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Sucrose and Metabolism Distribution Patterns in the Latices of Three Hevea brasiliensis *Clones: Effects of Tapping and Stimulation on the Tree Trunk*

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This study describes the sucrose balance between supply and demand in the bark of the rubber tree, along with concurrent latex metabolic activity. Experiments were designed using three Hevea brasiliensis *clones (PB 235, RRIM 600 and GT 1) in the same polyclonal plot at the Chachoengsao Rubber Research Centre (CRRC-DOA) in Thailand. Treatments were carried out on previously untapped trees (growth potential control), trees tapped without stimulation (½S d/3 6d/7 9m/12, physiological control), and trees tapped with ethephon stimulation (½S d/3 6d/7 9m/12 ET 2.5% 5/y and 12/y). Tapping had a marked effect on latex physiology in the whole trunk. Sucrose concentration was significantly reduced. The Latex Diagnosis Mapping (LDM) method was used to describe the shape and size of the latex regeneration area and of the metabolically active bark area. For the three clones, rubber production correlated with the estimated latex regeneration area. It took around 100 cm² of latex regeneration area to regenerate 1 g of rubber. As it assesses the impact of any tapping system on whole trunk latex physiology, the LDM method was used to develop new tapping systems, such as systems involving ethylene gas stimulation, micro-tapping cut systems, and multi-tapping cut systems.*

Key words: *Hevea brasiliensis*; latex; tapping; ethephon stimulation; clone; RRIM 600, PB 235; GT 1; latex diagnosis; mapping; sucrose; metabolism; inorganic phosphorus; latex regeneration; physiology

In Thailand, the rubber tree (*Hevea brasiliensis* Muell. Arg.) is one of the major economic crops and it is estimated that 10% of the country's population survive directly or indirectly on it. In terms of its economic potential, the rubber tree is not valued for latex production alone, but also for its wood, which provides significant income for farmers. Both rubber

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production and growth require assimilates derived from photosynthesis, mainly in the form of sucrose (Suc). As farmers' benefits rely on strict farming management to maintain an ideal balance between rubber production and plant growth, it is also worth understanding how regular tapping or stimulation affects tree growth. A negative relation has been found to exist between latex production and wood biomass creation[1-6].

Using radio-labelled isotopes, it was found that the flow area of a recently opened rubber tree extends for about 40 cm – 50 cm above and below the tapping cut[7]. In older trees, that flow area can extend up to 70 cm above the cut and to the whole area below the tapping cut. Turgor pressure measurements to determine the drained area reveals a pressure drop at 1.2 m below the tapping cut[8]. A bark area where rapid movement of latex near the region of the tapping cut is found to occur and is referred[9] to as the 'potential displacement area'. Suc latex content is depleted below and above the tapping cut as a consequence of the latex regeneration process[10]. However, none of the earlier studies concurrently described the suc supply/demand balance and the associated latex metabolic activity on the tree trunk.

This study describes and quantifies the sucrose balance between supply and demand in the latex-producing bark of the rubber tree, along with concurrent latex metabolic activity. Such a study can not be restricted to the tapped panel only, as other bark areas might be involved in or are at least affected by the latex regeneration process. Physiological analyses were therefore carried out on the untapped bark area too, in order to map latex metabolic activity and concurrent latex sucrose availability on the trunk. Ethephon stimulation was used as a physiological tool to study the influence of increased rubber production for enhanced latex regeneration, which then affected the metabolic characteristics of the latex sink. Our study used two parameters of latex diagnosis[11-15], Suc and inorganic phosphorus (P), to obtain results relative to latex carbohydrate partitioning described by Suc, and concurrent latex metabolic activity described by P, on the trunk.

MATERIALS AND METHODS

Planting Material

Experiments were set up on three *H. brasiliensis* clones (PB 235, RRIM 600 and GT 1) in the same polyclonal plot at the Chachoengsao Rubber Research Centre (CRRC-RRIT-DOA). All the trees were planted in 1993 in a 2.5 m × 7 m planting design (571 trees/ha). Tapping in each plot was started once the monoclonal plots were ready for tapping (*i.e.* 50% of the stand reaching a trunk girth of 50 cm, measured at 1 m from the ground) in May 1999 on PB 235 (*Experiment CHOE01*), October 1999 on RRIM 600 (*Experiment CHOE04*) and May 2000 on GT 1 (*Experiment CHOE05*), in relation to clonal growth potential. Depending on the trial, each treatment comprised 10-13 trees per clone. The experimental design was a 'One Tree Plot Design' (OTPD), where each tree under test was a one treatment replicate. Before the start of tapping, in each experiment, trees were selected uniformly from the normal population of each plot in terms of trunk girth, canopy phytosanitary status and trunk conformation. The treatments comprised untapped trees (growth potential control), trees tapped without stimulation (½S d/3 6d/7 9m/12, physiological control) and trees tapped with ethephon stimulation (½S d/3 6d/7 9m/12 Et 2.5% 5/y and 12/y). Tapping was stopped during the refoliation and high temperature/high water stress period from February to April, in accordance with RRIT-DOA tapping system recommendations for the Chachoengsao area. Tapped treatments were opened on panel

B0-1, 1.3 m from the ground, with a tapping cut of 30° slope. For the ethephon stimulated treatments, stimulation was carried out using panel application (Pa), corresponding to 0.6 g of stimulant per tree per stimulation (*i.e.* 15 mg a.i. per tree per stimulation).

Method

Sampled trees were selected to represent the treatments for girth and production (dry rubber production). Trunk girth and latex physiological parameters, measured by the latex diagnosis technique[12,15] were recorded monthly, for every tree. For this particular study, latex analysis was applied to the whole trunk of a single tree per treatment (untapped control, ½S d/3 6d/7, ½S d/3 6d/7 Et 2.5% 5/y and ½S d/3 6d/7 Et 2.5% 12/y), due to laboratory constraints. Latex Suc and inorganic P concentrations measured at different positions on the trunk, describe the available carbohydrate substrate and related metabolic activity of the latex, in response to each treatment (tapping and stimulation intensity). Latex sampling positions were drawn on the trunk one day prior to latex sampling, covering trunk areas below and above the tapping cut (±90 cm, *i.e.* up to 2 m above ground) on both tapped and untapped panels. Latex sample was collected in the morning of each scheduled tapping day, from 7.00 to 9.00 am. Tapping was delayed until the end of latex sampling to avoid any tapping effect on the latex analysis results. Sampling was carried out upwards from the lowermost line, first on the tapped panel and then on the untapped panel. Seven drops of latex were collected from each sampling position to measure latex Suc and inorganic P using the latex diagnosis technique, adapted to CRRC Latex Physiology Laboratory facilities[16]. Suc and P concentrations in the latex were expressed in millimols per litre of fresh latex (mM L^{-1}).

