

## ***Destabilisation of Lutoids in Hevea brasiliensis Latex During Early and Late Flow***

H.Y. YEANG\*<sup>#</sup> AND OTHMAN HASHIM\*

*Lutoid damage in latex exuding from the rubber tree has been associated with the onset of latex vessel plugging and the subsequent cessation of flow. The commonly held supposition that lutoid damage (and, by inference, latex vessel plugging) is most intense at the beginning of flow when the tree is tapped is re-examined. Damage to lutoids in latex collected at various intervals after tapping and just prior to flow cessation was assessed by the appearance of the bottom fraction of centrifuged latex and the bursting index of lutoids. Although increased bottom fraction damage was regularly observed in the initial latex fraction, the very extensive damage that has previously been reported to occur immediately after tapping was found to be uncommon in regularly tapped trees. Lutoid damage, as determined by the bursting index, fell briefly after the initial flow (1st min) and was lowest between the 4th and 15th minute. But thereafter, the bursting index increased steadily until cessation of flow, by which time, lutoid damage in the latex was most severe. Water potential was observed to increase as the latex became more dilute during the course of flow, but it was not clear to what extent lutoid damage could be attributed to the resulting osmotic shock. Based on the results of this study, a reassessment should be made of the common belief that the early flow latex is highly unstable and that latex that is exuded during the later flow is more stable.*

From the mid-1960s, damage to lutoids in the latex leading to latex destabilisation has been implicated in the progressive latex vessel plugging that takes place during the course of latex flow when the rubber tree is tapped. It has since been widely accepted that lutoid damage is most severe during early latex flow immediately after the tree is tapped and lutoid damage diminishes during the late flow<sup>1-8</sup>. Osmotic shock<sup>1</sup>, physical shear<sup>9</sup> and various other physiological factors<sup>2,6-8,10</sup> that act during the latex outflow from the tree are thought to be responsible for the rupture of lutoid and, possibly, other latex organelles as well. By this argument, latex-destabilising factors are released from the damaged lutoids and this leads

to the rapid formation of latex vessel plugs during the initial and early flow.

The above proposition first arose when Pakianathan *et al.*<sup>1</sup> reported that centrifugation of the early flow of Tjir 1 buddings revealed gross damage of the bottom fraction—the major constituent of which are the lutoids—whereas bottom fractions of the later flow fractions were relatively intact. The hypothesis received support from the work of Pujarnisclé *et al.*<sup>3</sup> and Ribaillier<sup>4</sup> who reported that lutoid damage, as determined by the ‘bursting index’ of lutoids, was highest in the initial and early flow and decreased in successive flow fractions. The hypothesis of severe lutoid damage in the early

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

<sup>#</sup> Corresponding author

flow has influenced thinking in the concepts of latex vessel plug formation during the course of latex flow. This viewpoint has also led many *Hevea* researchers to take precautions to avoid the early latex flow in the study of latex physiology in the belief that the more stable late flow latex gave a better representation of the latex *in situ*.

The present study examines luitoid damage at various intervals from the time of tapping until the cessation of flow. The widely held belief that severe luitoid damage is characteristic of the early flow is re-examined.

#### MATERIALS AND METHODS

Latex was collected from trees tapped on a half spiral alternate day system ( $\frac{1}{2}S$   $d/2$ ) and that were not subjected to ethephon stimulation. The clones used in the studies are specified in the presentation of the results below. All latex collections were made between July and September, *i.e.* outside of the wintering season or the period of refoliation that followed wintering. In the collection of sequential flow fractions after tapping, the time of tapping was taken as the time the first drop of latex was collected and all time measurements were made from this reference point. In all cases, the latex flow in the first 15 s which might contain bark debris was disregarded. The latices were kept chilled in the field before they were taken back to the laboratory for centrifugation. Latex centrifugation was carried out for 1 h on a Sorvall RC 2B high speed centrifuge at 19 000 r.p.m. (43 000 g) at 4°C - 7°C to give three main fractions: the rubber cream, the C-serum and the bottom fraction that consisted mainly of luitoids.

Ribaillier<sup>11</sup> introduced the concept of the 'bursting index' (*l'indice d'éclatement*) of luitoids to measure the extent of luitoid damage

in a latex sample. This measurement gives an indication of the proportion of luitoids in the latex that have 'burst' or suffered significant membrane damage. Since latex acid phosphatase is generally confined to the luitoids in stable latex, its release from the luitoids give an indication of luitoid integrity. The bursting index of luitoids is calculated as the activity of acid phosphatase present in the latex C-serum (*i.e.* released from damaged luitoids) expressed as a percentage of the total acid phosphatase activity of the latex<sup>4,11</sup>. Total acid phosphatase activity is determined after treating the latex with *Triton X-100* to solubilise the membranes of the luitoids and thus release their acid phosphatase content.

