THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Botany)

DEGREE

Botany

FIELD

Botany

DEPARTMENT

TITLE: Carbon Partitioning in *Hevea brasiliensis* Muell. Arg. : Dynamics among Functional Sinks (Latex Regeneration, Respiration, Growth, and Reserves) at Trunk Scale

NAME: Miss Unakorn Silpi

THIS THESIS HAS BEEN ACCEPTED BY

S. Thanisawanyangkura **THESIS ADVISOR**
(Assistant Professor Somprach Thanisawanyangura, Dr. Sci.)

W. Arirob. **COMMITTEE MEMBER**
(Assistant Professor Wallop Arirob, Dr. Agr. Sci.)

P. Kasemsap. **COMMITTEE MEMBER**
(Assistant Professor Poonpipope Kasemsap, Ph.D.)

COMMITTEE MEMBER
(Mr. Eric Gohet, Ph.D.)

COMMITTEE MEMBER
(Mr. André Lacointe, Ph.D.)

COMMITTEE MEMBER
(Mr. Thierry Améglio, Ph.D.)

COMMITTEE MEMBER
(Mr. Philippe Thaler, Ph.D.)

N. Juntawong **DEPARTMENT HEAD**
(Associate Professor Niran Juntawong, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON

Vinai Artkongham **DEAN**
(Associate Professor Vinai Artkongham, M.A.)

THESIS

CARBON PARTITIONING IN *HEVEA BRASILIENSIS* MUELL.ARG. : DYNAMICS AMONG FUNCTIONAL SINKS (LATEX REGENERATION, RESPIRATION, GROWTH, AND RESERVES) AT TRUNK SCALE

UNAKORN SILPI

A Thesis in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Botany)
Graduate School, Kasetsart University

ISBN 974-274-434-3

Unakorn Silpi 2005: Carbon Partitioning in *Hevea brasiliensis* Muell.Arg.: Dynamics among Functional Sinks (Latex Regeneration, Respiration, Growth, and Reserves) at Trunk Scale. Doctor of Philosophy (Botany), Major Field: Botany, Department of Botany. Thesis Advisor: Assistant Professor Somprach Thanisawanyangura, Dr.Sci. 156 pages.

ISBN 974-274-434-3

This study was devoted to the evaluation of diurnal and seasonal carbon flux distribution between different functional sinks. The results showed that tapping activity has a huge physiological effect at the whole trunk scale in any aspect involved with carbon utilization. In comparison with untapped tree, tapping is confirmed to modify the whole physiological behavior of the trunk. A huge depletion of the latex sucrose concentration at whole trunk scale was obviously demonstrated, but the spatial extension of the regeneration area was shown to depend on quantitative rubber production. The latex diagnosis approach allowed a rather precise description of the shape and size of the metabolically active bark area. The multi-clonal linear relation between rubber production and the estimated latex regeneration area indicates that the regeneration of 1 g of dry rubber requires an average of 100 cm^2 latex regeneration area. Tapping strongly affected radial growth. However, this effect seems to depend on the distance from the cut. Tapped tree exhibited a higher reserves deposition than untapped, suggesting that latex regeneration induces an extra demand for more reserves to be used for the future. This strong evidence indicates that reserves sink is not a mere passive buffer and the plant tends to keep reserves at a sufficient level, depending on the demand. Starch and sucrose are the main forms of reserved presented in wood tissue, with maximum deposition of reserves at defoliation and massive utilization occurred during annual leaf shedding – refoliation. The difference in pattern and amount of respiration activity due to tapping and ethephon stimulation was well addressed. Highly linear correlation between respiration activity and radius increment in untapped tree will allow establishing the growth coefficient and respiratory cost for wood production. No linear relationship between growth and respiration in tapped tree especially in tapped panel indicates that respiration is mainly devoted to latex production. This will allow estimating the total cost for rubber biosynthesis.

UNAKORN SILPI
Student's signature

Thesis Advisor's signature

1 / JUNE / 05

ACKNOWLEDGEMENTS

I would like to acknowledge the efforts of many people who contributed to the research and to this thesis in particular. Without them, this work would have never been undertaken.

I feel grateful to my thesis advisors, Asst. Prof. Sornprach Thanisawanyangkura, Asst. Prof. Dr. Wallop Arirob, Asst. Prof. Dr. Poonpipope Kasemsap, Dr. Eric Gohet, Dr. André Lacointe, Dr. Thierry Améglio and Dr. Philippe Thaler, for their direction, suggestion and encouragement, and to Mr. André Clémont-Demange for his fruitful comment and his enthusiastic support.

I would like to thank the staff of CRRC laboratory especially Mr. Arak and Mrs Pisamai Chantuma, Mrs. Pavinee Momnuan, the staff of PIAF laboratory, INRA, Clermont-Ferrand especially Mr. Francois-Alain Daudet and Mr. Boris Adam, and the staff of Ecotrop unit, CIRAD, Montpellier, particularly Mrs. Anne Clément and Dr. Michael Dingkuhn.

My heart feels grateful to friends and colleagues for their warm support during the works, especially Mr. Jate Sathornkich, Miss Natedao Musigamart, Miss Ornouma Duangngam, Mr. Jessada Phattaralerphong, Mr. Rungroj Jitthongchai, Mr. Hongkham Wongchannoi, Mr. Vuthiphan Wongmongkol, Miss Sopida Chitchuenchoei, Mr. Krissada Sangsing, Miss Supaluk Iamkham, Miss Duangrat Sathakhun, Miss Duangjai Waicharoeng, Miss Somya Tangtrakanpong, Miss Siriluck Liengprayoon, Mr. Phongphan Siripornpakdeekul and Mr. Prasut Kwanjai. Finally, my appreciation devotes to my family who always stand by me.

Much of this work has been supported by the French Embassy, CIRAD Montpellier, PIAF INRA, Clermont-Ferrand, and the Thai-French Joint Committee on Higher Education and Research.

Unakorn Silpi
June, 2005

TABLE OF CONTENTS

Page

LIST OF TABLES

Appendix Table

LIST OF FIGURES

LIST OF FIGURES (CONTINUE)

Figure **Page**

LIST OF FIGURES (CONTINUE)

Figure **Page**

LIST OF FIGURES (CONTINUE)

Appendix Figure **Page**

CARBON PARTITIONING IN *HEVEA BRASILIENSIS* MUELL. ARG. : DYNAMICS AMONG FUNCTIONAL SINKS (LATEX REGENERATION, RESPIRATION, GROWTH, AND RESERVES) AT TRUNK SCALE

INTRODUCTION

Among many problems related to the development of rubber productivity, the present work will deal with a major problem on the low productivity of rubber tree (*Hevea brasiliensis* Muell.Arg.). In the small-holding context, the relatively low rubber productivity is mainly due to non-adapted tapping systems and short tapping intervals generally practiced by small-holders. Considering the cost of labor in Thailand, rubber productivity can be almost doubled or tripled of the present level through the development of improved tapping systems based on a better understanding of the physiology of the latex cells and the eco-physiology of the tree as a whole.

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.

There is an apparent contradiction between the relatively low harvest index in rubber crop and the large impact of tapping on tree growth. Actually, the carbon economy of rubber tree as assessed by comparing carbohydrate consumption for rubber biosynthesis to the equivalent of carbohydrate accounted for by decrease in biomass increment is not balanced. The tapped tree seems to lose more carbohydrate in biomass than what is used for rubber regeneration. This apparent loss may arise from an under-evaluation of carbohydrate cost linked to tapping, an under-evaluation of biomass growth of tapped tree, or indirect effects of tapping on carbon acquisition. Base on this concept, the present work will focus on the dynamic aspect of major sinks (growth, respiration, latex production and reserves pool), either diurnal or seasonal scale, as the consequences of the use of ethylene stimulation and identify the phenomena that determine the relative partition of assimilates among them.

Experiments on a small number of adult trees subjected to various tapping systems will aim at estimating the trunk area influenced by tapping, quantify the carbon fluxes involved in latex production, wood production, respiration, reserve accumulation and mobilization. Measurements on trunk portions will include sugar and starch contents, trunk radius and diameter variations and CO_2 production by a gas exchange system. Experiments on a large number of trees in various locations and tapping systems will be aimed at estimating total biomass from allometric relationships and biomass allocated to latex production, i.e. in order to evaluate the harvest index at the season time.

The main objectives of this study were as following,

1. Assessment of the extent of the influence zone induced by tapping, by determining the variation of carbon flux, water flux and latex metabolism in accordance with the distance from the tapping cut.
2. Evaluation of diurnal and seasonal carbon flux distribution between different functional sinks, i.e. latex production, wood production, glucidic reserves and respiratory losses associated.
3. Assessment of total biomass and biomass allocated to latex production from allometric relationships, i.e. in order to evaluate the harvest index at the season time.
4. Assessment of the actual energy cost for latex production by comparing trunk respiration of tapped and non-tapped trees.
5. Evaluation of the cumulative effects of tapping on carbohydrate reserves (mobilization and quantity).

LITERATURE REVIEW

Laticiferous System and Its Demand for Substrate and Energy Supplies

1. Latex Cell: General View

Latex, the milky fluid obtained from tapping, is the cytoplasm coming from laticifer cells of rubber tree (*Hevea brasiliensis* Muell. Arg.), the most important commercial rubber producer from Euphorbiaceae family. Commercially exploited latex is produced from secondary laticifer in the phloem layer of trunk bark. Articulated, anastomosed laticiferous vessels are arranged in concentric ring surrounded by parenchyma and ray cells without plasmodesmata between the latex vessels and their surrounding (de Fay and Jacob, 1989). After the tree has been tapped, its bark is continually wounded and partly removed, but young phloem and cambium still remain. The underlying cells then differentiated wound phellogen. The substituted phelloderm and the surface cork thus form the new protective layer to cover the exploited bark. The number of laticiferous mantles in the renewal bark reaches that of virgin bark on the same tree after 1 or 2 years (de Fay and Jacob, 1989). The most obvious component in latex is rubber particle (cis-1,4 polyisoprene) which forms 25 to 45% of latex volume and varies in diameter from 60 nm to 6 µm. Colloidal stability of rubber particle in latex is maintained by negative charge on its phosphoglycoprotein monomembrane. (Gomez and Moir, 1979)

2. Latex Regeneration and Substrate Translocation to Laticifer Cell

Regularly tapping is known to activate of the entire mechanism involved in latex regeneration. This metabolism must be active enough in order to compensate the loss of cellular cytoplasm between each tapping. Three major steps to produce rubber includes the generation of acetyl-CoA, the conversion of acetyl-CoA to isopentenyl pyrophosphate (IPP) via mevalonic acid and the polymerization of IPP into rubber (Backhaus, 1985). Sucrose plays a role as precursor for rubber biosynthesis. When tapping starts, the level of latex sucrose decreases, with the greatest decrease in the most drained area of the bark (Tupy, 1973). The absence of plasmodesmata in mature laticifers implies that sucrose penetration into laticifer cell requires active transport across laticifer plasma membrane. The rate of sucrose absorption then depends on the energy supply (de Fay and Jacob, 1989).

Two transport routes that feed laticifers are suggested by de Fay and Jacob (1989) based on the functional organization model of the secretion tissue of H. brasieliensis. Vascular rays are the horizontal transport road for metabolites flowing to the laticifers from either the underlying wood or from functional sieve tubes. In addition to this short distance there is the long-distance vertical transport of assimilates from the canopy and perhaps also from the root system. This transport runs through conducting phloem. The simultaneous complementary existence of such long-and short-distance transport phenomena has been studied in detail in other plants (Zimmermann and Milburn, 1975).

3. Enhancement of the Latex Metabolism and Sucrose Loading by Ethylene Stimulation

Ethylene is well known to activate the respiration of fruits and other plant tissues (Reid and Pratt, 1970; Reid, 1972 and Abeles, 1973). This phenomenon may be mainly because of an increase activity of respiratory enzymes and the improvement of substrate supply to the treated tissue (Coupe and Chrestin, 1989). More details can be found in the works of Levy and Kedar (1970), Veen and Kwakkenbos (1984), Vreugdenhil, Oerlemans and Steeghs (1984), Satoh and Esahi (1984). The action of ethylene on permeability and transport could be found also in Jones (1968), Ridge and Osborne (1970) and Abeles and Leather (1971).

In *Hevea brasiliensis*, 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 using radioactive property of U14-C injected in the bark *in vivo* resulted in the present of radioactivity in cytosol and rubber fraction. The 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. Latex Diagnosis (LD) : Predictable 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 (Taussky and Shorr, 1953; Ashwell, 1957; 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 laticifer 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 also 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.

Other Major Sinks in Rubber Tree

1. Respiration

In biochemical sense, respiration refers to glycolysis, the oxidative pentose phosphate pathway, the tricarboxylic acid cycle (TCA cycle), the mitochondrial electron transport and the oxidative phosphorylation. The whole process of respiration involves the catabolism of sugar, or other substrates, the production of CO_2, and the consumption of O_2. Respiration provides two kinds of product, i.e. energy supply which is trapped during sugar breakdown in forms of reduced nucleotide (NADH, NADPH and $FADH_2$) and ATP. The other products are the intermediates that contribute to plant structure.

Respiration rate is expressed by many unit, whenever possible, the specific rate and the units will be mg CO_2 efflux. g^{-1} dry mass . h^{-1} (Amthor, 1989). It can be roughly estimated that nearly half the carbon assimilated in photosynthesis is lost to respiration. Respiration is not only a fix proportion of gross or apparent photosynthesis and it's not always operating at maximal efficiency. Nearly every factor that affects the growth and development of a crop will have some influence on respiration.

Many factors affect respiration rate i.e., growth rate and developmental stage, temperature, substrate level, tissue nitrogen content, photosynthesis and light environment, nitrogen assimilation rate, phloem transport, carbondioxide concentration (Amthor, 1989). Among them, temperature is one of the most significant abiotic factors. An increase in temperature results in an increase in respiration rate. The work of Hofstra and Hesketh (1969), Brown and Thomas (1980) reveals an exponential increase in leaf respiration with an increase in temperature to at least 40°C in some crops, i.e. bean, cotton, sorghum, maize and sugar beet. The rate of respiration rate is high during tissue development such as in young leaf, root or germinating seed. Once a leaf is fully expanded and growth ceases, the respiration rate for a given temperature usually remains rather constant or continue to decrease as a leaf ages. Near the end of the life cycle of a crop plant, tissues become senescence and metabolically inactive, and result in a probable decrease in the specific maintenance respiration rate as well. Tissue nitrogen content is also known to have a positive correlation with respiration (James, 1953). This relation might be due to the respiratory cost of turnover of proteins, since high nitrogen content implies high protein content (Beevers, 1974). However, in some case much of the nitrogen in plant tissue is not related to active respiration and this could be explained as the nitrogen are in storage compounds and /or high content of proteins are not undergoing turnover (Amthor, 1989).

According to the scope of the two-component functional model, carbon losses through respiratory activity include processes involved in both growth and maintenance. The growth loss (R_g) is that associated with synthesis of new biomass and coupled to growth rate while the maintenance loss (R_m) is that associated with maintenance of existing biomass i.e. maintaining of ion gradients, protein turnover and processes involved in physiological adaptation to a changing environment. Thus Rm is coupled to plant size or to plant protein content (Penning de Vries, 1972; Amthor, 1984).

1.1 Theory

McCree (1970) and Hesketh *et al.* (1971) were apparently the first who use the regression method with plants, McCree's original analysis used whole white clover plants (*Trifolium repens*), the functional model is directly applicable to his data and the two-component model was later developed in part due to his experiments. His method had described the respiration of whole plants with the following equation:

$$R = kP + cW \tag{1}$$

where

R = integrated 24h totals of respiration (g CO_2 m^{-2} ground d^{-1})
P = integrated 24h totals of gross photosynthesis (g CO_2 m^{-2}ground d^{-1})
W = dry mass of living tissue (g CO_2 equivalents m^{-2} ground)

Thornley (1970) then derived equation (1) by extending the analysis of the microbiologist Pirt (1965). During some time interval (Δt) an amount of substrate carbon (ΔS) is generated by photosynthesis. In a state where there is no net change in the amount of stored substrate from one time period to the next, ΔS is completely used during Δt. In this derivation it was assumed a priori that this substrate is used for either growth or maintenance. The growth component (ΔS_G) is composed of two parts as following

$$\Delta S_G = \Delta S_R + \Delta S_T \tag{2}$$

where

ΔS_R = substrate carbon that is completely respired to provide energy for the conversion of ΔS_T (carbon skeleton) into new structural phytomass.

The maintenance component (ΔS_M) is completely respired. Then,

$$\Delta S = \Delta S_R + \Delta S_T + \Delta S_M \tag{3}$$

and the growth conversion efficiency or true growth yield (YG) is given by

$$Y_G = \Delta S_T / (\Delta S_R + \Delta S_T) \tag{4}$$

The maintenance respiration coefficient (m) can be defined by

$$m = \Delta S_M / W\Delta t \tag{5}$$

and total respiration (R) by

$$R = (\Delta S_R + \Delta S_M) / \Delta t \tag{6}$$

Which, by applying the previous equations and the relationship $\Delta S = P\Delta t$ (where P is the average rate of gross photosynthesis during Δt), can also be written

$$R = (1 - Y_G)\, P + m Y_G W \tag{7}$$

Thornley's analysis yielded the following relationship for a steady state of substrate production and utilization

$$R = [(1 - Y_G) / Y_G]\, dW/dt + mW \tag{8}$$

where

dW/dt = growth rate of the plant in units of carbon per unit time

The coefficients of equation (1) and (8) are related as follows:

$k = 1 - Y_G$ and $c = m\, Y_G$ (Amthor, 1986)

The term $[(1 - Y_G)/ YG]$ has been called the growth coefficient, (G_R) and reflects the respiratory costs of biosynthesis. The first term on the right of both equation (1) and (2) has been referred to as growth respiration and the second as maintenance respiration although they do not have the same meaning in both formulations. When metabolic activity increases, both ion movement and protein turnover are intensified, which is expressed by an increase in *m*.

1.2 Q_{10} value

The Q_{10} factor is commonly used to describe responses to temperature. It is the factor by which the rate of a particular process increases for each 10^{o}C temperature increase. A Q_{10} of 1 represents no effect of temperature on the rate of interest, a Q_{10} of 2 means the rate doubles for each increase in temperature of 10 ^{o}C and so on. The Q_{10} of respiration is often about 2 in the physiologically relevant temperature (James, 1953). The rate of respiration at a given temperature was much higher during the day (artificially darken) than the night. Specific respiration rate at a given temperature was also higher during and following sunny days compared to cloudy days. Breez and Elston (1978) observed higher Q_{10}s when respiratory substrate levels were low, they found Q_{10}s ranging from 1.5 to 4.0, dependent on substrate levels and temperature range investigated. Over the temperature range of about 10 to 25 ^{o}C, field grown potato, alfafa and soybean had a Q_{10} near 2 (Thomas and Hill, 1949; Sale, 1974; and da Costa *et al*, 1986).

To conduct a measurement on respiratory activity in the rubber tree at the level of latex producing bark, it can be done in the same way as those who measured bark respiration either on stem or trunk as a component of diurnal and seasonal fluctuation of carbon budgets in trees (Linder and Troeng, 1981; Malkina *et al.*, 1985; Kakubari, 1989; Lacointe *et al.*, 1995). By partitioning respiration into growth and maintenance components using the relationship between respiration and growth, in different situations the respiration rate obtained can be attributed either only to maintenance, as the case during periods of dormancy (no radial growth) where the annual minimum value is obtained, or to both maintenance and growth in any other times of the year. As maintenance respiration increases approximately exponentially with increasing temperature on usual temperature range (Amthor, 1989). A Q_{10} relationship is used to derive from particular situations where only R_m is supposed to occur the estimated basic maintenance respiration for the times where both maintenance and growth respiration occur throughout the year. The difference between corrected R_m at actual time and total respiration brings about the value of non-maintenance respiration, which will be then, useful for estimating the conversion efficiency for wood production.

2. Radial Growth

2.1 Secondary growth of *Hevea brasiliensis*

Trunk tissue in *Hevea* is derived from the functioning of the vascular cambium which forms secondary xylem and phloem (de Fay and Jacob, 1989). Pattern of radial growth is correlated to the rate of the cambium activity. In some plants, the cambium is active throughout the entire life of the plant, i.e. the cambial cells divide continuously and the resulting cells undergo gradual differentiation to form the xylem and phloem elements, this type of activity is usually found in plants growing in tropical regions. In the conifers, intensively dividing cambial cells divide once every 4-6 days whereas apical meristematic cell may divide every 8-10 hours (Bannan, 1962). The length of the cell cycle (from mitosis to mitosis including division, synthesis and enlargement) investigated in *Pinus strobus* is about 10 days.

Like most of the tropical species (Rao and Rajput, 2001), before experience tapping radial growth of *Hevea* under conventional area is active for most of the years with an exception in some cases when water availability is limited (Chandrashekar *et al.*, 1996). The radial growth pattern of the rubber from juvenile stage until mature tree was well demonstrated by de Fay (1999). During the early stage, radial growth appears rhythmic, alternating between rapid growth period (1.5-1.7 mm / week) and slow growth (0.5 - 0.8 mm/week) in accordant with the rhythm of apical growth and resulting in multiple growth rings per year. From the second years, an annual rhythm tends to replace the endogenous rapid rhythm. At maturity (after 6 years) only one phase of radial growth is observed after re-foliation and flowering from March -April to November-December. (Rao, 1972; de Fay and Jacob, 1989). At the period of radial growth, the cambial area was about double to the time from December - January to March (de Fay, 1999). During the immature periods, several recent studies suggest that modifications in the microclimate as a result of intercropping have beneficial effects on photosynthesis and growth of rubber (Rodrigo *et al.*, 1997; Rodrigo *et al.*, 2000; Rodrigo *et al.*, 2001; Rodrigo *et al.*, 2005; Senevirathna *et al.*, 2003).

The laticifers exploited for rubber production are differentiated in the phloem. The laticifer differentiation is a rhythmic process and a ring of laticifers is produced each time. Hence the latex vessels in *Hevea* form concentric rings almost alternating with layer of other phloem tissue. During the active growth period, the rate of laticifer differentiation would be much higher than that during the rest of the year. Higher number of vessels row was associated with higher rate of cambial activity. Gomez *et al.* (1972) studied the influence of age of the tree on number of latex vessel rings and observed a linear relationship up to about 15 years.

2.2 Radial growth assessment

Plant radial growth can be monitored by measuring periodic changes in radius or diameter of tree stems or branches (Kozlowski, 1971). Changes in diameter of tree involve *two major components i.e.*, the additional and enlargement of cambial derivatives and the reversible changed in size resulting from hydration and thermal effects (Kozlowski, 1971, Améglio and Cruiziat 1992, Simonneau *et al.* 1993). Hence, the change in trunk or stem diameter is often used as an indicator for plant water status and its shrinkage behaviour is a common phenomenon when tree is under drought stress (Gensler and Diaz-Munoz, 1983; Garnier and Berger, 1986; Kozlowski, 1991, Zweifel and Hasler, 2001, Ueda and Shibata 2001). Linear Variable Differential Transformer (LVDTs) is nowadays a standard tool for monitoring changes in stem diameter variation with a sufficient resolution for monitoring changes in stem diameter. It is used in irrigation scheduling and in measurements of plant response to environmental constraints.

3. Reserves

3.1 Reserves viewed as a dynamics sink

Reserves are a key parameter to productivity of tree plantations, which has to be evaluated on a long term, taking into account the cumulative effects of cultivation practices and climate conditions. Due to seasonal changes in climatic conditions and to developmental variations in source-sink ratio, trees periodically have to sustain growth and other functions when the demand temporarily exceeds assimilates that are being produced. 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 mobilizable form. Their chemical nature (non-structural carbohydrates, NSC), location and dynamics have been documented for many temperate fruit and forest tree species (Glerum, 1980; Tromp, 1983; Kozlowski, 1992; Lacointe *et al.*, 1993a and 1993b; Witt and Sauter, 1994; Barbaroux and Bréda, 2002; Barbaroux *et al.*, 2003). Yet, factors and mechanisms involved in reserve accumulation or mobilization remain poorly understood. A common concept, used in most model of tree growth (review by Le Roux *et al.*, 2001), is that plant store unused carbohydrate during periods of excess production of assimilate, once current demands for maintenance, growth and reproduction are satisfied. On the contrary, reserve are mobilized when current demands exceed current assimilate supply, for example during bud-break in broad-leaved species (Lacointe *et al.*, 1993a; Barbaroux *et al.*, 2003). Therefore, reserves are considered as a passive buffer. However, this concept can not explain how a tree could cope with possible shortage of assimilate (e.g. in case of accidental defoliation) without the ability to accumulate reserve together with current use for maintenance and growth. This points out that reserve as a mere buffer receiving only excess carbohydrates is likely a too simple concept (Cannell and Dewar, 1994; Lacointe, 2000; Le Roux *et al.*, 2001). Moreover, recent works demonstrated that the ratio of growth to storage reserve rate was just slightly affected when assimilate supply was reduced (Lacointe et al., 2004) or that reserves accumulated under water deficit while growth was reduced (Barbaroux and Bréda, 2002), indicating that reserve might be also an active sink for carbohydrate. However, experimental studies on reserves variations as a function of changes in carbohydrate availability are still lacking. Indeed, actual data on dynamics of NSC content on adult tree and particularly tropical trees remain scarce (Bory and Clair-Maczulajtys, 1988; Mialet-Serra *et al.*, 2005)

3.2 Reserves study in rubber tree

Hevea brasiliensis does not differ from other tree species that stores carbohydrate reserves, mainly in the form of starch, in the perennial parts, i.e. tap root or large perennial root, stem and branch in wood and bark, with large amount at the stem-branch junction (de Fay and Jacob, 1989; 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 sytem (Sauter and Kloth, 1986). Parenchyma in the roots functions as an important site of storage (Gholz and Cropper, 1991). Seasonal depletion of starch occurs during the period of development of young leaves and flowers, but this phenomenon is limited only 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). In latex vessels, sucrose is the main form of stored sugar as well as the main form of translocated sugar (Tupy and Resing, 1968; Kuhn *et al*, 1999). Downward decrease of latex sucrose along the trunk in untapped tree probably reflects sugar concentration in solutes in the sieve tubes (Tupy, 1973).

Rubber exploitation by tapping results in an important sink in bark where the latex is withdrawn. This process uses 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, 1973). Previous works have shown that sucrose content within laticiferous vessels is often a factor limiting production (Gohet, 1996; Gohet *et al.*, 1996). Semi-quantitative studies (histo-cytological localisation) 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 actual carbohydrate content along the trunk according to tapping demand remains unknown so far.

Since 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.*, 2004).

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

Plant material

The carbon balance study in mature *Hevea brasiliensis* included 4 experimental approaches, i.e.

(1) the study of sucrose and metabolic status in latex producing bark by latex diagnosis(LD) mapping technique,

(2) the study of trunk radial growth and daily variation by LVDT and RS techniques,

(3) the assessment of bark respiratory activity by multi-chambers gas exchange system and

(4) the follow-up of glucidic reserves status in wood tissues.

The experiments were set up in 3 *Hevea brasiliensis* clones (PB 235, RRIM 600 and GT 1) on the same polyclonal plot of Chachoengsao Rubber Research Center (CRRC-RRIT-DOA ; 13° 39.56'N; 101° 26.29'E, 69 m from sea level), Thailand. The temperature ranged 17.6 -36.5 °C, the mean relative air humidity was 63.5%. The annual rainfall averaged 1280 mm year^{-1} (2001-2002). The soil type was Kabin Buri soil series (sandy clay loam - clay loam). Dry season lasted approximately 5 months (December-April). Tapping activity generally starts in May and stops at the end of January which allow 9 months of tapping and 3 months of resting period (no latex exploitation).

All trees in the experiments were planted in 1993 under a 2.5m x 7.0 m planting design (571 trees/ha). Tapping of each experiment was started when monoclonal plots were ready for tapping (i.e. 50% of the stand reaching a trunk girth of 50 cm, measured at 1.00 m from the ground) in May 1999 on PB235 (experiment CHOE01), October 1999 on RRIM600 (experiment CHOE04), May 2000 on GT1 (experiment CHOE05), and July 2001 on RRIM600 (experiment TE01) with respect to clonal growth potential. Depending on trial, each treatment comprised groups of 10-13 trees per clone. Experimental design was a "One Tree Plot Design" (OTPD), each tree under trial being one treatment replication. Before start of tapping of each experiment, trees were selected as homogenous, belonging to the normal population of each plot regarding trunk girth, canopy sanitary status and trunk conformation.

Treatments included untapped trees (**untapped** ; *growth potential control*), trees tapped without stimulation (***nil stim*** ; *physiological control*, tapped with half spiral cut, once every three days, 6 working days per week, 9 months of tapping per year ; 1/2S d/3 6d/7 9m/12 *nil stim*) and trees tapped with ethephon stimulation (**Et 5/y** and **Et 12/y** ; tapped with ehtephon stimulation 5 and 12 times per year ; 1/2S d/3 6d/7 9m/12 Et 2.5% 5/y and 12/y). Excluding experiment TE01, tapping was stopped from February to April, in accordance with RRIT-DOA tapping system recommendations for Chachoengsao area (tapping stops during refoliation and high temperature/high water stress period). For experiment TE01 the first tapping year stopped on the 6th of April 2002 (9 months tapping), the second tapping year resumed on 1st June 2002 and stopped on 29th March 2003 (10 months tapping). Tapped treatments were opened on panel B0-1 at 1.30 m from ground, with a tapping cut slope of 30°. Regarding ethephon stimulated treatments, stimulation was performed using panel application (Pa), corresponding to application of 0.6 g of stimulant per tree and per stimulation (i.e. 15 mg a.i. per tree per stimulation).

Methodology

1. Dynamics of Laticiferous System and Spatial Extension of Latex Regeneration Area (Experiment CHOE01, CHOE04, CHOE05)

Dry rubber production, trunk girth and latex physiological parameters measured using latex diagnosis technique (Jacob *et al*, 1985 and 1995) were recorded monthly, tree per tree. Regarding this particular study, latex diagnosis was applied to the whole trunk of one unique tree per treatment (untapped, *nil stim*, Et 5/y and Et 12/y). Latex concentrations of sucrose (Suc) and inorganic phosphorus (Pi) were measured at different trunk positions, aiming at describing the available carbohydrate substrate and the related metabolic activity of the latex in response to each treatment (tapping and stimulation intensity), at trunk scale. Sampled trees were selected as representative of treatments regarding girth and production. Latex sampling positions were drawn on the trunk one day prior latex sampling in order to cover trunk areas below and above the tapping cut (±90 cm, i.e. up to 2 m above ground), on both tapped and untapped panels (Fig.1). Latex collection was performed in the morning of scheduled tapping day, from 7.00 to 9.00 am. Tapping was delayed until end of latex sampling, to avoid any influence of tapping on latex analysis results. Sampling was performed upwards from the lowermost line, first on tapped panel, then on untapped panel. Seven drops of latex were collected from each sampling position to measure latex sucrose (Suc) and inorganic phosphorus (Pi) using latex diagnosis (LD) technique, adapted to CRRC Latex Diagnosis Laboratory facilities (Gohet and Chantuma, 1999). Sucrose (Suc) and inorganic phosphorus (Pi) latex concentrations were expressed in millimols per liter of fresh latex (mM.l^{-1}).

Figure 1 Latex diagnosis mapping; sampling positions were drawn on the trunk one day prior latex sampling in order to cover trunk areas below and above the tapping cut (± 90 cm, i.e. up to 2 m above ground)

(a) on tapped panel (b) untapped panel.

2. The Assessment of Trunk Radial Growth and Daily Variation (Experiment TE01)

Experiment was set up on clone RRIM600, included 2 untapped trees (**untapped**), 2 tapped tree without stimulation (***nil stim***)and 2 tapped trees with Ethephon stimulation 8/y (**Et 8/y**). Measurement of trunk radius variation was conducted simultaneously with the gas exchange measurement. Trunk diameter variation were measured continuously with linear variable differential transformers (LVDT: model D.F.2.5, Solartron Metrology, Massy, France) and trunk radius were measured by resistance system: RS (LM10-Potentiometers, Colvern^{TM}) (Fig.2). This study allowed, for the first time, continuous approach in fine scale to measure trunk respiration and radius variation at the same time in the rubber tree under tropical condition. RS sensor was also first used in a long-term field experiment in tropical conditions. This choice was motivated mainly to obtain differences in radial increment between both sides of the tree (whereas LVDT measures the diameter) and also because this device is much smaller than the LVDT, thus allowing enough space for the tree to be normally tapped (see in Fig.4). But the accuracy and reliability of the RS system was to be tested as compared to LVDT. The experiment was set up in December 2001 and ended in March 2003.

2.1 Principle of measurement

Water movement through the soil-plant-atmosphere system is the result from the existence of water potential gradients maintained by transpiration. By capillary effect, tension is created within the conducting system and water is drawn from the soil through the plant roots. From the hydraulic point of view, a plant behaves like a complex network of capacitance and resistance. Absorption from the roots usually lags behind transpiration. When transpiration declines in accordance with solar radiation, during the afternoon, absorption begins to exceed transpiration and the plant rehydrates. These internal water deficits are usually reduced or eliminated during late afternoon or during the night, in case that normal soil water supply exists.

As transpiration loss from leaf increases in the early morning hours, some water moves out of the living tissues surrounding the xylem and penetrates into the xylem in response to the water potential gradients that develop. This movement causes the trunk to shrink (due to the flexibility of plant cell walls). As transpiration rate slows in the late afternoon, the potential gradient reverses and some water flows back from the xylem to the living tissues, thus causes the trunk to swell. Trunk diameter fluctuations occur in response to the potential gradients within the trunk, which reflect the balance between tree water supply and demand.

2.2 Positions of LVDT and RS

Different positions on trunk surface were selected according to the prior result on trunk latex diagnosis mapping (Silpi *et al*, 2001). There were 4 RS sensors installed in each tapped tree i.e., 2 on tapped panel and 2 on the opposite panel at the same level. On tapped panel, one sensor was fixed above the cut *ca.* 1.5m from ground and one below the cut *ca.* 1.0m from ground. On the control-untapped tree, RS was installed at 1.1m from ground and one supplement LVDT was fixed closely as the reference for RS performance (Fig.3). To fix RS device on the trunk, a metallic rod was inserted horizontally deep inside the heartwood and the sensor was fixed with the rod, only a small needle was left free to move inwards or outwards according to trunk movement. The voltage signal received from both devices was monitored continuously (hourly scale) using CR10X logger (Campbell Scientific, Inc.). The correct position of the sensor needle was adjusted regularly depending on plant growth rate. Once a month, manual circumference measurement was conducted on each tree at 1.7 m by tape.

2.3 Data analysis

For the scaling of the over range output from RS Data, see detail in the appendix

Transformation of LVDT signal into trunk diameter

Trunk diameter at T_n (μm) = $[v_{Tn} / (V_{Tn} . 75)] - [v_{T0} / (V_{T0} . k)]$

when

v_{Tn} = LVDT signal output at time n

V_{Tn} = voltage given to LVDT sensor at time n

v_{T0} = LVDT signal output at time zero

V_{T0} = voltage given to LVDT sensor at time zero

k = transform coefficient value (75)

Transformation of RS signal into trunk radius

Trunk radius at T_n (μm) = $\{[(v_{Tn} . 10000)/ V_{Tn}] - ([v_{T0} . 10000)/ V_{T0}]\} . (-1)$

when

v_{Tn} = RS signal output at time n

V_{Tn} = voltage given to RS sensor at time n

v_{T0} = RS signal output at time zero

V_{T0} = voltage given to RS sensor at time zero

Figure 2 The feature of the two sensors for monitoring trunk radial increment

(a) RS (b) LVDT

3. Respiratory Activity of *Hevea brasiliensis* at Trunk Scale (Experiment TE01)

Bark respiratory activity of *Hevea brasiliensis* clone RRIM600 was monitored by the multi-channel gas exchange measurement similar to Lacointe *et al.* (1995). This approach was conducted at the same trees that were used for the trunk radial growth measurement. Ambient air was blown in cylindrical chambers and airflow was measured with mass flow meter (Tylan®, Millipore). The difference in the CO_2 concentration of the air before and after going from the chamber was measured with an infrared gas analyzer, differential mode (LI-6200, LI-COR Inc. or Binos®1004P, Fisher-Rosemount GmbH & Co). Whole chamber CO_2 exchange rate was calculated as the product of mass flow (mol. s^{-1}) and the difference in CO_2 concentration (μmol . mol^{-1}). Gas exchange data of each chamber was collected at 57 minutes interval. The experiment was conducted continuously from December 2001 until March 2003 to cover a complete annual growth cycle.

3.1 Chamber placement

The position on which the chambers were placed was decided by taking into account the variation regarding latex metabolic activity within trunk areas. For tapped treatments(***nil stim*** **and Et 8/y**), each tree received four chambers (cylindrical in shape, 10 cm in diameter, 4 cm in depth, volume *ca.* 300 cm^2), two were placed in tapped panel (the upper at *ca.* 35 cm above the cut and the lower at *ca.* 45 cm below the cut in December, 2001) and other two chambers were placed on untapped panel at the same position (Fig.3 and 4). These positions were expected to reflect different functions concerning latex regeneration process. For untapped tree, one chamber was placed on each tree at the same position where the tapping cut in tapped treatment was made. For each position that the chamber was placed on, the RS sensor was installed closely to the chamber in order to provide the growth data and to couple the respiration and radial growth activities in the same place.

