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Initial Physiological Changes in Hevea *Latex and Latex Flow Characteristics Associated with Intensive Tapping*

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Physiological changes associated with the onset of dryness in Hevea *were progressively monitored by inducing dryness through intensive tapping. Trees tapped intensively on S/2.d/1 and S/1.4d/1 showed no change in plugging index compared with controls on S/2.d/2 tapping. However, the panel turgor and initial flow rate of latex were reduced while latex stability, as determined by the Aerosol OT test, showed an increase. High speed centrifugation of latex from intensively tapped trees revealed various abnormalities; in particular, the bottom fraction was diminished. Dilution of the latex could be partly responsible for this, but a relative reduction in the density of bottom fraction particles appeared to have resulted in a high proportion of the particles not sedimenting after centrifugation. Latex polyphenol oxidase activity was progressively diminished in trees tapped intensively. Intensive tapping induced changes in the mineral composition of the latex non-rubbers. Copper, manganese and calcium were increased while nitrogen, magnesium, phosphorus and potassium and calcium contents were increased while nitrogen, magnesium, phosphorus and potassium contents decreased. The physiological changes observed are discussed in relation to the possible mechanisms leading to the onset of dryness.*

Over-exploitation of *Hevea* trees can lead to a cessation of yield and where the dryness persists, the physiological disorder known as 'brown bast' may develop. Extensive studies have been made on dryness and brown bast and several postulations such as depletion of latex constituents[1], impairment of phloem transport[2,3], wound reaction[4,5], or adverse water relations[6,7] have been put forward to explain the more immediate causes. A preliminary study of the effects of over-intensive tapping on the development of dryness has previously been reported[8]. The aim of the following investigation is to obtain a further understanding of the physiological changes in latex and latex flow characteristics associated with the onset and development of dryness. The induction of dryness through deliberate over-exploitation allows such physiological changes to be progressively monitored.

MATERIALS AND METHODS

Thirty RRIM 628 *(Panel A)* trees on S/2.d/2 tapping were selected on the basis of similarities in girth and yield. Ten trees each were tapped S/2.d/2 (tapping intensity : 100%), S/1.d/1 (tapping intensity: 400%) and S/1.4d/1 (tapping intensity 1600%; *i.e.* full spiral, four times daily). A randomised plot design was adopted in the study. With trees tapped four times a day (S/1.4d/1), observations and latex collection were made at the first tapping of the day together with the other two treatments.

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Yield was determined by volume measurement of individual trees. Tapping cuts were examined periodically for discoloration in the cortical region. The length of the dry portion of the cut was measured and dryness expressed as a percentage of the length of the tapping cut.

Plugging index was determined by measuring the yield volume of the first 5 min after tapping and the final volume when flow had ceased. Plugging index was then expressed as

$$\frac{\text{Rate of flow in first 5 min}}{\text{Final volume}} \times 100$$

according to Milford *et al*[9]. Latex samples pooled from trees of each treatment were collected periodically and analysed for dry rubber content (d.r.c.), total solids and mineral elements[10]. The entire yield from each tapping was collected, and from this the appropriate quantity was sampled for analysis.

Latices were collected from individual trees in chilled containers for high-speed centrifugation studies. The first 30 min flow of latex after tapping was collected for this purpose. The latices were centrifuged in translucent polycarbonate tubes for 1 h in a Sorval RC-28 centrifuge at 20 000 r.p.m. (49 000 g max) at 3°C–4°C. The centrifuge pattern, essentially comprising a rubber phase, a sediment ('bottom fraction') and a liquid phase (C-serum)[11,12] were examined visually and the bottom fraction subsequently removed and oven dried at 60°C to constant weight.

Latices were at times stained with neutral red before centrifugation as previously described[8,12].

In assays for the enzymes acid phosphatase (EC 3.1.3.2.) and polyphenol oxidase (EC 1.14.18.1.) and for soluble proteins in latex, four parts by volume of 0.125% Triton X-100 were first added to one part of latex (pooled from all trees in each treatment) to rupture vesicular components of latex[13]. The mixture was centrifuged as described above and the resultant serum phase that was analysed was thus a diluted mixture of C-serum and vesicle-bound sera, the latter being mainly B-serum from the lutoids.

Polyphenol oxidase activity was determined by the rate of change in optical density at 400 nm with catechol as the substrate in 0.1*M* citrate buffer at pH 6.0.

Acid phosphatase activity was determined by the change in optical density at 404 nm using p-nitrophenylphosphate as the substrate in 0.1*M* citrate buffer at pH 5.0[14]. Acid phosphatase activity in C-serum (*i.e.* the serum phase after centrifugation of undiluted latex) was also measured.

The 'bursting index' of lutoids in latex was expressed as

$$\frac{\text{Acid phosphatase activity in C-serum}}{\text{Acid phosphatase activity in serum from Triton X-100-treated latex}} \times 100$$

based on Ribaillier[15]. Whereas the dilution of whole latex by Triton X-100 was fixed (1:4), the actual dilution of the latex serum (mainly a mixture of B and C sera) varied according to the proportion of the serum constituent in the latex. A correction was therefore made in the calculation of the bursting index using the values of the latex d.r.c. to estimate the proportion of serum present.

Specific enzyme activity was expressed as the enzyme activity per milligramme protein. Protein concentration was deter-

mined by precipitating the protein with 5% trichloroacetic acid, redissolving in 0.25*N* sodium hydroxide and assaying by the Lowry reaction[16].

Polyacrylamide gel electrophoresis of the diluted serum at pH 8.3 was carried out based on the method of Ornstein and Davies[17,18] and at pH 4.5 according to Reisfeld *et al.*[19] as described previously[20]. The gels were first equilibrated for 30 min in the same buffer used for the enzyme reaction before staining for isoenzymes. Acid phosphatase isoenzymes were located following Work and Work[21]. Dihydroxyphenylalanine (DOPA) was used to locate polyphenol oxidase[22] isoenzymes.