In a preliminary experiment, two methods of determining the 'bursting index of luitoids' were compared. A fresh latex sample was undiluted or diluted with 10% to 19% distilled water to induce varying degrees of luitoid damage<sup>1</sup>. The bursting indices of the samples were then determined using the method of the International Rubber Research and Development Board (IRRDB)<sup>12</sup> which involved incubating the enzyme substrate, p-nitrophenyl phosphate, in latex (that had been pre-treated or not pre-treated with *Triton X-100*) and then quantitating the enzyme product in the serum recovered by acid coagulation of the latex. Bursting indices were determined on the same latex samples by an alternative method described by Yeang<sup>13</sup> where enzyme substrate incubation was carried out in the serum obtained by centrifugation of latex that had been pre-treated or not pre-treated with *Triton X-100*. The latter method was very similar to the original procedure that Ribaillier used<sup>14</sup>. As shown in *Figure 1*, the two methods showed similar trends. Except where indicated otherwise in the present study, data presented on the bursting index of luitoids were obtained using the method described by Yeang only.

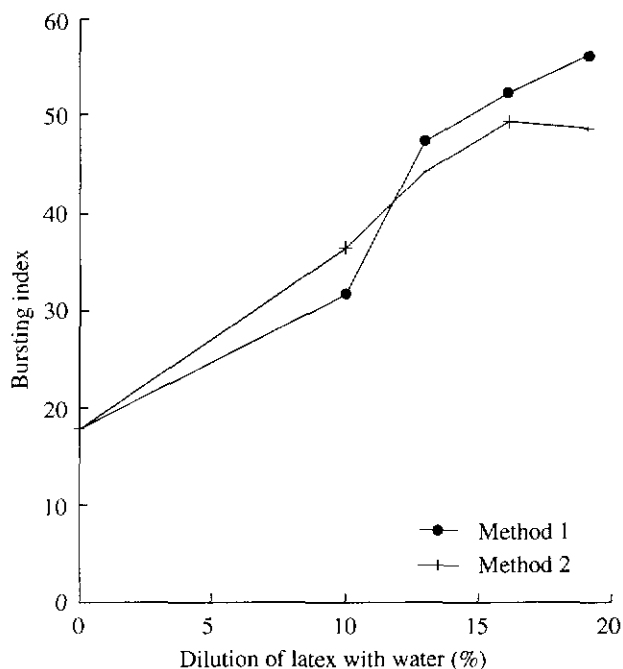


Figure 1. Comparison of the bursting index of lutoids as determined according to the IRRDB procedure<sup>12</sup> (Method 1) and by the method of Yeang<sup>13</sup> (Method 2). The test latex was undiluted or diluted 10% – 19% with distilled water. The extent of lutoid damage caused by latex dilution depends on the intrinsic state of the lutoids. In some experiments (results not presented), little increased lutoid damage was observed with this range of dilution.

The bursting indices and the appearance of the bottom fractions of early and late latex flow fractions were compared in 48 RRIM 600 trees (24 trees on renewed *Panel B1-1* and another 24 trees on renewed *Panel B1-2*). Latex fractions of 13 ml were collected at the time of tapping (initial fractions) and again 60 min after tapping (late fractions). To ensure a good representation of tapping panels and tapping days, half of the trees were on *Panel B0-1* tapping and the rest were tapped on *Panel B1-1*. The latex samplings were made over eight tapping days.

Changes in bottom fraction appearance, water potential and bursting indices in the course of latex flow were studied in greater detail on six individual buddings (three of PR 261 on virgin *Panel B0-1* and three of Tjir 1 on renewed *Panel B1-2*). Latex fractions (8 ml) were collected from 0.25, 4, 15, 30, and 50 min after tapping. After the 50th min collection, sequential 4 ml fractions were continually collected in calibrated test tubes until the flow ceased completely. The contents of the last two or three tubes were combined to give an 8 ml aliquot representing the latex

exuded just before complete flow cessation. On the average, flow ceased about 100 min after tapping.

In another study to examine how early and late flow latex bursting index measurements might be influenced by the method used to determine this parameter, sequential latex fractions were collected from the 5th, 15th, 30th, 60th, 90th and 120th min after tapping from two RRIM 600 trees (*Panel B1-1*). Bursting indices of the fractions were then determined using two methods (according to IRRDB<sup>12</sup> and to Yeang<sup>13</sup>).

Water potential of C-serum was determined using a Wescor HR 33T hygrometer to monitor the 'dilution reaction'<sup>15,16</sup> of the latex that occurs after tapping.

## RESULTS

### **Bottom Fraction Damage in RRIM 600 Early and Late Flow Latex Fractions**

Pakianathan *et al.*<sup>1</sup> reported that the early flow (first 16 ml) of latex exhibited gross bottom fraction damage to the extent that most of it would have disappeared. Examination of the latex centrifugation patterns of forty eight RRIM 600 trees showed that the condition of the bottom fraction of the initial 13 ml flow samples was comparable to that of the late flow latices collected at the 60th minute. There were no distinct differences in bottom fraction size or appearance (*Figure 2*). Significantly, there was not a single case of gross bottom fraction damage observed among the forty eight initial flow latex samples. Assays on the bursting index of lutoids gave an average value of 27.1% for the early flow latex and 32.2% for the late flow. Hence, the late flow samples had in fact 19% greater bottom fraction damage than the

initial flow samples. This difference was statistically significant ( $P < 0.001$ , paired t-test,  $df = 47$ ).