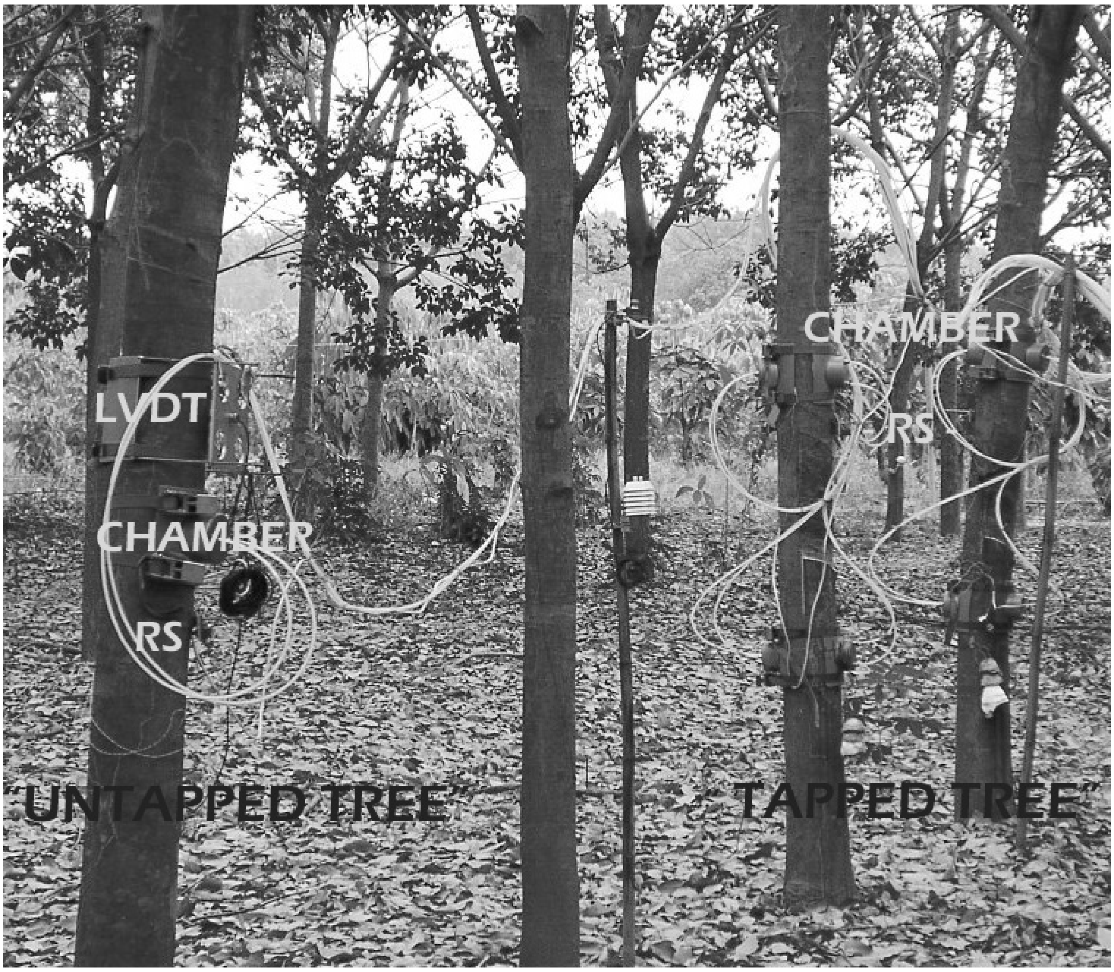


Figure 3 Position and number of respiratory chambers, LVDT and RS sensors on trunk bark of untapped and tapped trees. On the bark surface of tapped tree, the chambers were fixed above and below the cut at the distance 35 and 45 cm respectively from the cut in Dec. 01. In untapped tree the position was at 1.3 m from ground, i.e. at the distance where the tree was opened. These positions were fixed throughout the experimental period but the string was adjusted at times to allow trunk growth.

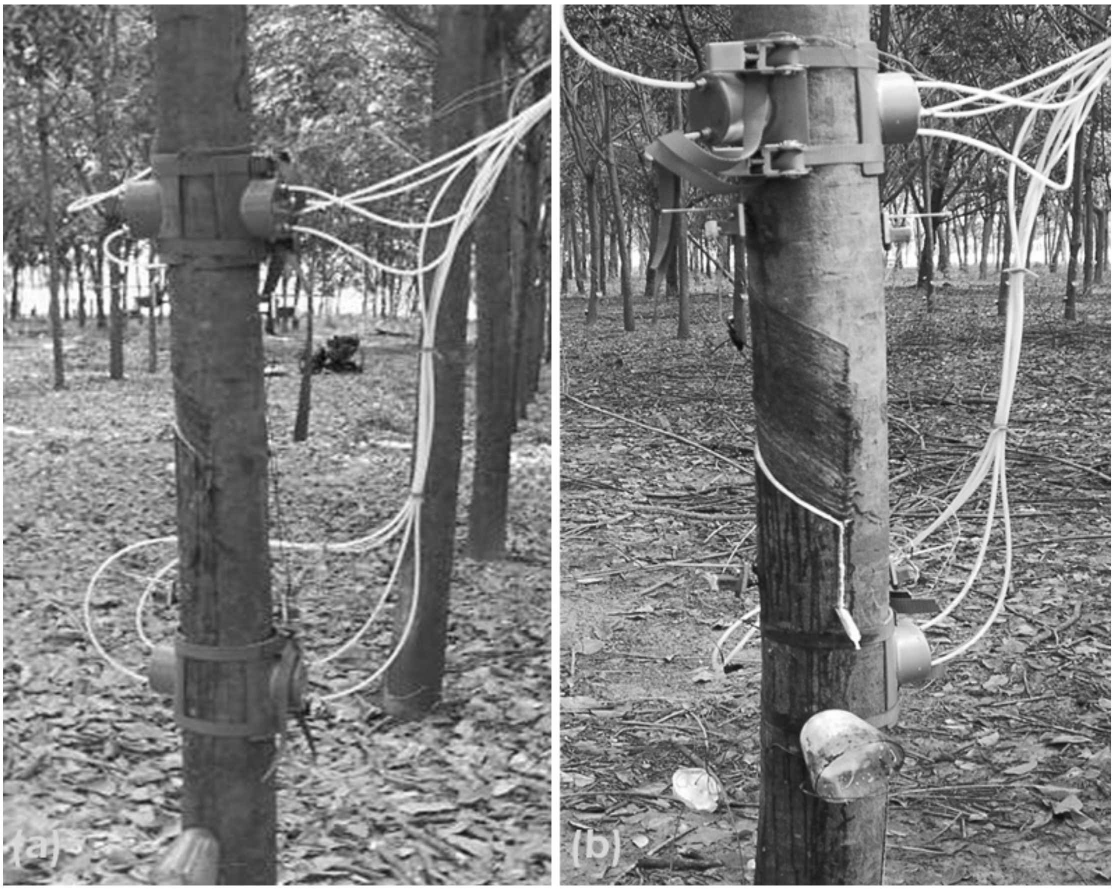


Figure 4 Tapped tree under experiment (plot TE01), showing that normal tapping still possible

(a) Dec. 01 (b) Sep. 02

3.2 Data analysis

There were three outputs obtained from the system, i.e. the CO_2 concentration (ppm), air flow rate ($l.mn^{-1}$) and the chamber temperature (°C). These outputs were further processed to calculate the respiration rate per unit bark area (nmol $CO_2. mn^{-1}. cm^{-1}$) at hourly and daily means. However, due to the system technical problem during the experiment, the original control system had to be replaced by a more appropriate system which led to some changes of the data format output. The data set from the substitute control system required some extra manipulation to be transformed into the same format as the original system (see details in the appendix).

The respiration (RS) intensity, expressed as μmol CO_2. mn^{-1} at chamber temperature (°C) was calculated from the following formula,

$$\text{RS intensity}_{\text{chamber Temp.}} = \{F \cdot [CO_{2\ \text{chamber}} - CO_{2\ \text{zero}}]\} / 22.4 \quad \mu\text{mol } CO_2 \cdot mn^{-1}$$

when

F = air flow rate ($l.mn^{-1}$)

$CO_{2\ \text{chamber}}$ = CO_2 concentration measured from chamber (ppm)

$CO_{2\ \text{zero}}$ = CO_2 concentration measured from ambient air (ppm) synchronized to the same time as $CO_{2\ \text{chamber}}$

The respiration intensity, extrapolated to ambient temperature(°C) and to reference 20 °C was calculated from the following formula,

$$\text{RS intensity}_{\text{ambient Temp.}} = \text{RS intensity}_{\text{chamber Temp.}} \cdot Q_{10}^{(\text{Tambient} - \text{Tchamber})/10}$$

$$\text{RS intensity}_{20\,°\text{C}} = \text{RS intensity}_{\text{chamber Temp.}} \cdot Q_{10}^{(20 - \text{Tchamber})/10}$$

(Q_{10} = 2.3)

The respiration rate at ambient Temp per unit bark area, expressed as nmol $CO_2.mn^-1.cm^{-1}$ was calculated from the following formula,

$$RS_{\text{ambient Temp}} = (\text{RS intensity}_{\text{ambient Temp.}} \cdot 1000) / \text{surface area of chamber}$$

(chamber' surface area = 86 cm^2)

The respiration rate per unit bark surface area based on 24 hour average (RS_{24h}), was calculated from the following formula,

$$RS_{24h\ \text{at T chamber}} = (\text{Avg. RS intensity}_{\text{chamber Temp avg. 24h}} \cdot 1000) / \text{chamber surface area}$$

$$RS_{24h\ \text{at T ambient}} = RS_{24h\ \text{at T chamber}} \cdot Q_{10}^{(\text{Tambient} - \text{Tchamber})/10}$$

4. Dynamics of Carbohydrate Reserves Based on Phenology and Latex Exploitation (Experiment TE01)

4.1 Principle of the enzymatic analysis

The glucose or fructose concentration in the medium is proportional to the NADPH formed during the reaction (Fig.5). The NADPH synthesis is observed, with a spectrophotometer, at 340 nm because NADPH absorbs at this wavelength contrarily to NADP. The Beer-Lambert law, a combination between the absorbance and the concentration at a fixed wavelength, is applied to evaluate NADPH concentration.

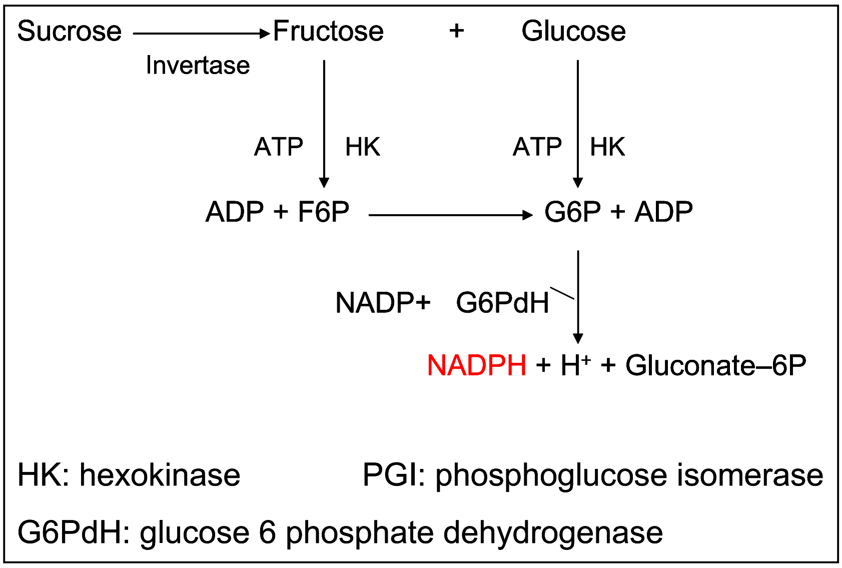


Figure 5 Principle of the enzymatic analysis, based on the biochemical reaction of glycolysis

4.2 Field sampling

Five periods of sampling were chosen according to the tree's phenology and latex production (Fig.6). The period (1) and (5) were considered as the starting of the rainy season and resting period for latex production. The period (2) was nearly at the end of rainy season and the peak of latex production. The period (3) was at the end of defoliation and the period (4) was the end of re-foliation when first annual leaves flush matured. At each sampling date, groups of 3 trees from each treatment were sampled. Alternation of sampled tree for each period was taken into account, in order to reduce the metabolic perturbation and necrosis hazard. Number and position of sampling are shown below.

Tapped treatments (***nil stim*** **and Et 8/y**): 12 samples from the base of the tree up to 2 m (6 samples on tapped panel, including renewed bark area, and 6 samples on untapped panel), 1 sample at 3 m. and 2 samples on taproot at 10 and 20 cm from soil surface.

Untapped treatment (**untapped/control**): 4 samples from the base of the tree up to 2 m (1 sample for every 50 cm), 1 sample at 3 m, 2 samples on taproot at 10 and 20 cm from soil surface. Thus, the total samples per replication are 37 samples.

4.3 Biochemical analysis

After the core sampling was made, the sample was soaked immediately in liquid N_2 and was kept in cryo-tube immersed in liquid nitrogen until transferred from the field to the laboratory and dried with freeze dryer. After, the sample was grinded and stored at -80 °C. The powder was re-dried in the oven for 2 hrs at 65°C. Soluble sugars were extracted from 18-23 mg aliquot samples with 1ml 80% EtOH during 30 min at 80°C, then centrifuged. This step was repeated twice, first with 80% EtOH and then with 50% EtOH and all the supernatants were pooled. The sediment which contains starch was filled with 0.5 ml 80% EtOH and kept at -80°C until analysis. The supernatant was filtered in the mini column added with mixture of polyvinyl polypyrrolidone and activated charcoal to eliminate pigments ad polyphenols. Ethanol was evaporated using vacuum dryer (Maxi Dry Plus, Heto,Denmark). Soluble sugars and starch were quantified by enzymatic analysis (Boehringer, 1988). Sucrose was transformed into glucose and fructose by invertase (β-fructofuranosidase), glucose and fructose were quantified using hexokinase, glucose-6-phosphate-dehydrogenase and phosphoglucose isomerase followed by spectro-photometry of NADPH at 340 nm. For starch analysis, after EtOH was evaporated, the sediment was hydrolysed with NaOH 0.02N during 1.5h at 90°C and then with α- amyloglucosidase for 1h at 50°C and then glucose was quantified as described above. The results were expressed as mg glucose equivalent per gram structural dry matter.

4.4 Data analysis

Glucose, fructose and sucrose quantity in a sample.

After calculation of coefficient from standard curve($k_{\text{glucose , fructose}} \sim 0.04$, $k_{\text{sucrose}} \sim 0.02$)

$A = k . Q$ μg

$Q\ \mu g = A / k$

Then

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

when

A = absorbance

V_{sample} = sample volume

Vr = volume used to dilute the sample after drying

M_{sample} = mass of sample

Glucose: $A_{sample} = DO2 - DO1$

Fructose: $A_{sample} = DO3 - DO2$

Sucrose: $A_{sample} = (DO2-DO1)_{sucrose} - (DO2-DO1)_{glucose}$

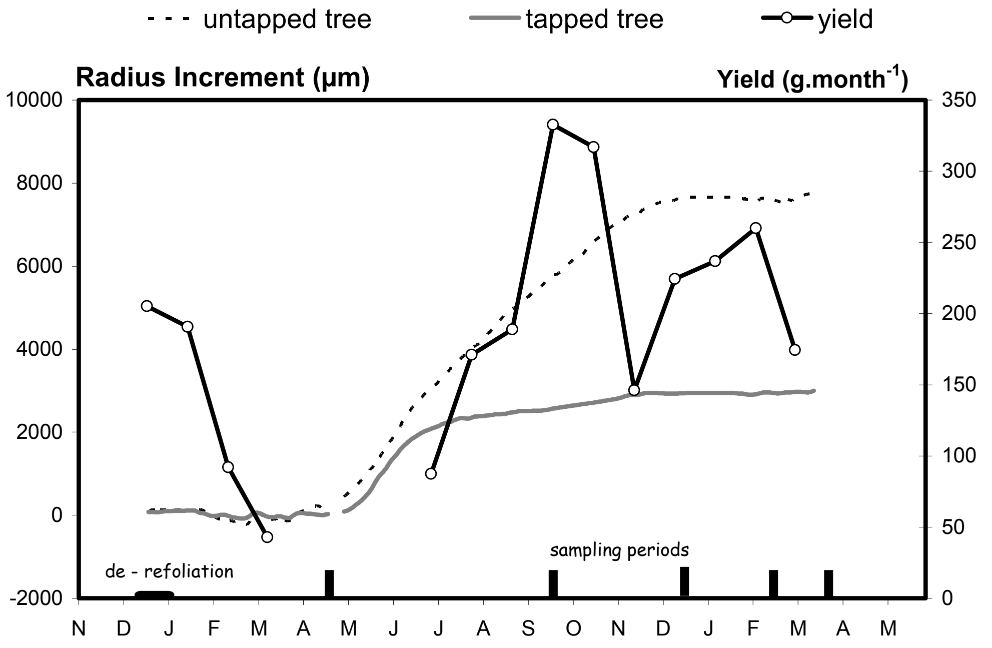


Figure 6 Five sampling periods chosen by taking into account the annual growth cycle and latex production. (1) May 15th 2002 (2) October 21st 2002 (3) January 21st 2003 (4) March 4th 2003 and (5) April 29th 2003

RESULTS

Dynamics of Laticiferous System and Spatial Extension of Latex Regeneration Area

1. Vertical Distribution of Latex Inorganic Phosphorus (Pi) and Sucrose (Suc) Contents.

Inorganic phosphorus (Pi) and sucrose (Suc) are the two main latex physiological parameters measured by latex diagnosis (LD) (Eschbach *et al.*, 1984; Jacob *et al.*, 1985). Correlation between these parameters and yield allows establishing latex clonal metabolic typology (Jacob *et al.*, 1995; Gohet *et al.*, 2001 and 2003), where clones GT1, RRIM600 and PB235 are respectively known as medium, medium-high and high metabolism clones.

The vertical distribution of the latex metabolic activity and available substrate for rubber biosynthesis, respectively estimated by latex inorganic phosphorous (Pi) and sucrose (Suc) contents in tapped and untapped panels, compared between untapped trees, tapped trees without stimulation and tapped trees with ethephon stimulation, of the three clones is shown in figure 7 and 8.

1.1 Tapped panel

On tapped panel, a significant change in the levels of the two parameters (Suc and Pi) within the studied area (± 90 cm from the tapping cut) is observed for all 3 clones between tapped and untapped trees.

Latex sucrose content (Suc) is significantly decreased by tapping in comparison with the equivalent sampling position in untapped trees, below and above the tapping cut. Maximum latex sucrose depletion mainly occurs in the bark regions surrounding the tapping cut (± 50 cm from the tapping cut). Ethephon stimulation generally enhances this drop of latex sucrose content. It is however remarkable that even the uppermost location of the tapped panel is dramatically affected by tapping and ethephon stimulation. In the lowest parts of the panel, difference between untapped trees and tapped trees is less important since latex sucrose content shows an increasing bottom-up gradient in untapped trees.

Conversely, latex inorganic phosphorus content (Pi) is significantly increased by tapping in comparison with the equivalent sampling positions in untapped trees, below and above the tapping cut. However, relative importance of this increase in latex Pi seems very much related to the clone. It is huge on clone PB235 (high metabolism clone), medium on clone RRIM600 (medium-high metabolism clone) and low on clone GT1 (medium metabolism clone) and therefore seems closely related to clonal latex metabolism. Increase in latex Pi mainly occurs in the bark area below or just above the tapping cut. Ethephon stimulation generally enhances this effect of tapping on latex Pi, especially below the tapping cut.

1.2 Untapped panel

On untapped panel, a significant change in the levels of the two parameters (Suc and Pi) within the studied area is likewise observed for all 3 clones between tapped and untapped trees.

Latex sucrose content (Suc) is significantly decreased by tapping in comparison with the equivalent sampling position in untapped trees. Maximum latex sucrose depletion mainly occurs in the uppermost bark regions of the untapped panel, due to the bottom-up increasing gradient of latex sucrose observed in untapped trees. Ethephon stimulation has little and non-constant effect on this decrease in latex sucrose content. Latex sucrose pattern is much more regular in the untapped panel than in the tapped panel, showing a general increasing bottom-up gradient whatever the clone and the tapping intensity.

Conversely, and as observed on tapped panel, latex inorganic phosphorus content (Pi) is significantly increased by tapping in untapped panel in comparison with the equivalent sampling positions in untapped trees. Relative importance of this increase in latex Pi seems very much related to the clone. It is high on clone PB 235 (high metabolism clone), medium on clone RRIM 600 (medium-high metabolism clone) and low on clone GT1 (medium metabolism clone) and therefore seems as well related to clonal latex metabolism. As on tapped panel, ethephon stimulation generally enhances this effect of ethephon stimulation on latex Pi, especially in bottom areas of the untapped panel. However, this effect is much less significant than on tapped panel and therefore seems poorly correlated with the clonal metabolism.

2. Latex Metabolic Status at Trunk Scale.

Regarding latex inorganic phosphorus, observed values are rather low compared to usual standards, especially on tapped GT1 and RRIM600. They nevertheless show a general increasing trend with increasing exploitation intensity. Observed low Pi values of clones GT1 and RRIM 600 may be explained by the period of sampling (end of August), as latex metabolism activation is still rather incomplete.

Such data, as it is well established that latex regeneration *stricto sensu* is a rather localized phenomenon, clearly show that tapping and subsequent latex regeneration modify completely the physiology of the rubber tree latex system, affecting the whole latex metabolism and concurrent latex carbohydrate availability at tree scale.

In Figures 9(a) (GT1), 9(b) (RRIM600) and 9(c) (PB235) show the metabolic latex diagnosis mapping ("LD" mapping) of the 12 sampled trees. Each value is the average of 3 sampling points of the same line. Shadowed areas represent the sampling positions where latex Pi concentration is found higher than the average trunk Pi concentration of the physiological control treatment (1/2 S d/3 6d/7 *nil stim*). Dark grey area: high Pi with the concurrent low suc, pale grey area: high Pi with the concurrent low suc, white area: area comparable to the physiological status of untapped tree, and dotted area: the renewed bark. Such representation clearly represents the extension of the metabolically active area and indicates that:

Table 1 The mean trunk values of latex sucrose and latex inorganic phosphorus observed on the 12 sampled trees. Latex sucrose average trunk concentration is confirmed dramatically decreased by tapping for all three clones, especially on clone PB235 (-62% to -66% for GT1, - 55% to −67% for RRIM600, -80 to 85% for PB235). Effect of stimulation is much less significant at trunk level.

Clone	Parameter (mM.l-1)	Untapped	Tapped nil stim	Tapped Et 5/y	Tapped Et 12/y
GT 1	Suc	41.5	15.7	15.4	14.1
	Pi	3.1	4.2	5.5	4.5
RRIM 600	Suc	24.3	7.9	10.8	7.9
	Pi	3.4	4.5	5.7	5.5
PB 235	Suc	35.0	7.3	6.4	5.2
	Pi	3.5	11.8	14.9	22.5

The most active trunk areas are always found on tapped panel, below the cut or just above the cut. Above the cut, clear and regular bottom-up decreasing gradients represent the decreasing latex metabolic activity when distance from the tapping cut increases.

On untapped panel, active areas are, if any, always located at the bottom of the panel. Nevertheless, metabolic activation of untapped panel is in most cases found low compared to equivalent positions in the tapped panel.

These active areas are extended by ethephon stimulation to higher areas of the two panels. A low activation area is generally found in the middle of the untapped panel followed by a higher activation area on the uppermost parts of this panel. This may reflect a spiral-oriented activation of the latex system following the acknowledged spiral alignment of latex and phloem tissues in the tree bark.

The concurrent comparison of the metabolic activity, estimated by Pi concentrations, and of corresponding sucrose concentrations can be used as a suitable method in order to describe the functioning of the latex system in the different bark regions of the trunk.

Areas with low Pi: such physiological profile characterizes a rather inactive latex metabolism, comparable to the physiological status of the untapped tree, although higher Pi and lower sucrose concentrations. These areas are usually located on the untapped panel (except the lower part) and on the higher parts of the tapped panel, above the tapping cut. These areas do not seem very much involved in the latex regeneration process.

Areas with high Pi and low Suc (dark gray in figure 9): such physiological profile characterizes an active latex metabolism where sucrose is actually used for latex regeneration (latex regeneration area). These areas are usually located on the tapped panel below the cut and may extend to the bark area above the cut and to the lowest parts of the untapped panel, near the ground.

Areas with high Pi and high Suc: such physiological profile characterizes an active latex metabolism, where sucrose accumulates (latex sugar importation areas) instead of being used for latex regeneration. When existing, these "buffer" areas are always found on the lowest parts of the tapped panel. Sucrose concentration is in such case always higher compared to the equivalent position in untapped panel, although higher latex metabolic activity, indicating an important importation sink effect. Such areas seem to represent an intermediary physiological status where metabolic activation is high enough for active sucrose importation but not high enough for latex regeneration. These areas are in the study-case wider in clone GT1 (medium metabolism) than in clone RRIM600 (medium-high metabolism) whereas they do not exist at all in clone PB235 (high metabolism). They exist only in case of metabolic activation with medium stimulation intensity (Et 5/y) and disappear when latex metabolic activation is increased by a higher stimulation intensity (Et 12/y).

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

Figure 9 shows the suspected latex regeneration areas in dark gray. On such basis, spatial extension of latex regeneration areas can be rather precisely estimated. Table 2 presents these estimated areas on both panels for the 3 studied clones, depending on exploitation intensity.

Table 2 Extension of the estimated latex regeneration areas (low Suc, high Pi) of the 9 tapped trees (height on tapped panel: hA, height on untapped panel: hB and corresponding total area expressed in cm^2, calculated by the product of the trunk semi-circumference at the moment of latex sampling and total height of this bark area. Associated rubber production is expressed in g.tree^{-1}.tapping^{-1}, and corresponds to the production of last month before latex sampling.

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

Comparison of the regeneration area, estimated by the product of the total height of latex regenerating bark and monthly dry rubber production at the moment of latex sampling, expressed in g.tree^{-1}.tapping^{-1} (g.t^{-1}.t^{-1}), shows a highly significant linear relation (r^2 = 0.84**) (Fig.10).

Estimated latex regeneration areas logically increases together with rubber production. Linear regression curve (P = 0.01 Area) indicates that regeneration of 1 gram of dry rubber requires, in average, *ca.* 100 cm^2 latex regeneration area., draining an equivalent latex volume of 3.0 ml (with a latex dry rubber content estimated to 33%). Regression has no constant term, as it is logical that no production implies no latex regeneration likewise. It is remarkable that the three clones fit to the study, which implies that the density of tapped latex vessels is not much different between these 3 clones.

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

$$V = A \times T$$

when

V = Volume (m^3), A = Area (m^2) and T = Thickness (m)

with: $V = 3.0$ ml ($3.10^{-6}\ m^3$) and $A = 100\ cm^2$ ($10^{-2}\ m^2$)

$$3.10^{-6} = 10^{-2}.\ T$$

then

$$T = 3.10^{-4}\ m = 300\ \mu m.$$

Calculated utile latex tissue thickness (T) is estimated to 300 µm. As latex vessel average diameter is 25 µm (Gomez, 1982), our estimation of the latex regeneration area leads to 12 (300/25) efficient latex rings, as each latex ring is composed of a unique latex vessel layer (De Fay, 1989). Gomez *et al.* (1972) reported a total number of latex rings near from 25 vessels in tapped bark of Wickham rubber clones. However, as usually only around 60% of the latex rings are usually tapped, as the 40% left are too near from the cambium and therefore remain untapped (Gomez, 1982). This leads to an actual number of tapped latex rings of 15, rather near from our estimation (12 tapped latex rings).

SUCROSE INORGANIC PHOSPHORUS

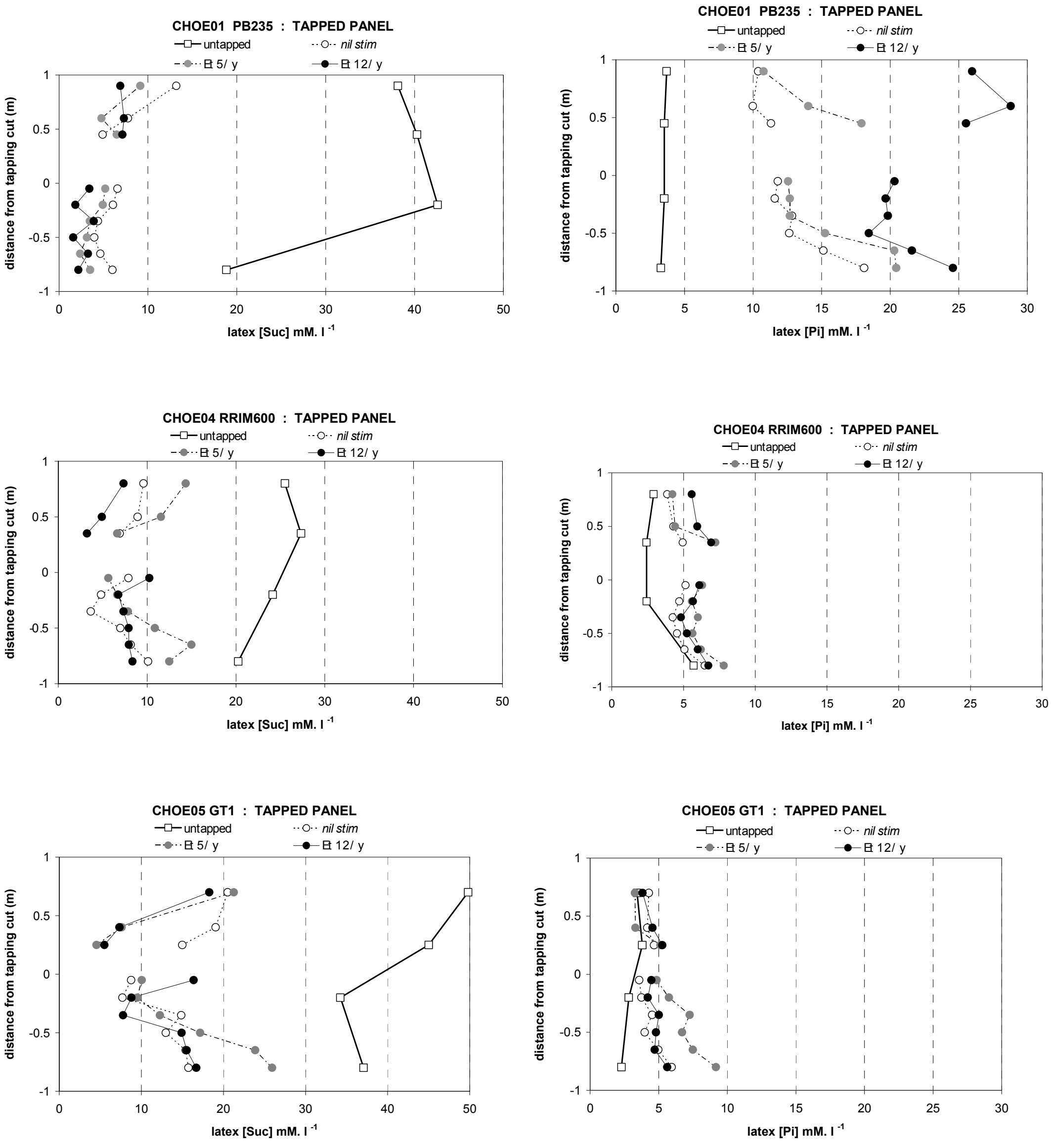


Figure 7 Vertical distribution of latex sucrose content ([Suc], mM.l^{-1}) and inorganic phosphorus content ([Pi], mM.l^{-1}) in tapped panel of clones PB235, RRIM600 and GT1, depending on distance from the tapping cut (±90 cm). Sampling on tapped trees is performed 5, 20, 35, 50, 65 and 80 cm below the cut. Height of sampling above the cut depends on the width of renewed bark, function of opening date of each clone: 10, 25 and 55 cm above renewed bark. Sampling on untapped tree is performed at 4 locations, equivalent to following positions on tapped trees: 20 and 80 cm below the cut, 10 and 55 cm above renewed bark.

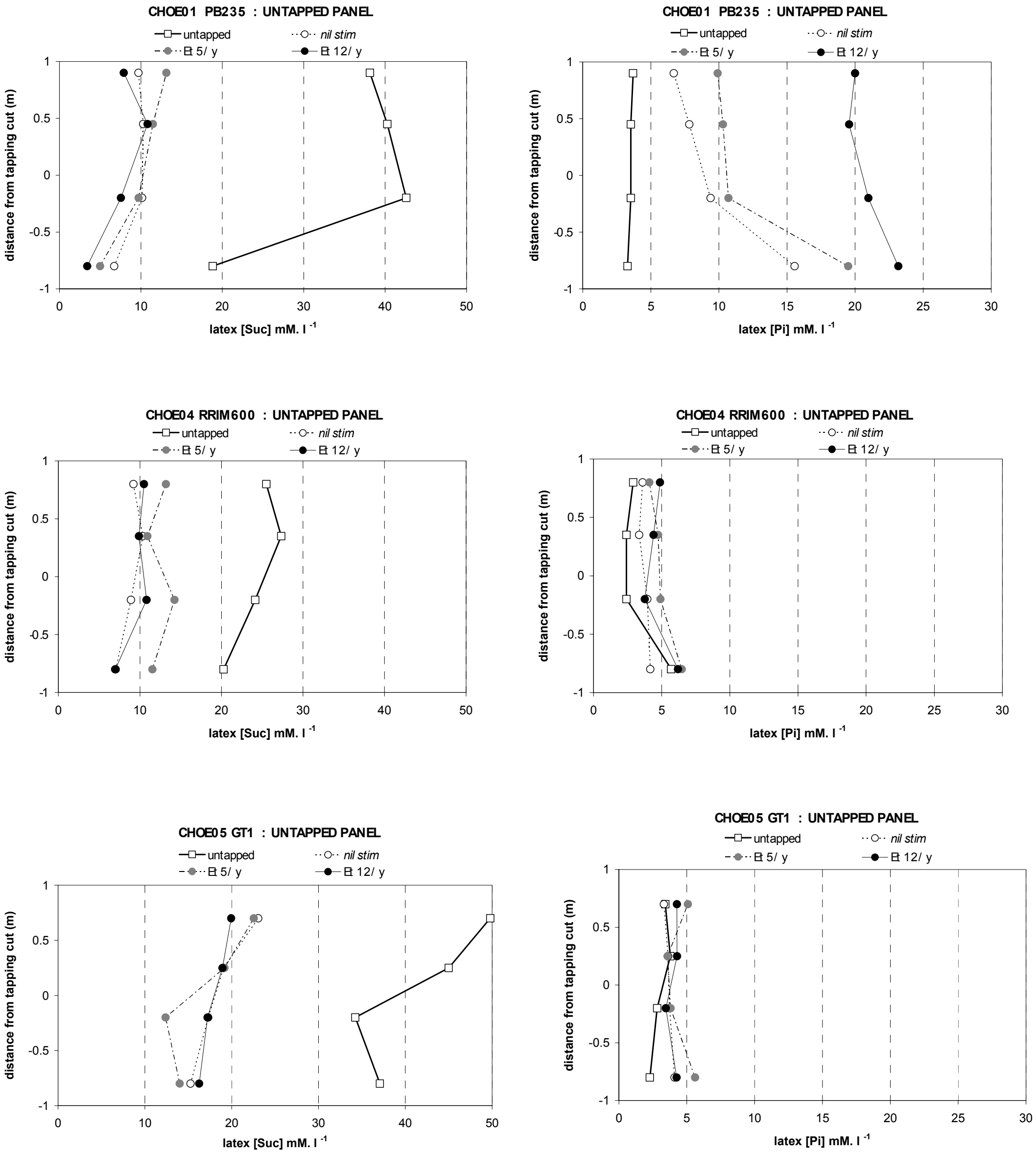


Figure 8 Vertical distribution of latex sucrose content ([Suc], mM.l^{-1}) and Latex inorganic phosphorus content ([Pi], mM.l^{-1}) in untapped panel of clones PB235, RRIM600 and GT1, depending on distance from the tapping cut (± 90 cm). Sampling is performed at 4 locations, equivalent to following positions on the tapped panel: 20 and 80 cm below the cut, 10 and 55 cm above renewed bark.