Stability of latices pooled for each treatment was determined by the Aerosol OT test as described by Yip and Gomez[23] using 0.4% or 0.5% Aerosol OT.

Turgor pressure measurements at the tapping cut before and 5 min after tapping were made using glass capillary manometers as described by Buttery and Boatman[24].

Two experiments were carried out. Enzyme assays were carried out only in the first experiment while latex stability and panel turgor pressure were investigated in the second experiment only. For studies that were carried out in both experiments, data from only the second experiment are presented as the results are generally similar in both experiments.

RESULTS

Yield and Dryness

A marked decline in yield was observed when the trees were tapped S/1.4d/1 (*Figure 1*). The average yield per tree after one month's intensive tapping was about 11% of S/2.d/2 control. Trees tapped S/1.d/1 also showed a progressive decline in yield when tapping intensity was increased from S/2.d/2 to S/1.d/1 (*Figure 1*). By the end of the experiment (*i.e.* after six weeks) the trees were yielding only about 53% of the S/2.d/2 controls.

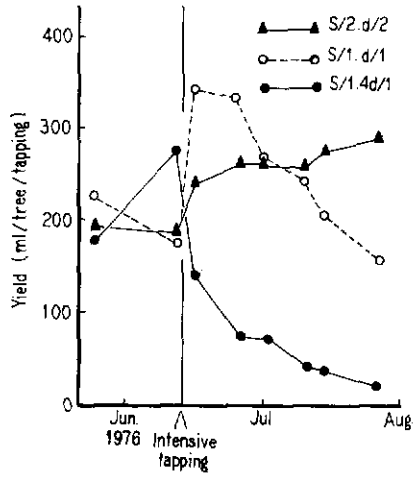

Figure 1. Effect of intensive tapping on yield expressed as latex volume.

The mean dryness of trees tapped S/1.4d/1 expressed as a percentage of the tapping cut length, was in the region of 70% at the termination of the experiment. The first totally dry trees were recorded half a month from commencement of intensive tapping (*Figure 2*) and five trees became totally dry by the end of the experiment. Of the trees tapped S/1.d/1, mean dryness of the tapping cut was 50% and five trees were totally dry when the experiment ended. The dry tapping cuts showed a slightly grey discoloration, but there was no splitting or flaking of the bark.

Plugging Index, Turgor Pressure and Latex Stability

Plugging index did not appear to be affected by intensive tapping. No signifi-

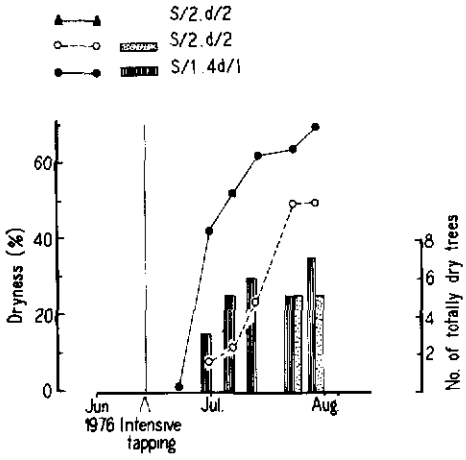

Figure 2. Effect of intensive tapping on dryness expressed as percentage of tapping cut length (graph) and as number (out of ten) of totally dry trees (histogram).

cant differences were observed between the three tapping intensities except for a single reading where the tress tapped S/1.4d/1 showed an increase *(Figure 3)*. However, the initial flow rate descreased with intensive tapping *(Figure 4)*. Turgor pressure measurements of the panel just below the tapping cut before tapping showed a decline with intensive tapping.

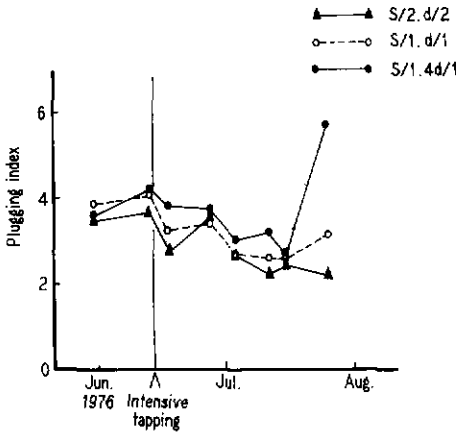

Figure 3. Effect of intensive tapping on plugging index.

This was particularly prominant in trees tapped S/1.4d/1. The decrease in turgor following tapping was similarly reduced in intensively tapped trees *(Figure 5)*.

Apparent latex stability, as determined by the Aerosol OT test after four to seven weeks of intensive tapping, markedly increased in latex from trees tapped S/1.4d/1. Latex from trees tapped S/1.d/1 also increased in stability, but to a lesser extent *(Figure 6)*. The disparity among the three treatments was considerably reduced when Aerosol OT concentration for the test was reduced from 0.5% to 0.4%. Latices from the S/1.4d/1 treatment was unaffected by the reduction in Aerosol OT concentration, but readings

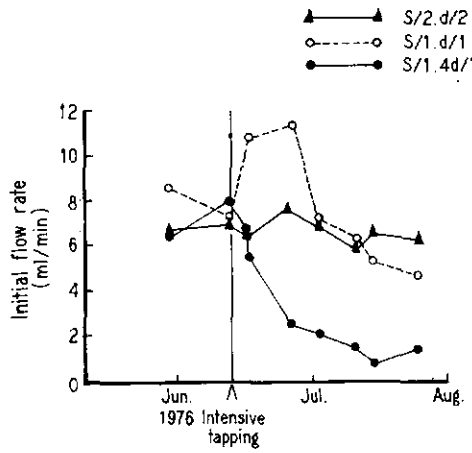

Figure 4. Effect of intensive tapping on initial rate of flow.

for the latices from the S/1.d/1 and S/2.d/2 treatments increased considerably. Nevertheless, the ranking in stability among latices from the three treatments remained unchanged *(Figure 6)*.

