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Increase in Carbohydrate Status in the Wood and Bark Tissues of *Hevea brasiliensis* by Double-cut Alternative Tapping System

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ABSTRACT

Starch and total soluble sugar (SS) status in rubber tree were investigated carbohydrate reserve related to tapping system. RRIM 600 clone were studied for three years in Chachoengsao Rubber Research Center (CRRC-RRIT-DOA). Trees were tapped using 1/2S d/2 (control, D/2), 1/2S d/3.ET 2.5%,8/y (D/3) and the DCA system (double-cut alternative tapping system). Bark and wood tissue were collected in October 2002 (period of high rainfall and high latex production) at various positions along trunks and were analyzed using starch enzymatic analysis technique. Together with a higher production of latex, DCA system had higher content in both starch and SS in wood. In bark, SS was also higher in DCA, whereas starch was not different among treatments. According to accumulation of starch on various part of tree, it was found that the starch decreased with height of samples up the trunk. Content in lower part of tree was higher than that in the top or crown part, but there was the decrease of starch in both wood and bark within the bark regeneration area or the area which has been already tapped, confirming the hypothesis that tapping affects the level and distribution pattern of carbohydrate within the trunk.

Key words: *Hevea brasiliensis*, latex, sucrose content, metabolic activity, carbohydrate, tapping systems

INTRODUCTION

The exploitation system is very specific, since in response to tapping (cutting trunk bark every two to five days), a secondary metabolism is induced which uses assimilates that are artificially derived from the other sinks (Templeton, 1969; Wycherley, 1976; Jacob *et al*., 1998).

First attempts to establish rubber tree carbon balance (Sethuraj, 1981; Gomez *et al*., 1989) seem to indicate that carbon availability is not likely to be limiting at the tree scale, but only locally, around the tapping panel (exploited trunk area). Previous works (Gohet,1996; Gohet *et al*., 1998; Jacob *et al*.,1998) have shown that sucrose content within laticiferous vessels is often a factor limiting production: intensive exploitation induces a shortage of intra-laticiferous sucrose, particularly when production is stimulated by the application of ethephon. On the other hand, some clones are able to maintain a relatively high level of sucrose despite a high production of latex. These clonal differences may be related to the ability to mobilise

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reserves as a consequence of differences in laticiferous sucrose loading capacity and/or availability of wood starch. First studies (histo-cytological localisation) by Gohet (1996) showed that the cumulative effect of tapping resulted in a shortage of starch within superficial wood layers behind the tapping panel, whereas starch accumulated above the tapping cut. Assessing formation, distribution and mobilisation of reserve metabolites, should thereby help to manage tapping systems.

The present study aims at assessing the impact of a new tapping system, double cut alternated system (DCA), designed to increase latex yield without any external input like ethephon stimulation. Thus, the aim of the present paper is to compare DCA to standard systems and to assess if the observed carbohydrate patterns relate to yield performance.

MATERIALS AND METHODS

Plant materials

The measurements and analyses were performed in RRIM600 clone, planted in Chachoengsao Rubber Research Center (CRRC) in 1992 and opened for tapping in May 2000. The tapping systems used were (i)1/2S d/2 (a half spiral cut tapped every two days) named D/2, (ii) 1/2S d/3 ET 2.5%, 8/y (a half spiral cut, one day in tapping followed by two days of rest, stimulated with ethephon, eight applications per year) named D/3 and (iii) Double Cut Alternate, 2x1/2S d/4 (ET2.5%, 2x4/y) named DCA. In the later system, there were two half spiral cuts, one on each side (panel) of the tree. Each cut is tapped every four days alternately, so that the tapping frequency at tree scale is D/2, whereas it is D/4 for each panel. In the present case, four ethephon applications per panel or eight applications per year were applied. Opening was made at 1.5 m on one side and at 0.8 m on the other side, so as keeping a large distance between tapped areas. The tapped panel in D/2 and D/3 is named panel A, and the untapped one is panel B. In DCA panel A is the low cut and panel B is the high cut (Figure1)

In each treatment, the five sampled trees were chosen as presenting homogeneous rubber production (quantity and dynamics) and girth compared to the average of each treatment.