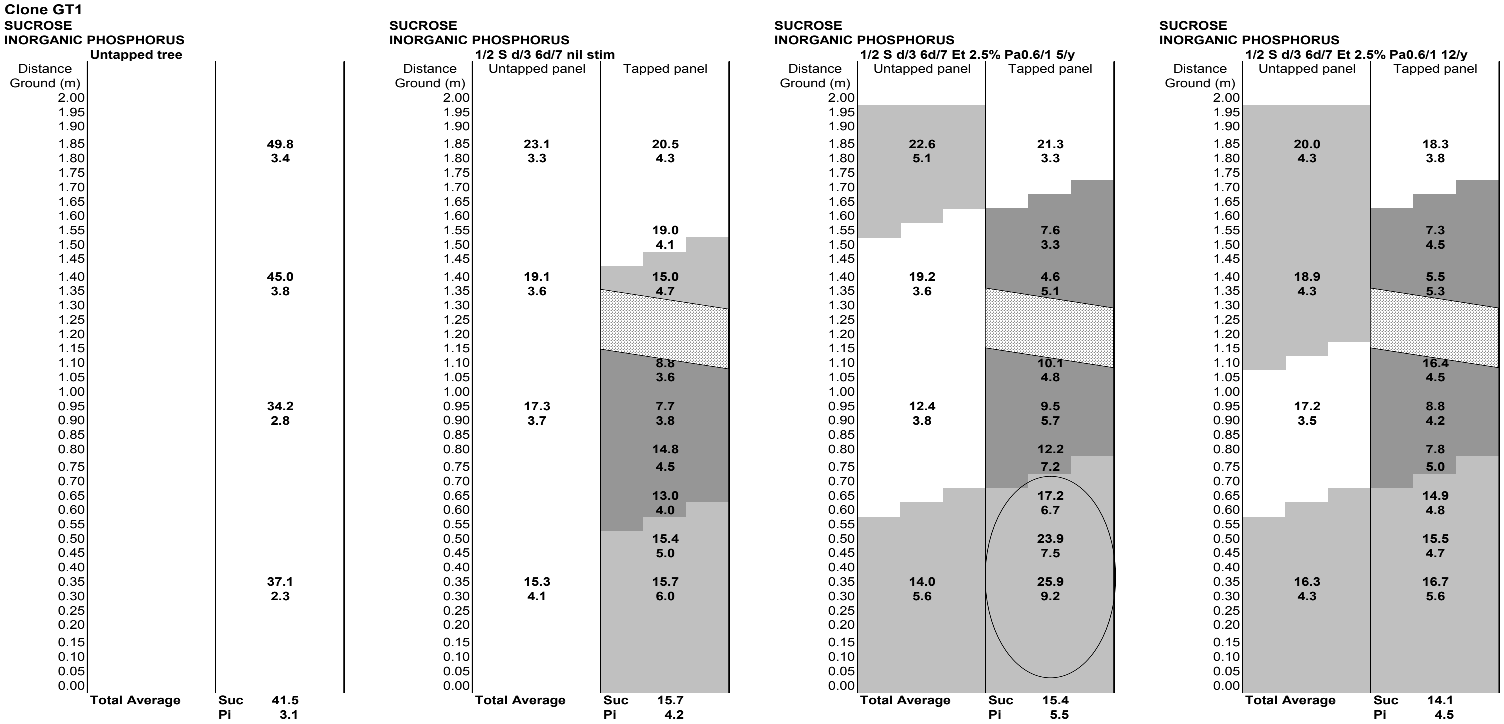


Figure 9 (a) The metabolic latex diagnosis mapping ("LD" mapping) of the 4 sampled trees clone GT1. Each value is the average of 3 sampling points of the same line. Shadowed areas represent the sampling positions where latex Pi concentration is found higher than the average trunk Pi concentration of the physiological control treatment (*nil stim*). The circled area shows an "sugar importation area", dark grey area: high Pi with the concurrent low suc, pale grey area: high Pi with the concurrent low suc, white area: area comparable to the physiological status of untapped tree, dotted area: the renewed bark

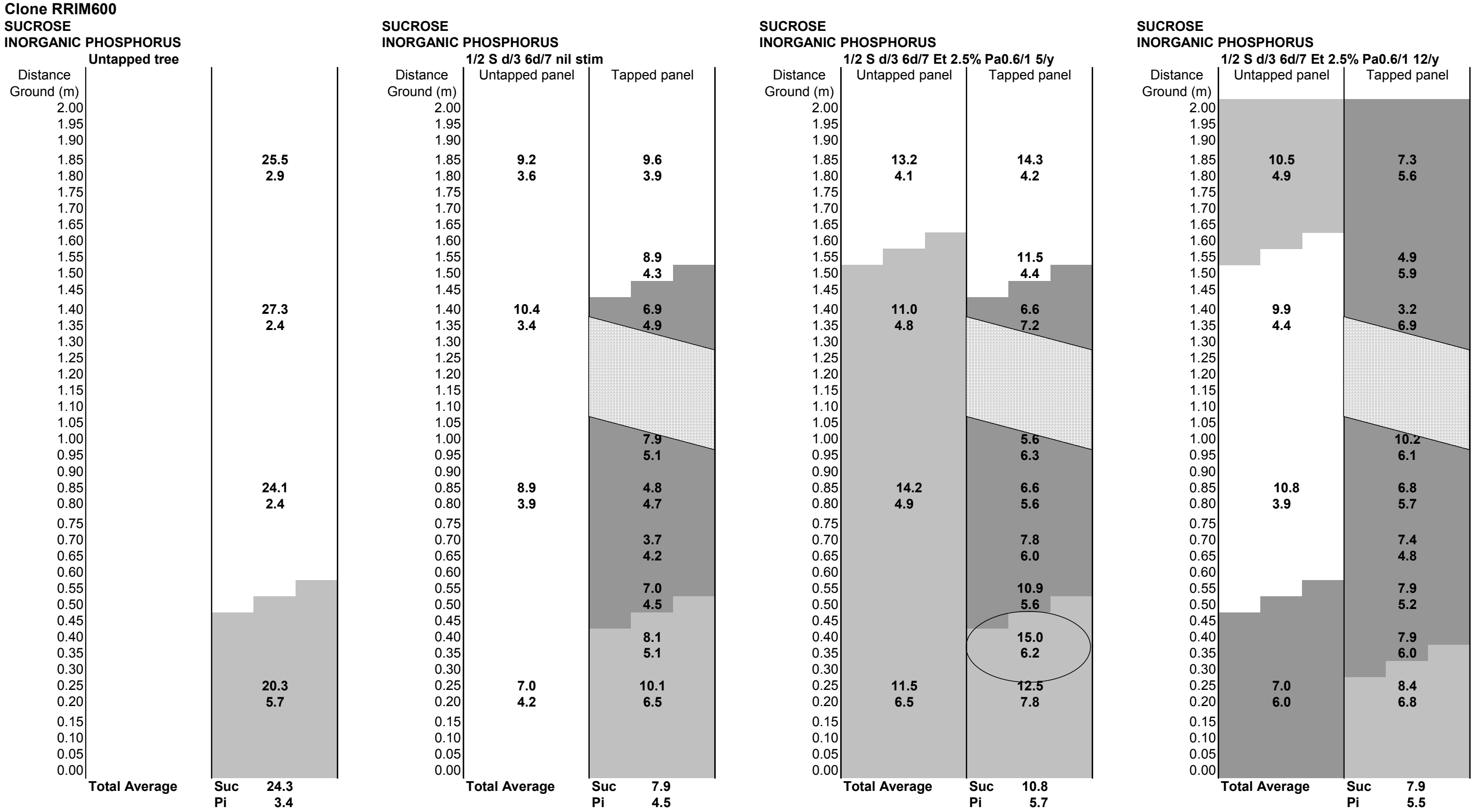
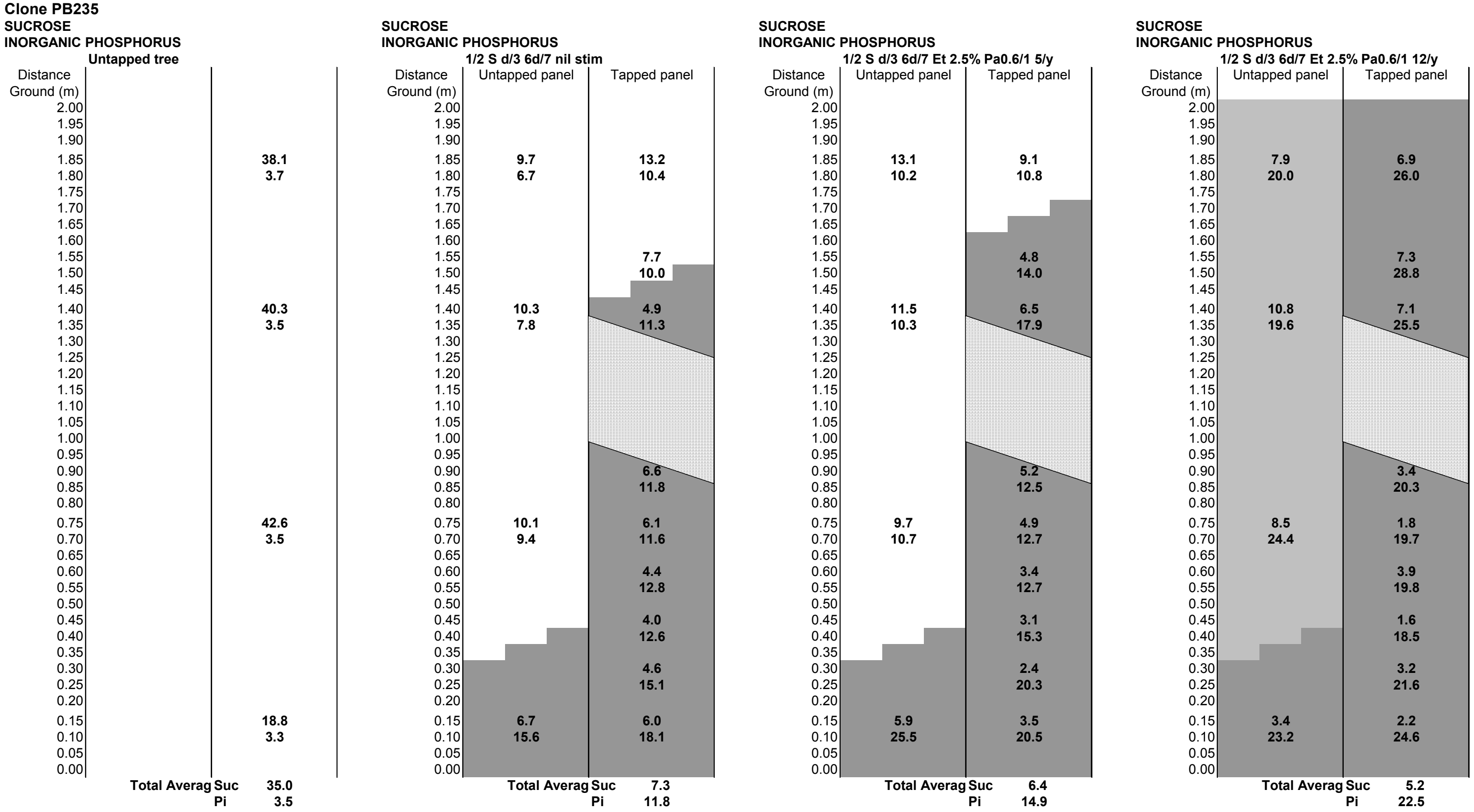


Figure 9(cont'd) (b) clone RRIM600



<u>Figure 9 (cont'd)</u> (c) clone PB235

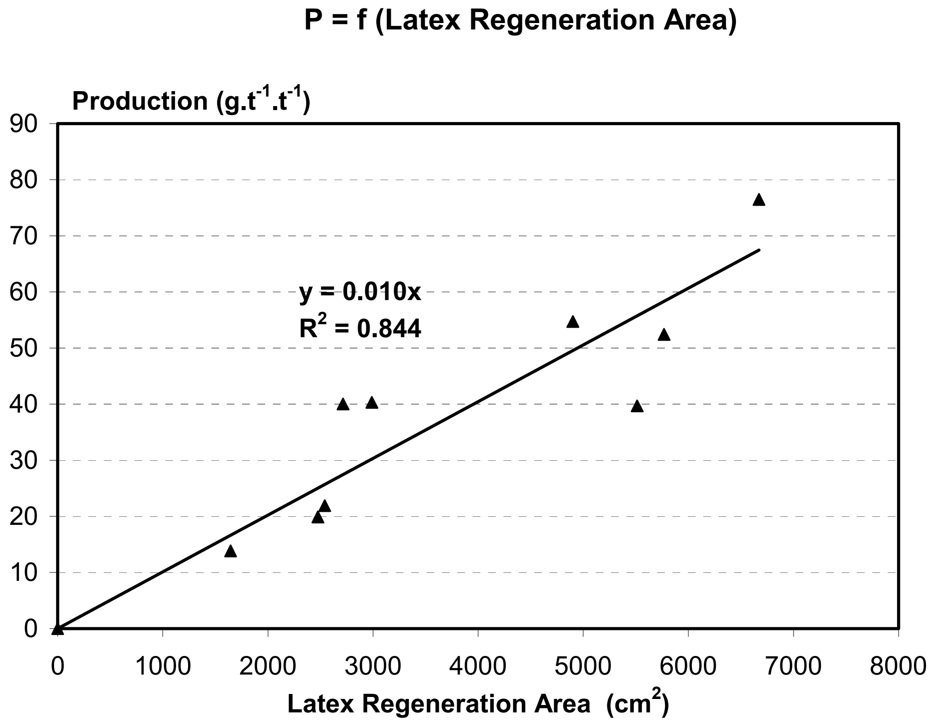


Figure 10 Multi-clonal linear relation between rubber production (month before latex sampling), expressed in g.tree^{-1}.tapping^{-1} (g.t^{-1}.t^{-1}), and the estimated latex regeneration area, expressed in cm^{2}. Regression has no constant term, as no production is characterized by absence of latex regeneration (untapped tree).

The Assessment of Trunk Radial Growth and Daily Variation

Both LVDT and RS sensor functioned well for long term continuous measurement of trunk radius under tropical condition. However, the measurement from RS sensor was overestimated when compared with trunk circumference measured by tape or with trunk diameter measured by LVDT sensor. Thus, data obtained from RS sensor needed to be adjusted with tape measurement (see in the appendix) in order to get the actual range without smoothing out the changes at fine scale. Nevertheless, RS sensor was proved to be a suitable method to follow long term radius movement, regarding its consistency, cost and the nature of the sensor that can measure directly the radius and require less space as compared to LVDT. This is particularly useful in the rubber tree where the two panels don't have the same growth rate. However, RS was not well adapted for monitoring the reversible radius movement, unlike LVDT, since the contractions were not accurately recorded.

Thus the use of both type of sensors together allowed to assess for the first time reversible changes at daily scale for untapped trees and to get a very precise figure of radius dynamics according to tapping and season.

1. Untapped Tree

1.1 Seasonal radius increment

Continuous measurement of trunk radius variation by RS sensor was investigated over one complete year (December 2001 – March 2003) (Fig.11a). The seasonal pattern of trunk radial changes of untapped tree showed no growth from December – February, which is the period of annual leaf shedding and re-foliation, and there was a slight and unsteady growth from late February to mid April. Significant continuous growth started from mid April until November. Thus, radial growth resumed after the beginning of rainy season and stopped at the end of rainy season. Radial growth rates was the highest in May – June with the growth rate between 40 - 60 µm d^{-1}, and then decreased regularly, although day to day fluctuations were significant. For one complete year, the radius of untapped tree increased about 6 – 7.5 mm. Transformed into circumference increment, these results (3.8 – 4.7 cm per year) were consistent with average growth rate reported for the same clone in the same location. Radius variation was highly variable in late February – March, this was explained by the occasional rainfall that occurred during dry season.

1.2 Daily radius movement

Daily radius variation of untapped rubber trees was described by the data obtained from LVDT, a more standard tool for measuring diameter variation, which was used in this experiment to check the validity of RS. Daily variation consists of reversible and irreversible movement and can be described into three phases as following (Downes *et al*, 1999) (figure 12). The *contraction phase* is defined as the period between the morning maximum and the daily minimum. During that phase as transpiration loss from the leaf increases, some water moves out the surrounding tissues to enter the xylem, resulting in trunk shrinkage. The *recovery phase* is defined as the period between the daily minimum until the radius reaches the morning maximum again. During that phase as transpiration slows down, the potential gradient reverses some water flows back to the surrounding tissues, resulting in trunk swelling. Finally, the *trunk radius increment* is defined as the period when the radius exceeded the morning maximum until the successive maximum. During that phase, measured increments consist in radius increment alone. The morning maximum occurred between 6 – 8 am and the daily minimum occurred between 3 – 6 p.m.

During the period of high radial growth (June, figure 13), the range of trunk radius changes due to water movements (*ca.* 90μm.d^{-1}) exceeded the range of radius increment itself (*ca.* 40μm.d^{-1}).

Figure 13 shows the daily radius variation at 3 different periods i.e., (1) during de-foliation in January 2002, (2) after the tree completed re-foliation in February and March 2002, and (3) during growing season in June 2002. In period (1) the range of daily movement was quite small, about 25 μm in both directions. This was due to the absence of growth and to the low leaf area, driving low transpiration rates and thereby slight changes in water potentials between compartments.

In period (2) the daily fluctuation (contraction and recovery phase) was about 4 times more, (100 μm). The radius increment in this period was not regular, reflecting the water supply from the soil that depended on the occasional rainfall. The lag between transpiration demand, due to full leaf area and high temperatures, and water supply from the soil was likely to create high differences in water potential between xylem and surrounding tissues. Thereby high waters fluxes to the xylem could be recovered only once the transpiration slowed down in late afternoon and night. During some days, a complete recover was not possible, resulting in a net shrinkage of the trunk.

In period (3) the daily variation was about 100 μm and the radius increment in this period was rather regular and the value was around 40 μm per day. Daily contractions were of lesser range than during February-March because frequent rainfalls allowed better water supply from the soil and thereby a smaller lag between absorption of water by the roots and transpiration.

2. **Radial Growth after Tapping Activity and Ethephon Stimulation**

2.1 The effect of tapping on radial growth

Although the negative impact of tapping on radial growth is well known at seasonal scale, information about its dynamics at short time-scale remains scarce. The use of continuous and accurate measurements by RS systems was thus appropriate to better understand how tapping affects radial growth.

Under Chachoengsao Rubber Research Center (CRRC) conditions, generally tapping starts in May and stops at the end of January. This allows 9 months of tapping and 3 months of resting period. In our plot (TE01), the first tapping year started on the 4th July 2001 and stopped on the 6th April 2002 (9 months of tapping). The second tapping year resumed on the 1st June 2002 and stopped on the 29th March 2003 (10 months of tapping following 2 months of rest). Before tapping resumed, the radial growth pattern of tapped tree was similar to untapped i.e., nearly no growth from December 2001 to early April 2002, then the annual growing period started in late April after the beginning of rainy season. In May the tree reached the maximum growth rate and it was between 20 - 60 μm $.day^{-1}$. After tapping started, a strong impact was noticeable on radial growth rate as the rate was dropped sharply in all positions a few weeks after tapping resumed

(Fig.14-16). This is a concrete evidence showing that the effect of tapping is not limited only to one specific area but extend to the whole trunk level.

However, use of RS sensors allowed to record differences in the growth patterns according to the four positions along the trunk where the RS sensors were placed on and to see how radial growth in each position was affected by the tapping activity.

The area below the cut in tapped panel showed quick decrease and almost no growth after 2 months of tapping. This area, the closest to the tapping cut is considered as the place where most of latex regeneration occurs. On the opposite, in the most distant area - above the cut in untapped panel - this decrease was slower and some slight growth was still observed after 5 months of tapping. For the two other positions (above the cut-tapped panel and below the cut-untapped panel), the decrease of growth rate was in between (Fig. 17). This showed that the effect of tapping on trunk radial growth was not homogeneous, but related to the distance from the tapping cut.

Cumulative radius increment for 1 year of all tapped trees, either non ET or ET treatment, showed a bottom – up increasing trend for radius increment (Fig.18). The upper positions (above the cut) had about 20-35 % higher in cumulative growth than the lower positions. Generally the untapped panel – above the cut showed the highest cumulative increment except the C2 tree which the highest increment was on the opposite side but in this tree all positions had remarkable low increments as compared to other trees. The tapped panel – below the cut gave the lowest in radial increment except the B2 tree.

2.2 The effect of ethephon stimulation on radial growth

It is quite difficult to see in this experiment the effect of ethephon on radial growth clearly since there was only slight difference between the yields of the two treatments, due to the fact that the trees were in the second year of tapping only. Anyway it has to note that the 2 trees in Et 8/y treatment had very different behavior for wood production. It seemed that the balance between wood and latex production in C1 tree is much better than C2, thus allow the tree to produce more biomass while maintain the comparable yield (Fig.19).

3. Competition between Wood and Latex Production

Figure 19 (a) presented the estimated cumulative wood and latex biomass of the average of 12 trees of each treatment from May 2001 to March 2003. For almost 2 years, untapped, non ET and ET treatments produced total biomass 72.6, 60.7, and 64.8 kg / tree respectively. For wood biomass non ET and ET produced 51.1 and 52.9 kg / tree respectively. For latex biomass, non ET and ET produced 9.6 and 11.8 kg / tree respectively. Thus the proportion of latex: wood in non ET and ET was 1: 5.3 and 1: 4.5

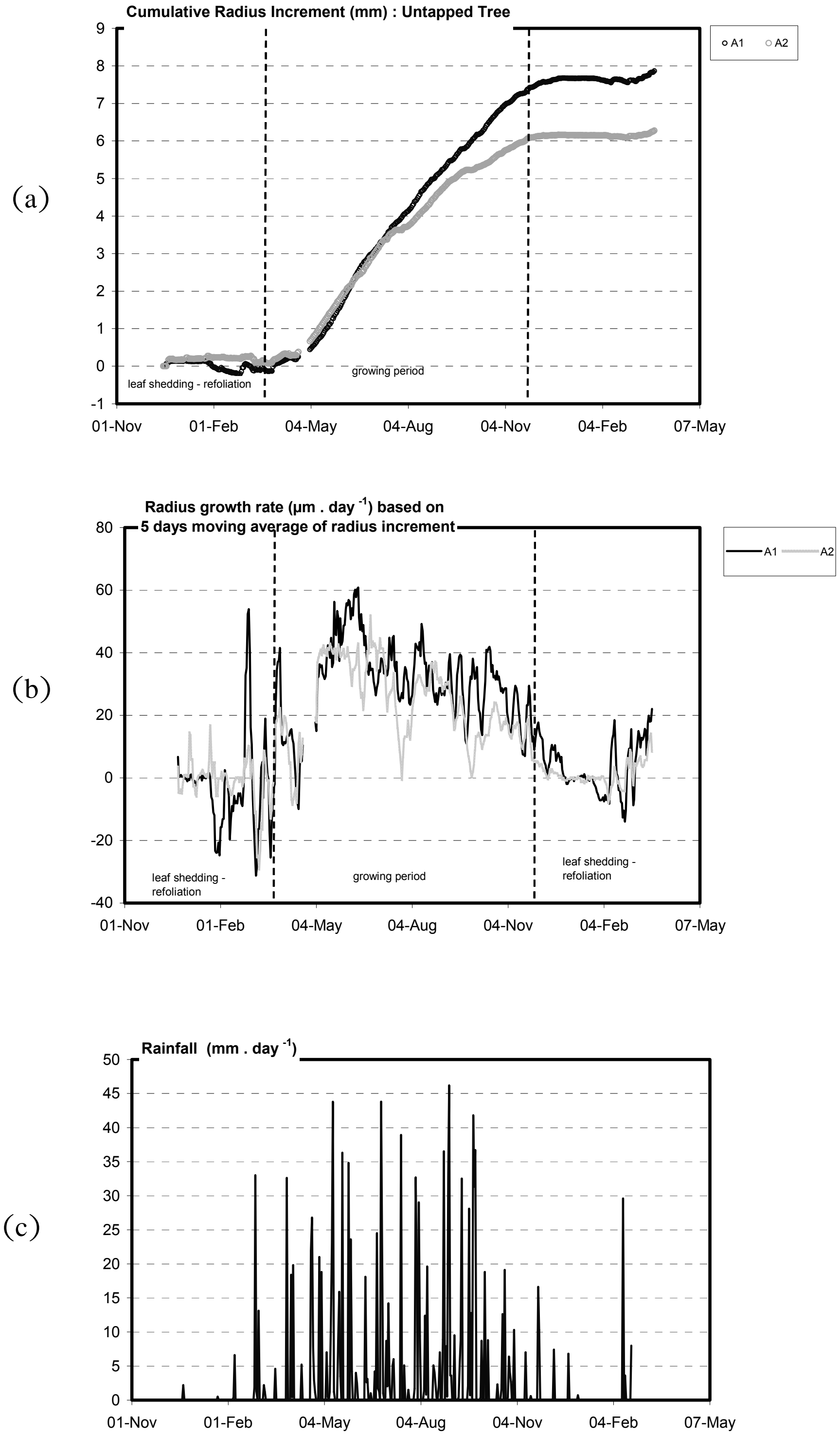


Figure 11 (a) Seasonal radial growth increment (µm) of untapped tree (A1, A2) (b) Radial growth rate (µm. day^{-1}) based on 5 days moving average of radial increment and (c) Daily rainfall (mm.day^{-1})

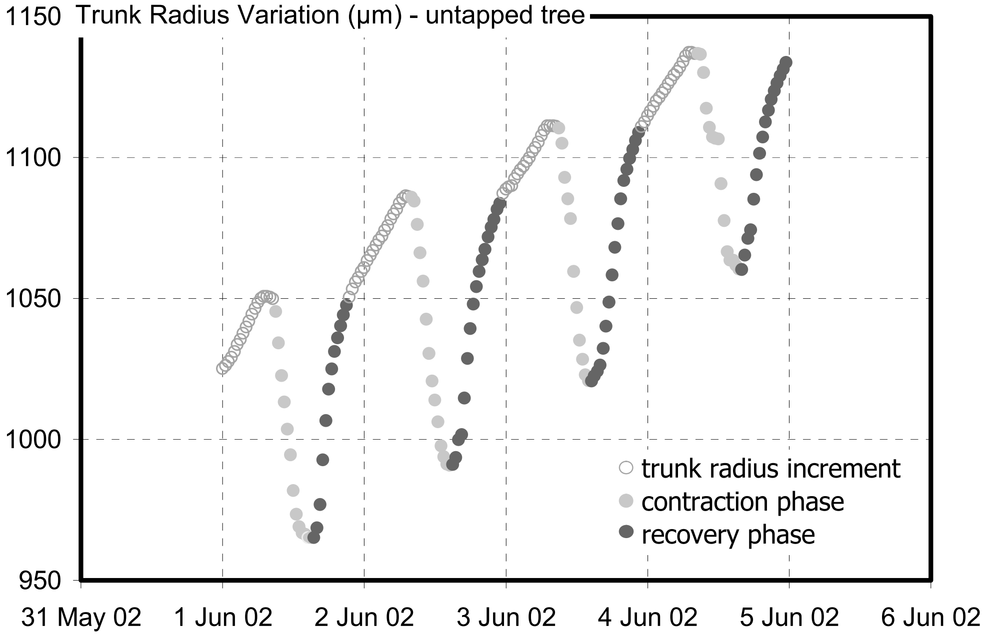


Figure 12 Pattern of daily radius variation

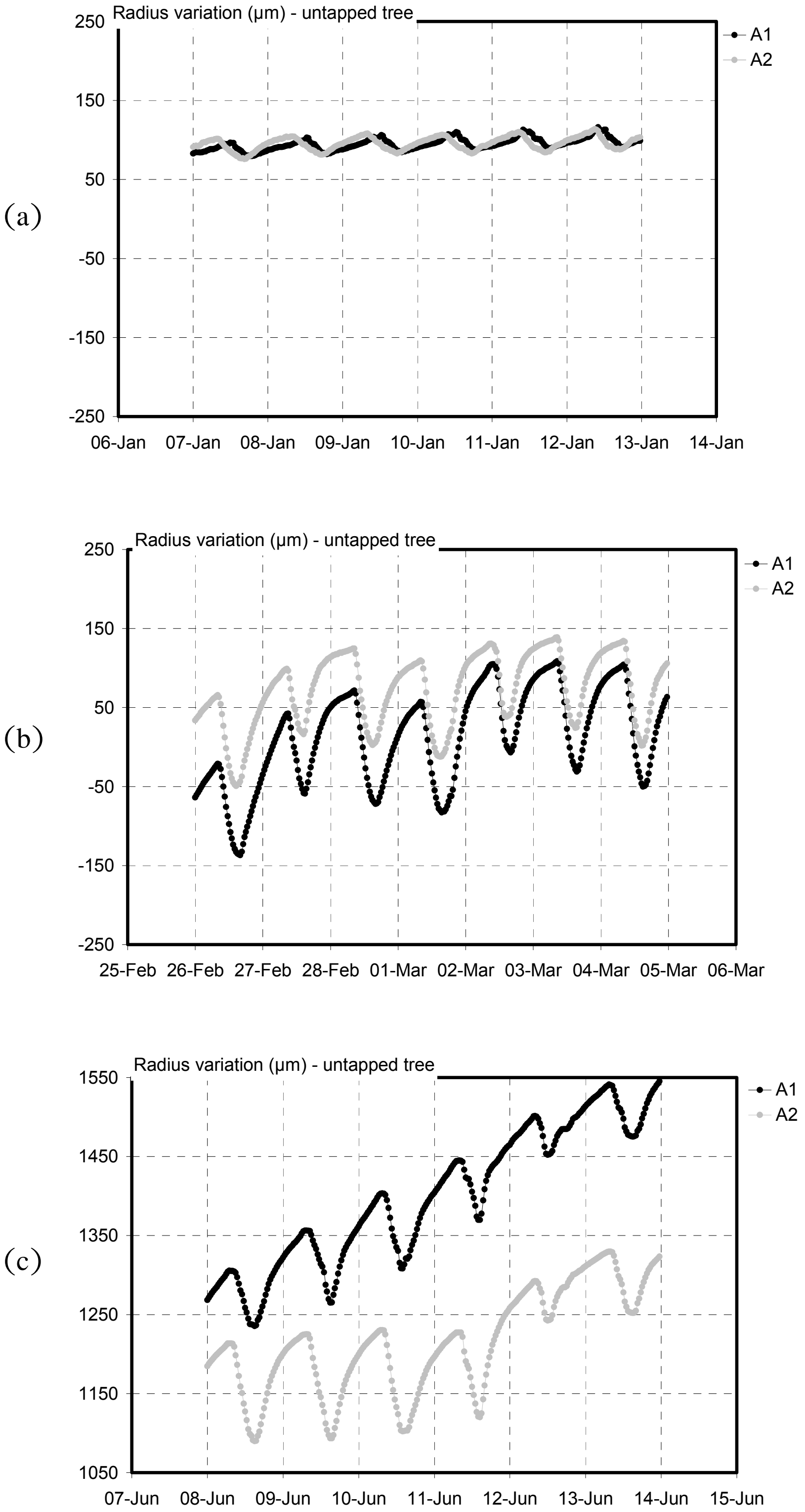


Figure 13 Daily radius fluctuations (µm) observed from LVDT measurement in Untapped trees at (a) defoliation (b) dry season (c) vegetative season

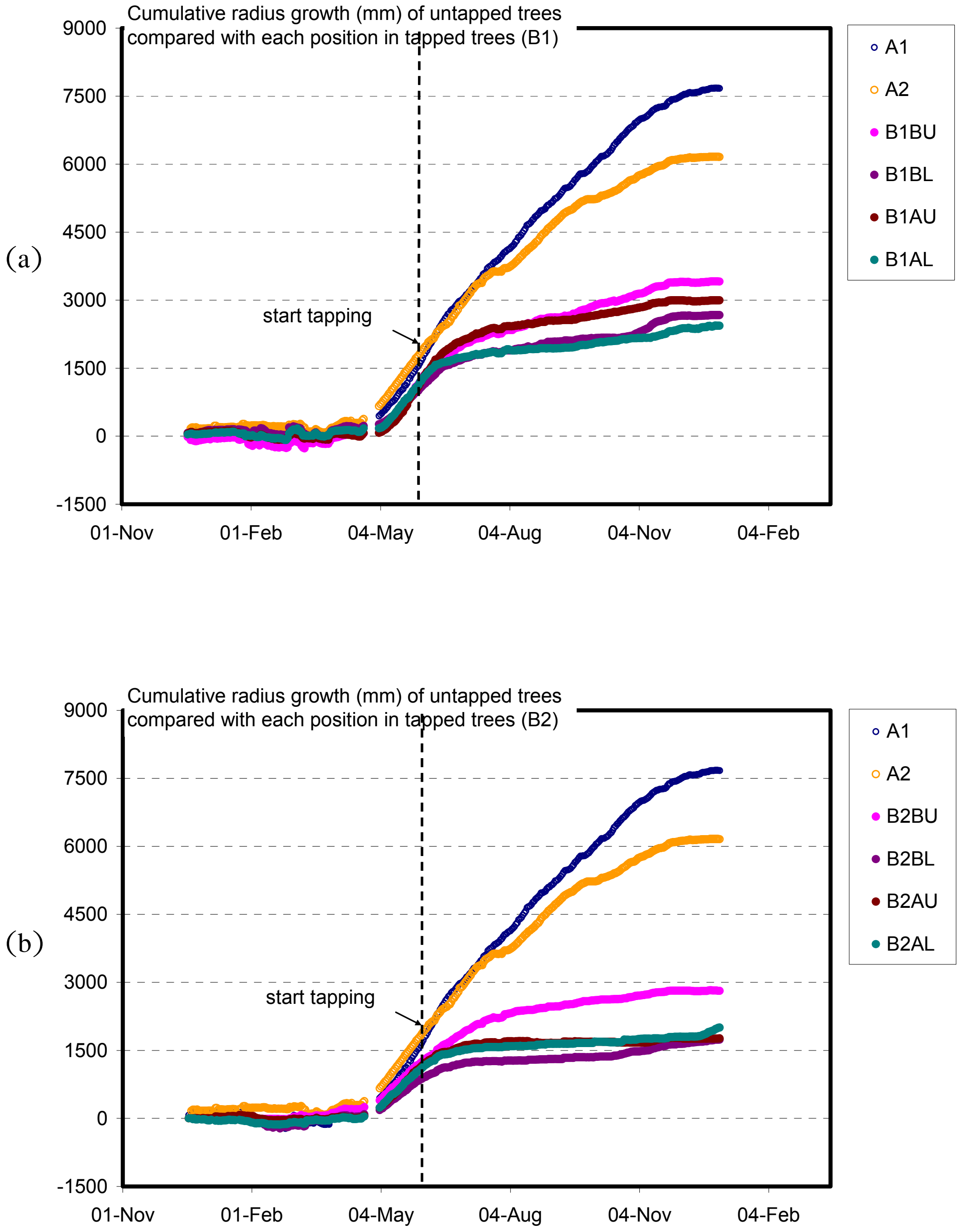


Figure 14 Comparison of cumulative radial growth of untapped with all positions of *nil stim* treatment (a) B1 (b) B2

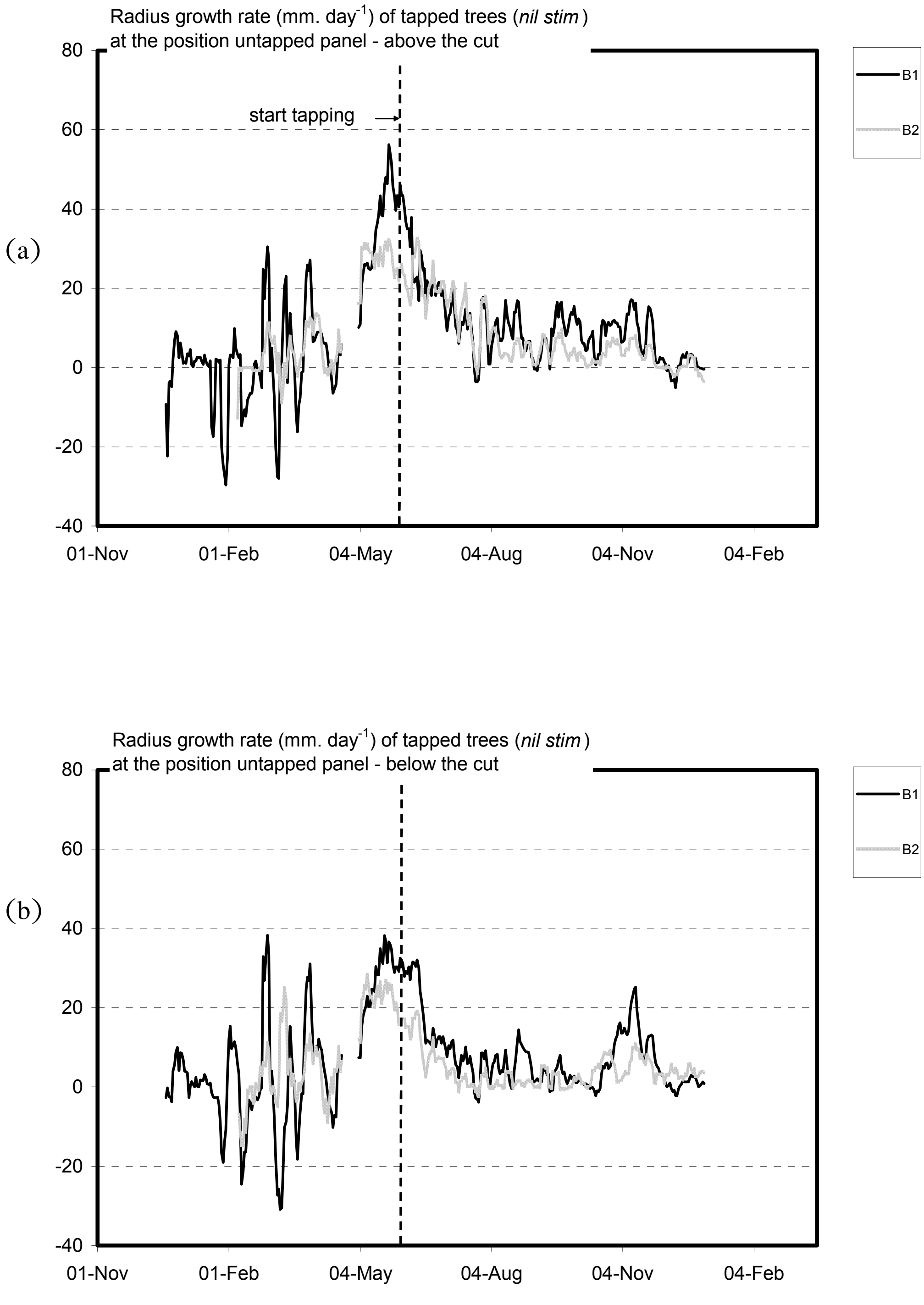
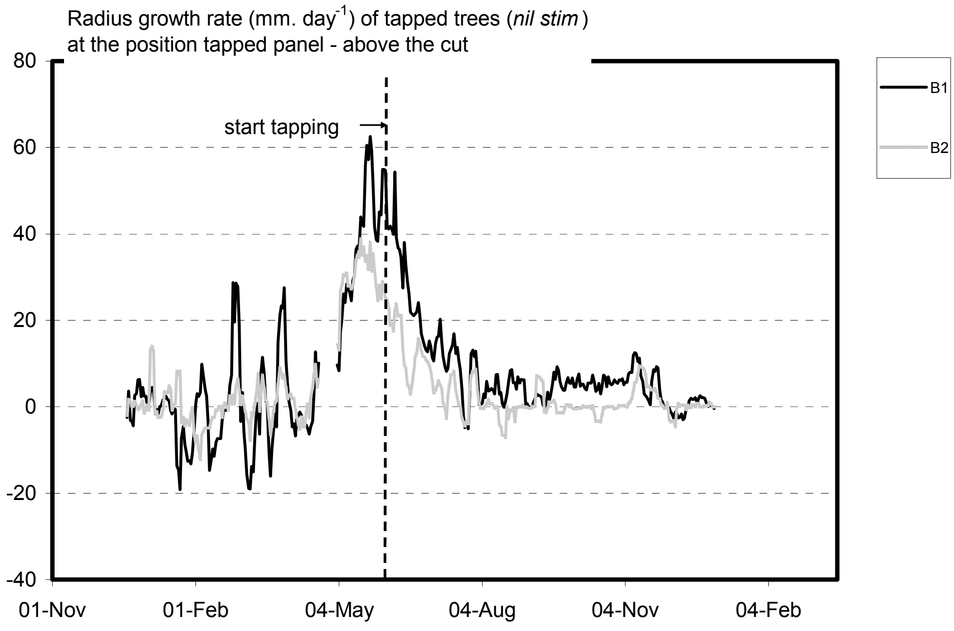