RESULTS

Vertical Distribution of Latex Inorganic Phosphorus and Sucrose Content

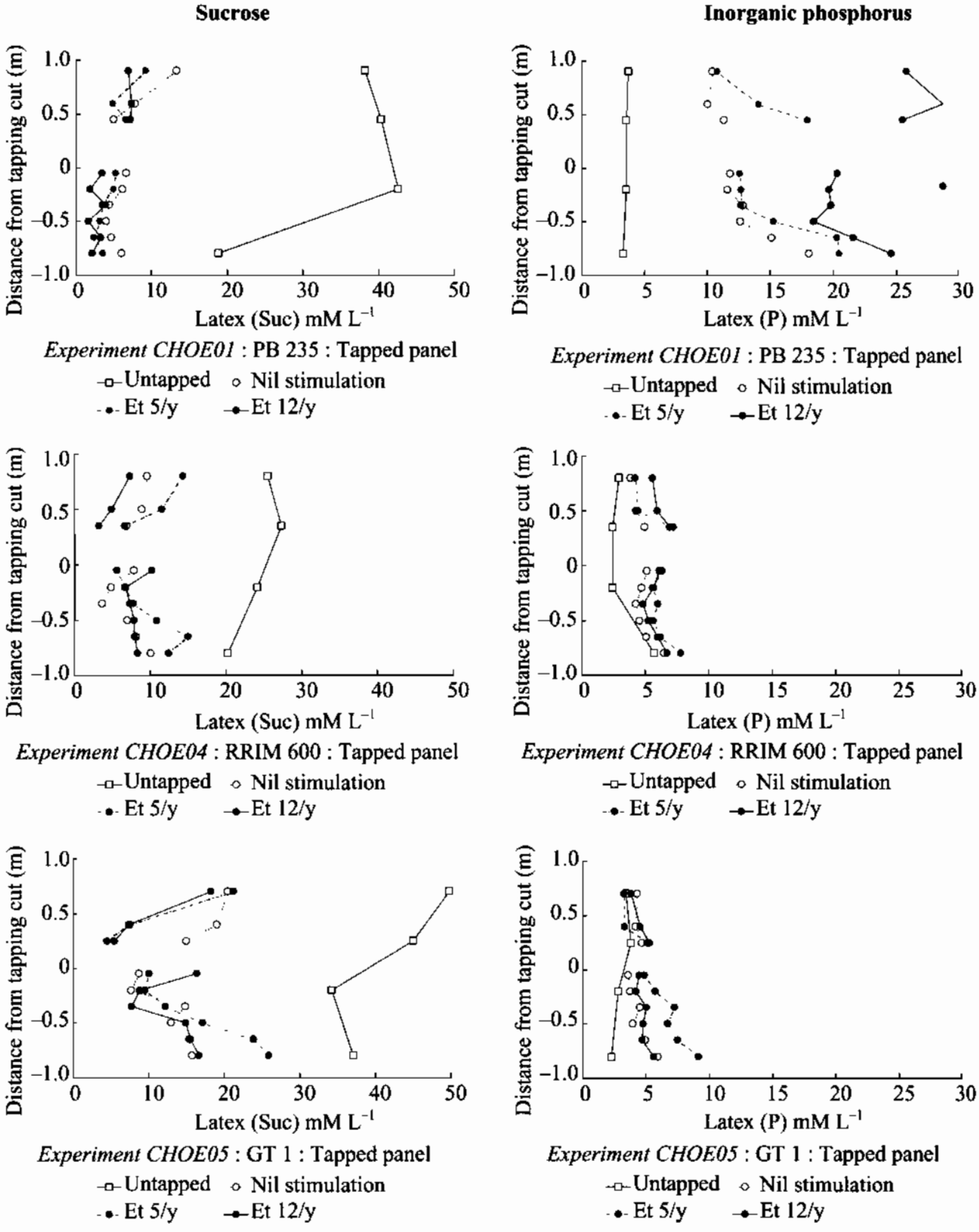
Inorganic P and Suc are two latex physiological parameters measured by latex diagnosis[11–15]. Correlations between these parameters and yield make it possible to establish a clonal latex metabolic typology[17–20], where clones GT 1, RRIM 600 and PB 235 are known as medium, medium-high and high-metabolism clones, respectively.

The vertical distribution of latex P and latex Suc on tapped and untapped panels, compared between untapped trees, tapped trees without stimulation and tapped trees with ethephon stimulation, is shown in *Figures 1* and *2* for all three clones.

Tapped panel. On the tapped panel (*Figure 1*) a significant change was found in the levels of the two parameters (Suc and P) within the studied area (±90 cm from the tapping cut), for all three clones, between tapped and untapped trees.

Latex Suc was significantly reduced by tapping when compared to the equivalent sampling position in untapped trees, below and above the tapping cut. Maximum latex Suc depletion occurred in the bark regions surrounding the tapping cut (±50 cm from the tapping cut). Ethephon stimulation generally enhanced the drop in latex Suc. It was however surprising that even the uppermost location of the tapped panel was dramatically affected by tapping and ethephon stimulation. In the lowest parts of the panel, the difference between untapped trees and tapped trees was smaller as latex Suc displayed an increasing bottom-up gradient in untapped trees.

Conversely, latex P was significantly increased by tapping, when compared to the



Sampling on tapped trees was carried out 5, 20, 35, 50, 65 and 80 cm below the cut. The height of sampling above the cut depended on the width of renewed bark, which depended on the opening date for each clone: 10, 25 and 55 cm above renewed bark. Sampling on untapped trees was carried out at four positions, equivalent to the following positions on tapped trees: 20 and 80 cm below the cut, 10 and 55 cm above renewed bark.

Figure 1. Vertical distribution of latex sucrose content [(Suc), mM L^{-1}] and inorganic phosphorus content [(P)], mM L^{-1}) on the tapped panel of clones PB 235, RRIM 600 and GT 1, depending on the distance from the tapping cut (± 90 cm).