Methods

Sampling was performed in October 2002 (year 3 of tapping), period of high rainfall and high latex yield. Samples consisted in 0.5 cm diameter, 5 cm long cores, including 1cm of bark and 4 cm of wood, which were immediately separated. Sixteen samples were taken from the base of the tree up to 3 m (8 samples on tapped panel or panel A, including renewing bark area, and 8 samples on panel B) (Figure 1).

After the core sampling was made, it was soaked immediately in liquid nitrogen and was kept in cryo-tube immersed in liquid nitrogen until transfer to the laboratory and stored at -80 °C, until freeze-drying using a -50 °C freeze-dryer (Telstar Cryodos, Spain). Thereafter, the samples were blended using ball-blender MM200 (Retsch, Germany), ball diameter 7 mm. Storage after this step until extraction and chemical analysis was at -80 °C.

Biochemical analysis

The powder of wood and bark was re-dried in the oven for 2 hours at 65°C. Soluble sugars were extracted from 20 mg samples with 80% ethanol during 30 min at 80°C, then centrifuged. This step was repeated twice, first with 80% ethanol and then with 50% ethanol and all the supernatants were pooled. The sediment, which contained starch, was filled with 80% ethanol and kept at -80 °C until analysis. The supernatant was filtered in crushed glass mini columns added with a mixture of polyvinyl polypyrrolidone and activated charcoal to eliminate pigments and polyphenols. Ethanol was

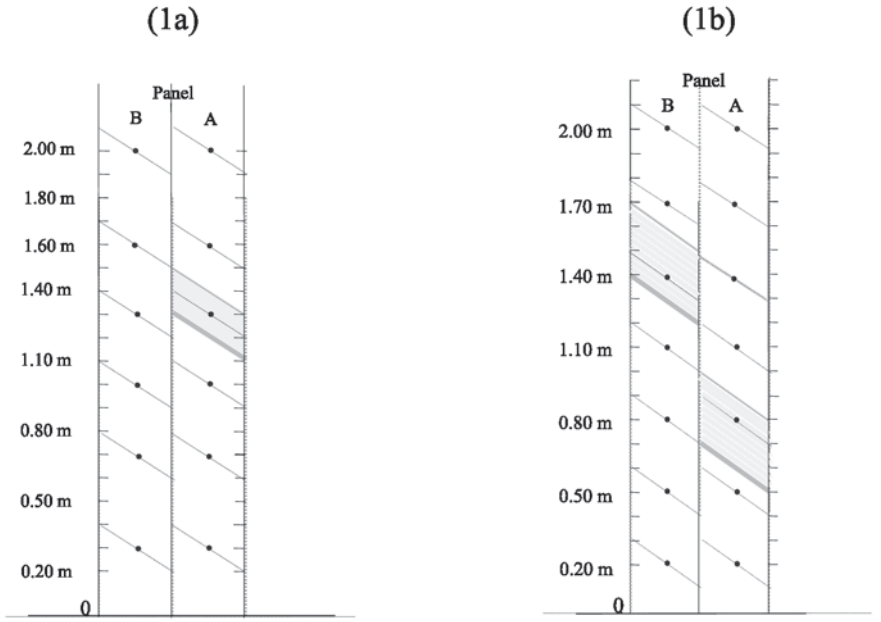

Figure 1 Diagram of rubber trees sampled on both panel A and B tapped (1a) treatment 1/2S d/2, 1/2S d/3 and (1b) DCA system.

evaporated using a vacuum dryer (Maxi Dry Plus, Heto, Denmark). Soluble sugars (SS) and starch were quantified by enzymatic analysis. Sucrose was transformed into glucose and fructose by invertase (β-fructofuranosidase). The glucose and fructose were quantified using hexokinase, glucose-6-phosphate-dehydrogenase and phosphoglucose isomerase followed by spectrophotometry of resulting NADPH at 340 nm. For starch analysis, after the ethanol was evaporated, the sediment was hydrolysed with NaOH 0.02N for 1.5h at 90°C, then with α-amyloglucosidase for 1 h at 50°C and then glucose was quantified as described above. The results were expressed as mg glucose equivalent per gram of structural dry matter (mg_G/g_{SDM}, mg Glu equi./g structural DM). Sum of starch and soluble sugar represented the total non-structural carbohydrate (TNC) (Boehringer, 1984).