### **Bottom Fraction Damage in Sequential Latex Flow Fractions of PR 261, Tjir 1 and RRIM 600**

Information on lutoid damage in the latex exuded just before complete cessation of flow has rarely been reported in the past. In another study, therefore, sequential flow fractions were collected from six individual trees until the actual cessation of flow. Three of the trees were of the clone PR 261 (*Trees 1 to 3*) and the other three trees were of the clone Tjir 1 (*Trees 4 to 6*).

*Estimation of bottom fraction damage by visual assessment.* The sequential latex fractions collected from the time of tapping to the cessation of flow were centrifuged. The centrifuged latices, as exemplified by flow fractions from *Tree 5*, are shown in *Figure 3*. Of all the latex samples collected from the six trees, distinct visual bottom fraction damage as described by Pakianathan *et al.*<sup>1</sup> was observed in only one sample from PR 261 (*Tree 3*). This was the 'initial fraction' collected 15 s after tapping; similar bottom fraction damage was absent in the next sample collected at the 4th min (an 'early flow' fraction) and in all samples subsequent to that. Severe bottom fraction damage was also absent in all flow fractions (even the 'initial fractions') from the other five trees, including three trees of the clone Tjir 1, a high plugging clone that Pakianathan used originally in his experiments. Hence, the bottom fraction damage in the initial flow observed in *Tree 3* is atypical.

In all six trees, the bottom fractions of the later flow fractions (collected 50 min and later) had irregular edges not seen in the earlier

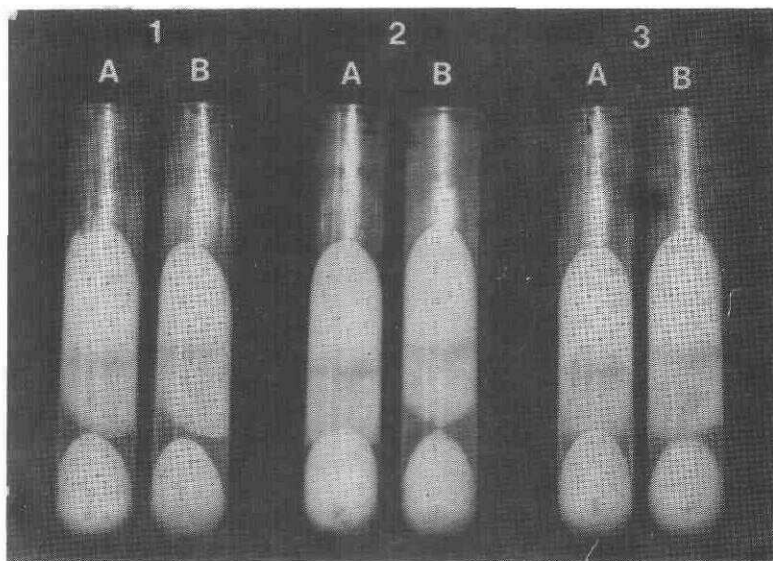


Figure 2. Centrifugation of latices from three RRIM 600 trees (1, 2 and 3) collected immediately after tapping (A) and collected 60 min after tapping from the same trees (B).

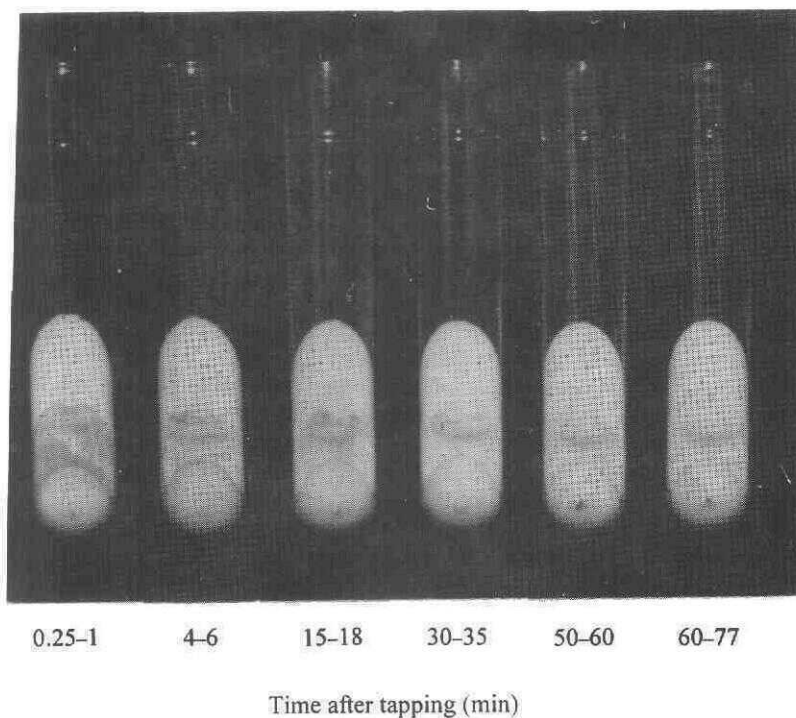


Figure 3. Centrifugation of 8 ml latex fractions collected from Tree 5 (Tjir 1) at different intervals after tapping. The last fraction includes latex collected just before flow cessation.

