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# SERUM PROTEIN PATTERNS IN HEALTHY AND BROWN BAST AFFECTED TREES OF *HEVEA*

By

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ABSTRACT

*Serum protein patterns of healthy and brown bast affected Hevea trees of six clones viz, RRIC 7, RRIC 45, RRIC 100, RRIC 101, RRIM 600 and PB 86, were investigated. Total protein content in serum decreased noticeably in all six clones when affected by brown bast, the highest reduction being 50% in PB 86. Gel electrophoretic separation of serum proteins complemented these findings, with the disappearance of a number of bands and reduced intensity of the remaining bands. Protein content at different heights showed a high degree of reduction extending upwards from the tapping panel in clone RRIC 101 whilst in RRIM 600 it extended downward from the tapping panel. Proteins are indicative of the spread of brown bast to neighbouring tissues at varying degrees although they may not result in a noticeable change in external appearance or in latex yields.*

## INTRODUCTION

Brown bast is generally considered a physiological disorder of the *Hevea brasiliensis* tree, caused by over extraction of latex. Although this disorder does not lead to the death of the tree, the yield losses caused by it, particularly those of more vigorous high yielders, which are apparently more susceptible to brown bast, have been of considerable concern to planters. Attempts have been made to understand the exact cause of this condition since the 1920s. Rands (1921) and Rhodes (1930) held the view that it is intimately linked with the phenomenon of wound healing in woody plants. It has also been attributed to nutritional stress (Vollema 1949, Compangon *et. al*, 1953 as quoted by Paranjothy *et. al*. 1975), repeated removal of large volumes of latex leading to fluctuations of water available to the bark (Sharples & Lambourne, 1924) and a strong dilution reaction during latex flow (Frey Wyssling, 1932). In a recent paper Paranjothy et *al* (1975) have presented evidence from electron-microscopy to show that it is brought about by instability of lutoids within the latex vessels, leading finally to coagulation of vessel contents. According to them, it originates in and spreads along the vessels, sometimes to considerable distances from the tapping cut. In a majority of the brown bast affected trees, however, the disorder is limited to an area close to the tapping cut, possibly confined to the drainage area or its neighbourhood (Paranjothy *et. al*, 1975).

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In some of our studies on characterization of *Hevea* clones, based on serum protein patterns, we observed that a reduction in protein content is often associated with brown bast. This was further explored to obtain more information on spread of brown bast, and also to see if it can be used as an early warning signal of the disease and if it has any association with its cause.

## MATERIAL & METHODS

Collection of samples : All latex samples were taken from Dartonfield Group, Agalawatta and Eladuwa Estate, Matugama. They were collected in ice and brought into the laboratory for preparation of A-serum by acid coagulation. A-serum is latex serum obtained by acid coagulation of *Hevea* latex.

In the study on protein content at different heights latex samples were collected both from healthy and brown bast affected trees at a) 10cm, b) 50 cm (normal tapping panel), c) 70 cm, d) opposite the normal tapping cut, e) 120 cm, and f) 170 cm above ground level.

A-serum : Preparation of A-serum was carried out as described by Walujono and Suseno (1973). To 20 ml of latex, 10 ml of 2% acetic acid was added and left for about 2 h; the resulting coagulum was pressed by a glass rod to obtain clear A-serum.

Protein content : Determination of protein content in A-serum was carried out by the method of Lowry *et al* (1951). One ml of clear A-serum was used for digestion.

Electrophoresis : Proteins in A-serum was separated by polyacrylamide gel electrophoresis at pH 7.2, O.1M tris buffer, with small pore gel, prepared by the method of Davis (1964). Running time was 50—60 min on a Shandon gel electrophoresis apparatus. Gels were fixed in 12.5% trichloro acetic acid (TCA) solution for 2h. They were then stained in 1% Coomassie Brilliant Blue in 12.5% TCA for 48 h as described by Chrambach *et al* (1967). Gels were preserved in 7% acetic acid.

## RESULTS

Protein content in A-serum : A reduction in protein content was observed in brown bast trees of all clones examined, compared to healthy trees (Table 1). Clone PB 86 which is less susceptible to brown bast, compared to RRIC 101, showed the highest reduction in protein content (50%) followed by RRIC 100 which had a reduction of 38.4%. Clone RRIM 600 showed the lowest reduction.

