DYNAMICS OF CARBOHYDRATE RESERVES AS RELATED TO TAPPING IN RUBBER TREE

INTRODUCTION

In Thailand, natural rubber is mostly produced by smallholdings, whose general small size has hindered implementation of tapping systems physiologically balanced with the potential of the rubber tree. Thai rubber smallholders generally use high tapping frequencies like 2d/3 (two days in tapping follow by one day rest), 3d/4 (three days in tapping follow by one day rest), 5d/6 (five days in tapping follow by one day rest) or even d/1 (daily tapping), associated with shortened tapping cut (1/3S, one-third spiral cut). Consequence of these intensive tapping systems is a general low productivity (output/tapper/day), leading to rather low tappers and planters incomes, as well as high TPD (tapping panel dryness) rates and short life-cycle of plantations. High frequency tapping system result in insufficient time for latex regeneration between consecutive tappings, reducing output per tree per tapping (Jacob *et al*., 1995, d′Auzac *et al*., 1997) and preventing the use of ethephon stimulation because of insufficient latex sugar content (Tupy and Primot 1976, Low and Gomez, 1982). High tapping panel dryness rates (Anekachai, 1989) is also a severe problem on production. Another concern with the generalized use of 1/3S of spiral by farmer is that when the last third shorts to be tapped, the virgin bar is surrounded by two areas of regenerating bark. Thus, it is like a huge “island bark” likely to have a low yield potential. Trends to open the trees to early, when they are not big enough aggravate the situation.

Thus, the issue to be addressed by research is not simple. As reducing the tapping frequency is not an option due to the small size of farms, how to increase rubber yield per tapping in very intensive systems? This would require increase of the absolute yield per tree.

For long, yield in 1/2S d/2 (one half spiral cut and tapping every 2 days) has been considered as the potential yield, only to be approached by other tapping system,

but the challenge is to propose a system that would exceed this potential. Moreover, as such system is meant to meet farmers' requirements, it should not involve any costly additional input.

The new tapping system named "DCA (Double Cut Alternative)" was designed to such a purpose. The principle of DCA, is to increase the latex regeneration time by splitting the tapping on two different tapping cuts (opposite panels), tapped alternately (t,t), avoiding as much as possible competition between these two cuts by maintaining, at all time, sufficient vertical distance (at least 75-80 cm) between their respective latex regeneration area. DCA involves "alternate" tapping but may also represent an "alternative" to currently used intensive tapping systems. It has been designed from former experimental studies on alternate tapping in Côte d'Ivoire (Cirad, unpublished data) and in Thailand (Anekachai, 1989), as well as from physiological studies regarding latex regeneration process and spatial extension.

Background physiological hypotheses on which lay the principles of DCA tapping systems have been validated: limitation of yield potential of very high tapping frequency tapping systems is mainly caused by limitation of regeneration time between consecutive tappings (Jacob *et al.,* 1988a, 1988b, 1988c, 1995).

However, before being able to recommend such a system, DCA long-term effects have to be assessed. The need to take into account the cumulative effects, from one year to another, of cultivation practices and climate conditions on the plant functioning is specific to perennial crops, which productivity has to be evaluated on a long term. Particularly, mobilization of carbohydrate reserves are likely to be of first importance as regard to competition between rubber production and the other functions sinks.

Tapping brings about a major change in the global carbon pool through the activation of latex metabolism. The regeneration process for latex induces the translocation of sucrose as their initial precursor to the new sink (the cells near the cut)

and a direct competition for carbohydrate assimilate exists between rubber production and growth. The extent of competition depends on the laticiferous sink size: it appears to be high when the laticiferous sink is high and conversely. Laticiferous sink size/activity depends on both clone and tapping system, so that there should be interactions between photosynthate accumulation, partition and utilization in latex and tree development. Assessing the time-evolution of reserve metabolites at the whole tree scale, will allow understand the antagonism between latex production and primary growth in order to preserve a balanced partition of assimilates between these two sinks, key for a high and sustainable productivity of rubber plantations (Wycherley, 1976; Gohet, 1996).

During the vegetative season, deciduous trees accumulate carbohydrate reserves, mainly as starch that constitutes the source of carbohydrate for maintenance respiration during wintering and for refoliation thereafter. For a large number of temperate forest and fruit trees, many researches have demonstrated the huge influence of carbohydrate reserves on production, metabolism, growth and resistance of trees to different stresses, showing that the accumulation of non-structural carbohydrates is particularly sensitive to environment and cultivation practices (Lacointe *et al.,* 1993; Kozlowski, 1992; Frossard and Lacointe, 1988; Glerum, 1980; Ziegler, 1964, Tromp, 1983; Kozlowski and Keller, 1966). However, works concerning tropical trees remain scarce (Bory and Clair-Maczulajtys, 1991).

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 (Jacob *et al*., 1998; Gohet, 1996; Gohet *et al*., 1998) have shown that sucrose content within laticiferous vessels is often a major factor limiting production: intensive exploitation induces a shortage of intra-laticiferous sucrose, particularly when production is stimulated by the application of ethylene generators. On the other hand, some clones are able to maintain a relatively high level of sucrose despite a high production of latex (Gohet, 1996; Gohet *et al*., 1998). These clonal differences may be related to the ability to mobilise 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) have shown 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 mobilization of reserve metabolites, should thereby help at managing tapping systems adapted to different agro-climatic conditions (location and size of cuts, panel management, tapping and stimulation frequency, tapping rest periods).

Experiments on various tapping systems, including the DCA system, will aim at estimating the trunk area influenced by tapping, quantify the carbon fluxes involved in latex production, reserve accumulation and mobilization.

The main objectives of this study were as following,

1. To assess carbohydrate reserve dynamics as related to tapping system parameters.
2. To assess the impact of DCA tapping system on trunk metabolism.
3. To provide tools to design adapted tapping systems.
4. To provide tools for rubber breeding.

LITERATURE REVIEW

Regularly tapping is known to activate to the entire mechanism involved in latex regeneration and sucrose content within laticiferous vessels is often a limiting production.

1. Latex Diagnosis (LD) : Predictive Tools for Yield Potential and Actual Exploitation Status

Latex diagnosis (LD) is the biochemical and biophysical analysis of some parameters from latex cytoplasm that provide useful data on the state of health of the laticiferous system. The analysis bases on the colorimetric reaction (Ashwell, 1957; Taussky and Shorr, 1953; Boyne and Ellman, 1972). The choice of the parameters to be analyzed depends on the degree of correlation, which can be established between these parameters and production under certain condition.

Latex sucrose content indicates the balance between the sugar uptake and utilization (catabolism to rubber). It indicates the strength of the laticiferous sink and also the sucrose loading capability of the producing tissues. A high sucrose content in latex may indicate good loading of the laticiferous cell which maybe accompanied by an active metabolism. Nevertheless, high sugar content in latex may also indicate low metabolic utilization of this sugar and hence finally low productivity.

Latex inorganic phosphorus content reflects the energy metabolism of the latex cells, hence the capability for activating the glucidic metabolism and all the processes of energy transfers (adenylates phosphates) and of redox potential (NAD(P)H), involved in the isoprene synthesis. It may derive in situ from the hydrolysis of phosphorylated molecules including that of the inorganic pyrophosphate (PPi) produced by the rubber transferase that is responsible for the lengthening of the polyisoprenic chain (Lynen, 1969).

Latex thiols content reflects the scavenging potential of molecules such as cystein, methionin and glutathion involved in the neutralization of the reactive oxygen form (ROS), therefore acting on the homeostasis of the latex cells.

2. Distribution Pattern of Latex Sucrose Content and Concurrent Metabolic Activity at the Trunk Level

The sucrose balance between supply and utilization in the latex producing bark of the rubber tree conciliate the latex metabolic activity. Such a study cannot be restricted to the only tapped panel, as some other bark areas may be as well involved or at least affected by the latex regeneration process. Physiological analyses are therefore carried out as well on the untapped bark area, in order to map the latex metabolic activity and the concurrent latex sucrose availability at the trunk level. Lustinec and Resing (1965) found, using radio-labeled isotopes, that the flow area of recently opened rubber tree was distributed about 40-50 cm above and below the tapping cut. On older trees, this area could extend up to 70 cm above the cut and to the whole area below the tapping cut. Buttery and Boatman (1966), using turgor pressure measurements to determine drained area, reported a pressure drop down to 1.20 m below the tapping cut. Pakianathan *et al.* (1975) termed "Potential displacement area" a bark area where rapid movement of latex near the region of the tapping cut can occur. Tupy (1973a) showed that sucrose latex content was depleted below and above the tapping cut as a consequence of latex regeneration process. Nevertheless, none of these works concurrently described the sucrose supply/ utilization balance and the associated latex metabolic activity at the trunk level. We tried here to apply the Latex Diagnosis technique (Eschbach *et al.,* 1984; Jacob *et al*. 1985, 1988a, 1988b, 1995) in order to get the first results on carbohydrate partitioning and the concurrent latex metabolic activity at the trunk level.

3. Enhancement of Latex Metabolism and Sucrose Loading

In *Hevea brasiliensis* Muell. Arg., stimulation by ethylene has a pronounced effect on sucrose level and metabolism. Lacrotte *et al.* (1985) studied the influence of

ethylene on sugar content of laticiferous cells in *Hevea brasiliensis* Muell. Arg. Using radioactive property of U^{14}-C injected in the bark in vivo resulted in the presence of radioactivity in cytosol and rubber fraction. His result showed that stimulation led to an increased flux of sucrose into the latex and suggested that ehtylene probably acted on an ATPase proton pump that simultaneously caused a rise in latex pH and pumping of sucrose from the neighbouring phloem. Coupe and Chrestin (1989) emphasized that the enhancement of rubber biosynthesis upon ethylene treatment is based on the increased synthesis of proteins, especially plasmalemmic ATPase and mitochodrial enzymes. The functioning of ATPase leads to an alkalinization of latex cytosol and thus activates the functioning of invertase, the key enzyme of glycolysis in latex cell. Activation of glycolysis leads to overproduction of pyruvate and ATP and the availability of these molecules allows the enhancement of rubber biosynthesis through mevalonic acid pathway.

4. Carbohydrate Reserves: Key Parameter to Productivity of Tree Plantations

Reserves are a key parameter to productivity of tree plantations, which has to be evaluated on a long term, taking into accounted the cumulative effects of cultivation practices and climate condition.

Among carbohydrate compound in the tree, starch is the major form stored in the perennial part, i.e. tap root or large perennial root, stem and branch in wood and bark, with large amount at the stem-branch junction. Leaf also contains high concentration of carbohydrate but the proportion is small when compared to the total amount present in plant. Sucrose is also the main form of stored sugar in plant as well as the main form of translocated sugar (Kuhn *et al,* 1999). The main carbohydrate storage tissue in woody plants is the ray parenchyma, which forms a continuous system throughout the branches, stems and structural roots, interconnected by plasmodesmata to the plant's symplastic system (Sauter and Kloth, 1986). Parenchyma in the roots seems to be an important site of storage (Gholz and Cropper, 1991). The functional role of carbohydrate reserves is to supplement current assimilates at times when there is an unusually large demand for assimilates. These

crucial periods are times at leaf refoliation when rapid and timely is important to suppress competitors and to maximize seasonal light interception, period of seed filling or periods of repair after biotic or abiotic damage (Cannell and Dewar, 1994). This infers the availability of carbohydrate reserves, defined as resources accumulated in mobilization form.

The need to take into account the cumulative effects, from one year to another, of cultivation practices and climate conditions on the plant functioning is specific to perennial crops, which productivity has to be evaluated on a long term. During the vegetative season, deciduous trees accumulate carbohydrate reserves, mainly as starch that constitutes the source of carbohydrate for maintenance respiration during wintering and for refoliation thereafter. For a large number of temperate forest and fruit trees, many researches have demonstrated the huge influence of carbohydrate reserves on production, metabolism, growth and resistance of trees to different stresses, showing that the accumulation of non-structural carbohydrates is particularly sensitive to environment and cultivation practices (Lacointe *et al.,* 1993; Kozlowski, 1992; Frossard and Lacointe, 1988; Glerum, 1980; Ziegler, 1964, Tromp, 1983; Kozlowski and Keller, 1966). However, works concerning tropical trees remain scarce (Bory and Clair-Maczulajtys, 1991, Mialet-Serra *et al.* 2005)).

However, starch acts as both a long-term and short-term storage polysaccharide in plants. It is accumulated during active photosynthesis and then mobilized and exported as sucrose for respiration. Differences in starch concentrations could indicate different rates of production, or shifts in allocation.
(Ludovici *et al*.,2002)

5. Seasonal Dynamics of Carbohydrate Reserves Based on Phenological Development

The seasonal cycles of reserve carbohydrates in tropical agroforestry trees found that concentrations of sugar and total reserve carbohydrates (sugar + starch) were highest during the dry season when growth is stopped. Starch had two maxima,

one early in the dry season, and one early in the wet season. All carbohydrate values decreased as active growth resumed during the wet season. In several species, carbohydrate concentration in the lower boles decreased during reproductive growth, especially during the last phase of fruit maturation. (Latt *et al*., 2001)

Seasonal fluxes of carbohydrates in conifer reflected the hypothesized use and starch storage patterns. Starch concentrations peaked in the spring (March – June) in all tissues measured; however, minimum concentrations in aboveground tissue occurred in late winter (October – January) while minimum concentrations in below ground tissue occurred in late fall (Ludovici *et al*., 2002). Carbohydrate storage serves to buffer the tree during periods of low C gain relative to C use. Excess sugars accumulate as non-structural starch when C production exceeds growth demands, and conversely provide a buffer when consumption is greater than current production.

The drier part of the year causes a cessation of growth in tropical trees. NSC concentrations (Non soluble carbohydrate) increase in all plant compartments during the drier part of the year and this increase is largely due to starch, with sugars affected only little. Hence the NSC enrichment is not associated with osmotic adjustment, but reflects a true carbon surplus. During the rainy season, NSC pools are moderately reduced, which coincide with resumed tree growth and new leaf production. Surprisingly, variations in NSC concentrations are not or only loosely associated with the different tree reproductive phenologies, but rather mirror moisture availability. (Korner, 2003)

Regarding, peach trees (*Prunus persical* L. Batsch), the ability of trees to mobilize their carbohydrate reserves in response to scion-trunk girdling, which prevents photosynthate transport toward the roots (Jordan and Habib, 1996). Girdling induces a NSC-I (insoluble non-structural carbohydrates) depletion in roots and rootstock-trunk bark and a NSC-I accumulation in leaves and shoots. On the contrary, the NSC-S (soluble non-structural carbohydrates) concentrations of the organs located both above and below girding are not significantly affected by the treatment. In early spring, trees have high amounts of reserves (10-15% of the total dry matter), mainly

starch, which are only partially mobilized. Thus the oldest starch (namely that stored the previous summer) is usually only partially used since mobilization follows the LIFO (Last in First Out) rule (Lacointe *et al*.,1993). Therefore, little carbon is mobilized from starch that is one or more years old and substantial amounts of starch may be available at any time to act as mobilizable safety reserves in case of deficient photosynthetic carbon supply. This old starch might be effectively used for metabolism during stress, whatever the season and even beyond the phenological stages. Like winter and spring, when mobilization usually occurs is still uncertain.

The new concept is that reserves are not considered any more as a passive buffer. Reserve storage has its own sink strength, just like growth, in other words reserves are not a mere passive buffer, and the plant normally 'manages' to keep reserves at a sufficiently high level, possibly at the expense of growth if resources are limiting. (LeRoux *et al*., 2002).This allows enough reserves to be available when required, e.g. for refoliation, either normal or accidental (e.g. after insects or fire attack). Any growth activity and possibly any metabolic activity, including latex production is accompanied by some reserve storage activity, however there may be a time lag due to maturation of new storage cells (the ones that are provided by growth, e.g. ray parenchyma cells), so that storage often continues a few weeks after growth has stopped.

In the rubber tree, a decrease in starch reserves in the trunk occurs during the period of development of young leaves and flowers (de Fay and Jacob, 1989) precise that this is histological observation, no quantitative analysis has been done. In fact, this phenomenon is limited to surface wood and an increase in sugar content of laticifers during leaf renewal may also be explained by this phenomenon which involves complex hydrolytic processes (de Fay and Jacob, 1989).

Starch content in the superficial trunk tissues (wood and bark) changes in relationship to apical and radial activity: there is almost no starch in the first 2 mm. of xylem and in phloem during leaf growth, and thereafter, there is a clear correlation between the width of the low - starch area and the cambium activity. However, in

October – November starch is low in cambium and cambial activity stops whereas leaves continue to function and starch to accumulate in xylem (de Fay, 1999).

Rubber tree showed a along the trunk of rubber tree a bottom-up decreasing gradient of starch was observed. Seasonal variation has been reported to affect the overall content; highest content always occurred at leaf shedding and the lowest content occurred at refoliation. This was obvious evidence showing that the peak consumption of reserve was used for the annual refoliation followed by flowering and fruiting (Silpi *et al.,* 2007).

6. Enzyme Activities Linked to Hydrolysis of Starch

Starch acts as both a long-term and short-term storage polysaccharide in plants. It is accumulated during active photosynthesis and then mobilized and exported as sucrose for respiration. Differences in starch concentrations could indicate different rates of production, or shifts in allocation.

The dynamics of carbohydrates within rubber trunk, are processed in bark and wood along the year. The soluble sugars and starch content were analysed. Silpi *et al.* (2007) found that a higher starch storage within some xylem areas in tapped trees as compared to untapped trees. Starch content within xylem also varied according to phenology. Moreover, starch and soluble sugar metabolism was deeply affected by nearness to the tapping cut and showed differences in starch patterns along the trunk in xylem and also in latex sucrose content.

In complement to this analytic approach it seems interesting to study more directly the processes involved in variations in starch and soluble sugar contents measured in the concerned tissues. Hydrolysis of starch reserves and the export of the resulting soluble sugars require the activity of specific enzymes. Total amylases are commonly involved in starch reserves mobilization (Witt and Sauter, 1994a, 1994b and Sissons and MacGregor,1994). The resulting sugars could be either used locally

or exported to other tissues in the form of sucrose. The intensity of this export largely depends on the activity of sucrose-phosphate synthase (SPS) (Wardlaw and Willenbrink, 1994), controlling sucrose synthesis within the cell.

The previous worked (Schrader and Sauter, 2002) reported that the activity of SPS (sucrose-phosphate synthase) in poplar wood increases dramatically in autumn in parallel with leaf fall, reaches a maximum level in winter at the time of the starch to sugar conversion and declines in spring during starch resynthesis and mobilization. In summer, the activity of SPS remains at a very low level. The SPS from poplar wood also seems to play an important role in sucrose biosynthesis for cold acclimation in autumn and winter in the wood parenchyma tissue. Low temperature promote this starch-to-sugar-conversion (Sauter,1988). In spring starch is first resynthesised and then mobilised during bud break. Hauch and Magel (1998) investigated the SPS and SuSy (sucrose syntethase) in the trunk wood of Robinia, particularly in relation to heartwood formation. Witt and Sauter (1994) found that the enzymes of the starch/sucrose interconversion were rarely investigated in the wood of perennial plant organs in spite of importance of this pathway for survival in moderate or cold climates.

Starch is hydrolysed to glucose, maltose and dextrin by total amylase (α-amylase and β-amylase) and other related enzymes. Specifically, amylase catalyses the hydrolysis of α-1,4 glucosidic linkages in amylose and amylopectin. It is suggested that these amylase isoforms are located in amyloplasts and take part in starch degradation. Volence *et al.,* (1991) found that endoamylase activity consistently increase at periods of starch degradation in alfalfa taproots. The amylase activity, which could be extracted from poplar wood grains, is involved in the degradation of these starch grains, and that the bind in to the grain surface is a prerequisite of the amylolytic activity (Witt *et al*., 1995).

Rubber exploitation by tapping results in an important sink in bark where the latex is withdrawn. This process user assimilate artificially derived from the other sinks (Templeton, 1969; Wycherley, 1976). Sucrose concentration in this sink region is depressed with respect to its level in more distant sites of the bark from the tapping,

and that this decrease is caused mainly by sucrose utilization in metabolic processes involved in latex regeneration (Tupy, 1973a, 1973b). Previous works have shown that sucrose content within laticiferous vessels is often a factor limiting production (Gohet, 1996; Gohet *et al.*,1998 and 2003). Semi-quantitative studies (histo-cytological localization) by Gohet (1996) and de Fay (1999) 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. However, the extension of changes in latex metabolic and latex sucrose, actual carbohydrate content along the trunk including starch hydrolysis and sugar export according to tapping frequencies particularly DCA system demand remains unknown so far.

Nevertheless, tapping intensity is commonly modulated by changing tapping frequency or when production is stimulated by the application of ethylene generators. Thereby carbohydrate availability can be artificially modulated too and this provides an interesting tool to study carbohydrate dynamics within the tree (Gohet, 1996; Silpi *et al.,* 2007). Moreover, assessing the time-evolution of reserve metabolites at the whole tree scale, will allow a better understanding the antagonism between latex production and primary growth in order to maintain a balanced partition of assimilates between these two sinks, key for a high and sustainable productivity of rubber plantations (Wycherley,1976; Gohet, 1996).

MATERIALS AND METHODS

1. Plant Materials

Field research activities were conducted on rubber trees at Chachoengsao Rubber Research Center (CRRC-DOA, 13.41 °N; 101.04 °E, 69 m above from mean sea level), Thailand. Temperature ranged 17.6-36.5 °C, mean relative air humidity (RH) was 63.5%. Annual rainfall averaged 1,291 mm year^{-1} (average 15 years). The soil type was Kabin Buri soil series (sandy clay loam – clay loam). Dry season lasted about 5 months, from December to April. In general, tapping starts in May and stops at the end of January, allowing 9 months of tapping and 3 months of resting period for latex production. All trees on RRIM600 clone in the experiment were planted in 1992 under a 2.5 m x 7.0 m planting design (571 trees/ha) and opened for tapping in May 2000. Tapping of experiment was started when rubber trees were ready for tapping (i.e. 50% of stand reaching a trunk girth of 50 cm, measure at 1.00 m. from the ground) in May 2000. Trees in trial were selected before opening for homogenous girth.

2. Methodologies

Two experiments were set. Experiment 1 is a “one tree plot design” (OTPD) comprising 10 replications per treatment. The tapping systems used were Control (untapped trees), 1/2S d/2 (a half spiral cut tapped every two days, D/2), 1/2S d/4 ET 2.5%. 6/y (a half spiral cut, one day in tapping followed by three days of rest, stimulated with ethephon, six applications per year, D/4) and Double Cut Alternate, 2x1/2S d/4 no stimulated. named DCA. (Fig.1). Experiment 2 is a “randomised complete block” comprising 4 replications per treatment. Four tapping systems used were control (untapped treatment was add in years 5), 1/2S d/2 (D/2, a half spiral cut alternate daily), 1/2S d/3 ET 2.5%.8/y (D/3, a half spiral cut one day in tapping followed by two days of rest, stimulated with ethepon, eight applications per year) and DCA (Double Cut Alternate, 2x1/2S d/4 (t,t) ET2.5% 2x4/y(8/y), two half spiral cuts, each cut tapped alternately every four days, stimulated with ethepon, four

applications per panel or eight applications per year) (Fig. 2). Carbohydrate reserves and latex diagnosis were allowed to work on Experiment 1 and 2, respectively.

