Rubber Science, **26**(1): 23-35, 2013

TAPPING INDUCED BIOMASS LOSS IN NATURAL RUBBER (*HEVEA BRASILIENSIS*) TREES: PUTATIVE FACTORS EXPLAINING THE UNKNOWN MECHANISM

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Received : 29 January 2013 Accepted : 9 April 2013

Annamalainathan, K., Jacob, J., Vinod, K.K., Thomas, K.U., Sreelatha, S., Sumesh, K.V. and Suryakumar, M. (2013). Tapping induced biomass loss in natural rubber (*Hevea brasiliensis*) trees: Putative factors explaining the unknown mechanism. *Rubber Science*, **26**(1): 23-35.

The mechanisms of biomass loss in Para rubber tree (*Hevea brasiliensis*) due to regular harvesting of latex have not been studied in detail. Annual shoot biomass increment of five different clones of natural rubber tree that were tapped under two different frequencies for twelve years have been monitored. Accrual of biomass was lesser in tapped trees when compared to untapped trees. Trees began losing biomass since the very first year of tapping. Higher the intensity of tapping there was corresponding more loss of shoot biomass. After twelve years of tapping, the most popular high yielding clone, RRII 105 lost around 39 per cent of shoot biomass with reference to biomass of untapped trees. Two clones, RRII 300 and PB 235 lost relatively smaller amount of biomass. The possible mechanisms of tapping mediated biomass loss that was not accounted either by removal of latex or standing biomass known as 'k factor' were explored. In tapped trees, non-phosphorylative alternative respiration was found significantly increased in and around tapping panel region due to continuous tapping. Those clones which recorded increased rate of alternative oxidase (AOX) activity tended to lose more shoot biomass. Tapped trees had higher amount of accumulated carbohydrates in the soft bark tissues and increased ATP level in the latex indicating increased sink activity induced by tapping process. A large amount of ATP and other resources like sugars and proteins were lost through the latex. All these putative factors accounted for the missing biomass in tapped trees.

Keywords: Alternative respiration, ATP, Biomass, Tapping

INTRODUCTION

Tapping, the process of harvesting latex from rubber trees is essentially a process of controlled wounding (Thomas *et al*., 1995). This process is known to enhance the metabolic activities of laticiferous sink tissues in order to regenerate the components of the latex between successive tapping as well as for wound healing (Chrestin *et al*., 1989). It has been known for long time that a negative relationship exists between latex production and wood

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biomass accumulation. Higher the rubber yield, greater would be the loss in girth increment and hence smaller be the increase in shoot dry weight. The negative impact of tapping on tree growth was much larger during the periods of increased latex production (Annamalainathan *et al.*, 1998; Silpi *et al.*, 2006). Tapping process creates a strong 'extra sink' leading to continuous regeneration of latex in the laticifers tissues that utilizes assimilates derived from leaf photosynthesis and other sinks (Jacob *et al.*, 1998; Chantuma *et al.*, 2009). Therefore, latex biosynthesis and biomass production compete for the same carbon sources in the tree, because sucrose is the starting precursor for the synthesis of *cis*-polyisoprene which is also the major source of energy for the growth metabolism (Jacob *et al.*, 1998). The additional demand for latex regeneration disrupts the natural allocation of carbohydrates and other metabolites in tapped trees. The dynamics of carbohydrate mobilization and its relation to latex metabolic profiles in the bark tissue under different tapping systems have been reported (Silpi *et al.*, 2007). However, due to this induced sink activity there was no decline in carbohydrates contents in and around latex flow area, but an increase in the carbohydrate content of trunk wood as compared to untapped trees (Silpi *et al.*, 2007).

The biomass of a tapped tree is lesser than that of an untapped tree in all the clones studied (Sethuraj, 1992; Annamalainathan *et al.*, 1998) because of the induced channelization of carbon resources for latex regeneration that are otherwise meant for biomass production. Interestingly, the quantum of biomass lost from tapped trees varies widely among the clones. Templeton (1968) showed seven fold difference between clones for the tapping mediated biomass loss. Although, this clonal variation can be accounted to differences in their genetic yield potential and maintenance respiration, the actual reduction in biomass is only partially accounted for the rubber harvest through latex. This missing biomass, not accounted either by latex or standing biomass is known as 'k factor' (Sethuraj, 1992). The mechanisms responsible for the k factor in a tapped tree are not thoroughly understood. One possible assumption is that large quantities of carbon resources get drained along with the harvested latex, adversely affecting the biomass of the tree. Other than carbohydrates large amount of proteins, lipids, RNA, minerals *etc.* are drained along with the latex harvested (Chua, 1967).