RESULTS AND DISCUSSION

Latex yield of DCA (5.62 kg/tree/year) was actually higher than that of D/2 (4.40 kg/tree/year) and D/3 (3.85 kg/tree/year).

1. Composition of total non structural carbohydrate (TNC)

Starch was the major form of TNC, with a lower proportion of sucrose. Glucose and fructose accounted for a negligible proportion. As the most species, starch was the major TNC, and changes in TNC were mainly due to changes in starch concentration. Whereas sucrose was the dominate soluble sugar (SS). Our results liked the previous work, which reported for beech and oak (Barbaroux *et al.,* 2003), poplar (Witt and Sauter, 1994) and Walnut (Lacointe *et al*., 1993).

At trunk scale, mean TNC was higher in wood (58.8-70.2 mg_G/g_{SDM}) than in bark (43.3-50.4 mg_G/g_{SDM}). This resulted showed higher starch concentration in wood. On the other hand, SS in wood (6.5-9.6 mg_G/g_{SDM}) was lower than in bark (12.3-18.6 mg_G/g_{SDM}). As a result proportion of SS to TNC was much higher in bark than in wood (Table 1).

Table 1 Relation between dry latex yield (kg_{DM}/tree/years), non structural carbohydrate (TNC, starch and total soluble sugar, SS) (mg_G/g_{SDM}) measured inside wood and bark tissue from May 2000 - December 2002.

Tapping system	Yield (kg_{DM}/t/y)	Carbohydrate (mg_G/g_{SDM})					
		Starch		SS		TNC	
		Wood	Bark	Wood	Bark	Wood	Bark
1/2S d/2	4.40 b	63.6 a	31.0 a	6.5 bc	12.3 bc	70.2 a	43.3 b
1/2S d/3.ET	3.85 bc	51.2 b	33.6 a	7.6 b	15.8 b	58.8 b	49.5 a
DCA	5.62 a	60.4 a	31.8 a	9.6 a	18.6 a	70.0 a	50.4 a

Treatments with same letters are not significantly different. DMRT Test $P<0.05$

Mean TNC content in wood was higher in D/2 and DCA (70.2 and 70.0 mg_G/g_{SDM} respectively) than in D/3 (58.8 mg_G/g_{SDM}). Difference between D/2 and D/3 was a result of higher starch content in D/2, whereas SS did not differ significantly. On the contrary, in DCA both starch and SS were higher than in D/3.

In bark, starch content did not change among treatments, whereas SS was higher in DCA than in the two other treatments. Although neither starch nor SS differed significantly between D/2 and D/3, the resultant TNC was higher in D/3. Thereby TNC in bark was the same in D/3 and in DCA.

Proportion of SS to TNC was higher in DCA, medium in D/3 and lower in D/2 for both bark and wood. Concentration of TNC in bark as compared to wood was higher in D/3 than in the two other treatments.

Thus the trend was a higher TNC for the higher production of latex. For DCA, this was proved true not only for starch in wood - as in D/2 - , but also for SS in both bark and wood.

Laticiferous vessels, where latex is produced, are located within the phloem, which was a part of the bark (Bobilioff, 1923; Gomez,1982; De Fay and Jacob, 1989). Therefore carbohydrate resources in the bark are likely to be directly involved in latex regeneration whereas carbohydrate resources in the wood parenchyma may represent a more distant tank, connected to bark through medullar rays. Similarly, sucrose as the transport form of carbohydrate and the basic metabolite for rubber biosynthesis, maybe considered as the directly available carbohydrate source, whereas starch is the reserve compound, which has to be hydrolysed into soluble sugar to be used. Thereby, we propose the following classification: starch in wood is the reserve compartment, whereas SS in wood is the transport compartment. In bark, starch is the local reserve compartment and SS is the ready-to-use compartment.

This experiment confirmed that enhancing carbohydrate demand for latex regeneration enhanced the sink strength of reserve, as represented by starch content in wood. DCA and D/2 had both higher latex yield and higher reserve.

Moreover, in DCA mobilization of reserves towards transport form seemed also higher since SS in wood was higher than in D/2.

In bark, the local reserve tank, represented by starch, seemed to behave as a buffer, as its content was the same among treatments with different yield.