First opening has been performed on both experiments. ½ S d/2 (D/2) have been opened on panel B0-1 at 1.50m from ground, ½ S d/3 (D/4) treatment at 1.30m from ground and ½ S d/4 (D/4) treatment at 1.20m from ground. Double cut treatments (DCA : 2 x ½ S d/4 (t,t)) have been opened simultaneously on panel B0-1 at 0.75m from ground and on panel B0-2 at 1.50m from ground. Panel management schedule after 5 years of tapping is presented in (Fig. 1 and 2). In each treatment, sampled trees were chosen as representing homogeneous rubber production (quantity and dynamics) and girth compared to the average of each treatment.

Both experiments are tapped 7d/7 9m/12, as refoliation and dry season, associated with very high temperatures, prevent economic tapping in February, March and April in Chachoengsao area. Stimulation is performed using 2.5% ethephon concentration (0.7 g/tree/application) applied to the bark under regeneration just above the tapping cut on 1 centimetre : ET 2.5% Pa 0.7 (1) according to Lukman (1983). Stimulant applications are evenly distributed from May to December.

Tapping involves periodically cutting bark on the trunk, and hence severing latex vessels. It is performed at a 30 ° angle from the horizontal, from high on the left of the tree to low on its right, exposing the maximum number of latex vessels per length of incision. The same cut is regularly reopened by excising at each tapping a new, thin shaving of bark from the sloping cut. As a result, latex down flows immediately along the cut into a cup attached to the trunk. The flow progressively diminishes, and eventually stops after one to three hours as severed vessel ends become plugged by caps of latex coagulum. In these experiments, tapping was performed a single panel by used half-spiral cut, i.e. with the tapping cut spiraling over half of the trunk circumference, thus delimiting two sides on the trunk respectively referred to as ‘tapped panel’ or Panel A and ‘untapped panel’ or Panel B. After some years, when all of the virgin bark on Panel A has been used, tapping would shift to Panel B. In DCA 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. Opening was made at 1.5 m on one side and at 0.75 m on the other side, so as keeping a large distance between tapped areas. The tapped panel in D/2 and D/4 is named panel A, and the untapped one is panel B. In DCA, panel A is cut in year 1- year 4 at the low cut and year 5 is cut at the high cut. For panel B of DCA is cut at the high cut.

The area where the bark removed during the previous tapping year was under regeneration is named the renewing bark area. Then tapping was resumed. Latex production was collected from field and weighted every 4 weeks for each tree, and its DW estimated according to 85 % mean total solid concentration. Mean tree girth at 1m high at the beginning of experiment (May 2000) was 49.2 cm, ranging 46.5-51.6 cm. The study presents results obtained during the first 5 years of tapping.

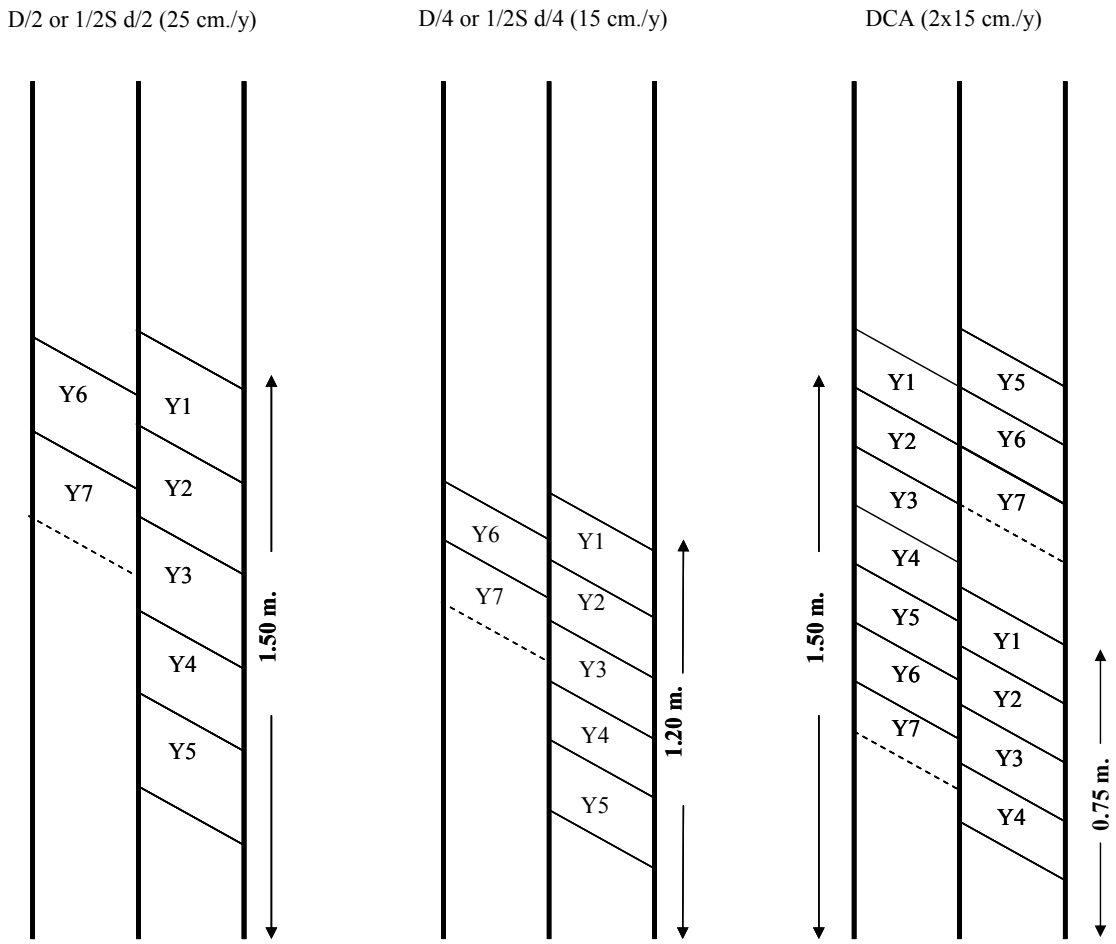

Figure 1 Tapping panel management of tapping system on experiment 1 (1). D/2 or 1/2S d/2 (2). D/4 or 1/2S d/4 ET 2.5%, 6/y and (3). DCA (Double cut alternative system, 2x1/2S d/4 (t,t)).

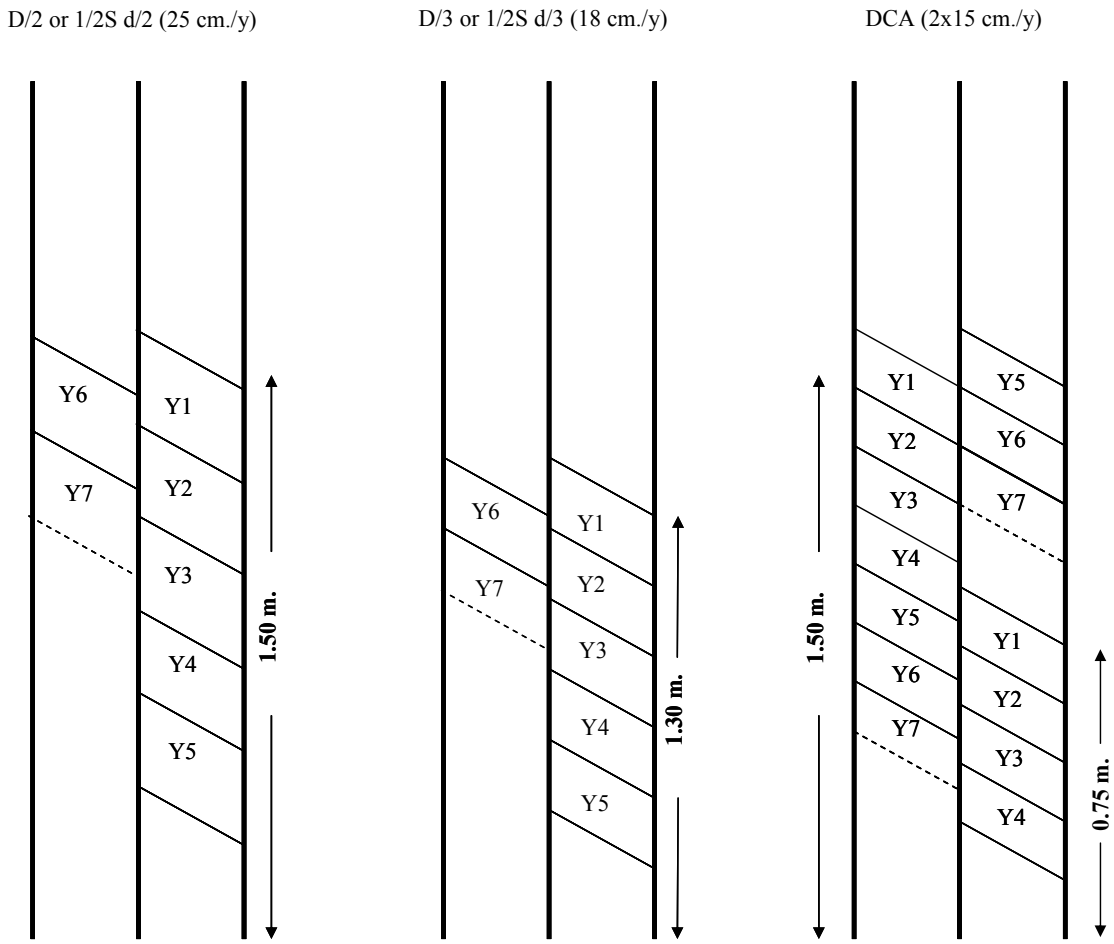

Figure 2 Tapping panel management of tapping system (1). D/2 or 1/2S d/2 (2). D/3 or 1/2S d/3 ET 2.5%, 8/y and (3). DCA (Double cut alternative system, 2x1/2S d/4 (t,t)) ET2.5% Pa 0.7 (1) 2 x 4/Y.

2.1 Field sampling

2.1.1 Latex diagnosis

Latex diagnosis was applied to the whole trunk of four trees per treatments of experiment 2 during September 2003 and October 2004 (2 years). Latex sucrose and inorganic phosphorus concentration were measured at different trunk positions from ground level to 2.0 m high on both panel, distance between two positions were 15 cm (Fig.2). Latex collection was performed in the morning, from

6.00 to 7.00 am on each tapping day. An iron punch (1 mm diameter, 2 cm long) was punched into the bark until reaching the wood. Puncture was followed by the insertion of a polyethylene tube into the hole in order to collect latex in a sampling hemolysis tube. Sampling was performed upwards from the basal level until 2.0 m level from ground, first on panel A then on panel B (Fig.2). On each tree, ten latex drops were collected from each sampling position to measure latex concentration.

2.1.2 Carbohydrate reserves of rubber trees

Sampling was performed during 2 years (2003-2004 or year 4 and 5 of tapping) according to season (climate, the annual growth cycle and with latex production cycle) i.e. (1). defoliation period (5 February 2003 and 19 January 2004 - dry season, still tapping, leafless stage, no radial growth, (2). refoliation period (6 March 2003 and 20 February 2004 – dry season, tapping rest, end of refoliation when the first flush matured, no radial growth), (3). start of tapping or low production period (2 May 2003 and 11 May 2004– end of dry season, resting period for tapping, beginning of growing period) and (4). high production period (28 October 2003 and 18 October 2004 – rainy season, high latex production period, growth continuing). Each tapping treatment included 12 trees (treatment replications). In each treatment, the sampled trees were chosen as presenting homogeneous rubber production (quantity and dynamics) and girth compared to the average of each treatment. Along the trunk, samples were taken at 20, 50, 80,110, 140, 170, and 200 from ground (7 samples on tapped panel or panel A, including renewing bark area, and 7 samples on panel B) and 4 samples were taken every 1 m. from 3 m. to top on a main axis. In root, 2 samples on taproot were performed at 10 and 30 cm. and 2 samples along a large lateral root (Fig. 3). At each sampling date, groups of 3 trees from each treatment were sampled. Samples consisted in 0.5 cm diameter, 5 cm long cores, including 1 cm of bark and 4 cm of wood. They were made with a wood auger. Wood and bark were separated. Sample trees were alternated, in order to reduce the metabolic perturbation and necrosis hazard due to core-sampling from one period to the next one. After each core was sampled, 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 ^{o}C, before freeze-drying using a -50 ^{o}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 ^{o}C. 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.

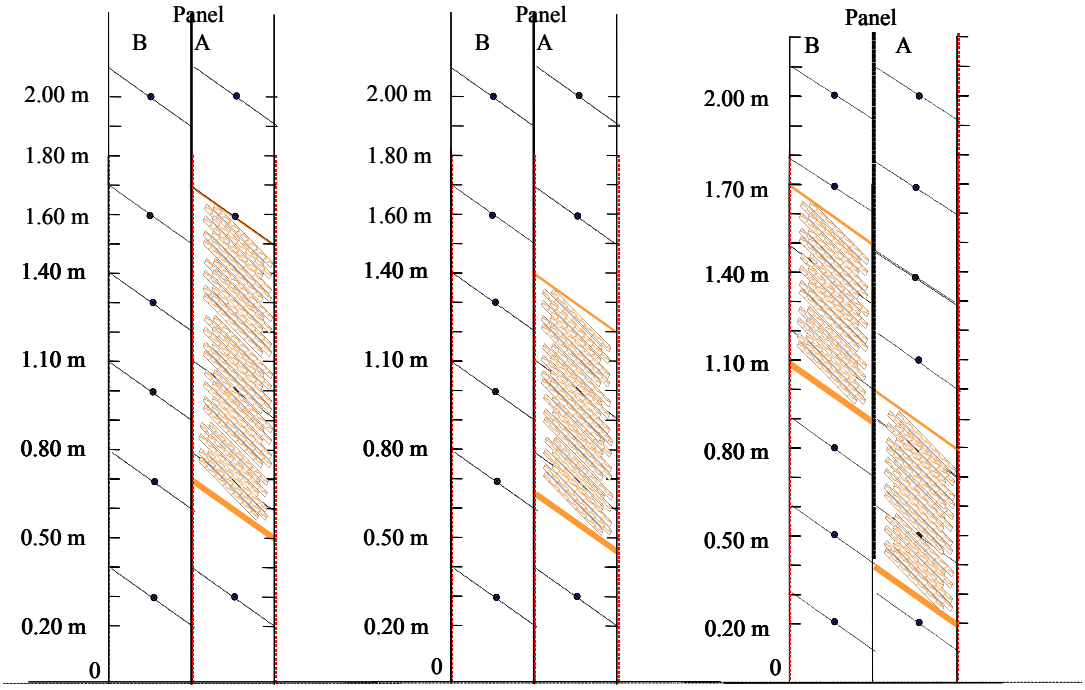

Figure 3 Diagram of sampling were taken on each treatment (1) D/2 or 1/2S d/2 (2). D/4 or 1/2S d/4 ET 2.5%, 6/y and (3) DCA (double cut alternative system) or 2x1/2S d/4 (t,t).

2.1.3 Hydrolysis of starch reserves

Samples were collected from 2 clones: PB 235 (high metabolism and high production) and RRIM 600 (medium metabolism and medium production) (Gohet *et al.*, 2003), during low production periods (started to tapping in May 2004). Samples were collected along the trunk from the bottom up to 3.0 m. from the ground. The samples diameter was 0.5 cm and 5 cm long, which consisted of 1 cm of bark and 4 cm of wood. After coring, the samples, soaked immediately in liquid nitrogen, were kept in cryo-tube and immersed in liquid nitrogen until transfer to the laboratory and then stored at -80 °C. Each rubber clone was separated in two sample groups. The first group name dry wood and dry bark, were applied freeze-drying using a -50 °C freeze-dryer (Telstar Cryodos®, Spain) and blended using ball-blender MM200 (Retsch, Germany); the ball diameter was 7 mm. Then, samples stored at -80 °C until extraction and biochemical analysis were conducted. Another sample group (fresh wood and fresh bark) was simply stored at -80 °C without blending.

2.2 Biochemical analysis

2.2.1 Latex metabolic activity

Latex metabolic activity was analysed by using latex diagnosis (LD) technique (Eschbach *et al.* 1984, Jacob *et al.* 1985, 1988a, 1988b, 1995) adapted to the CRRC Latex Diagnosis Laboratory facilities (Gohet and Chantuma, 1999). Only the results concerning inorganic phosphorus (Pi, indicator of latex metabolic activity) and sucrose (Suc, precursor molecule of the latex rubber synthesis) are presented and discussed hereafter. The concentrations measured for these two major physiological parameters are expressed in millimols per liter of fresh latex ($mM.l^{-1}$).

2.2.2 Carbohydrate Reserves

Powder 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 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 spectro-photometry 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_{Glu}/g_{SDM}, mg Glu equi./g structural DM). Sum of starch and soluble sugar represented the total non-structural carbohydrate (TNC) (Boehringer, 1984).

2.2.3 Hydrolysis of starch reserves

Blend 150 mg FW (or 75 mg DW) in liquid Nitrogen with 10% PVPP (polyvinylpolypyrrolidone) (12% for bark). Put the powder in a beaker and add 800 µl (for FW, or 1,000 for DW) of extraction buffer (Hepes pH 7.0, KOH 50 mM, DTT (1,4-dithio-dl-threitol) 10 mM , $MgCl_2$ 5 mM and EDTA 1 mM), mix slowly, keep for 5 min.on ice, put in a micro tube and then centrifuge at 10,000 rpm, 4 °C, 10 min. Take the supernatant for, total amylases and SPS activities. For cell wall invertase: rinse the pellet 3 times with 500 µl extraction buffer pH 7.0 and centrifuge each time 10,000 rpm, 4°C, 5 min. Resuspend the pellet in 500 µl cell wall invertase

extraction buffer pH 5.0 (K_2HPO_4 70 mM and acid citric 40 mM, NaCl 1 M) for 12 h at 4 °C. Centrifuge at 10,000 rpm, 4ºC, 10 min. Collect the supernatant in a clean micro tube. Protein assay by Bradford method, the standard curve (2-20 µg/100 µl) take 100 µl of the standard solution, add 700 µl H_2O and 200 µl Bradford reactive (BioRad ref), mix well. For the extracts: take 10 µl of extract (50 µl for the cell wall invertase extract), add 790 µl (750 µl for the cell wall invertase extract) H_2O and 200 µl Bradford reactive, mix well. Wait for 5 minutes before measuring the OD at 595 nm (Bradford, 1976). Total amylase assay, Add 325 µl Hepes/KOH (100 mM , pH 8.0) to 125 µl 2 % w/v, amylopectin. Pre-incubate separately 50 µl of extract and the assay buffer, 5 min at 30°C. For the control (blank), heat 50 µl extract 5 min. at 100 ºC. To start the reaction, add 450 µl of the assay buffer (Hepes + amylopectin) to the extract and control (blank). Incubate 1.5 h at 30° C. To stop the reaction heat all samples 5 min at 100°C. The measurement of glucose formed was carried out by the Nelson method (Nelson, 1944), using glucose as standard (glucose concentration between 0-1000µM). Nelson reaction solution consists (Cuprosodic solution: 15 % CuSO4 to 100 ml, Sodic solution (anhydric Na_2CO_3 25 g/L, $NaHCO_3$ 20 g/L, sodico-potassic tartrate 25 g/L, anhydric Na_2SO_4 200 g/L) and Arseniomolybdic solution: ammonium molybdate 50 g/L, H_2SO_4 42 ml/L, AsO_4HNa_2,7 H_2O 6 g/L (adapted from Sauter *et al.*, 1993). 100 µl of incubation medium (or standard) is mixed with 100µl of cuprosodic solution and boiled 15 min. at 100°C. After cooling tubes in ice, 100 µl of arseniomolydic solution is added and mixed. 1 ml of H_2O is added and the absorbance is read absorbance at 520 nm after 10 min. Sucrose phosphate syntase (SPS - EC.2.4.1.14), add 50 µl of 4X SPS buffer (400 mM Hepes pH 7.5 NaOH , 100 mM $MgCl_2$, 20 mM F6P, 100 mM G6P, UDPG 80 mM and H_2O) to 100 µl H_2O and pre-incubate 3 min. at 30 ºC. For the control of each sample (blank) add 50 µl of boiled extract (4 min. at 100 ºC), and for the measurement, add 50 µl of extract, pre-incubate 3 min. at 30 ºC. Incubate all the micro tubes 20 min at 30 °C. To stop the reaction, heat the micro tubes 4 min at 100 ºC. Then cool it on ice (freeze if needed for storage) and centrifuge it, use 150 µl of supernatant to determine the quantity of UDP produced by enzymatic way. Add 845 µl UDP buffer (100 mM Tris pH 7.5 HCL 150 µL, 10 mM $MgCl_2$, 0.3 mM NADH and 0.8 mM PEP) to 150 µL of SPS reaction medium. Add 5 µl of pyruvate kinase/lactate dehydrogenase (450 U PK/450

U LDH, Boehninger). Mix and centrifuge quickly. Incubate 60 min. at 30 °C and read the OD at 340 nm. (adapted from Schrader and Sauter, 2002 ; Hauch and Magel, 1998. For SPS assay, do not freeze the extract (Geigenberger *et al*.,1999). Cell wall invertase (CWI - EC 3.2.1.25), for the control (blank), heat 50 µl extract 5 min. at 100 ºC. Add 50 µl extract to 150 µl H_2O (200 µl final volume). Pre-incubate the 2X invertase assay buffer (70 mM K_2HPO_4, 40 mM citric acid, pH 5.0, 50 mM sucrose) and the extracts 3 min. at 26ºC (30°C). Add 200 µl 2X invertase assay buffer to each extract and mix gently. Incubate 20 min. at 26 °C and stop the reaction by heating 5 min. at 100 ºC. The glucose formed is measured with the Nelson method. (adapted from Roitsch *et al*., 1995).

2.3 Data analysis

2.3.1 Latex diagnosis (LD), the concentrations measured for these two major physiological parameters (inorganic phosphorus [Pi] and sucrose [Suc]) are expressed in millimols per liter of fresh latex ($mM.l^{-1}$).

$$[Pi] = OD \times K \times [(FLW + w1 + w2)/FLW]$$
$$[Suc] = OD \times K \times [(FLW + w1 + w2)/FLW]$$

K = coefficient of the standard curve
FLW = fresh latex weight in grammes
w1 = weight of water per tube (grammes)
w2 = weight of TCA 20% used to induce coagulation in each tube

2.3.2 Non structural carbohydrate (TNC): glucose, fructose and sucrose [Q mg/g] analyse quantity in samples.

$$A = k.\ Q\ \mu g$$
$$Q\ mg/g = \frac{A_{samples}.\ Vr(ml) \times 1000}{k.\ V_{sample}(\mu l).\ M_{sample}\ (mg)} \quad mg/g\ DM$$

k = coefficient of the standard curve

A = absorbance

V_{sample} = sample volume

Vr = volume use to dilute the sample after drying

M_{sample} = mass of sample

Glucose: A_{sample} = OD2-OD1

Fructose: A_{sample} = OD3-OD2

Sucrose: $A_{sample} = (OD2-OD1)_{sample} - (OD2-OD1)_{glucose}$

2.3.3 Enzyme activity

1. Protein

$$[\text{Protein}] = kA/\upsilon \quad \mu g/\mu l$$

k = coefficient of the standard curve

A = OD_{sample}

υ = sample volume (µl)

2. Total amylase

$$[\text{Total amylase}] = \frac{A \times \text{total reaction media}}{k \times [\text{Prot.}] \times \upsilon \times \text{incubate time (min)}} \quad \mu M/min/\mu g$$

k = coefficient of the standard curve

A = $OD_{sample} - OD_{blank}$

υ = sample volume (µl)

total reaction media = $5x10^{-4}$

3. SPS (Sucrose Phosphate Synthase) is expressed in [NADH]

$$[NADH] = \frac{A \times \text{total reaction media } (10^{-3}) \times NADH \times (200/150)}{k \times \upsilon \times \text{incubate time}}$$

NADH = µM/min/µg

k = coefficient of the standard curve (=6.23)

A = $OD_{sample} - OD_{blank}$

υ = sample volume (µl)

total reaction media = 10^{-3}

4. Cell wall invertase (CWI)

$$[CWI] = \frac{A \times \text{total reaction media}}{k \times 2 \times [Prot.] \times \upsilon \times \text{incubate time(min)}} \quad \mu M/min/\mu g$$

k = coefficient of the standard curve

A = $OD_{sample} - OD_{blank}$

υ = sample volume (µl)

total reaction media = $4x10^{-4}$

2.4 Stat analysis

2.4.1 Rubber yield and latex diagnosis, experiment 2 is a "randomised complete block" comprising 4 replications and 6 treatments (A). 1/2S d/2, (B). 1/3S d/2 ET2.5% 4/Y, (C). 1/2S d/3 ET 2.5% 4/Y, (D). 1/2S d/3 ET 2.5% 6/Y, (E). 1/2S d/3 ET 2.5% 8/Y and (F). DCA 2 x 1/2S d/4 (t,t) ET2.5% 2 x 4/Y). Results from treatments B, C and D are not presented here. As a matter of fact, the objectives of these treatments are not in line with the aim of this presentation.