In an untapped tree, there is no loss of latex, therefore, comparatively low metabolic activity in laticifers (Jacob *et al.*, 1998). Tapping process stimulates metabolic activities and respiration rates in the bark tissues. Maintenance respiration is generally high in tissues with high metabolic activity (Ryan *et al.*, 1994). Thus there may be more loss of biomass through respiration in the high yielding clones that is not reflected in the rubber yield. Annamalainathan *et al.* (2001; 2009) have found that a tapped tree recorded high respiration rate including increased alternative respiration, a key component of the non-phosphorylating electron transport pathway, than an untapped tree. The non-phosphorylating electron transport pathway shares electron from the ubiquinone of electron transport chain in mitochondria and not coupled to ATP synthesis. Therefore, we hypothesize that the 'k factor' could possibly be accounted to an increase in the non-phosphorylating alternative respiratory activity. However, clonal differences for the wound induced alternate respiration and the resultant competition for carbon sources between growth process and latex production have not been thoroughly investigated so far. Therefore, this study evaluates five different clones which were subjected to two different latex tapping frequencies together with untapped trees as control for twelve years from the commencement of tapping, to understand the impact of wounding frequency, drainage of resources in the latex on annual biomass accumulation.

MATERIALS AND METHODS

Plant material

Five *Hevea* clones, *viz.* RRII 105, RRII 300, PB 235, PB 260 and PB 311 planted during 1987 at the *Hevea* Breeding Sub-station at Nettana located in the state of Karnataka of India (12° 43′ N and 75° 42′ E latitude and longitude, respectively) were used in this study. For each clone, plots with twenty five trees each (planted in factorial randomized blocks with three replications) were individually tapped under two tapping systems *viz.* S/2 d2 system (d2, tapped from a half spiral cut once in two days) and S/2 d3 system 6d/7 (d3, tapped from a half spiral cut once in three days, two tappings per week) as described by Vijayakumar *et al.* (2009) from the year 1999 onwards. Another set of trees, a plot of 25 trees for each clone, were left untapped throughout the study period. Observations were continued for twelve consecutive years until 2011. During the period the trees were subjected to standard agronomic management.

Shoot biomass estimation

For accounting the annual shoot biomass, trunk girth value of all the trees under each treatment were measured at a height of 150 cm from bud union of tree. The shoot dry biomass (W) of tapped and untapped trees was calculated using the Shorrocks regression model (Shorrocks *et al.*, 1965), $W=0.002604\ G^{2.7826}$ where, G is the mean trunk girth at 150cm from bud union.

Dry rubber yield

Latex volume (mL) and dry rubber content of latex (drc) were recorded from the tapped plots on all tapping days to estimate average annual dry rubber yield (kg ha^{-1} yr^{-1}). The latex volume of the individual tree was also recorded on the day of sample collection for the biochemical studies.

Assay of respiratory activities

Six to ten trees each from the tapped and untapped plots of every clone were randomly selected for biochemical investigations during summer (March-April) and post- monsoon (November) seasons every year starting from 2000 onwards. Bark samples were collected from just below the tapping panel from the tapped trees. Bark samples were also collected from the untapped trees at the corresponding height as in tapped trees. A very thin slice of 150 mg fresh laticifer-enriched soft bark tissue just adjacent to cambium approximately of 0.5 mm uniform thickness was used for the measurement of dark respiration using a Clarke type oxygen electrode (Hansatech, UK) as described by Lambers *et al.* (1983) and modified by Annamalainathan *et al.* (1998). The assay buffer (pH 7.2) contained 10 mM KH_2PO_4, 10 mM NaCl, 2 mM $MgSO_4$, 0.1 per cent BSA and 100 mM sucrose. The cytochrome and alternative pathways of respiration were measured by adding appropriate inhibitors. The alternative pathway was inhibited in soft bark tissue after incubating the tissue in 3 mM salicyl hydroxamic acid (SHAM) for ten minutes as described by Millenaar *et al.* (1998). The requirement of optimum concentration of SHAM (stock solution in methoxyethanol) for maximum inhibition of the alternative pathway was standardized. To inhibit cytochrome pathway the tissue was incubated with a range of KCN from 50 to 500 µM and at 500 µM of KCN maximum inhibition was found. The respiration was measured after 10 minutes of pre incubation with the inhibitors.

Biochemical components

Total sugars and starch from the soft bark tissue were extracted in 80 per cent ethanol and estimated by the method described by Scott and Melvin (1953) and McCready *et al.* (1950) respectively. Standards were also treated in the same way as samples. Fresh latex was also sampled from all the experimental tapped trees in the morning hours just five minutes after tapping for biochemical estimation.

Soluble protein content of the latex C- serum was extracted in 50 mM Tris buffer (pH 7.4) and the protein contents in the latex serum was estimated by Lowry's method (Lowry *et al.*, 1951).