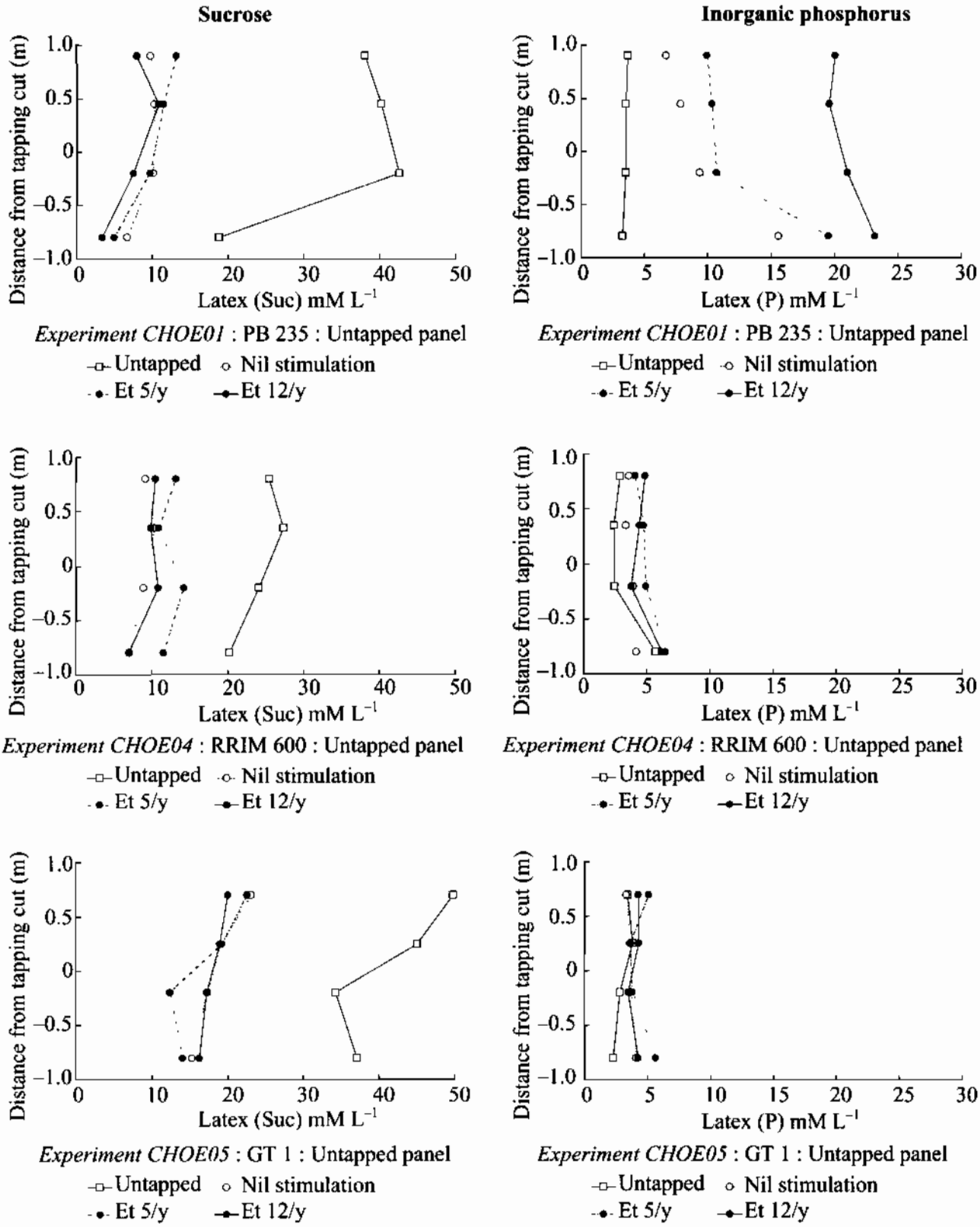


Figure 2. Vertical distribution of latex sucrose content [(Suc), mM L ^{1}] and latex inorganic phosphorus content [(P), mM L^{-1}] on the untapped panel of clones PB 235, RRIM 600 and GT 1, depending on the distance from the tapping cut (± 90 cm). Sampling was carried out at four positions, equivalent to the following positions on the tapped panel: 20 cm and 80 cm below the cut, 10 cm and 55 cm above renewed bark.

equivalent sampling positions in untapped trees, below and above the tapping cut. However, the relative size of the increase in latex P seemed to be closely related to the clone. The increase in latex P was greatest in clone PB 235 (high-metabolism clone), moderate in clone RRIM 600 (medium-high metabolism clone) and low in clone GT 1 (medium-metabolism clone), and therefore seemed closely related to clonal latex metabolism. The increase in latex P mainly occurred in the bark area below or just above the tapping cut. Ethephon stimulation generally enhanced the effect of ethephon stimulation on latex P, especially below the tapping cut.

Untapped panel. On the untapped panel (*Figure 2*), a significant change was observed in the levels of the two parameters (Suc and P) within the studied area, for all three clones between tapped and untapped trees.

Latex Suc was significantly reduced by tapping, when compared to the equivalent sampling position in untapped trees. Maximum latex Suc depletion mainly occurred in the uppermost bark regions of the untapped panel, due to the increasing bottom-up gradient for latex Suc observed in untapped trees. The effect of ethephon stimulation on the decrease in latex Suc was slight and not constant. The latex Suc pattern was much more regular on the untapped panel than on the tapped panel, displaying a general increasing bottom-up gradient irrespective of the clone and the tapping intensity.

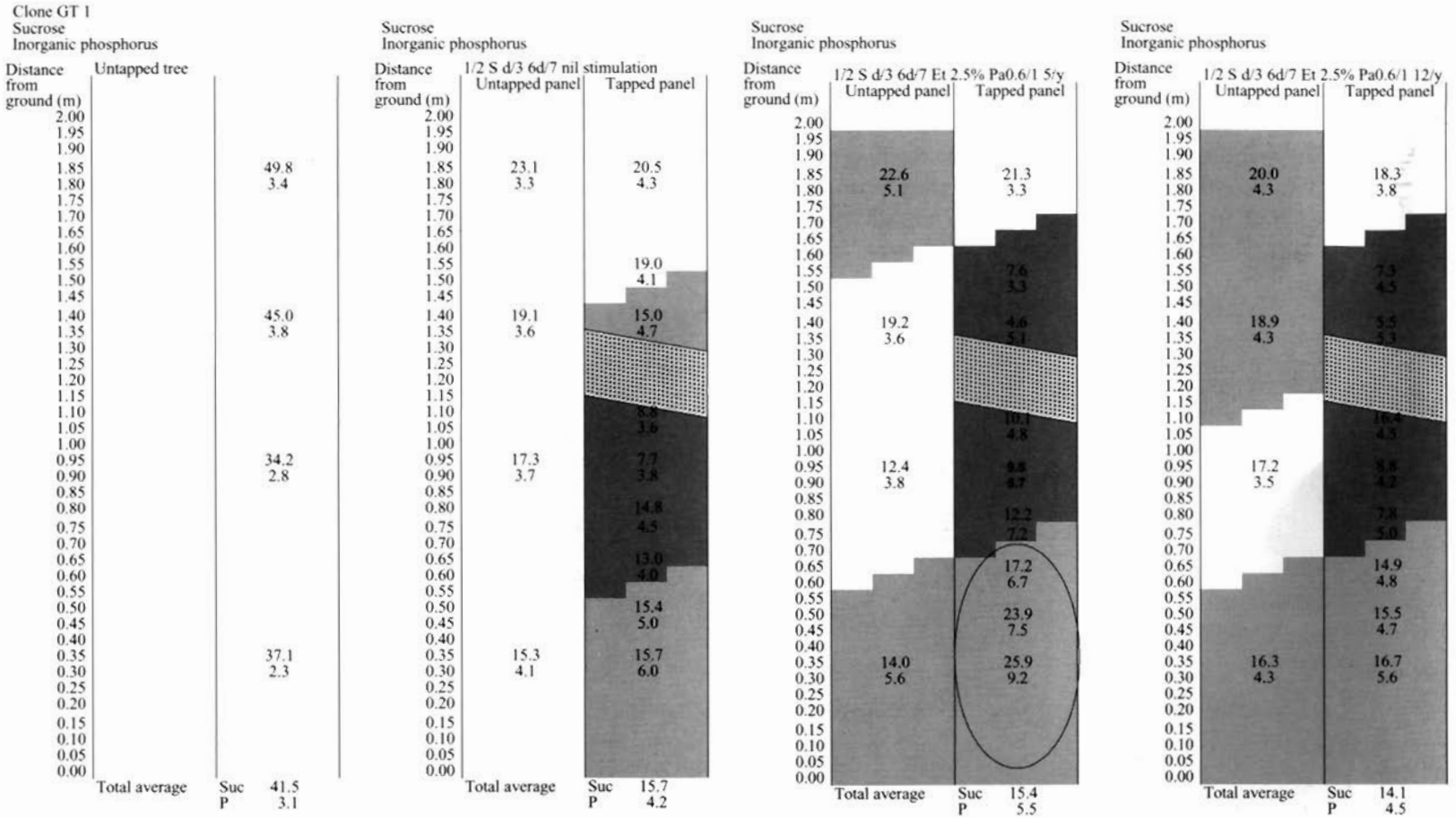
Conversely, as observed on the tapped panel, latex P was significantly increased by tapping on the untapped panel, when compared to the equivalent sampling positions in untapped trees. The relative size of the increase in latex P seemed to be very closely related to the clone. It was greatest in clone PB 235 (high-metabolism clone), moderate in clone RRIM 600 (medium-high metabolism clone) and low in clone GT 1 (medium-metabolism clone), and therefore seemed related to clonal latex metabolism. As on the tapped panel, ethephon stimulation generally enhanced the effect of ethephon stimulation on latex P, especially in the lower areas of the untapped panel. However, the effect was much less significant than on the tapped panel, and therefore seemed weakly correlated with clonal metabolism.