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2.4.2 Carbohydrate reserve, results were processed in a two-step procedure. First, a 4-way analysis of variance (ANOVA), where the 4 factors were tapping treatment (control, D/2, D/4 and DCA), panel (A vs B), sampling height above ground

level (0-2m.), and season (9 sampling dates), was performed. However, we focus only main factors for the first information. It was difficult to impress interaction factors although these factors had significant difference. Data set was subsequently processed as a complete design in a 3-way ANOVA by combined treatments and panel for all dates and distance from ground. SAS version 8.0 (962500628) was used in both cases.

RESULTS

1. Effects of Tapping Systems on Rubber Production and Latex Concentration

During first 3 years of tapping, rubber production was significantly higher in DCA than D/2 and D/3. The production of DCA reached 124-129% of the corresponding production of control D/2 (Table 1). After 5 years of tapping, cumulative production of DCA reached 114% of the corresponding production of control D/2 (Table 1). Highest differences between the two tappings were obtained during the first 3 years of tapping. Advantage of DCA strategy was limited during years 4 and 5. DCA production became even lower than D/2 in year 5. This seemed to result from a bottleneck in sucrose supply appearing in year 4, due to crossover of DCA panels (creation of a "full spiral"). Although D/3 with stimulation was considered the physiological control that relies on optimization of time for latex regeneration and increased metabolism by ethylene, production was limited by actual number of tapping days.

Mostly, sucrose content in low cut of DCA was less than in high cut but on the contrary inorganic phosphorus (Pi) was higher in low cut than in high cut. In year 5, panel change over resulted in a low metabolic activity (low Pi) (Table 2).

Table 1 Rubber production expressed in kg/tree (kg/t).

	Year 1		Year 2		Year 3		Year 4		Year 5		Cumulated	
	kg/t	% A	kg/t	% A	kg/t	% A	kg/t	% A	kg/t	% A	kg/t	% A
D/2	2.4 b	100	3.6 b	100	4.4 b	100	4.6 a	100	3.8 a	100	18.7 b	100
D/3 ET 8/y	2.7 ab	114	3.3 b	91	3.8 bc	87	3.5 c	77	2.4 c	65	15.8 c	84
DCA,ET2x4/Y	3.1 a	129	4.5 a	124	5.6 a	128	4.6 a	101	3.5 a	93	21.2 a	114

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

Note: Tapping day of each year start from May to February of year after, for example: year 1 (May 2000- February 2001), year 2 (May 2001- February 2002), year 3 (May 2002- February 2003), year 4 (May 2003- February 2004) and year 5 (May 2004- February 2005).

Table 2 Sucrose concentration ([Suc], mM/litre of latex) and Inorganic phosphorus concentration ([Pi], mM/litre of latex) collected 5 cm under tapping cut.

Treatment	Year 1		Year 2		Year 3		Year 4		Year 5	
	[Suc]	[Pi]	[Suc]	[Pi]	[Suc]	[Pi]	[Suc]	[Pi]	[Suc]	[Pi]
D/2	10.0 bc	19 de	7.7 a	17	8.0 b	20 abc	5.4 b	18 ab	10.5 a	19 b
D/3 ET 8/y	9.9 bc	27 ab	6.4 ab	16	8.0 b	16 de	4.5 b	17 ab	5.2 b	17 c
DCA,ET2x(4/Y)	14.1 a	23 c	6.3 ab	19	8.3 b	22 a	7.5 a	16 b	9.0 a	25 a
Panel A, Y1-Y4: low cut Y5 : high cut	11.9 b	24 bc	7.3 a	19	10.2 a	21 ab	6.1 b	21 a	11.1 a	22 b
Panel B Y1-Y4: high cut Y5 : low cut	16.3 a	21 cd	5.4 b	19	6.4 c	23 a	8.9 a	11 c	7.0 b	28 a

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

Note: Latex collections were done under the tapping cut (the middle and 5 cm from the cut).

2. The Effect of Tapping Systems on Latex inorganic Phosphorus and Sucrose

In year 4 of tapping, sucrose content at trunk scale level was not significantly different among treatments but inorganic phosphorus (Pi) was significantly higher in DCA than in D/2 and D/3 (Table 3).

In year 5 onward, sucrose was not significantly different between D/2 and DCA but Pi was higher in D/2 than in DCA. Whereas D/3 had least sucrose and Pi as compared to D/2 and DCA (Table 3).

Table 3 Average trunk contents of latex sucrose and latex inorganic phosphorus in different tapping system.

Treatment	Year 4 (September 2003)		Year 5 (October 2004)	
	[Suc] mML^{-1}	[Pi] mML^{-1}	[Suc] mML^{-1}	[Pi] mML^{-1}
Control	-	-	24.04 ± 1.18 a	8.57 ± 0.33 c
D/2	12.67 ± 0.47	10.10 ± 0.57 b	14.46 ± 0.87 b	16.15 ± 0.94 a
D/3 ET 8/y	12.60 ± 0.83	12.32 ± 0.63 b	8.52 ± 0.66 c	13.02 ± 0.88 b
DCA ET2x(4/y)	13.34 ± 0.83	19.66 ± 0.63 a	15.62 ± 0.79 b	11.73 ± 0.60 b

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

Control treatment was untapped, thus latex metabolic activity was low. Accordingly, this treatment had high sucrose and on the contrary low Pi (Table 4). D/2 and D/3 were tapped only on panel A. After year 4 onward, sucrose concentration and inorganic phosphorus (Pi) of D/2 and D/3 were not significantly different. DCA was tapped in both panels and sucrose and Pi of DCA were not different between the 2 panels.

In year 4 of tapping, sucrose was not significantly different among treatments and between 2 panels. Nevertheless, both panels of DCA had significantly higher Pi than D/2 and D/3.

In year 5 of tapping, D/2 and DCA had significantly higher sucrose than D/3. Panel A of D/2 and DCA were not different in sucrose and Pi, but in panel B (untapped panel) Pi was higher in D/2 than in DCA (tapped panel) (Table 4).

Table 4 Sucrose concentration ([Suc], mM/litre of latex) and Inorganic phosphorus concentration ([Pi], mM/litre of latex) on tapped panel and untapped panel.

Treatment	Panel	Year 4 (September 2003)		Year 5 (October 2004)	
		[Suc] mML^{-1}	[Pi] mML^{-1}	[Suc] mML^{-1}	[Pi] mML^{-1}
Control	A	-	-	24.04 a	8.57 c
D/2	A	14.15	11.62 b	15.85 ab	13.38 ab
D/2	B	11.85	9.25 b	13.83 b	17.42 a
D/3 ET 8/y	A	13.62	11.15 b	9.72 c	11.93 b
D/3 ET 8/y	B	11.95	13.00 b	7.87 c	13.61 ab
DCA ET2x(4/y)	A	13.07	19.82 a	17.12 ab	12.63 b[1/]
DCA ET2x(4/y)	B	13.60	19.47 a	14.11 b	10.83 bc

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

Note : 1/ Panel A was cut in Y1-Y4 on low cut and the new high cut was changed over in year 5.

3. Vertical Distribution of Latex inorganic Phosphorus and Sucrose Along the Trunk and Comparison of Panels for Different Tapping Systems

The vertical distribution of the latex metabolic activity and available substrate for rubber biosynthesis, respectively estimated by latex inorganic phosphorus (Pi) and sucrose (Suc) contents in tapped and untapped panels, compared between tapping system : Control (untapped trees), D/2, D/3 and DCA (Fig. 4, 5, 6 and 7).

The control (untapped trees) had a significantly higher sucrose content than tapped treatments with a slight increasing bottom-up gradient along the trunk (Fig. 6). Conversely, Pi concentration was less in control that in tapped treatments, with also a less marked vertical gradient (Fig. 6 and 7).

On tapped panel (panel A) of D/2 and D/3, Sucrose and Pi pattern were affected by the occurrence of the tapping cut. D/2 and D/3 showed the same trends for sucrose and Pi. In Tapped panel (panel A), sucrose in year 4 onward had higher concentration in the bottom part. Within the regeneration areas, below and above the tapping cut, sucrose concentration was dramatically decreased, whereas it was high at 2 m from ground (Fig. 4 and 6). However, in year 5 of tapping: there was a significant difference among trapped treatments. The uppermost parts of D/2 had significantly higher sucrose content than that of D/3. Conversely, inorganic phosphorus content (Pi) was higher in location below tapping cut (latex regeneration) than above tapping cut. In untapped panel (panel B), the gradient of sucrose and Pi were more regular than in tapped panel.

In year 4, DCA treatment was tapped in both panel but the tapping cut was low cut in panel A and high in panel B. On panel A (low cut), sucrose was significantly higher above tapping cut than below tapping cut, whereas mean Pi content was not significantly different between the 2 areas. However, Pi was the highest at the place just above tapping cut. On panel B, sucrose was lower in latex regeneration area, where below tapping cut. Pi gradient was larger below cut than above cut (Fig. 4 and 5). In year 5 of tapping, all bark in panel A low cut was consumed so that the tapping cut was changed over to high level, 1.50 m from ground. Meanwhile, the original high cut in panel B was consumed at 15 cm average per year, so at that time this tapping cut reached 90 cm from ground. On panel A, sucrose was low at bottom part and dramatically increased in the “island bark” (the bark between 2 tapping cuts) However, sucrose was disturbed near the place below new tapping cut and sucrose was higher on the uppermost parts. Pi showed a decreasing bottom-up gradient along the trunk. On panel B, the overall pattern of sucrose and Pi was more steady than on panel A. As a whole, sucrose content of DCA was not significantly different from D/2 but DCA showed less Pi than D/2 (Fig. 6 and 7).

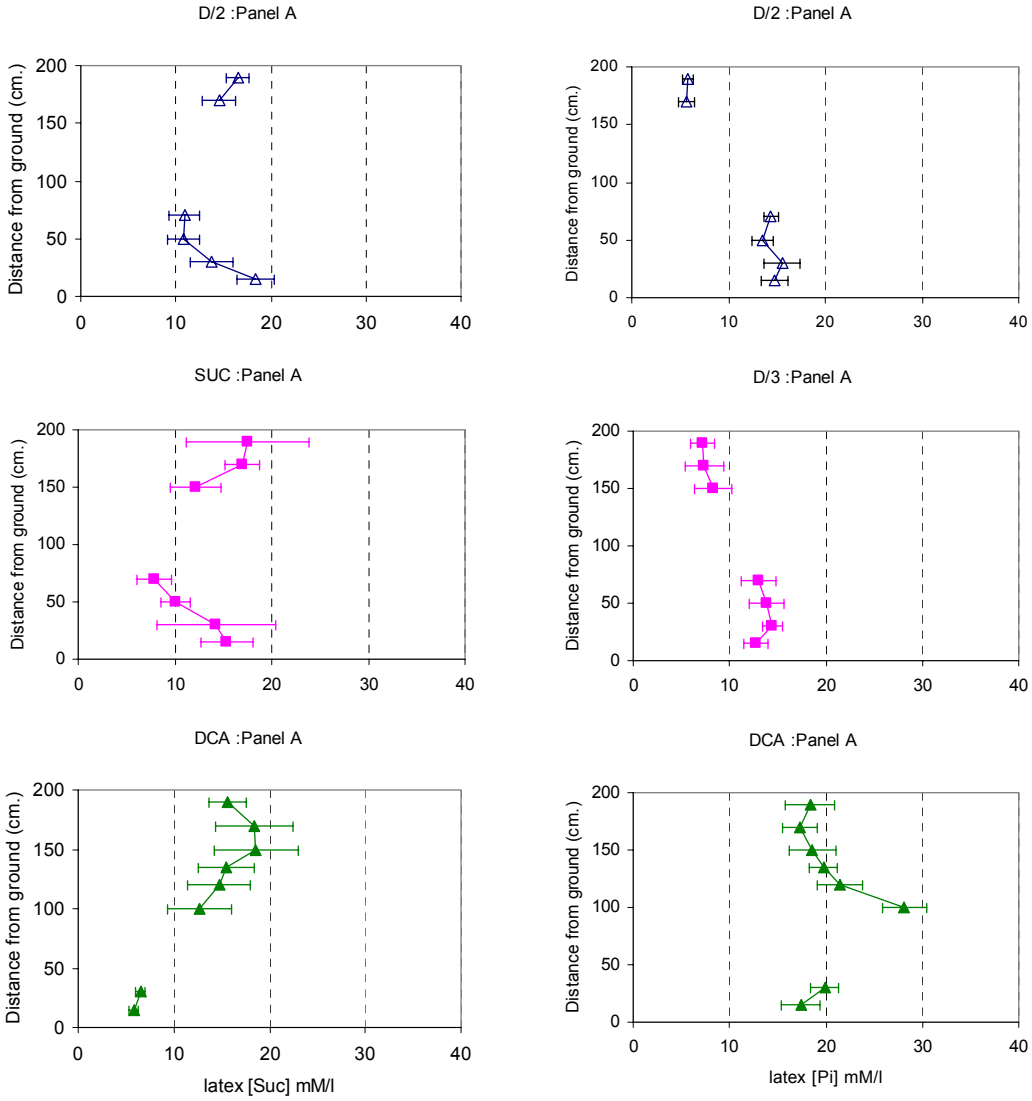

Figure 4 Vertical distribution of latex sucrose content ([Suc], $mM.l^{-1}$) and inorganic phosphorus content ([Pi], $mM.l^{-1}$) after 4 year of tapping (September 2003) in panel A (tapped panel) of RRIM600 clone, depending on distance from ground. Sampling on tapped trees is performed every 15 cm from bottom to 200 cm above the ground. Number of samples depended on position and width of renew bark [bark consumption of each treatment, no data in these areas].

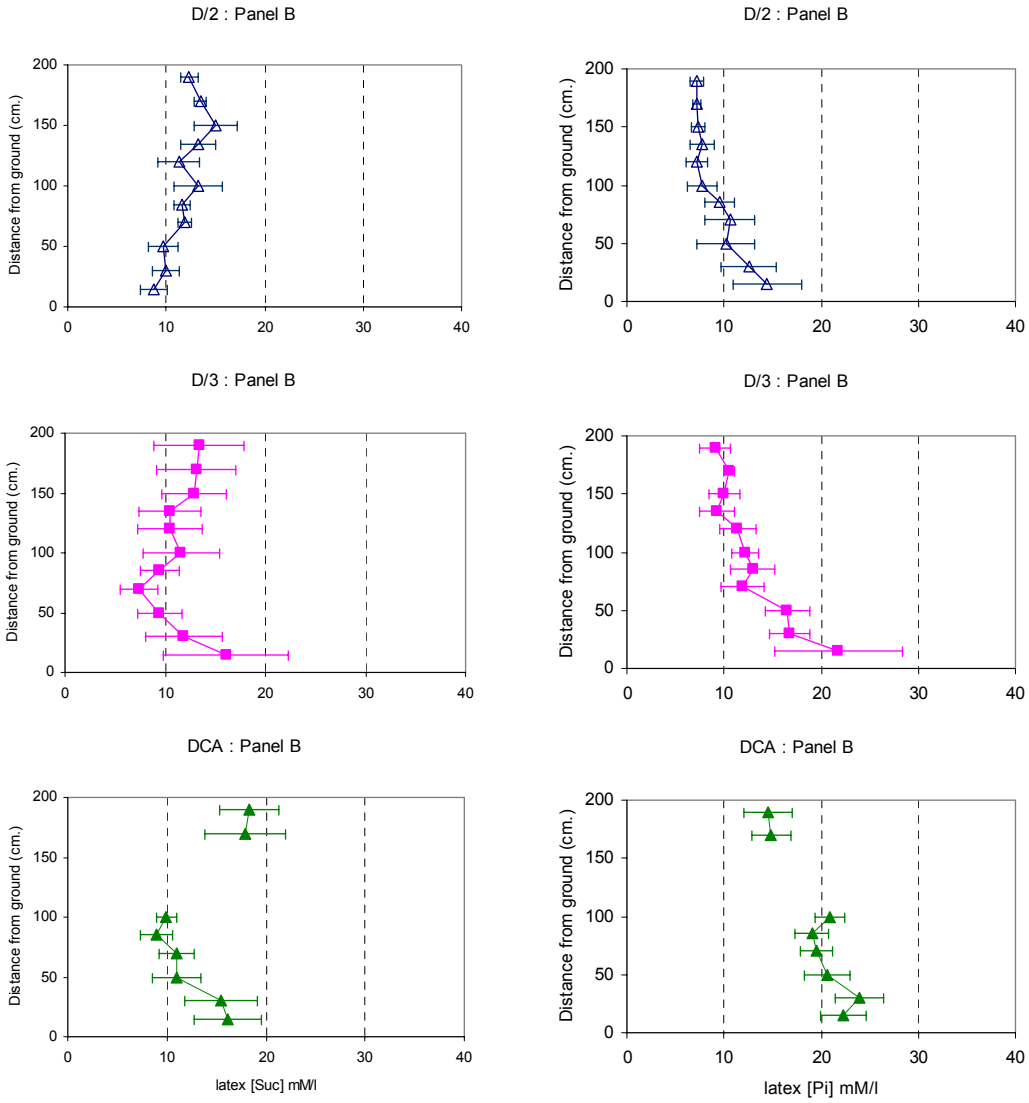

Figure 5 Vertical distribution of latex sucrose content ([Suc], $mM.l^{-1}$) and inorganic phosphorus content ([Pi], $mM.l^{-1}$) after 4 year of tapping (September 2003) in panel B (untapped panel for D/2 and D/3, tapped panel for DCA)of RRIM600 clone, depending on distance from ground. Sampling on tapped trees is performed every 15 cm from bottom to 200 cm above the ground. Number of samples depended on position and width of renew bark [bark consumption of each treatment, no data in these areas].

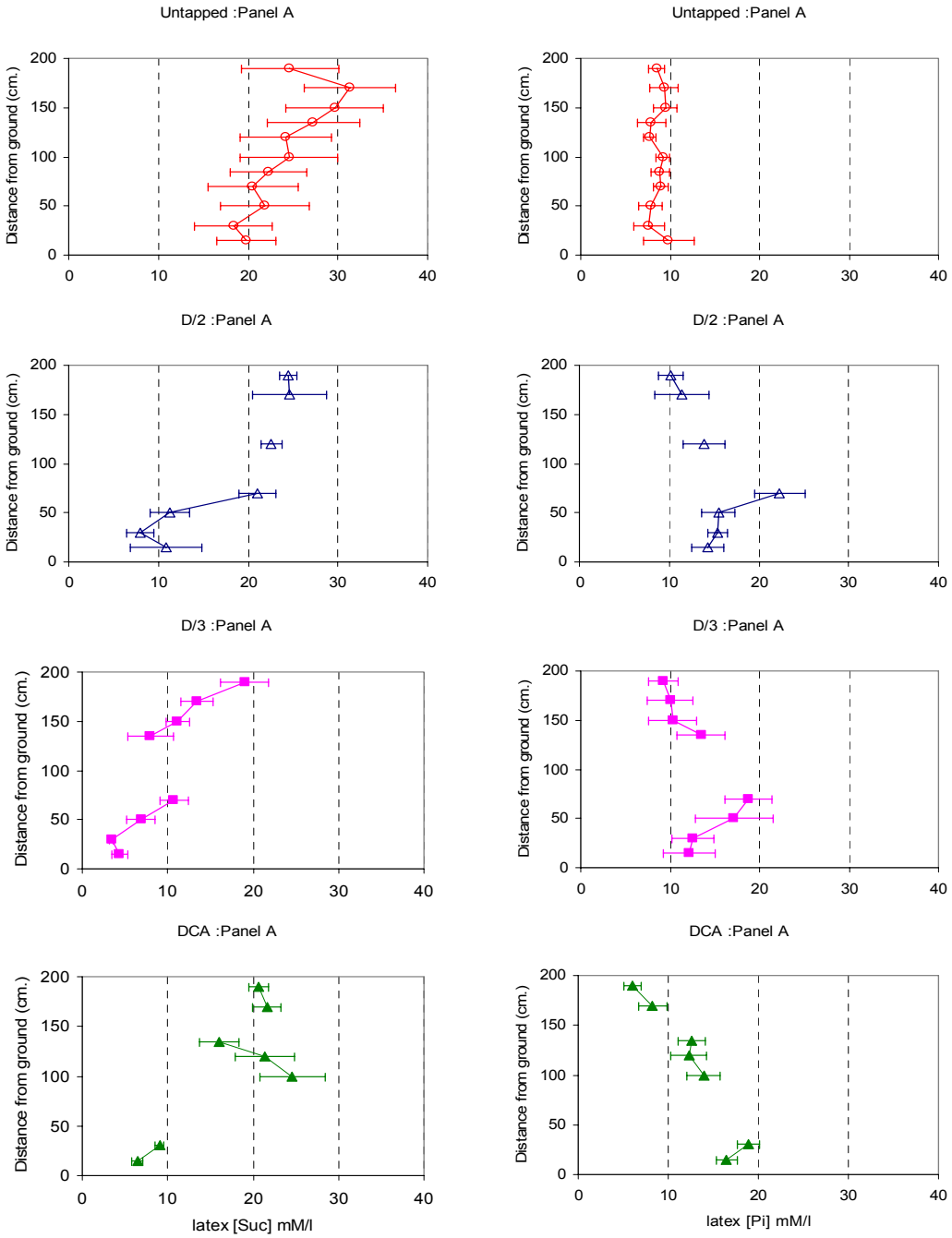

Figure 6 Vertical distribution of latex sucrose content ([Suc], $mM.l^{-1}$) and inorganic phosphorus content ([Pi], $mM.l^{-1}$) after 5 year of tapping (October 2004) in panel A (tapped panel) of RRIM600 clone, depending on distance from ground. Sampling on tapped trees is performed every 15 cm from bottom to 200 cm above the ground. Number of samples depended on position and width of renew bark [bark consumption of each treatment, no data in these areas].

