Note that the neighbouring tannin cells do not show any abnormality.

Figure 6 illustrates macerated latex vessels containing bag-like tyloses fitting the lumen of the vessel and as yet showing no signs of lignification. *Figure 7* shows another example of macerated latex vessels showing a group of tyloses which are already lignified.

Figure 8 shows the occurrence of globules in tannin cells from affected tissue. The globules are white in colour in this kind of preparation and give a vacuolated appearance to the tannin cells showing this phenomenon. Normal tannin cells found in the same section do not exhibit this phenomenon.

Electron Microscopic Observations

Figure 9 shows a mosaic montage of latex vessels from a tree which was showing a depression of turgor pressure and low rates of latex flow[15]. The partial emptiness of the vessel is interesting. Rubber particle stability is high and destabilisation phenomena are not easily visualised.

Figure 10 shows a mosaic montage of a latex vessel from bark which was already showing tylose formation. Three tyloses can be seen in the section. Their lignified walls indicate continuous growth of the wall over a period of time. The latex in the vessel however, is coagulated.

Figure 11 shows a typical sign of latex instability frequently observed in trees showing signs of partial dryness or precoagulation phenomenon on the tapping cut. The individual integrity of the rubber particles has been in jeopardy. The region adjacent to the wall also shows some cytological depletion of normal constituents of the laticifer.

DISCUSSION

It has been argued by Paranjothy *et al.*[7] that brown bast is a disease of the laticiferous system. Since then further investigations[8-11] have shown that tylose or tylosoid formation is observed in bark showing partial dryness and irreversible forms of dryness.

Indications of cell division, the presence of nuclei in tylosoid units and their gradual lignification are evidence presented in this paper pointing to the activities in the living tissue. A question related to de-differentiation of laticifers into smaller units which then re-differentiate into sclerified elements is worth keeping in mind for further investigations as it is not clear that all sclerified elements formed in the latex vessels arise through tylose formation.

From the growth of the multi-layered wall of the tyloses as seen in electron micrographs, the question of latex vessel death has to be raised and approached with caution. Although these vessels contained coagulated latex, the tyloses were able to secrete many layers in their walls which indicated an open supply line for wall-forming materials. However, in those trees where affected bark sloughs off, these phenomena may go unnoticed or may even be absent.

In the xylem, tyloses are well known to occur as a response to injury or water stress and occasionally as a response to infection[16,17,18]. Perhaps current research on *Hevea* would throw further light on the problem.

Tyloses in the latex vessels of *Hevea* trees suffering from dryness were discovered in our laboratories in 1962[19] but it remained obscure till 1975[7]. Since then, more work has been carried out on the subject[8-11].

Our histological studies were plagued by problems related to technical difficulties in the demonstration of these structures in delicate diseased tissue and by now most of the technical problems have been overcome as illustrated in this paper. However, the mode of origin, frequency of occurrence and the triggering factors remain as the three main problems related to tylose formation in latex vessels requiring further study and analysis. That the tyloses would form effective blockages in the laticiferous system still containing at least some

Figure 5. Section through lignified tyloses in latex vessels. Note nuclei in them. Safranin and fast green technique. Magnification 256×.

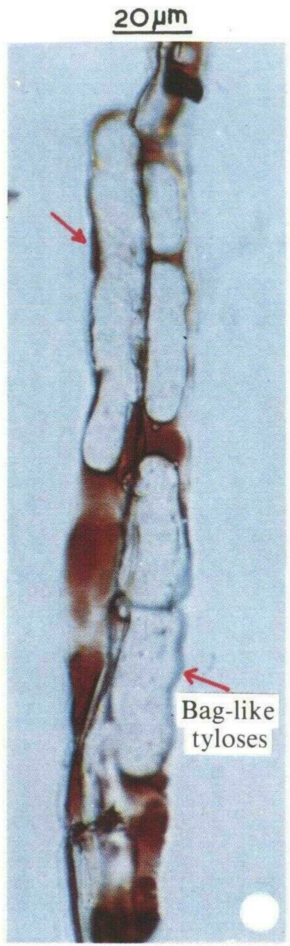

Figure 6. Macerated latex vessels showing bag-like tyloses. Stained in Sudan III after maceration in sodium hypochloride solution. Magnification 500×.

