Physiological Changes in Hevea brasiliensis Tapping Panels During the Induction of Dryness by Interruption of Phloem Transport I. Changes in Latex

S. E. CHUA

Buddings of Tjir 1 were ring-barked above and below the tapping panel, rapidly inducing dryness at the tapping cut. Associated physiological changes included the production of a latex with low content of rubber hydrocarbon and total solids and a subnormal pH, the tapping cuts showed a grey discolouration near the phloem tissue. Ultracentrifuged samples of latex showed heavy flocculation of 'bottom fraction' particles, an indication of excessive dilution of the latex in the tissues isolated by ring-barking. Paper chromatographic techniques revealed no evidence of apparent depletion of amino acids, organic acids or sugars in the tissues.

The disorder known as brown bast of *Hevea* has an extensive literature; experimental evidence, however, is not proportionately adequate. DIJKMAN (1951) reviewed previous opinions on the nature and cause of the condition. Over-exploitation was believed to lead to excessive losses of latex and depletion of assimilates in the bark. Rubber regeneration became inadequate and extremely dilute latex, which did not coagulate easily, was obtained. The exhausted tissues turned dry eventually.

In recent years the Institute has pursued deliberately an experimental approach to the disease, the investigations highlighting the complex of physiological and pathological factors involved. Studies on the onset of dryness have been difficult because it has proved impossible to forecast generally which trees within a given planting would go dry. Compilation of case histories for each tree is very laborious and the alternative approach adopted was to deliberately induce dryness in healthy trees by the artificial interruption of phloem transport. The results obtained may not reflect the physiological changes accompanying the onset of the disorder, but a comparison of the experimental trees with known cases of brown bast provides a basis for scientific deduction of the mechanisms involved.

MATERIALS AND METHODS

Twelve trees of Tjir 1 were selected for this study, four each being allocated to Treatments 1 and 2, and control. Treatment 1 consisted of ring-barking the trees above and below the tapping panels. Treatment 2 was the complete isolation of the tapping cuts by incisions to the wood round the tapping panels.

The trees were tapped alternate daily on a half-spiral cut (S/2.d/2.100%) and the tapping cuts were examined for discolouration of the phloem tissues. The length of the non-latex bearing tissues at the cut was measured in inches. Volumes and total solids content of latex were determined after each tapping.

Latex collected from each tree, in tubes packed in crushed ice, was ultracentrifuged; samples from trees within each treatment were pooled before centrifuging. The latex of each treatment was collected in successive samples of 15 ml each and poured into Lustroid (celluloid) tubes fitted with air-tight caps. The samples were centrifuged at 26000 rev/min in a Spinco Model L ultracentrifuge for 1 hour. The temperature was maintained at 5°C.

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Qualitative determinations of amino acids, sugars and organic acids of the latex were carried out by paper chromatography. For amino acid determination, 10 ml aliquots of latex from each treatment were used for extraction. The latex was slowly mixed with 120 ml of 80% ethanol with constant stirring so as to coagulate the hydrocarbon; the extract was filtered and the alcohol evaporated to dryness at 30–40°C under reduced pressure. Because the presence of inorganic salts seemed to form hydrophylic centres which extracted water from the solvent, rendering the chromatograms uninterpretable, the extracts were desalted by the solvent extraction technique of SMITH (1960). The dried extracts were dissolved in acetone containing 5% of 6N HCl and filtered; the filtrate was evaporated under reduced pressure at 30–40°C to approximately 0.5 ml.

The aqueous extract was spotted on a sheet of Whatman No. 1 paper with a platinum wire with a terminal loop of 2–3 mm diameter, holding 3–4 µl of extract. One-dimensional chromatograms were made by the ascending method, using butanol-acetic acid solvent (120 ml n-butanol, 30 ml acetic acid and 50 ml distilled water).

The amino acid spots were detected by a ninhydrin spray consisting of 0.3 gm ninhydrin dissolved in 95 ml acetone with 5 ml of collidine added to improve resolution and differentiation.

For the chromatographic examination of sugars, 10 ml aliquots of latex were coagulated with ethanol, filtered and evaporated under reduced pressure to approximately 10 ml. This volume was clarified by adding 10 ml of 0.3 n barium hydroxide and 10 ml of 5% zinc sulphate solution, filtered again and the filtrate evaporated under reduced pressure to 0.5 ml.