Latex Metabolic Status on the Trunk

On the trunk, the average latex Suc was found to be dramatically reduced by tapping for all three clones, especially for clone PB 235 (–62% to –66% for GT 1; –55% to –67% for RRIM 600; –80% to –85% for PB 235). The stimulation effect was much less significant.

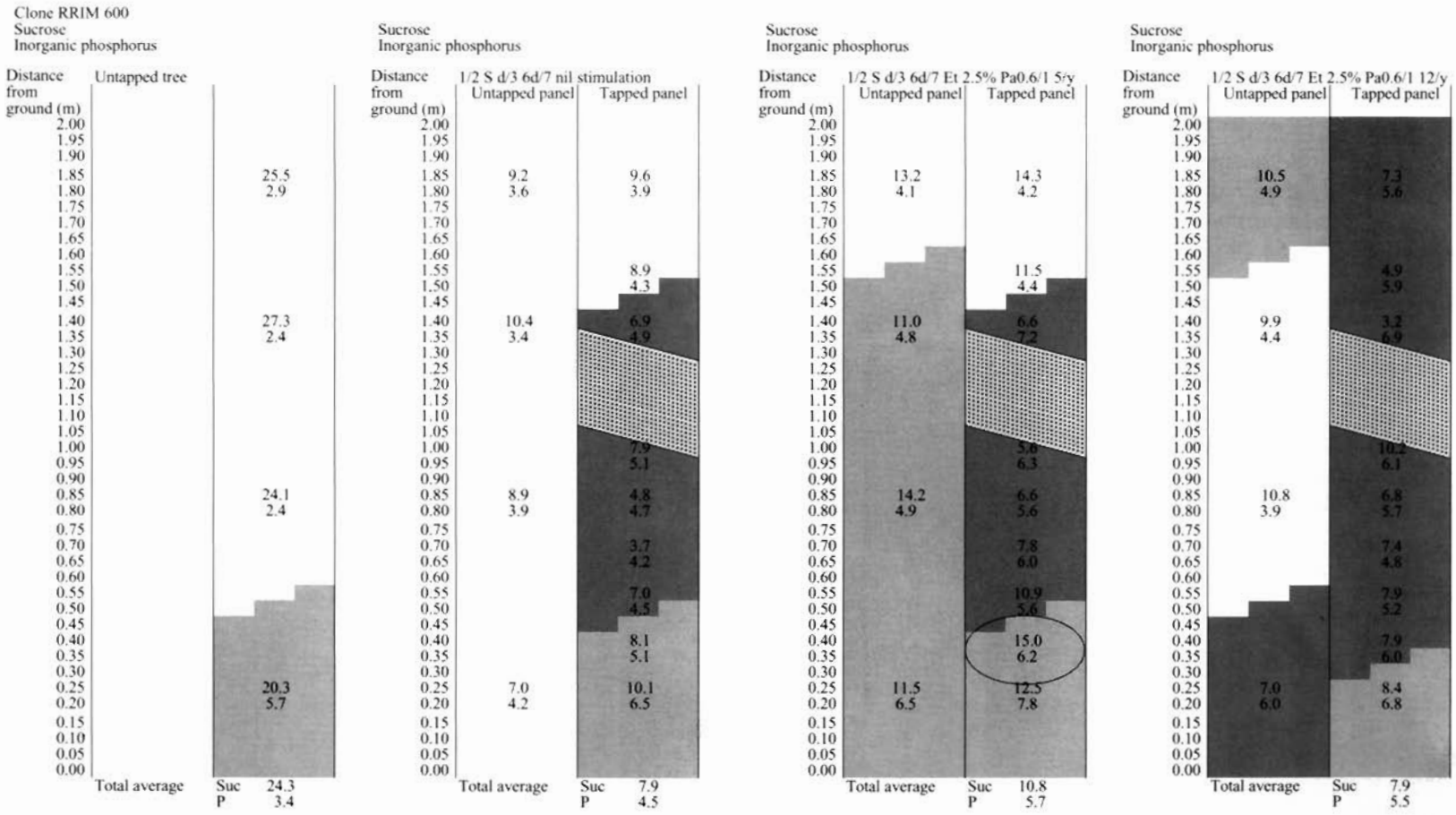
For latex P, the observed values were low compared to the usual standards, especially for tapped GT 1 and RRIM 600. They nevertheless displayed a general increasing trend as the exploitation intensity increased. The low P values found for clones GT 1 and RRIM 600 might be explained by the sampling period (end of August), as latex metabolism activation was still rather incomplete. As it has been well established that latex regeneration *stricto sensu* is a rather localised phenomenon, these data clearly showed that tapping and subsequent latex regeneration completely modified the physiology of the rubber tree latex system, affecting the whole latex metabolism and concurrent latex carbohydrate availability on a tree scale.

Figures 3a (GT 1), *3b* (RRIM 600) and *3c* (PB 235) show latex sampling positions of the LDM for the 12 trees sampled. Each value is the average of 3 sampling points along the same line. The shaded areas are the sampling positions where latex P concentration was found to be higher than the average trunk P



