THE EFFECT OF INTENSIVE TAPPING ON INDUCTION OF TAPPING PANEL DRYNESS AND ASSOCIATED BIOCHEMICAL CHANGES IN TWO CLONES OF HEVEA

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ABSTRACT

Randomness in the incidence of Tapping Panel Dryness (TPD) in any field makes it difficult to quantify clonal susceptibility to this syndrome. In a preliminary study, using GT1 as a reference clone, the susceptibility of RRII 105 to the syndrome was quantified by an index based on the period required to induce total dryness of the tapping cut under a high intensity tapping system. The incidence of TPD was more rapid in RRII 105; the rate of incidence of TPD for clone RRII 105 was 1.92 times greater than that found for clone GT1. The possibility of using this an an index to classify clones according to susceptibility to TPD is discussed.

Biochemical parameters such as bursting index, total and free acid phosphatase activity, proteins, sugars and lipids were monitored in the experimental trees. The pattern of changes markedly varied between the two clones studied. A lower bursting index and higher levels of sugars and proteins were observed in the initial months in the latex of the intensively tapped trees of the susceptible clone. The possibility of using these parameters also, for early prediction of susceptibility to TPD, is discussed.

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Introduction

Tapping Panel Dryness (TPD), also known as 'brown bast', is a physiological syndrome affecting productivity of certain trees of *Hevea brasiliensis*. The reasons for the random incidence of the syndrome, within a stand of trees, are not well understood despite many years of research (1). Variations in the intensity of incidence of TPD have been attributed to genetic variations (2), nutritional imbalance (3) and intensity of exploitation (4,5,6). Usually, high yielding clones are more susceptible to TPD compared to medium and low yielding clones (2). High intensity of tapping leads to high incidence of TPD (7). Chua (4) gave evidence to show that both tapping intensity and tapping frequency are important in causing TPD. Paranjothy (6) reported very fast induction of TPD in clone RRIM 628 by subjecting trees to full spiral four times daily tapping. Overstimulation also leads to increased incidence of TPD (8).

Quantitative studies on the incidence of TPD are often difficult on account of the randomness of the incidence. Many times, in well laid out experiments, the treatment effects become statistically insignificant because of this. Further, the time taken for useful comparisons of genotypes for their susceptibility is also quite long under normal tapping systems. In view of these circumstances, an experiment was designed to see if intensive tapping and regular monitoring of individual trees for TPD could be used as a method to quantify the relative susceptibility of *Hevea* clones to the syndrome.

Study of biochemical changes in the laticiferous system subjected to conditions favourable for the induction of TPD would be useful to understand the mechanisms involved in the development of the syndrome. Increased bursting index of lutoids has been observed in the latices of trees with dry cut syndrome (9). High bursting index is often associated with lutoid membrane lipid composition (10). It has been reported that regular tapping results in a decrease in sucrose level in the latex and of reserve polysaccharides in the bark (11, 12). The decrease becomes more severe with increase in the intensity of exploitation (12,13). When a tree hardly produces any more latex, sucrose accumulates in the tree owing to non-utilization (8). Paranjothy (6) reported gradual decline in total solids and percentage of dry rubber content (drc) in the latices of intensively tapped trees. Nitrogen and other inorganic elements were found to increase. In the study reported here, total and free acid phosphatase activities, bursting index, sugars, soluble proteins and various lipids were estimated in the latex samples obtained from intensively and normally tapped trees.

Materials and methods

The experiment was conducted in the Central Experimental Station of the Rubber Research Institute of India, Chethackal (19° 22′ N, 76° 50′E). Two stands of unopened trees of clones RRII 105 and GT1 of the same age in one field, which had attained a tappable girth of 50cm at 125cm height, were selected for the study. In each clone, 24 randomly selected trees were subjected to an intensive tapping system of ½S d/0.5 6d/7. The trees were tapped between 6.00 and 7.00 hours in the morning, and 16.00 and 17.00 hours in the evening. The latices obtained from morning and evening tappings were cup-coagulated separately. The daily yield data of individual trees from morning and evening tappings were collected. The remaining trees were subjected to normal tapping of ½S d/2 6d/7. All the trees were rain-guarded during the rainy season. Plugging index and drc were determined at fortnightly intervals. The symptoms of TPD were monitored at monthly intervals. The time taken to reach the stage of practically no latex yield was taken as the time taken to reach complete dryness of the tapping cut. The trees were also checked visually for the occurrence of TPD. The experiment was started in February 1989 and data were collected until March 1990.