One-dimensional chromatograms were developed by the ascending method after spotting the extracts on sheets of Whatman No. 1 paper, using phenol-water (160 g phenol dissolved in 40 ml distilled water) as a solvent. Sugars were detected by first spraying the paper with silver nitrate solution (0.1 ml saturated solution of $AgNO_3$ in water, in 100 ml acetone) and then, after air-drying the acetone, spraying with 0.5% sodium hydroxide in ethanol.

For organic acids, 10 ml aliquots of latex were coagulated with ethanol, filtered and the alcohol evaporated to approximately 10 ml as before; solid ammonium sulphate was added to near saturation and the sample was acidified to pH1 with sulphuric acid. The precipitated proteins and higher fatty acids were filtered off and the aqueous filtrate extracted with twice its volume of ether. The undissociated acids passed into the ether layer, which was neutralised with ammonium hydroxide and evaporated to 0.5 ml under reduced pressure.

The extracts were spotted on Whatman No. 1 papers and one-dimensional chromatograms were developed by the ascending method. The solvents used were butanol-acetic acid and ethanol-ammonia (180 ml ethanol, 10 ml of concentrated ammonia solution and 10 ml of distilled water).

The organic acid spots were detected either by using iodine vapour or by dipping the papers in bromo-cresol green (0.1% w/v in 99.5% ethanol) to which N sodium hydroxide had been added until it turned blue-green; one part by volume of this solution was mixed before use with 4 parts of acetone. Organic acids showed up as yellow spots on a blue or green back-ground. Although dryness of the panel was induced in the treated trees, at the end of the nine month experimental period, all the trees were living and apparently healthy except for the induced dryness. Subsequently the cuts callused over and the renewed bark of the treated panels are now yielding.

RESULTS

General Physical Observations

When isolation cuts were made on the trees of Treatment 1 and 2 the latex flowed profusely and, in order to reduce bleeding, 70% ethanol was sprayed on the cuts to coagulate the latex quickly. Had the bleeding not been stopped, the isolated panel would have gone completely dry immediately. Under continued S/2.d/2.100% tapping the yield of treated trees dropped rapidly from an average of 160 ml of latex to about 25 ml; the yield later