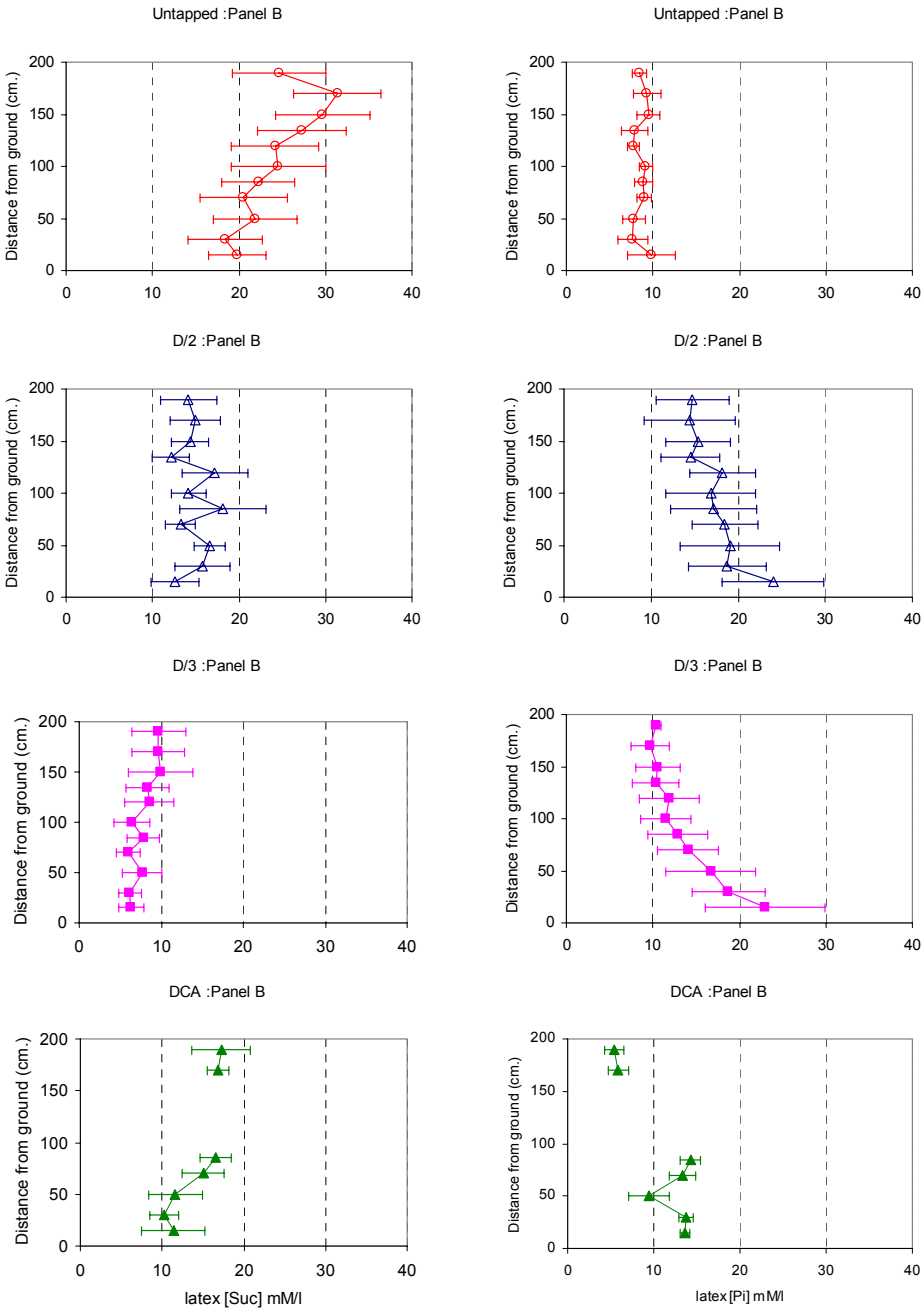

Figure 7 Vertical distribution of latex sucrose content ([Suc], $mM.l^{-1}$) and inorganic phosphorus content ([Pi], $mM.l^{-1}$) after 5 year of tapping (October 2004) in panel B (untapped panel for D/2 and D/3 , tapped panel for DCA) of RRIM600 clone, depending on distance from ground. Sampling on tapped trees is performed every 15 cm from bottom to 200 cm above the ground. Number of samples depended on position and width of renew bark [bark consumption of each treatment, no data in these areas].

4. Spatial Extension of Latex Regeneration Area and Relation with Rubber Production

The latex metabolic status within the trunk bark was evaluated by comparing inorganic phosphorus (Pi, $mM.l^{-1}$) values measured in each sampling position (Fig 8). The average Pi values were 19.7 $mM.l^{-1}$, 12.3 $mM.l^{-1}$ and 10.1 $mM.l^{-1}$ on DCA system, D/3 and D/2, respectively. As this Pi value was considered a good indicator of latex metabolic activity, the result confirmed that DCA enhanced latex metabolic activation inside the trunk as a whole.

On D/2 and D/3 had no significant latex metabolic activity between on tapped panel A compared to untapped panel B. On DCA trees, the metabolism was enhanced in tapped panel A (at 0.75 m from ground) and tapped panel B (1.50 m from ground) as Pi varied from 14.6 to 25.0 $mM.l^{-1}$ (mean 19.7 $mM.l^{-1}$). Whereas on D/2 and D/3 trees the metabolism was lower, as Pi varied in the range from 5.7 to 15.5 $mM.l^{-1}$ (mean 10.1 $mM.l^{-1}$) and from 7.7 to 21.3 $mM.l^{-1}$(mean 12.3 $mM.l^{-1}$), respectively. Therefore, DCA was significantly increased the size of this latex metabolically active area in both panel A and panel B (Fig 8).

The sucrose on panel A was 13.5 $mM.l^{-1}$, 11.5 $mM.l^{-1}$ and 5.5 $mM.l^{-1}$ on D/2, D/3 and DCA system, respectively (Fig. 9). In the area located below the cut of tapped panel B (high cut) of DCA system, the sucrose content of 12.1 $mM.l^{-1}$ was not significantly different compared with other tapping systems.

These average sucrose concentrations were negatively correlated with the estimated sizes of their respective latex regeneration areas, as well as with the average latex metabolic activity (Pi) and the rubber productions since the start of the experiment (May 2000 – February 2004) observed on the concerned trees (Table 5). The total area with both a low latex sugar content and a high latex metabolic activity (high Pi level) could therefore be considered as the bark area where latex regeneration actually takes place and thus could easily be identified using the latex diagnosis technique. It was thus possible to estimate quite precisely its size and its shape. The

extension of this latex regeneration area (low latex sucrose + high latex Pi) could be estimated at about 0.29 m^2, 0.32 m^2 and 0.42 m^2 on D/2, D/3 and DCA system respectively, which included the involved areas above and below the tapping cut both on panel A and panel B. The average latex sucrose content in tapped panel A and B was higher in DCA (13.3 $mM.l^{-1}$) than in other treatments (12.6 –12.7 $mM.l^{-1}$) (Fig. 9).

Latex sucrose content and latex Pi content of D/2 and D/3 (Fig. 10) were negatively correlated. As the metabolic activity was higher, an increase in latex metabolic activity (higher Pi) was mainly due to the increase in the latex regeneration process that required increased sucrose consumption. A higher latex metabolic activity increased the sucrose consumption and latex sucrose content therefore decreased. The latex system mostly functions as a utilization sink. Conversely, with DCA system, latex sucrose content and latex Pi content were both negatively correlated and positively correlated. Negative correlation regarded the latex system functioning as a utilization sink. The positive correlation was as higher latex metabolic activity (higher Pi) enhanced sucrose importation into the latex cells. The latex system mostly functions as an accumulation sink.

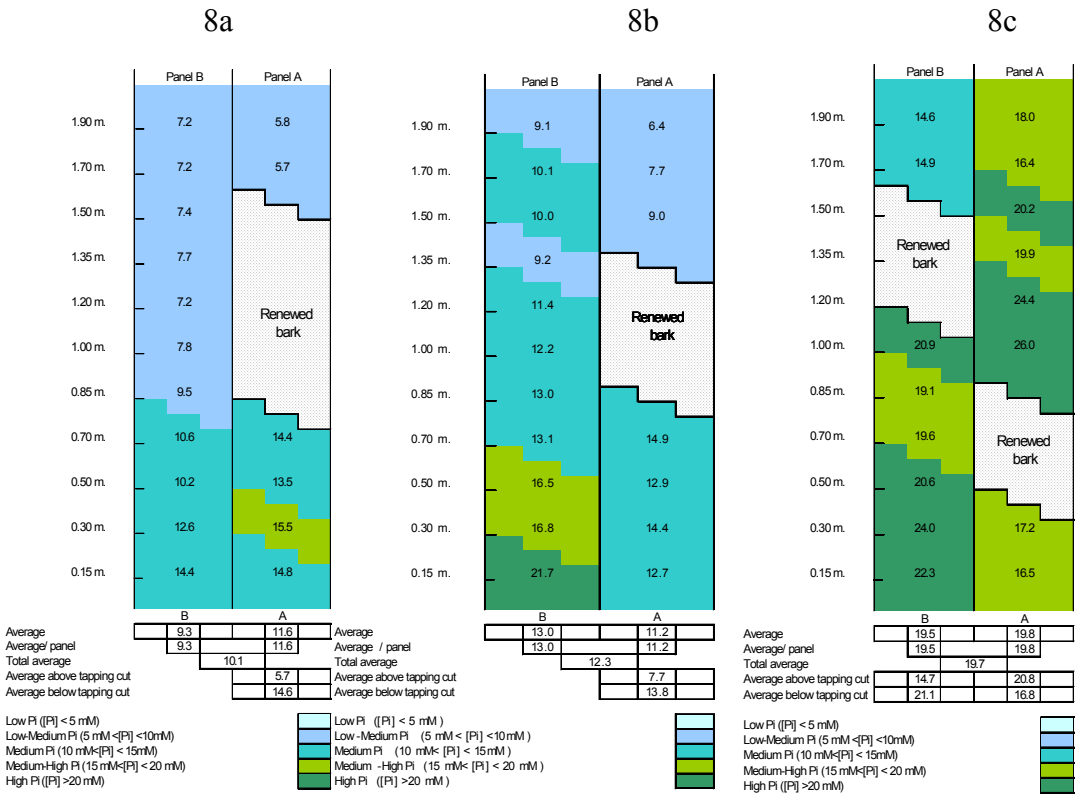

Figure 8 Latex metabolic activity areas determined by latex Pi level (Average of 4 replications per treatment) (8a) D/2, (8b) D/3 and (8c) DCA system.

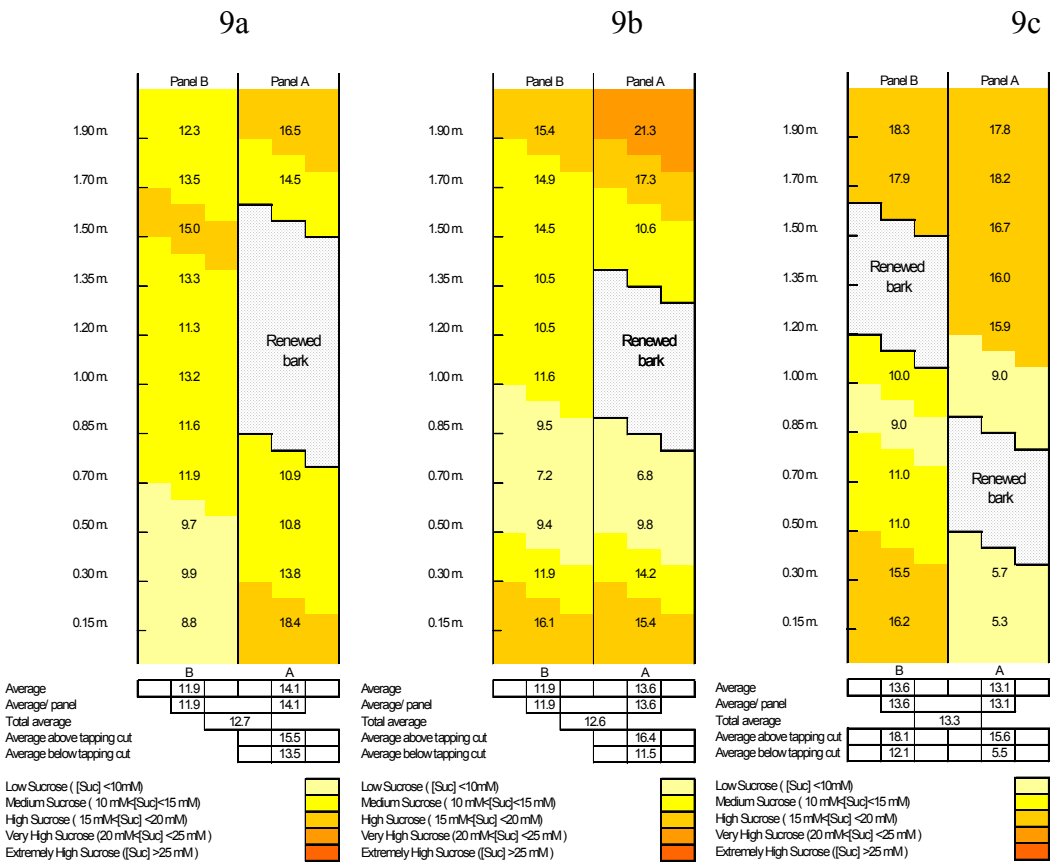

Figure 9 Latex sucrose content distribution in the lower part of the trunk (average of 4 replications per treatment). The estimated latex regeneration area was limited by low Sucrose and high Pi. (9a) D/2, (9b) D/3 and (9c) DCA system.

Table 5 Relation between estimated size of latex regeneration area, production and average metabolic parameters (Suc, Pi) measured inside latex of panel (A) of different tapping systems.

Tapping system[1/]	Latex regeneration area (m^2)	Average [Suc] concentration Panel A[4/] ($mM.l.latex^{-1}$)	Average [Pi] concentration Panel A ($mM.l.latex^{-1}$)	Average production ($g.tree^{-1}.tapping^{-1}$)	Production[2/] ($kg.tree^{-1}.year^{-1}$)
D/2	0.29	14.1	11.5	33.1	3.75
D/3	0.32	13.6	11.2	43.7	3.33
ET 8/y	(10%)[3/]	(-4%)	(-3%)	(32%)	(-11%)
DCA, ET	0.42	13.1	19.6	42.3	4.45
2x(4/Y)	(45%)	(-5%)	(71%)	(28%)	(19%)

Note: 1/ D/3: 1/2S d/3 ET 2.5% and DCA: 2x1/2S d/4(t,t) ET2.5% 2x4/y (8/y)

2/ Rubber production during May 2002 – February 2004.

3/ (-) percentage compared with D/2

4/ Latex collections were done under the tapping cut (the middle and 5 cm from the cut).

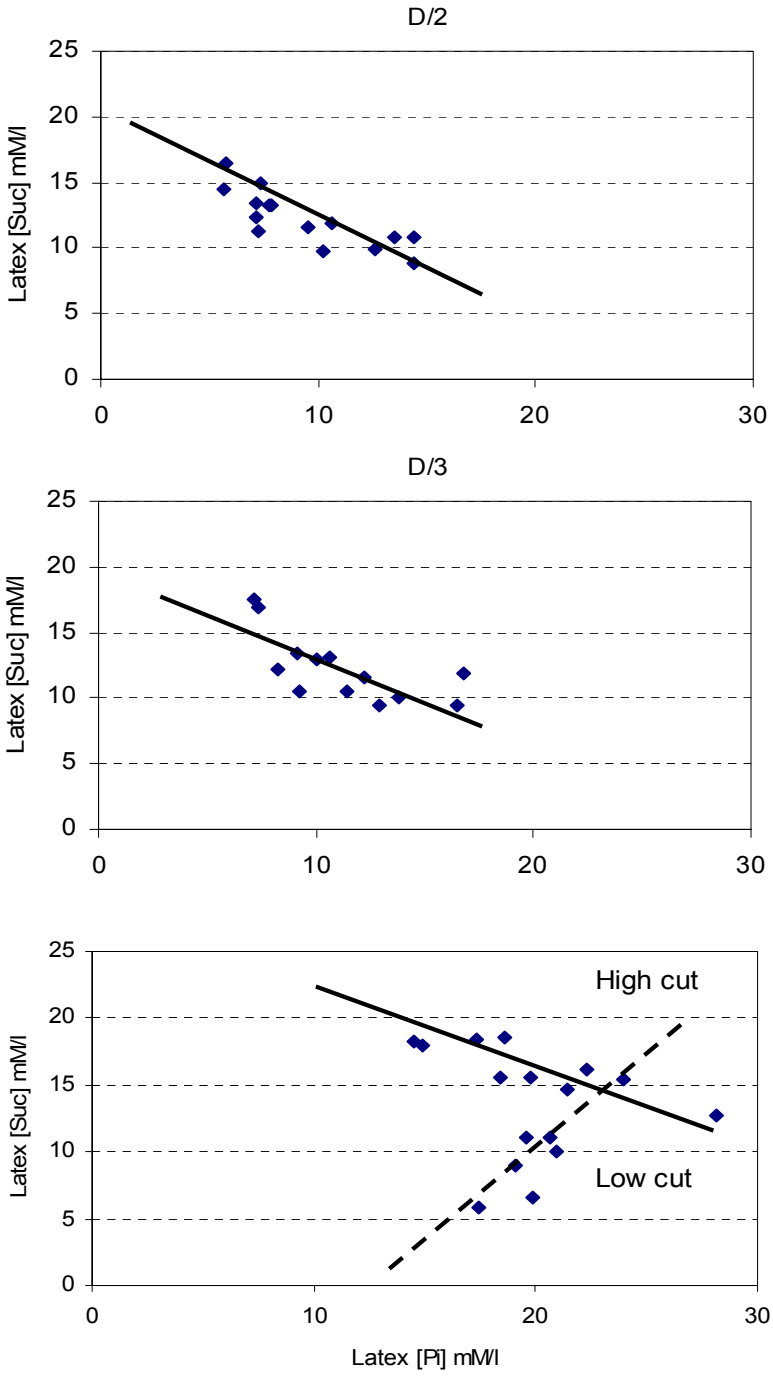

Figure 10 Relationship between latex sucrose content (Suc) and latex inorganic phosphorus content (Pi): all sampling positions.

5. Non-structural Carbohydrate in Trunk Wood

5.1 Concentration of non-structural carbohydrate in trunk wood

Starch was the major component in trunk wood, accounting for 79% of TNC (total non-structural carbohydrate) in control or untapped treatment (Table 6). SS (soluble sugar) was almost made of sucrose only. Glucose and fructose accounted for a negligible proportion except at refoliation, when SS was the highest in refoliation (February 2003 and January 2004) and starch was the least (Table 8).

5.2 Effects of tapping and panel on carbohydrate reserve

Mean TNC at tree scale was significantly higher in tapped treatments (D/2, D/4 and DCA) than in control (Appendix Table 1 and Table 6). This was a result of higher starch content. DCA had the highest starch and TNC, D2 and D4 were medium and control treatment was the least TNC. But only D4 had a higher SS content than others. Consequently, starch accounted for 81 %, 80% and 82 % of TNC in D/2, D/4 and DCA respectively.

In D/2 and D/4 the untapped side of the tree (panel B) had significantly difference higher starch and TNC than the tapped one (panel A). However, the latter had still higher content than control (Table 7). DCA, there was no difference between the two sides of the tree (panel A and panel B) which were both tapped. They had the same content in starch and TNC than the untapped panel of D/2 and D/4, and therefore higher content than the tapped panel of these classical tapped treatments.

The untapped side of D/4 (panel B) had significantly higher SS than the tapped one (panel A). Nevertheless, there was no significant difference between panels within D/2 and DCA.

Table 6 Main effect of treatment on non-structural carbohydrate concentrations (mg_{Glu}/g_{SDM}) in trunk wood, as averaged for all dates and distances from ground (0-2 m.).

Treatment	Starch	SS	TNC
Control	51.64 c	13.99 b	65.63 c
D/2	59.40 b	14.19 b	73.59 b
D/4	59.83 b	15.08 a	74.92 b
DCA	62.98 a	14.25 b	77.23 a
F Statistic	27.40	8.03	29.16
P	.0001	.0001	.0001

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

Table 7 Analysis of variance by combined treatments and panel of D/2 and D/4 (Tapped on panel A and untapped on panel B) for all dates and distance from ground (0-2m.). Mean concentration of non-structural carbohydrates (mg_{Glu}/g_{SDM}) in trunk wood.

Treatment	Starch	SS	TNC
D/2 x Panel A	57.95 b	14.22 bc	71.27 b
D/2 x Panel B	61.76 a	14.15 c	75.91 a
D/4 x Panel A	57.95 b	14.78 b	72.73 b
D/4 x Panel B	61.70 a	15.39 a	77.10 a
F statistic	7.78	7.91	9.54
P	.0001	.0001	.0001

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

5.3 Seasonal variation of starch, SS and TNC concentration

The overall pattern (Table 8 and Fig. 11), during the first year of observation, the highest TNC concentration was recorded at leaf-fall (February 2003) followed by a huge drop just after complete refoliation (March 2003) whatever the treatment. A net deposition occurred mainly from May 2003 to leaf-fall (January 2004), i.e. the period, including the rainy season, when both radial growth and (for tapped trees) latex regeneration occurred. SS and starch had opposite trend. In high production to defoliation stage (February 2003, October 2003 and February 2004, October 2004), starch was high and SS was low, conversely just after leaf-fall, in March, starch was low and SS high (Table 8). However, variations in TNC were mainly accounted for by variations in starch.

During the second year of observation (2004), the drop in starch and TNC content after refoliation was of lower extant than the previous year. Starch content ranged 43.05-57.38 mg_{Glu}/g_{SDM} in February 2004 whereas it ranged 12.77-32.21 mg_{Glu}/g_{SDM} in March 2003. During the following vegetative season (May 2004 to October 2004) the increase in starch and TNC were not as strong as the previous year.

Although the overall pattern was similar for all treatments, the date x treatment interaction was significant, indicating that dynamics differed among treatments at some periods. Along 2 years, mean total TNC concentration of control ranged 31.5-81.2 mg_{Glu}/g_{SDM} (Fig. 11). Starch accumulated extensively in February 2003 (leaf fall) and was responsible for the drop in TNC after that. TNC increased regularly from March 2003 (refoliation) to October 2003 (high rainfall). Starch decreased between October 2003 and leaf fall, whereas SS increased sharply, thus TNC was stable. SS Peaked at leaf fall for both years.

At most periods, starch and TNC were higher in tapped treatments (D/2, D/4 and DCA) than in untapped treatment. DCA had the highest starch and TNC and then D2 and D4 were medium order. Along 2 years, average total TNC concentration

(mg_{Glu}/g_{SDM}) ranged 33.7-97.0, 41.2-92.2 and 41.7-96.4 for D/2, D/4 and DCA respectively (Fig. 11).

Dynamics of starch and SS significantly differed among treatments (Appendix Table 1). Thus, differences between treatments were not the same along the year (Fig. 11). At leaf fall (in February 2003), starch and TNC peaked for all treatments. DCA was significantly higher than control. Starch and TNC at the refoliation period (March 2003) decreased more in control and D/2 than in D/4 and DCA. Difference of carbohydrate concentration between DCA and control was high (19.27 mg_{Glu}/g_{SDM}). Minimal annual concentration was recorded at that time for all treatments but DCA. During dry season and tapping rest (May 2003), starch and TNC increased slightly for all treatments but DCA, which reached annual minimal concentrations. Difference of carbohydrate concentration among treatments was low. The increase in starch during the period of high growth and high latex production (October 2003) was the highest for DCA but difference between treatment was little. However, SS decreased much more for control than for tapped treatments (among the latter it decreased significantly only in D/2). Consequently, DCA and D/4 showed higher TNC than D/2 and control.