fractions. This is indicative of flocculation and, hence, instability of the lutoids. The bottom fraction of the later flow fractions also appeared opaque and white (as compared to the translucent bottom fractions from the early flow fractions). These characteristics of the late flow bottom fraction resemble those of latex that has been destabilised by the addition water to induce lutoid damage (Figure 4).

*Estimation of bottom fraction damage by the bursting index.* The latex flow rate from the cut and the bursting index of lutoids of various fractions for the six trees are shown in Figure 5. Generally, bursting index in the 'initial flow' fraction collected immediately after tapping (during the first minute, 15 s from taping) was moderately higher than that of the 'early fraction' collected at the 4th or 15th minute. In only one of the trees (PR 261, Tree 3) was bursting index of the initial fraction very high (Figure 5), where severe bottom fraction damage was observed as mentioned above. The bursting index of the immediate fraction that followed (4th minute) was low.

In all six trees, bursting index of the subsequent latex fractions after the 4th or 15th minute rose as the latex flow rate from the tapping cut decreased progressively until flow ceased (Figure 5). This is in agreement with the above-mentioned observations of instability of the bottom fraction towards the cessation of latex flow. Relatively high bursting indices were encountered at the end of flow, with some values exceeding 50. From a visual assessment of the bottom fraction of these latex samples, it did not appear that more than half of the lutoids had actually ruptured as might be implied from the term 'bursting index'. It is likely that the high bursting indices had resulted from the leakage of acid phosphatase from destabilised lutoids, even though the lutoids themselves had not disintegrated<sup>17</sup>.

*Changes in water potential and latex viscosity.* As latex flow progressed, C-serum water potential increased (i.e.  $\Psi$  became less negative), indicating a dilution of the latex. The change in water potential occurred in tandem with the progressive decrease in latex total solids content, the latter being mainly due to the decrease of the rubber content of the latex (Figure 6). Despite the latex of the late fractions being more dilute, the late flow latex was often visibly more viscous as evident from the amounts of latex adhering on to the side of the latex collection tubes when their contents were emptied (Figure 7).

#### **Further examination of the Trend in the Bursting Index of Lutoids During Latex Flow**

To investigate if the bursting index data obtained in the present study were influenced by the methodology used for this measurement, an experiment was carried out comparing the bursting index of sequential flow fractions from two RRIM 600 trees using both the IRRDB method<sup>12</sup> as well as the method described by Yeang<sup>13</sup>. As shown in Figure 8, the general trends in bursting index using either method were similar. With the two trees, bursting index readings increased markedly towards the end of flow. Since the first fraction was collected 5 min after tapping (and no earlier 'initial fraction' was collected), the fall in bursting index normally occurring shortly after tapping (as seen in the Tjir 1 and PR 261 trees) was not evident.

#### **DISCUSSION**

The rate of plugging is influenced by the length of tapping cut<sup>18</sup>. With shorter cuts, higher turgor is maintained for a longer period of time and lutoid damage through shear can be expected to be correspondingly greater. The present study pertains to the half spiral tapping cut that is commonly adopted in commercial latex exploitation.