Figure 15 Radial growth rate of *nil stim* − untapped panel based on 5 day moving average of radius increment (a) above the cut (b) below the cut

(a)



(b)

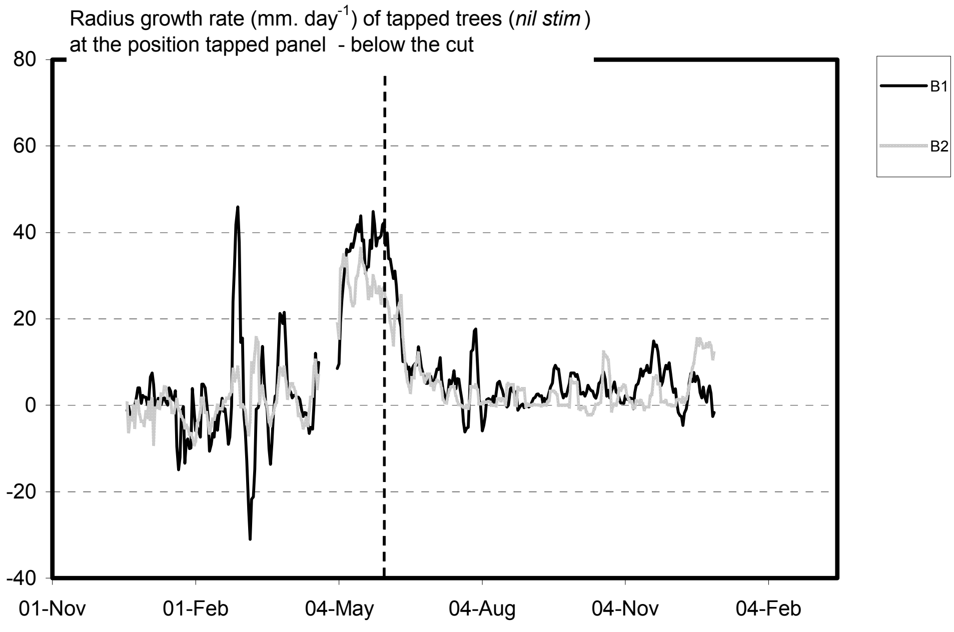
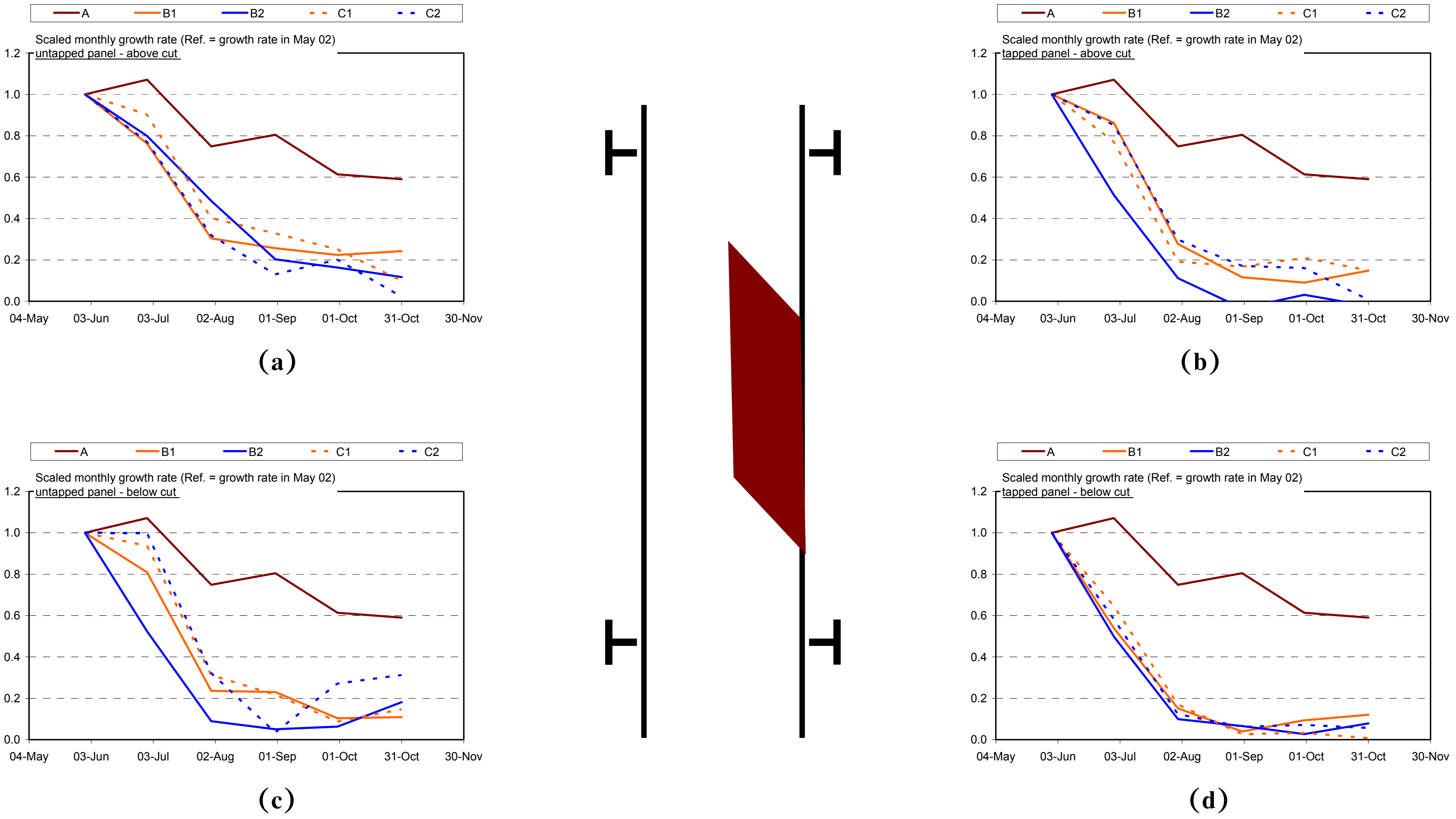
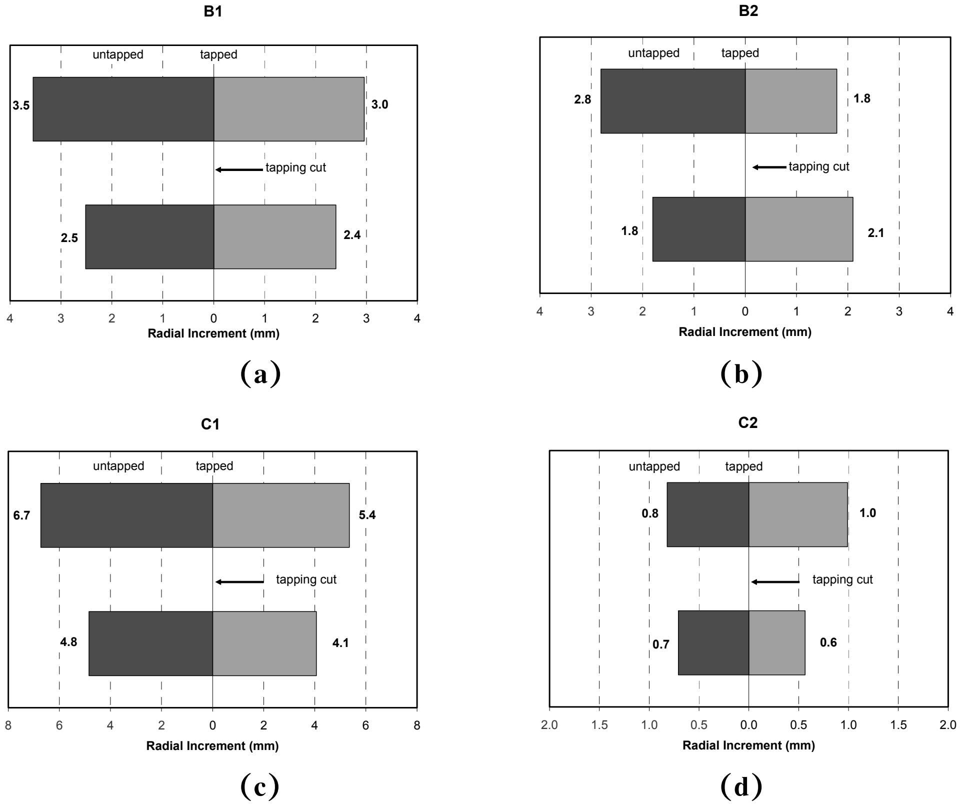


Figure 16 Radial growth rate of *nil stim* – tapped panel based on 5 day moving average of radius increment (a) above the cut (b) below the cut



(a) (b) (c) (d)

Figure 17 Comparison of scaled monthly growth rate at different position of the trunk (A = untapped, B1, B2 = *nil stim*, C1, C2 = Et 8/y), reference is the growth rate in May 2002 , (a) untapped panel – above the cut, (b) tapped panel – above the cut, (c) untapped panel – below the cut and (d) tapped panel – below the cut



(a) (b) (c) (d)

Figure 18 Comparison of one year cumulative radial growth (8th Feb.02 – 9th Jan. 03) at different position on the tree
(a), (b) = *nil stim* (c),(d) = Et 8/y

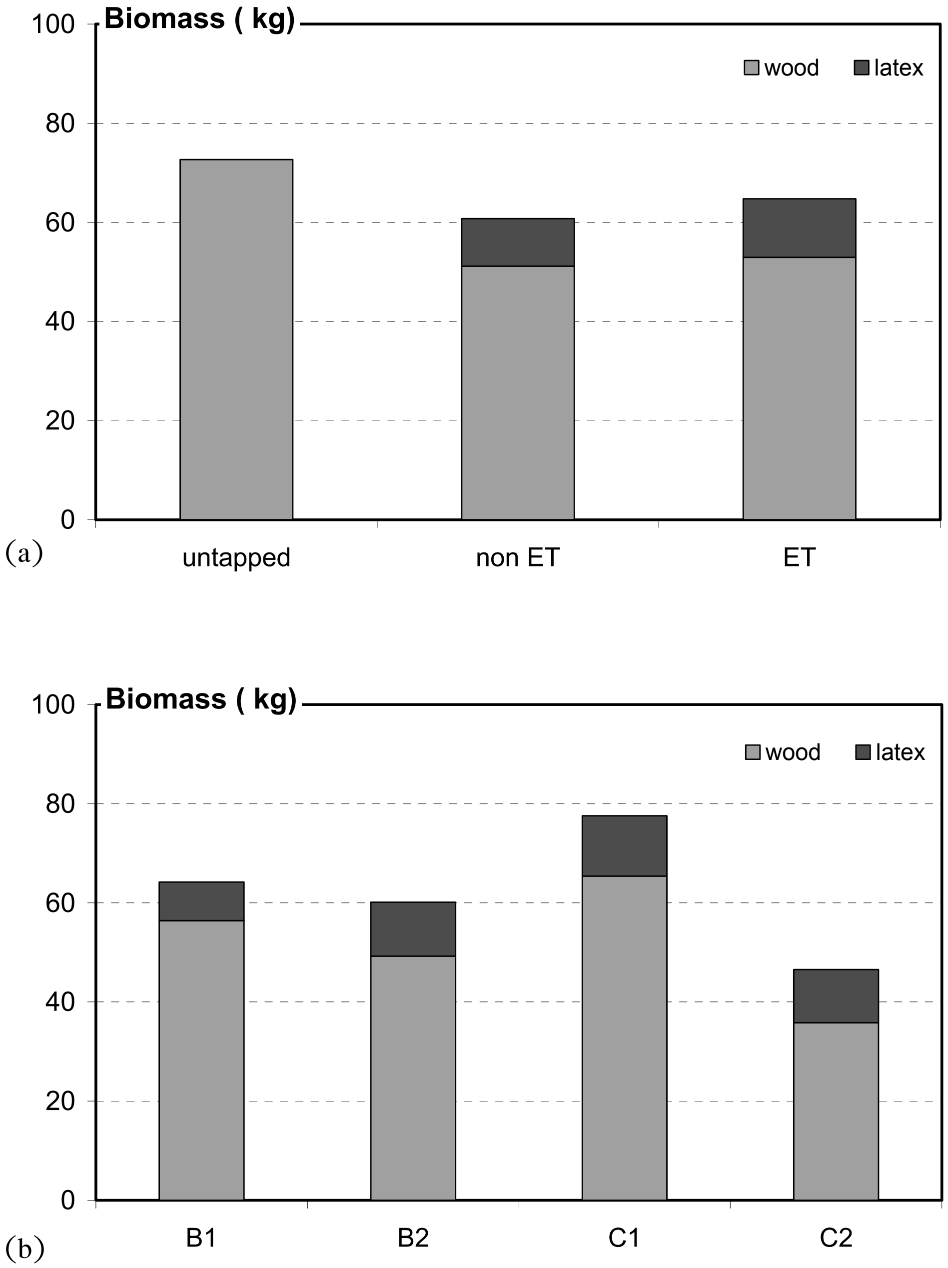


Figure 19 Estimation of cumulative wood and latex biomass produced from May 2001 – March 2003. Wood biomass was calculated from circumference at 1.7 m (tape measurement). Latex biomass was calculated by multiplying the cumulative yield (kg) with the calorific value 2.5.

(a) Average from 12 trees for each treatment in plot TE01

(b) Each tapped trees under fine radial growth measurement

Respiratory Activity of *Hevea brasiliensis* at Trunk Scale

1. Seasonal Variation of Bark Respiratory Activity

The information of bark respiratory activity presented in this chapter is based on the calculation of 24 hours mean at ambient temperature (°C). The Q_{10} value for extrapolated respiration was 2.3, which was the value from the experiments in the walnut tree *(Juglans regia L.).*

1.1 Overall trend

The total respiration rates (nmol.min^{-1}.cm^{-2}) of untapped, *nil stim*, and Et 8/y treatments from December 2001 - March 2003 were shown in figure 20. No data were obtained from April - mid September 2002 due to the technical problem of the control system.

All treatments gave similar trend for seasonal variation i.e., high respiratory activity during vegetative season followed by long term decreasing trend with short term fluctuation until reached the minimal rate at leaf shedding - refoliation period (mid December-February). Annual variation of bark respiration ranged 5 - 40 nmol.min^{-1}.cm^{-2} for the investigated trees. The occasional increase of respiration rate during the long-term decreasing trend fitted with the daily radius increment rate. Highly positive linear correlation between respiration activity and radius increment in untapped tree was found. In tapped tree, the degree of correlation was different depending on the chamber position. The position with the degree of correlation closed to those of untapped tree was the position situated the furthest to the tapping cut i.e., untapped panel-above cut position. The position without clear linear correlation between the 2 parameters was the position in tapped panel - below the cut. Day to day variability was higher in tapped trees, particularly those stimulated, than in untapped trees. High radial growth rate from April - September observed by RS measurement suggested a strong respiratory activity of this period even though no respiration data were recorded at that time.

1.2 Annual minimum respiration period

The minimum respiration of the year occurred at leaf shedding – re foliation period. Under Chachoengsao condition (mean annual rain fall 2001-2002: 1280 mm. year^{-1}), annual leaf shedding was noticed from mid December and the complete re-foliation state was observed at the end of February (Fig. 20). No true dormancy period was noticed since the tree re-foliated just after de-foliation had completed. In some trees, the two phenomena overlapped i.e. some branches did not complete de -foliation while the others had started to re-foliate.

Both tapped and untapped treatments shared a common trend of having two periods of minimum respiration; the first minimum was noticed during leaf shedding, when a significant number of leaves remained. This was during 29th December, 2001 – 3rd January, 2002 in the first year and around 11th – 14th January, 2003 in the second year. After this, a small increase of respiration was observed and continued about 3 weeks and then started a decreasing trend again until reaching the second minimum. The increase of respiration had started before bud break was noticed and then reached the peak during the stage of leaf expansion. Simultaneous increase in respiratory activity and re-foliation process suggests the requirement of energy for leaf expanding especially the translocation of reserve sugar in wood to the site of new leaves. When re-foliation process completed, the respiration rate remained in a decreasing trend until it reached the second minimum when negative radius variation was also found. This shrinkage phenomenon in concomitance to low respiration rate at the period of fully expanded canopy may suggest a stress situation when soil water content was not enough to refill the tree after transpiration, due to the absence of rainfall.

Concerning the quantitative aspect, untapped tree had the lowest minimum respiration. Since no radial growth was observed at that period, the minimum respiration implied the maintenance respiration of the untapped rubber tree. The values from the 2 replicas was 6.0 and 11.4 nmol.min^{-1}.cm^{-2} for the first minimum (table 3) and 3.9 and 6.3 nmol.min^{-1}.cm^{-2} for the second minimum (table 4). Tapped trees showed higher respiration rate at the same period. First minimum respiration for *nil stim* treatment was 9.5 and 11.1 nmol.min^{-1}.cm^{-2} and the second minimum was 7.2 and 9.3 nmol.min^{-1}.cm^{-2}. For Et 8/y treatment the first minimum was 9.6 and 12.4 nmol.min^{-1}. cm^{-2} and the second minimum was 8.3 and 9.5 nmol.min^{-1}.cm^{-2} (table 3 and 4). Thus, the difference between untapped and tapped tree was particularly clear, the second minimal respiration of untapped trees representing about 60% of value of tapped trees (table 4). Among tapped trees, three out of four showed higher respiration in tapped panel. Since tapping activity continued at that period, the respiration rate of tapped tree could account mainly for the maintenance cost and also the associated respiration for latex regeneration process. The difference in respiration between untapped and tapped tree plus the actual latex production will be further analyzed to estimate the actual respiration required for the latex regeneration process.

1.3 Trend during tapping season: September 2002 – January 2003

Due to technical problems, respiration was measured only from September 2002, when growth was already very low for tapped trees.

Untapped tree

The two replications were quite similar regarding the range and trend along the years. Their respiration rates were less fluctuating as compared to tapped treatments. Maximum respiration occurred on September 28th for A1 (36.4 nmol.min^{-1}.cm^{-2}) and September 23rd for A2 (34.9 nmol.min^{-1}.cm^{-2}). From September to November the respiration ranged 15 – 35 nmol.min^{-1}.cm^{-2}, then it decreased to 10 -20 nmol.min^{-1}.cm^{-2} in December and then in January it ranged 6 -12 nmol.min^{-1}.cm^{-2} (Fig. 22).

Tapped tree – nil stim treatment

Unlike untapped tree, respiration rate within *nil stim* treatment was more heterogeneous depending on which panel or position the chamber was placed on. It seemed that the respiration rate of the positions on untapped panel was closer to each other than in tapped panel, regarding range and trend. With high peaks occurring from time to time, bark respiration on untapped panel ranged 15 – 30 nmol.min^{-1}.cm^{-2} during September to November, ranged 15 – 20 nmol.min^{-1}.cm^{-2} in December and then ranged 10 – 15 nmol.min^{-1}.cm^{-2} in January (Fig. 23). On tapped panel, there was more difference as mentioned before. At the above cut position, the respiration rate of the two replications ranged 15 – 30 nmol.min^{-1}.cm^{-2} from September to November, and ranged 14-20 nmol.min^{-1}.cm^{-2} in December. In January B1A> ranged 8-14 nmol.min^{-1}.cm^{-2} while in B2A> it ranged 10-18 nmol.min^{-1}.cm^{-2}. The positions below the cut generally gave lower value for the minimum with an exceptional high peak (more than 35 nmol.min^{-1}.cm^{-2}) in September. From September to November it ranged 10-25 for both replications, then in December it ranged 8-14 and 14-18 nmol.min^{-1}.cm^{-2} for B1A< and B2A< respectively. In January it ranged 6-10 and 10 -16 nmol.min^{-1}.cm^{-2} for B1A< and B2A< respectively (Fig. 24).

Table 3 Year 2002 the 1st minimum respiration rate per unit bark area (nmol.min^{-1}.cm^{-2}) at ambient temperature (°C), the value was the average from 29th Dec. 01 – 3rd Jan. 02.

Treatment	Respiration rate					
	Tree		Panel		Position	
Untapped	A1	6.0(+ 0.67)	-	6.0 (+ 0.67)	-	6.0 (+ 0.67)
	A2	11.4 (+ 1.08)	-	11.4 (+ 1.08)	-	11.4 (+ 1.08)
nil stim	B1	9.5 (+ 0.89)	A	9.9 (+ 0.81)	A<	9.6 (+ 0.75)
					A<	10.3 (+ 0.74)
			B	9.1 (+ 0.78)	B>	9.1 (+ 0.73)
					B<	9.0 (+ 0.90)
	B2	11.1(+ 1.92)	A	12.8 (+ 0.95)	A>	13.5 (+ 0.72)
					A<	12.1 (+ 0.57)
			B	9.5 (+ 0.77)	B>	10.1 (+ 0.41)
					B<	8.8 (+ 0.32)
Et 8/y	C1	9.6 (+ 0.96)	A	9.2 (+ 1.11)	A>	10.2 (+ 0.52)
					A<	8.2 (+ 0.42)
			B	10.1 (+ 0.53)	B>	10.4 (+ 0.47)
					B<	9.8 (+ 0.47)
	C2	12.4(+ 1.88)	A	13.2 (+ 1.71)	A>	14.5 (+ 1.07)
					A<	11.8 (+ 0.84)
			B	11.6 (+ 1.75)	B>	13.1 (+ 0.84)
					B<	10.0 (+ 0.48)

Table 4 Year 2002 the 2nd minimum respiration rate per unit bark area (nmol.min^{-1}.cm^{-2}) at ambient temperature (°C), the value was the average from 22nd – 27th Feb.

Treatment				Respiration rate		
		Tree		Panel		Position
Untapped	A1	3.9 (+ 0.24)	–	3.9 (+ 0.24)	–	3.9 (+ 0.24)
	A2	6.3 (+ 0.63)	–	6.3 (+ 0.63)	–	6.3 (+ 0.63)
nil stim	B1	7.2 (+ 0.99)	A	7.7 (+ 0.63)	A>	7.4 (+0.54)
					A<	8.1 (+ 0.56)
			B	6.6 (+ 0.94)	B>	5.8 (+ 0.48)
					B<	7.3 (+ 0.60)
	B2	9.3 (+ 1.64)	A	10.6 (+ 0.85)	A>	11.3 (+ 0.66)
					A<	10.0 (+ 0.50)
			B	7.9 (+ 0.91)	B>	8.7 (+ 0.48)
					B<	7.1 (+ 0.48)
Et 8/y	C1	9.5 (+ 0.76)	A	9.7 (+ 0.90)	A>	10.5 (+ 0.61)
					A<	9.6 (+ 0.57)
			B	9.4 (+ 0.56)	B>	9.0 (+ 0.35)
					B<	7.0 (+ 0.43)
	C2	8.3 (+ 1.60)	A	8.3 (+ 1.46)	A>	9.7 (+ 0.48)
					A<	10.1 (+ 0.54)
			B	8.4 (+ 1.80)	B>	9.0 (+ 0.37)
					B<	6.8 (+ 0.47)

Both panels, either above or below the cut, had peak of respiration on these 6 periods: September 23rd – 25th, October 23rd, October 29th, November 1st, November 12th and November 24th – 26th. The values were mostly between 25 – 30 nmol.min^{-1}.cm^{-2} except a few exception (B1B< on November 1st: 33.5 nmol.min^{-1}.cm^{-2}, B2B< on September 24th: 33.2 nmol.min^{-1}.cm^{-2}, B1A< on September 23rd: 37.4 nmol.min^{-1}.cm^{-2}, B2A< on September 23rd: 34.2 nmol.min^{-1}.cm^{-2})

Tapped tree – Et 8/y treatment

The pattern of respiration was close to *nil stim* but higher in value and degree of fluctuation i.e., more peaks were observed especially at the below cut position of both panels. Most of the respiration peaks occurred at the same dates for *nil stim* and Et 8/y treatments. From October, generally the respiration rate in untapped panel was higher than tapped panel especially at the below cut position. Difference between replication (C1 and C2) could be seen easily at the position above the cut (for both panels). In tapped panel, this difference was so obvious in December with higher respiration on C2A>. But in untapped panel it was the contrary, higher respiration had been found in C1B> since September which seemed to fit with growth rate data. For the positions below the cut (for both panels) the range was quite similar between replications.

With high peaks occurred from time to time (more frequent than *nil stim*), respiration in untapped panel – above cut position from September to November ranged 20-40 and 15-30 nmol.min^{-1}.cm^{-2} for C1 and C2 respectively. Then in December it ranged 20 – 30 nmol.min^{-1}.cm^{-2} (C1) and 15-20 nmol.min^{-1}.cm^{-2} (C2) and then in January it ranged 15-20 nmol.min^{-1}.cm^{-2} (C1) and 10-15 nmol.min^{-1}.cm^{-2} (C2). For the below cut position it ranged 15-40 nmol.min^{-1}.cm^{-2}(C1) and 15-30 nmol.min^{-1}.cm^{-2} (C2) from September to November, ranged 20 - 30 nmol.min^{-1}.cm^{-2} (C1,C2) in December and then ranged 10-20 nmol.min^{-1}.cm^{-2} (C1) and 17-25 nmol.min^{-1}.cm^{-2} (C2) in January (Fig. 25).

For tapped panel, the respiration of the below cut positions were similar for both replications, it ranged 15 – 35 nmol.min^{-1}.cm^{-2} from September to November, 15 – 25 nmol.min^{-1}.cm^{-2} in December, and 10-20 nmol.min^{-1}.cm^{-2} in January. For the above cut it was different, from September to November C1A> ranged 5-35 nmol.min^{-1}.cm^{-2} while C2A> ranged 15-30 nmol.min^{-1}.cm^{-2}, in December it ranged 6-20 nmol.min^{-1}.cm^{-2}for C1A> and 15-20 nmol.min^{-1}.cm^{-2} for C2A>. In January it ranged 4-16 nmol.min^{-1}.cm^{-2} for C1 and 10-16 nmol.min^{-1}.cm^{-2} for C2 (Fig. 26).

Concerning high peaks, all positions in Et 8/y shared the common periods for high peaks respiration like in *nil stim* treatment (with higher value up to 40 nmol.min^{-1}.cm^{-2}). Besides these periods, there was also other dates i.e. September 29th, October 8th

– 10th, December 6th, 11th, 19th – 20th, 24th – 26th, and January 17th – 19th. More peaks were found on the below cut than the above cut positions. The major high peaks common found for *nil stim* and Et 8/y treatments during September, October and November were fitted with the weather condition. Peak of raining occurred during September 18th - 24th, 30th – October 6th, October 19th- 31st, and November 23rd – 24th. Nevertheless, high peak of respiration on September 29th seemed to be an exception. Since September 28th was the stimulation day and the trees were tapped on the following day. High peak on September 29th was so clear on tapped panel (above or below the cut) and could be seen also on untapped panel. In *nil stim* treatment this peak was noticed also but not with the same scale. Another support was the period with high peak on tapped panel-below cut during December 19th – 20th (the 18th: the stimulation day, the 19th: the tapping day), and January 17th -19th (the 16th: the stimulation day, the 18th: the tapping day). These two periods occurred during dry season and de-foliation time.

2. Difference in Respiration Rate between Panel and Position

2.1 Between position (above and below the cut)

The figure 27 and 28 showed the difference in respiration between above and below the cut in tapped treatments from September 2002 to January 2003, calculated by subtracting the 'below cut' respiration out of the 'above cut' respiration. In *nil stim* treatment, this resulted in positive value for tapped panel and negative value for untapped panel, implied that the 'below the cut' area respired less than the 'above the cut' area in tapped panel but reacted contrary in untapped panel. But this did not correlate with the difference in radial growth at the same period. The delta ranged up to 5 $nmol.min^{-1}.cm^{-2}$ different for both panel. In Et 8/y treatment the delta range was double, up to 10 $nmol.min^{-1}.cm^{-2}$. This indicates that ethylene stimulation changes the pattern of respiration along the trunk, bringing to more heterogeneity.

The two replications did not have the same trend. In tapped panel C1, the respiration was higher above than below cut from September to October and then from November to January the below cut respired more than the above cut. For C2 the below cut respired more than the above cut for the whole period. In untapped panel C1, the respiration was higher above than below cut and in C2 after mid November it was higher in the below cut than above cut.

2.2 Between panel (tapped and untapped panel)

The figure 29 and 30 showed the difference in respiration between tapped and untapped panel in tapped treatments from September 2002 to January 2003, calculated by subtracting the 'untapped panel' respiration out of the ' tapped panel'. Most of the results showed negative value with the delta up to 10 nmol.min^{-1}.cm^{-2} in *nil stim* and up to 30 nmol.min^{-1}.cm^{-2} in Et 8/y treatment. This implied that the 'untapped panel' respired more than the 'tapped panel' during vegetative season.

3. Relationship between Respiration and Radial growth

3.1 Untapped tree

The rate of respiration and radial growth were calculated together for linear correlation. Based on the moving average of 7 successive days, high degree of positive linear correlation ($r^2 > 0.8$) was found in untapped tree, for both replications (Fig. 31). This will allow establishing the growth coefficient and respiratory cost for wood production in the next step. The annual minimum respiration rate of untapped tree observed from the year 2002 (table 3 and 4) suggested the minimum value from 4 – 11 nmol.min^{-1}.cm^{-2}. These values were close to the constant in the linear equation that described the respiration rate (y) as the function of the radial growth rate (x) i.e. $y = 0.7022x + 12.483$ and $y = 0.5512x + 11.07$ for untapped tree replication 1 and 2. The constant value implied the minimum respiration rate. The high degree of correlation in untapped tree showed that radial growth was the main component of respiration in this period.

3.2 The effect of tapping on tapped tree

There was also the correlation between respiration and radial growth in the untapped panel above cut (Fig. 32a and b) but the degree of correlation was much lower. On tapped panel below the cut there was no correlation between respiration and radial growth rate. Concerning this, tapping activity is shown in this experiment to modify the relationship between respiration and radial growth since latex regeneration is one more factor involved.

3.3 Diurnal variations in the days after stimulation

Figure 36 compared the diurnal pattern of respiration rate between *nil stim* and Et 8/y treatments at the position tapped panel-below the cut on 5 different periods (Fig. 36 a-e). The aim was to follow the effect of ethephon on respiration especially on the first tapping day after stimulation and the following day. After the day of stimulation which is normally followed by the tapping day, we observed an increase in daily respiration of stimulated trees especially the daily minimum respiration. Higher in daily minimum respiration remained until the day after tapping.

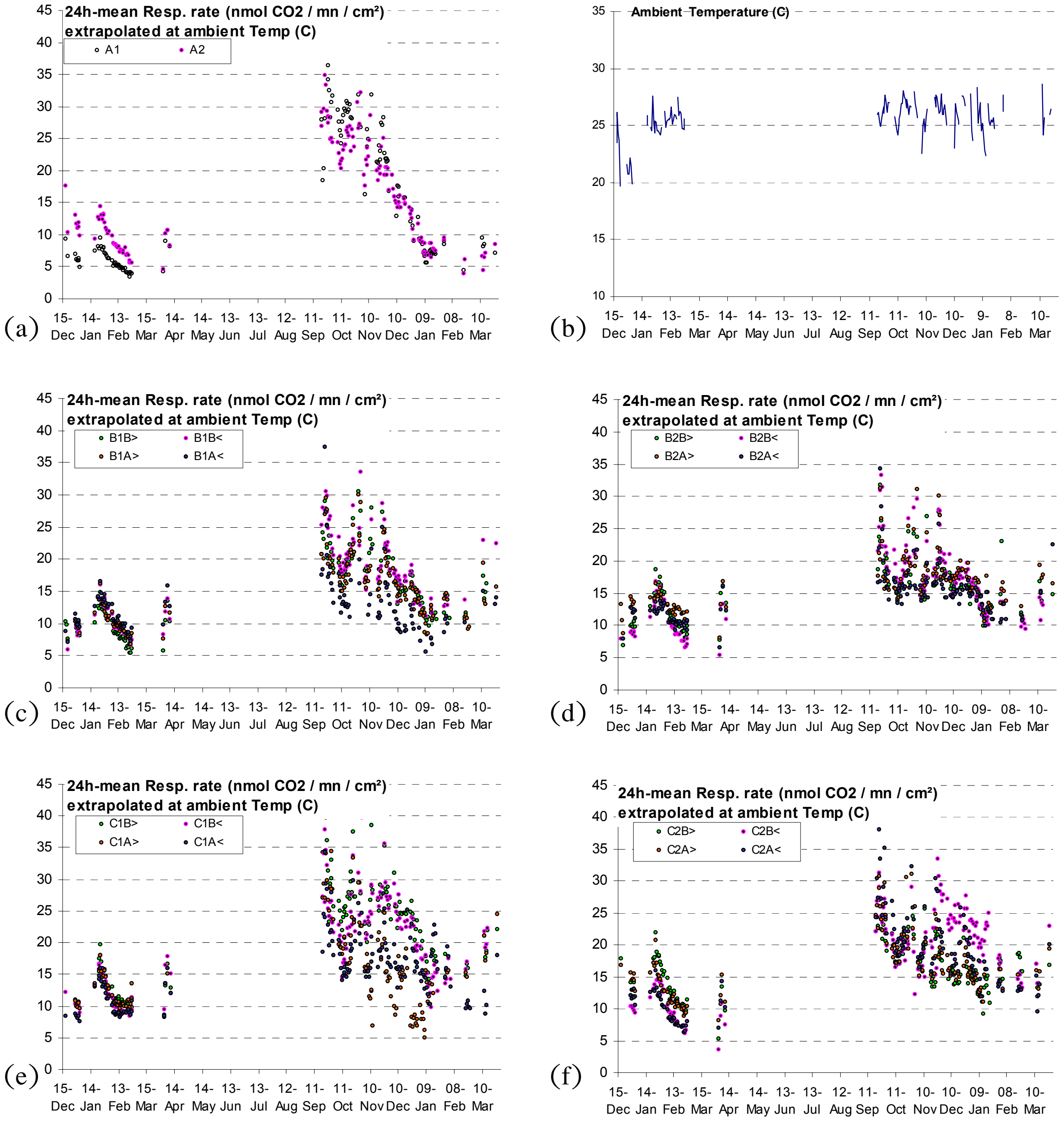


Figure 20 Annual variation (24h mean) for respiration rate (nmolCO$_2$ .mn^{-1}.cm^{-2}) extrapolated at ambient temperature(°C). Data were gathered during Dec. 01– Mar. 03. No data during Apr.– Aug. 02 due to technical problem.