The time taken by each tree to reach the stage of complete TPD was recorded. The data was analysed statistically to compare clonal susceptibility to TPD. The data was also examined to deduce any possible relationships between TPD and yield or drc, (before the onset of TPD).

For biochemical studies, six trees of both clones were selected from intensively and normally tapped trees. Bursting index was determined by the method of Ribaillier (14). Reducing sugars were estimated according to Nelson (15) and Somogyi (16). Total sugars were estimated by the same method after acid hydrolysis. Total soluble protein content in C-serum was estimated according to the method of Lowry (17). Total lipids were extracted according to Bligh and Dyer (18) and estimated gravimetrically. The amount of phospholipids and triglycerides were estimated according to Hasma and Subramaniam (19). For the estimation of sterol, the diethyl ether eluent was made up to a known volume and aliquots were evaporated and dissolved in acetic acid. The colour was developed by adding acetic anhydride and sulphuric acid (4:1) reagent and measured at 625nm using β-sitosterol as standard. The parameters were estimated at monthly intervals during the initial five months of the experiment. Lipids were estimated only during the months of March and April.

Results and discussion

The cumulative incidence of TPD at monthly intervals for clones RRII 105 and GT1 are presented in Figure 1. The incidence of TPD was faster in RRII 105, the rate flattening off towards the end of the period. By contrast, in GT1 incidence was slower and the rate was linear throughout the period of study. The linear equations worked out for clones RRII 105 and GT1 respectively are:

$$Y_1 = -80.4 + 0.6062\ X_1$$
$$Y_2^1 = -53.4 + 0.3150\ X_2^1$$

Where X_1 and X_2 are the number of days, and Y_1 and Y_2 are the cumulative percentage of trees affected with TPD. The slopes of the two curves were very different from each other, the gradient being higher for clone RRII 105.

The ratio of incidence of TPD in RRII 105 to that in GT1 is 1.92. Such a ratio using a standard reference clone which is relatively resistant to TPD, can be used as an index to describe susceptibility of *Hevea* clones to this syndrome.

The results show that quantitative comparisons for TPD susceptibility between clones can be made within one year by adopting intensive tapping systems. The availability of monthly data on TPD incidence from stands under normal tapping systems might also be useful for the quantification of TPD using the method described above.

It is widely believed that high yielding trees are more susceptible to TPD (1). The coefficients of correlation between the time to reach TPD by individual trees and yield and drc are presented in Table 1. Only the affected trees were considered for this analysis. The mean daily yield did not show any significant relationship with TPD either during the entire productive period or during the initial 75 per cent of this period. However it may be noted that the 'r' values, though not significant, were higher in the case of the susceptible clone RRII 105. The mean drc was found to be significantly and negatively related to time to TPD in RRII 105, when the initial 75 per cent of the productive period was considered.

Data on changes in the various biochemical parameters studied are presented in Tables 2 and 3. In clone RRII 105, the bursting index of latex obtained from intensively tapped trees was low in the initial two months when compared to normally tapped trees, although this difference was subsequently reversed. In clone GT1, a lowering of the bursting index was seen in the month of April but subsequently the intensively tapped trees had higher values. Intensive tapping did not cause much difference in the total acid phosphatase activity in either clone although the activity was higher in clone RRII 105. A similar trend was found in the case of free acid phosphatase up to the month of May, but by June the free acid phosphatase activity decreased in the intensively tapped trees of both clones. Examination of the data shows that considerable reduction in bound acid phosphatase activity did not occur up to June for clone RRII 105 but on the other hand it was low in the intensively tapped trees of GT1 when compared to the values for normally tapped trees. A marked reduction of lutoids might have occured after this period.

The soluble protein content of the C-serum was found to be slightly higher over the initial two months of observation in clone RRII 105. In May, this increase was around 25 per cent but in June, the difference was absent. The above mentioned differences were not clear cut in the case of clone GT1. The higher nitrogen value reported by Paranjothy (6), in the latex of intensively tapped trees might be true only under conditions of faster induction of TPD.