The ATP concentration in latex was determined luminometrically (Stratec Electronic GmbH, Brikenfeld, Germany) as described by Amalou *et al.* (1992). The ATP content was quantified using a bioluminescent assay kit (Sigma FL-AA) which contain luciferin- luciferase enzyme.

Data were analysed using standard statistical procedures (analysis of variance; ANOVA). All differences were tested for statistical significance using Duncan's multiple range test.

RESULTS AND DISCUSSION

Shoot biomass increment and rubber yield

The annual increment in shoot biomass and annual mean rubber yield in five clones are presented in Fig. 1. When compared to an untapped tree biomass accumulation of a tapped tree was less and shoot biomass loss was gradually increasing from first year of tapping onwards. Further, there were clonal differences in the biomass incremental pattern. The annual shoot biomass increment was always higher in the PB clones than in RRII clones. The clone PB 235 had significantly higher biomass in the untapped trees among the *Hevea* clones studied. After commencement of tapping, PB 235 and PB 260 were found accumulating

Table 1. **Standing shoot biomass in 22-year old untapped and tapped trees of *Hevea brasiliensis*. Annual mean yield of 12 years is also given for two tapping systems, d2 and d3. Values with different letters indicate significant difference at P< 0.05**

Clone	Standing dry shoot biomass (kg tree^{-1})			Biomass loss intapped *vis-à-vis* untapped trees (%)		Annual mean rubber yield (kg ha^{-1}yr^{-1})	
	UT	d2	d3	d2	d3	d2	d3
RRII 105	694^{d} ± 58	422^{f} ± 39	494^{ef} ± 37	39	29	1983^{a} ±115	1684^{b} ±91
RRII 300	528^{e} ± 32	412^{f} ± 31	534^{e} ± 42	22	-1	997^{c} ±53	1183^{c} ±82
PB 235	1175^{a} ± 72	820^{c} ± 79	870^{c} ± 81	30	26	2160^{a} ±134	2068^{a} ±137
PB 260	959^{b} ± 62	668^{d} ± 38	661^{d} ± 39	30	31	1912^{a} ±128	1921^{a} ±143
PB 311	765^{cd} ± 40	558^{e} ± 44	577^{e} ± 37	27	25	1892^{ab} ±150	1967^{a} ±160

Means are followed by (±) standard error; Treatment means followed by same letters are significantly not different at P< 0.05 by Duncan's multiple range test; UT, untapped trees; d2, S/2 d2 system of tapping; d3, S/2 d3 system of tapping. For details of notations please see materials and methods