At leaf fall stage (January 2004), starch and TNC peaked for all tapped treatments whereas it decreased for control. Difference between tapped treatments and control was the highest recorded along the year. At refoliation stage (February 2004), there was a sharp decrease in starch for tapped treatments and to a lower extent for control. However, starch remained higher in D/2 and DCA than in D/4 and control. As a whole, starch did not decrease as much as during the similar period the previous year. During dry season and tapping rest, between February 2004 and May 2004, tapped treatments had higher starch and TNC than control. Between May 2004 to October 2004 starch and TNC increased more for control than for tapped treatments (not as the previous year). The additional sampling date in August 2004 showed that during the period of high growth for tapped trees (May to August), starch decreased in tapped trees, whereas it increased in control. As a whole, in October 2004 TNC levels were the close to those of 2003, and D/2 had higher TNC than DCA.

SS showed larger variations in control and D/4 than in D/2 and DCA. Particularly SS evolution in D/4 was opposed to that in other treatments in May and October 2004.

Table 8 Mean concentration of non-structural carbohydrate (mg_{Glu}/g_{SDM}) in trunk wood, per date, for all treatments and locations (0-2 m.).

DATE	Starch	SS	TNC
5 February 2003	70.32 b	15.79 bc	86.12 b
6 March 2003	31.72 f	16.93 a	48.65 f
2 May 2003	38.44 e	13.29 d	51.73 f
28 October 2003	65.61 c	11.55 e	77.16 d
19 January 2004	76.04 a	16.54 ab	92.58 a
20 February 2004	61.09 d	16.10 b	77.19 d
11 May 2004	65.60 c	13.30 d	78.91 d
6 August 2004	59.10 d	11.09 e	70.19 e
18 October 2004	67.13 bc	15.28 c	82.41 c
F Statistic	161.96	71.84	162.06
P	.0001	.0001	.0001

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

Note : Defoliation period = 5 February 2003 and 19 January 2004
Refoliation period = 6 March 2003 and 20 February 2004
Start tapping or low production period = 2 May 2003 and 11 May 2004
High production period = 28 October 2003 and 18 October 2004

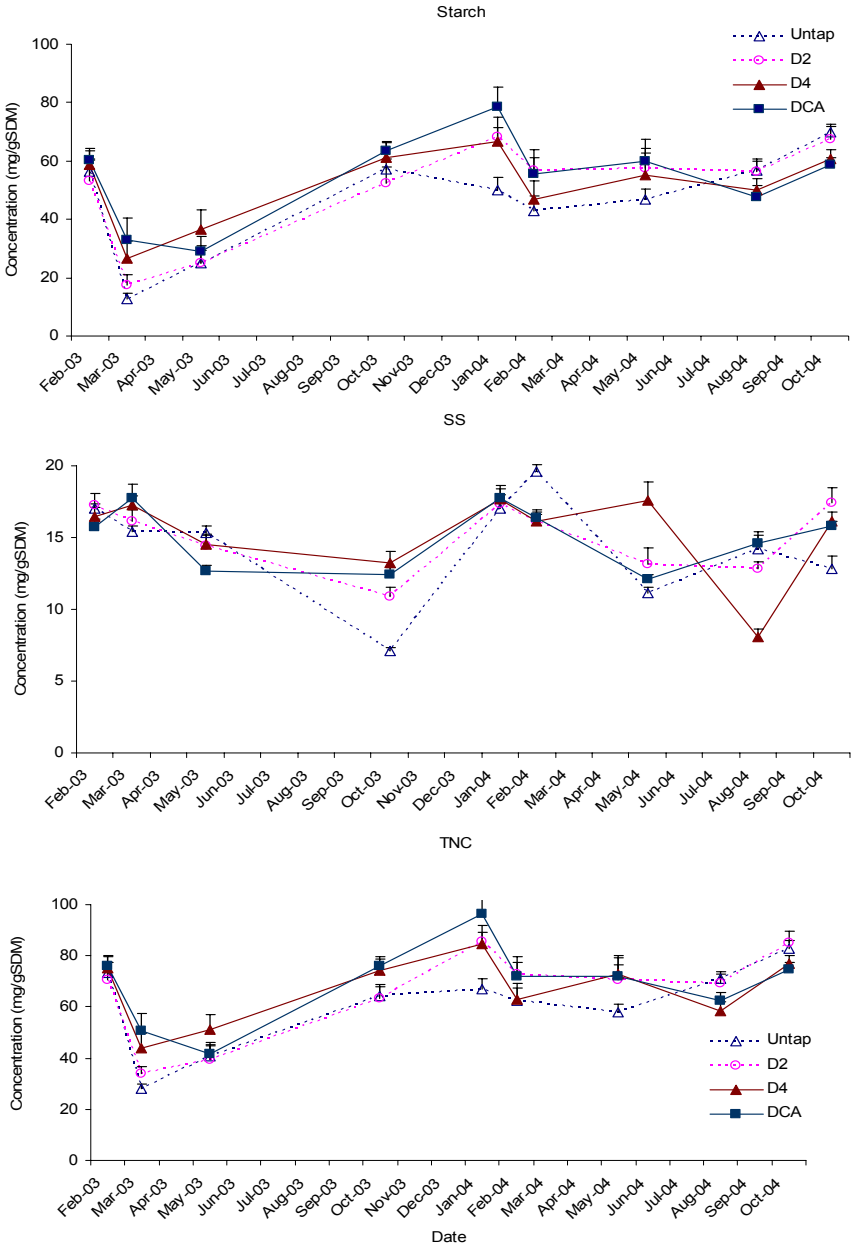

Figure 11 Mean carbohydrate concentration (mg_{Glu}/g_{SDM}) in trunk wood, up to 600 cm, at 9 sampling dates. February 2003 and January 2004– leafless stage, March 2003 and February 2004 – at the end of refoliation, May 2003 and May 2004 – resting period for tapping, October 2003 and October 2004 – high latex production period. starch; SS, total soluble sugars; TNC, total non-structural carbohydrates.

5.4 Effect of tapping on vertical pattern of carbohydrate

Distance from ground had a very significant effect on starch, SS and TNC (Appendix Table 1). Distance x Treatment interaction was significant, indicating that vertical patterns differed among treatments.

Control, there was a decreasing bottom-up starch gradient along the trunk (Fig. 12). This gradient was larger in the lower part of the trunk (20-110 cm from ground) than in the upper part (150-300 cm from ground). Whereas, the overall trend of SS was a slight increasing bottom-up gradient along the axis (Fig. 13). Such SS gradient was opposite to starch, but the range was lower (2.5 mg_{Glu}/g_{SDM} difference between 20 cm to 300 cm from ground). Therefore, vertical patterns in TNC of control mainly relied on changes in starch along the trunk. In D/2 and D/4 which only panel A was tapped, vertical distribution patterns of starch were much irregular with large variations related to the location of the tapping cut in panel A (Fig. 12). Nevertheless, there was an overall significant decreasing bottom-up gradient along the trunk. The vertical gradient was locally disturbed by the presence of the tapping cut at 80-110 cm. distance from ground, with a trend to accumulate starch in wood of previously tapped area, where bark is regenerating. However, vertical patterns in the untapped panel (B) of D/2 and D/4 was closer to that of control although the gradient was less marked in the lower part and more irregular. Starch content remained higher in panel B of tapped trees than in control all along the trunk. The overall trend of SS was a slight increasing bottom-up gradient along the axis. Such SS gradient was opposite to starch (Fig. 13). It was similar in D/2 than in control, but there was a clear impact of the tapping cut in D/4. SS was the highest at 90 cm in tapped panel and the least at the same height in the opposite untapped panel. Starch and SS in DCA (Fig. 12 and 13) were no different between panel A and panel B, both panels being tapped. The overall gradients of starch and SS had the same trend as in control. However, the SS gradient between 20-200 cm from ground was lower than in control, starch content being much higher all along this part of the trunk for DCA (between 60 to 78 mg_{Glu}/g_{SDM}).

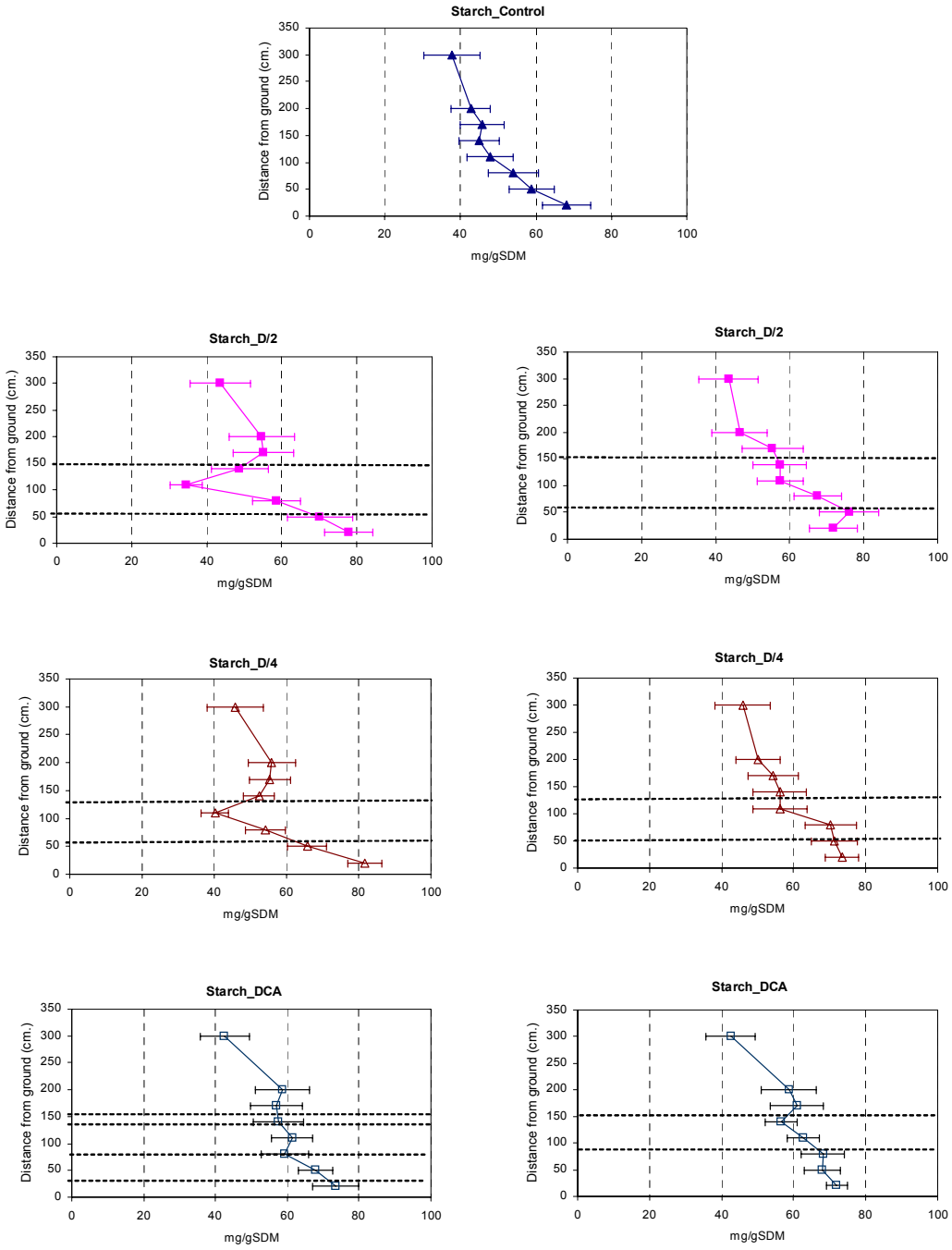

Figure 12 Vertical distribution of starch on panel A (left side) and starch on panel B (right side) in trunk wood. Panel A was tapped in D/2 and D/4 whereas panel B was untapped. Both panel A and B were tapped in DCA, average from 9 dates.

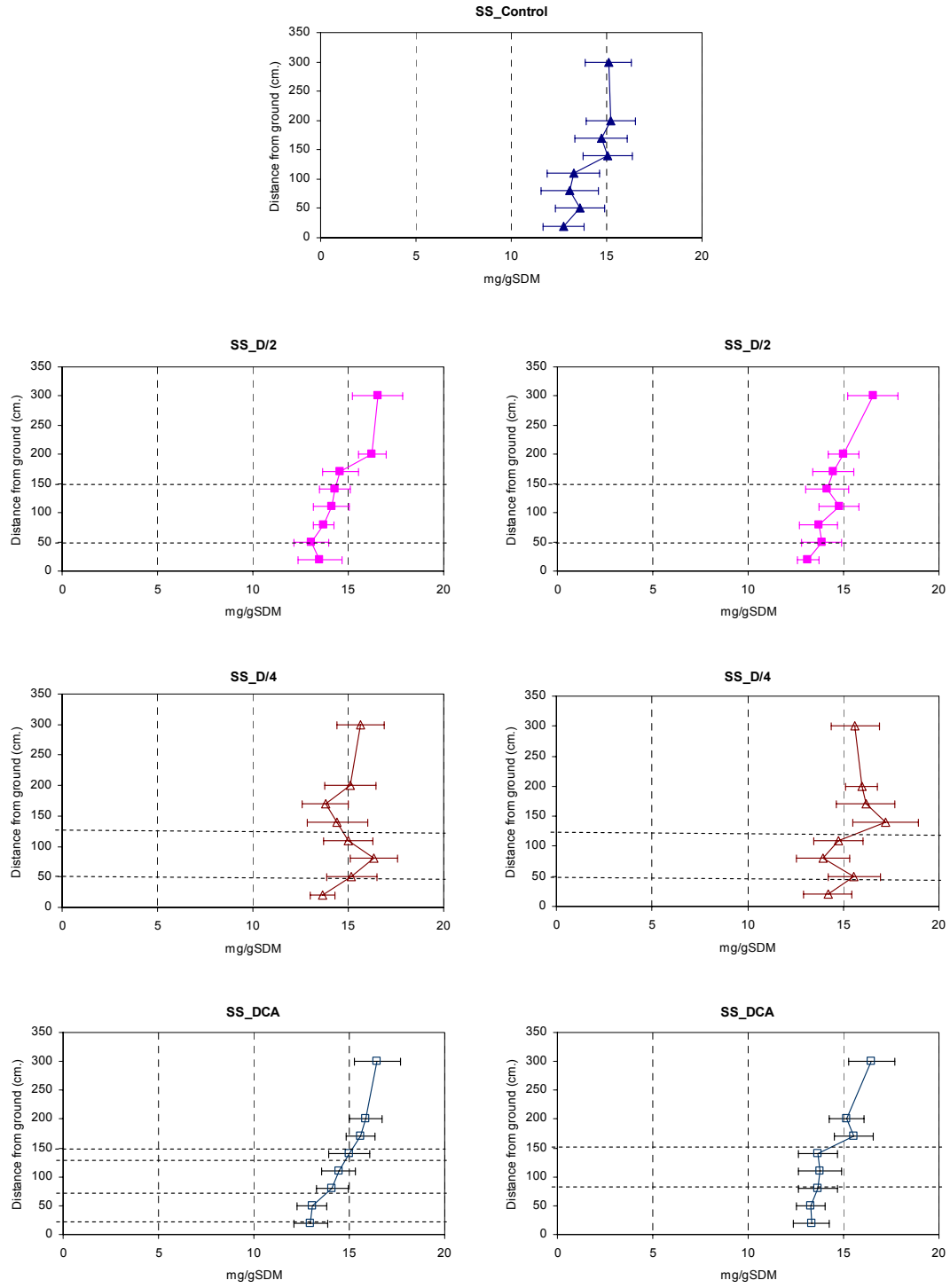

Figure 13 Vertical distribution of SS, total soluble sugar on panel A (left side) and SS on panel B (right side) in trunk wood. Panel A was tapped in D/2 and D/4 whereas panel B was untapped. Both panel A and B were tapped in DCA, average from 9 dates.

Table 9 Difference in starch concentration between tapped treatments and control along the trunk wood in percent**.**

Height (cm.)	Starch (%) Wood in Panel A			Starch (%) Wood in Panel B		
	D/2	D/4	DCA	D/2	D/4	DCA
600	-3	-5	-6	-3	-5	-6
500	6	2	7	6	2	7
400	1	7	15	1	7	15
300	15	21	13	15	21	13
200	28	31	37	9	17	37
170	21	21	25	21	19	33
140	8	16	28	28	25	26
110	-28	-16	28	20	17	31
80	8	0	10	25	30	26
50	19	12	15	29	21	15
20	14	20	8	6	8	6

Note: The shadowed areas represent the renew bark.

Table 10 Differences in soluble sugar concentration between tapped treatments and control along the trunk wood in percent**.**

Height (cm.)	SS (%) Wood in Panel A			SS (%) Wood in Panel B		
	D/2	D/4	DCA	D/2	D/4	DCA
600	10	5	4	10	5	4
500	5	2	0	5	2	0
400	11	7	8	11	7	8
300	10	4	9	10	4	9
200	7	-1	4	-1	5	0
170	-1	-6	6	-2	10	6
140	-5	-4	0	-6	14	-9
110	7	13	9	11	11	4
80	5	25	8	5	7	5
50	-4	12	-4	2	14	-2
20	6	7	2	3	11	4

Note: The shadowed areas represent the renew bark.

6. Non-structural Carbohydrate in Trunk Bark

6.1 Concentration of non-structural carbohydrate in trunk bark

SS was the major component of trunk bark, accounting for 73 % of TNC in control treatment (Table 11). SS was almost made of sucrose only. Mean SS was higher in bark (20.0 mg_{Glu}/g_{SDM}) than in wood but mean starch content was much lower (8.4 mg_{Glu}/g_{SDM}). Consequently, mean TNC was lower in bark (28.4 mg_{Glu}/g_{SDM}) than in wood.

6.2 Effect of tapping and panel on carbohydrate reserve

SS content was lower in D/4 and DCA than in control and D/2, whereas starch was higher in DCA than in other treatments. Consequently, TNC was lower in D/4 than in other treatments (Appendix Table 2 and Table 11). SS accounted for 72 %, 70 % and 69 % of TNC in D/2, D/4 and DCA respectively.

Tapped treatments of D/2 and D/4 showed significantly higher starch, SS and TNC in panel A (tapped panel) than in panel B (untapped panel). Thus, it was the contrary to what happened in wood. Whereas in DCA, there was no significant difference between the two panels, which were both tapped. Among all panel x treatment combinations, panel A of D/2 had the highest TNC (Table 12).

Table 11 Main effect of treatment on non-structural carbohydrate concentrations (mg_{Glu}/g_{SDM}) in trunk bark, as averaged for all dates and distances from ground (0-2 m.).

Treatment	Starch	SS	TNC
Control	8.34 b	20.13 a	28.91 a
D/2	8.26 b	20.71 a	28.97 a
D/4	8.16 b	18.66 b	26.82 b
DCA	8.80 a	19.32 b	28.12 a
F Statistic	4.04	15.72	9.94
P	.0073	.0001	.0001

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

Table 12 Three-way Analysis of variance by combined treatments and panel of control (untapped tree on panel A), D/2 and D/4 (Tapped on panel A and untapped on panel B) and DCA (both panel A and B were tapped), for all dates and distance from ground (0-2m.). Mean concentration of non-structural carbohydrates (mg_{Glu}/g_{SDM}) in trunk bark.

Treatment	Starch	SS	TNC
D/2 x Panel A	8.65 a	21.61 a	30.26 a
D/2 x Panel B	7.87 b	19.80 b	27.67 b
D/4 x Panel A	8.72 a	19.29 b	28.02 b
D/4 x Panel B	7.59 b	18.03 c	25.62 c
F statistic	10.63	25.52	27.14
P	.0001	.0001	.0001

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

6.3 Seasonal variation of starch, SS and TNC concentration

The overall pattern for TNC (Table 13 and Fig. 14) was the same as in wood. The highest TNC concentration was recorded at leaf-fall (February 2003) followed by a huge drop just after complete refoliation (March 2003). A net deposition occurred mainly from May 2003 to next leaf-fall (January 2004). TNC concentration dropped again after complete refoliation (February 2004) whatever the treatment. From February 2004 to May 2004 (dry season and tapping rest) to high production (October 2004) there was a steady increase. Thus, contrary to results in wood, the seasonal pattern was the same for TNC along the 2 years of observation.

In bark, SS and starch had same trend, except that during the first year most SS deposition occurred earlier (between March and May 2003) than main deposition of starch (between May and October 2003). SS and starch were high in high production stage (October 2003 and 2004) and leaf fall (February 2003 and January 2004), conversely just after leaf-fall, in refoliation period (March 2003 and February 2004), SS and starch were low (Fig. 14). Both changes in SS and starch contributed significantly to variations in TNC along time.

Interaction between date and treatment effects was significant, indicating differences in dynamics according to treatments (Appendix Table 2). As a result differences among treatments were not the same along time. However, differences between taped and untapped treatments were not as clear as in wood.

Starch dynamics was very close for D/2 and control, except in October 2004. As a whole, variations of starch in DCA where more irregular than in other treatments. Particularly its content dropped at leaf fall in January 2004, whereas it peaked for all other treatments. During the period of high latex production (October), DCA had a much higher content than control. In 2003 the same observation was true for D/4, whereas it was the case for D/2 in 2004 (Fig. 14).

SS content changed less along time in control and D/2 than in D/4 and DCA. D/2 had the highest content almost all the time, particularly during the periods of high latex production (October). Differences between treatments were the least SS after refoliation, when SS content was low for all. In August 2004 SS content dropped in D/4 contrary to other treatments (Fig. 14).

Along the year, mean total TNC concentration ranged 26.6-33.0, 27.9-34.8, 24.7-30.7 and 27.2-31.4 mg_{Glu}/g_{SDM} for control, D/2, D/4 and DCA respectively (Fig. 14). There was little difference between treatments for TNC dynamics. Differences were recorded in January 2004 (leaf fall) when DCA had lower content than others, in August 2004 when D/4 had lower content than others and in October 2004 (high latex production) when D/2 had a higher content than other treatments.

Interaction between date and treatment effects was significant, indicating differences in dynamics according to treatments (Appendix Table 2). As a result differences among treatments were not the same along time. However, differences between taped and untapped treatments was not as clear as in wood.

Starch dynamics was very close for D/2 and control, except in October 2004. As a whole variations of starch in DCA where more irregular than in other treatments. Particularly, its content dropped at leaf fall in January 2004, whereas it peaked for all other treatments. During the period of high latex production (October), DCA had a much higher content than control. In 2003 the same observation was true for D/4, whereas it was the case for D/2 in 2004 (Fig. 14). SS content changed less along time in control and D/2 than in D/4 and DCA. D/2 had the highest SS content almost all the time, particularly during the periods of high latex production (October). Differences between treatments were the least SS after refoliation, when SS content was low for all. In August 2004 SS content dropped in D/4 contrary to other treatments.

Along the year, mean total TNC concentration ranged 26.6-33.0, 27.9-34.8, 24.7-30.7 and 27.2-31.4 mg_{Glu}/g_{SDM} for control, D/2, D/4 and DCA respectively (Fig.

12). There was little difference between treatments for TNC dynamics. Differences were recorded in January 2004 (leaf fall) when DCA was lower content than others, in August 2004 when D/4 was lower content than others whereas D/2 had a higher TNC content than other treatments in October 2004 (high latex production).

Table 13 Mean concentration of non-structural carbohydrate (mg_{Glu}/g_{SDM}) in trunk bark, per date, for all treatments and locations (0-2 m.).