The data show a marked increase in the level of total sugars in the latex of intensively tapped trees of clone RRII 105 but the contrary for GT1 where the sugar level was slightly lower. However, by July the difference in the level of total sugars was abolished in RRII 105. Variation in sugar content is mainly accounted for by non-reducing sugars. The observation of higher levels of sugars reported here in the intensively tapped trees of clone RRII 105 is not in accord with earlier reports (12, 13). However, the results obtained in clone GT1 are in agreement with these reports. The induction of high level of sugars in the latex might be an indication of faster induction of TPD.

The data on total lipids and its three fractions (Table 3), shows that all fractions of lipids in general increased due to intensive tapping in both the rubber cream as well as in the bottom fractions of latex for both of the clones. However, the changes in triglycerides and phospholipids in the rubber cream of intensively tapped trees of GT1 were small. This clone, which is less susceptible to TPD induction, was found to contain higher levels of different lipids in its bottom fraction.

The present study, though preliminary, shows that with the help of properly laid out intensive tapping experiments, quantitative data on variations in clonal susceptibilities to TPD can be collected within a short time using a relatively small number of trees. A general consensus on the choice of a reference clone and on other conditions of the experiment would be useful. The higher levels of sugars and proteins and the initial decrease in bursting index found in the latex of the intensively tapped trees of the susceptible clone indicate that it might be possible to use these biochemical parameters as well for early prediction of susceptibility to TPD.

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References

1. Sethuraj, M.R., A conceptual analysis of the brown bast syndrome, *IRRDB Symposium*, Paris, 1988, 247-251.

2. Sivakumaran, S., Haridas, G. and Abraham, P.D., Problem of tree dryness with high yielding precocious clones and methods to exploit such clones, ref 1, 253-267.

3. Pushpadas, M.V., Kochappan Nair K., Krishnakumari, M and Karthikakutty Amma, M., Brown bast and nutrition: A case study., *Rubb. Board Bull.*, 1975, 12 3, 83-88.

4. Chua, S.E., Physiological aspects of exploitation., *Planters Bull. Rub. Res. Inst. Malaysia*, 1965, 80, 139-143.

5. Ng, E.K., Abraham, P.D., P'ng, J.C. and Lee, C.K., Exploitation of modern *Hevea* clones., *J. Rubb. Res. Inst. Malaysia*, 1969, 21 3, 292.

6. Paranjothy, K., Gomez, J.B. and Yeang, H.Y., Physiological aspects of brown bast development., *Proc. Int. Rubb. Conf.*, Kuala Lumpur, 1975, 181-202.

7. Chua, S.E., Physiological changes in *Hevea* trees under intensive tapping., *J. Rubb. Res. Inst. Malaysia*, 1967, **20**, 100-105.

8. Cretin, H., Contribution a 1'etude des facteurs limitant la production du latex d'*Hevea brasiliensis*, in *Rapport d'eleve de deuxieme annee, Orstom*, Cote d'Ivoire, 1978.

9. Chrestin, M., Jacob, J.L. and D'Auzac, J., Biochemical basis for cessation of latex flow and occurence of physiological Bark Dryness., *Proc. Int. Rubb. Conf.*, Kuala Lumpur, 1985, 20-42.

10. Sherief, P.M. and Sethuraj, M.R., The role of lipids and Proteins in the mechanism of Latex vessel plugging in *Hevea brasiliensis*., *Physiol. Plant.*, 1978, 42, 351-353.

11. Tupy, J., The level and distribution pattern of latex sucrose along the trunk of *Hevea brasiliensis* as affected by the sink region induced by latex tapping., *Physiol. Veg.*, 1973, 11 1, 1-11.

12. Low, F.C. and Gomez, J.B., Carbohydrate status of exploited *Hevea*. Pt 111. Non-structural carbohydrates in the bark., *J. Rubb. Res. Inst. Malaysia*, 1984, 32, 82-102.

13. Bealing, F.J. and Chua, S.E., Output; composition and metabolic activity of *Hevea* latex in relation to tapping intensity and the onset of brown bast., *J. Rubb. Res. Inst. Malaysia*, 1972, **23**, 204.

14. Ribaillier, D., Action *in vitro* de certain ions mineraux et composes organiques sur la stabilite des lutoides du latex d'*Hevea*., *Rev. Gen. Caout. Plast.*, 1980, 45 12, 1395-1398.

15. Nelson, N., A photometric adaptation of the Somogyi method for the determination of glucose., *J. Biol. Chem.*, 1944, **153**, 375.