(**a**) untapped (A1,A2) (**c–d**) *nil stim* (B1,B2) (**e–f**) Et 8/y (C1,C2)

Positions on tapped panel above the cut = A>, below the cut = A<

Positions on untapped panel above the cut = B>, below the cut = B<

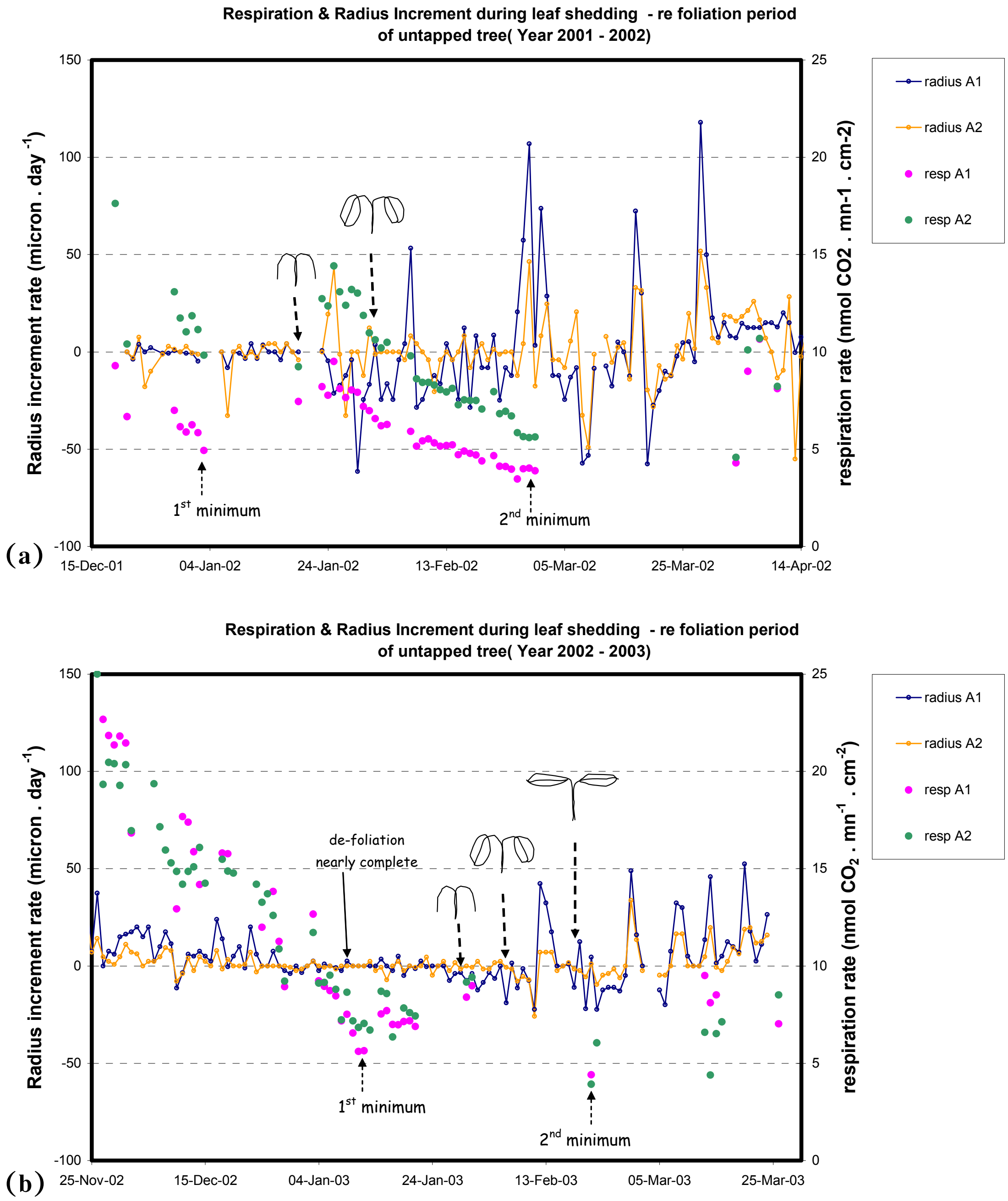
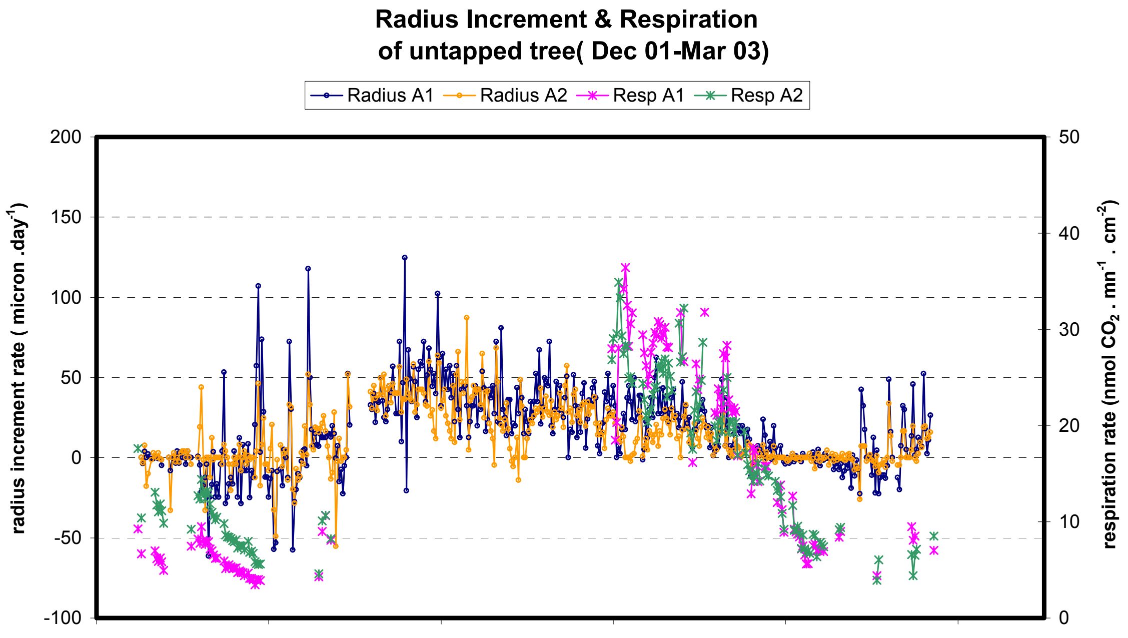
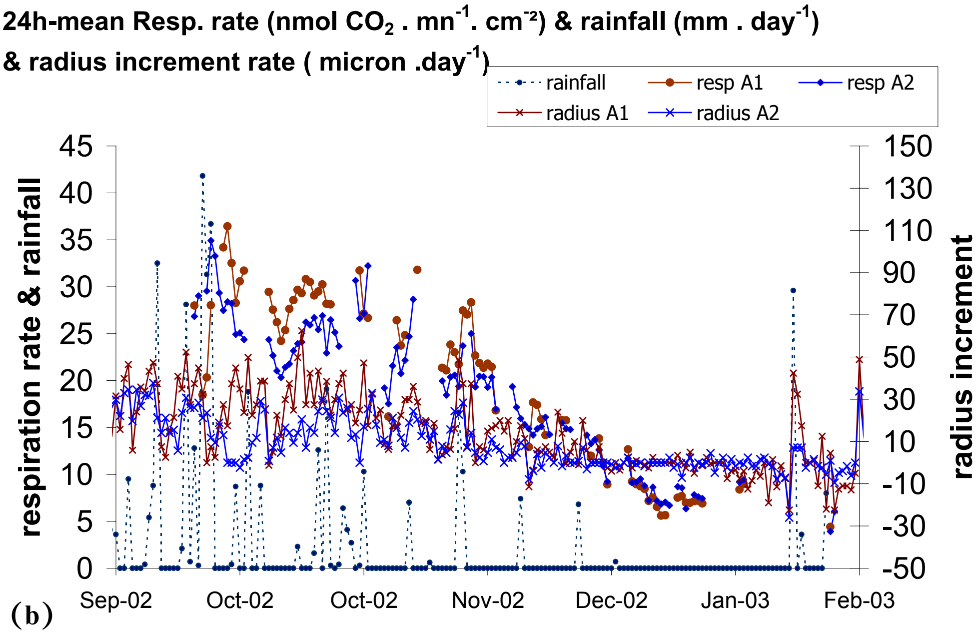


Figure 21 Evolution of respiration and radial growth rate during defoliation – re foliation period.

(a) year 2001-2002 (b) 2002-2003



(a)



(b)

Figure 22 Untapped treatment (A1, A2)

(a) Radial growth rate (μm.day^{-1}) from RS measurement and respiration (nmol.min^{-1}.cm^{-2}) from Dec. 01 – Mar. 03

(b) Respiration rate, radial growth and daily rainfall from Sep. 02 – Feb. 03

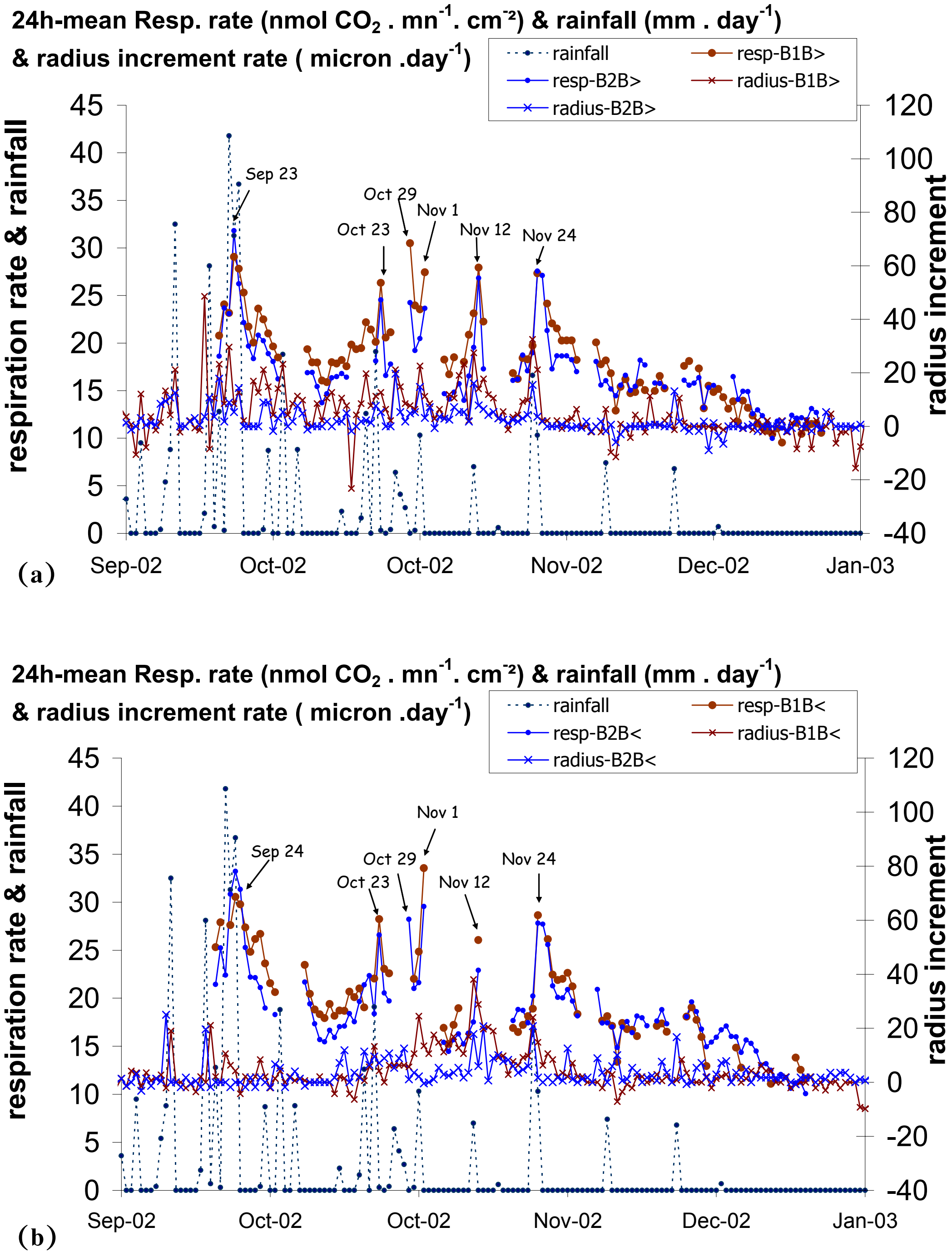


Figure 23 *nil stim* treatment; respiration, radial growth rate and rainfall data for the position in untapped panel

(a) above cut (B1B> , B2B>) **(b)** below cut (B1B< ,B2B<)

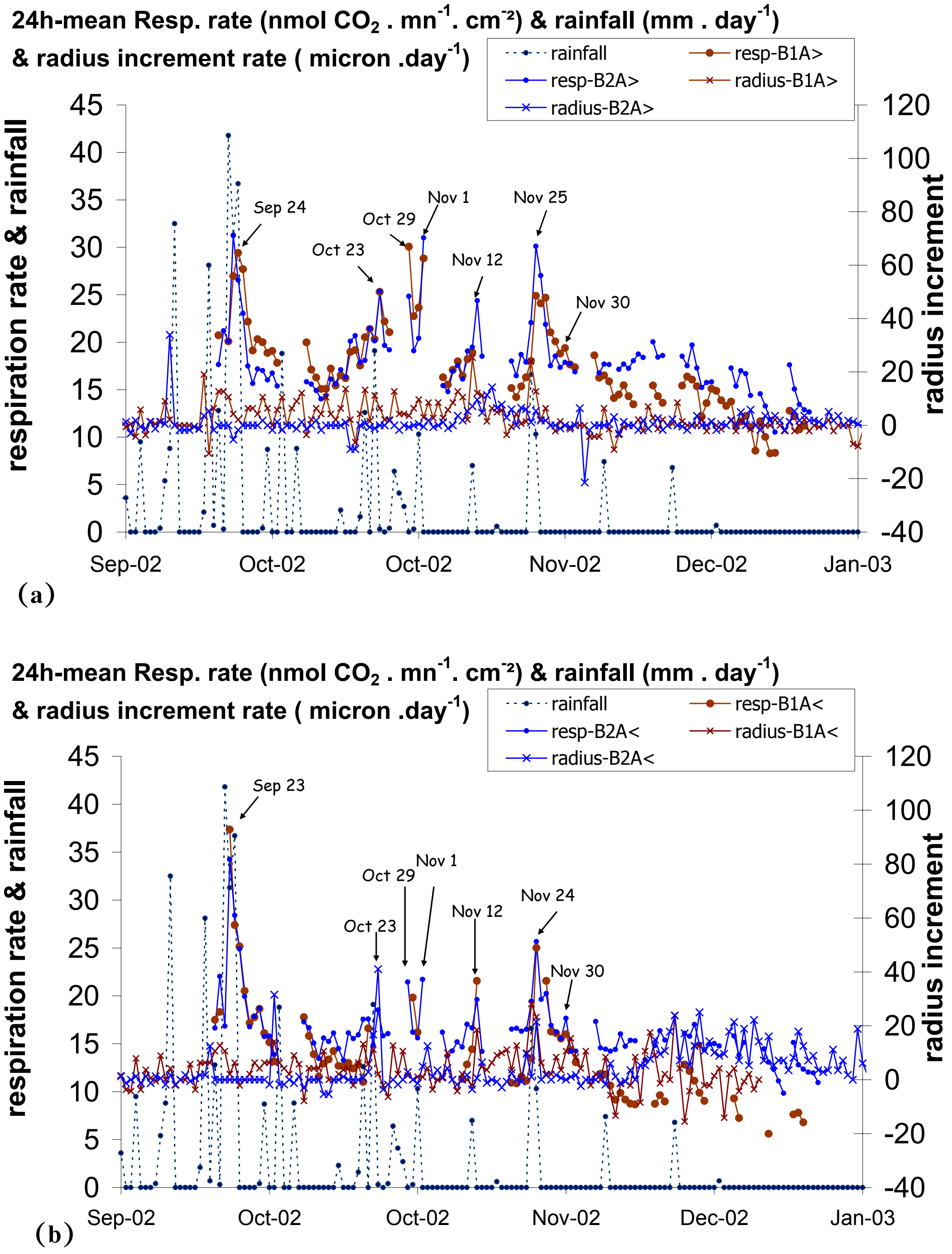


Figure 24 *nil stim* treatment; respiration, radial growth rate and rainfall data for the position in tapped panel

(a) above cut (B1A> , B2A>) **(b)** below cut (B1A< , B2A<)

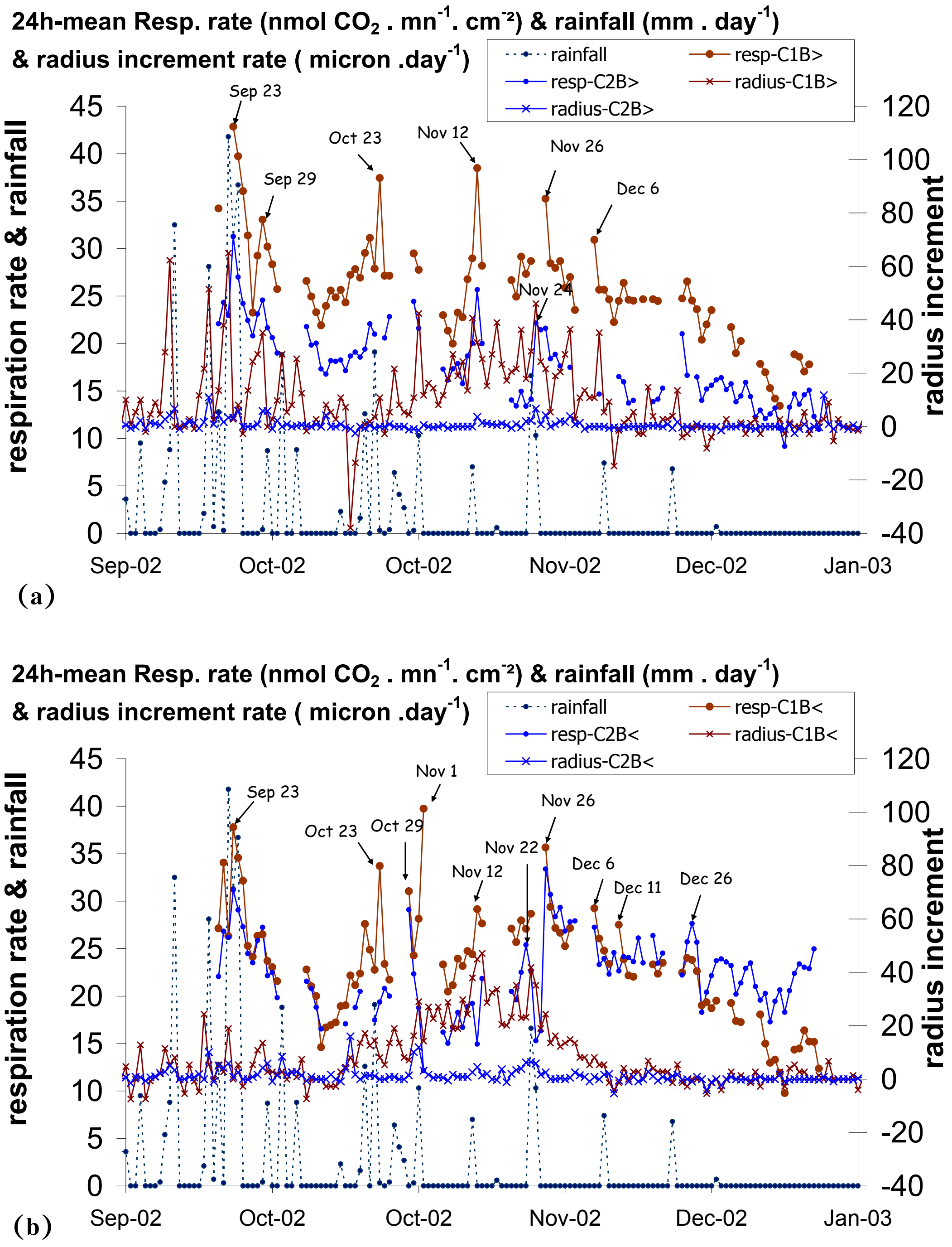


Figure 25 Et 8/y treatment; respiration, radial growth rate and rainfall data for the position in untapped panel

(a) above cut (C1B>, C2B>) **(b)** below cut (C1B< and C2B<)

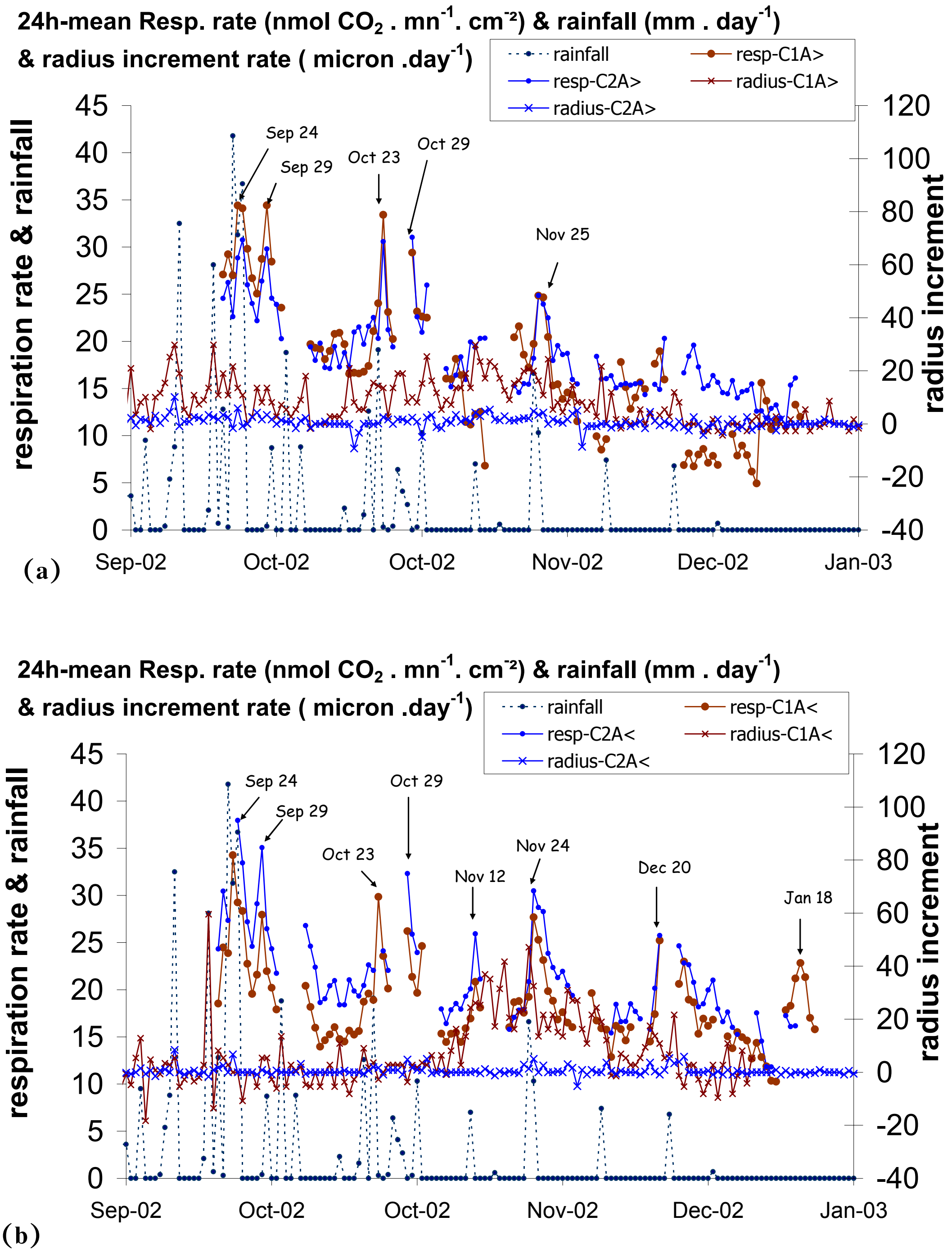
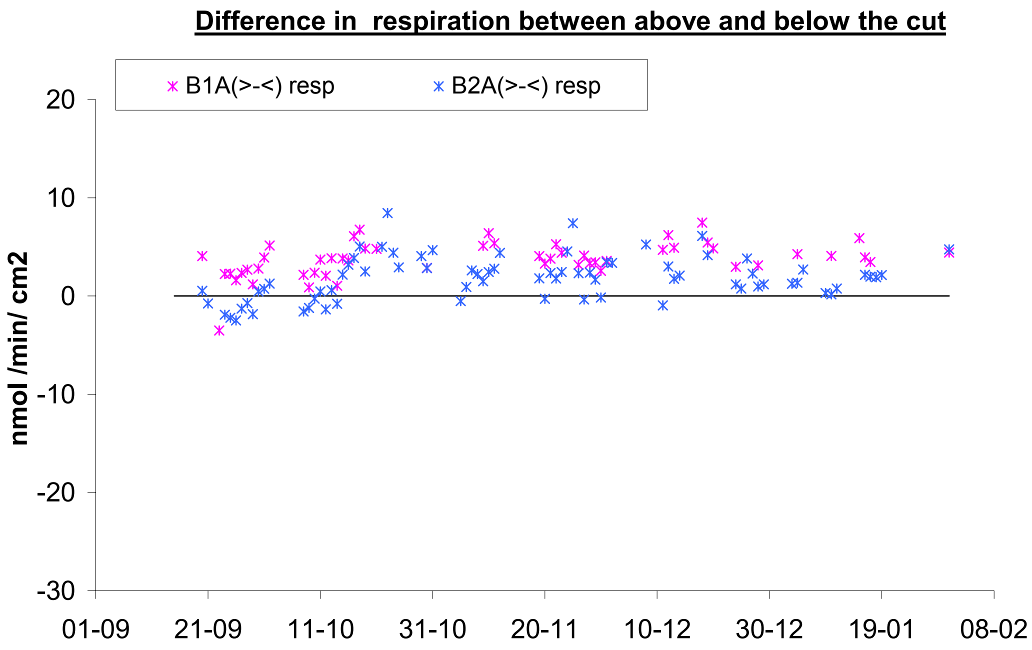
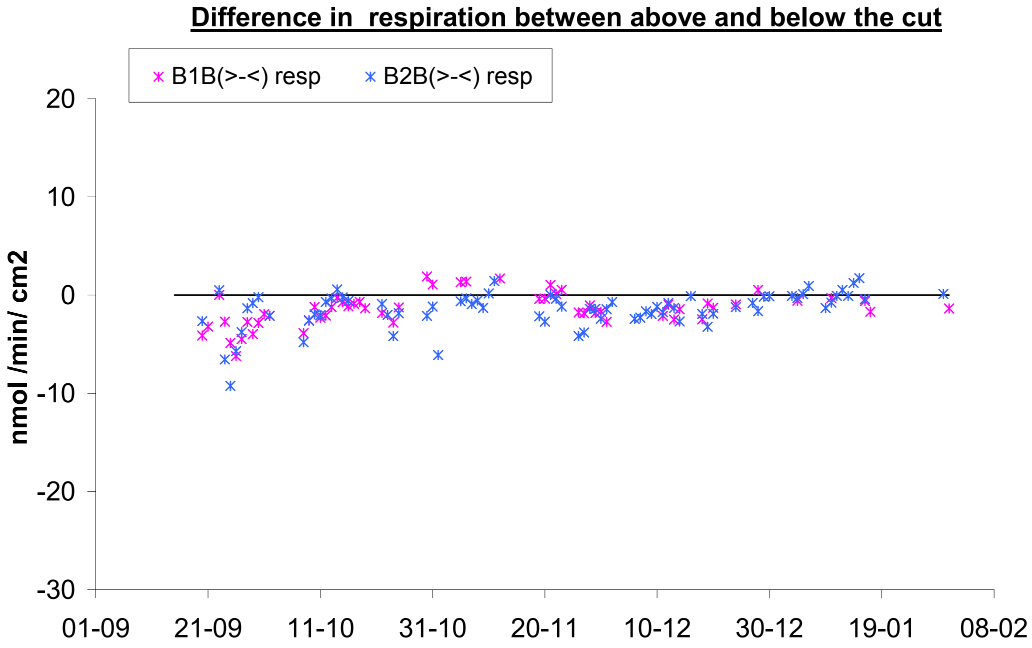


Figure 26 Et 8/y treatment; respiration, radial growth rate and rainfall data for the position in tapped panel

(a) above cut (C1A>, C2A>) **(b)** below cut (C1A<, C2A<)



(a)



(b)

Figure 27 Difference in respiration between above and below the cut of *nil stim* treatment B1, B2

(a) tapped panel **(b)** untapped panel

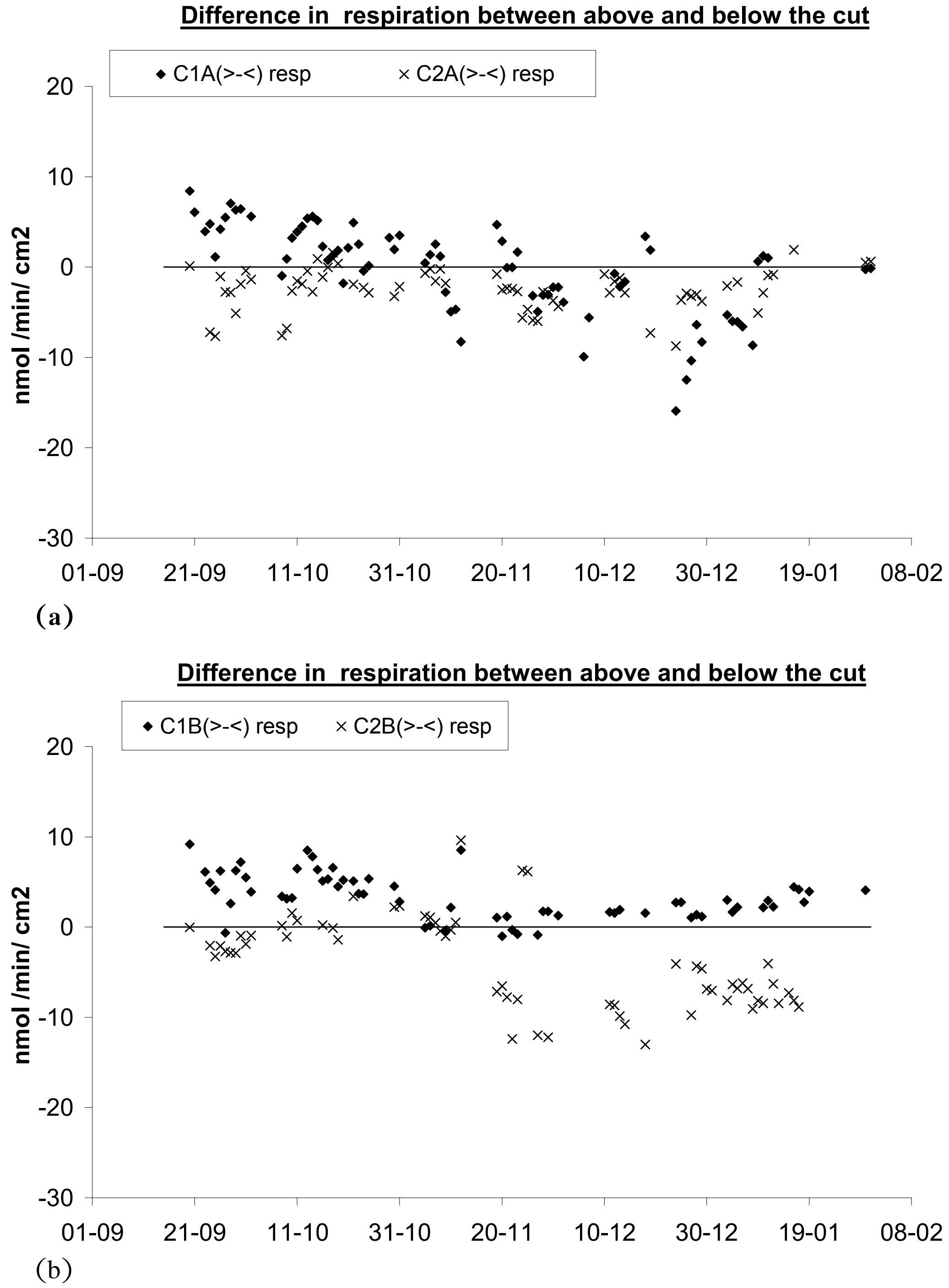
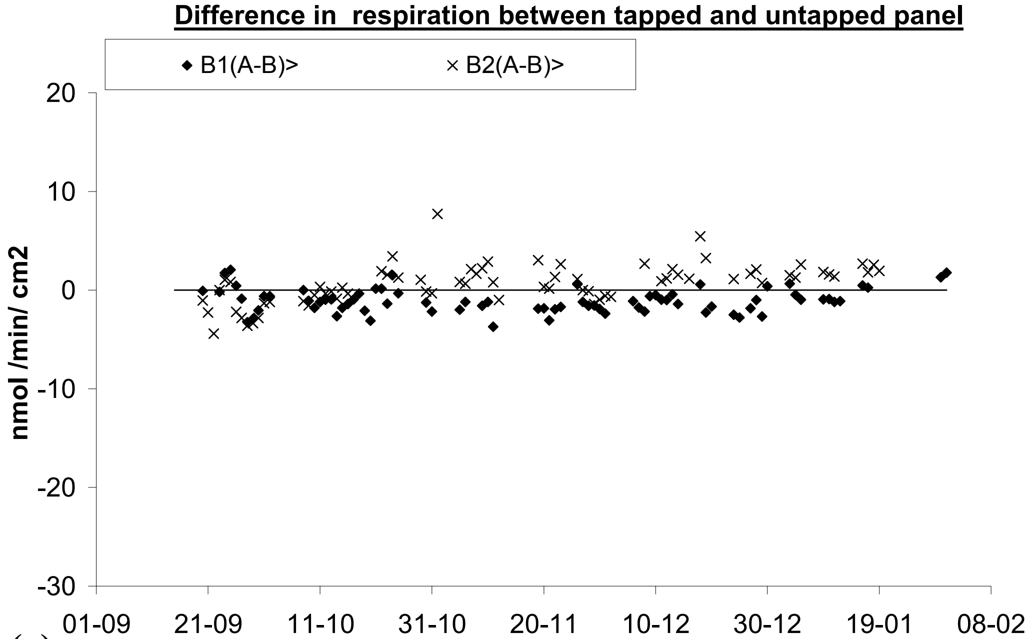
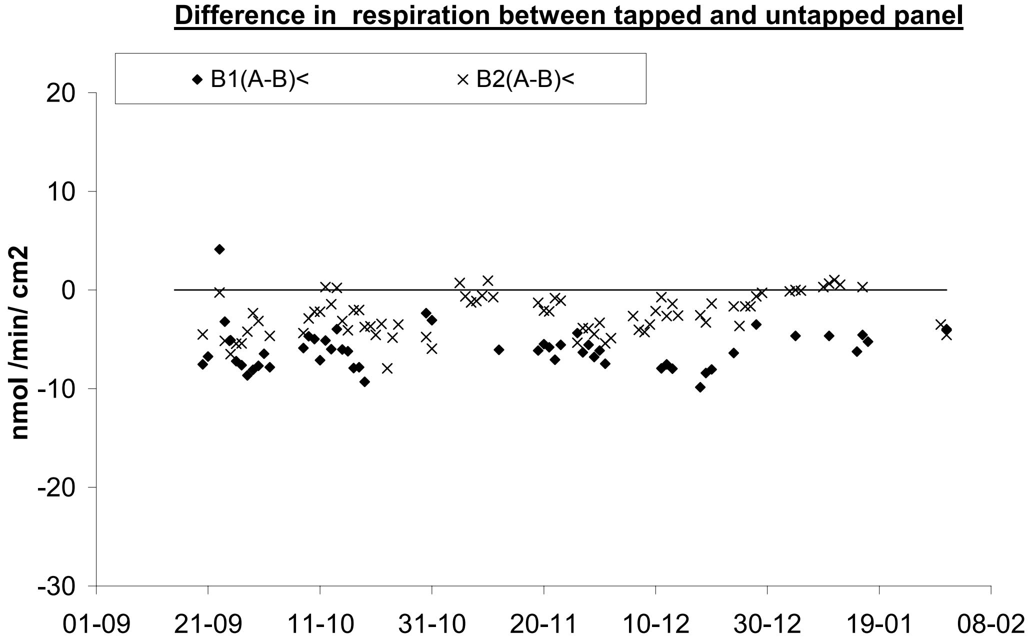


Figure 28 Difference in respiration between above and below the cut of Et 8/y treatment C1,C2

(a) tapped panel **(b)** untapped panel



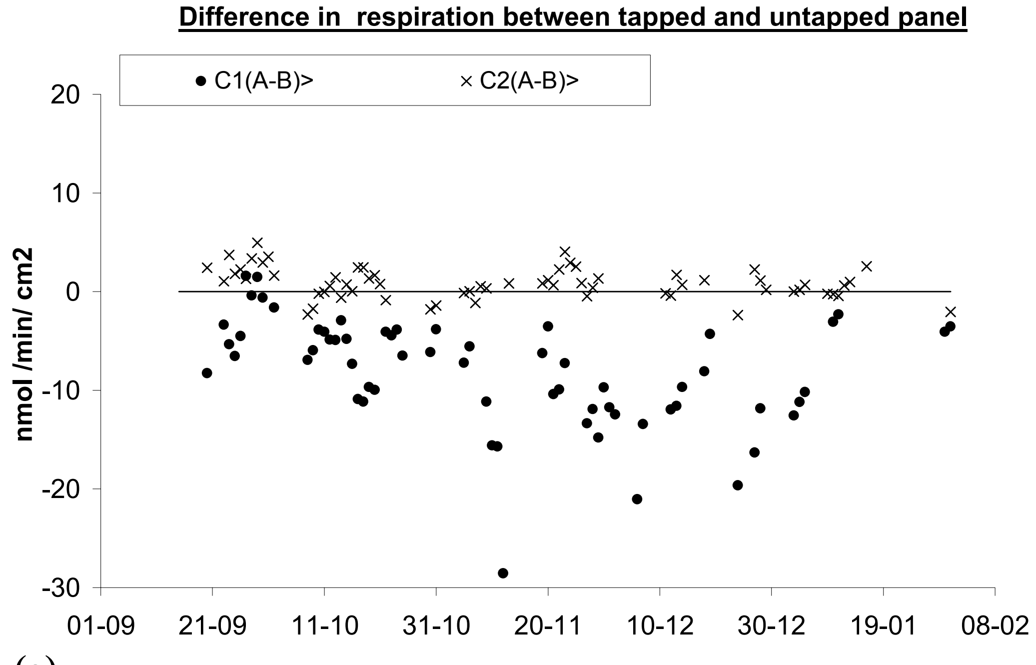
(a)



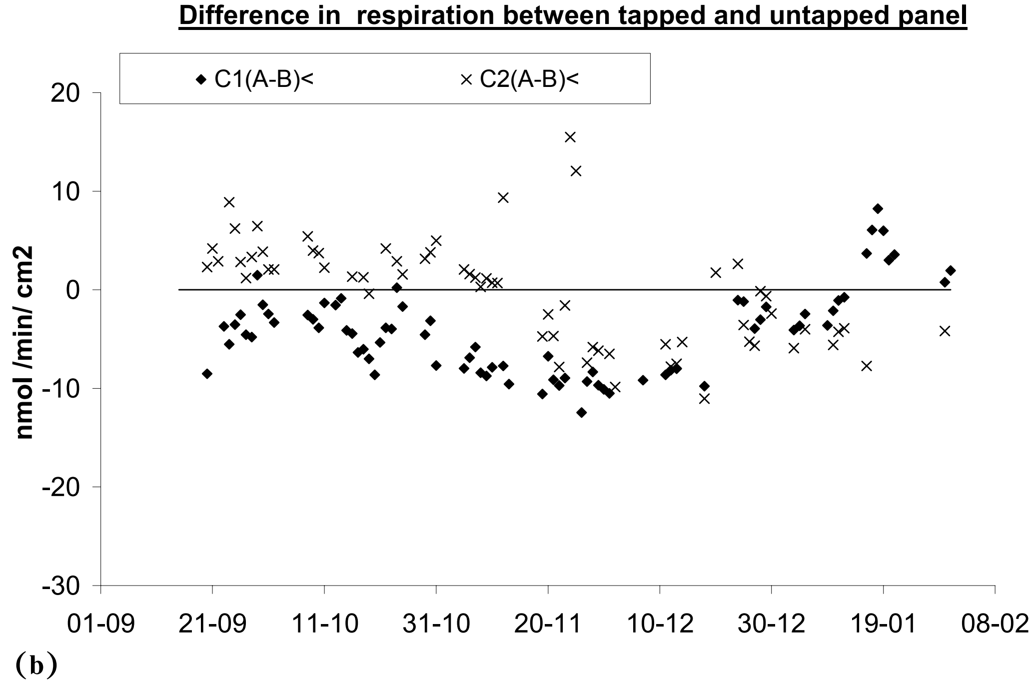
(b)