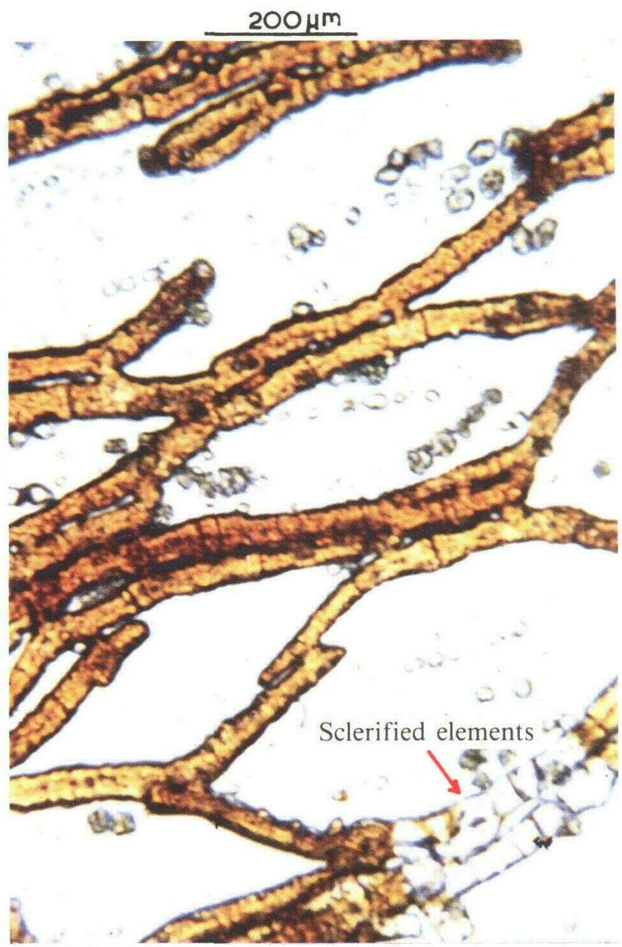

Figure 7. Macerated latex vessels showing sclerified elements. Stained in Sudan III. Magnification 100×.

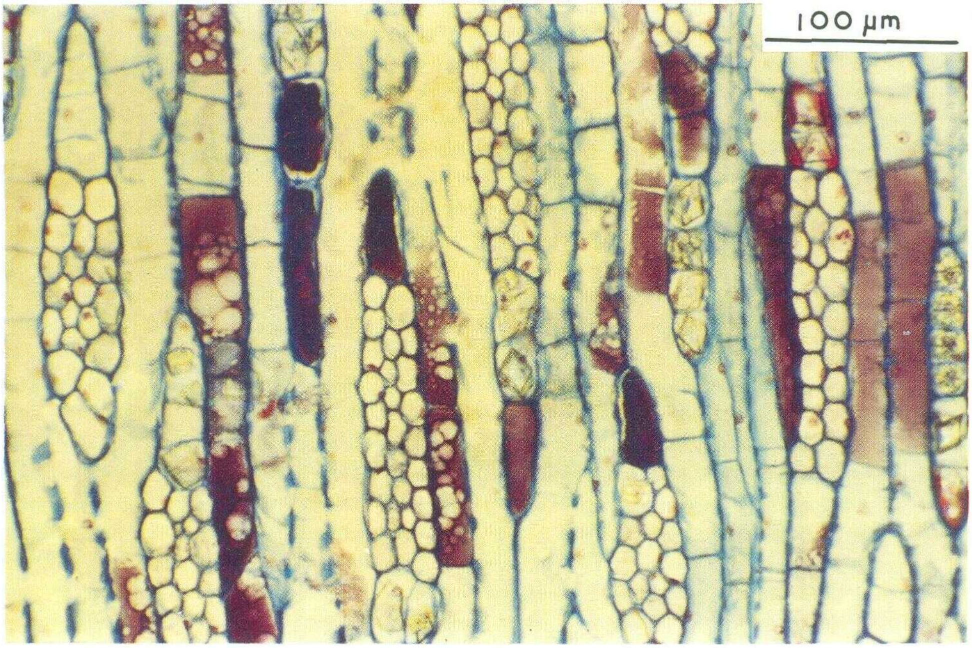

Figure 8. Occurrence of clear globules in tannin cells from affected tissue. All tannin cells do not display this phenomenon. Stained in safranin and fast green. Magnification 256×.

liquid latex is fairly certain. That they sclerify to form lignified elements in the tissue is very evident. It can be considered that the purpose of tylose formation is to prevent fluid loss from the laticiferous system. Questions such as to whether these are formed as a response to repeated, frequent injury, water stress or infection as in the case of xylem[16,17,18] require further experimental investigation and study to elucidate.