16. Somogyi, M., A reagent for the copper iodometric determination of very small amounts of sugar., *J. Biol. Chem.*, 1937, **117**, 771.

17. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the Folin-Ciocalteu reagent., *J. Biol. Chem*, 1951, **193**, 265.

18. Bligh, E.G. and Dyer, W.J., A rapid method of total lipid extraction and purification., *Cana. J. Biochem. and Physiol.*, 1959, **37**, 911-917.

19. Hasma, H. and Subramaniam, A., Composition of lipids in latex of *Hevea brasiliensis* clone RRIM 501., *J. Nat. Rubb. Res.*, 1986, **1**, 30-40.

Table 1: *Correlation coefficients of time taken to reach tapping panel dryness with treewise yield and dry rubber content (drc) in clones RRII 105 and GT 1 subjected to intensive tapping (½S d/0.5 6d/7; panel BO-1)*

Clone	Yield[a]	Yield[b]	DRC[c]	DRC[d]
RRII 105	-0.3760[ns]	-0.4213[ns]	0.2353[ns]	-0.4493*
GT 1	-0.1687[ns]	-0.0985[ns]	-0.2631[ns]	-0.4165[ns]

a - Yield/day/tree during the entire productive period.
b - Yield/day/tree during the initial 75 per cent of productive period.
c - Mean drc during Februarry to May 1989.
d - Mean drc during the initial 75 per cent of the productive period.

Table 2: *Progressive changes in biochemical parameters in the latices of intensively (½S d/0.5 6d/7) and normally (½S d/2 6d/7) tapped trees of RRII 105 and GT 1 (panel BO-1)*

Parameters	Clones	March		April		May		June		July	
		N	I	N	I	N	I	N	I	N	I
Bursting index	RRII 105	23.16	15.30	27.44	16.45	26.23	29.29	11.44	18.88	-*	
	GT 1	27.44	25.99	26.19	5.93	35.39	42.12	12.36	44.30		
Total acid phosphatase activity (µ moles of p-nitro phenol liberated/min/ml latex)	RRII 105	7.49	7.85	6.82	8.22	3.68	3.78	6.08	7.44	-	
	GT 1	3.47	3.01	3.09	2.71	1.39	1.25	2.43	2.23		
Free acid phosphatase activity (µ moles of p-nitro phenol liberated/min/ml latex)	RRII 105	1.68	1.24	1.20	1.30	0.96	1.03	0.70	0.29	-	
	GT 1	0.95	0.76	0.57	0.73	0.48	0.49	1.48	0.99		
Total soluble proteins	RRII 105	10.66	12.50	10.32	12.02	12.26	16.69	9.15	8.75	-	
(mg/ml C-Serum)	GT 1	11.53	10.68	10.99	11.35	9.50	10.33	10.97	10.78		
Total sugars	RRII 105	13.53	24.30	12.84	28.05	8.88	19.30	15.60	19.73	18.24	19.00
(mg/ml C-Serum)	GT 1	12.39	8.47	9.60	9.17	11.91	10.59	14.53	12.54	14.20	10.03
Reducing sugars	RRII 105	3.82	6.24	6.07	8.54	3.65	4.27	4.99	5.69	3.17	3.14
(mg/ml C-Serum)	GT 1	3.27	3.85	5.50	5.32	5.82	4.17	3.31	4.15	3.00	3.24
Non-reducing sugars	RRII 105	9.69	18.06	6.77	20.50	5.23	14.98	7.69	14.04	15.07	15.86
(mg/ml C-Serum)	GT 1	9.13	4.62	4.10	3.85	6.09	5.75	11.22	8.39	11.20	6.79

N - normal tapping I - intensive tapping * - not recorded

Table 3: *Lipid composition in the rubber cream and bottom fraction of latices of intensively (½S d/0.5 6d/7) and normally tapped (½S d/2 6d/7) trees of clones RRII 105 and GT 1 (Panel BO-1) in mg/g dry weight*