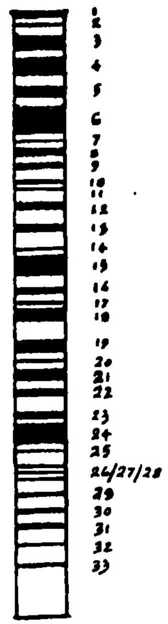


Fig. 1. Typical diagram of protein patterns in A–serum of *Hevea* clones, prepared after screening 70 *Hevea* clones by acrylamide gel electrophoresis (Prematillake, 1981).

SERUM PROTEIN PATTERNS IN HEALTHY AND BROWN BAST AFFECTED TREES OF *HEVEA*

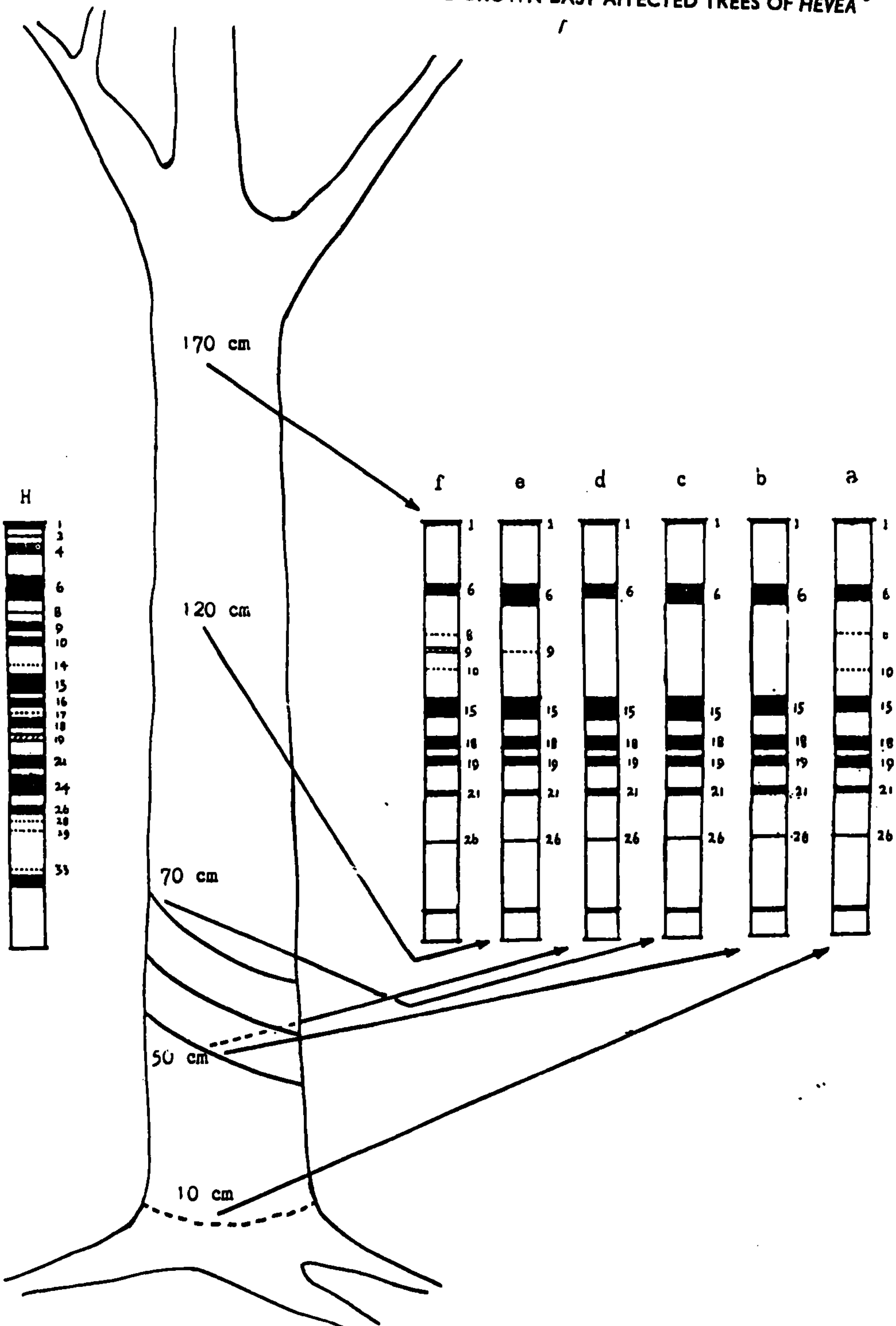


Fig. 2. A—serum protein patterns at different height levels of a brown bast affected tree of clone RRIC 101. Note the disappearance of bands 8, 9 & 10 in the affected area.