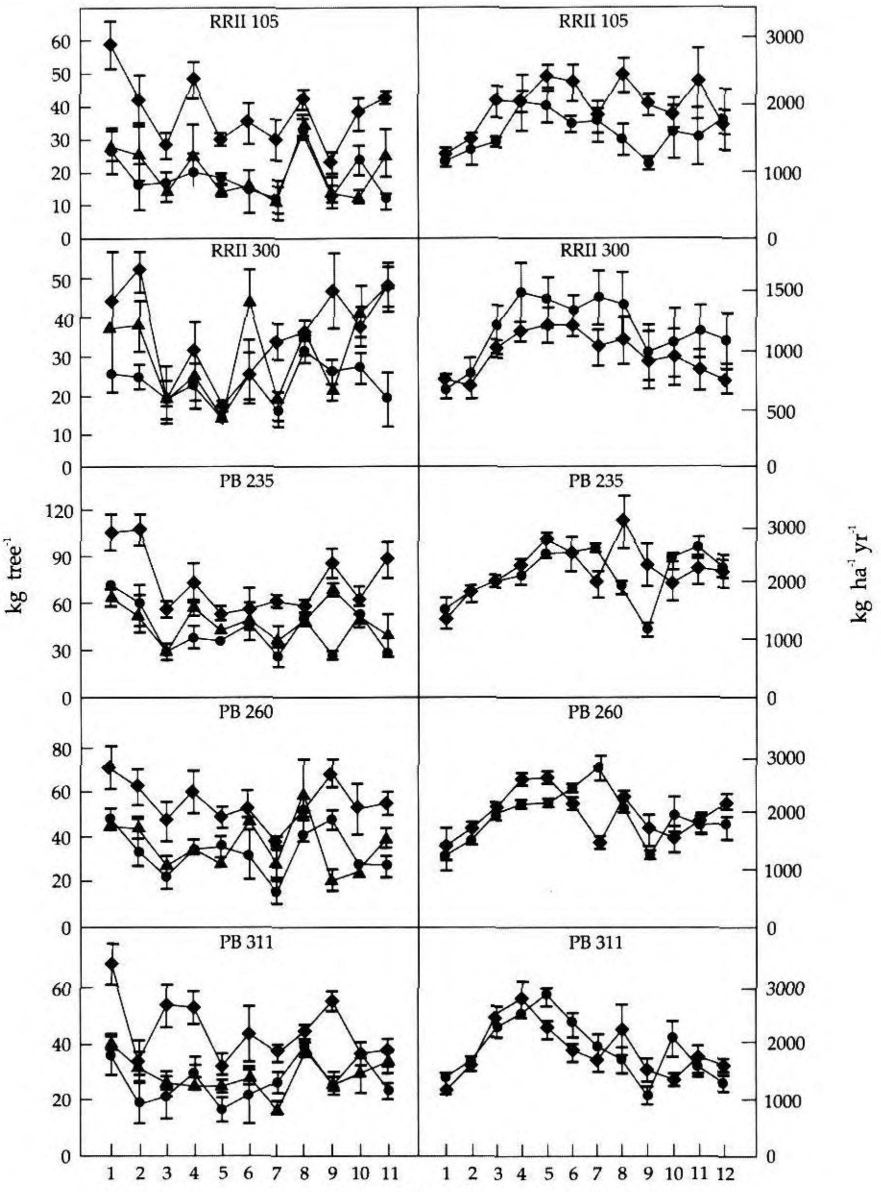

Fig. 1. Annual shoot biomass accumulation (kg tree^{-1}) of untapped and tapped trees (d2 and d3) and mean annual rubber yield (kg ha^{-1} yr^{-1}) under d2 and d3 systems in five rubber clones from the first year of tapping onwards. The notations, d2 and d3 are explained in materials and methods. Error bars indicate standard error, n =75 trees

significantly higher annual biomass than other clones. After twelve years of tapping, the trees tapped under the d2 system lost more biomass than trees under the d3 system (Table 1) in all the clones except PB 260. In general there was no significant difference in biomass loss between d2 and d3 systems of tapping in clones PB 260 and PB 311. However, the popular clone RRII 105 was found to be more tapping sensitive as it lost around 39 per cent of shoot biomass after 12 years of tapping under d2 system whereas, the d3 system lost only 29 per cent. Interestingly, RRII 300 under d3 system of tapping could maintain the growth similar to untapped trees with no biomass loss.

Comparison of the annual rubber yield pattern of five clones under d2 and d3 systems of tapping revealed that the clone PB 235 was superior in yield potential under both the tapping systems followed by PB 260 and PB 311 (Table 1). Rubber yield under d2 system of tapping was better than d3 system only in clone, RRII 105, while d3 system of tapping yielded more rubber than d2 in RRII 300 (Fig. 1). For the other clones there was no significant difference in rubber yield between the two tapping systems. RRII 105 is a high yielding rubber clone in traditional rubber growing tracts of India. On the contrary, RRII 300 has consistently been ranked as a poor rubber yielder in South Karnataka conditions.

There was significant reduction in radial increment of trunk and corresponding loss of biomass in tapped trees of *Hevea brasiliensis* when compared to trees that are left untapped, corroborating the hypothesis that there was competition for resources between the process of growth and latex biosynthesis. Harvesting latex by wounding the bark in the tree and subsequent wound healing are natural phenomenan involving various physiological mechanisms operating in a tapped tree vis-à-vis an untapped tree. Furthermore, the replacement of the harvested latex is necessity of the trees, and all these processes are high energy and resource demanding (Jacob *et al.*, 1998). Therefore, more the frequency of wounding, more would be the stress for the trees. Expression of rubber producing efficiency of *Hevea* trees as the ratio of the weight of dry rubber formed to total dry weight accumulation in the tree is an indication of partition of assimilates between the two physiological pathways of plant growth and rubber yield. Apparently the results indicated that the percentage of allocation of carbon resources for these two physiological processes seems to be genetically determined, as evidenced by the strong positive correlation between the extent of biomass loss and annual dry rubber yield in different clones (Fig. 2). In PB clones, the tapping induced shoot biomass loss was comparatively smaller than RRII clones and showed better yield performance for a given tapping frequency. Interestingly, the clone, RRII 105 was shown to be more sensitive to tapping under d2 system, because this clone had to lose almost 39 per cent of shoot biomass to yield for a comparable quantity of latex as in PB 235 and PB 260. RRII 105 is a popular high yielding rubber clone in traditional rubber growing tracts in India (Kerala and southern district of Tamil Nadu) however, it was shown to be a medium yielder in coastal Karnataka region (Vinod *et al.*, 2010) where this study took place. However, RRII 300, a low yielding clone was found to be almost tapping insensitive under a low frequency tapping.