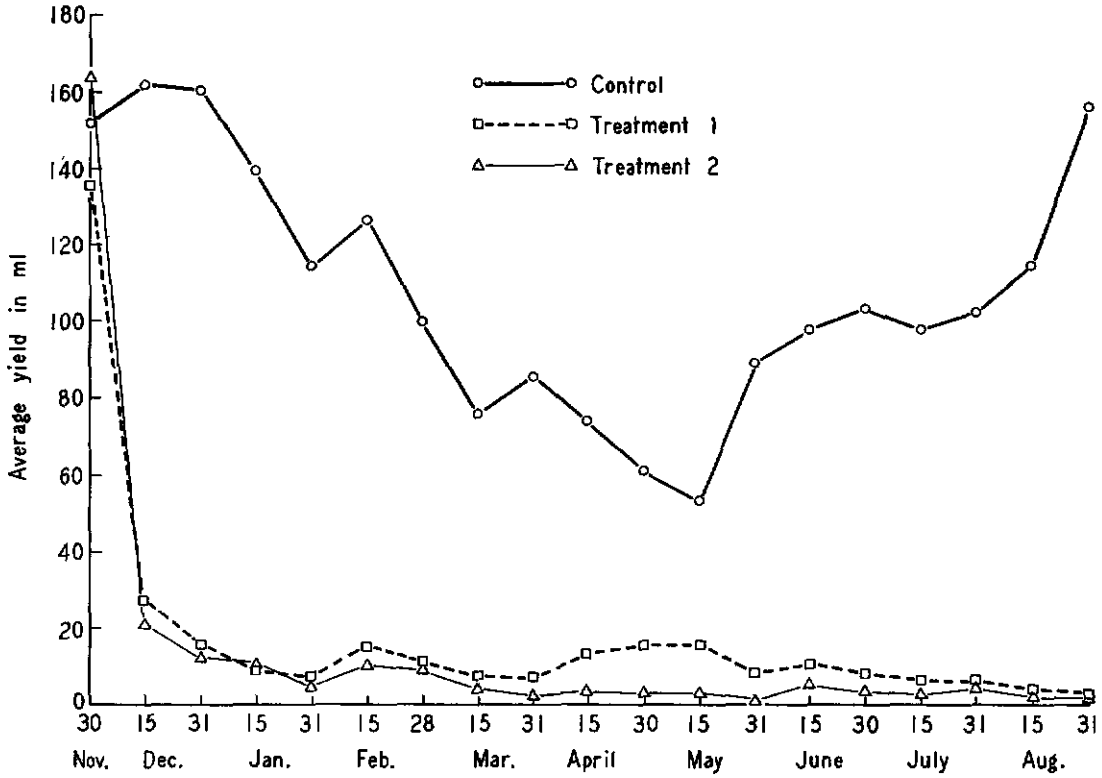


Figure 1. Effect of interruption of phloem transport on yield.

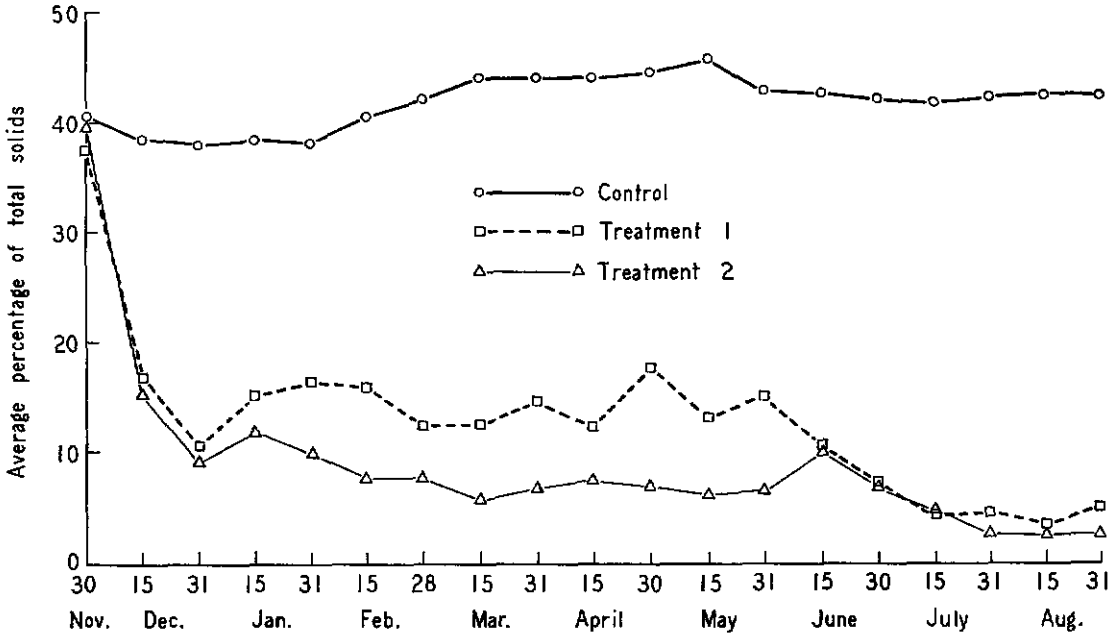
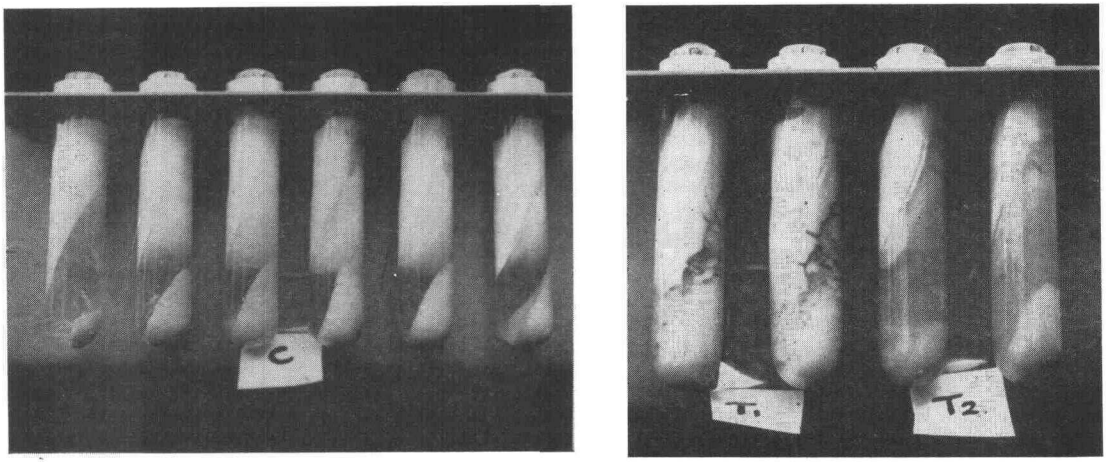