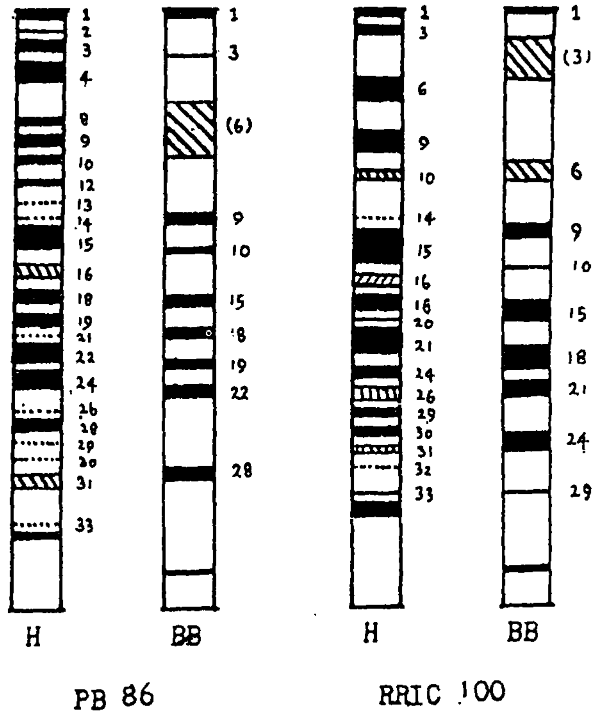


Fig. 3. Variation of protein pattern in A-serum of healthy and brown bast affected trees of clones PB 86 and RRIC 100. Note the disappearance bands 13 & 8 in brown bast trees of PB 86 & RRIC 100 respectively, and the reduced intensity of some of the remaining bands.

Table 1. *Protein content in A-serum of brown bast affected and healthy trees of six clones.*

| Clone | Protein content % wt | | % reduction brown bast trees |
|---|---|---|---|
| | Healthy | Brown bast | |
| RRIC 7 | 0.803 | 0.550 | 31.5 |
| RRIC 45 | 0.723 | 0.639 | 11.6 |
| RRIC 100 | 0.796 | 0.490 | 38.4 |
| RRIC 101 | 0.586 | 0.499 | 14.8 |
| RRIM 600 | 0.735 | 0.718 | 2.3 |
| PB 86 | 0.753 | 0.376 | 50.0 |

Protein content at different height levels : Protein content was also monitored in A-serum of latex collected at different heights and the results are given in Table 2. A high degree of reduction in protein content in the tapping cut area and above it, was observed in brown bast affected trees of RRIC 101 : the reduction was also found to be nearly uniform at positions c, d, e, and f. In clone RRIM 600 the degree of reduction in protein content was comparatively low. In contrast to RRIC 101, the highest reduction in protein content in RRIM 600 was found to be in the tapping cut area and below it whilst the degree of reduction above the tapping panel was comparatively low. Clone RRIC 100 showed a reduction of 32.0% at 30 cm above the tapping panel whilst clone PB 86 showed no reduction in protein content at 30 cm above the tapping panel. PB 86 in fact had a higher protein content, an increase of 11.7% at this height.

Table 2. *The degree of reduction in protein content of brown bast affected trees at different height levels as compared to corresponding positions of healthy trees*

| Clone | Percentage reduction | | | | | |
|---|---|---|---|---|---|---|
| | *a* | *b* | *c* | *d* | *e* | *f* |
| RRIC 101 | 4.2 | 14.4 | 35.6 | 36.6 | 43.4 | 39.4 |
| RRIM 600 | 20.5 | 22.0 | 22.1 | 8.7 | 5.3 | 8.0 |

(*a*, 10cm; *b*, tapping cut; *c*, opposite side of tapping cut ; *d*, 70 cm ; e, 120 cm; and *f*, 170 cm).