Figure 29 Difference in respiration between tapped and untapped panel of *nil sim* treatment B1, B2

(a) above the cut (b) below the cut



(a)



(b)

Figure 30 Difference in respiration between tapped and untapped panel of Et 8/y treatment C1, C2

(a) above the cut (b) below the cut

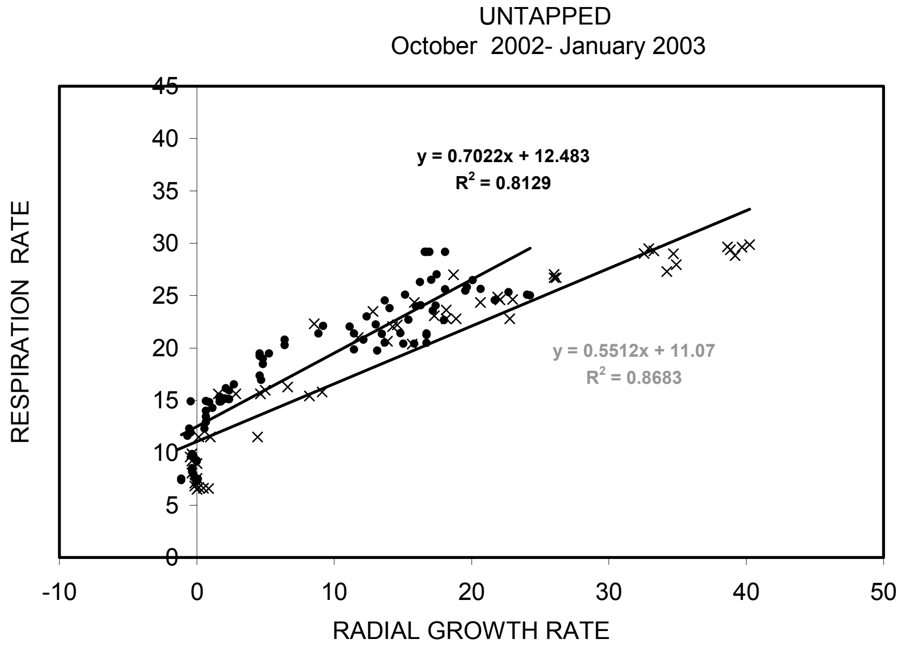


Figure 31 Relationship between respiration rate (nmol CO_2 . min^{-1} . cm^{-2}) and radial growth rate (µm . day^{-1}) of untapped tree , calculated from moving average over 7 successive days from 11th Oct. 02 -14th Jan. 03

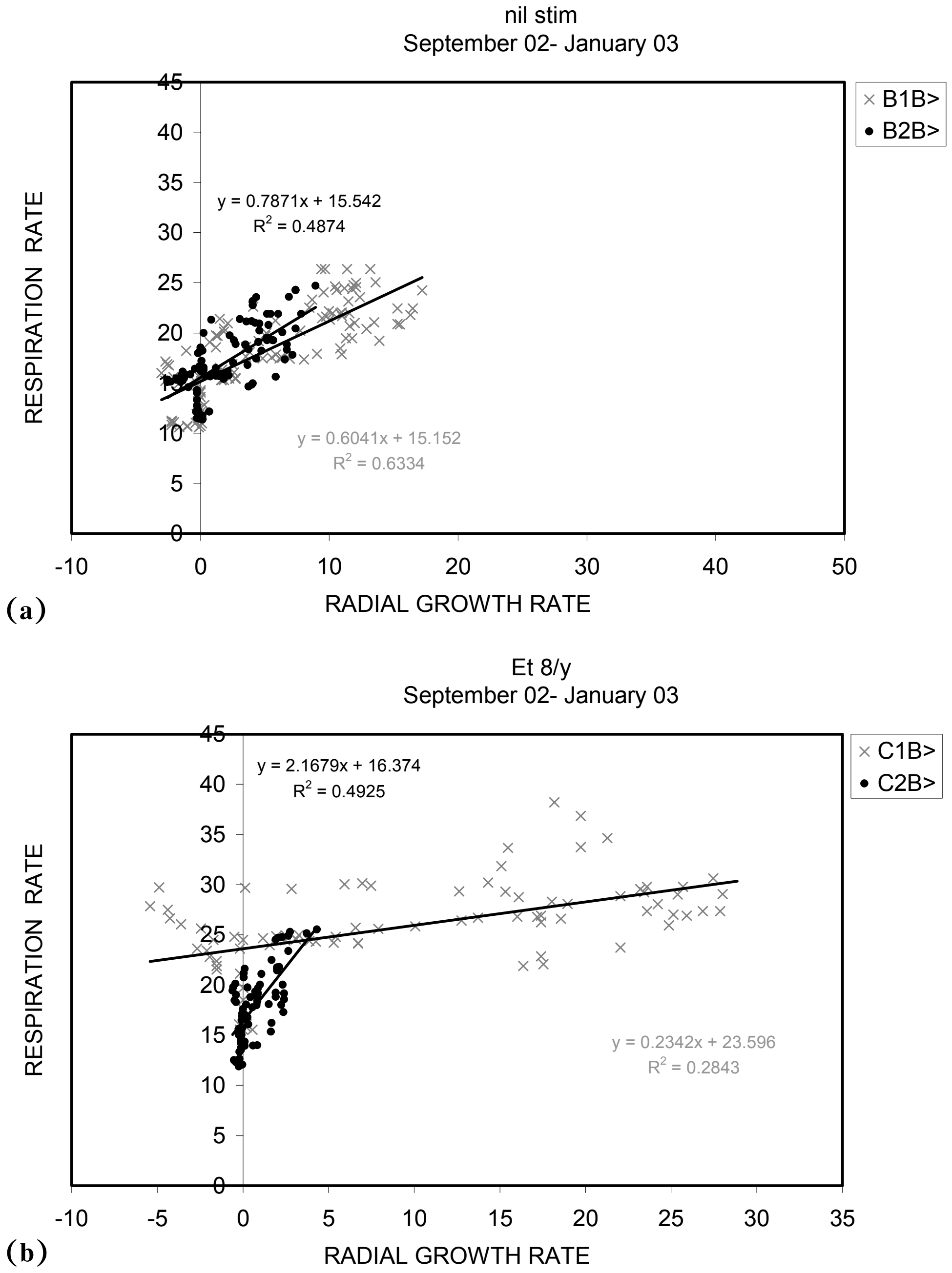


Figure 32 Relationship between respiration (nmol CO_2 . min^{-1} . cm^{-2})and radial growth rate (µm . day^{-1})of untapped panel – above the cut, calculated from moving average over 7 successive days(20 Sep. 02 -14 Jan. 03)

(a) *nil stim* **(b)** Et 8/y

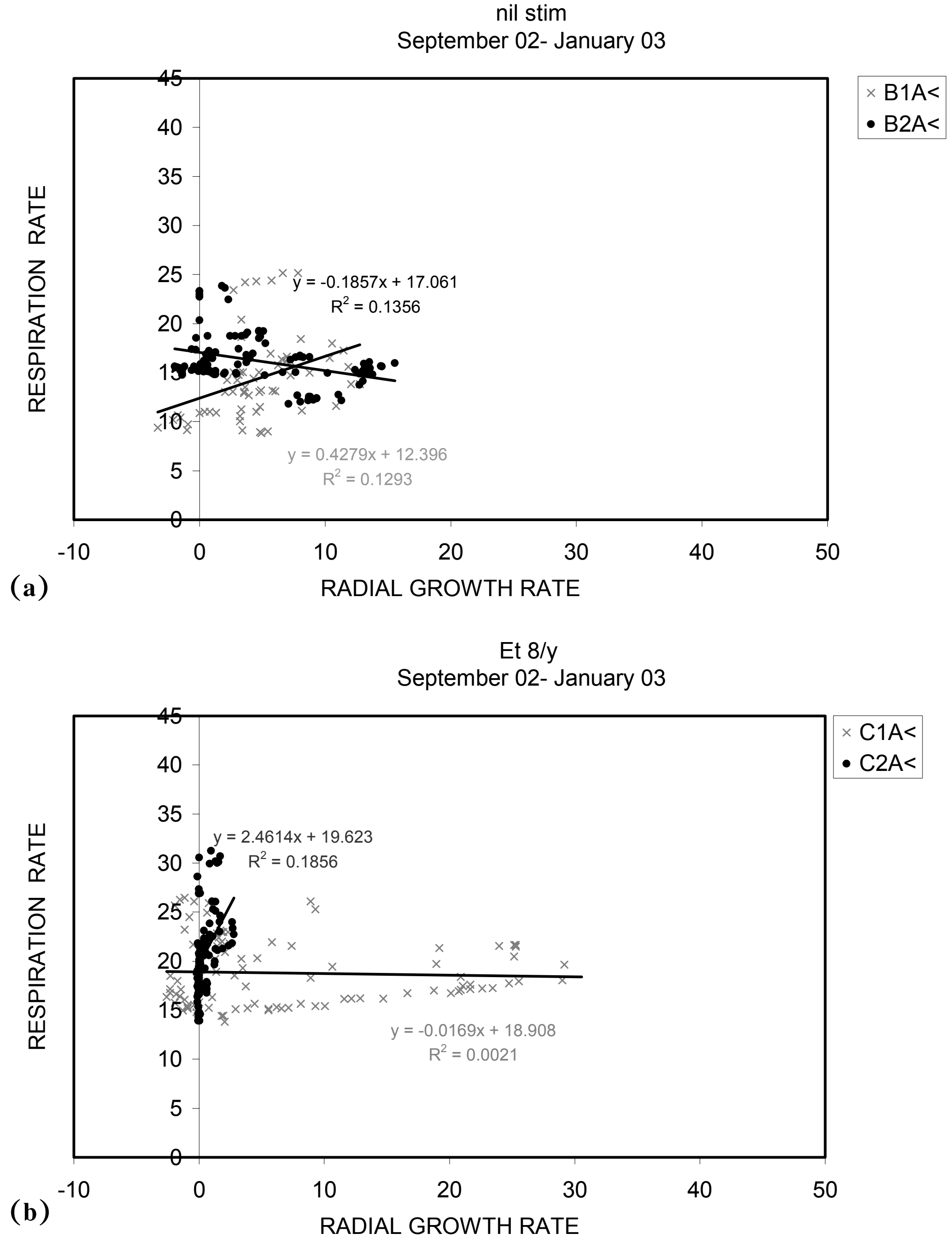


Figure 33 Relationship between respiration (nmol CO_2 . min^{-1} . cm^{-2}) and radial growth rate (µm . day^{-1}) of tapped panel : below the cut, calculated from moving average over 7 successive days (20 Sep. 02 -14 Jan. 03)

(a) *nil stim* **(b)** Et 8/y

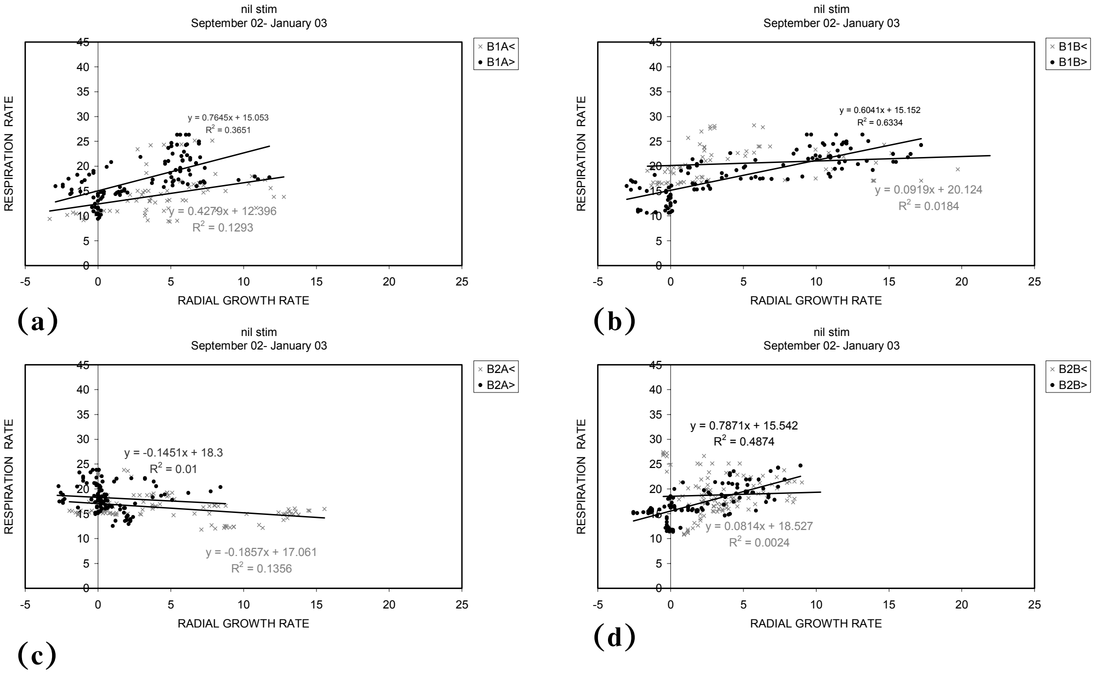


Figure 34 Relationship between respiration rate (nmol CO_2 . min^{-1} . cm^{-2}) and radial growth rate (µm . day^{-1}) of *nil stim*, calculated from moving average over 7 successive days from 20 Sep. 02 -14 Jan. 03, compared between above (>) and below (<) the cut.

(a) B1 – tapped panel **(b)** B1 – untapped panel

(c) B2– tapped panel **(d)** B2 – untapped panel

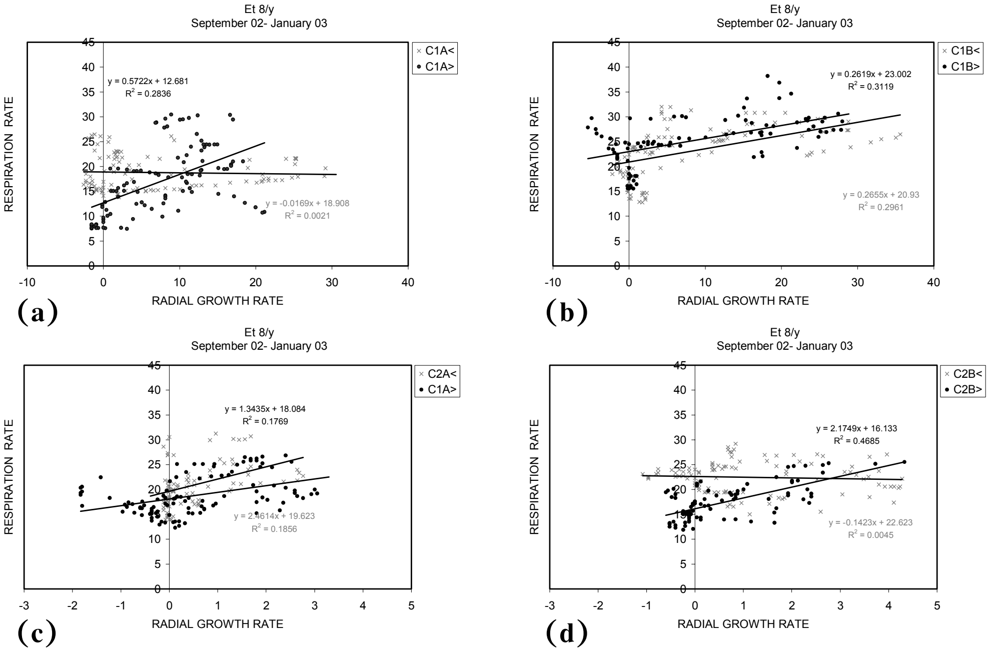
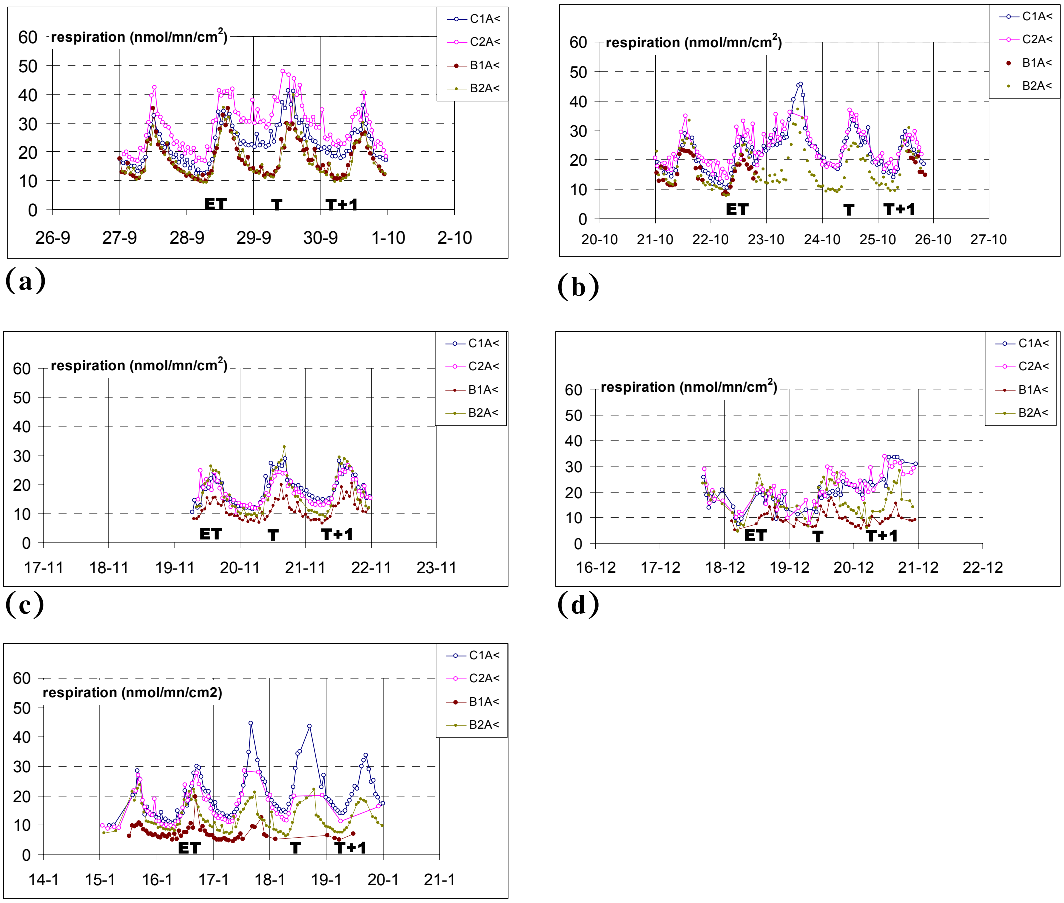


Figure 35 Relationship between respiration rate (nmol CO_2 . $min^{-1}.cm^{-2}$) and radial growth rate (µm . day^{-1}) of Et 8/y, calculated from moving average over 7 successive days from 20 September 2002 -14 January 2003, compared between above (>) and below(<) the cut.

(a) C1 : tapped panel **(b)** C1 : untapped panel

(c) C2 : tapped panel **(d)** C2 : untapped panel



(a) **(b)** **(c)** **(d)** **(e)**

Figure 36 Diurnal pattern of respiration (nmol.min^{-1}.cm^{-2}), extrapolated to ambient temperature, compared between *nil stim* and Et 8/y treatments at the position tapped panel – below the cut to track the effect of stimulation on respiration. **ET** = the day of ethephon stimulation, **T** = the taping day, **T+1** = the day after tapping.

(a) Sep. 02 **(b)** Oct. 02 **(c)** Nov. 02 **(d)** Dec.02 **(e)** Jan. 03

Dynamics of Carbohydrate Reserves based on Phenology and Latex Exploitation

1. The Main Feature of the Annual Cycle

Glucidic reserves content i.e., starch, sucrose, glucose, and fructose in wood tissue of the rubber tree (shoot and root parts) were determined periodically along the year, taking into account the tree phenology and latex production.

Reserves found in the wood of rubber tree were mainly in the form of starch, with a lower proportion of sucrose.Glucose and fructose accounted for a negligible proportion except at re-foliation, when TSS was the highest (table 5) and starch the lowest. Thus, seasonal changes in non-structural carbohydrates (NSC) were mainly due to changes in starch content. Seasonal fluctuation of glucidic reserves in wood tissue emphasises the role of starch and sucrose in starch- sugars interconversion. Mean total NSC content along the year ranged 23.4- 79.0, 25.2- 113.5, and 32.5-104.2 mg Glu equivalent. g structural DM^{-1} for control, *nil stim* and Et 8/y respectively.

Reserves deposition reached their maximum content at leaf fall January and the lowest NSC occurred just after complete refoliation (March) whatever the treatment (Fig. 37). Annual maximum consumption occurred during the formation of new leaf. A net deposition occurred mainly from April-May to defoliation (January). During this period, including the rainy season, radial growth was significant and trees in *nil stim* and *ET* treatments were tapped. From March to April-May (dry season and tapping rest), there were slight changes.

2. Effect of Tapping and Stimulation

Compared to untapped, tapped tree (*nil stim* and *ET*) exhibited a higher content of total carbohydrate for all seasons (Fig. 37), either in shoot or root, particularly in October (high latex production period). At the end of the tapping rest periods when trunk radial growth just started (May 02 and April 03), NSC was not very different among treatments all along the trunk, ranging 20-65 mg Glu equivalent. g structural DM^{-1}. However, it was higher in taproot for both tapped treatments and also slightly higher for ET above the tapping cut (50 and 90 cm).

In October, period of high rainfall and high latex production with significant growth, there was clearly higher NSC content for tapped treatments all along the taproot-trunk axis, although NSC increased for untapped tree too, except in lower locations (50 cm below cut and taproot). The largest difference between treatments (52.0, 94.4 and 107.6 mg Glu equivalent. g structural DM^{-1} for control, Et 8/y and *nil stim* respectively) was recorded at 50 cm below cut, i.e. within the latex regeneration area.

In January, at leaf fall, NSC peaked for *control* all along the trunk, whereas for *nil stim* and Et 8/y it was not very different from the contents recorded in October for both tapped treatments, and even sometimes lower (190 cm above cut and taproot for *ET*). Difference between treatments was the lowest along the year, but in most locations tapped treatments still had higher NSC than *control*.

After refoliation (March), NSC decreased sharply for all treatments and locations, reaching annual minimal contents. In most locations, there was almost no difference between treatments except at 50 cm below the cut where content remained quite high in tapped trees. Between March 03 and April 03 (dry season and tapping rest) there was a slight increase in NSC for all treatments.

However, in taproot there was no increase between May and October but NSC content was higher in tapped treatments *nil stim* than in control all year long. For Et 8/y, NSC content in taproot decreased between October and leaf-fall. In general, there were less seasonal variations in NSC in taproot than in trunk.

3. Vertical Distribution of Starch and TSS Content

3.1 Starch

In general, a striking figure for all treatments was a common bottom-up decreasing gradient of starch along the trunk. This gradient was more consistent in untapped tree and modified by tapping and ethephon stimulation. Figure 38 shows the vertical distribution pattern for starch and TSS at four periods i.e. tapping rest (May 02), high production (Oct. 02), defoliation (Jan. 03) and refoliation (Mar. 03). For control, the larger gradient was found in May which was twice the gradient occurring at the other times. This gradient was mainly due to low content in the upper part, but it was much less marked after starch pool had been refilled, in October and also at defoliation. After massive utilization of starch for annual refoliaton, a decreasing bottom-up gradient along the trunk was observed again. Therefore differences in starch content between May and refoliation was larger in the upper part of the trunk and this suggests that, although no preference for starch mobilization but at refill period the plant tendes to give priority to the lower part of the trunk.

For both tapped treatments, vertical distribution patterns of starch were much irregular with wide variations related to the location of the tapping cut. Nevertheless, there was an overall decreasing bottom-up gradient along the trunk (without taproot) after refoliation and in May except at the location 20 cm above the cut where starch content remained high. This was the area of regenerating bark (where bark was removed during the previous tapping year).

In *nil stim* trees, tapping activity significantly modified reserves dynamics in terms of quantity and pattern. When tapping activity was not resumed in May the distribution was the same trend as in control tree except in the area of the renewed bark where the peak content occurred (Fig.38). However, this was not observed in untapped panel (Fig. 40a.). Regardless this area, then compare with the untapped tree, we could observe nearly the same pattern and range at the comparable distance of untapped tree. The content in October was higher than untapped tree for almost positions. This is the period when radial growth was still active in untapped trees but almost stopped for tapped trees and generally it is the time when maximum yield is obtained. At leaf shedding, the starch content reached the highest content with fewer gradients along the trunk. Logically, the starch reserve of this period should reach its annual maximum before a large amount will be consumed for the refoliation. At refoliation, the total trend showed an obvious bottom-up gradient. High concentration at 50 cm below the cut was significantly different from the untapped tree. After tapping was stopped by the end of March, last set of trees were sampled in April 03, the overall gradient remained the same and high concentration at the position below the cut was observed. However, no peak content was found within the renewed area, as it was found the year before. In Et 8/y treatment (Fig. 38 c), the trend in May 02 was similar to *nil stim*, but the total content was higher. In October, except at the lower part of the trunk, starch content was closed to the content in control tree, thus much lower than in *nil stim*. At leaf shedding, it seemed that the starch content in low value area (refer to the content in October) was refilled everywhere except only the renewed bark area. It has to be noted that the sampling day was just 6 days after ethephon stimulation. This might be a reason for the low starch content at the renewed bark area since ethylene could be involved in the starch hydrolysis. The total starch content at refoliation was found low, except in the lower part of the trunk, similar to *nil stim*. Thus, conversely to untapped tree, there was a steep bottom-up decreasing gradient, but not regular, actually the gradient slope is the same as in untapped tree above (equivalent to the above cut position in tapped tree), whereas there is a sharp step at the cut level. In untapped panel(data not shown), strong decreasing gradient from bottom to top in all sampling periods was found except in Oct. 02 and the trend was more consistent as compared to tapped panel. Starch content was higher than in *nil stim* during resting period as if the effect of ethrel had induced a prolongated sink effect for starch, which was not used for latex regeneration during this period. In October, in the contrary to tapped panel, starch remained higher below the cut in stimulated than in *nil stim* trees. There was also no accumulation above the cut at leaf shedding.

The different etween tapped and untapped panel (half side of the trunk opposite to the tapping cut) is also remarkable (Fig. 40). In untapped panel, starch content was also affected by tapping since it was different from control, but it was also different from tapping panel. During rest period following the first tapping year (May 02) starch pattern was almost the same as in control, the area opposite to bark regeneration presented no starch peak, contrary to tapped panel. At the following peak production period (October) starch in untapped panel was clearly in between tapped panel and control. It was almost the same as in tapped panel at leaf fall, but thereafter the decrease following re-foliation was lower than in tapped panel so that content remained higher, except at the uppermost location.

3.2 TSS

TSS showed no clear gradient along the axis and few changes along the year for control, except higher content in root, particularly at leaf fall. Therefore, changes in NSC mainly relied on changes in starch all along the trunk. However, differences among treatment occurred also for TSS when focusing at specific periods. In control tree, the only period when the TSS content was significantly lower than other periods was in October (Fig. 38 d).

TSS varied more in both time and space for tapped treatments, the content increased from May to leaf fall but mainly below the tapping cut. When latex production was high (October), TSS was higher all along the trunk in Et 8/y, medium in *nil stim* and lower in *control*, whereas it was the contrary during tapping rest (Fig. 39 a - b). At refoliation, TSS content was much lower above the cut in Et 8/y than in *nil stim*, particularly in the highest position. Actually, an increasing bottom-up gradient was recorded along the trunk for *nil stim*, whereas such gradient was noticeable only from 90 cm below to 20 cm above the cut for Et (Fig. 38 e-f). Logically, this seemed to correspond to the decreasing gradient of starch content.

TSS was always the highest within the renewing area during tapping season for Et 8/y (Fig. 38 f), and this corresponded to low starch content. This strengthens the hypothesis that ethylene enhances starch hydrolysis but that resulting TSS could not be used within the renewing area where they accumulated.

Table 5 Content of starch, sucrose and glucose+fructose (mg Glu equivalent . g structural DM^{-1}) at time of stop tapping (May 02), maximum yield (Oct. 02), defoliation (Jan.03) and refoliation (Mar.03) at 3 different positions (± 50 cm from the cut of tapped panel, taproot).

Organ	Treat.	mg Glu equivalent . g structural DM^{-1}											
		May 02			Oct 02			Jan 03			Mar 03		
		starch	suc	glu+fru	starch	suc	glu+fru	starch	suc	glu+fru	starch	suc	glu+fru
		avg.	avg.	avg.	avg.	avg.	avg.	avg.	avg.	avg.	avg.	avg.	avg.
shoot - 50 cm above cut	cont.	25.1±1.3	11.3±1.7	2.4±0.8	52.3±10.6	8.6±1.8	0	52.0±8.9	14.6±3.3	1.1±0.7	29.2±19.2	14.4±3.9	2.7±0.9
	nil stim	26.0±5.5	10.8±0.8	1.4±0.6	76.9±6.8	10.5±3.2	0	75.1±15.2	16.2±2.6	0.04±0.4	33.7±5.0	18.3±3.1	2.6±0.6
	Et 8/y	55.2±6.1	8.9±1.2	0	66.0±18.6	16.7±4.8	0	74.3±4.2	12.7±1.5	1.1±0.9	28.6±12.1	13.7±3.5	3.5±0.4
shoot - 50 cm below cut	cont.	38.4±1.9	12.7±1.8	0.6±0.4	43.5±7.5	8.5±2.8	0	62.0±13.2	16.2±3.4	0	35.1±12.1	12.5±2.6	2.6±0.6
	nil stim	33.3±9.3	9.4±0.4	0.2±1.0	95.7±10.2	11.9±2.3	0	78.6±15.2	17.8±1.1	0.2±0.4	57.2±16.9	14.2±0.9	0.9±0.4
	Et 8/y	45.9±4.7	9.6±2.0	0.1±1.2	79.2±16.4	15.2±3.5	0	81.5±6.8	17.4±1.1	0.2±0.2	62.8±7.2	12.9±3.5	0.9±0.3
root- 10cm below ground	cont.	29.0±17.5	16.9±2.4	0.2±0.8	36.1±9.4	9.7±1.0	0	57.6±10.8	21.1±4.9	0.3±0.9	38.5±16.4	11.5±1.5	1.8±1.7
	nil stim	58.0±21.8	13.5±2.9	0	53.1±6.7	20.3±0.8	0	75.3±9.3	21.0±1.2	0.2±0.6	48.2±14.7	19.3±3.4	0
	Et 8/y	74.0±16.2	16.7±3.8	0	67.2±7.1	17.5±4.7	0	45.6±4.1	12.1±4.8	5.4±5.0	31.8±7.7	17.9±4.7	0.6±0.5

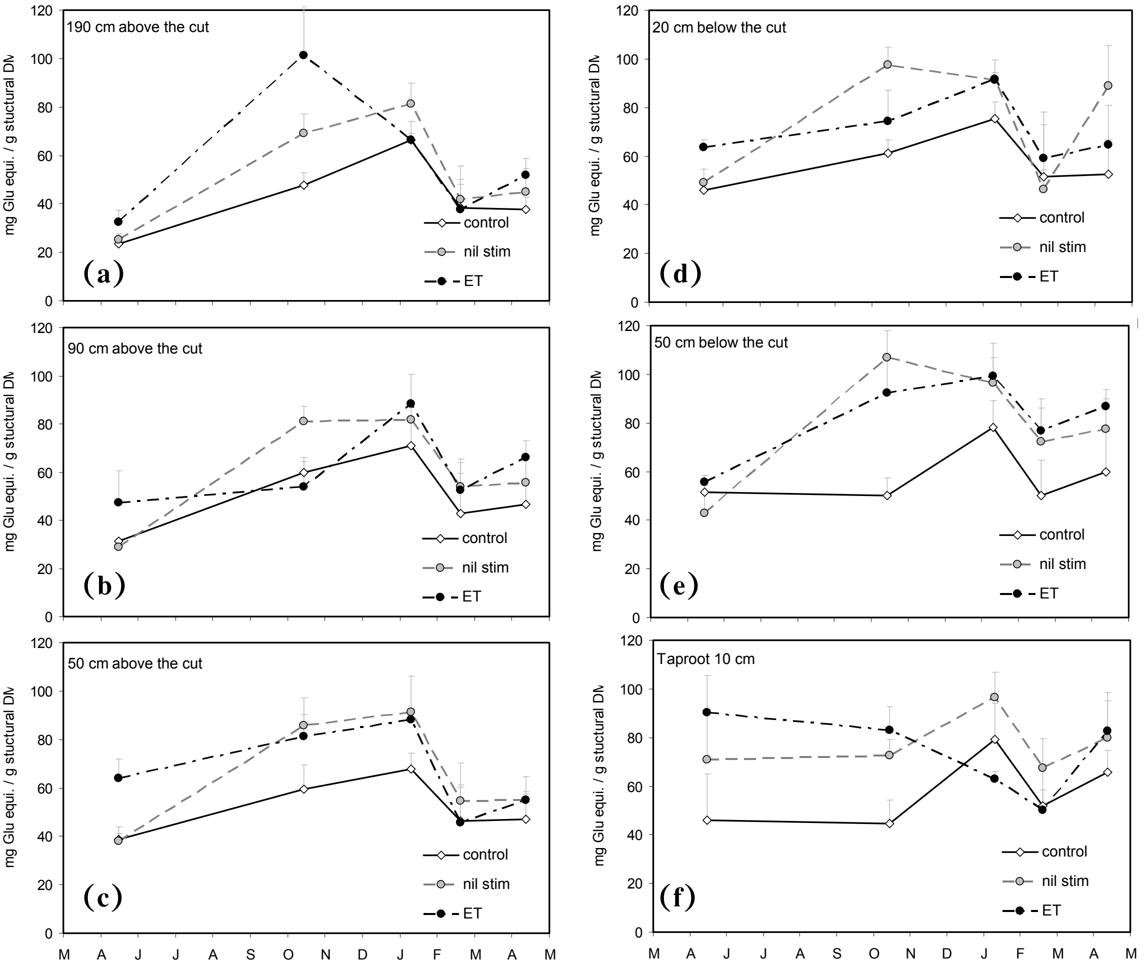


Figure 37 Evolution of total non-structural carbohydrate, NSC (mg Glu equivalent . g structural DM^{-1}) along the year at various positions from the cut; compared between untapped (control) tapped without stimulation (*nil stim*), and tapped with ethephon stimulation 8 times /y (ET).

(a) – (e) different positions from above ground part (f) taproot

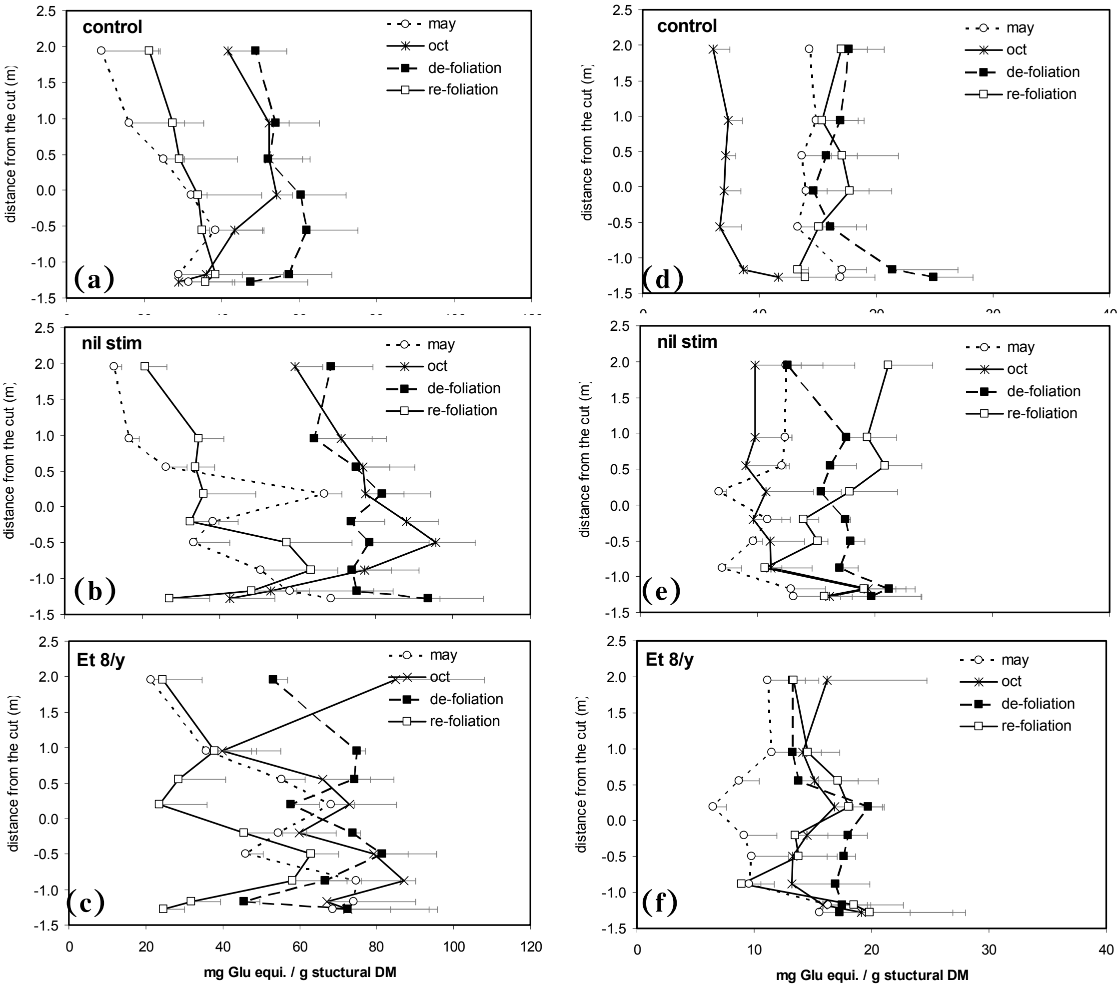


Figure 38 Vertical distribution of starch and total soluble sugars (TSS) content (mg Glu equivalent . g structural DM^{-1}) from -20 cm below ground up to 3 m along the trunk, compared between control and tapped panels of *nil stim* and Et 8/y treatments, four periods includes May (stop tapping),October (high production), end of January (completely defoliated) and early of March (completely refoliated).