		RRII 105		GT 1	
		Normal	Intensive	Normal	Intensive
Total lipids	r	43.28	50.29	43.17	46.46
	b	144.18	158.43	197.70	216.00
Sterols	r	3.97	6.54	5.01	7.18
	b	28.35	33.16	37.84	42.77
Triglycerides	r	5.55	7.16	6.04	6.58
	b	39.12	48.92	60.04	67.95
Phospho lipids	r	6.96	8.74	9.52	8.85
	b	16.01	29.92	21.68	40.17

r - rubber cream

b - bottom fraction

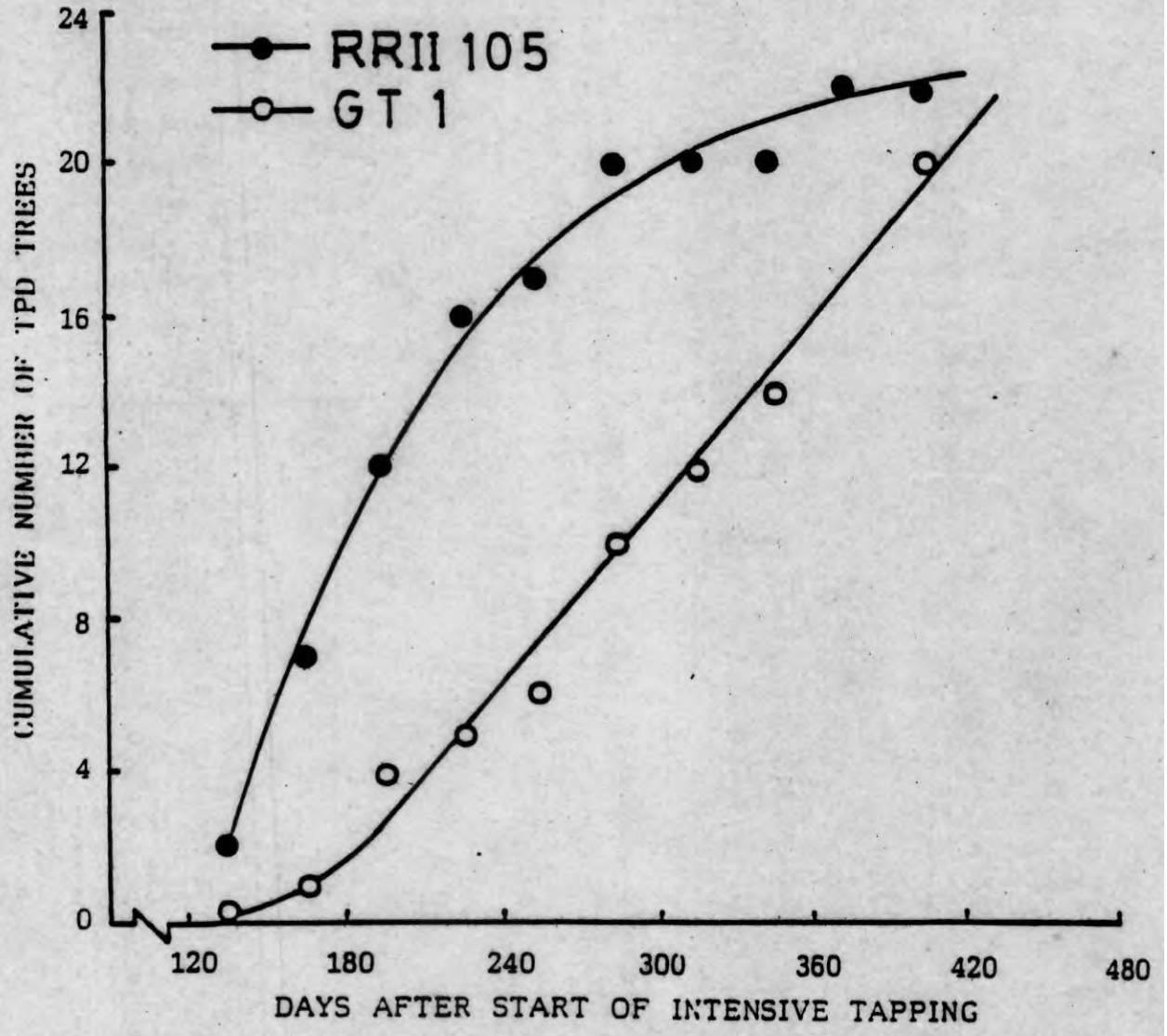

Figure 1: *Cumulative incidence of tapping panel dryness in intensively tapped (½S d/0.5 6d/7) trees of clones RRII 105 and GT 1 in Panel BO-1*