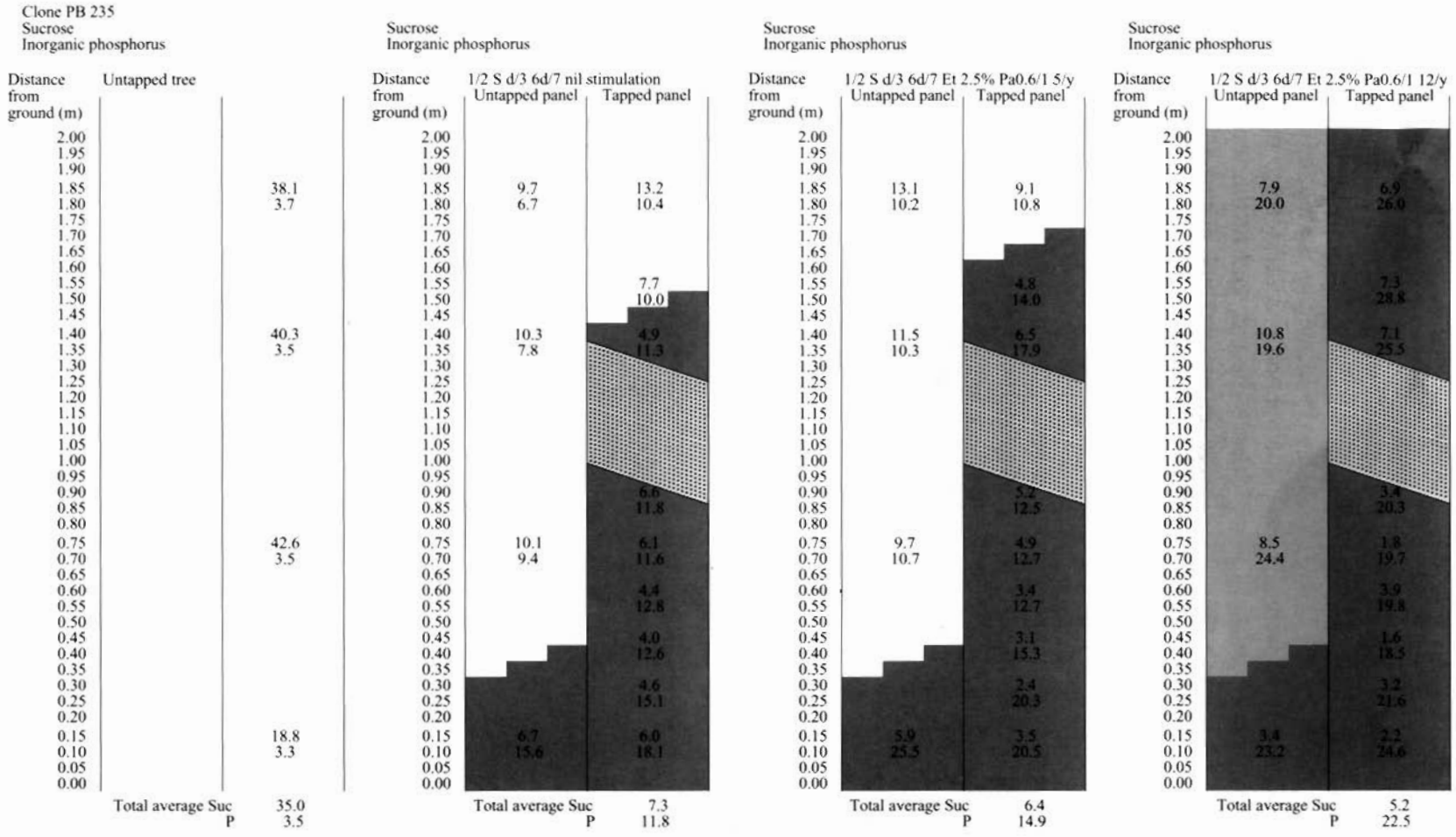
Each value is the average of three sampling points along the same line. The shaded areas are the sampling positions where latex P concentration was found to be higher than the average trunk P concentration of the physiological control treatment (1/2 S d/3 6d/7 *nil stimulation*). The circled area shows a 'sugar import area'; Dark grey area: high P with concurrent low Suc (Regeneration); Pale grey area: high P area (Metabolic activation); White area: area comparable to the physiological status of the untapped tree; Dotted area: renewed bark.

Figure 3a. GT 1: Latex diagnosis mapping for four sampled trees.



Each value is the average of three sampling points along the same line. The shaded areas are the sampling positions where latex P concentration was found to be higher than the average trunk P concentration of the physiological control treatment (1/2 S d/3 6d/7 *nil stimulation*). The circled area shows a 'sugar import area'; Dark grey area: high P with concurrent low Suc (Regeneration); Pale grey area: high P area (Metabolic activation); White area: area comparable to the physiological status of the untapped tree; Dotted area: renewed bark.

Figure 3b. Clone: RRIM 600: Latex diagnosis mapping for four sampled trees.



Each value is the average of three sampling points along the same line. The shaded areas are the sampling positions where latex P concentration was found to be higher than the average trunk P concentration of the physiological control treatment (1/2 S d/3 6d/7 *nil stimulation*). Dark grey area: high P with concurrent low Suc (Regeneration); Pale grey area: high P area (Metabolic activation); White area: area comparable to the physiological status of the untapped tree; Dotted area: renewed bark.

Figure 3c. Clone PB 235: Latex diagnosis mapping for four sampled trees.

concentration in the physiological control treatment (1/2 S d/3 6d/7 nil stimulation).

Such representation clearly shows the extension of the metabolically active area:

- The most active trunk areas (highest P) were always found on the tapped panel, below the cut or just above the cut. Above the cut, clear and regular decreasing of P from the bottom-upward gradient reflected by decreasing latex metabolic activity as the distance from the tapping cut increased.
- On the untapped panel, any active areas (high P) were always located at the bottom of the panel. Nevertheless, metabolic activation was found in most cases to have a low level when compared to equivalent positions on the tapped panel.
- These active areas were extended by ethephon stimulation to higher areas of the two panels.
- A low activation area was generally found in the middle of the untapped panel, followed by a higher activation area in the uppermost parts of the same panel. That might reflect spiral-oriented activation of the latex system, following the acknowledged spiral alignment of latex and phloem tissues in the bark of the tree.

A combined comparison of metabolic activity, estimated by latex P concentrations, and of corresponding Suc concentrations proved to be a suitable method for describing the functioning of the latex system in the different bark regions of the trunk:

- Areas with low P: Such a physiological profile characterised relatively inactive latex metabolism, which was comparable to the physiological status of the untapped tree. These areas were usually located on the untapped panel (excluding its lowest part) and in the higher parts of the tapped panel, above the tapping cut.
- Areas with high P and low Suc: Such a physiological profile characterised active latex metabolism where sucrose was actually used for latex regeneration (latex regeneration area). These areas were usually located on the tapped panel below the cut and could extend to the bark area above the cut, and to the lowest part of the untapped panel, near the ground.
- Areas with high P and high Suc: Such a physiological profile characterised active latex metabolism, where sucrose accumulated (latex sugar import areas) instead of being used for latex regeneration. When present, these 'buffer' areas were found on the lowest part of the tapped panel. In such cases, sucrose concentration was always higher when compared to the equivalent position on the untapped panel, although the latter had higher latex metabolic activity, creating a substantial import sink effect. Such areas seemed to correspond to an intermediate physiological status where metabolic activation was high enough for active sucrose import but not high enough for latex regeneration. These areas were wider in clone GT 1 (medium-metabolism clone) than in clone RRIM 600 (medium-high metabolism clone), whilst they did not exist in clone PB 235 (high-metabolism clone). They only existed in the case of metabolic activation with medium stimulation intensity (Et 5/y) and disappeared when latex metabolic activation was increased by a higher stimulation intensity (Et 12/y).

Spatial Extension of the Latex Regeneration Area—Relation with Rubber Production

Figures 3a, b and *c* show the probable latex regeneration areas in dark grey. On such a basis, spatial extension of latex regeneration areas could be fairly precisely estimated. *Table 2* shows the estimated areas on both

TABLE 1. MEAN TRUNK VALUES FOR LATEX SUCROSE AND LATEX INORGANIC PHOSPHORUS OBSERVED IN THE 12 SAMPLED TREES.