Protein patterns in A-serum : Protein patterns obtained by polyacrylamide gel electrophoresis of A-serum of latex at different heights of clone RRIC 101 is given in Fig. 2. It was observed that bands 8,9 and 10 were completely absent in A-serum of brown bast trees in RRIC 101, at height b(tapping panel) c(opposite tapping panel) and d(slightly above tapping panel), compared to the typical protein pattern for healthy trees of RRIC 101. They were found at reduced intensity at positions a and f. At position a too, band No. 9 was completely absent.

Protein patterns of two other popular clones *viz.* PB 86 and RRIC 100 were also investigated, when affected by brown bast. Their protein patterns are given in Fig. 3. In PB 86, bands 4, 5, 6 and 7 disappeared forming a diffused band, band 8, 12, 13, 14, 16, 21, 24, 26, 29, 30, 31 and 33 completely disappeared whilst the intensity of band Nos. 3, 9, 10 and 19 were reduced. In clone RRIC 100, it was seen that bands 14, 16, 20, 26, 30, 31, 32 and 31 and 33 disappeared completely whilst the intensity of bands 6, 9, 10, 18 and 29 were reduced. When compared to the typical protein map for healthy trees, band No. 3 appeared to be diffused.

## DISCUSSION

The results of this investigation show that the protein content in *Hevea* latex is considerably reduced when the tree is affected by brown bast. This was true for all the six clones examined. A reduction in protein content in latex of brown bast affected trees has not been reported before. Reductions in protein contents have been reported in several plant varieties under water stress condition. Mature leaves of water-deficient, Bermuda grass have been reported to contain a lower protein content than well-watered control plants (Barnett and Naylor, 1966). Similar observations have been reported in sugar beet (Shah and Loomis 1965). In *Zea mays*, water stress has caused a shift of ribosomes from polymeric to the monomeric form, starting about 30 min after stress initiation and when the water potential of the tissue began to decrease measurably. Release of stress has caused the ribosomes to revert from monometric to polymetic form after a lag period (Hsiao, 1970). Dehydration of germinating wheat embryo has been found to inactivate m-RNA and to arrest protein biosynthesis (Chen et. *al*, 1968). Stressed tissues of tobacco leaves have shown reduced capacity to incorporate L-leucine C 14 into protein. The reduction was about 50% (Ben-Zioni et. *al*. 1967). One other significant feature observed in plant tissues under water stress is proline accumulation (Chen et *al* 1964, Kemble and Macpherson, 1954; Prusakova, 1960; Barnett and Naylor, 1966; Levy, 1980). Brown bast has not been linked with water stress although some workers are now beginning to think so (Yapa, 1984). However, the effect of such water stress situation on protein content in *Hevea* serum is not known.

The disappearance of some of the protein bands and the reduction in intensity of some of the remainder in brown bast latex, are complementary to findings on protein content in A-serum of this study. The degree of reduction in protein content of brown bast affected trees at different heights seems to be an indication of the spread of this physiological disorder (Table 2). A higher degree of reduction in protein content extends upwards from the tapping panel in clone RRIC 101 whilst in RRIM 600 it extends downwards from the tapping panel (Table 2). Since reduced protein content is a characteristic of brown bast tissue, these observations seem to show the possible spread of brown bast. According to Paranjothy et *al* (1975), in a majority of the brown bast trees, the disorder is limited to an area close to the tapping cut and it is likely that in these cases the development is confined to the drainage area or its neighbourhood. The protein pattern obtained by gel electrophoresis (Fig. 2) also provides an indication of the extent of spread of brown bast. Although the yield of the bark of the opposite side of the tapping panel (panel B, position C of Fig. 2) seems to be unaffected, the high degree of reduction in protein content in both RRIC 101 and RRIM 600, seem to suggest that panel B is also affected. It has been reported that brown bast originates in and spreads along the vessels (Paranjothy *et al*, 1975), where it was also observed that the disease does not generally spread from virgin bark to regenerated bark or from one regenerated panel to another. However, the results of this study on protein levels, indicate that brown bast does spread to neighbouring tissues in varying degrees, although it may not result in a noticeable change in the external appearance or the latex yields of neighbouring tissue. This makes it even more important to find an early warning signal for the detection of the onset of brown bast and its subsequent spread.

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