High Speed Centrifugation

High speed centrifugation of latices from intensively tapped trees showed various abnormalities *(Figure 7)*. The rubber phase was significantly reduced.

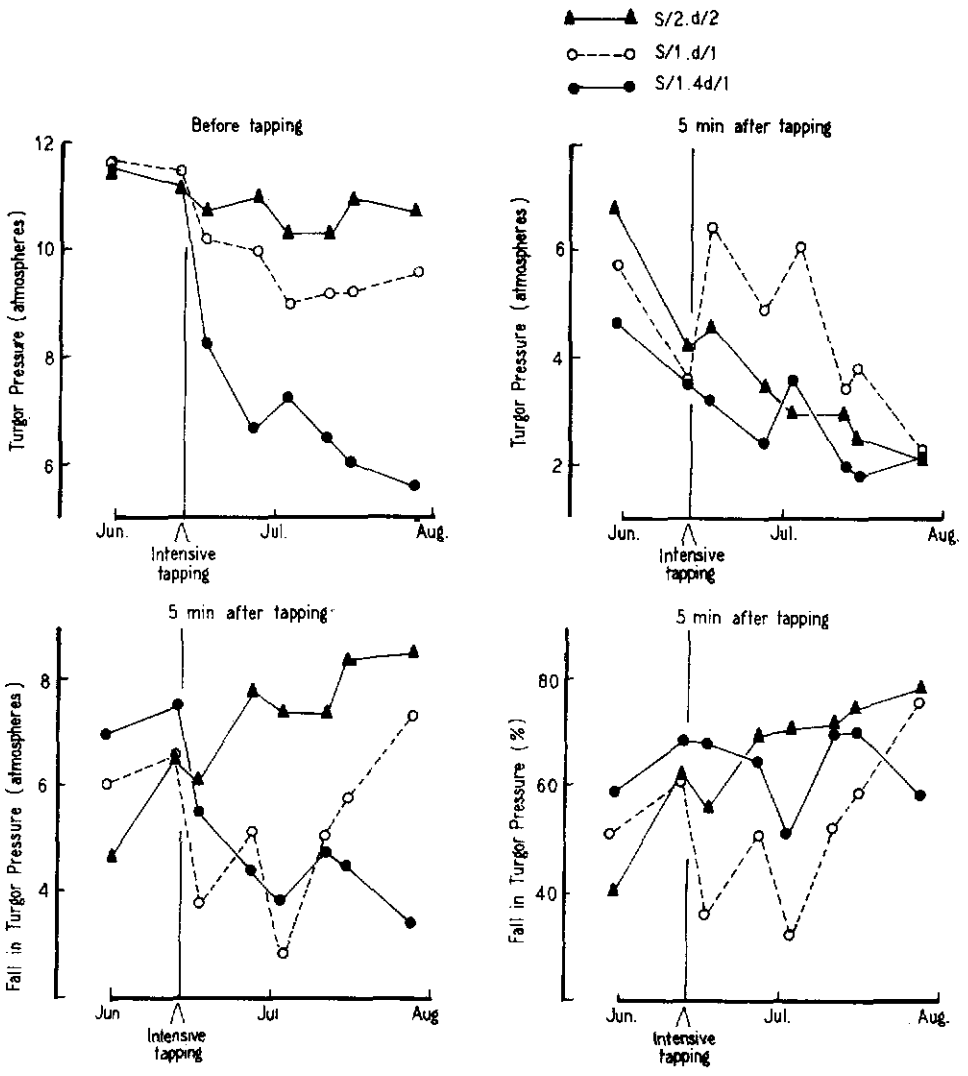

Figure 5. Effects of intensive tapping on turgor pressure at the tapping cut expressed in atmospheres and fall in turgor pressure 5 min after tapping expressed as percentage of turgor pressure before tapping.

The Frey-Wyssling layer was seen to be indistinct, distorted and displaced and sometimes absent. The most notable effect of intensive tapping, however, lay in the reduction of an intact bottom fraction. The reduction followed a progressive trend, and was more prominent in latices from trees tapped S/1.4d/1 than trees tapped S/1.d/1. Dry weight measurements of the bottom fraction confirmed this visual observation *(Figure 8)*.

Notwithstanding the effects of dilution associated with low d.r.c., the reduction in bottom fraction was, in part at least, caused by a reduction in the apparent density of bottom fraction particles. This resulted in a high proportion of them not

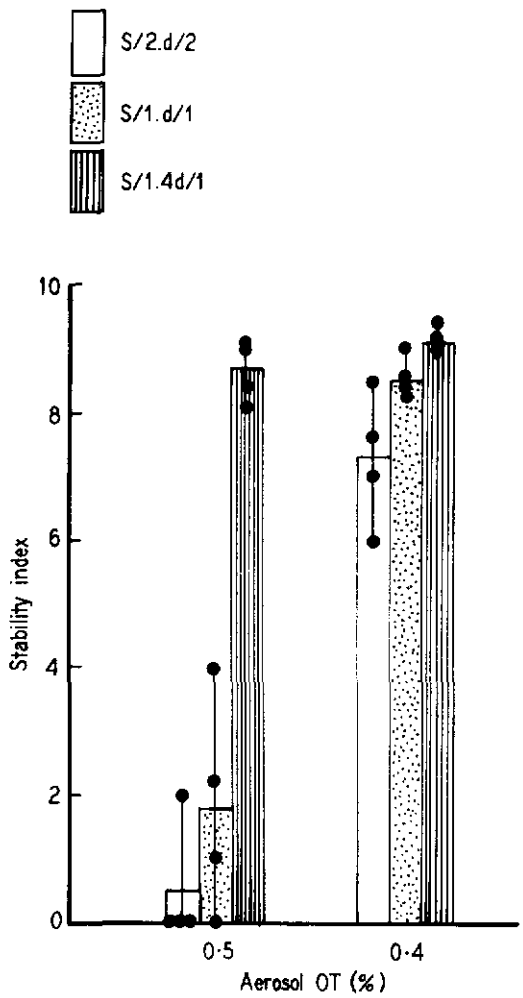

Figure 6. Effect of intensive tapping on stability index as determined using 0.5% and 0.4% Aerosol OT in the test mixtures. Histograms are the means of results from four determinations (which are also presented individually) carried out 30–50 days from the commencement of intensive tapping.