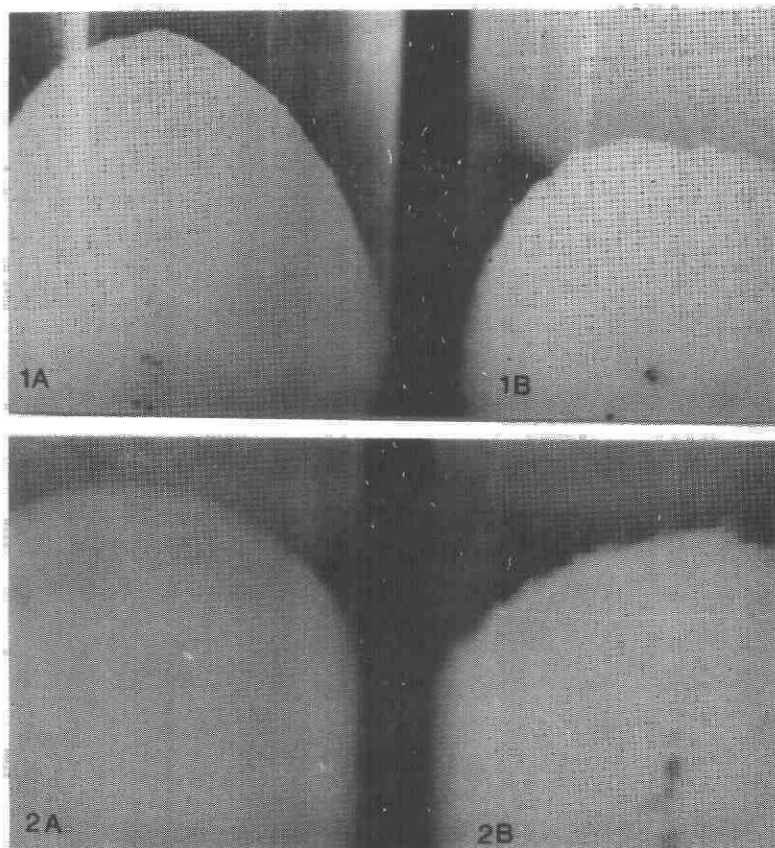


Figure 4. Bottom fractions of centrifuged latexes from the early and late flow compared with bottom fractions from early flow latex that was undiluted or diluted with distilled water.

- (1A) Latex collected 15 min after tapping.
- (1B) Latex collected at the cessation of flow.
- (2A) Undiluted latex.
- (2B) Latex diluted 18% with distilled water.

Samples 1B and 2B show irregular edges of the bottom fraction indicative of lutoid instability.

The size of the latex bottom fraction alone does not always give a reliable indication of its physiological integrity and well-being of the lutoids. Damaged lutoids tend to flocculate, adhering with one another and with rubber particles. Such destabilised bottom fraction has a jagged rather than smooth outline (Figure 4).

Where lutoid damage is not severe, there is no diminution of the bottom fraction size. Lutoid damage in its early stages can in fact give rise to a slight *increase* in the size of the bottom fraction because of the flocculence. Where lutoid damage is more extensive, the bottom fraction is reduced in size partly because of