Once tyloses are formed in the latex vessels, the latex contained in those vessels are found to be destabilised and coagulation *in situ* and blocked vessels result. Therefore, it is obvious that preventive measures should be aimed at events preceding tylose formation in the tissue. As far as we can surmise from present data, the instability of latex *in situ* is a matter for consideration *a priori.*

Evidence from electron microscopy indicates that in some latex vessels, a partial emptiness develops which manifests itself in the form of low turgor pressure. It is possible that the turgor generation mechanism which involves the general ionic balance in the fluid contents of the tissue and the transportation phenomena in latex-bearing tissue is irreversibly impaired in such instances. From the observation of a low dry rubber content in such latex vessels, it may even be surmised that the biosynthesis of latex is perhaps adversely affected.

Factors leading to instability of latex may arise from perpetual deficiencies of water in the phloem tissue or the excessive presence of destabilising molecules or the absence of stabilising molecules in the colloidal system of latex. More research needs to be done but a start has been made in the recognition of the differences in levels of incidence in various precocious clones in certain agroclimatological regions[20]. Considerable information exists on the vacuo-lysosomal complex of *Hevea* latex, the lutoids[10]. Although a theoretical connection exists between these factors, practical demonstrations are still lacking.

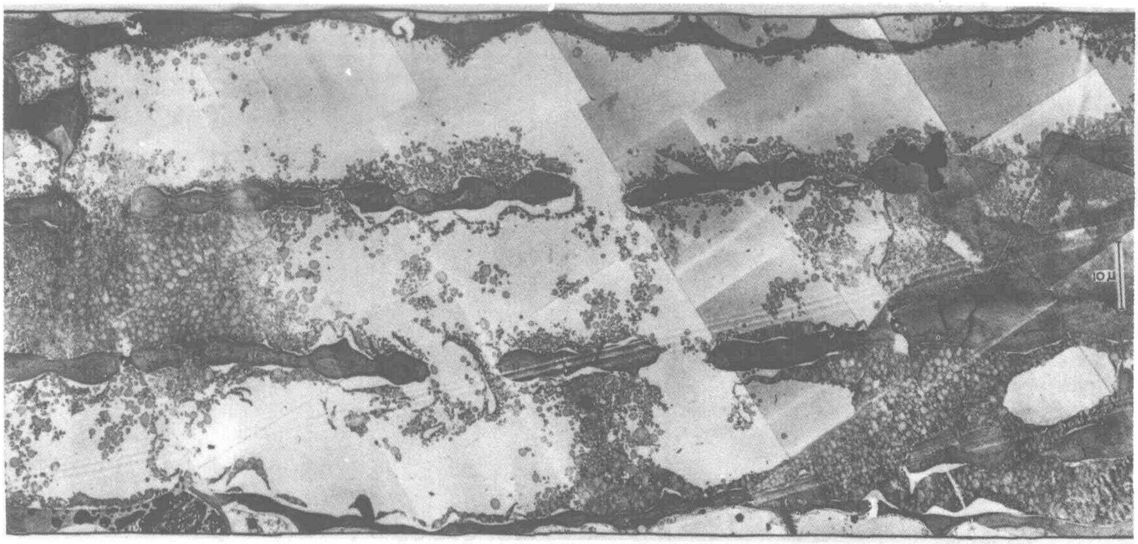

Figure 9. Montage of electron micrographs from a tree showing low turgor pressure. Note the partial emptiness of the vessel. Magnification 850×.