sedimenting after centrifugation. The unsedimented material, appearing as a fine dispersion beneath the rubber phase, could be identified as bottom fraction particles and distinguished from the rubber phase by staining with neutral red, whereby it was stained pink similar to that of the bottom fraction. The bottom fraction in unstained latex, which was of a cream colour in normal latex and was discolored a pale grey, and assumed a gel-like appearance following intensive tapping.

Acid Phosphatase and Polyphenol Oxidase Activity

Acid phosphatase activity in C-serum and in serum from Triton X-100-treated latex was determined to assess the degree of bottom fraction damage using as an index the ratio of the enzyme in C-serum to enzyme in serum from Triton X-100 diluted latex (the 'bursting index')[15]. There appeared to be a tendency towards enhanced lutoid bursting when tapping intensity was increased *(Figure 9)*.

Polyacrylamide gel electrophoresis at acid and at alkaline pH showed no difference in the acid phosphatase zymograms between the tapping treatments (results not presented).

Polyphenol oxidase activity (and specific activity) in latex was progressively depressed by intensive tapping, this depression being more prominent in latex from trees tapped S/1.4d/1 *(Figure 10)*. Electrophoresis at acid pH revealed a distinct reduction in the staining of a major component in the zymogram on intensive tapping *(Figure 11a)*, while electrophoresis at alkaline pH showed only very minor changes in the zymogram *(Figure 11b)*, indicating that a cationic component of the isoenzymes was largely responsible for the quantitative change in enzyme activity.

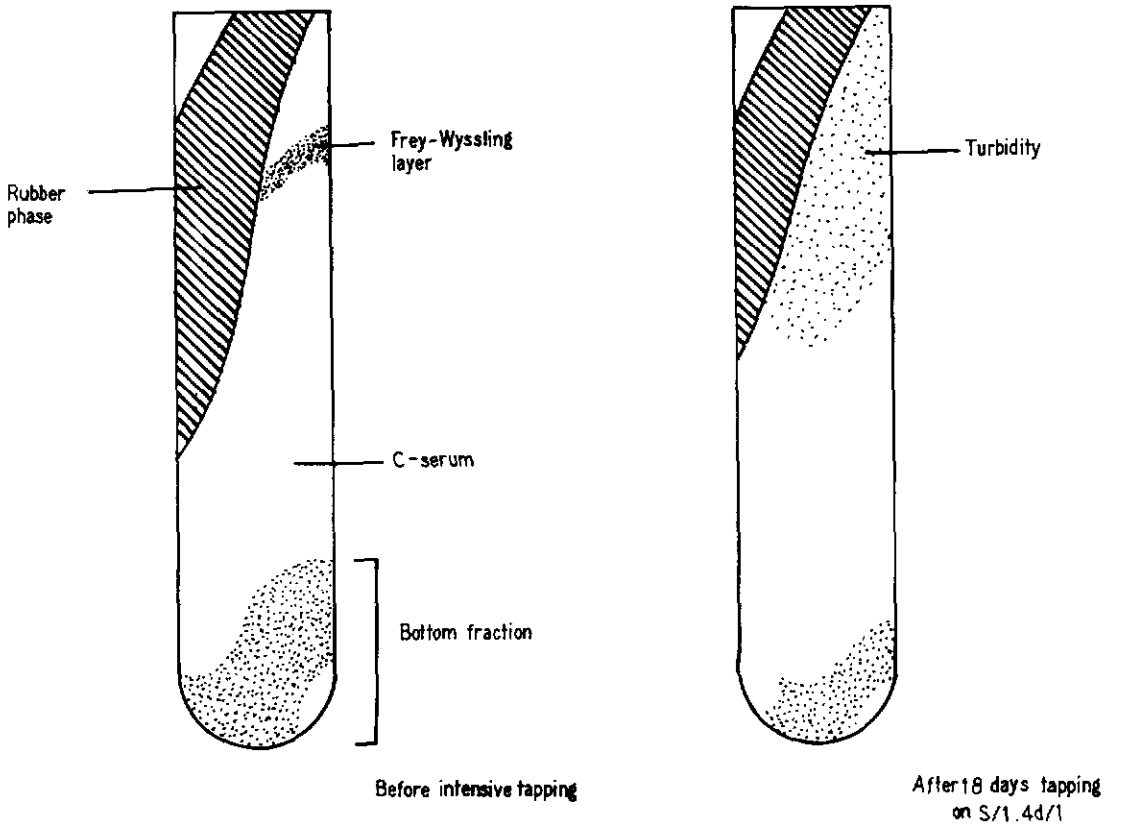

Figure 7. Effect of intensive tapping on latex ultracentrifugation pattern.

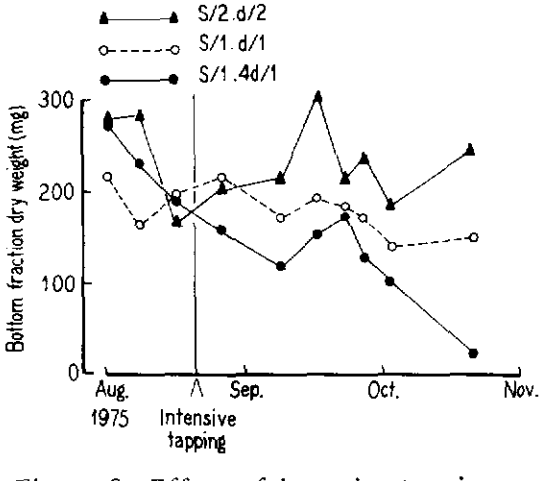

Figure 8. Effect of intensive tapping on bottom fraction dry weight.