(a) -(c) starch (d) - (f) TSS

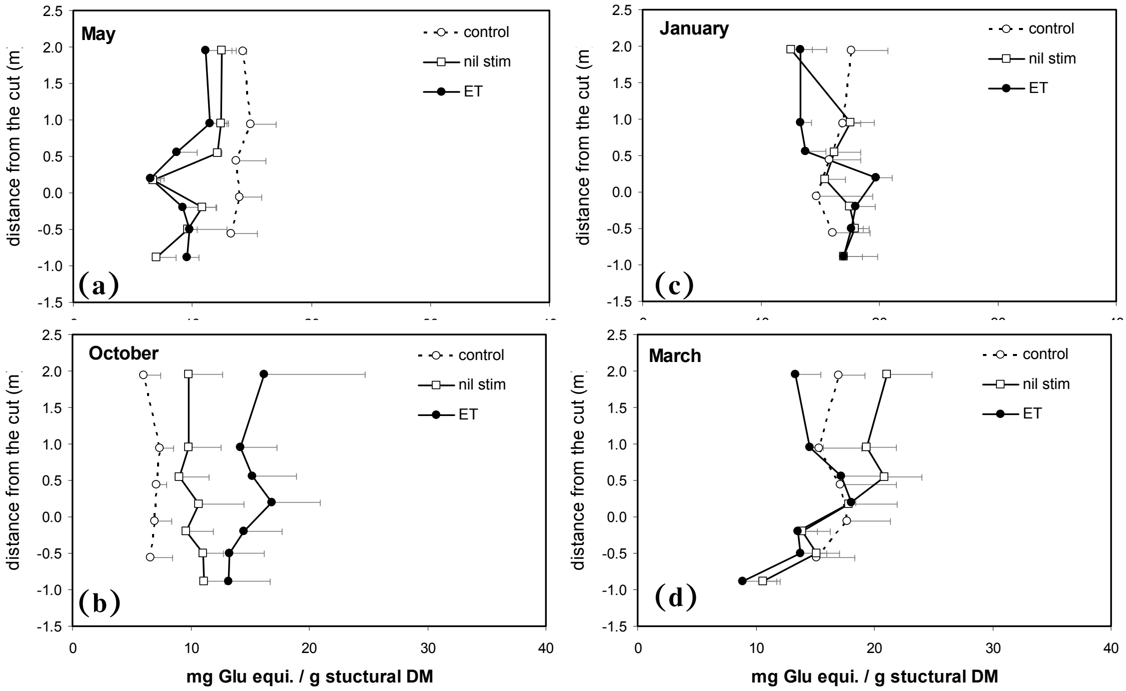


Figure 39 Vertical distribution of total soluble sugars content,TSS (mg Glu equivalent . g structural DM^{-1}) along the trunk up to 3 m above ground, compared between untapped treatment (control) and tapped panel of tapped treatments (*nil stim* and Et 8/y), four periods includes May (stop tapping), October (high production), end of January (completely defoliated) and early of March (completely refoliated)

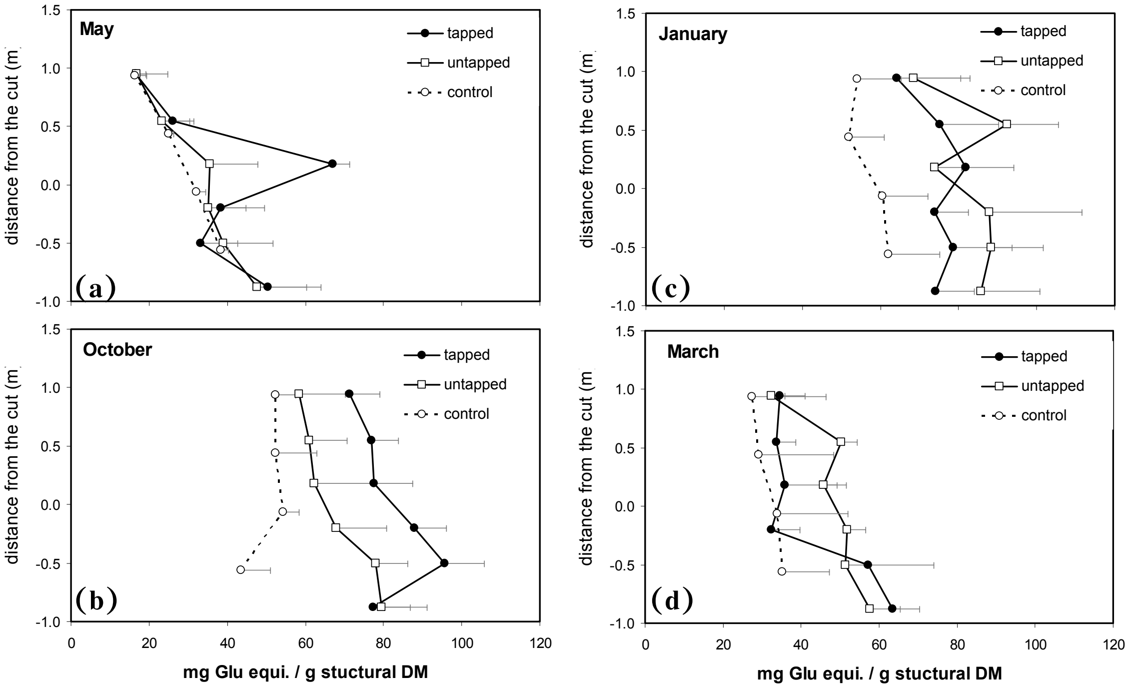


Figure 40 Vertical distribution of starch content (mg Glu equivalent . g structural DM^{-1}) compared between untapped treatment (control) and both panels of *nil stim* treatment (tapped and untapped panel), four periods includes May (stop tapping), October (high production), end of January (completely defoliated) and early of March (completely refoliated)

DISCUSSION

In Thailand, the rubber tree (*Hevea brasiliensis* Muell. Arg.) is one of the major economic crops, as it directly or indirectly supports around 10 % of the country population. Concerning on economic value, not only latex production, but also wood timber brings about a significant income to the farmers. It is well known that a negative relation exists between latex production and wood biomass creation (Templeton, 1968; Wycherley, 1976; Sethuraj, 1981 and 1985; Gohet, 1996; Gohet *et al*., 1996). Both rubber production and growth require assimilates from photosynthesis, mainly in the form of sucrose. From this view, it is worth understanding the influence of tapping system (with / without stimulation) on the change in carbon partitioning within the tree (growth, respiration, reserves deposition and mobilization) since farmers' benefit relies on a suitable management in order to keep a suitable balance between rubber production and plant growth.

Dynamics of Laticiferous System and Spatial Extension of Latex Regeneration Area

The aim of this study is to describe and quantify the sucrose balance between supply and utilization in the latex producing bark of the rubber tree, as well as the concurrent latex metabolic activity. Such a study cannot be restricted to the only tapped panel, as some other bark areas may be likewise 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. Ethephon stimulation is used as a physiological tool in order to study the influence of an increased rubber production, and therefore an enhanced latex regeneration, on the metabolic characteristics of the latex sink.

The latex sink strength induced by tapping is confirmed in this experiment to modify the whole physiological behavior of the tree. The difference between panels seemed to be less important as compared to the treatments. In fact, the effect of tapping is better reviewed in the untapped panel than in tapped panel because of the disturbance related to bark injury.

In comparison with untapped tree, tapping, with or without ethephon stimulation, induces a huge depletion (−60% down to −90%) of the latex sucrose concentration at whole trunk scale, even in areas which are not concerned by latex regeneration *stricto sensu*. Although proved a rather local process, whose spatial expansion is shown to correlate to the quantitative rubber production, latex regeneration induced by tapping has a huge effect at whole tree scale, at least on latex sugar reserves. This is in accordance with the important negative effect of rubber production on growth and biomass creation of the tapped rubber tree. As a matter of fact, many authors mentioned that the biomass loss of tapped trees in comparison with untapped controls could not be accounted by the rubber production only (Templeton 1969; Wycherley 1976; Sethuraj 1981 and 1985; Gohet 1996; Gohet *et al.* 1996).

As it occurs in latex, a general decrease of sugar reserves at whole tree level in other storage sinks may therefore be suspected. Such a depleting effect of rubber production on whole tree sugar reserves could be a possible explanation for the apparent discrepancy between rubber production and its huge effect on tree growth.

Results of the current study regarding the latex metabolic activity are based on the comparative description of latex sucrose (Suc) content and concurrent latex inorganic phosphorus (Pi) content in several areas of the trunk bark of *Hevea brasiliensis*, with a large pattern of exploitation intensities and on three different clones. They confirm previous studies about the latex regeneration area (d'Auzac and Pujarniscle, 1961; Tupý, 1969, 1973a, 1973b, 1985). Tapping creates a significant depletion of latex sucrose content in tapped panel, as a consequence of its consumption for rubber regeneration. This depression of latex sucrose content is increased by the use of ethephon stimulation, as the rubber production and therefore latex regeneration likewise increases. Accurate estimation of the latex metabolic activity by the level of latex inorganic phosphorus (Pi) is confirmed.

The concurrent analysis of latex sucrose (Suc) and latex inorganic phosphorus (Pi) levels allows a rather precise and easy description of the shape and size of the metabolically active bark area (area with high Pi): Depending on exploitation intensity, this high metabolic activity area extends to the whole tapped panel, including bark areas above the tapping cut, but also to the basal level of the untapped panel. According to the associated sucrose level, this high metabolic activity area can be divided in two distinct secondary areas:

An area with concurrent low Suc and high Pi, close to the tapping cut, which can be considered as the actual latex regeneration area. In the study-case and whatever the clone, this area increases by 100 cm^2 when production at the moment of the sampling increases by 1 $g.t^{-1}.t^{-1}$. Spatial extension of this area is clearly related to clonal latex metabolic typology, as its expansion requires lower intensification of exploitation when clonal latex metabolism is more active (Gohet *et al.*, 2003)

An area with concurrent high Suc and high Pi, more distant to the tapping cut, that represent highly active sucrose importation areas, whose duty is still unknown (accumulation of sugars for next latex regeneration?) but that do not seem to participate to the latex regeneration process *stricto sensu*.

The location of the latex regeneration bark area mostly on tapped panel, below and above the tapping cut, confirms former results by several authors but obtained using very different methods like using radio-labeled isotopes (Lustinec and Resing, 1965; Lustinec *et al.*, 1969) or turgor pressure measurements (Buttery and Boatman, 1966; Pakianathan *et al.*, 1976).

However, the calculation for the number of the efficient latex rings seems to underestimate the utile latex tissue thickness (T). This is because, each latex ring is not a continuously entire ring, but comprise of anastomosed latex vessels to form a network of draining system. This would allowed the actual number of latex rings more than the estimated value (12) which would be more close to that reported by Gomez (1982)

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

A frequent bottom-up increasing gradient of latex sucrose concentration along the trunk is observed on untapped trees or on untapped panel of tapped trees. This confirms the results obtained by Tupý (1973a), who showed the existence of such a gradient on PR107 untapped trees and suggested therefore that latex sucrose loading might be in equilibrium with the phloem sap concentration gradient (vertical long distance translocation of sucrose in phloem sieve tubes). If proved, such equilibrium would have been a finding in favor of a symplastic latex sucrose loading, not depending on latex metabolism, plasmalemma ATPase activity and H^+/sucrose plasmalemma symporters (Patrick, 1990). Nevertheless, the absence of functional plasmodesmata between laticifers and neighboring parenchyma cells makes this hypothesis of symplastic latex sucrose loading rather improbable (de Fay *et al.* 1989). For this reason, high sugar content of latex extracted from untapped trees, as well as bottom-up increasing concentration gradients in rather non active bark areas (untapped tree, untapped panel of tapped trees), remain quite unexplained regarding sugar loading mechanisms.

As a matter of fact, significant clues are in favor of an apoplastic latex sucrose loading: Presence of sucrose accumulation areas on lower parts of tapped panel of stimulated clones GT1 and RRIM 600 reflects a probable massive activation of the proton-sucrose transmembrane symport to the laticiferous system (Patrick, 1990; Lacrotte, 1991; Patrick and Offler, 1996). It is however impossible to conclude if this increased sink effect in metabolically active bark areas is due:

- to the direct effect of ethylene released following ethephon stimulation.
- to the increased rubber production and subsequent increased metabolic activation resulting from this stimulation, or
- to these two factors interacting together.

Likewise, former studies have shown that such enhancement of sucrose importation could also be found in untapped panel (Silpi *et al.* 2001) and were significantly related to latex metabolic activation in these bark areas. Relative importance of this sink effect in bark regions located outside the latex regeneration area *stricto sensu* might be another clue explaining discrepancy between rubber production and associated negative effect on the growth and biomass creation of the tapped rubber tree: All carbohydrates stored inside latex cells, even in areas outside the latex regeneration area, are no longer available for any other metabolisms and in particular for primary biomass creation (i.e. growth). Moreover, such increased sink strength might also be responsible for some still unexplained findings: In particular, overexploitation may lead to a decrease in production but not to any concurrent growth recovery (Gohet 1996; Gohet *et al.* 1996), as latex sink effect might still be important in such case.

This study presents the first concurrent delimitation and quantification of the latex metabolic activity inside the bark of the untapped and tapped rubber tree at trunk scale, using simple biochemical parameters. Latex diagnosis (Eschbach *et al.* 1984, Jacob *et al.* 1985, 1988a, 1988b, 1995) is thus proved to be a very useful and powerful tool in order to study the physiology of the latex producing bark of *Hevea brasiliensis*. "Latex diagnosis mapping" or "LD mapping" technique allows latex sampling in any part of the trunk bark, as tapping is not required (puncture latex sampling), and is moreover much simpler and more economic compared to the methods previously used to study the delimitation of the regeneration area, like formerly using radio-labeled isotopes (Lustinec and Resing 1965; Lustinec *et al.*, 1969) or turgor pressure measurements (Buttery and Boatman 1966; Pakianathan *et al.*, 1976). Moreover, former methods only describe size and shape of the latex regeneration area but cannot help in quantifying the metabolic activities of the different bark areas involved or affected by the latex regeneration process.

In addition, histological studies and quantitative analysis of carbohydrates of bark and wood should be performed in order to assess the existing relations between the latex sugar content and the carbohydrates content of the surrounding bark tissues and wood (soluble sugars, starch...). This data, supplemented by a planed survey of bark respiration and biomass increment, should help in a better understanding of the physiological mechanisms involved in regulation of assimilates partition (wood creation, rubber production, respiration and carbohydrates storage) of tapped *Hevea brasiliensis* and its response to exploitation intensification.

Such research methodologies could also be used in order to optimize the tapping systems recommendations (high tapping frequencies, reduced tapping frequencies compensated by ethephon stimulation...) and also provide new ideas for finding and/or optimizing new tapping systems like ethylene gas stimulated systems, micro-tapping cut systems and multi-tapping cut systems, as based on a better physiological knowledge of rubber tree functioning.

The Assessment of Trunk Radial Growth and Daily Variation

Trunk radial growth and daily radius variation of *Hevea brasiliensis* clone RRIM600 (plot TE01) was conducted in parallel with gas exchange experiment to allow fine continuous measurement of trunk radius or diameter variation. For the first time, this continuous approach was done in the rubber tree under tropical condition. Radial variations integrate not only irreversible growth but also reversible within the study area that results from daily transpiration and also likely latex exploitation by tapping.

1. Dynamics of Radius Variation in the Untapped Rubber Tree

Our experimental site is the marginal area for rubber cultivation, with the annual rainfall below 1300 mm. year^{-1}. Nevertheless the distribution of rainfall about 8 months per year makes it sufficient to grow rubber although water is still a major limiting factor for growth. The driest period arrives with the period of leaf regeneration and this response into, not only growth stop but also trunk shrinkage. Such result reinforced the hypothesis that radial growth decrease during periods of competition of water among sink organs. Trunk shrinkage is commonly observed in dry season at leaf fall- re-foliation period when soil moisture content was low (Devakumar *et al.*, 1999; Chandrashekar *et al.*, 1996). It is found even in the area when annual rainfall is more than 2400 mm but the distribution over the year was very short. At this period, change in canopy component due to leaf fall alters the micro-climate by providing more light to penetrate to ground surface which then change the soil temperature and vapour pressure deficit, and evapotranspirative loss of soil moisture (Takenaka 1994; Barthelemy *et al.*, 1991). When newly form leaves arrived at this extreme environment they enhanced the rate of water loss through transpiration. During period of drought stress, the trunk of rubber tree remained in a shrunken condition and did not show any radius growth until soil water condition was replenished by sufficient rainfall in April. However, a rapid and significant trunk expansion could be noticed in the day just after an occasional rainfall. This expansion should not be considered as growth since it was part of the complete restoration of turgor pressure which lost during the severe stress conditions (Chandrashekar *et al.* 1996; Kozlowski, 1971).

Several workers have found that radial growth of *Hevea* started to decrease whereas leaf is still functioning and no apparent limiting factors (Chandrashekar *et al*, 1996; de Fay, 1999). According to de Fay (1999), water stress could not be considered as the sole parameter for radial growth rest since the later started before the dry season. In our experiment, we observed that growth decrease in October and November in favour of reserves storage (data not shown).

2. The Effect of Tapping on Radial Growth

The use of RS allowed recording radius variations in corresponds with different positions along the trunk. In global view, growth rates of all observed positions, either tapped or untapped panel, dropped sharply after 2 weeks (4 tappings). Such response showed that the effect is not limited only to the so-called ‘drained area’ i.e. the area below or above the cut of tapped panel (PA-below) but also to the whole trunk scale in relation with the distance from the tapping cut. Maximal growth reduction occurred in the below cut area of tapped panel where latex regeneration process was thought to be the most active (Tupý, 1973a; Silpi *et al*, 2001 and 2004). Long term growth reduction could be envisaged as a result of carbon limitation mainly due to high consumption of sucrose for latex regeneration. Bark regeneration is also a carbon consuming process and tapping is thought to activate the vascular cambium (de Fay and Jacob, 1989). In the study of Thomas *et* al. (1995), after 2 weeks of tapping the cambial derivatives were produced in large numbers and these cells the differentiation was initiated after about 1 week. New differentiated sieve tubes in the regeneration bark had a larger diameter than those produced from the cambium in the uncut area.

By the works of Gooding (1952) with dendrometer and Buttery and Boatman (1966) with manometer they concluded that the mechanism of latex flow was connected with a sharp drop in turgor pressure in secondary phloem and probably more particularly in the laticiferous tissue. As tapping activity continued, the ability of phloem tissue to recover by maintain the optimal turgor pressure enough for may not accomplish successfully and hence impede the process of cell enlargement. This might particularly true in the area below the cut where radial growth was found poorest. Since this area was known as the active area for latex regeneration with fairly low sucrose content (less than 10 mM l^{-1}) in latex mantles (Silpi *et al*, 2001 and 2004), this might contribute to the low osmotic turgor pressure (low osmoticum content).

Although growth reduction was found at the whole trunk level, but the higher growth rate in the above cut portion as compared to the below cut might be explained, to some extent, by the girdling effect. Since every time the tree is tapped, bark is continually wounded and partly removed. The part which remains on the tapping panel consists of young phloem and the cambium, which means the translocation of current assimilates from the canopy is partly obstructed by mean of tapping. Daudet *et al.* (2005) observed an accelerated radial growth rate of walnut stem at the level above girdling while the area that was isolated by double girdling displayed almost complete stop of radial growth. Daudet *et al.* (2005) pointed out the importance of current photo-assimilate flux for active radial growth. However, laticiferous tissue is also supplied by the vascular rays which appear to be a major zone of horizontal translocation between the wood and the phloem. From our observation on the dynamics of wood reserves content (data not shown), a major change of starch in tapped tree occurred around the cut but the reserves content of the tapped tree was higher than untapped. Wood reserves play an important role in supplying laticiferous tissue with sugars that are precursors of the synthesis of rubber. According to de Fay and Jacob (1989), the functional organization model of the secretion tissue of *Hevea brasileinsis* implies the existence of complex interrelations between vertical and horizontal translocation and thus vascular rays are the horizontal transport road for metabolites flowing to the laticifers and which come either from the underlying wood or from functional sieve tubes.

Respiratory Activity of *Hevea brasiliensis* at Trunk Scale

Carbon loss by respiration is substantially required for providing energy and intermediates for plant. Thus, the study of carbon economy in rubber can not be completed without this part. It is well known in rubber tree that the carbohydrate consumption for rubber biosynthesis to the equivalent of carbohydrate accounted for by decrease in biomass increment is not balanced, tapped tree seems to lose more carbohydrate in biomass than what is used for rubber regeneration. Hence, the aim of this study is to describe and quantify the component that would account for the actual cost of the two main functional sinks in the rubber tree i.e. the radial growth and the latex production, as the consequences of tapping and the use of ethephon stimulation.

In this study, the difference in pattern and amount of respiration activity of untapped and tapped tree (*nil stim* and Et 8/y) was well addressed. The main outcome from this work was that we could obtain the elements required for establishing the mathematics equations that allow estimating the respiration cost for wood and latex production, this part will be done in the next publication.

From the concept of the functional model of respiration (McCree, 1970; Amthor, 1984), bark respiration activity during the leaf shedding period (annual minimum respiration) in untapped tree (control) represented the maintenance loss (R_m). By using the Q_{10} relationship, this component will be applied for the whole year to separate the non maintenance component into growth and latex production associated respiration. From the relationship between respiration and radial growth, highly linear correlation was observed in untapped tree and this will allow establishing the growth coefficient and respiratory cost for the wood production.

For tapped tree, we observed no linear relationship between growth and respiration especially in tapped panel. This indicates that the respiration is mainly devoted to latex production and since the latex regeneration process strongly affected the respiration rate, the scope of the two-component functional model itself would not be sufficient to identify the third component loss i.e. R_L which is the loss associated with latex regeneration. Thus, we can propose the extended model specifically for rubber, adapted from the two-component model as below,

$$R_T = R_m + R_g + R_L$$

when R_T refers to the total respiration, R_m and R_g refer to the respiration associated to maintenance and growth, and R_L refer to respiration devoted to latex regeneration. Thus R_L can be termed as

$$R_L = b \, . \, \Delta L/\Delta t$$

when $\Delta L/\Delta t$ is the rate of latex production per time unit and b is the latex regeneration coefficient. These equations will allow estimating the total cost for rubber biosynthesis.

Dynamics of Carbohydrate Reserves Based on Phenological Development and Latex Exploitation

The evaluation of glucidic reserves at seasonal scale to assess the cumulative effects of tapping and ethephon stimulation on reserves is of our interest in order to complete the whole frame of carbon balance study. This work provides the first quantitative analysis of carbohydrate reserves in the rubber tree. Analysing starch, sucrose, fructose and glucose content from 4 cm long wood core samples was proved appropriately to assess both spatial and temporal variations of carbohydrate reserves of rubber trees at trunk scale. Consistent patterns were demonstrated in relation to seasonal developmental changes, but also to diversion of assimilates by tapping.

1. Seasonal Pattern of Reserves

Similarly to most trees, starch was the major non-structural carbohydrates (NSC) found in within trunk wood sample and the content was comparable to data reported for beech and oak (Barbaroux *et al.*, 2003), poplar (Witt and Sauter, 1994), walnut (Lacointe, 1993) and lower to content in coconut (Mialet-Serra *et al.*, 2005). Sucrose content was the major soluble sugar and its content was more stable than starch among location and along the year. Significant content in monosaccharides was found only in trunks in March, after complete re-foliation.

Seasonal pattern of carbohydrate in control (untapped) trees were consistent with previous results on deciduous trees (Lacointe, 1993; Witt and Sauter, 1994; Barbaroux *et al.*, 2003): total carbohydrate decreased sharply after re-foliation, indicating net mobilization, either for direct incorporation in new shoot (including leaf and flower) or to sustain increased growth respiration (Lacointe, 1993). Although one major difference with temperate conditions was that, bud burst occurring almost without time lag following leaf fall, the leafless period lasted no longer than 2 weeks.

During the dry season, total carbohydrate content varied few, while radial growth remained very low. Although rubber leaves are considered to reach their maximum photosynthetic potential 20-25 days after complete expansion (Samsuddin and Impens, 1979), water stress and high temperatures likely limited actual assimilation during this period (Sangsing *et al.*, 2004). Thereafter, net deposition of reserves was recorded until defoliation (January), while radial growth was steady until November. Thereby, reserve deposition and radial growth were concomitant during 6 months, when climatic conditions were favourable, similarly to temperate species. However, carbohydrate deposition occurred only when radial growth ceased (November) in the lowest part of the trunk and in taproot. Together with the bottom-up decreasing gradient of starch after re-foliation this indicated a source-sink distance effect: more reserves were depleted closer to the canopy when growing shoots were net sinks, whereas refilling took more time for the basal locations when leaves were net sources (Lacointe, 1993). Such results were in accordance to histological studies by de Fay (1999) demonstrating that when rubber cambium was active, starch was depleted in the very nearby xylem, whereas it increased in deeper xylem. During leaf growth, this author found almost no starch in proximal parenchyma. On the contrary, when cambium stopped function, starch started to increase in the newly created tissues (mainly xylem). Large seasonal variations in starch while sucrose content remained quite stable indicated that sucrose may act as a buffer compartment. When more sucrose from parenchyma was used or transported, more starch was hydrolysed to compensate for the loss of sucrose. Such results are consistent with girdling experiments by Jordan (1996) showing that when phloem transport breaks down, peach trees maintain stable soluble sugar content whereas insoluble sugar content drops. Vertical distribution along the trunk confirmed such trend since there was almost no gradient of TSS, whereas starch was lower closer to canopy. However, in our experiment sucrose content increased more than starch along the vegetative season in taproot. The transient significant level of monosaccharides recorded after re-foliation have also been reported during periods of high starch mobilization by Witt and Sauter (1994) and could indicate that certain steps in the pathway of sucrose synthesis or sucrose loading into vessels are limiting for carbohydrate export from wood.

2. The Effect of Tapping and Ethephon Stimulation on Reserves

Tapping had a huge impact on carbohydrate contents in trunk and taproot. Average content, vertical distribution and seasonal dynamics were dramatically affected. Higher concentration for the total carbohydrate in tapped tree was the most striking feature from this work, underlying the strong impact of latex sink strength on whole plant carbohydrates pool. Sucrose content within laticiferous vessels actually drops following tapping and the ability to mobilize carbohydrate, partly from reserves; to cope with this consumption is a key factor in rubber exploitation.

By mean of tapping it creates a regular wound that leads the tree to be under stress. Such type of stress is well known to increase respiration and thereby carbohydrate demand. The additional use of ethylene generator is likely to increase such an effect, since ethylene is a stress hormone (Abeles, 1985; Chen and Patterson, 1985; Kimmerer and Kozlowski, 1982). Thus tapping demand was initially expected to induce a depletion of reserves. Gohet (1996) found such a depletion of starch in proximal parenchyma within the latex regeneration area of tapped trees. Such result seemed in contradiction to the present increase in NSC content. However, the study by Gohet relied on histological data within the outermost 2 mm parenchyma, whereas our study was based on quantitative chemical analysis on 4 cm long cores. Therefore, we can infer that tapping depletes starch in the proximal parenchyma due to increased transfer of sucrose to laticiferous vessels, but that the sink effect induces an increase in NSC in the deeper parenchyma. Moreover, the highest positive difference between tapped trees and control was measured in October, while carbohydrate demand for latex regeneration was the highest too. Thereby, we conclude that diverting carbohydrate through tapping had a positive effect on carbohydrate deposition within trunk parenchyma of rubber trees, mainly in starch form. Although the pattern was more or less clear according to locations, this increased deposition between May and October was recorded all along the trunk, even 190 cm above the cut, far from the latex regeneration area (Silpi *et al.*, 2004). Since very little carbohydrate additional deposition occurred in tapped trees between October and leaf-fall, whereas a steady deposition was recorded for control, we may infer that reserve capacity was already full in tapped trees at that time.

Although regeneration of latex is thought to be a rather local phenomenon but the extent of the area perturbated in its carbohydrate metabolism was much larger. The particular significant was the pattern observed in untapped panel, which was quite similar to that observed in tapped panel. Two explanations for this phenomenon could be (1) the tapping activity enhances the sink strength into the trunk area by creating a parallel demand for more future reserves or (2) since there is much less radial growth in that area, then more carbohydrate is accumulated as reserves.

The effects of tapping were enhanced by ethephon stimulation, since for many parameters; the patterns for tapped trees (*nil stim*) were in between untapped and stimulated trees. This gives useful information to better understand the effect of tapping on carbohydrate reserves dynamics, which could be used as tools to predict the long term effect of new tapping system, for instance: the double cut alternative system (DCA), on whole tree functioning and production (Gohet and Chantuma, 2003). Moreover, the additional changes induced by the artificial sink represented by tapping provide a new approach and interesting elements to analyze processes of reserves deposition and mobilization in trees. More information to better understand reserves dynamics, its co-ordination to other sinks could be obtained by follow the annual dynamics within more various sink size, tracing the translocation from the parenchyma to laticiferous system.

3. Vertical Distribution of Starch and TSS

Vertical pattern of both starch and TSS were much more irregular in tapped treatments than in control and this irregularity was enhanced by the use of ethephon. The most dramatic change was the peak of starch in May 2002 within the regeneration area. Such accumulation of starch above the cut was also reported by Gohet (1996). Two hypotheses can explain this pattern, (1) since bark was removed, including a large part of functioning conductive tissues, this area was likely largely disconnected to the rest of the tree, so that its reserves were less mobilizable when necessary, for example for re-foliation that occurred the previous February and which depleted starch in the other parts of the trunk, (2) bark regeneration process increases local demand and this could induce local increase of reserves accordingly to present findings that reserve level tend to adjust to current demand. Converse ranking in TSS content between tapping rest period and production peak period resulted from a decrease in TSS all along the trunk in control and concomitant increase for stimulated treatment, content in tapped trees without stimulation (*nil stim*) remaining stable meanwhile. Such pattern could result from the effect of ethylene, which enhances carbohydrate mobilization in trees (Eklund, 1998) and particularly increases latex metabolism (d'Auzac *et al.*, 1997) and incorporation of sucrose into rubber (Lacrotte *et al.*, 1985). Our results showed that the effect of Ethephon stimulation was wide and not limited to the proximity of the tapping cut where stimulant paste was applied. In October, the occurrence of higher TSS 50 cm below the cut was related to higher latex metabolism within this area, considered as directly involved in latex regeneration (Silpi, 2004).

Higher starch recorded all along the untapped panel even in its uppermost location during high production period, showed that the effect of tapping was not limited to the trunk area directly regenerating latex. On the other hand, lower decrease in this side following re-foliation may be the consequence of lesser consumption of carbohydrate. Actually, the medium part of the tapped panel was likely to be source of carbohydrate for both growth of new shoots and latex regeneration, whereas untapped panel was likely involved in growth only.

CONCLUSION

This study provides information of diurnal and seasonal carbon flux distribution between different functional sinks inside the rubber tree, i.e. latex production, wood production, glucidic reserves and respiratory losses associated. It's the first time that such a multi-disciplinary study on carbon budget has been conducted in the mature, rubber tree under tropical condition *in situ*.

Under "normal" or "untapped" condition, the rubber tree is like other trees when carbon fixed from photosynthesis has been partitioned among utilized and stored sinks. In long term view , the tree has its ability to maintain the balance between the *use* of carbon through respiration process to provide energy and intermediates that is required for plant growth and the *reserves pool* that maintain in a sufficient levels to supplement the current assimilates whenever a huge demand like refoliation occurs.

However, once the tree is tapped, the newly artificial sink is in fact against the tree's natural behavior hence not only creating new sink but also the tree's stress. Thus, at least 2 phenomena occur. i.e. the activation of latex system that create a huge demand for rubber biosynthesis precursor, and the loss in total biomass of the tapped tree when compared with untapped tree.

This study was devoted to the evaluation of diurnal and seasonal carbon flux distribution between different functional sinks, i.e. latex production, wood production, glucidic reserves and respiratory losses associated. The results showed that tapping activity has a huge physiological effect at the whole trunk scale in any aspect involved with carbon utilization. In comparison with untapped tree, tapping is confirmed to modify the whole physiological behavior of the trunk. A huge depletion of the latex sucrose concentration at whole trunk scale was obviously demonstrated, but the spatial extension of the regeneration area was shown to depend on quantitative rubber production. The latex diagnosis approach (LD) allowed a rather precise description of the shape and size of the metabolically active bark area. The multi-clonal linear relation between rubber production and the estimated latex regeneration area indicates that the regeneration of 1 g of dry rubber requires an average of 100 cm^2 latex regeneration area.

Tapping strongly affected radial growth i.e. radial growth rate was nearly stopped a few weeks after tapping resumed. Anyway, this effect seems to depend on the distance from the cut. Tapped tree exhibited a higher reserves deposition than untapped, suggesting that latex regeneration induces an extra demand for more reserves to be used for the future. This strong evidence support the concept that reserves sink is not a mere passive buffer and the plant tends to keep reserves at a sufficient level, depending on the demand. Massive utilization of reserves occurred during annual leaf shedding −re foliation. Starch and sucrose are the main forms of reserved presented in wood tissue, maximum deposition of total carbohydrate content was found at the annual defoliation period.

The difference in pattern and amount of respiration activity due to tapping and ethephon stimulation was well addressed. Highly linear correlation between respiration activity and radius increment in untapped tree will allow establishing the growth coefficient and respiratory cost for wood production. No linear relationship between growth and respiration in tapped tree especially in tapped panel indicates that respiration is mainly devoted to latex production. This will allow estimating the total cost for rubber biosynthesis. Concerning the diurnal scale, higher respiration rate found especially for the daily minimum value after the stimulation day and the following tapping day suggest the metabolic activity involving mainly in the regeneration process for latex and cell component, the active transport of sucrose into laticiferous system. Even the pattern of respiration in stimulation treatment was close to non stimulated one but it was higher in value and degree of fluctuation.

LITERATURE CITED

Abels, F.B. and G.R. Leather. 1971. Abscission: control of cellulose secretion by ethylene. **Planta.** 97: 87.

Abels, F.B. 1973. **Ethylene in Plant Biology.** Academic Press, NewYork.

Ameglio, T., and P. Cruiziat. 1992. Daily variations of stem and branch diameter: Short review from a developed example, pp. 193-204. In T.K. Karalis, ed. **Mechanics of Swelling NATO ASI series vol. H64.** Springer-Verlag Berlin Heidelberg.

Amthor, J.S., 1984. The role of maintenance respiration in plant growth. **Plant Cell Environ**. 7: 561-569.

Amthor, J.S., 1986. Evolution and applicability of a whole plant respiration model. **J. theor. Biol.** 122: 473-490.

Amthor, J.S. 1989. **Respiration and Crop Productivity.** Springer-Verlag, New York. 215 p.

ap Rees, T. 1988. Hexose phosphate metabolism by nonphotosynthetic tissues of higher plants, pp.1-33. In J. Preiss, ed. **The Biochemistry of Plants, Vol.14, Carbohydrate.** Academic Press, Sandiego.

Ashwell. 1957. Colorimetric analysis of sugars. **Methods in Enzymology.** 3: 73-105.

Backhaus, R.A. 1985. Rubber formation in plant: A mini review. **Isr. J. Bot.** 34:283-293.

Bannan, M.W. 1962. The vascular cambium and tree-ring development, pp. 3-21. In T.T. Kozlowski, ed. **Tree Growth.** Ronald Press, New York.

Barbaroux, C. and N. Bréda. 2002. Contrasting distribution and seasonal dynamics of carbohydrate reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beach trees. **Tree Physiol.** 22: 1201-1210.

Barbaroux, C., N. Bréda, and E. Dufrene. 2003. Distribution of above-ground and below-ground carbohydrate reserves in adult trees of two contrasting broad-leave species (*Quercus petraea* and *Fagus sylvatica*). **New Phytologist.** 157: 605-615.

Barthelemy, D., C. Edelin and F. Halle. 1991. Canopy architecture, pp. 1-20. *In* Raghavendra, A.S. ed. **Physiology of Trees.** John Wiley, New York.

Beevers, H. 1974. Conceptual developments in metabolic control, 1924-1974. **Plant Physiol.** 54: 437-442.

Boehringer, S.A. 1988. D-glucose *In* **Chimie alimentaire. Méthodes enzymatique pour l'analyse agro-alimentaire.** Boehringer Mannheim France, notice n° 273-CA, 4p.

Bory, G. and D. Clair-Maczulajtys. 1988. L'étude des métabolites de réserve. **Bulletin de la Société de Botanique de France**. 135: 5-8.

Boyne, A.F. and G.L. Ellman. 1972. A methodology for analysis of tissue sulfhydril components. **Anal. Biochem.** 46: 639-653.

Brown, K.W. and J.C. Thomas. 1980. The influence of water stress preconditioning on dark respiration. **Physiol. Plant.** 49: 205-209.