Clone	Parameter (mM L^{-1})	Untapped	Tapped *(Nil stimulation)*	Tapped (Et 5/y)	Tapped (Et 12/y)
GT 1	Suc	41.5	15.7	15.4	14.1
	P	3.1	4.2	5.5	4.5
RRIM 600	Suc	24.3	7.9	10.8	7.9
	P	3.4	4.5	5.7	5.5
PB 235	Suc	35.0	7.3	6.4	5.2
	P	3.5	11.8	14.9	22.5

TABLE 2. EXTENSION OF THE ESTIMATED LATEX REGENERATION AREAS (LOW SUC, HIGH P) IN THE NINE TAPPED TREES[a]

Clone	Ethephon stimulation	Height of tapped panel (h_A,cm)	Height of untapped panel (h_B, cm)	Total height (h_A+h_B, cm)	Trunk semi-circumference (cm)	Area (cm^2)	Production (g/t/t)
GT 1	Nil stimulation						
	(0/y)	60	0	60	27.4	1644	13.7
	5/y	95	0	95	26.1	2475	19.9
	12/y	95	0	95	26.8	2541	21.9
RRIM 600	Nil stimulation						
	(0/y)	100	0	100	29.9	2990	40.3
	5/y	100	0	100	27.2	2715	40.0
	12/y	160	40	200	28.9	5770	52.4
PB 235	Nil stimulation						
	(0/y)	150	40	190	25.8	4902	54.1
	5/y	170	40	210	26.3	5513	39.7
	12/y	200	40	240	27.8	6672	76.5

[a]Height on tapped panel: h_A; height on untapped panel: h_B; and corresponding total area expressed in cm^2. Resulting rubber production is expressed in g/t/t and corresponds to production in the last month before latex sampling.

panels for the three clones studied, depending on exploitation intensity.

A comparison of the regeneration area, estimated by the product of the total height of latex regenerating bark and monthly dry rubber production at the moment of latex sampling, expressed in g/t/t, showed a highly significant linear relation ($r^2 = 0.84^{**}$) (*Figure 4*).

Estimated latex regeneration areas logically increased together with rubber production. The linear regression curve (P = 0.01 Area) indicated that it took, on average, a 100 cm^2 latex regeneration area, draining an equivalent latex volume of 3 mL (with a latex DRC estimated at 33%), to regenerate 1 gram of dry rubber. The regression equation had no constant term, it was also logical that no production implied

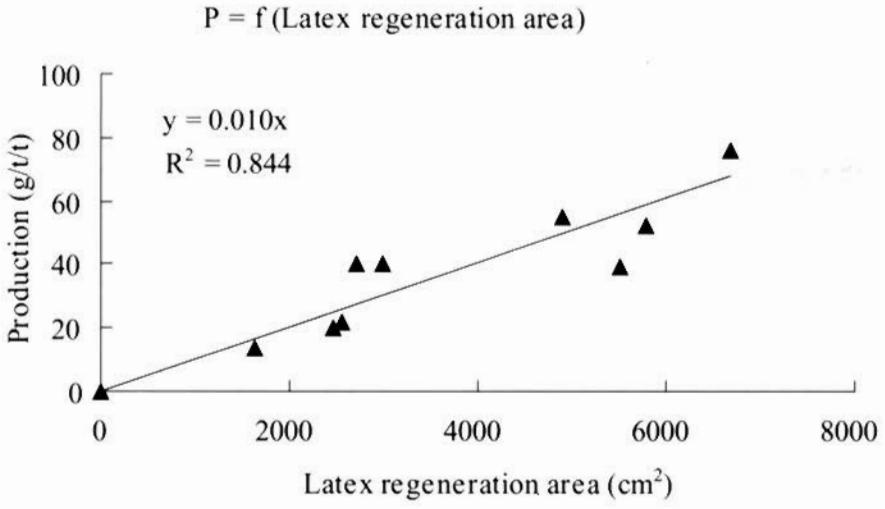


Figure 4. Multi-clonal linear regression between rubber production (the last month before latex sampling), expressed in g/t/t, and the estimated latex regeneration area, expressed in cm².

no latex regeneration. It was noteworthy that the three clones fitted the same curve, implying that the density of tapped latex vessels did not vary greatly between the three clones and that tapping depth was comparable in the three experimental plots.

In order to check the accuracy of the proposed relation, it was possible to estimate the useful thickness of latex tissues (T), allowing equivalence between the estimated regeneration area and the corresponding drained latex volume, as shown below:

$$V = A \times T \qquad \ldots 1$$

where V is the volume (m^3); A is the area (m^2) and T is the thickness. Thus V = 3 mL (3.1^{-6} m^3); A = 100 cm^2 (10^{-2} m^2); and T is 3.1^{-6} / 10^{-2}. T$\Longleftrightarrow$T = 3.1^{-4} m = 300 µm.

The calculated useful latex tissue thickness (T) was estimated at 300 µm. As the average diameter of the latex vessel including membranes was 25 µm[21], and the internal diameter excluding membranes was approximately 20 µm[22], our estimation of the latex regeneration area amounted to 15 (300/20) efficient latex rings, as each latex ring consisted of a single latex vessel layer[22].

An average of 25 latex rings has been reported in the tapped bark of most Wickham *H. brasiliensis* clones[23]. It has further been stated that around 60% of the latex rings are routinely tapped under normal tapping practice, as the remaining 40% are near the cambium and therefore remain untapped[21], concluding that 15 latex rings are actually tapped, which corresponds to our estimation.

DISCUSSION AND CONCLUSION

It was confirmed that the latex sink induced by tapping modified the whole physiological behaviour of the tree.

In comparison with untapped trees, tapping, with or without ethephon stimulation, induced massive depletion (–60% to –90%) of latex Suc concentration on a whole trunk scale, even in areas which were not concerned with latex regeneration *stricto sensu*. Latex

regeneration induced by tapping had a significant physiological effect on a whole tree scale, at least on latex sugar reserves, although it proved to be a rather local process. This was in accordance with the major negative effect of rubber production on growth and biomass creation in tapped rubber trees. In fact, other studies have reported that the biomass loss in tapped trees, when compared to untapped controls, could not be explained by rubber production only[1–6].

Tapping was thus confirmed to modify the whole physiological behaviour of the tree. As it occurred in latex, a general decrease in sugar reserves on a whole tree scale in other storage sinks might therefore be suspected. Such a depleting effect of rubber production on whole tree sugar reserves might be a possible explanation for the apparent discrepancy between rubber production and its huge effect on tree growth.

The results of this study on latex metabolic activity were based on a comparative description of latex Suc content and concurrent latex inorganic P content in several areas of the trunk bark of *H. brasiliensis*, with a broad pattern of exploitation intensities and on three different clones. They confirmed previous studies on the latex regeneration area[10,24–27]. Tapping created a significant depletion of latex sucrose content in the tapped panel, due to its consumption for rubber regeneration. Such a depression in latex Suc content was increased by the use of ethephon stimulation, as rubber production, and therefore latex regeneration, increased. Accurate estimation of latex metabolic activity through the level of latex P was confirmed.