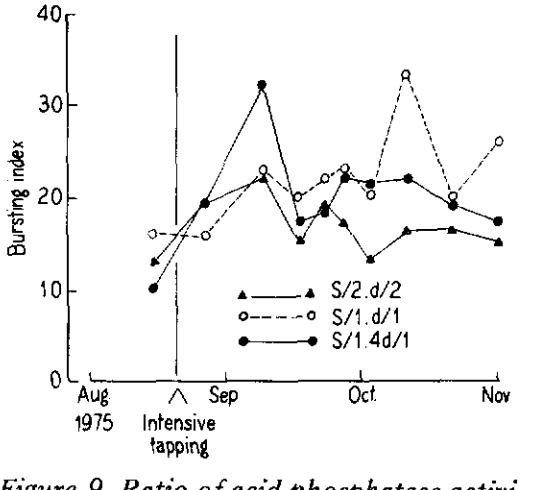

Figure 9. Ratio of acid phosphatase activity in C-serum to activity in serum derived from Triton X-100-diluted latex (the 'bursting index').

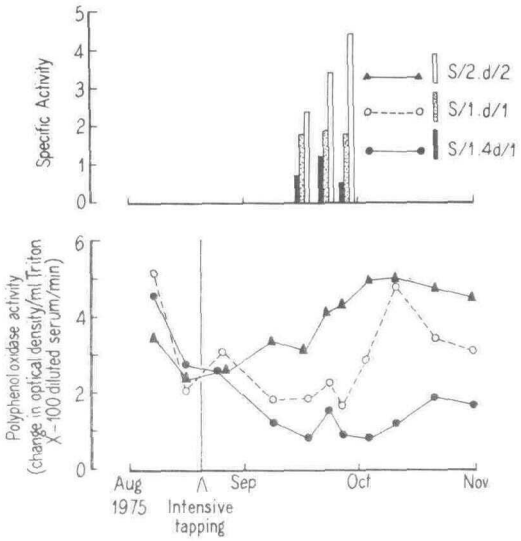

Figure 10. Polyphenol oxidase activity and specific activity in serum derived from Triton X-100-diluted latex.

Latex Total Solids, Dry Rubber Content and Minerals

Total solids and d.r.c. declined progressively in latex from intensively tapped trees when compared to controls *(Figure 12).*

In the assessment of latex mineral content, the d.r.c. of the latex sample has a significant bearing on mineral concentration when this is expressed as a proportion of dry weight or a proportion of fresh weight of whole latex. This is because rubber particles constitute a fairly large fraction of whole latex; hence, the lower the d.r.c., the higher would be the proportion of the non-rubber phase where most of the minerals are found. In this

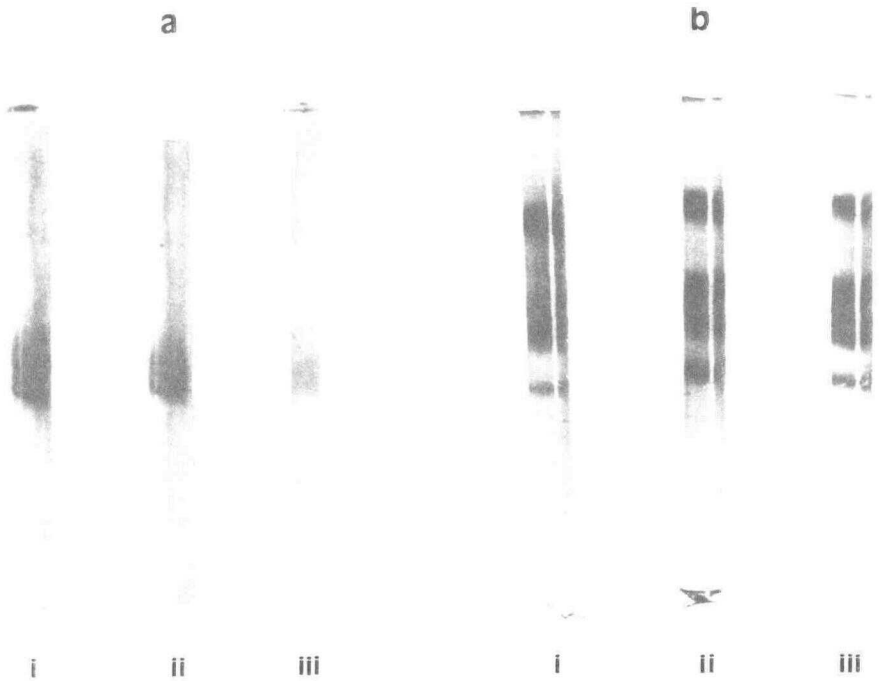

Figure 11. Polyphenol oxidase isoenzymes in serum derived from Triton X-100-diluted latex after five weeks of intensive tapping. (a) Electrophoresis at acid pH showing decrease in major isoenzyme component with increasing tapping intensity: (i) S/2.d/2; (ii) S/1.d/1; (iii) S/1.4d/1. (b) Electrophoresis at alkaline pH revealing only very minor changes.