DATE	Starch	SS	TNC
5 February 2003	6.74 e	23.78 a	30.52 c
6 March 2003	3.83 f	17.08 f	20.91 g
2 May 2003	3.46 f	21.95 bc	25.41 e
28 October 2003	11.56 b	20.99 c	33.08 ab
19 January 2004	11.54 b	22.50 b	34.04 a
20 February 2004	7.88 d	15.84 g	23.71 f
11 May 2004	8.33 d	16.75 fg	25.08 e
6 August 2004	9.58 c	18.49 e	28.06 d
18 October 2004	12.57 a	19.60 d	32.17 b
F Statistic	213.30	61.42	90.30
P	.0001	.0001	.0001

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

Note : Defoliation period = 5 February 2003 and 19 January 2004

Refoliation period = 6 March 2003 and 20 February 2004

Start tapping or low production period = 2 May 2003 and 11 May 2004

High production period = 28 October 2003 and 18 October 2004

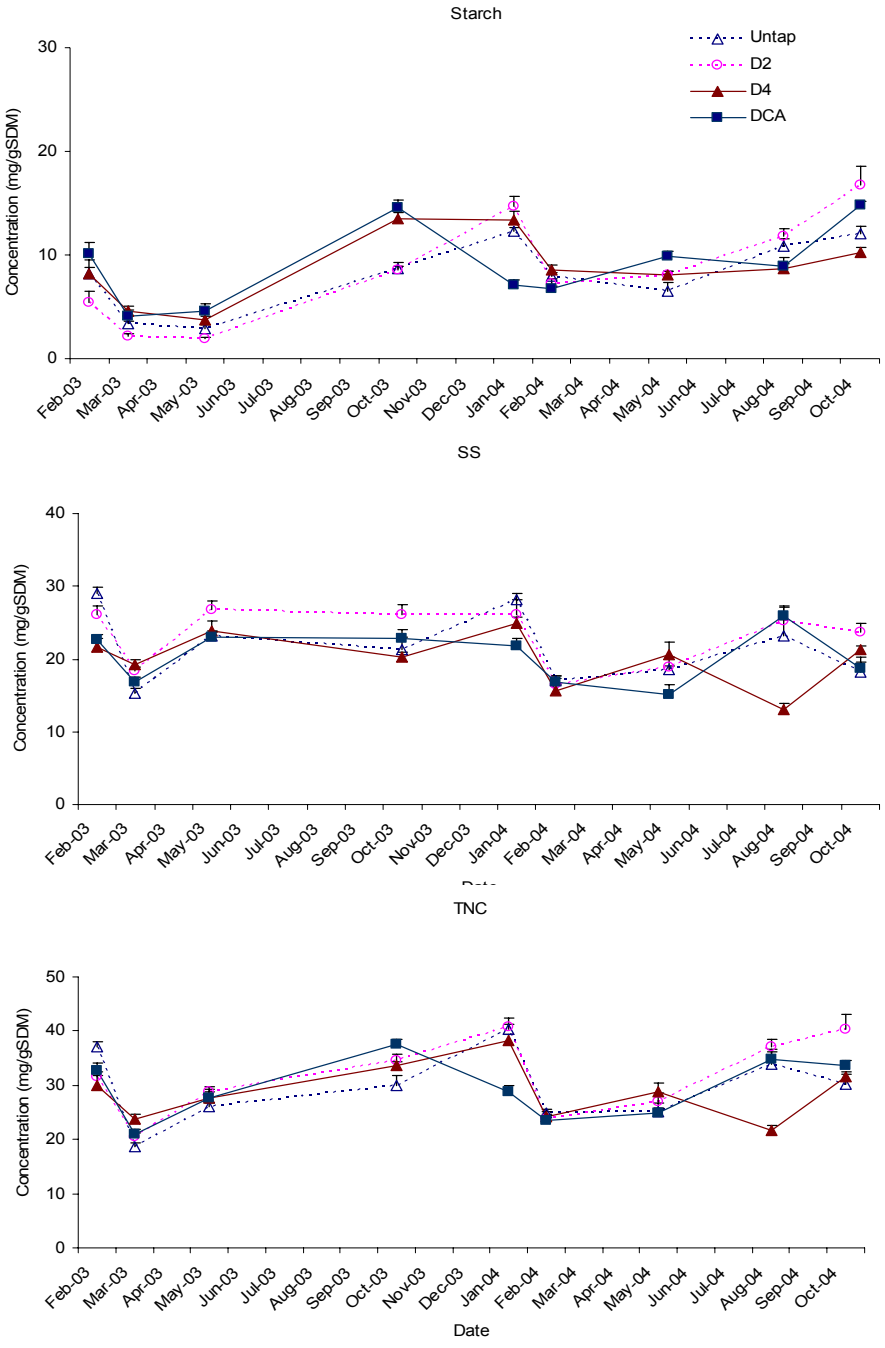

Figure 14 Mean carbohydrate concentration (mg_{Glu}/g_{SDM}) in trunk bark on panel A, up to 600 cm, at 9 sampling dates. February – leafless stage, March – at the end of refoliation, May – resting period for tapping, October – high latex production period. Starch; SS, total soluble sugars; TNC, total non-structural carbohydrates.

6.4 Effects of tapping on vertical pattern of carbohydrate

Distance from ground showed a highly significant effect on SS and starch (Fig. 15 and 16). Vertical variations in TNC were almost the same as variations in SS. The vertical patterns are detailed hereafter; SS content of control treatment did not change along the trunk between 20 to 150 cm from ground. It increased between 150 to 300 cm from ground (Fig. 15 and Table 14) whereas SS vertical distribution patterns of D/2 and D/4 were much irregular with large variations related to the location of the tapping cut in panel A. Nevertheless, there was an overall increasing bottom-up gradient along the trunk. SS accumulated at 80-110 cm distance from ground, where bark is regenerating. In panel B (untapped) trend was the same for both treatments and similar to control. SS in tapped panel of D/4 showed the same trend in both wood and bark. In panel A, SS of DCA was the same pattern as control, although this panel was tapped. In the other side (panel B), which was tapped too, there was a trend to accumulate SS in the bark regeneration area (110-150 cm from ground), although it was not as clear as in treatments with one cut only (D/2 and D/4).

Starch content of control treatment was a slight decreasing bottom-up gradient along the trunk (Fig. 16 and Table 15). In panel A of D/2 was high variability and no clear trend was shown. However, in D/4 the pattern was clearly opposite to SS pattern, starch being depleted between 80 to 110 cm from ground in the bark regenerating area. There was no gradient starch in panel B (untapped) for both treatments. Starch of DCA tended to be depleted in the bark regenerating area of each panel, but variability within location was high.

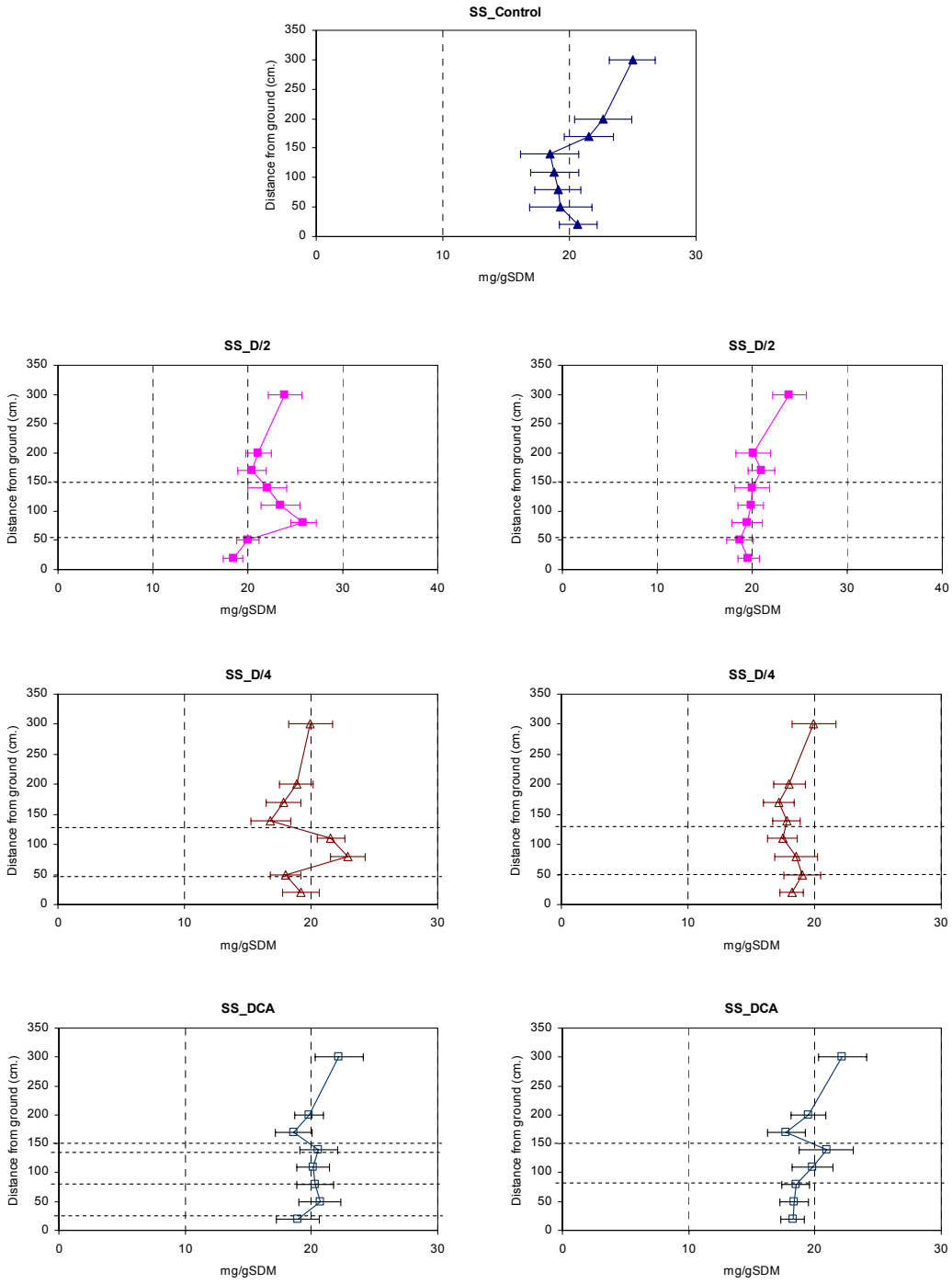

Figure 15 Vertical distribution of SS, total soluble sugars on panel A (left side) and SS on panel B (right side) in trunk bark, average from 9 dates.

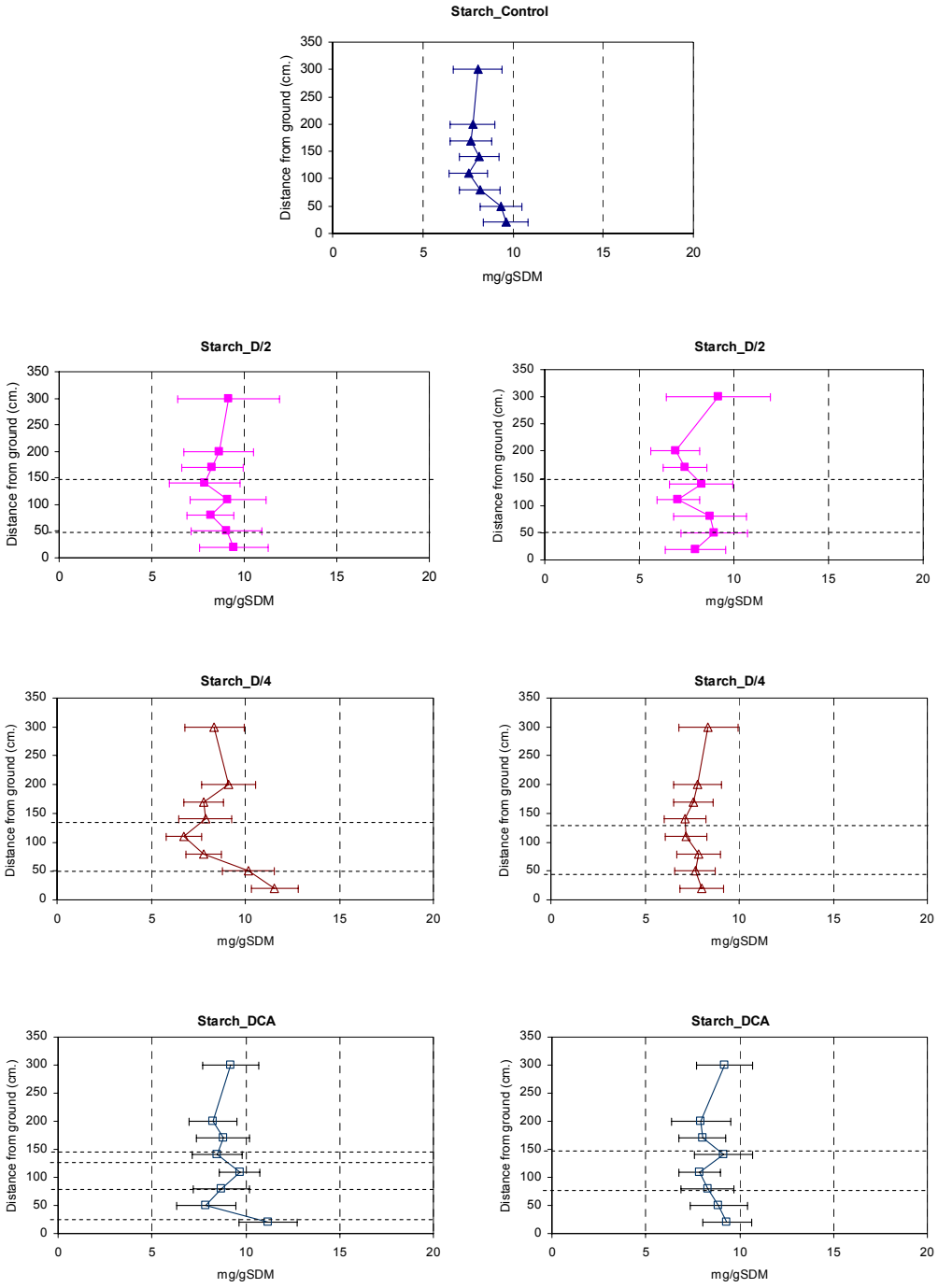

Figure 16 Vertical distribution of starch on panel A (left side) and starch on panel B (right side) in trunk bark, average from 9 dates.

Table 14 Difference in starch concentration between tapped treatments and control along the trunk bark in percent**.**

Height (cm.)	Starch (%) Bark in Panel A			Starch (%) Bark in Panel B		
	D/2	D/4	DCA	D/2	D/4	DCA
600	16	17	30	-10	-10	0
500	16	22	19	-3	3	0
400	-16	6	2	-18	4	0
300	14	4	14	0	-9	0
200	11	18	6	-16	-6	-4
170	8	2	15	-16	-14	-9
140	-3	-3	4	-2	-16	8
110	21	-10	29	-27	-26	-19
80	0	-4	6	0	-10	-5
50	-3	9	-16	14	-3	13
20	-2	20	16	-29	-28	-17

Note: The shadowed areas represent the renew bark.

Table 15 Difference in soluble sugar concentration between tapped treatments and control along the trunk bark in percent**.**

Height (cm.)	SS (%) Bark in Panel A			SS (%) Bark in Panel B		
	D/2	D/4	DCA	D/2	D/4	DCA
600	12	-6	-11	26	6	-4
500	8	-8	-7	16	-2	0
400	10	-15	-15	30	0	0
300	-4	-20	-11	8	-10	0
200	-7	-17	-13	2	-9	-2
170	-5	-17	-14	13	-8	-5
140	20	-9	12	-3	-14	2
110	25	15	7	-1	-13	-1
80	35	20	6	-4	-9	-9
50	4	-7	7	-10	-8	-11
20	-11	-7	-8	4	-4	-4

Note: The shadowed areas represent the renew bark.

7. Non-structural Carbohydrate in Taproot and Lateral Root

Root samples (taproot and lateral root) were separated to 2 parts: inner part and outer part of root. In inner part of root, mean starch, SS and TNC concentration were no significantly different among treatments (control, D/2, D/4 and DCA). Starch was the major component of inner part of root, accounting for 76-78% of TNC. (Appendix Table 3 and Table 16). Outer part of root, SS was the major component, accounting for 65-77 % of TNC. SS was no significant among treatments, whereas mean starch and TNC were significantly higher in DCA, D/4 and control than in D/2 (Table 16).

7.1 Effect of kind of roots on carbohydrate

In inner part of root, starch and TNC concentration were significantly higher in lateral root, both samples, than in taproot at 10 cm. and taproot at 30 cm. from ground. In the opposite, SS in taproots was significantly higher than lateral root. (Table 17).

Outer part of root, SS concentration was significantly higher in taproot at 10 cm and taproot at 30 cm than in lateral root. But starch in outer part of root was the same as in inner part. Lateral root had higher starch than both taproots (Table 17).

Table 16 Mean concentration of non-structural carbohydrates (mg_{Glu}/g_{SDM}) in inner part of root and outer part of root, as related to tapping treatment, for all dates and kind of roots homogeneous groups.

Treatment	inner part of root			outer part of root		
	Starch	SS	TNC	Starch	SS	TNC
Control	61.11	16.50	77.61	14.29 a	29.52	43.81 a
D/2	57.19	17.82	75.00	8.53 c	29.34	36.03 b
D/4	54.19	16.93	71.13	10.90 b	27.50	40.24 a
DCA	55.00	15.61	70.61	14.32 a	26.32	40.64 a
F statistic	2.08	2.51	2.43	18.28	1.17	6.67
P	.1043	.0599	.0663	.0001	.3206	.0003

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

Table 17 Mean concentration of non-structural carbohydrates (mg_{Glu}/g_{SDM}) in inner part of root and outer part of root, as related to kind of roots, for all dates and treatments.

	inner part of root			outer part of root		
Roots	Starch	SS	TNC	Starch	SS	TNC
Taproot at 10 cm.	52.69 b	17.31 b	70.00 b	10.72 b	29.00 a	39.72
Taproot at 30 cm.	50.92 b	18.74 a	69.66 b	11.69 b	30.45 a	42.14
Lateral root	67.00 a	14.10 c	81.10 a	13.36 a	25.39 b	38.74
F Statistic	22.50	22.45	12.44	5.92	6.94	2.28
P	.0001	.0001	.0001	.0032	.0012	.1044

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

7.2 Seasonal variation of carbohydrate in taproot and lateral root

The TNC overall pattern of both inner part and outer part of root were the same trend of seasonal variation (Table 18). The highest TNC concentration was recorded at leaf-fall (February 2003) followed by a huge drop just after complete refoliation (March 2003) whatever the treatment. A net deposition occurred mainly from May 2003 to leaf-fall (January 2004). TNC concentration was steady from refoliation (February 2004) to high production (October 2004).

Table 18 Mean concentration of non-structural carbohydrate (mg_{Glu}/g_{SDM}) in inner part and outer part of root, by date, for all treatments and locations.

Date	Inner part of root			Outer part of root		
	Starch	SS	TNC	Starch	SS	TNC
5 February 2003	64.88 ab	18.13 ab	83.01 a	6.42 d	41.09 a	47.52 a
6 March 2003	44.85 d	18.49 ab	63.34 c	9.58 bc	24.35 cd	33.92 c
2 May 2003	52.74 cd	12.52 e	65.26 c	7.40 cd	30.84 b	38.25 bc
28 October 2003	71.00 a	13.61 de	84.61 a	17.14 a	29.37 bc	46.51 a
19 January 2004	57.21 bc	20.21 a	77.42 a	14.26 a	33.73 b	48.00 a
20 February 2004	59.64 bc	19.99 a	79.63 a	9.37 bc	24.72 cd	34.10 c
11 May 2004	58.40 bc	17.02 bc	75.42 ab	15.08 a	25.13 cd	40.21 b
6 August 2004	51.31 cd	15.92 bcd	67.23 bc	11.41 b	25.49 cd	36.90 bc
18 October 2004	51.79 cd	14.57 cde	66.36 bc	15.20 a	21.77 d	36.96 bc
F Statistic	5.93	10.06	6.50	12.81	8.53	7.86
P	.0001	.0001	.0001	.0001	.0001	.0001

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

8. Hydrolysis of Starch Reserves and the Export of Soluble Sugars in Trunk

Total amylase enzyme is commonly involved in starch reserves mobilization (Witt and Sauter, 1994 and Sissons and MacGregor, 1994). For this experiment, the analytical chemistry method was modified from other trees: walnut and peach to rubber trees. The first step in the conversion between starch-sucrose is hydrolysis of starch by total amylase (α-amylase and β-amylase). The measurement of amylase activity consists to determine the amount of hydrolysis products formed (reducing sugar) according to time. As enzymes are catalysts the velocity of the reaction would be expected to be proportional to the concentration of the enzyme quantity and the incubation time. Then it was important to verify the linearity area to after work in these optimal conditions. These conditions were controlled for the other enzymes too. For the study of incubation time, with the same concentration of substrate (saturating) different incubation times were applied for a same volume of extract (50 µl) (Fig. 17). Therefore, the suitable volume of the extract and the incubation time to measure total amylase activity was 50 µl of extract and 90 minutes (Fig.18).

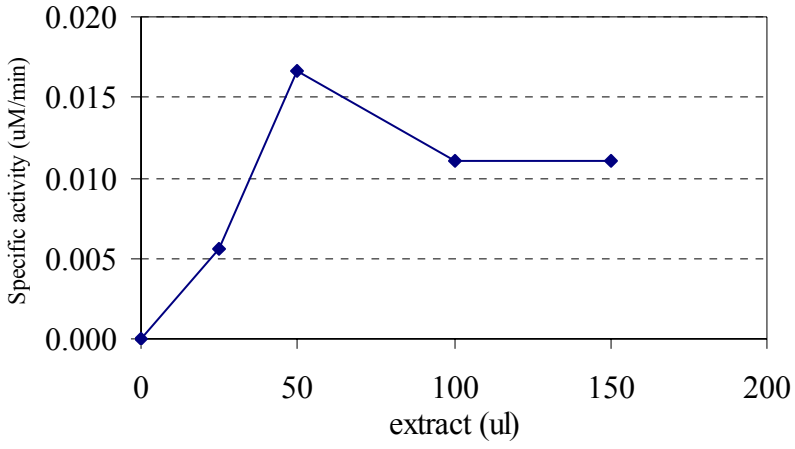

Figure 17 Influence of the volume of the extract on the total amylase activity (100 mM Hepes pH 8.0, KOH)

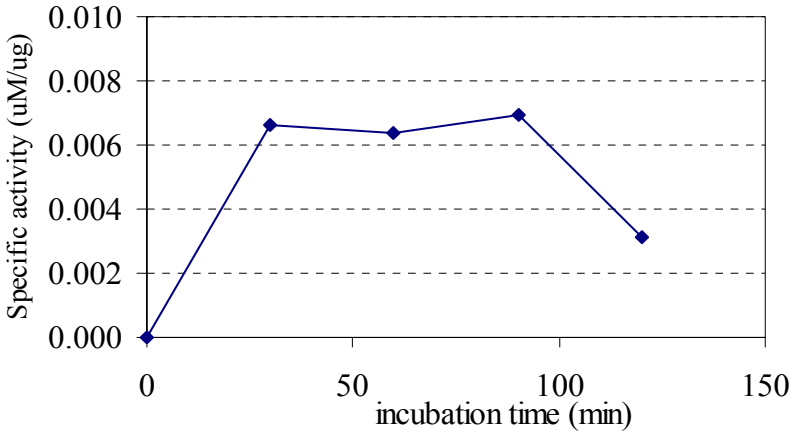

Figure 18 Influence of incubation time on total amylase activity (100 mM Hepes pH 8.0 KOH).