The combined analysis of latex Suc and latex P levels enabled a precise and easy description of the shape and size of the metabolically active bark area (area with high P). Depending on exploitation intensity, the high metabolic activity area extended to the whole tapped panel, including bark areas above the tapping cut, and also to the basal level of the untapped panel. Depending on the associated Suc level, this high metabolic activity area could be divided into two distinct secondary areas:

- An area with both low Suc and high P, close to the tapping cut which could be considered as the actual latex regeneration area. In the study case, irrespective of the clone, that area increased by 100 cm^2 when production at the time of sampling increased by 1 g/t/t. Spatial extension of the area was clearly related to the clonal latex metabolic typology, as its expansion required less intense exploitation when the clonal latex metabolism was more active[19].
- An area with both high Suc and high P, more distant from the tapping cut, corresponding to a highly active sucrose import area, whose function is still unknown (accumulation of sugars for later latex regeneration?) but it did not seem to participate in the latex regeneration process *stricto sensu*.

The fact that the latex regeneration bark area was mostly located on the tapped panel, below and above the tapping cut, confirmed earlier results from other studies using different methods, such as radio-labelled isotopes[7,28] or turgor pressure measurements[8–9].

Nevertheless, this study also demonstrated that using ethephon stimulation (or more generally, higher rubber production) could extend the latex regeneration area at least to the basal level of the opposite untapped panel.

A frequently increasing bottom-upward gradient for latex Suc concentration along the trunk was observed on untapped trees or on the untapped panel of tapped trees. This confirmed the results on untapped trees[10] of PR 107, and

it was suggested that latex sucrose loading might be in equilibrium with the phloem sap concentration gradient (vertical long-distance translocation of sucrose in phloem sieve tubes). If proved, such equilibrium would argue in favour of symplastic latex sucrose loading, not depending on latex metabolism, plasmalemma ATPase activity and H^+/sucrose plasmalemma symporters[29]. However, the absence of functional plasmodesmata between laticifers and neighbouring parenchyma cells made the hypothesis of symplastic latex sucrose loading rather improbable[22]. For that reason, a high Suc content in latex extracted from untapped trees, as well as increasing bottom-upward concentration gradients in rather inactive bark areas (untapped tree, untapped panel of tapped trees), remained unexplained as far as sugar loading mechanisms were concerned.

We found significant clues supporting apoplastic latex Suc loading: the presence of Suc accumulation areas on the lower parts of tapped panels on stimulated GT 1 and RRIM 600 clones reflected probable massive activation of the proton-sucrose transmembrane symport to the laticiferous system[29–31]. However, it was impossible to conclude if the increased sink effect in metabolically active bark areas was due to:

- The direct effect of ethylene released following ethephon stimulation
- Increased rubber production and subsequent increased metabolic activation resulting from such stimulation
- The two factors interacting together.

Likewise, earlier studies have showed that such enhancement of Suc import could also be found on the untapped panel[32] and was significantly related to latex metabolic activation in those bark areas. The relative extent of this sink effect in bark regions located outside the latex regeneration area *stricto sensu* might be another clue explaining the discrepancy between rubber production and the associated negative effect on growth and biomass creation in tapped rubber trees. All carbohydrates stored inside latex cells, even in areas outside the latex regeneration area, were no longer available for any other metabolism; in particular, for primary biomass creation (*i.e.* growth). Moreover, such increased sink strength might also have been responsible for some still unexplained findings. In particular, over-exploitation might have led to a decrease in production but not to any concurrent growth recovery[1–2], as the latex sink effect might still have been substantial in such a case.

This study presents the first combined delimitation and quantification of latex metabolic activity inside the bark of untapped and tapped rubber trees on the whole trunk, using simple biochemical parameters. The LDM technique is thus proved to be a very useful and powerful tool for studying the physiology of the latex producing bark of *H. brasiliensis*. It enables latex sampling in any part of the trunk bark, as tapping is not required (puncture latex sampling), and is also much simpler and economical when compared to the methods previously used to study the limits of the regeneration area, like the formerly used radio-labelled isotopes[7,28] or turgor pressure measurements[8–9]. Moreover, earlier methods only described the size and shape of the latex regeneration area, but could not help in quantifying the metabolic activities of the different bark areas involved in or affected by the latex regeneration process.

Further histological studies and quantitative analyses of carbohydrates in bark and wood are required to assess the relations existing between latex sugar content and the carbohydrate content of the surrounding bark tissues and wood (soluble sugars, starch and the like). These data, along with a scheduled survey of bark respiration and biomass increment, should provide a clearer understanding of the

physiological mechanisms involved in the regulation of assimilate partitioning (wood creation, rubber production, respiration and carbohydrate storage) in tapped *H. brasiliensis* and its response to exploitation intensification.

Such research methodologies could also be used to provide new ideas for finding and/or optimising new tapping systems, such as ethylene gas stimulation systems, micro-tapping cut systems and multi-tapping cut systems, based on better physiological knowledge of functioning in the whole rubber tree.

Indeed, LDM may provide some important clues to explain previously unexplained results. For instance, preliminary LDM results on double-cut alternative tapping system experiments resulting in a significant increase in production[18,33] have concurrently shown a significant increase in the latex regeneration area, providing initial explanations for the extra yield obtained[34].

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REFERENCES

1. GOHET, E. (1996) The Production of Latex by *Hevea brasiliensis*. Relation with Growth. Influence of Different Factors: Clonal Origin, Hormonal Stimulation, Carbohydrate Reserves. Ph.D. Thesis, Montpellier II University, France.

2. GOHET, E., PRÉVÔT, J.C., ESCHBACH, J.M., CLÉMENT, A. AND JACOB, J.L. (1996) Clone, Croissance et Stimulation, Facteurs de la Production de Latex chez *Hevea brasiliensis*. *Plantations, Recherche, Développement*, **3(1)**, 30–38.

3. SETHURAJ, M.R. (1981) Yield Components in *Hevea brasiliensis*. *Plant Cell Environ.*, **4**, 81–83.

4. SETHURAJ, M.R. (1985) Physiology of Growth and Yield in *Hevea brasiliensis*. *Proc. Int. Rubb. Conf. Kuala Lumpur 1985* (*Rajarao J.C. and Amin L.L. eds.*), **5**, p. 3–19. Kuala Lumpur: Rubber Research Institute of Malaysia.

5. TEMPLETON, J.K. (1969) Partition of Assimilates. *J. Rub. Res. Inst. Malaya*, **21(3)**, 259–273.

6. WYCHERLEY, P.R. (1976) Tapping and Partition. *J. Rubb. Res. Inst. Malaysia*, **24(4)**, 169–194.

7. LUSTINEC, J. AND RESING, W.L. (1965) Methods for Delimitation of the Flow Area by Micro-tapping and Radio-isotopes. *Plant. Bull. Rubber Res. Inst. Malaya*, **80**, 144–149.

8. BUTTERY, B.R. AND BOATMAN, S.G. (1966) Manometric Measurement of Turgor Pressures in Laticiferous Phloem Tissues. *J. Exp. Bot.*, **17**, 283–296.

9. PAKIANATHAN, S.W., WAIN, R.L. AND NG, E.K. (1975) Studies on Displacement Area on Tapping Immature *Hevea* Trees. *Proc. Int. Rubber Conf., Rubber Research Institute Malaysia, Kuala Lumpur, 1976*, 225–248.

10. TUPY, J. (1973) The Level and Distribution Pattern of Latex Sucrose along the Trunk of *Hevea brasiliensis* Muell. Arg. as Affected by the Sink Region Induced by Latex Tapping. *Physiol. Vég.*, **11(1)**, 1–11.