Figure 2. Effect of phloem interruption on total solids of latex.



A *B*

Figure 3. Ultracentrifugal separation to show damage to non-rubber bottom fraction particles (C=Control and T1 and T2=Treatments 1 and 2 respectively).

decreased gradually until, at the end of 9 months, the panels were almost completely dry (*Figure 1*). The yield of control trees showed only a drop during the wintering period. Total solids in the latex of treated trees showed a similar decrease below controls (*Figure 2*) but the reduction was noticeably greater in Treatment 1 than in Treatment 2. Control and Treatment 1 trees showed an increase in total solids during the wintering months. The flow of latex from the cuts on treated trees was patchy, the latex was watery and the inner phloem tissue showed grey discolouration; the pH of the latex from trees of Treatments 1 and 2 fell from 6.8 to 6.3 during this period.

Ultracentrifuge studies

When sequential 15 ml samples of latex from the control trees were centrifuged there was some flocculation of the non-rubber 'bottom fraction' particles observed in the first, second and third samples (*Figure 3A*); in the fourth, fifth and sixth tubes, the particles were stable and had not flocculated. Similar samples from Treatments 1 and 2 showed heavy flocculation of the non-rubber 'bottom fraction' particles and a large reduction in the volume of the rubber hydrocarbon layer (*Figure 3B*) was observed in centrifuged latex.

Paper Chromatographic observations

Quantitative estimation of amino acids, organic acids and sugars was not attempted but the number and size of spots were recorded. Six to seven sugars, 13 to 18 amino acids and four to six organic acids were regularly observed in latex of both the treated and control trees. No significant changes were seen when dryness was induced, except for an increase in the size of the organic acid spots.

DISCUSSION

Interrupting phloem transport gave rise to grey discolouration of the inner phloem tissues, low total solids contents, watery latex and the development of dry patches in tapping cuts. These symptoms are similar to those of naturally occurring brown bast trees described by RANDS (1921), but such similarities are often deceptive. However, the physical characteristics of the latex suggest that mechanically interrupting the phloem depletes the supply of substrates essential for rubber biosynthesis. The watery latex is an indication of cytoplas-

mic dilution, a condition that might be expected to cause the observed flocculation of 'bottom fraction' of latex from treated trees. PAKIANATHAN *et al.*, (1966) have shown that when latex samples are collected in cold hypertonic solutions of potassium chloride, sodium sulphate, mannitol or sucrose and buffered to pH 7, no obvious damage to or flocculation of 'bottom fraction' particles occurs. They suggest that extreme particle damage must occur after the latex left the tree and it might be the result of osmotic shock caused by the considerable dilution occurring in the early stages of flow. The 'bottom fraction' flocculation seen in the first two or three sequential samples from the control trees in the present experiment might be due to this normal dilution effect, but there has been no direct evidence that the dryness of the isolated panels has resulted from the excessive dilution of latex flowing from treated trees.

From the chromatographic observations, the slight increase in organic acid content may indicate an increase in respiratory activity of the isolated panels. Since there is no increase or decrease in either the number and intensity of amino acids and sugar spots obtained it might be assumed that there is lateral movement of organic solutes such as soluble carbohydrates from the wood to the isolated panels.

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Botanical Division
Rubber Research Institute of Malaya
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