Tapping and respiratory activities

Tapping under both d2 and d3 systems showed an increased rate of cytochrome c (cyt c)

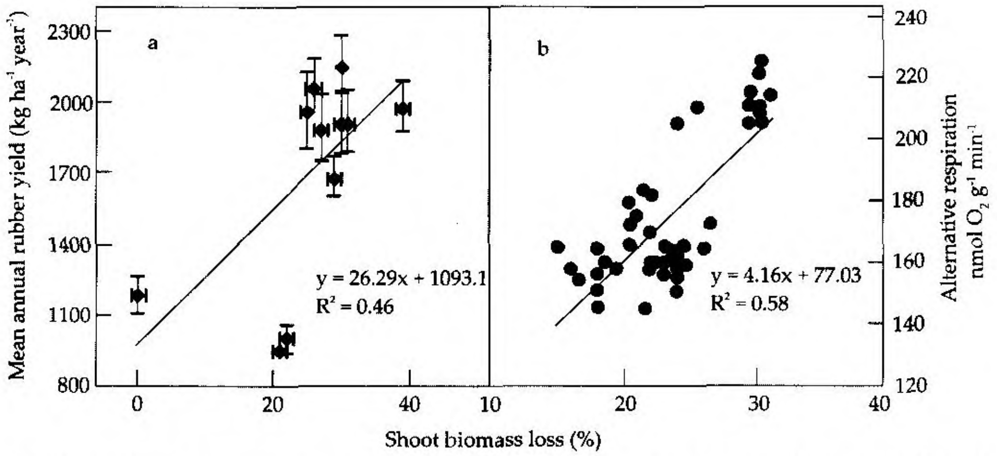

Fig. 2. Relationship between percentage of shoot biomass loss in tapped trees and annual rubber yield (kg $ha^{-1}yr^{-1}$) and alternative respiration measured as oxygen uptake O_2 in five different clones. ± Bars indicate SE, n= 75 trees in the case of shoot biomass (nmol O_2 g^{-1} min^{-1} and rubber yield and n=6 for each clone and every season in the case of respiratory measurements

mediated respiration (Table 2). Soft bark tissue of tapped trees recorded about two fold increase in cyt c activity when compared to untapped trees. Among the five clones PB 235 recorded very high rate of cyt c mediated respiratory activity in tapped trees. Trees tapped under d2 system had more cyt c activity than trees subjected to d3 system of tapping in all the clones, except RRII 300. Similar to cyt c activity, alternative oxidase (AOX) mediated respiration in soft bark tissue was 2 to 2.5 fold higher among tapped trees than untapped in all the clones (Table 2). Alternative respiration or cyanide insensitive respiratory activity is due to wound induced activation of alternative oxidase (AOX), in which the net productivity of ATP is only one third of cyt c activity (Lambers *et al.*, 1983). The popular clone, RRII 105 recorded significantly higher rate

Table 2. **Rate of dark respiration (cyt c and alternative oxidase mediated activities) measured as oxygen uptake (nmol O_2 g^{-1} min^{-1}) in soft bark tissue of untapped and tapped trees in five clones**

Clone	cyt-c activity (nmol O_2 g^{-1} min^{-1})			Alternative respiration (nmol O_2 g^{-1} min^{-1})		
	UT	d2	d3	UT	d2	d3
RRII 105	198.0^{g}	408.6^{bc}	$373.0c^{de}$	85.0^{gh}	229.0^{a}	186.0^{bc}
RRII 300	253.0^{f}	356.0^{de}	$364.0c^{de}$	86.4^{fg}	142.0^{cd}	127.0^{ef}
PB 235	202.0^{g}	484.0^{a}	448.0^{ab}	75.0^{ghi}	197.0^{b}	169.0^{cd}
PB 260	199.0^{g}	$368.0c^{de}$	337.0^{e}	63.6^{i}	166.0^{cd}	161.0^{cd}
PB 311	167.0^{g}	398.0^{cd}	349.0^{de}	76.4^{hi}	177.0^{bc}	147.0^{de}

Means followed by same letters indicate non-significant difference at $P< 0.05$ by Duncan's multiple range test; UT, untapped trees; d2, S/2 d2 system of tapping; d3, S/2 d3 system of tapping of alternative respiration upon tapping when compared to other clones whereas RRII 300 recorded the lowest activity. All the PB clones recorded medium range of AOX mediated respiration. The rate of AOX mediated respiration was significantly higher in trees under d2 tapping system than d3 system in all the clones except PB 260, in which the activity was on par. This result indicated that in general, high frequency of wounding induces increased AOX activity and thereby increased loss of substrates through non-phosphorylative pathway of respiration. A positive association between the rate of AOX activity and shoot biomass loss suggests that higher AOX activity might reduce shoot biomass (Fig. 2b).