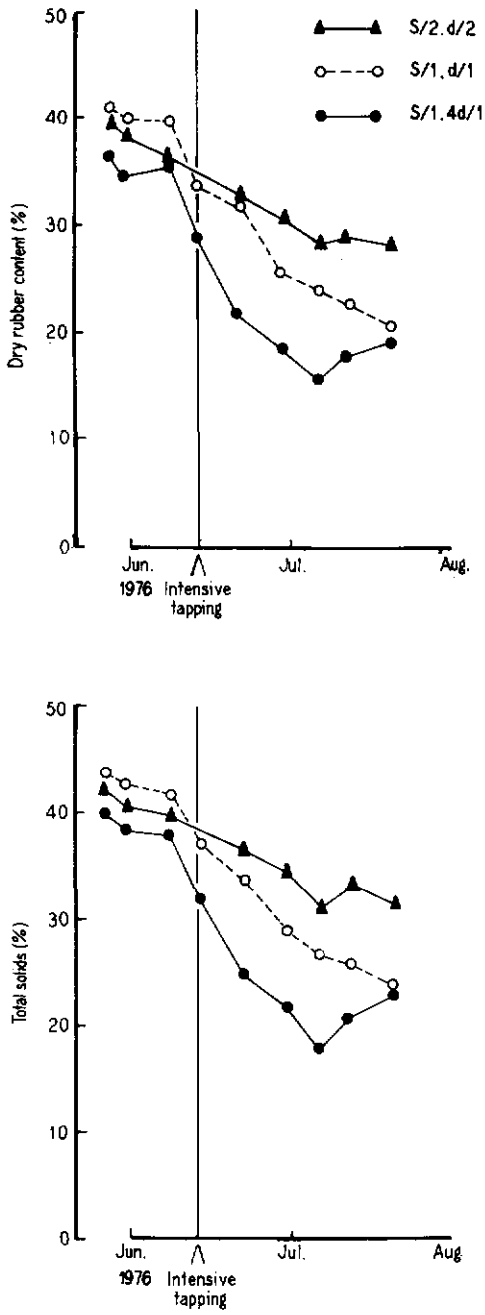

Figure 12. Changes in dry rubber content and total solids of latex with intensive tapping.

study, therefore, latex mineral content is expressed as concentration in the non-rubber phase of latex (fresh weight) to circumvent the d.r.c. effect and so enable a more objective assessment of the actual physiological concentrations of the various mineral elements.

In any case, calculating mineral concentrations on the basis of the fresh weight of whole latex, a common practice, gave essentially similar results (not presented).

Nitrogen, magnesium, phosphorus and potassium (the first of two experiments only) decreased in the latex non-rubber phase relative to control when subjected to intensive tapping. Copper, manganese (second experiment only) and calcium (second experiment only) in the non-rubber phase increased with intensive tapping. *Figure 13* shows results from the second experiment. In the instances above where an increase or decrease induced by intensive tapping was observed in only one of two experiments, the minerals concerned showed no significant change in the other experiment. On no occasion were opposing trends observed.

Intensive tapping on S/1.d/1 was extended over a period of nine months in the first experiment to monitor long-term changes in total solids, d.r.c. and latex minerals (results not presented). Changes that had occurred during the initial phase of the experiment were generally maintained throughout the extended period. Only in copper did the differences diminish with the extended period of intensive tapping.

DISCUSSION

Intensive tapping rapidly brought about dryness of the tapping cut with trees tapped S/1.4d/1 being distinctly more severely affected than those on S/1.d/1

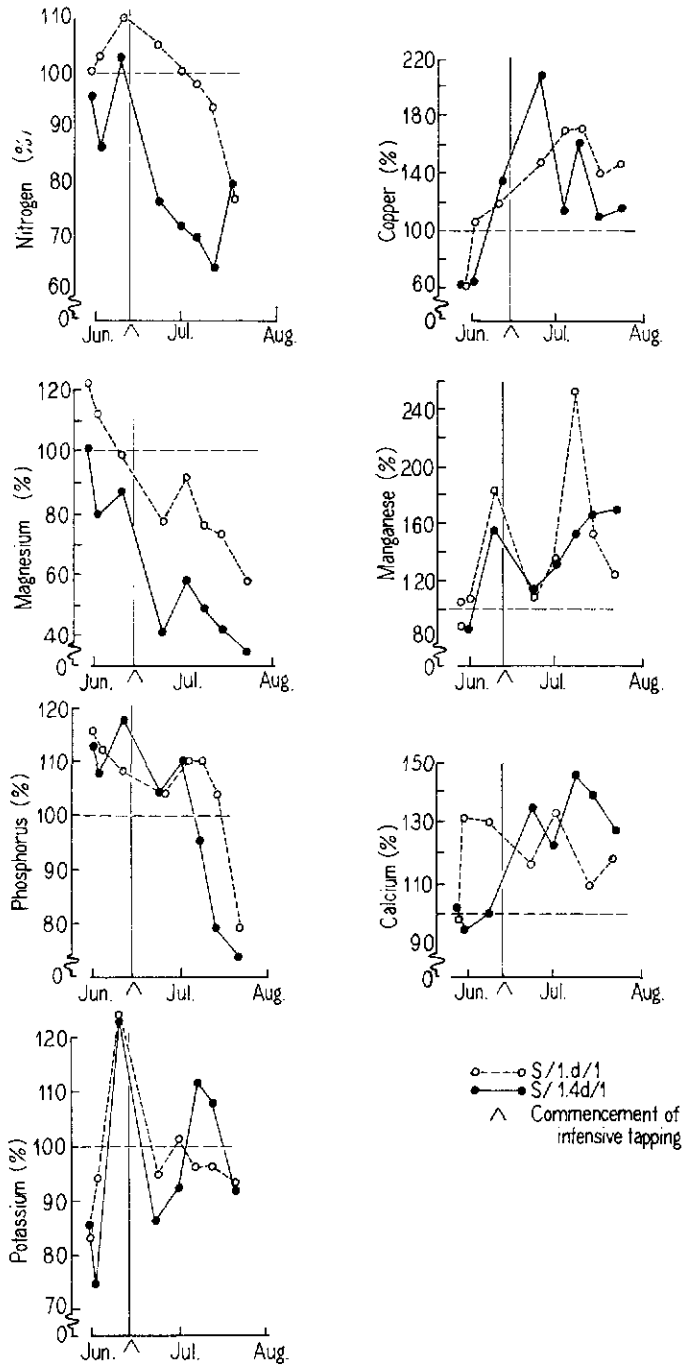

Figure 13. Changes in latex mineral content with intensive tapping. Mineral concentration is expressed as proportion of the latex non-rubber phase (fresh weight) and presented as percentage of S/2.d/2 control.

tapping. These trends were observed when dryness was expressed either as a proportion of dry length of the tapping cut or as number of totally dry trees.