The ready-to-use SS in bark was higher in DCA despite a higher consumption for latex regeneration, indicating a high reserve mobilization capacity with this system. On the contrary, D/2 had the lowest SS in bark and in wood together with high starch in wood and medium latex yield. This could indicate that reserve mobilization is limiting in this tapping system.

With D/3 system, accumulation of reserve in wood seemed the limiting point, as indicated by the lowest starch, but not SS, in wood and the highest proportion of TNC in bark as compared to wood.

Higher TNC in bark in D/3 stimulated than in D/2 non-stimulated may be due to the effect of ethylene stimulation, as ethylene enhances carbohydrate mobilization in trees (Eklund, 1998) and particularly increases latex metabolism (d' Auzac,1997).

2. Vertical distribution (Figure 2 and 3)

The general pattern was a decreasing bottom-up gradient in starch concentration along the trunk. The direction of this gradient was in accordance with results on other species (Kozlowski, 1992). For SS, there was no clear gradient. Tapping had a huge impact on vertical distribution of carbohydrate in trunk, both for starch and SS.

In wood, the impact was particularly marked for D/3 and for DCA. In D/3, the difference between tapped and untapped panel was very clear: higher content in the bottom part and lower content in the bark regeneration area above the cut, in tapped panel, instead of just a slight bottom-up decreasing gradient in untapped panel. In DCA the occurrence of two cuts at different height on each side of the tree allowed to confirm that the vertical variations in starch content were due to tapping. Lowest content were recorded in the renewing bark area of each panel, situated at different height. On the contrary, below the cut, in what is considered as the main regeneration area for latex, starch content was the highest, particularly in panel B, where the cut was still high enough. There was less change in SS content along the trunk in wood, but the trend was opposite to starch, with a slight peak of SS in the renewing bark area for tapped panel in D/3 and in both panels in DCA. In D/2, patterns were less consistent.

In bark there was no clear vertical gradient for starch but important variations related to tapping. Starch was lower in the renewed bark, located in panel A for the three treatments, and also in the second renewing area located in panel B for DCA only. Surprisingly there was also a decrease at the same height in panel B in D/3, whereas there was no tapping in this panel. Nevertheless, the decrease was less important than in the tapped panel. SS concentration did not show clear vertical trend in D/2 with almost no change along the trunk, and a slight increasing bottom-up gradient in D/3. Thereby, in the renewing bark SS was not much lower, and in some case higher, than starch, whereas it was lower anywhere else. In DCA there was a clearer vertical gradient together with opposite vertical variations in starch and SS within the renewing bark.

These results seemed opposite to histological studies by Gohet (1996) showing a decrease in starch just below the tapping cut and an accumulation above it, within the renewing bark area. However, the study by Gohet relied on histological data within the outermost 2 mm parenchyma, whereas our study was based on quantitative chemical analysis on 4 cm-long cores of wood and 1 cm cores of bark. Therefore, we can infer that tapping depletes starch in the proximal parenchyma due to increased transfer of sucrose to laticiferous vessels, but that the sink effect induces an increase in TNC in the deeper parenchyma.

DCA showed clearly that the highest concentrations of starch are measured below the tapping cut in both wood and bark, strengthening the hypothesis that enhancing carbohydrate demand for latex regeneration enhances the sink strength of carbohydrate reserve too.

Lower starch content in wood within the bark regeneration area maybe explained by (i) no latex regeneration in this area, (ii) a poor vascular connection due to the removal of functional phloem by tapping.