Biochemical composition of soft bark tissue and latex

The concentration of carbohydrates in soft bark tissues, accounted as total soluble sugars and starch content, was higher in tapped trees than in untapped trees (Table 3). Significant differences were observed in carbohydrate concentration between two seasons, summer and post-monsoon especially under d3 system of tapping. The difference was high during post-monsoon season than during summer in the case of total sugars, but not much seasonal differences was observed for starch content of the bark tissues. Among the clones, during summer, total sugar content was high for RRII 300 under d2 system of tapping, while PB 235 maintained high but similar levels of sugar content under both systems of tapping. There was no great difference of sugar content among untapped trees of all the clones in both the seasons. During summer, starch content of the soft bark tissues remained largely similar across clones and tapping treatments; however, during post-monsoon season the tapped

Table 3. **Soluble sugars and starch contents of soft bark tissue on fresh weight basis of untapped and tapped trees of *Hevea brasiliensis* during summer and post monsoon seasons**

Clone	Total soluble sugars (mg g^{-1})					
	Summer			Post-Monsoon		
	UT	d2	d3	UT	d2	d3
RRII105	28.3^{i}	34.0^{d-g}	35.5^{bcd}	27.9^{f}	36.4^{bcd}	38.8^{a}
RRII300	30.2^{hi}	39.0^{a}	34.4^{cde}	32.3^{e}	38.3^{ab}	36.4^{bcd}
PB 235	31.6^{fgh}	37.2^{abc}	37.3^{ab}	29.4^{f}	37.9^{ab}	38.8^{a}
PB 260	31.1^{gh}	$34.7b^{-e}$	35.4^{bcd}	29.2^{f}	36.3^{bcd}	37.0^{abc}
PB 311	32.0^{e-h}	$34.0d^{-g}$	33.8^{d-g}	29.1^{f}	34.5^{d}	35.7^{cd}
	Starch content (mg g^{-1})					
RRII105	58.8^{b}	61.0^{ab}	63.9^{ab}	61.8^{ef}	70.3^{abc}	72.2^{ab}
RRII300	58.0^{b}	63.2^{ab}	61.0^{ab}	59.3^{fg}	67.5^{c}	66.9^{cd}
PB 235	55.9^{b}	62.0^{ab}	67.6^{ab}	57.2^{g}	63.2^{de}	73.7^{a}
PB 260	58.7^{b}	66.7^{ab}	65.2^{ab}	62.0^{ef}	68.6^{bc}	68.7^{bc}
PB 311	63.9^{ab}	68.0^{a}	70.3^{a}	60.8^{efg}	63.6^{de}	68.5^{bc}

Means followed by same letters indicate non-significant difference at $P< 0.05$ by Duncan's multiple range test; Comparisons were made within a season; UT, untapped; d2, S/2 d2 system of tapping; d3, S/2 d3 system of tapping