The initial flow rate after tapping was depressed in trees exploited intensively, reflecting the low turgor pressure of the laticifer system of the trees. The fall in turgor following tapping was similarly low. Low turgor was probably a direct result of the excessive withdrawal of latex associated with the intensive tapping systems. Studies by Sethuraj *et al.*[25] similarly revealed low initial flow rates and panel turgor associated with increased tapping intensity. Changes in plugging index were also found to be insignificant, as in the findings in the present study.

Disruption of the Frey-Wyssling layer and the changes in size, colouration and texture of the bottom fraction of centrifuged latex observed with intensive tapping are indicative of an alteration of the properties of latex. The changes in colour in the bottom fraction from cream to pale grey could have arisen from a decrease in the amount of Frey-Wyssling particles sedimented with the bottom fraction. This would be consistent with the observed disruption or disappearance of the Frey-Wyssling layer in the latex of intensively tapped trees. Such changes in the Frey-Wyssling layer might also explain the decrease in activity of latex polyphenol oxidase, an enzyme associated with Frey-Wyssling complexes[26]. The function of these complexes in latex is poorly understood and their influence on the physiology of *Hevea* – in the present context, with respect to dryness – needs to be investigated further.

Dilution of the latex (associated with the low d.r.c.) could be partly responsible for the diminution of the bottom fraction. On the other hand, the reduction in the bottom fraction could be indicative of lutoid damage as ruptured lutoids do not sediment[15]. The increase in the lutoid bursting index is in agreement with this proposition. The reduction of the rubber content of latex from intensively tapped trees resulting in a 'thin' latex cannot be explained simply as an aqueous dilution of the latex. This was evident from the analyses of mineral content which showed both increase and decrease in contents of different minerals in the non-rubber phase. In a separate study, it has been found that the osmotic pressure of C-serum in latices from trees tapped intensively on S/2.4d/1(800%) was depressed only 7% after three weeks even though the d.r.c. and total solids had decreased by an order of 35%[27]. This is again inconsistent with the concept of a simple aqueous dilution of the latex.

Previous work has indicated latex instability (and in particular instability of the bottom fraction) as an important factor in the onset and development of dryness[8]. Latex instability is also evident from reports of pre-coagulation on the tapping cuts of incipient dry trees[5,6]. However, unequivocal evidence in support of a crucial role of latex instability in the onset of dryness arising from intensive tapping is lacking in the present study. Although intensive tapping gave rise to a reduction in the intact bottom fraction of centrifuged latex and increased the bursting index of lutoids, latex vessel plugging was essentially unchanged while the Aerosol OT test actually indicated an *increase* in the stability of latices from intensively tapped trees. In addition, the activity of polyphenol oxidase, an enzyme thought to be associated with latex coagulation[28], was depressed when tapping intensity was increased. Certainly, the increase in latex

composition of certain minerals in the non-rubber phase (e.g. copper) and decrease in other minerals (e.g. nitrogen) would constitute a deviation from the normal balance of mineral elements in the latex. However, there is little evidence to suggest that this imbalance had resulted in a critical instability of latex which subsequently gave rise to dryness. It has been found previously that in RRIM 628 trees tapped fairly intensively on S/2.d/1 (100%), partial dryness was associated with decreases in latex potassium and phosphorus contents (expressed as proportion of dry weight of whole latex)[29]. In the present experiments, both minerals were increased, rather than decreased, with intensive tapping when similarly expressed as proportional dry weight of whole latex (results not presented). Calcium and magnesium ions, when added in sufficient amounts, can destabilise latex[30]. Magnesium concentrations in both experiments, were decreased when expressed on the basis of the latex non-rubber phase while intensively tapped trees in only one of the two experiments carried out showed an increase in calcium concentration.

It must be borne in mind, nevertheless, that the drastic effects of intensive tapping on the physiology of the tree may render experimental results misleading and their interpretation in the context of conventional tapping difficult. For example, the marked decreases in latex magnesium and dry rubber contents when trees are tapped intensively give rise to a latex that is less predisposed to gelling. Hence, the apparent increase in latex stability shown by the Aerosol OT test does not rule out the possibility that de-stabilising factors could, in fact, have actually increased rather than decreased in the latex. Accordingly, the plugging index might well have been higher had there not been the large decrease in latex magnesium and d.r.c. Notwithstanding these possible reasons for the apparent high stability of latex, it does not appear that latex instability is not an important consideration in the development of dryness when trees are tapped over-intensively as in the present experiments. On the other hand, bearing in view that the initial flow rate, panel turgor, latex d.r.c. and latex total solids were severely depressed by intensive tapping, the possibility of exhaustion – either of specific latex constituents or depletion of latex within the laticifer system itself – being the immediate cause of dryness in the present experimental system should not be ignored. Though instability of latex does not seem to be a precipitating factor in the development of dryness in over-intensively tapped trees, its role in dryness in conventionally tapped trees cannot be dismissed as dryness, as these two instances are not fully comparable in many respects.

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REFERENCES

1. PUSHPDAS, M.V., NAIR, K.K., KRISHNAKUMARI, M. AND AMMA, M.K. (1975) Brown Bast and Nutrition: A Case Study. *Rubb. Bd Bull. (India)*, 12(3),83.

2. HORNE, A.S. (1925) Further Observations on the Phloem Necrosis (Brown Bast Diseases) in *Hevea brasiliensis*. *Trop. Agric. (Ceylon)*, 64(6), 328.

3. CHUA, S.E. (1967) Physiological Changes in *Hevea* Trees under Intensive Tapping. *J. Rubb. Res. Inst. Malaya*, 20(2), 100.