11. ESCHBACH, J.M., ROUSSEL, D., VAN DE SYPE, H., JACOB, J.L. AND d' AUZAC, J. (1984) Relationship between Yield and Clonal Physiological Characteristics of Latex from *Hevea brasiliensis*. *Physiol. Vég.*, **22**, 295–304.

12. JACOB, J.L., ESCHBACH, J.M., PRÉVÔT, J.C., ROUSSEL, D., LACROTTE, R., CHRESTIN, H. AND d'AUZAC, J. (1985) Physiological Basis for Latex Diagnosis of Functioning of the Laticiferous System in Rubber Trees. *Proc. Int. Rubb. Conf. Kuala Lumpur 1985* (*Rajarao J.C. and Amin L.L. eds.*). **5**, p. 43–65. Kuala Lumpur: Rubber Research Institute of Malaysia.

13. JACOB, J.L., SERRES, E., PRÉVÔT, J.C., LACROTTE, R., CLÉMENT-VIDAL, A., ESCHBACH, J.M. AND OMONT, H. (1988) Mise au Point du Diagnostic Latex. *Agritrop.*, **12**, 97–118.

14. JACOB, J.L., PRÉVÔT, J.C. AND CLÉMENT-VIDAL, A. (1988) Physiological Parameters of Latex from *Hevea brasiliensis*: Their Uses in the Study of the Laticiferous System: Typology of Functioning of Production Mechanisms: Effect of stimulation. *Proceedings of the IRRDB Rubber Physiology and Exploitation Meeting. Hainan, China, 9-12 December 1986*, (*Pan Yanqing and Zhao Canwen, eds.*) p. 136–157 Hainan: South China Academy of Tropical Crops.

15. JACOB, J.L., PREVOT, J.C., LACROTTE, R. AND ESCHBACH, J.M. (1995) Le Diagnostic Latex. *Plantations, Recherche, Développement*, **2(2)**, 34–37.

16. GOHET, E. AND CHANTUMA, P. (1999) Micro-Latex Diagnosis: Standard Analysis Procedure. Chachoengsao Rubber Research Center. RRIT-DOA-MOAC, November 1999.

17. GOHET, E., DIAN, K., PRÉVÔT, J.C., OBOUAYEBA, S., KÉLI, J.Z., d'AUZAC, J. AND JACOB, J.L. (1997) Relation between Latex Sugar Content, Latex Metabolic Activity and Yield Potential of *Hevea brasiliensis* Clones. *Symposium IRRDB. IRRDB Annual meeting, Ho Chi Minh City, Vietnam, 11–13 October 1997.*

18. GOHET. E. AND CHANTUMA, P. (2003) Reduced Tapping Frequency and DCA Tapping Systems: Research towards Improvement of Thailand Rubber Plantations Productivity. *IRRDB Symposium. IRRDB Annual Meeting, Chiang Mai, Thailand, 15–16 September 2003.*

19. GOHET, E., CHANTUMA, P., LACOTE, R., OBOUAYEBA, S., DIAN, K., CLEMENT-DEMANGE, A., DADANG KURNIA AND ESCHBACH, J.M. (2003) Latex Clonal Typology of *Hevea brasiliensis*: Physiological Modelling of Yield Potential and Clonal Response to Ethephon Stimulation. *Proceedings of the IRRDB International Workshop on Exploitation Technology, Kottayam, Kerala, India, 15–18 December 2003.*

20. JACOB, J.L., PRÉVÔT, J.C., LACROTTE, R., CLÉMENT, A., SERRES, E, AND GOHET, E. (1995) Typologie Clonale du Fonctionnement des Laticifères chez *Hevea brasiliensis*. *Plantations, Recherche, Développement*, **2(5)**, 43–49.

21. GOMEZ, J.B. (1982) *Anatomy of* Hevea *and its Influence on Latex Production, MRRDB Monograph No. 7*. Kuala Lumpur: Malaysian Rubber Research Development Board, pp. 75.

22. DE FAY, E., HEBANT, C. AND JACOB, J.L. (1989) Cytology and Cytochemistry of the Laticiferous System, *Physiology of Rubber Tree Latex* (*d'Auzac, J., Jacob, J.L. and Chrestin, H., eds.*), 15–29. Boca Raton, Florida: CRC Press Inc.

23. GOMEZ, J.B., NARAYANAN, R. AND CHEN, K.T. (1972) Some Structural Factors Affecting the Productivity of *Hevea brasiliensis*. I. Quantitative Determination of the Laticiferous Tissue. *J. Rubb. Res. Inst. Malaya*, **23(3)**, 193–204.

24. D'AUZAC, J. AND PUJARNISCLE, S. (1961) Aperçu sur L'étude des Glucides de L'hévéa

et sur leur Variation. *Rev. gén. Caout. Plast.*, **38**, 1131–1139.

25. TUPY, J. (1969) Stimulatory Effects of 2,4-dichlorophenoxy Acetic Acid and of 1-Naphthylacetic Acid on Sucrose Level, Invertase Activity and Sucrose Utilization in the Latex of *Hevea brasiliensis*. *Planta*, **88**, 144–153.

26. TUPY, J. (1973) The Sucrose Mobilizing Effect of Auxins in *Hevea brasiliensis* Muell. Arg.; Dependence on the Metabolic Activity of the Treated Tissue. *Physiol. Vég.*, **11(1)**, 13–23.

27. TUPY, J. (1985) Some Aspects of Sucrose Transport and Utilization in Latex Producing Bark of *Hevea brasiliensis* Muell. Arg. *Biol. Plant.*, **27(1)**, 51–64.

28. LUSTINEC, J., SIMMER, J. AND RESING, W.L. (1969) Trunk Contraction of *Hevea brasiliensis* due to Tapping. *Biol. Plant.*, **11**, 236–244.

29. PATRICK, J.W. (1990) Sieve Element Unloading: Cellular Pathway, Mechanism and Control. *Physiol. Plant.*, **78**, 298–308.

30. LACROTTE, R. (1991) Study of the Relations between Latex Sucrose Content and Rubber Production. Approach of Sucrose Loading Mechanisms Within the Laticiferous Cells of *Hevea brasiliensis*. Ph.D. Thesis, Montpellier II University, France.

31. PATRICK, J.W. AND OFFLER, C. (1996) Post-sieve Element Transport of Photoassimilates in Sink Regions. *J. Exp. Bot.*, **48**, 1165–1177.

32. SILPI, U., CHANTUMA, P., KOSAISAWE, J., THANISAWANYANGKURA, S. AND GOHET, E. (2001) Distribution Pattern of Latex Sucrose and Metabolic Activity in Response to Tapping and Ethrel Stimulation in Latex Producing Bark of *Hevea brasiliensis* Müll. Arg., *Annual IRRDB Symposium (J. Sainte-Beuve, ed.)*. Montpellier, France: CIRAD.

33. GOHET, E. AND CHANTUMA, P. (2003) 'Double Cut Alternative' Tapping System (DCA): Towards Improvement of Yield and Labour Productivity of Thailand Rubber Smallholdings. *IRRDB Workshop Exploitation Technology, Kottayam, India, 15-18 December 2003*.

34. CHANTUMA, P. AND GOHET, E. (2004) Latex Diagnosis Mapping on Double Cut Alternative Tapping System (unpublished).