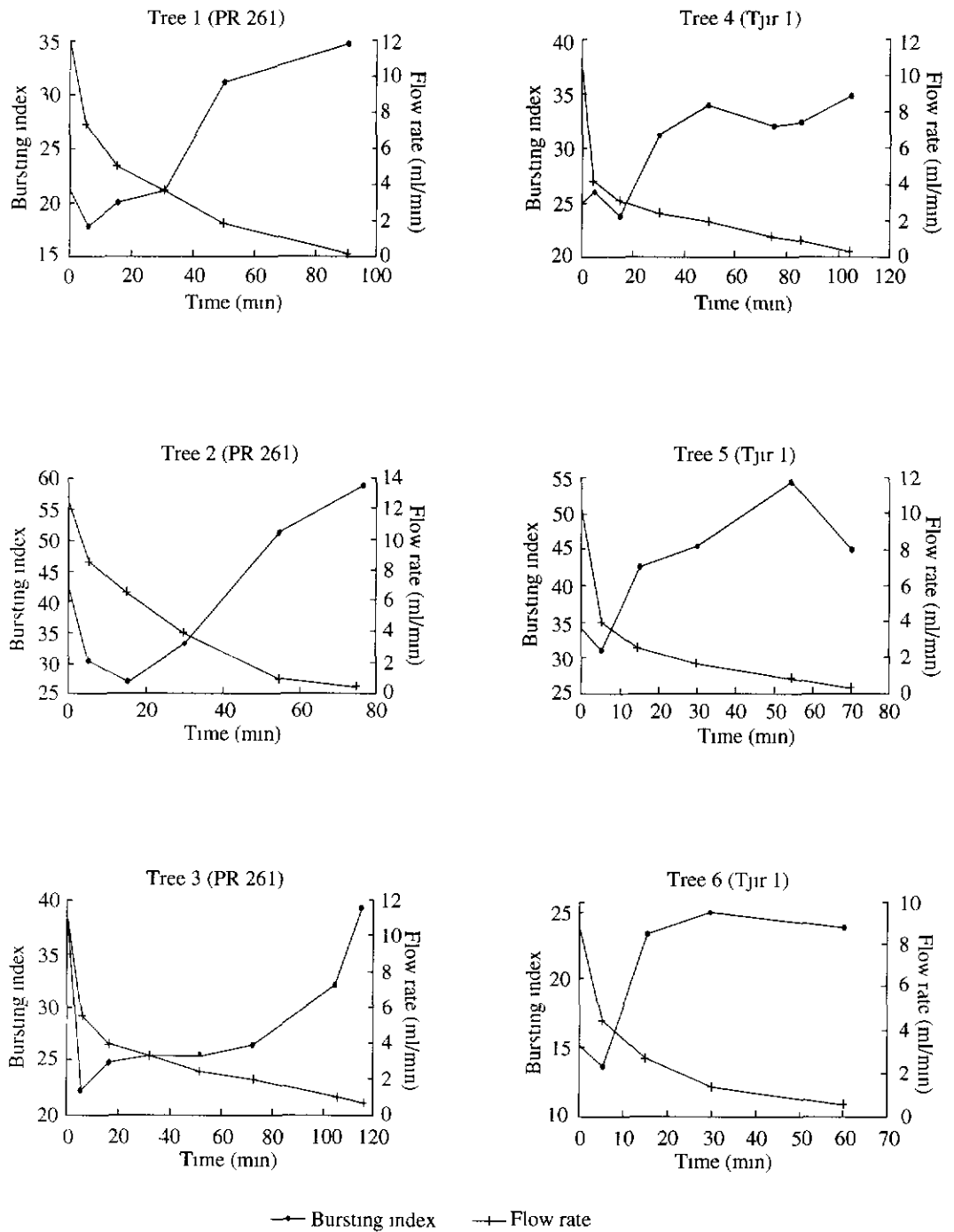


Figure 5 Latex bursting index and flow rate of six trees at various intervals after tapping

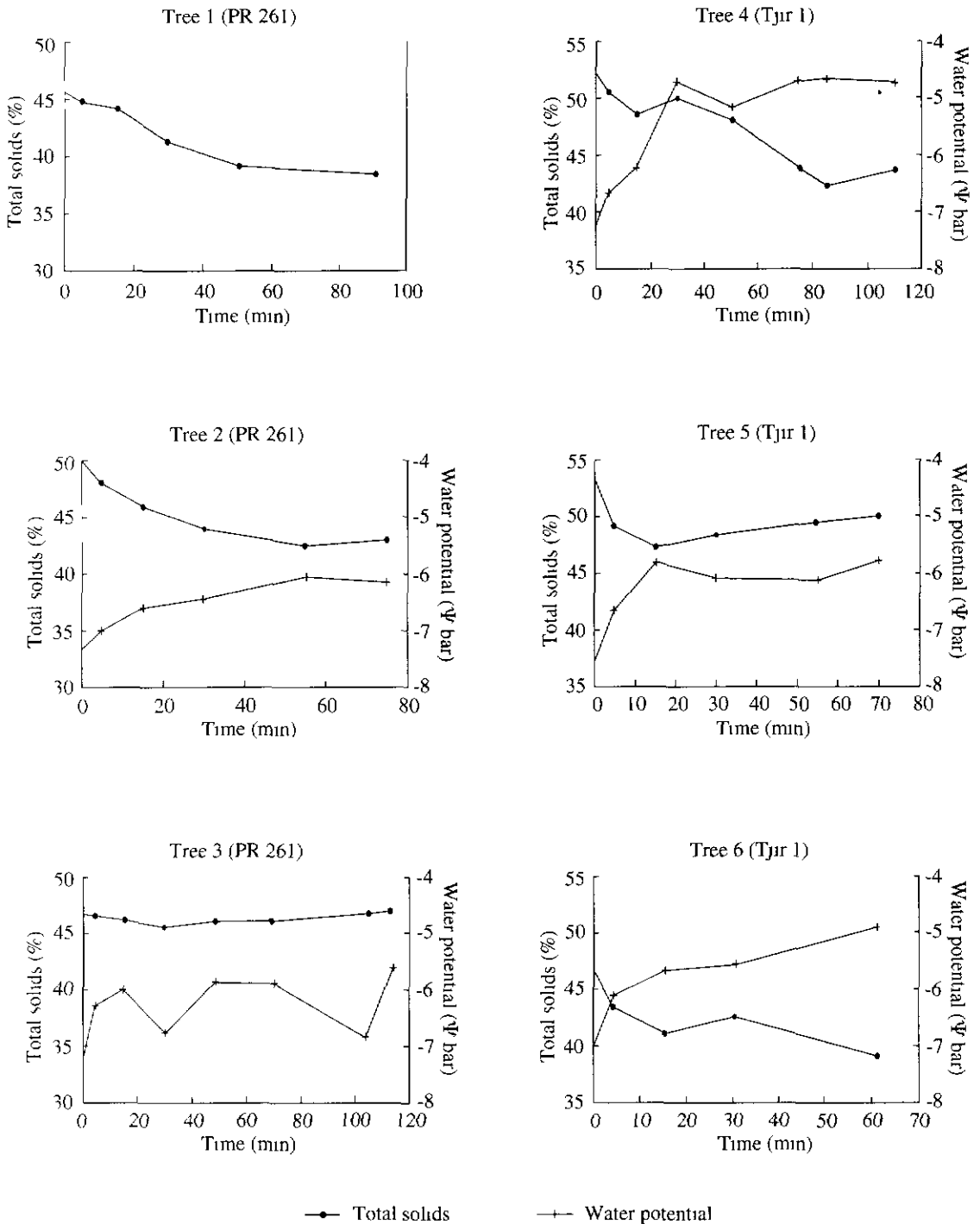
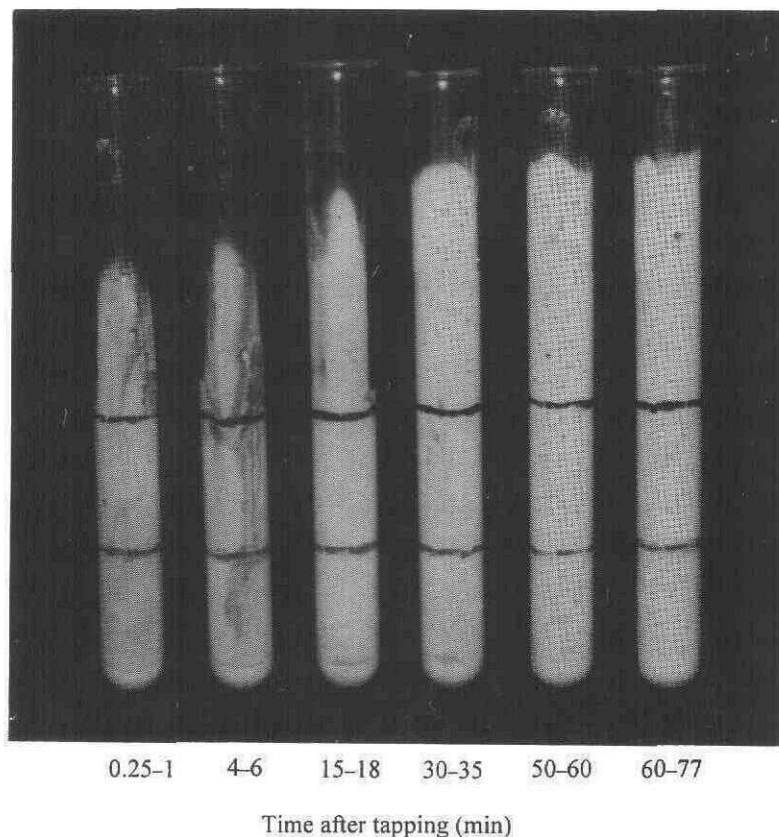