4. RHODES, E. (1930) Brown Bast. Some Considerations as to Its Nature. *J. Rubb. Res. Inst. Malaya*, 2(1), 1.

5. RANDS, R.D. (1921) Brown Bast Disease of Plantation Rubber. Its Cause and Prevention. *Archf. Rubbercult. Ned-India*, 5, 223.

6. SHARPIES, A. AND LAMBOURNE, J. (1924) Field Experiments Relating to Brown Bast Disease of *Hevea brasiliensis*. *Malay. Agric. J.*, 12, 290.

7. TAYLOR, R.A. (1926) A Note on Brown Bast. *Trop. Agric. (Ceylon)*, 72, 323.

8. PARANJOTHY, K., GOMEZ, J.B. AND YEANG, H.Y. (1976) Physiological Aspects of Brown Bast Development. *Proc. Int. Rubb. Conf. Kuala Lumpur 1975*, Volume II, 181.

9. MILFORD, G.F.J., PAARDEKOOPER, E.C. AND HO, C.Y. (1968) Latex Vessel Plugging, Its Importance to Yield and Clonal Behaviour. *J. Rubb. Res. Inst. Malaya*, 21, 274.

10. RUBBER RESEARCH INSTITUTE OF MALAYSIA (1973) *Manual of Laboratory Methods for Chemical Analysis of* Hevea brasiliensis *Rubber*. Kuala Lumpur: Rubber Research Institute of Malaysia.

11. COOK, A.S. AND SEKHAR, B.C. (1953) Fractions from *Hevea brasiliensis* Latex Centrifuged at 59 000 g. *J. Rubb. Res. Inst. Malaysia*, 14, 163.

12. MOIR, G.F.J. (1959) Ultracentrifugation and Staining of *Hevea* Latex. *Nature*, 184(4699), 1629.

13. BEALING, F.J. AND CHUA, S.E. (1972) Output, Composition and Metabolic Activity of *Hevea* Latex in Relation to Tapping Intensity and the Onset of Brown Bast. *J. Rubb. Res. Inst. Malaya*, 23(3), 204.

14. SIGMA CHEMICAL CO. (1974) The Colorimetric Determination of Phosphatase. *Sigma Tech. Bull. No. 104*. St. Louis, U.S.A.: Sigma Chemical Co.

15. RIBAILLIER, D. (1968) Action in vitro de certains ion mineraux et composes organiques sur la stabilite des lutoids du latex d'*Hevea*. *Rev. gen. Caoutch. Plastq.*, 45, 1935.

16. LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. AND RANDALL, R.J. (1951) Protein Measurement with the Folin Phenol Reagent. *J. biol. Chem*, 193, 265.

17. ORNSTEIN, L. (1964) Disc Electrophoresis I. Background and Theory. *Ann. N.Y. Acad. Sci.*, 121(2), 321.

18. DAVIES, B.J. (1964) Disc Electrophoresis II. Method and Application to Human Serum Protein. *Ann. N.Y. Acad. Sci.*, 121(2), 404.

19. REISFIELD, B.A., LEWIS, U.J. AND WILLIAMS, D.E. (1962) Disk Electrophoresis of Basic Proteins and Peptides in Polyacrylamide Gels. *Nature*, 195, 281.

20. YEANG, H.Y., GHANDIMATHI, H. AND PARANJOTHY, K. (1977) Protein and Enzyme Variation in Some *Hevea* Cultivars. *J. Rubb. Res. Inst. Malaysia*, 25(1), 9.

21. WORK, T.S. AND WORK, E. (Ed) (1969) *Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. 1. North Holland Publishing Co.

22. NYE, T.G. KERN, C.R. AND AIDRICH, R.F. (1968) Polyacrylamide Gel Electrophoresis of *Solanum tuberesum*. I. Total Soluble Proteins and Polyphenol Oxidase Activity. *Phytochem.*, 7, 741.

23. YIP, E. AND GOMEZ, J.B. (1976) Stability of Fresh *Hevea* Latex in Relation to Latex Vessel Plugging. *Proc. Int. Rubb.*

Conf. Kuala Lumpur 1975, Volume II, 203.

24. BUTTERY, B.R. AND BOATMAN, S.G. (1966) Manometric Measurement of Turgor Pressures in Laticeferous Phloem Tissues. *J. exp. Bot.*, 12, 129.

25. SETHURAJ, M.R., USHA NAIR, N., GEORGE, M.J. AND MANI, K.T. (1977) Physiology of Latex Flow in *Hevea brasiliensis* as Influenced by Intensive Tapping. *J. Rubb. Res. Inst. Sri. Lanka*, 54, 221.

26. COUPE, N., PUJARNISCLE, S. AND D'AUZAC, J. (1971) Compartimentation de diverse oxydo reductase (peroxydase, O-diphenoloxydase et malate dehydrogenase) dans le latex d'*Hevea brasiliensis* (Kunth), Mull. Arg. *Physiol. Veg.*, 10(3), 459.

27. PARANJOTHY, K. (1981) Unpublished results. Rubber Research Institute of Malaysia.

28. BRZOZOWSKA-HANOWER, J., HANOWER, P. AND LIORET, C. (1978) Etude du macanisme de la coagulation du latex d'*Hevea brasiliensis* (Kunth) Mull. Arg. II. Systems enzymetiques impliques dans de processus 1. Phenol oxydases. *Physiol. Veg.*, 16(2), 231.

29. PARANJOTHY, K., YEANG, H.Y. AND CHOW, C.S. (1979) Faktor-faktor Berhubung dengan Perkembangan Kulit Separa Kering. *J. Sains I.P.G.M.*, 3(1), 1.

30. BELMAS, R. (1952) Contribution to the Physical Chemistry of *Hevea* Latex. I. Metal Cations in Latex. *Rubb. Chem. Technol.*, 25, 124.