The sucrose phosphate synthase (SPS) seems to play an important role in sucrose biosynthesis in the wood parenchyma tissue. The resulting sugars could be either used locally or exported to other tissues in the form of sucrose. The intensity of this export largely depends on the activity of SPS (Wardlaw and Willenbrink, 1994). The activity of SPS was measured by quantifying the UDP produced linked to the sucrose phosphate synthesized according to time and quantity of extract. The quantity of extract was varied from 0 to 80 µl and the maximum velocity of SPS was measured with saturating concentration of substrates (Fig. 19). On other hand, the incubation time was varied and the maximum velocity was measured in the same condition with 50 µl of extract (Fig. 20). Thus, the suitable concentration to have the optimal SPS activity in our experimental conditions was 50 µl for the extract volume and 30 minutes for the appropriated incubation time.

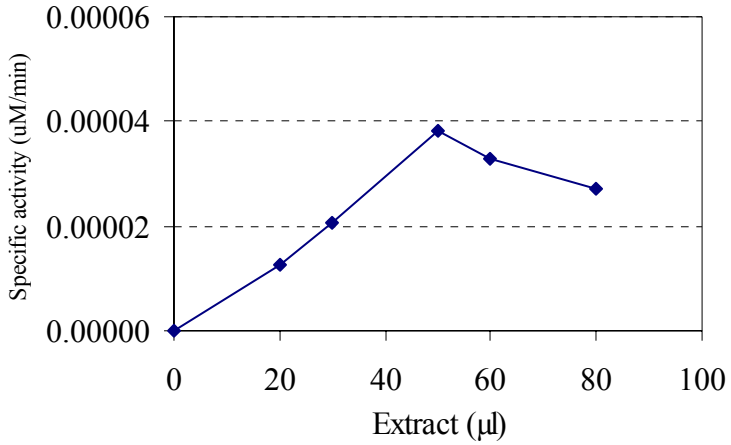

Figure 19 Influence of enzyme concencentration on Sucrose Phosphate Synthase (SPS) activity (4X SPS buffer: 400 mM Hepes pH 7.5 NaOH**,** 100 mM $MgCl_2$, 20 mM F6 P, 100 mM G6P, UDPG 80 mM and H_2O).

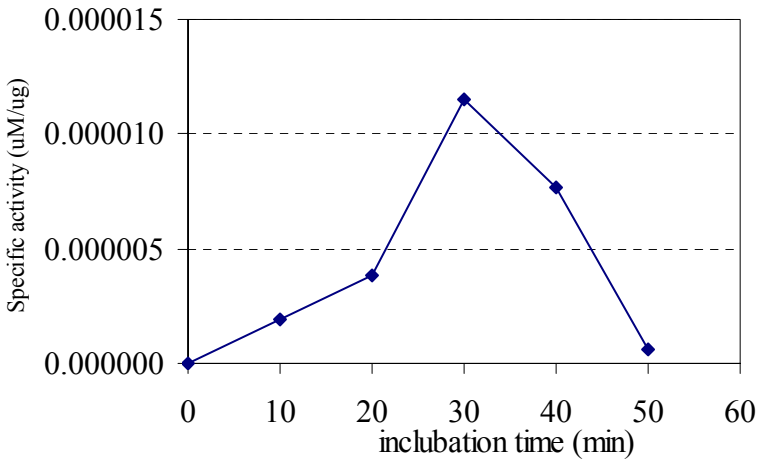

Figure 20 Influence of time incubation on Sucrose Phosphate Synthase (SPS) activity (4X SPS buffer: 400 mM Hepes pH 7.5 NaOH **,** 100 mM $MgCl_2$, 20 mM F6P, 100 mM G6P, UDPG 80 mM and H_2O).

Cell wall invertase (CWI) is involved in the sucrose transport in cell in hexose form and allow to evaluate the source-sink relationships between different organs or compartment. The extract was varied in volume from 0-60 µl and the maximum velocity of CWI at soluble content 50 µl (Fig. 21). And the maximum velocity was carried out with an incubation time of 20 minutes (Fig. 22).

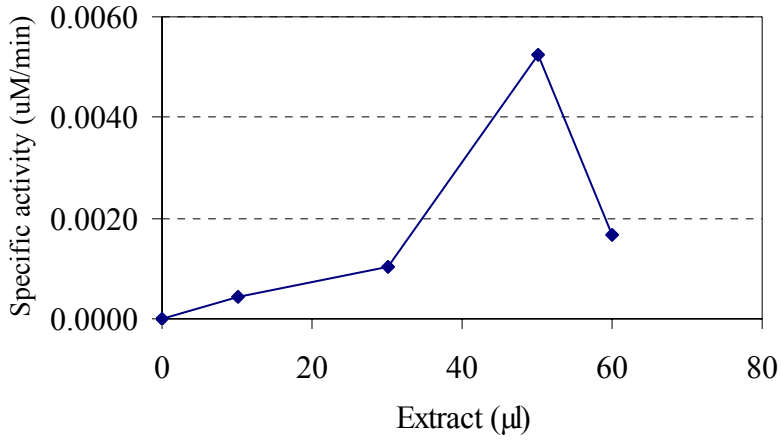

Figure 21 Cell Wall Invertase (CWI) activity related to the extract volume of enzyme. (the 2x invertase assay buffer: 70 mM K_2HPO_4, 40 mM citric acid, pH 5.0, 50 mM sucrose).

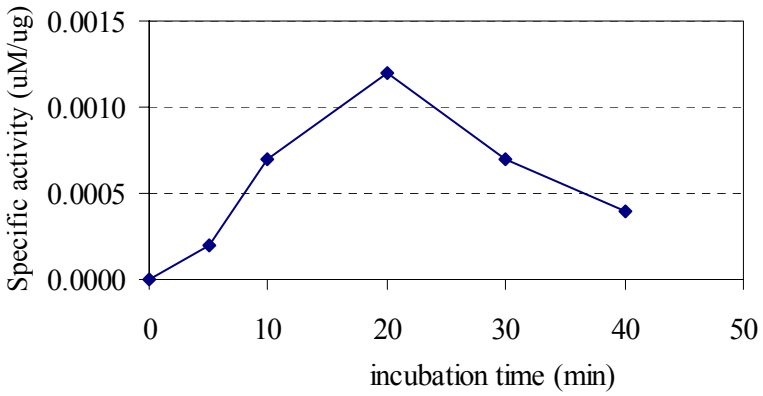

Figure 22 Cell Wall Invertase (CWI) activity related to incubation time 0 - 60 minutes. (the 2x invertase assay buffer: 70 mM K_2HPO_4, 40 mM citric acid, pH 5.0, 50 mM sucrose).

9. Enzymatic Activities as Related to Rubber Clones

In order to study physiological mechanism of sugar synthesis and hydrolysis, different enzyme activities were measured: total amylase, sucrose-phosphate synthase (SPS) and cell wall invertase in 2 clones (PB 235 and RRIM 600) and 4 kinds of samples (fresh wood, fresh bark, dry wood and dry bark samples).

Total amylase specific activity in fresh wood samples was similar between PB 235 and RRIM 600 (0.0090 μM/min/mg and 0.0085 μM/min/mg respectively) in our experimental conditions. According to types of samples, fresh wood had higher total amylase activity than dry wood samples.

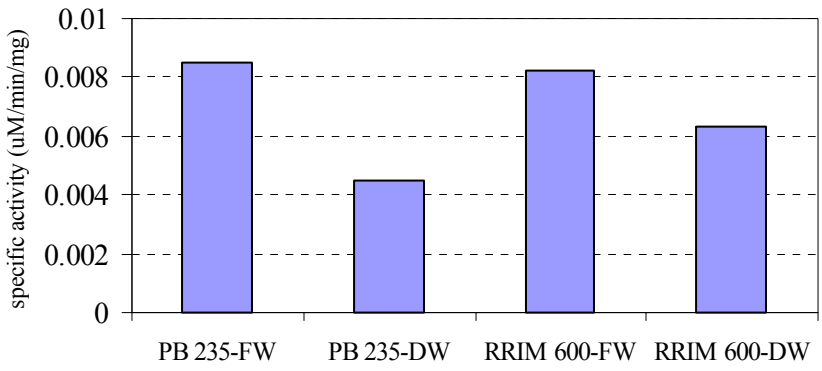

Figure 23 Total amylase activity in PB 235 and RRIM 600 (FW – fresh wood, DW – dry wood).

The activity of sucrose-phosphate synthase reflected both intensity of sucrose export and synthesis. The SPS was mainly found in fresh wood samples. SPS activity was higher in PB 235 (0.050 μM/min/mg) than in RRIM 600 (0.016 μM/min/mg) (Fig. 24).

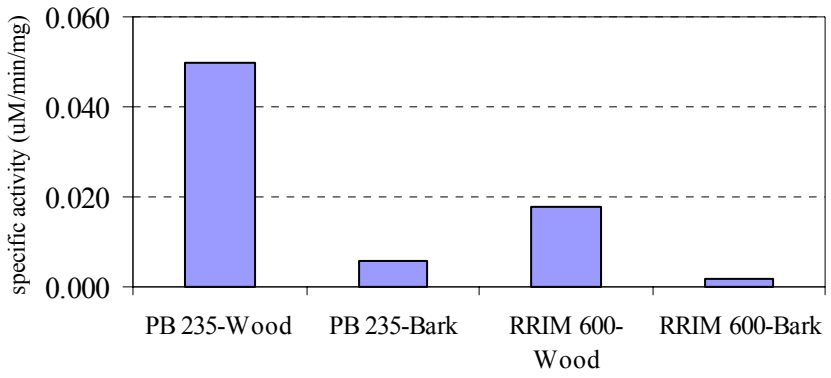

Figure 24 Sucrose Phosphate Synthase (SPS) activity in the wood and the bark of clones PB 235 and RRIM 600.

As other enzymes, invertase activity was mainly found in fresh sample. Therefore, the invertase was analysed in wood and bark of fresh samples and it showed that invertase activities in wood samples were higher than in the bark (Fig. 25). There was only invertase activity in PB 235 clone.

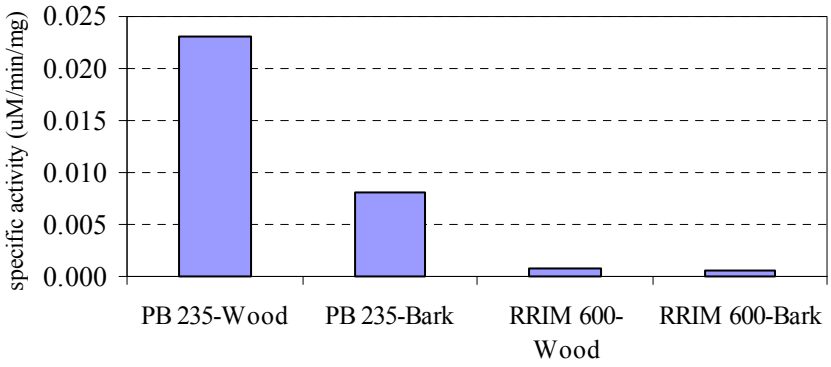

Figure 25 Cell wall invertase (CWI) activity in PB 235 and RRIM 600 (fresh wood and bark).

DISCUSSION

1. Rubber Production

The results confirm that DCA tapping system can provide a significant improvement of yield compared to D/2 (½ S d/2) control. Highest differences between the two tapping strategies were obtained during the first 3 years of tapping. Advantage of DCA strategy is limited during years 4 and 5.

So far, some systems have been developed and they proved more profitable than standard system (D/2) because they provided higher yield per tapping day, thanks to the use of ethylene stimulation. But none of the available systems gave higher absolute yield per tree than the standard (Gohet and Chantuma, 2003a and 2003b). If higher tapping frequencies were used, they had to be associated with shorter tapping cut (1/3 S) in order not to overexploit the trees. Conversely, use of ethylene stimulation for increasing yield per tapping day had to be associated to lower tapping frequencies for the same reason (Gohet and Chantuma, 2003a and 2003b). Stimulated 1/2S d/2 proved not sustainable (Anekachai, 1989).

Thus, not only DCA constitute a promising tapping system for smallholders, but our results showed that D/2 did not really reach the yield limit of a rubber tree. DCA results proved that limitation of yield potential can be alleviated when spacing the harvest by splitting the same tapping intensity at tree scale on two different cuts, which are to be tapped alternately and to be opened on different locations on the trunk.

The good results obtained with DCA tapping system are obviously linked to the extension of latex regeneration time between two consecutive tapping days, as each cut is actually tapped in d/4 frequency. Alternate tapping of each cut, with an appropriate location of the two cuts on opposite panels B0-1 and B0-2, associated with different heights of the two cuts (spaced by 75-80 cm), together minimize the

competition between the two cuts regarding latex regeneration (Lustinec and Resing, 1965, Buttery and Boatman, 1966, Tupy, 1973a, Pakianathan *et al*.,1975 and Silpi *et al*., 2001a, 2001b and 2006a).

However, there were changes along time according to the location of the two tapping cuts. From year 4 onwards, advantage of DCA strategy appeared quite limited, and DCA production became even lower than control in year 5. This seemed to result from a bottleneck in sucrose supply appearing in year 4, due to crossover of DCA panels (creation of a "full spiral"). In year 5, this yield limitation was increased by the panel change over, resulting in a low metabolic activity of the new high cut after change over.

Such striking results enhance the importance of understanding the physiological bases of DCA system. This is a necessity to further improve this system (as many combinations of number of cuts, length of the cuts and tapping frequencies can be envisaged). It is also a necessity to forecast the long term effect of DCA on tree functioning, in order to assess the sustainability of the system. Particularly, the cause of the decrease in rubber yield in DCA in year 5, seemingly related to a "bottleneck effect" is to be explained.

Moreover, as developed herein, it seems that there is a positive interaction between the two cuts according to latex metabolic parameters and mobilization of carbohydrate resources.

2. Vertical Distribution of Latex Inorganic Phosphorus (Pi) and Sucrose Along the Trunk

These results confirmed that control (untapped treatment) was significant higher sucrose than tapped treatments. Contrarily, the inorganic phosphorus (Pi) gradient of tapped trees less differed along the trunk. In tapped treatments, D/2 and D/3 were the same trend of sucrose and Pi which the metabolism seem to stimulated by using intensive tapping system in D/2 and especially that in D/3 was stimulated by

ethephon stimulation (Jacob *et al*., 1998a, 1998b and Gohet, 1996). In low cut of DCA was less sucrose content than in high cut but conversely Pi was higher in low cut than high cut. The benefit of 2 cuts, high cut imported sugar in the uppermost parts, whereas low cut stimulated metabolic activity.

In year 4 of tapping (September 2003), Pi at trunk scale level was highly significant higher in DCA than D/2 and D/3. Whereas, sucrose content was not significantly different among treatments. DCA induced a progressive and significant increase of latex Pi in comparison with the single cut D/2 and D/3, confirming a significant metabolic interaction between two tapping cut of DCA.

In year 5, this yield limitation is increased by the panel change over, resulting in a low metabolic activity of the new high cut after change over. However, it is expected that year 5 should be the worst production year of DCA system as the metabolism of the new high cut will be more and more activated. The bottleneck regarding sucrose supply of the lower DCA cut should decrease as well in the near future, because of enhancement of bark regeneration. Better perspectives are then expected from year 6 onwards, thus maintaining the comparative advantage of DCA tapping strategy over the control single cut strategy.

DCA results higher latex regeneration activity and thus higher sucrose consumption for rubber synthesis compared with D/2 and D/3. Because latex sucrose content in both tapped panel A and B was high, this reflects a higher latex regeneration activity, and thus a higher sucrose consumption for rubber synthesis inside DCA. The competition between the two DCA tapping cuts for latex carbohydrate supply remains quite low (Gohet and Chantuma, 2003a, 2003b). The location of the latex regeneration bark area mostly on tapped panel (A), below and above the tapping cut, also confirmed previous works by several authors which were obtained using very different methods like using radio-labeled isotopes (Lustinec and Resing, 1965, 1968, Lustinec *et al*., 1969), turgor pressure measurements (Buttery and Boatman,1966, Pakianathan *et al*., 1975) and latex diagnosis mapping (Silpi *et al*., 2001a, 2001b).

3. Carbohydrate Reserves in Rubber Trees

This study confirmed the results obtained previously in the same agronomic conditions (same location and same clone) during a one-year study in 2002 (Silpi *et al.* 2007). Starch was the major form of reserves in trunk wood, with sucrose as the only significant form of soluble sugar. Mean TNC concentration within trunk wood sample was comparable to data reported for beech and oak (Barbaroux *et al*. 2002 and 2003), poplar (Witt and Sauter, 1994a, 1994b), walnut (Lacointe *et al*. 1993). Changes in starch were responsible for most the changes in TNC along time and according to location in the trunk. It is confirmed also that TNC was higher in tapped treatments than in the untapped control. This was also found for almost all the sampling period over two years of experiment.

It revealed that additional carbohydrate demand created by regeneration of latex did not deplete wood reserves, but resulted in increase of such reserves. Once these main findings confirmed, the present study provides additional results of two types, some differences or more precise results were obtained with the same treatments and kind of sample as in Silpi *et al*. (2007). We also extended the investigations to different tapping systems, including the DCA type and we analysed carbohydrate contents in bark additionally to wood.

Effect of tapping and panel was clear in tapped treatments. Starch tended to accumulate in trunk wood opposite to tapping cut. Panel B of D/2 and D/4 was higher in starch than panel A of the same trees and than untapped control. It showed that sink effect was created on panel B by tapping panel A. As panel B was not directly involved in latex regeneration, starch accumulated.

Both tapped panels of DCA had the same content in starch and TNC and they were as high as the untapped panel of D/2 and D/4, and therefore higher than the tapped panel of these classical tapped treatments. DCA, the more productive system, could induce a positive interaction between the 2 tapped panels. Tapping on panel A created a sink effect on panel B and tapping on panel B created a sink effect on panel

A. Therefore, as in DCA, more carbon resources were available in the vicinity of tapping cuts. Not only latex regeneration can be higher, but metabolic profile were better. So, the system is likely sustainable.

We confirmed the seasonal variations observed by Silpi *et al*. (2007) in year 1. Starch was found to accumulate along the vegetative season and to drop after refoliation whereas SS tended to change in an opposite way but with a less clear pattern. However, as we investigated two consecutive years, we were able to assess inter-annual variability. Although the main pattern was confirmed, we found that inter-annual variability was large.

In untapped trees or control, peak starch content was similar in 2003 and 2004 than in 2002 (Silpi *et al*., 2007), but drop following refoliation was larger in 2003 and lower in 2004. Thereafter, the increase in starch content between May and October was much steeper in 2003 than in 2004, although finally starch content was higher in October 2004. For tapped treatments, the trend of starch was the same, with a less clear annual pattern in 2004. There was also a high variability of D/4 in year 2. Such variability maybe related to stimulation, as after application of stimulant (6 times a year), steep changes are known to occur in trunk metabolism (Jacob *et al*., 1985). Starch mean concentration in untapped control in year 2 was 2 times higher than in the previous research (Silpi *et al*., 2007). In addition starch decreased between October and leaf fall in control in year 1, whereas leaf fall marked a peak for all treatments in Silpi *et al*. (2007). Such annual differences may be related to climate (Appendix Table 5), as the dry season started early in November 2003, leaf fall occurred earlier in January 2004 than in normal years like February 2002, February 2003. As leaf-fall/refoliation process was also staggered over a longer period in 2004, some trees may have been not completely defoliated in February 2004 and not fully refoliated in March 2004. This may have hidden differences in starch content as related to phenology. However, the higher starch content recorded in May 2004, a period when all the trees were fully refoliated for months, showed that there was actually a difference between the two years of the experiment.

Clearly, 2003 differed from average climate in April, when unusual high rains were recorded, but total annual rain was close to the average (1,287 mm, as compared to 15 years average, 1,291 mm). On the other hand, 2004 was a dry year (1,061 mm). For both years rain was lower than average in October and November, but 2004 was low in May and September too (Appendix table 5). As a whole, our results indicated that during a dry year, seasonal pattern of starch content was less clear than during a more normal year.

Additional sampling in August 2004, in the middle of the rainy season, showed interesting differences among treatments. Starch and TNC content increased only in untapped control at that time. Results by Silpi *et al*. (2006b) showed that radial growth of tapped trees was almost stopped at that time, whereas it was steady in untapped control. Thereby, a steady radial growth maybe beneficial to accumulation of starch in trunk wood.

As the exploited part of rubber tree is the trunk bark, where rubber biosynthesis actually occurs, it was important to assess carbohydrate dynamics in this tissue, in addition to wood. Similarly to results obtained on beech and oak species (Barbaroux *et al*., 2003), SS was more concentrated than starch in bark. But contrary to what was found by these authors, lower total carbohydrate contents were recorded in bark than in wood. Thus, despite the occurrence of specific laticiferous tissue and the related metabolic activity, rubber trees do not particularly accumulate carbohydrate in bark.

Within laticiferous cells, tapping induces a strong decrease in sucrose content (Jacob *et al*., 1985 and 1998, Gohet 1996, Gohet *et al*., 1998) linked to regeneration of exported latex, therefore, we could expect clear differences in bark carbohydrate content between tapped and untapped trees. At the whole trunk scale, this was not marked. Only the stimulated treatment (D/4) had significantly less TNC in bark. SS content could not be related to yield, but possibly to laticiferous metabolism, as DCA and stimulated D/4 had both lower SS in bark and higher Pi (metabolic activity) in latex.

However, tapped panel in D/2 and D/4 had higher content in both SS and starch than untapped panel. Thus, contrary to what was recorded in wood, carbohydrate in bark was higher in the area closer to the tapping cut. We can conclude that tapping created a local sink effect for carbohydrate in bark. This can explain also why at trunk scale only DCA had higher starch than untapped control, as both sides of the tree beneficiated from this sink effect. However, this was not observed for SS.

Seasonal dynamics confirmed this sink effect, as it was both in October 2003 and 2004, peak for latex yield that difference in bark starch between tapped treatments and untapped control was the highest, although this was true the two years only for DCA.

Along the trunk, TNC of untapped control in bark did not change significantly. However, the relative proportion of starch and SS changed largely in tapped trees. Within renewing bark SS was higher than in the surrounding area and starch was lower. This opposite trend for starch and SS was particularly marked in D/4. As TNC content was not lower in the renewing bark, it is likely that this area was well supplied with carbohydrate, despite the necessity to restore phloem connections following bark scrapping. The lowest starch/SS ratio could be related to either the sink effect for SS created by bark regeneration or to the lack of well developed parenchyma to store starch in the renewing tissue. It would be interesting to study carbohydrate evolution along the years between tapping and complete bark regeneration.

Comparison of seasonal dynamics of SS and starch in wood and bark also provided information on the relative role of the four carbohydrate components: SS in wood, SS in bark, starch in wood and starch in bark. In control trees, SS in wood appeared clearly opposite to the three others, particularly at refoliation, when it increased whereas SS in bark decreased together with starch in both wood and bark (Appendix Fig. 1).