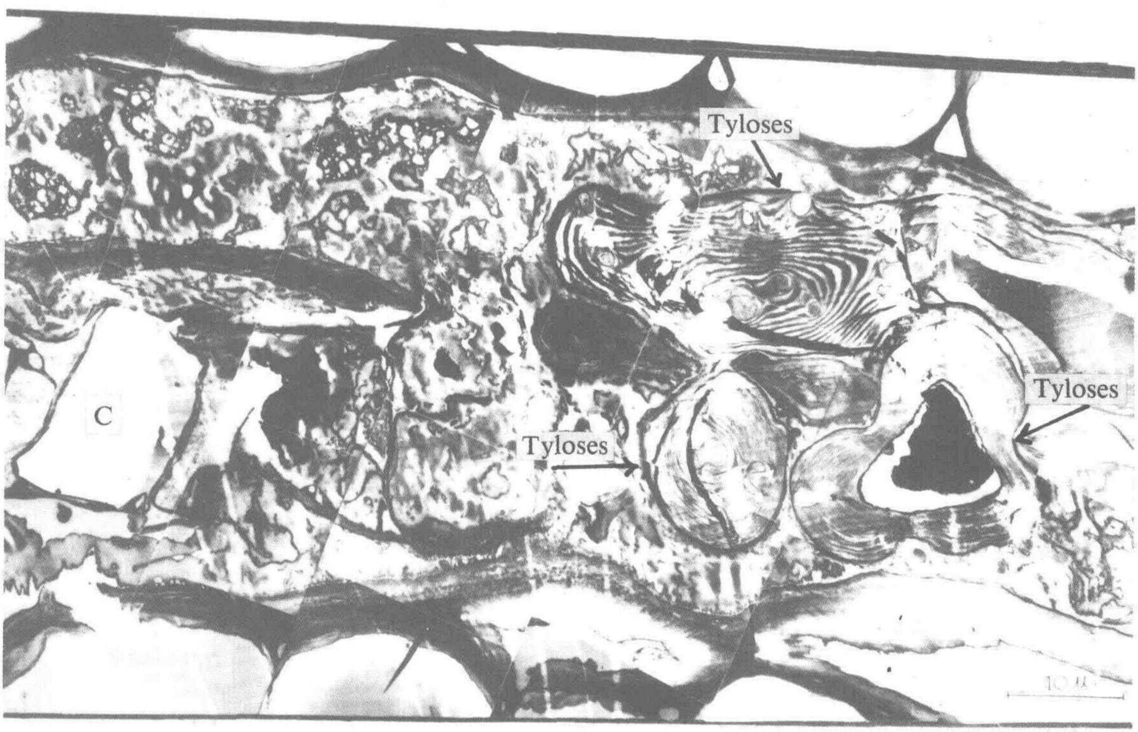

Figure 10. Montage of electron micrographs from a latex vessel with tyloses. 'C' denotes a calcium oxalate crystal. Magnification 1500×.

Figure 11. Electron micrograph of a latex vessel from a tree showing partial dryness. Note the instability of the rubber particles. Magnification 50 000×.

As discussed in an earlier paper[12], the primary causation for brown bast may be a wound reaction as the severity of the disease is related to the intensity of repeated wounding of the tree. The expression of the trauma is seen in long rested brown bast trees in the form of excessive meristematic activity, adventitious xylem formation and certain changes in the laticiferous system and adjacent tissue. From all studies conducted so far, the basic change in the laticiferous system appears to be the development of latex instability prior to the formation of anatomical symptoms of the disease. Admittedly, tylose formation, gummosis and other phenomena are triggered by physiological changes in the tissue, which are yet to be discovered.

Jacob and Prevot[10] take the view that bark dryness can be due to many causes. While they accept that over-stimulation and over-exploitation can cause bark dryness, the histologic symptoms are those caused by traumatic or infectious attack.

Events such as acceleration of phenolic metabolism, cell necrosis and wound gum formation, sclerification of certain cells, adventitious xylem formation, general increase in meristematic activity can all be caused by changes in basic hormone levels or induced enhancement of hormone levels or metabolic levels and this in turn can be accentuated by the wound reaction the tree is subjected to. It would therefore be possible that the entire latex outflow area may not be affected in the expression of the wound reaction but an intensively regenerating zone of bark in the drainage area may be the central point from which the wound reaction phenomena emanate. Investigators of brown bast physiology are therefore advised to look for changes in the 'maximum regeneration zone' for the earliest signs of change.

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