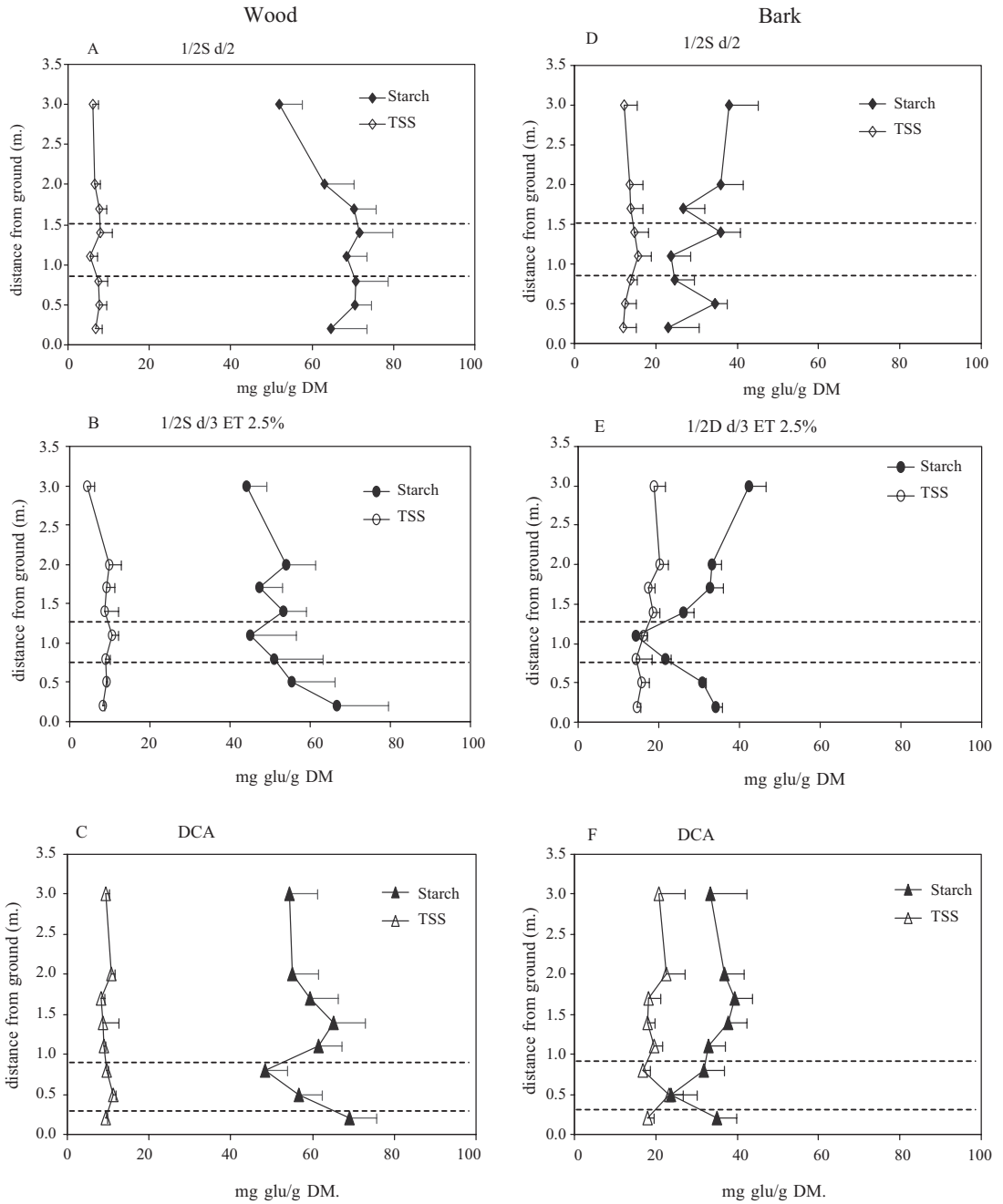

Figure 2 Vertical distribution of starch and SS along the trunk up to 3 m from ground level in wood (2A, 2B and 2C) and bark (2D, 2E and 2F) on panel A of RRIM 600 clone, compared among tapping systems.