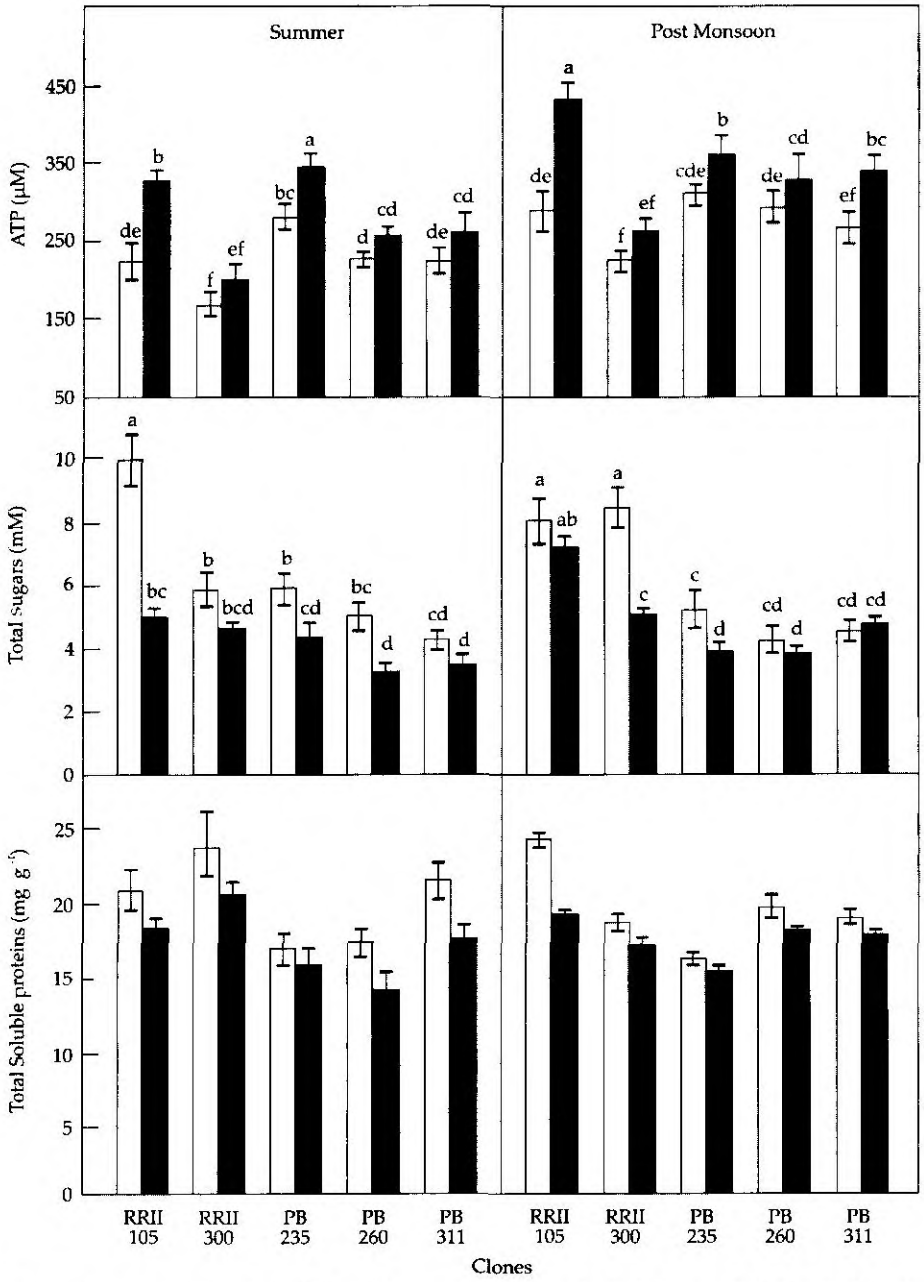

Fig. 3. Latex C-serum constitution of *Hevea* clones under d2 (unshaded bars) and d3 (shaded bars) tapping systems during summer and post-monsoon seasons, showing profiles of ATP content (µM), total soluble sugars (mM) and total soluble proteins (mg g^{-1}). Bars with same letters are not significantly different at $P < 0.05$ by Duncan's multiple range test

trees had significantly higher starch accumulation than the untapped trees.

Diagnostic results of latex biochemical pattern are depicted in Fig. 3. The trees tapped under d3 system had higher ATP content than those tapped under d2 system irrespective of the clones and seasons. Increased rate of respiratory activities in latex flow area was correspondingly supported by an increased ATP level in the latex, implying tapping induced increased sink activity. The clones, PB 235 and RRII 105 recorded higher levels of ATP under d3 system, with significantly higher level in RRII 105 during post-monsoon season (430 µM). The clone PB 235, however, had stable ATP level under both the seasons (339 and 359 µM). The ATP content in latex was very low in RRII 300. Observations on latex C-serum composition of total soluble sugars and soluble protein showed elevated levels of these constituents in trees under d2 system than those under d3 system, irrespective of clones and seasons. Higher levels of total sugars and soluble protein were found in RRII 105 and RRII 300 across seasons and tapping systems, while PB 235 and PB 260 had lower levels of these constituents.

The ATP content in latex apparently indicates the energy status of trunk, although in a tapped tree a large portion of ATP and other resources are drained through the latex harvested. Earlier reports have shown that high yielding rubber clones always recorded higher amount of ATP in latex than low yielding clones (Sreelatha *et al.*, 2004). There was clonal difference in loss of resources due to tapping which corroborated with yield of the clones. Therefore, in tapped trees there seems to be loss of ATP that is continuously produced and subsequently lost through tapping process. However, present observations suggest that ATP is sustainably replaced only under low frequency tapping as indicated by the higher ATP content in d3 tapping system than d2.

Biochemical responses predisposing biomass loss due to tapping

In rubber tapping process triggered an increased sink activity in the tree trunk as evidenced from increased carbohydrate accumulation in bark tissues in almost all the clones. Among the carbohydrates, starch may act more as a local buffer resource with higher concentration in the vicinity of tapping panel whereas, soluble sugars in the bark tissue may act as a ready substrate for rubber biosynthesis as well as other metabolic activities. Chantuma *et al.* (2009) have found an increased storage of carbohydrates in trunk together with a reduction in trunk radial growth in tapped trees. This can be interpreted as a probable build-up of carbon reserves for rubber biosynthesis and wound healing process at the cost of growth, in order to meet the increased demand induced by latex tapping. Moreover, latex biosynthesis is a reaction that competes with the wound healing process as a defence mechanism in tapped trees, could demand for higher allocation of carbon resources to sustain the defence (Christiansen *et al.*, 1987). The concomitant increase in carbon storage and decrease in tree biomass do not necessarily mean that carbon is limiting rather tree growth is of secondary priority compared to rubber biosynthesis and stress mitigation response in tapped trees. However, a genotype which maintains higher growth as well as yield is a preferred character. A clone which loses relatively less biomass upon tapping with sustainable yield is a preferred Latex-Timber (L-T) clone, because such a clone fetches additional income at the end of the economic life period through their timber value. Hence, the present study revealed that the clones PB 235 and PB 260 which consistently with higher biomass as well as better yield are good L-T clones for the south Karnataka region.