Our interpretation is based on changes in source-sink activities and in the active pathways for carbohydrate along the year. When new shoot, with leaf, were

developing, they were net sinks, the only carbohydrate source to sustain their demand being starch reserve. Therefore, it is easy to understand the decrease in starch content in both wood and bark at that time. Starch was hydrolysed and sucrose, the transport form of sugars, was synthesized. However, SS increased in wood but not in bark because at that time the only functional pathway towards developing shoots was xylem, located within wood (Lacointe *et al*., 1993). Latter on, during vegetative season, when functional phloem was connected again to the new leaf, which constituted a net source, bark could be directly supplied with sucrose, so that both starch and SS content in bark increased. Along the vegetative season, starch reserves in wood were completed again, whereas SS content dropped back to its base level, as no more transport occurred in the xylem. Such interpretation is reinforced by the observed lag between rise in sucrose in bark and rise in starch in wood. As the pathway for sucrose from leaf to wood is phloem, it is not surprising to see accumulation of sucrose in bark before starch was actually synthesized in wood.

Moreover, as starch in wood was more variable than the other components, we consider that it constitutes the long term reserve tank, the one actually sustaining carbohydrate demand, when direct photosynthetic supply is not enough. Conversely, wood SS, as the transport component, varied less, as in analogy, the level in a pipe connecting tanks tends to be more stable that the level in the tanks.

In bark, starch content was lower than in wood, but it varied in the same way and in the same range. Thus, it was likely a local reserve compartment. Conversely, SS content was higher, but with little variation. Although we have no information on how much SS was located in active phloem, parenchyma and the laticiferous vessels respectively, we can infer from the relatively high content and low variations that SS in bark was a ready-to-use component, which tended to be full all the time, rather than a reserve buffer.

When trees were tapped, opposition between changes in SS in wood and changes in the other components were not always as clear as in control. This means also that SS in bark, starch in bark and starch in wood did not always vary in the same

way in tapped trees (Appendix Fig. 1 and 2). However, our results were not clear enough to define a clear trend. We can nevertheless infer that following tapping more lateral transfer of sucrose occurs from wood to bark, through vascular rays, known to be of first importance for the supply of laticiferous tissues with carbohydrate (Hébant and de Fay, 1980). As the pattern recorded in untapped trees was explained mainly by the occurrence of vertical sucrose transfer in xylem at refoliation and in phloem the rest of time, superimposing a significant horizontal transfer is likely to make the pattern less marked, as observed.

Contrary to results recorded in many other tree species (Barbaroux *et al*, 2002, Lacointe *et al*., 1993), root and particularly the taproot was not more concentrated in carbohydrate than trunk. TNC content in root and trunk wood was in the same range, whereas for beech and oak (Barbaroux *et al*., 2003) there was more than twice more TNC in root than in shoot. However, the starch/SS ratio was a bit lower in root than in trunk. Within root, the fact that lateral root had more starch than taproot showed that the later was not a specific storage organ. The outer part of the root, although it did not develop distinct 'bark' had the same pattern as the trunk bark, with SS as the major carbohydrate, and a lower TNC than inner root. Such high concentration of SS as compared to starch was not the same as in oak and beech (Barbaroux *et al*., 2003). Contribution of root to carbohydrate supply for refoliation was not clear. The first year, pattern was the same as in trunk (drop in starch a refoliation), but the second year, starch decreased at leaf fall, whereas SS increased, and never recovered along the vegetative season, so that in October 2004, root starch was much lower than in October 2003. As a whole our results support the view that root does not play the major role in carbohydrate budget of rubber tree as related to phenology.

Although the effect of tapping was not significant, it is interesting to notice that ranking in starch and TNC was contrary in root than in shoot. Control had the highest TNC in root and DCA the lowest. In the same way, TNC was higher in root than in trunk for control, whereas it was the contrary for DCA. In D/2 and D/4 it was almost the same. Hence, it looks like a part of the higher TNC content recorded in trunk of tapped trees may come from the root.

A major conclusion from our result is to support the concept of reserves as a competing sink. Whereas previous concept was that reserve pool receive C when all other needs (growth, maintenance, reproduction) are fulfilled, in this new concept reserve is an active sink receiving C in parallel to other sinks and not with lower priority. Moreover, reserve can be considered a dynamic sink, as when an artificial sink is created by tapping, there is a parallel increase in reserve pool to cover it.

4. Hydrolysis of Starch Reserves and the Export of Soluble Sugars

As a first step to analyse enzyme involved in carbohydrate metabolism, we adapted biochemical procedure to rubber wood sample. We determined the appropriate enzyme concentration and incubation time to measure the initial rate of enzyme reactions according to Michaelis-Menten kinetic for total amylase, sucrose phosphate synthase (SPS) and cell wall invertase (CWI). The comparison of different sample could be achieved only with this control conditions. The suitable volume of the extract and incubation time to measure activity was 50 µl of extract and 90 minutes, 50 µl and 30 minutes and 50 µl and 20 minutes for total amylase, SPS and CWI respectively.

SPS activity was higher in PB 235 than in RRIM 600 because PB235 has high metabolism and high productivity clone whereas RRIM600 has medium metabolism and medium productivity (Gohet *et al.,* 2003). However, these data were only one date in the year. These preliminary results let suppose that the sucrose is synthesized from mobilization of reserve in wood and exported towards the sinks. The difference in SPS activity found between PB 235 and RRIM 600 must be confirmed and related to starch and sugar content in wood. However, this tendency did not explain the low content of sucrose found in latex vessels of clone PB235.

This research allowed assessing latex physiology, carbohydrate reserve dynamics and starch hydrolysis and sugar transport, these parameters involved in the production. Moreover, these parameters also allowed to provide tools to design and/or adapted tapping systems. The feasibility and sustainability of DCA tapping system

implementation to understand on trunk metabolism, our main conclusion should be that an improved knowledge in rubber tree physiology, involving latex physiology but also whole plant ecophysiology, might lead, as well as plant breeding, to significant improvement of rubber tree production and/or labour productivity. Nevertheless, our studies produced new knowledge should result in design of new tapping strategies or techniques, allowing to optimize both latex production and rubber wood production.

CONCLUSION

This investigation provides information of diurnal and seasonal carbon flux to improve latex production. Tapping system like DCA increased yield potential during the first three years of tapping compare with D/2 control. However, this limitation of yield potential of DCA can be alleviated when spacing the tappings by splitting the same tapping intensity at tree scale on two different tapping cuts, which are to be tapped alternately and to be opened on different locations on the trunk (opposite panels, vertical distance of at least 75-80 cm), in order to minimise their possible competition for latex regeneration and carbohydrate supply. This strategy seems to induce a metabolic synergy of the two DCA cuts, whose latex physiological profile is shown improved in comparison with the single cut equivalent system.

Nevertheless, the production of DCA in long term (5 years onward) could be maintain the same quantity as D/2. From our results after 4 years onward, DCA and D/2 seem to enlarge regeneration area and import sugar area. Laticiferous sink size/activity depends on tapping system, so that there should be interactions between photosynthate accumulation, partition and utilization in latex and tree development. DCA not only increased carbon pool in rubber trunk but also widen the trunk area involed in latex regeneration.

Latex metabolic activity, based on the comparative evolution of latex sucrose content and concurrent latex inorganic phosphorus content in several areas of the trunk bark of *Hevea brasiliensis* confirm the bark production area. Regular tapping thus created a significant depression of sucrose content of latex in tapped panel, as a consequence of its consumption for rubber regeneration. This depression of latex sucrose content was increased by the use of DCA, as the rubber production and therefore latex regeneration increased as well. Accurate estimation of the latex metabolic activity using the measurement of inorganic phosphorus (Pi) was also confirmed.

The concurrent analysis of latex sucrose and latex Pi levels thus allows a precise and easy description of the shape and size of the metabolically active bark area (area with high Pi): This high metabolic activity area extends to the whole tapped panel, including above and below the tapping cut. According to the concurrent sucrose level, this high metabolic activity area could be divided in two distinct secondary areas: A first area, with concurrent low sucrose and high Pi, close to the tapping cut, that could be considered as the actual latex regeneration area and second area, with concurrent high sucrose and high Pi, more distant to the tapping cut, that represents a highly active sucrose importation area, whose duty is still unknown (sugar reserve for next latex regeneration).

Seasonal dynamics allow to confirm this sink effect, as it was in October, peak for latex yield. Starch was found to accumulate along the vegetative season and to drop after refoliation. Whereas SS tend to change in an opposite way but with a less clear pattern TNC was higher in tapped treatment than in the untapped control. Starch tends to accumulate in trunk wood opposite to tapping according to a sink effect was created on opposite tapping panel. DCA, the more productive system, could induce a positive interaction between the two panels. The carbohydrate demand created by regeneration of latex did not deplete wood reserves, but on the contrary resulted in an increase of such reserves. Since DCA more carbon resource were able in the neighborhood of tapping cuts and induce metabolic profile, so the system is likely sustainable.

Starch was hydrolysed and sucrose, the transport form of sugars, was synthesized. Starch in wood was more variable than the other components, behavior as the long term reserve tank. Conversely, wood SS, as the transport component. Starch content in bark likely a local reserve compartment whereas SS was a ready to used.

Other major aspects that require further investigation are (i). carbohydrate of mass from concentration and biomass of the different components, (ii). SS content is located in each components: active phloem, parenchyma and the laticiferous vessels,

(iii) pathways and activities of sucrose transport from parenchyma into laticiferous vessels, (iv). tapping management improve reserve mobilization and thereby long-term production, (v) carbohydrate reserve relate to TPD (tapped panel dryness) trees and other symtoms affect to rubber production, (vi). investigate the mature period trees by food reserve instead of measurement the rubber trunk size (girth at 1.50 m. is 50 cm.), (vii). quantify relationship between sucrose content in latex, bark and wood and (viii). study the functioning of the enzyme involve in the sugar mobilization during high and low production periods relate to under more or less productive tapping systems and rubber clones.

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APPENDIX

Appendix Table 1 4-way analysis of variance of the effect of treatments (control, D2, D4 and DCA), distance from ground (0-2m.) and sampling date (9 dates) on starch, soluble sugars (SS) and total non-structural carbohydrates (TNC) in trunk wood.

	Degrees of freedom	Starch F	Starch p	SS F	SS p	TNC F	TNC P
Treatment	3	27.40	.0001	8.03	.0001	29.16	.0001
Panel	1	16.38	.0001	0.09	.7705	16.74	.0001
Distance	6	74.50	.0001	10.11	.0001	64.14	.0001
Date	8	161.96	.0001	71.84	.0001	162.06	.0001
TreatmentxDistance	18	2.98	.0001	1.19	.2601	2.94	.0001
TreatmentxDate	24	6.12	.0001	15.14	.0001	6.66	.0001
TreatmentxPanel	2	1.16	.3138	2.51	.0822	1.64	.1952
PanelxDistance	6	10.48	.0001	1.39	.2137	9.88	.0001
PanelxDate	8	13.12	.0001	2.56	.0091	12.51	.0001
DistancexDate	48	3.26	.0001	1.34	.0643	2.86	.0001
Trt.xPanelxDiatance	12	2.19	.0104	1.91	.0300	2.26	.0080
Trt.xPanelxDate	16	4.56	.0001	1.19	.2683	4.17	.0001
PanelxDistancexDate	48	1.45	.0267	1.13	.2587	1.49	.0180
Trt.xPanelxDistance xDate	240	1.45	.0001	1.01	.4549	1.37	.0007

Note: Defoliation period = 5 February 2003 and 19 January 2004
Refoliation period = 6 March 2003 and 20 February 2004
Start tapping or low production period = 2 May 2003 and 11 May 2004
High production period = 28 October 2003 and 18 October 2004

Appendix Table 2 4-way Analysis of variance of the effect of treatments (control, D2, D4 and DCA), distance from ground (0-2m.) and sampling date (9 dates) on starch, soluble sugars (SS) and total non-structural carbohydrates (TNC) in trunk bark.

	Degrees of freedom	Starch F	Starch p	SS F	SS p	TNC F	TNC p
Treatment	3	4.04	.0073	15.72	.0001	9.94	.0001
Panel	1	26.96	.0001	24.07	.0001	36.26	.0001
Distance	6	9.90	.0001	4.15	.0004	2.18	.0428
Date	8	213.3	.0001	61.42	.0001	90.30	.0001
Treatment x Distance	18	1.41	.1174	3.34	.0001	2.21	.0027
Treatment x Date	24	20.77	.0001	11.83	.0001	12.59	.0001
Treatment x Panel	2	1.06	.3457	1.33	.2645	1.21	.2992
Panel x Distance	6	3.90	.0007	4.99	.0001	2.53	.0194
Panel x Date	8	5.33	.0001	1.45	.1718	0.84	.5713
Distance x Date	48	1.31	.0824	1.59	.0076	1.24	.1353
Trt. x Panel x Distance	12	2.03	.0194	1.75	.0528	0.67	.7782
Trt. x Panel x Date	16	2.26	.0031	2.52	.0009	1.64	.0538
Panel x Distance x Date	48	1.50	.0176	1.60	.0070	1.66	.0038
Trt. x Panel x Distance x Date	240	1.38	.0007	1.42	.0002	1.23	.0214

Note : Defoliation period = 5 February 2003 and 19 January 2004
Refoliation period = 6 March 2003 and 20 February 2004
Start tapping or low production period = 2 May 2003 and 11 May 2004
High production period = 28 October 2003 and 18 October 2004

Appendix Table 3 Analysis of variance of the effect of combined treatments, inner part of root and sampling date on starch, soluble sugars (SS) and total non-structural carbohydrates (TNC).

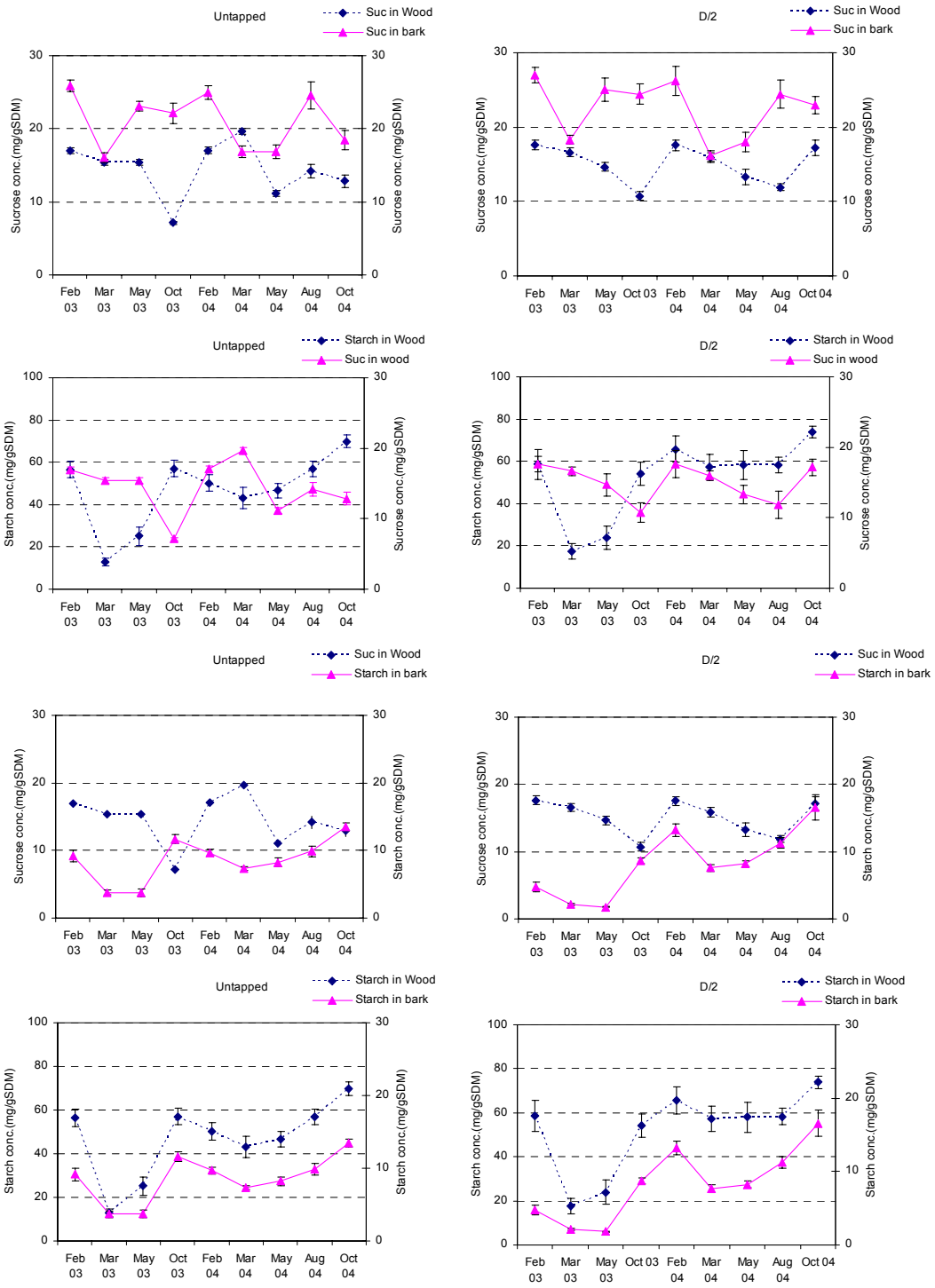
	Degrees of freedom	Starch F	Starch p	SS F	SS p	TNC F	TNC p
Treatment	3	2.08	.1043	2.51	.0599	2.43	.0663
Root	2	22.50	.0001	22.45	.0001	12.44	.0001
Date	8	5.93	.0001	10.06	.0001	6.50	.0001
Treatment x Root	6	3.12	.0059	1.87	.0872	3.30	.0040
Treatment x Date	24	3.64	.0001	3.92	.0001	3.92	.0001
Root x Date	16	1.97	.0164	0.80	.6796	1.80	.0325
Treatment x Root x Date	48	1.74	.0041	0.83	.7772	1.60	.0132

Appendix Table 4 Analysis of variance of the effect of combined treatments, outer part of root and sampling date on starch, soluble sugars (SS) and total non-structural carbohydrates (TNC).

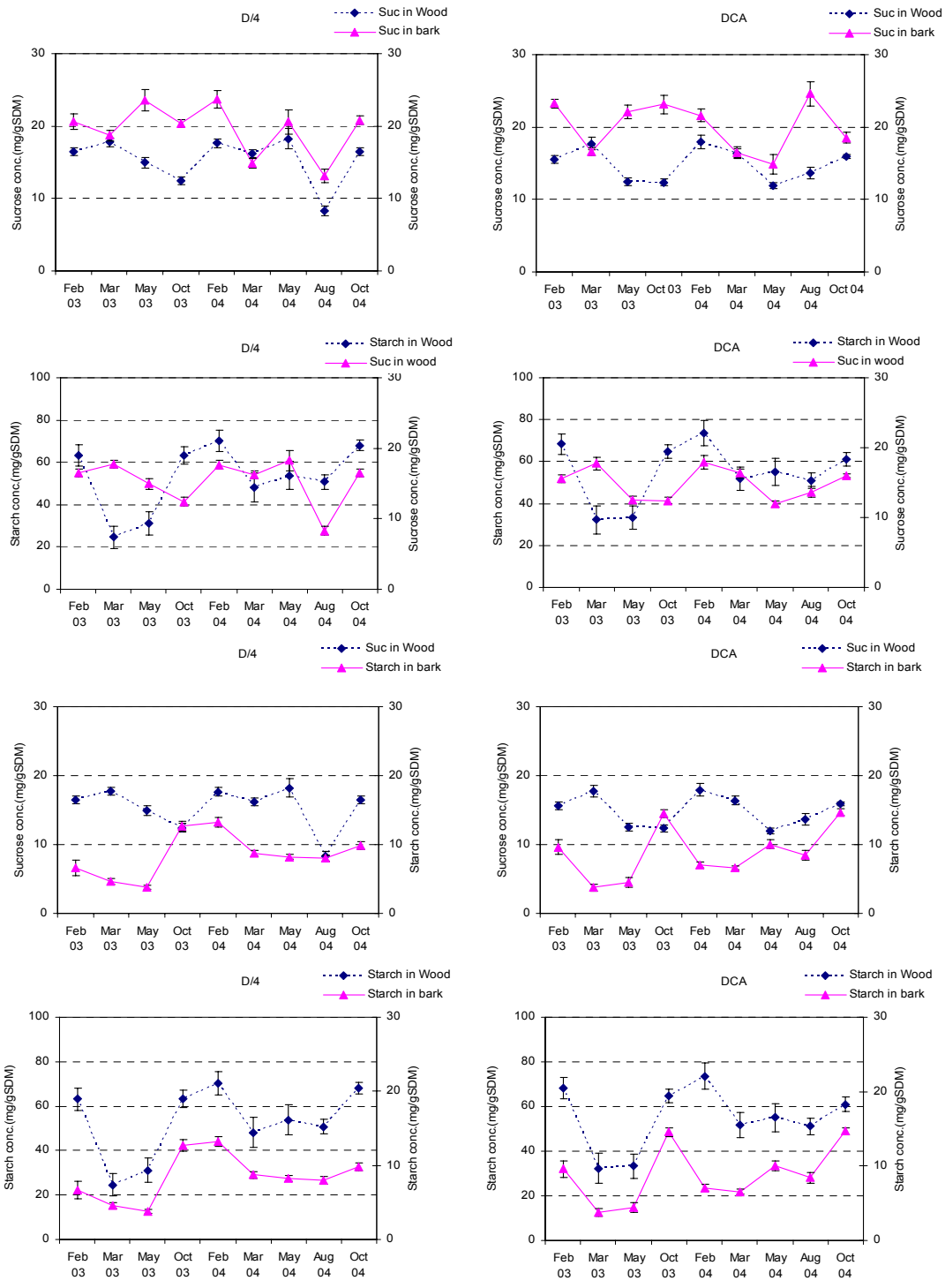
	Degrees of freedom	Starch F	Starch p	SS F	SS p	TNC F	TNC p
Treatment	3	18.28	.0001	1.17	.3206	6.67	.0003
Root	2	5.92	.0032	6.94	.0012	2.28	.1044
Date	8	12.81	.0001	8.53	.0001	7.86	.0001
Treatment x Root	6	1.07	.3821	1.19	.3142	0.72	.6304
Treatment x Date	24	7.16	.0001	2.86	.0001	5.95	.0001
Root x Date	16	2.36	.0031	1.09	.3658	1.26	.2230
Treatment x Root x Date	47	2.12	.0002	0.94	.5889	1.23	.1632

Appendix Table 5 Rain fall (mm.) in year 2002, 2003, 2004 and average 15 years at Chachoengsao Rubber Research Center.

	Rain fall (mm.)			
	2002	2003	2004	Avg 15 y
Jan	0.5	0.0	15.8	10.0
Feb	43.2	48.0	27.9	27.0
Mar	53.7	230.3	91.4	86.0
Apr	144.1	70.1	99.9	101.5
May	215.0	150.2	91.9	153.7
Jun	131.2	188.9	180.2	132.5
Jul	137.7	159.8	123.5	148.5
Aug	229.6	104.7	166.3	175.9
Sep	223.1	249.8	183.0	247.6
Oct	87.5	85.7	81.7	178.8
Nov	34.5	0	0	23.3
Dec	14.9	0	0	6.1
Total	1,315	1,288	1,062	1,291



Appendix Figure 1 Comparison of seasonal dynamics of SS and starch in wood and bark provided information on the relative role of the four carbohydrate components: SS in wood, SS in bark, starch in wood and starch in bark. (left side: control or untapped tree, right side: D/2 (1/2S d/2))



Appendix Figure 2 Comparison of seasonal dynamics of SS and starch in wood and bark provided information on the relative role of the four carbohydrate components: SS in wood, SS in bark, starch in wood and starch in bark. (left side: D/4 treatment, right side: DCA treatment).

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