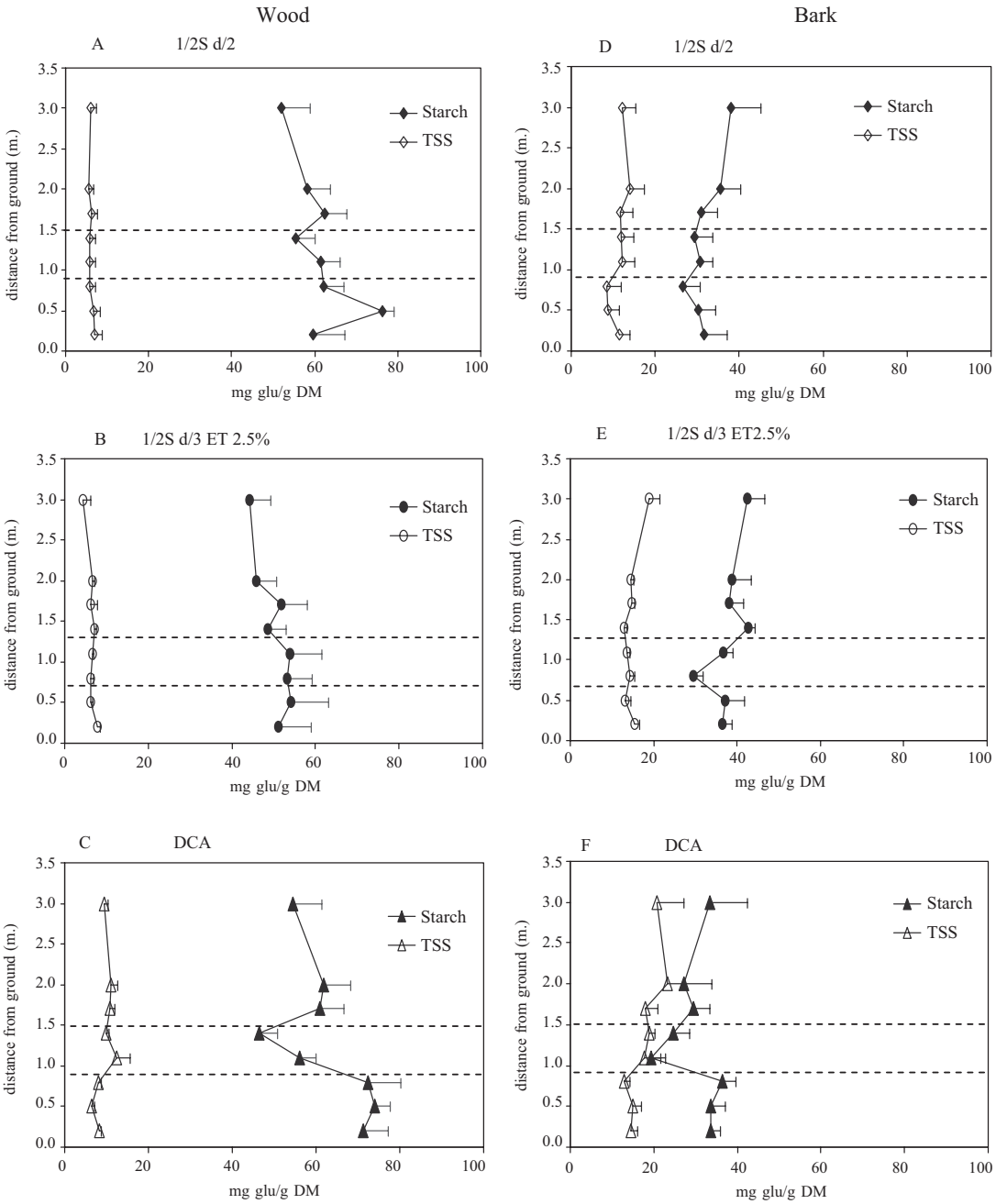

Figure 3 Vertical distribution of starch and SS along the trunk up to 3 m from ground level in wood (3A, 3B and 3C) and bark (3D, 3E and 3F) on panel B of RRIM 600 clone, compared among tapping systems.

CONCLUSION

Analysis of starch and soluble sugars in core samples of wood and bark of rubber trees proved an interesting tool to relate reserve dynamics to latex yield. Double cut alternate system (DCA), in which a longer time is allowed for regeneration of latex and where latex is drained alternately from two separated areas on each side of the trunk seemed the more able to accumulate carbohydrate reserves around the latex regeneration area and to mobilize such reserves. This may explain why DCA has both the highest production and the best metabolic profile (Gohet and Chantuma, 2003a, 2003b) as compared to classical systems using only one tapping cut. Control system 1/2S D/2 seemed able to accumulate starch, but less able to mobilize them, whereas 1/2S D/3 system with ethephon stimulation seemed on the contrary less able to accumulate starch.

From the present results obtained during the period of highest latex yield, study of carbohydrate reserve dynamics could be extended to the complete year cycle to describe not only the impact of tapping, but also interactions with phenology of the tree. Moreover, it would be of first interest to add an untapped control to the study.

Two other major aspects that require further investigation are (i) the enzymatic processes controlling starch synthesis and hydrolysis as well as sucrose synthesis, and (ii) the sucrose transport pathways and activity from parenchyma into laticiferous vessels.

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