Figure 6 Latex total solids content and C-serum water potential of six trees at various intervals after tapping. Water potential readings were not taken for Tree 1



*Figure 7. Appearance of latex collection tubes after pouring out latices that were collected (from Tree 3) at various intervals from the time of tapping. Varying amounts of latex remain adhered to the sides of the tubes. The last fraction includes latex collected just before flow cessation.*

outright luitoid rupture and partly because rubber particles adhere to luitoid membranes and such luitoids fail to sediment upon centrifugation. To complement visual assessment of the bottom fraction, another way to assess luitoid damage is by the bursting index. Both approaches were undertaken in the present study.

The term 'late flow' is often loosely defined in previous studies. For example, a 'late flow' fraction could be the fraction collected as early as 30 min after tapping<sup>5</sup>. In the study on the forty-eight RRIM 600 trees, latex collected at

the 60th minute from tapping was designated the 'late flow' although latex flow lasted about two hours. The results of this study showed that the initial 13 ml of latex collected after tapping did not show greater luitoid damage as compared with the 13 ml sample collected at the 60th minute, both by visual inspection of the bottom fraction of centrifuged latex and from the bursting index of luitoids. These results are therefore not in agreement with the prevalent belief that the initial flow latex fraction is highly unstable or that it is characterised by extensive bottom fraction