In a tapped tree, the prime channel through which carbon resources are drained out through latex harvested that contains predominantly rubber particles suspended in a liquid medium called C-serum, which is basically the laticifer cytoplasm that contains soluble sugars, proteins, fats, minerals *etc.* Investigations on drainage of cytoplasmic resources such as soluble sugars and proteins through the C-serum of latex revealed that loss of metabolic resources (except ATP) is more under high frequency tapping (d2 system) in almost all the clones. RRII 105, a potential high yielding clone, however lost more resources than other clones since this clone had high latex drainage under high frequency tapping (d2) as well as more concentration of soluble sugars and proteins than under low frequency tapping (d3) system. This partially answers the very high magnitude of biomass loss in this clone under d2 system than any other clones. Previous reports also indicated that extraction of rubber is not the only reason for biomass loss (d'Auzac and Pujarniscle, 1961; Chua, 1967) but also due to subsequent loss of vital growth factors and resources through latex serum on tapping. The effect of this loss increases manifold with the increasing intensity of tapping and further gets worsened with increased productivity per unit length of tapping cut. Chua, (1967) had observed that large quantities of proteins, RNA and energy-rich phosphate materials are lost in the latex serum and this possibly lead to a growth depression, since the formation of poly isoprene is a high energy demanding process which requires four ATP molecules for every isoprene molecule formed. Our previous study indicated that latex tapping mediated wounding process causes production of enormous amount of reactive oxygen species (ROS) in the tissues in and around the wounding area. Subsequent signalling response to the wounding stress activates the scavenging mechanisms in the bark tissue (Krishnakumar *et al.*, 2001). In this way a lot of energy is spent for wound healing process in tapped trees.

The balance sheet of 'k factor'

The enhanced respiratory activity in bark tissues of tapped trees, as observed in this study, may sequel to a concomitant increase of respiratory activities in the laticiferous tissues in order to supply the required ATP for the anabolic process. To support the induced metabolic process in the trunk, photosynthates are being supplied as substrate for enhanced respiration as well as latex biosynthetic reactions (Jacob *et al.*, 1989). The tapping process, therefore, not only drain the vital resources through latex, but also causes loss of photosynthates through increased respiration which can have a bearing on the biomass of the trees. It has been reported that not only the tapping cut area but also the entire latex flow region of the tapped trees recorded higher rate of dark respiration. The magnitude of respiration in the tapping cut area was significantly higher than an untapped tree (Annamalainathan *et al.*, 1998). The process of tapping induces enhanced sink activity resulting in the production of more NADH and ATP. However, all the energy currencies are not utilized for either rubber biosynthetic process or other growth process since a sizeable amount of these molecules are drained through harvested latex. In tapped trees it is likely that lot of energy is spent additionally to maintain the tissue. Water is one of the largely ignored components lost through the latex. The estimated cost of maintaining plant biomass ranges from 20 to 60 per cent of photosynthates produced per day in woody species (Ryan *et al.*, 1994) and a major part of the maintenance respiration is associated with protein turn over and maintenance of ion gradients across membranes. It is very likely that in a tapped tree the protein turnover is increased due to increased latex biosynthetic process and a portion of that lost through latex harvest. These are all various factors responsible for the 'k factor' in tapped trees.

CONCLUSION

In this study we explain how the process of tapping enhances the sink demand in tapping panel area and induces metabolic activities related to tapping leading to increased rate of alternative respiration and drainage of high amount of resources in latex. There were significant clonal differences existing for tapping induced respiratory activities and therefore to the loss of resources. High yielding clones recorded increased respiratory activities in laticiferous tissue and proportionately lost more sugars, proteins and ATP along with high latex volume. The clones which showed higher rate of non-phosphorylating alternative respiration tended to lose more shoot biomass. However, there could be other unaccountable tapping induced responses that may be responsible for the tapping induced missing biomass in natural rubber tree.

ACKNOWLEDGEMENTS

The authors thank Dr. K.R. Vijayakumar, former Director of Training, Rubber Board, Dr. R. Krishnakumar, Joint Director (Crop Physiology), RRII and Dr. T R. Chandrasekar, Senior Scientist, HBSS, Nettana for extending help and support during this investigation. The authors also thank Mr. P. Aneesh, Assistant Statistician, RRII for his help in statistical analysis of data.

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