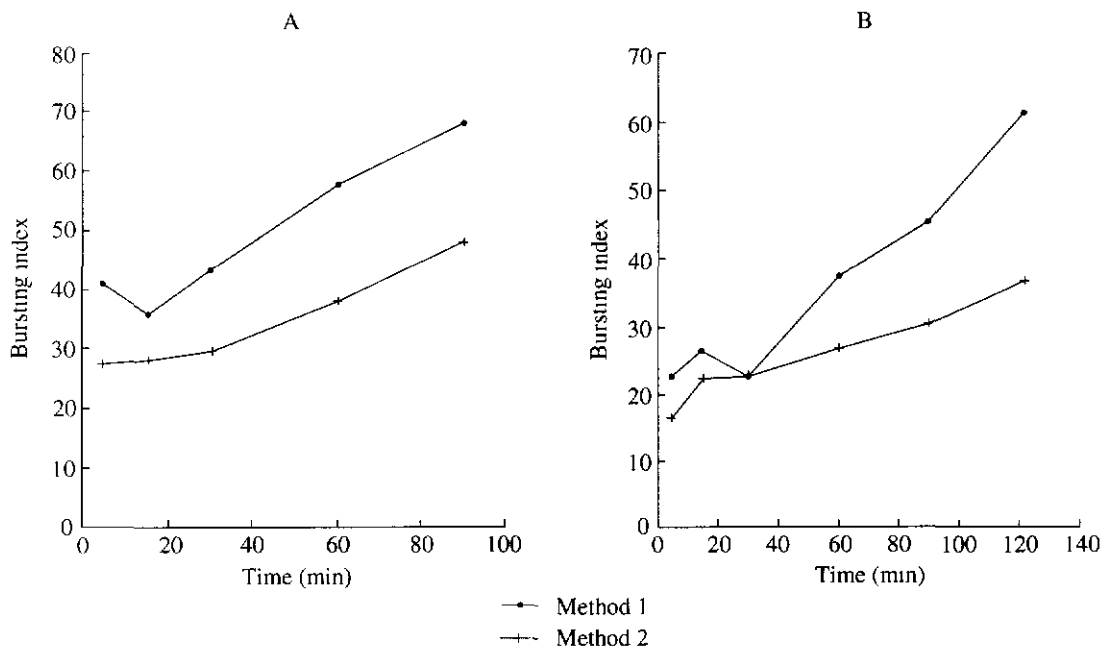


Figure 8. Comparison of bursting index of sequential latex fractions collected from two RRIM 600 trees (A and B) as determined according to the IRRDB procedure<sup>12</sup> (Method 1) and by the method of Yeang<sup>13</sup> (Method 2).

damage. Indeed, there is evidence that the later flow (at the 60th min) is less stable.

As in the case with the early and late latex fractions of RRIM 600 trees, the results obtained in the experiments with the PR 261 and Tjir 1 trees cast doubt to the commonly held belief<sup>1-8</sup>, based on early reports<sup>1,3,4</sup>, that latex during the early flow (first two 15 ml - 20 ml fractions) is more unstable than that during the late flow. From the results of the present study, bursting index in the initial flow (1st minute) could be moderately high and there was normally a brief fall immediately in the subsequent flow. However, bursting index

began to increase after the 4th to 15th min and this increment persisted until latex flow ceased. Therefore, for the greater part of latex flow after tapping, the bursting index assumed an increasing trend.

The well-known 'dilution effect'<sup>15-16</sup> was observed as latex flow progressed. The inflow of water into latex vessels might cause an osmotic shock that can destabilise lutoids<sup>1</sup> although it is not clear to what extent overall lutoid damage might be attributed to this latex dilution. Southorn<sup>2</sup> thought that the dilution factor contributed only partially to lutoid destabilisation while Pujarniscle<sup>3</sup> and

Raghavendra *et al.*<sup>19</sup> felt that the change in osmotic potential during flow could not have destabilised lutoids very significantly.

As lutoids contain latex-destabilising factors<sup>2,5-8,20-23</sup>, the release of such factors from destabilised lutoids can induce instability of the whole latex. An indication of the instability of the late flow latex was frequently seen from the relative latex viscosities of the early and late flow fractions; the late flow fractions were often visibly more viscous (*Figure 7*). This is consistent with the observation by Pujarniscle *et al.* that the latex collected just prior to cessation of flow had a greater tendency to agglutinate<sup>3</sup>. Latex of this nature can be expected to be predisposed to latex vessel plug formation.

Notwithstanding the observations of unstable early flow by Pujarniscle<sup>3</sup> and by Ribaillier<sup>4</sup>, the latter has in fact presented results to the contrary in an earlier report<sup>24</sup> where it was the late flow latex, rather than early flow from conventionally tapped trees, that showed markedly elevated bursting index measurements. The variation in bottom fraction damage during the course of latex flow observed in the present study is also in agreement with results of Raghavendra *et al.*<sup>19</sup> They found that, after an initial fall, the bursting index increased steadily from the 10th minute to the 60th minute. Similar trends have been reported more recently by Dian *et al.*<sup>25</sup> who further noted that increased lutoid damage in the later fractions was more marked in trees with symptoms of tapping panel dryness.

It may seem surprising that lutoid damage should be so prevalent towards the end of flow when latex exudation from the tapping cut is sluggish (*Figure 5*). Indeed, the effects of physical shear<sup>9</sup> or rate of latex dilution<sup>1</sup> that contribute to lutoid damage are expected to diminish with reduced flow rate. However, it should be noted that latex exudation from the

tapping cut at the end of flow is derived from only a very small number of yielding latex vessels that have not yet been sealed off by the process of latex vessel plugging. The rate of flow per individual latex vessel is therefore relatively very high.

## CONCLUSIONS

From the above studies on regularly tapped RRIM 600, PR 261 and Tjir 1 trees that were not ethephon-stimulated, it may be concluded that:

- While lutoid damage in latex from the initial flow (first minute) obtained immediately after tapping is moderately elevated, there is typically no extensive damage of the latex bottom fraction either in the initial flow or in the early (e.g. fourth minute) latex flow that follows.
- For the greater part of the latex flow after tapping (subsequent to the 4th - 15th min), lutoid damage assumes an increasing trend until the cessation of flow. Hence, latex collected during the later flow is less stable than that from the early flow and lutoid damage occurring towards the end of latex flow could be implicated in the mechanisms leading to the cessation of latex exudation.

## ACKNOWLEDGEMENT

Field and laboratory assistance from Choo Chin Loong, V. Monyrajan, Latifah Abdullah, Rosli Ismail and Fatimah Kamillah Omar is gratefully acknowledged.

*Date of receipt: March 1996*

*Date of acceptance: June 1996*

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