Buttery B.R. and S.G. Boatman. 1966. Manometric measurement of turgor pressures in laticiferous phloem tissues. **J. Exp. Bot.** 17: 283-296.

Cannell, M.G.R. and R.C. Dewar. 1994. Carbon allocation in trees: a review of concepts for modelling. *In* **Advances in Ecological Research.** 25: 59-104.

Chandrashekar, T.R., J.G Marattukalam and M.A. Nazeer, 1996. Growth reduction of *Hevea brasiliensis* to heat and drought stress under dry subhumid climatics conditions. **Indian Journal of Natural Rubber Research.** 9(1): 1-5.

Coupe, M. and H. Chrestin, 1989. Physio-chemical and biochemical mechanisms of hormonal (ethylene) stimulation, pp. 295-319. In J. d'Auzac, J.L. Jacob and H. Chrestin, eds. **Physiology of Rubber Tree Latex.** CRC Press, Florida.

d'Auzac, J. and S. Pujarniscle. 1961. Aperçu sur l'étude des glucides de l'hévéa et sur leur variation. **Rev. gén. Caout. Plast.** 38: 1131-1139.

da Costa, J.M.N., N.J. Rosenberg, and S.B. Verma. 1986. Respiratory release of CO_2 in alfafa and soybean under field conditiions. **Agricultural and Forest Meteorology.** 37: 143-157.

Daudet, F.A., T. Améglio, H. Cochard, O. Archilla and A. Lacointe. 2005. Experimental analysis of the role of water and carbon in tree stem diameter variations. **J. Exp. Bot.** 56(409): 135-144.

de Fay, E., C. Hebant and J.L. Jacob. 1989. Cytology and cytochemistry of the laticiferous system, pp. 15-29. *In* J. d'Auzac, J.L. Jacob and H. Chrestin, eds. **Physiology of Rubber Tree Latex.** CRC Press, Florida.

de Fay, E. and J.L. Jacob. 1989. Anatomical organization of the laticiferous system in the bark, pp. 3-14. *In* J. d'Auzac, J.L. Jacob and H. Chrestin, eds. **Physiology of Rubber Tree Latex.** CRC Press, Florida.

de Fay E. 1999. **Rhythmic cambial growth and its relationships with apical rhythmic growth in *Hevea brasiliensis* , *Theobroma cacao* and *Terminalia superba* under natural cultivation conditions.** Ph.D. thesis, Universite de Nancy I.

Devakumar, A.S., P.G. Prakash, M.B.M. Sathik and J. Jacob. 1999. Drought alters the canopy architecture and micro-climate of *Hevea brasiliensis* trees. **Trees.** 13:161-167.

Downes, G., C. Beadle and D. Worledge. 1999. Daily stem growth patterns in irrigated *Eucalyptus globules* and *E. nitens* in relation to climate. **Trees.** 14: 102-111.

Eschbach, J.M., D. Roussel, H. Van De Sype, J.L. Jacob and J. d' Auzac. 1984. Relationship between yield and clonal physiological characteristics of latex from *Hevea brasiliensis*. **Physiol. vég.** 22: 295-304.

Garnier, E. and A. Berger. 1986. Effect of water stress on stem diameter changes of peach trees growing in the field. **Journal of Applied Ecology.** 23: 193-209.

Gensler, W. and F. Diaz-Munoz. 1983. Simultaneous stem diameter expansions and apoplastic electropotential variations following irrigation or rainfall in cotton. **Crop Sci.** 23: 920-923.

Gholz, H.L. and W.P. Cropper. 1991. Carbohydrate dynamics in mature *Pinus elliottii* var. elliotti trees. **Can. J. For. Res.** 21: 1742-1747.

Glerum, C. 1980. Food sinks and food reserves of trees in temperate climates. **New Zealand Journal of Forestry Science.** 1 : 176-185.

Gohet, E. 1996. **La production de latex par *Hevea brasiliensis*. Relations avec la croissance. Influence de différents facteurs : origine clonale, stimulation hormonale, réserves hydrocarbonées.** Thèse de Doctorat d'Université. Université Montpellier II - Sciences et Techniques du Languedoc.

Gohet, E., J.C. Prévôt, J.M. Eschbach, A. Clément and J.L. Jacob. 1996. Clone, croissance et stimulation, facteurs de la production de latex chez *Hevea brasiliensis*. **Plantations, Recherche, Développement.** 3(1): 30-38.

Gohet, E. and P. Chantuma. 1999. **Micro Latex Diagnosis.** (Standard Analysis Procedure) Microdiagnostic Latex Training. Chachoengsao Rubber Research Center, RRIT-DOA-MOAC. November 1999.

Gohet, E. and P. Chantuma. 2003. Reduced tapping frequency and DCA tapping Systems: Research towards improvement of Thailand rubber plantations productivity. *In* **IRRDB Symposium.** IRRDB Annual meeting, Chiang Mai, Thailand, 15-16 September 2003.

Gohet, E. P. Chantuma, R. Lacote, S. Obouayeba, K. Dian, A. Clement-Demange, K. Dadang and J.M. Eschbach. 2003. Latex Clonal typology of *Hevea brasiliensis*: Physiological modeling of yield potential and clonal response to ethephon stimulation. *In* **Proceedings of the IRRDB International Workshop on Exploitation Technology.** Kottayam, Kerala, India, 15-18 December 2003.

Gomez, J.B., R. Narayanan and K.T. Chen. 1972. Some structural factors affecting the productivity of *Hevea brasiliensis.* I. Quantitative determination of the laticiferous tissue. **J. Rubb. Res. Inst. Malaya.** 23: 193-203.

Gomez, J.B. and G.F.J. Moir. 1979. The ultracytology of latex vessels in *Hevea brasiliensis.* **Malaysian Rubber Research and Development board, Monogr. No. 4.** Rubber Research Institute Malaysia, Kuala Lumpur.

Gomez, J.B. 1982. **Anatomy of *Hevea* and its Influence on Latex Production**. Malaysian Rubber Research Development Board Eds., 75 pages.

Gooding, E.G.B. 1952. Studies in the physiology of latex II. Latex flow on tapping *Hevea brasiliensis* associated changes in trunk diameter and latex concentration. **New Phytol.** 51: 11-29.

Grossman, Y. L. and T.M. DeJong. 1994. Carbohydrate requirements for dark respiration by peach vegetative organs. **Tree Physiol.** 14: 37-48.

Hesketh, J.D., D.N. Baker, and W.G. Duncan. 1971. Simulation of growth and yield in cotton: respiration and the carbon balance. **Crop Sci.** 11: 394-398.

Hofstra, G. and J.D. Hesketh. 1969. Effects of temperature on the gas exchange of leaves in the light and dark. **Planta.** 85: 228-237.

Jacob, J.L., J.M. Eschbach, J.C. Prévôt, D. Roussel, R. Lacrotte, H Chrestin and J. d' Auzac. 1985. Physiological basis for latex diagnosis of functioning of the laticiferous system in rubber trees, pp. 43-65. In J.C. Rajarao and L.L. Amin, eds. **Proceedings International Rubber Conference, Kuala Lumper, 1985, vol. 5.** Rubber Research Institute of Malaya, Kuala Lumpur, Malaysia.

Jacob, J.L., E. Serres, J.C. Prévôt, R. Lacrotte, A. Clément-Vidal., J.M. Eschbach and H. Omont. 1988a. Mise au point du diagnostic latex. **Agritrop.** 12: 97-118.

Jacob, J.L., J.C. Prévôt and A. Clément-Vidal. 1988b. Physiological parameters of latex from *Hevea brasiliensis*: their uses in the study of the laticiferous system: Typology of functioning of production mechanisms: Effect of stimulation. pp, 136-157. *In* **Proceedings of the IRRDB Rubber Physiology and Exploitation Meeting.** Hainan, China, 9-12 December 1986, Pan Yanqing and Zhao Canwen, eds. Hainan, China, South China Academy of Tropical crops.

Jacob, J.L., J.C. Prevot, R. Lacrotte and J.M. Eschbach. 1995. Le diagnostic latex. **Plantations, Recherche, Développement.** 2(2): 34-37.

James, W.O. 1953. **Plant Respiration.** Clarendon Press, Oxford.

Jones, R.L. 1968. Ethylene-enhanced of α-amylase from barley aleurone cells. **Plant. Physiol.** 43: 442.

Kakubari, Y. 1989. Diurnal and seasonal fluctuations in the bark respiration of standing *Quercus acutissima* trees in Nakaizu, Japan, pp. 529-531. In E. Dreyer et al., eds. **Forest Tree Physiology.** Elsevier, INRA, France.

Kozlowski, T.T. 1971. **Growth and development of trees**. Volume 2. Academic Press, New York, 514 pp.

Kozlowski, T.T., P.J. Kramer and S.G. Pallardy. 1991. **The physiological ecology of woody plants.** Academic Press, Tokyo.

Kozlowski, T.T. 1992. Carbohydrate sources and sinks in woody plants. **Botanical Review**. 58: 107-222.

Kuhn, C., L. Barker, L. Burkle and W.B. Frommer. 1999. Update on sucrose transport in higher plants. **J. Exp. Bot.** 50: 935-953.

Lacointe, A., A. Kajji, F.A. Daudet, P. Archer and J.S. Frossard. 1993a. Mobilization of carbon reserves in young walnut trees. **Acta Bot. Gallica.** 140(4): 435-441.

Lacointe, A., A. Kajji, T. Améglio, F.A. Daudet, P. Cruiziat, P. Archer and J.S. Frossard. 1993b. Storage and mobilization of carbon reserves in walnut and its consequences on the water status during winter. **Acta Hort.** 311: 201-209.

Lacointe, A., K. Abdellah, F.A. Daudet, P. Archer and J.S. Frossard. 1995. Seasonal variation of photosynthetic carbon flow rate into young walnut and its partitioning among the plant organs and functions. **J. Plant Physiol.** 146: 222-230.

Lacointe, A. 2000. Carbon allocation among tree organs: A review of basic processes and representation in functional-structural tree models. **Ann. For. Sci.** 57: 521-533.

Lacointe, A., E. Deleens, T. Améglio, B. Sainte-Joanis, C. Lelarge, M. Vandame, G.C. Song, F.A. Daudet. 2004. Testing the branch autonomy theory: a $^{13}C/^{14}C$ double-labelling experiment on differentially shaded branches. **Plant, Cell and Environment.** 27: 1159-1168.

Lacrotte, R., H. Van De Sype and H. Chrestin. 1985. Influence de ethylene sur l'utilisation du saccharose exogene par la laticiferes d' *Hevea brasileinsis*: proposition d'un mécanisme d' action. **Physiol. Veg.** 23: 187.

Lacrotte, R. 1991. **Etude des relations entre la teneur en sucres du latex et la production. Approche des mécanismes du chargement en saccharose des laticifères d'*Hevea brasiliensis* Muell. Arg.** Thèse de Doctorat d'Université. Université Montpellier II - Sciences et Techniques du Languedoc.

Le Roux, X., A. Lacointe, A. Escobar-Gutiérrez, S. Le Dizès. 2001. Carbon-based models of individual tree growth: A critical appraisal. **Ann. For. Sci.** 58: 469-506.

Levy, D. and N. Kedar. 1970. Effect of ethrel on growth and bulb initiation in onion. **Hortic. Sci.** 5: 80.

Linder, S. and E. Troeng. 1981. The seasonal Variation in Stem and Coarse Root Respiration of a 20-year-old Scots pine (*Pinus sylvestris* L.), pp. 125-139. In W. Tranquillini, ed. **Radial Growth in Trees.** Mitt. Forstl. Bundes-Versuchsanst (Wien).

Lustinec, J. and W.L. Resing. 1965. Methods for delimitation of the flow area by micro-tapping and radio-isotopes. **Plant. Bull. Rubber Res. Inst. Malaya.** 80: 144-149.

Lustinec, J., J. Simmer and W.L. Resing. 1969. Trunk contraction of *Hevea brasiliensis* due to tapping. **Biol. Plant.** 11: 236-244.

Lynen, F. 1969. Biochemical problem of rubber synthesis. **J. Rubber Res. Inst. Malaya.** 21: 389.

Malkina, I.S., A.M. Yakshina and Y.L. Tsel's Niker. 1985. Relationship between CO_2 evolution by the trunk and gas exchange of the leaves in oak. Translated from **Fiziologiya Rastenii.** 32(4): 769-776.

McCree, K.J. 1970. An equation for the rate of respiration of white clover plants grown under controlled conditions, pp. 221-229. In I. Setlik ed. **Prediction and Measurement of Photosynthetic Productivity.** Center for Agricultural Publishing and documentation.

Mialet-Serra, I., A. Clement, N. Sonderegger, O. Roupsard, C. Jourdan, J.P. Labouisse and M. Dingkuhn. 2005. Assimilate storage in vegetative organs of coconut (*Cocos nucifera* L.). **Expl. Agric.** 41: 161-174.

Pakianathan S.W., R.L. Wain and E.K. Ng. 1976. Studies on displacement area on tapping immature *Hevea* trees. *In* **Proceeding of the International Rubber Conference, Kuala Lumpur 1975.** 2: 225-246.

Patrick, J.W. 1990. Sieve element unloading: cellular pathway, mechanism and control. **Physiol. Plant.** 78: 298-308.

Patrick, J.W. and C. Offler. 1996. Post-sieve element transport of photoassimilates in sink regions. **J. Exp. Bot.** 48: 1165-1177.

Penning de Vries, F.W.T. 1972. Respiration and growth, pp. 327-347. In A.P. Rees, K.E. Cockshull, D.W. Hand and R.J. Hurd, eds. **Crop Processes in Controlled Environments.** Academic Press, London.

Pirt, S.J. 1965. The maintenance energy of bacteria in growing cultures. In **Proceedings of the Royal Society of London, Series B.** 163: 224-231.

Rao, A.N. 1972. Periodic changes in the cambium activity of *Hevea brasiliensis*. **J. Indian Bot. Soc.** 51: 13.

Rao, K.S., and K.S. Rajput. 2001. Relationship between seasonal cambial activity , development of xylem and phenology in *Azadirachta indica* growing in different forests of Gujarat State. **Ann. For. Sci.** 58: 691-698.

Reid, M.S. and H.K. Pratt. 1970. Ethylene and respiration climacteria. **Nature.** 226: 976.

Reid, M.S. 1972. Effect of ethylene on potato tube respiration. **Plant Physiol.** 49: 252

Ridge, I. and D.J. Osborne. 1970. Hydroxyproline and peroxidases in cell walls of *Pisum sativum*: regulation by ethylene. **J. Exp. Bot.** 21: 843.

Rodrigo, V.H.L., C.M. Stirling, Z. Teklehaimanot and A. Nugawela. 1997. The effect of planting density on growth and development of component crops in rubber/banana intercropping systems. **Field Crops Res.** 52: 95-108.

Rodrigo, V.H.L., A. Nugawela , A. Sivanathan, W.R.G. Witharama and W.K. Jayasinghe. 2000. Rubber cum sugarcane intercropping a suitable cropping system for the farmer in the immediate zone of Sri Lanka. **J. Rubber Res. Inst. Sri Lanka.** 83: 62-74.

Rodrigo, V.H.L., C.M. Stirling, Z. Teklehaimanot and A. Nugawela. 2001. Intercropping with banana to improve fractional interception and radiation-use efficiency of immature rubber plantations. **Field Crops Res.** 69: 237-249.

Rodrigo, V.H.L, C.M. Stirling, Z. Teklehaimanot, R.K. Samarasekera and P.D. Pathirana. 2005. Interplanting banana at high densities with immature rubber crop for improved water use. **Agron. Sustain. Dev.** 25: 45-54.

Sale, P.J.M. 1974. Productivity of vegetable crops in a region of high solar input. III. Carbon balance of potato crops. Aust. **J. Plant Physiol.** 1: 283-296.

Satoh, S. and Y. Esahi. 1984. Changes in ethylene production and in 1-amino-cyclopropane-1-carboxylic acid (ACC) and malonyl-ACC contents of cocklebur seeds during their development. **Plant Cell Physiol.** 25: 1277

Sauter, J.J. and S. Kloth. 1986. Plasmodesmatal frequency and radial translocation rates in ray cells of popular (Populus X canadensis Moench, “ robusta”). **Planta.** 168: 377-380.

Senevirathna, A.M.W.K., C.M. Stirling and V.H.L. Rodrigo. 2003. Growth , photosynthetic performance and shade adaptation of rubber (*Hevea brasiliensis*) grown in natural shade. **Tree Physiol.** 23: 705-712.

Sethuraj, M.R. 1981. Yield components in *Hevea brasiliensis*. **Plant Cell Environ.** 4: 81-83.

Sethuraj, M.R. 1985. Physiology of growth and yield in *Hevea brasiliensis*, pp. 3-19. Rubb. Res Inst. Malaysia, ed. *In* **Proc Int. Rubb. Conf.** Kuala Lumpur, Malaysia.

Silpi, U., P. Chantuma, J. Kosaisawe, S. Thanisawanyangkura and E. Gohet. 2001. Distribution pattern of latex sucrose and metabolic activity in response to tapping and ethrel stimulation in latex producing bark of *Hevea brasiliensis* Muell. Arg. *In* **Annual IRRDB Symposium** (J. Sainte-Beuve, ed.). CIRAD, Montpellier, France.

Silpi, U., P. Chantuma, P. Kasemsap, P. Thaler, S. Thanisawanyangkura, A. Lacointe, T. Améglio and E. Gohet. 2004. Spatial distribution of sucrose and metabolic activity in the laticiferous tissue of three Hevea brasiliensis clones : Effects of tapping and ethephon stimulation at trunk scale. In **IRRDB Annual Conference.** Kunming, Yunnan China.

Simonneau, T., R. Habib, J.P. Goutouly and J.G. Huguet. 1993. Diurnal changes in stem diameter depend upon variations in water content: Direct evidence in peach trees. **J. Exp. Bot.** 44: 615-621.

Takenaka, A. 1994. A simulation of tree architecture development based on growth responses to local light environment. **J. Plant Res.** 107: 321-330.

Taussky, H.H. and E. Shorr. 1953. A micro colorimetric method for the determination of inorganic phosphorous. **J. Biol. Chem.** 202: 675-685.

Templeton, J.K. 1968. Growth studies in *Hevea brasiliensis*. I. Growth analysis up to seven years after budgrafting. **J. Rubber Res. Inst. Malaya.** 20: 136-146.

Templeton, J.K. 1969. Partition of assimilates. **J. Rubber Res. Inst. Malaya.** 21: 259-263.

Thomas, M.D. and G.R. Hill. 1949. Photosynthesis under field conditions, pp. 19-52. In J. Franck and W.E. Loomis, eds. **Photosynthesis in Plants.** Iowa State College Press, Iowa.

Thomas, V., D. Premakumari, C.P. Reghu, A.O.N. Panikkar and C.K. Sarawathy Amma. 1995. Anatomical and histochemical aspects of bark regeneration in *Hevea brasiliensis*. **Annals of Botany**. 75: 421-426.

Thornley, J.H.M. 1970. Respiration, growth and maintenance in plants. **Nature.** 227: 304-305.

Tromp, J. 1983. Nutrient reserves in roots of fruit trees, in particular carbohydrates and nitrogen. **Plant Soil.** 71: 401-413.

Tupý, J. and W.L. Resing. 1968. Anaerobic respiration in latex of *Hevea brasiliensis*: substrate and limiting factors. **Biologia Plantarum.** 10: 72-80.

Tupý, J. 1969. Stimulatory effects of 2,4-dichlorophenoxy acetic acid and of 1-naphthylacetic acid on sucrose level, invertase activity and sucrose utilization in the latex of *Hevea brasiliensis*. **Planta.** 88: 144-153.

Tupý, J. 1973a. The level and distribution pattern of latex sucrose along the trunk of *Hevea brasiliensis* Muell. Arg. as affected by the sink region induced by latex tapping. **Physiol. Veg.** 11(1): 1-11.

Tupý, J. 1973b. The sucrose mobilizing effect of auxins in *Hevea brasiliensis* Muell. Arg.; dependence on the metabolic activity of the treated tissue. **Physiol. vég.** 11(1): 13-23.

Tupý, J. 1985. Some aspects of sucrose transport and utilization in latex producing bark of *Hevea brasiliensis* Muell. Arg. **Biol. Plant.** 27(1): 51-64.

Ueda, M. and E. Shibata. 2001. Diurnal changes in branch diameter as indicator of water status of Hinoki cypress *Chamaecyparis obtuse*. **Trees.** 15: 315-318.

Veen, H. and A.A.M. Kwakkenbos. 1984. Role of ethylene in distribution of assimilates in cut carnation. **J. Plant Physiol.** 115: 389.

Vreugdenhil, D., A.P.C. Oerlemans and M.H.G. Steeghs. 1984. Hormonal regulation of tuber induction in radish (Raphanus sativus), Role of ethylene. **Physiol. Plant.** 62: 175.

Witt, W. and J.J. Sauter. 1994. Starch metabolism in poplar wood ray cells during spring mobilization and summer deposition. **Physiologia Plantarum**. 92: 9-16.

Wycherley, P.R. 1976. Tapping and partition. **Journal of the Rubber Research Institute of Malaysia.** 24: 169-194.

Zimmermann, M.H. and J.A. Milburn. 1975. Transports in plants. I. Phloem transport. Encyclopedia of **Plant Physiology**. Springer Verlag, Berlin.

Zweifel, R. and R. Hasler. 2001. Dynamics of water storage in mature subalpine *Picea abies*: temporal and spatial patterns of change in stem radius. **Tree Physiol.** 21: 61-569.

APPENDIX

The Scaling Method for Validating the Over Range Output from RS System

Problematic

For the first time, RS sensor was used under long-term field experiment in tropical conditions. This choice was motivated mainly to obtain differences in radial increment between both sides of the rubber tree (*Hevea brasiliensis* Muell.Arg.) and also because this device is much smaller than the standard LVDT, thus allowing enough space for the tree to be normally tapped. But the accuracy and reliability of the RS system was to be tested as compared to LVDT.

The output signal received from the system showed the potential of RS to follow radial growth for more than 1 year. However, in the control tree (untapped) where both LVDT and RS were installed in parallel reveal the incompatible result between the two systems. Though having the same pattern of growth, but the range was not the same. Data from RS seemed to over-estimate the radius increment (appendix table 1). Since LVDT is a more standard tool for trunk radius measurement and its result fit well with the manual measurement by tape.

The origin of this issue comes from a finding that, the radial growth from the two systems, LVDT and RS, were not compatible to each other. Since LVDT is a more standard tool for trunk radius measurement and its result fit well with the manual measurement by tape, from this point of view growth data from RS seemed to over-estimate the radius increment (appendix table 1). However, in tapped tree only radial growth measured by RS and tape measurement were available. Thus, the result from RS measurement needed to be scaled by the monthly data from tape. The steps of the scaling method are as following.

Scaling Factor and Correction of Cumulative Radial Growth (mm)

1. Calculate the radius increment per cm vertical (RI_v) and relative radius increment (RRI). This calculation referred to 2 reference times i.e., May 2001 (T_0) and November 2003 (T_1) when the untapped trees circumferences were measured both at 1.0 and 1.7 m.

From appendix table 1,
Diff. at 1.0 = the difference of trunk radius at 1.0 m by time (T_1-T_0)
Diff. at 1.7 = the difference of trunk radius at 1.7 m by time (T_1-T_0)
Diff. $_{1.0\ -\ 1.7}$ = the difference of trunk increment between the 2 positions
Then, the radius increment in µm per cm vertical (RI_v) is calculated by
RI_v (µm. cm^{-1}) = Diff. $_{1.0\ -\ 1.7}$. 10000 / 70
From the average of 12 trees, the value of RI_v equals to 8 µm. cm^{-1}

Then, the relative radius increment per cm vertical per cm of increment (RRI) is calculated by
RRI (µm. cm^{-1}. cm^{-1}) = (RI_v) / average (Diff. at 1.0, Diff. at 1.7)
From the average of 12 trees, the value of RRI equals to 4 µm. cm^{-1}. cm^{-1}

The value of RRI is used to extrapolate the radius increment in six RS - experiment trees at 1.5 m from the monthly measurement by tape at 1.7m in order to be comparable with the measurement by RS system at 1.5 m. Since the radial growth starts after mid April and ends before the annual defoliation in late December, so the comparative period was selected from 11th Mar. to 17th Dec. 02.

2. Extrapolate the radius increment by tape at 1.5 m, $RI_{1.5\ tape}$, (the position where RS sensors - above the cut were installed) for the 6 trees in RS experiment (2 untapped, 2 *nil stim* and 2 ET 8/y) from tape - measuring circumference at 1.7 m.
$RI_{1.5\ tape}$ = $RI_{1.7\ tape}$ + $RI_{1.7\ tape}$ x (delta distance 1.7 ,1.5) / 10000

3. Calculate the scaling factor as the proportion of RI by tape and by RS
Scaling factor = $RI_{by\ tape}$ / $RI_{by\ RS}$ when RI referred to the total radius increment from March 11 to December 17, 2002 (table 2).

After, the scaling factor of each tree (appendix table 2) was used to scale the radial growth data originally obtained from RS measurement (appendix figure 1). Although in tapped tree there were 4 positions of RS measurement but we use the same scaling factor in order to maintain the different between positions within the same tree.

Calculation of Radius Growth Rate ($mm.day^{-1}$) Based on 5 Days Moving Average of Radius Increment

Since in this experiment, there are some periods when some data were missing and that sometime a kind of irrational data occurred some time. To obtain a meaningful radius growth rate, it is then required to calculate from the moving average of radius growth data (appendix figure 2). In this experiment, the radial growth rate (RGR) of each position was calculated as following.

$$RGR = RG_{5d-Tn} - RG_{5d-Tn-1}$$

when

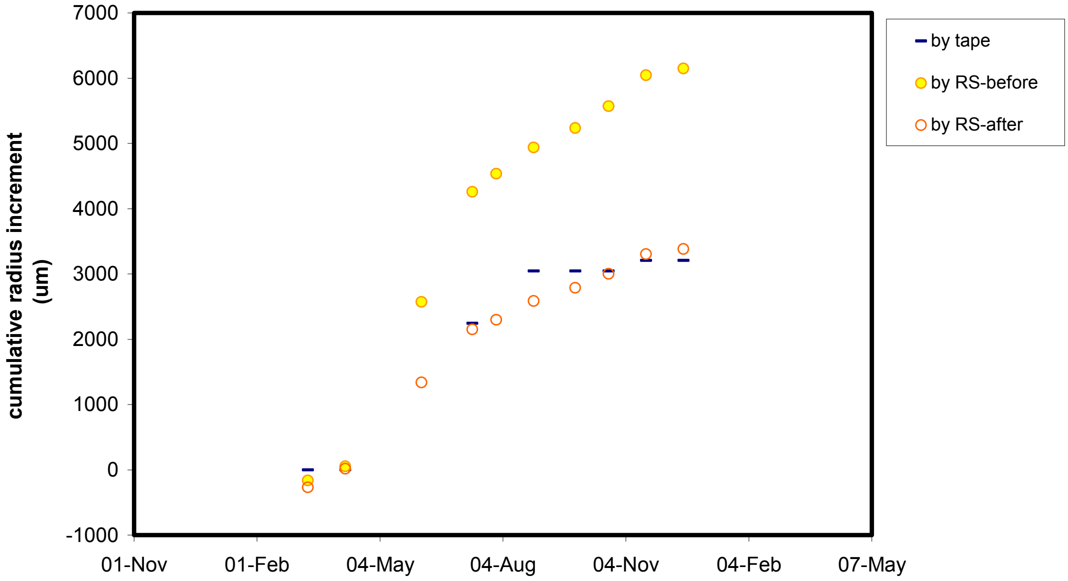
$RG_{5d-T1-1}$, RG_{5d-T1} = the 5 days moving average radial growth from day T_n and T_{n-1} respectively

Appendix Table 1 The radius increment by time, RI $_{(T1\ -\ T0)}$ (µm) and the relative radius increment, RRI (µm/cm of vertical distance / cm of increment). This calculation is based on the circumference measurement by tape meter at 1.0 m and 1.7 m in May 01 (T_0) and Nov. 03 (T_1) of 12 untapped trees.

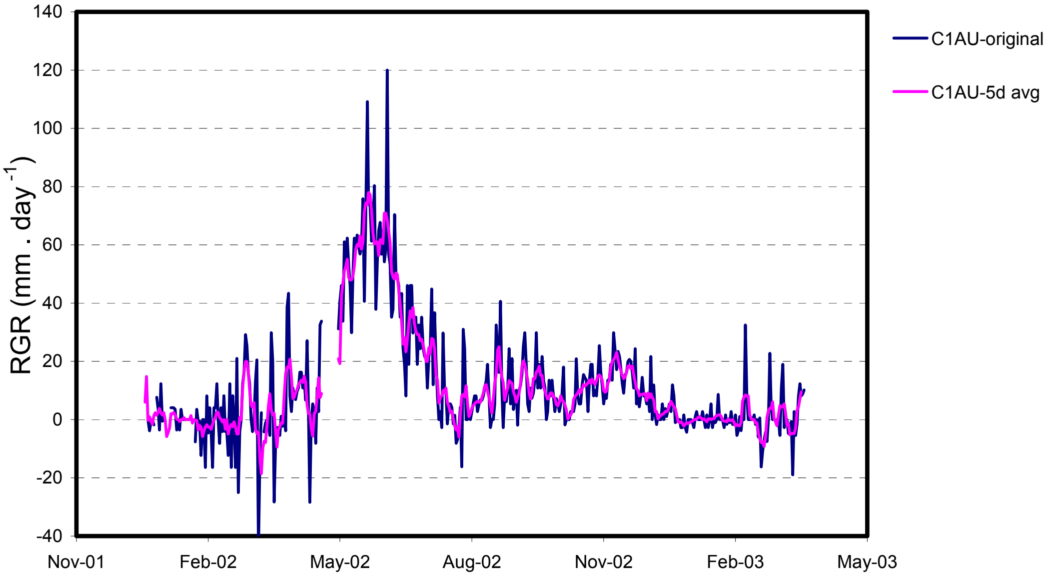
	Trunk radius (cm) measured by tape				Diff.	Diff.	Diff.		RI $_v$	RRI
	Nov. 03 (T_1) Untapped tree		May 01 (T_0) Untapped tree		at 1.0 m	at 1.7 m	1.0-1.7		(µm. cm^{-1})	(µm. cm^{-1}. cm^{-1})
TREE-REP.	1.0 m	1.7 m	1.0 m	1.7 m						
1	9.55	9.25	7.51	7.21	2.04	2.04	0.000		0.0	0.0
2	9.25	8.77	7.38	7.00	1.86	1.77	0.095		13.6	7.5
3	8.55	8.05	7.16	6.76	1.38	1.29	0.095		13.6	10.2
4	9.39	8.79	7.61	7.13	1.78	1.66	0.127		18.2	10.6
5	9.69	9.26	7.50	7.21	2.20	2.05	0.143		20.5	9.6
6	10.35	9.77	7.58	7.18	2.77	2.59	0.175		25.0	9.3
7	9.23	8.88	7.51	7.13	1.72	1.75	-0.032		-4.5	-2.6
8	9.15	8.79	7.50	7.10	1.66	1.69	-0.032		-4.5	-2.7
9	9.61	9.07	7.81	7.38	1.80	1.69	0.111		15.9	9.1
10	8.28	7.88	7.34	6.92	0.94	0.95	-0.016		-2.3	-2.4
11	9.58	9.25	7.27	7.00	2.31	2.24	0.064		9.1	4.0
12	9.41	9.06	7.54	7.13	1.86	1.93	-0.064		-9.1	-4.8
AVG.	9.3	8.9	7.5	7.1	1.86	1.80	0.06			
									8.0	4.0
						Std. Err.	**0.02**	**Std. Err.**	**3.3**	**1.7**

Appendix Table 2 The scaling factor of each individual tree under the RS experiment, its calculation based on the proportion of total radius increment (µm) from the extrapolation at 1.5 m from tape measurement (RI $_{\text{by tape}}$) and from RS measurement (RI $_{\text{by RS}}$) from 11th Mar. – 17 Dec. 02.

	untapped		*nil stim*		ET 8/y	
	Rep.1	Rep.2	Rep.1	Rep.2	Rep.1	Rep.2
RI $_{\text{by tape}}$	7659.23	6029.61	3208.48	2245.94	5935.69	962.54
RI $_{\text{by RS}}$	12571.48	10455.63	6312.05	5231.99	9113.90	4756.57
Scaling factor	0.61	0.58	0.51	0.43	0.65	0.20



Appendix Figure 1 The result after scaling process. This figure shows the cumulative radius increment from March 11 – December 17, 2003 from the 3 data sets i.e., by tape measurement , by RS before and after adjustment



Appendix Figure 2 Comparison of 2 ways to calculate RGR, i.e. from direct daily radius increment and from 5 days moving average of radius increment

The Use of Macro Files to Manipulate Respiration Data

Problematic

When numbers of analog signals are collected continuously together in long term study like the one of the multi-channel gas exchange measurement, it is necessary to have also an efficient way to process data in order to avoid errors and save time. The use of macro file is the solution for this purpose. Especially in our case when the original control system had to be replaced by a more appropriate system which led to some changes of the data format output. One of the main problems is that the analog signals from the substitution system contain some ‘noise’ that have to be removed. The other difficulty is to combine the chamber temperature data that was logged separately with another logger and after, transforms all signals into the same format as the original system so that we can compare the data from the two systems.

Briefly, there are 4 macro files used in the data manipulation. The first macro (called **ClearData_final**) is aimed at removing the unwanted signal (or noise) for flow and CO_2 efflux data and then averaging the voltage signal for each hour. Next, the second macro (called **ClearDataAverage_et_Temperature_to_ASC**) combined temperature data and converted all signal data into informative values. These two macro files were used only for the substitution system (KU-siemens) in order to have the similar output file format as the one from the original control system (Daisylab-INRA). Next macro (called **Siemens-Resp-Template**) created a template excel file containing data for a particular period (for example, 1 week), then separated data of each chamber into an individual worksheet and built some required figures. Last macro (called **Summary**) created an excel file containing all data from the experiment at daily scale. Following are the detail and format of each macro file.

Macro file : ClearData_final

```
------------------------------------------------------------
Global CO2Column As Integer
Global FlowColumn As Integer
Global ChamberColumn As Integer
```

```
Global nom As String
------------------------------------------------------------
Sub RawToTxt()
ThisWorkbook.ActiveSheet.EnableCalculation = False
repdef$ = ″C:\siemens\19chamber\rawdata″: ′ To be updated according to actual computer
configuration
rep$ = InputBox(″Name of folder containing all the DAT files to be processed:″, ″Clearing
Raw data files″, repdef$) & ″\″
For y = 2002 To 2004:
    If Dir(rep$ & ″*″ & y & ″*.dat″) <> ″″ Then
        For m = 1 To 12:  ′month
            If Dir(rep$ & ″*_″ & m & ″_″ & y & ″*.dat″) <> ″″ Then
                For d = 1 To 31: ′Day
                    nom = rep$ & d & ″_″ & m & ″_″ & y
                    If Dir(nom & ″*.dat″) <> ″″ Then
                        Application.StatusBar = ″processing files ″ & nom & ″_X.dat″
                        CurrentLine = 1
                        For n = 1 To 4:  ′ 4 = max number of files for d day
                            nom2 = nom & ″_″ & n & ″.dat″
                            If Dir(nom2) <> ″″ Then
                                Workbooks.OpenText FileName:=nom2, DataType:=xlDelimited,
                                        Comma:=True, Space:=False, _
                                        FieldInfo:=Array(Array(1,9), Array(2, 1), Array(3, 3),
                                        Array(4, 1), Array(5, 1), Array(6, 1), Array(7, 9), _
                                        Array(8, 1), Array(9, 9), Array(10, 1))
                                nl = ActiveWorkbook.ActiveSheet.UsedRange.Rows.Count
                                ActiveWorkbook.ActiveSheet.UsedRange.Copy
Destination:=ThisWorkbook.ActiveSheet.Cells(CurrentLine, 1)
                                ActiveWorkbook.Close
                                CurrentLine = CurrentLine + nl
                            End If
                        Next n
```

```
            ThisWorkbook.SaveAs FileName:=nom & ″_.txt″,
FileFormat:=xlCSVWindows
            Call ClearData
            ThisWorkbook.SaveAs FileName:=nom & ″_ClearDat_Average.txt″,
FileFormat:=xlCSVWindows
            ThisWorkbook.ActiveSheet.UsedRange.Clear
          End If
        Next d
      End If
    Next m
  End If
Next y
End Sub
----------------------------------------------------------
Function ClearData()
′ ClearData Macro
′ Macro recorded 21/11/02 by JJJ
′ Keyboard Shortcut: Ctrl+Shift+D
ChamberColumn = 4
  CO2Column = 5
  FlowColumn = 6
    Application.ScreenUpdating = False
    Call CutMaxMin(6250, 625, 30000, 12000)  ′flowmax, flowmin,co2max,
                                              co2min
    ThisWorkbook.SaveAs FileName:=nom & ″_CutMaxMin.txt″,
                   FileFormat:=xlCSVWindows
    ′Call CutByPercentileOld(0.25, 0.9, FlowColumn) ′cut flow
    ′Call CutByPercentileOld(0.25, 0.9, CO2Column) ′cut CO2
    Call CutByPercentile(0.25, 0.9, FlowColumn, 0.25, 0.9, CO2Column)
    ThisWorkbook.SaveAs FileName:=nom & ″_CutByPercentile.txt″,
                   FileFormat:=xlCSVWindows
    Call ClearBadChambers_And_Average
  Application.ScreenUpdating = True
```

```
  Application.StatusBar = ″Ready″
End Function
-----------------------------------------------------------
Function CutMaxMin(FlowMax As Integer, FlowMin As Integer, CO2Max As Integer,
              CO2Min As Integer)
      Application.StatusBar = ″Cutting : Flow > ″ & FlowMax & ″ and < ″ & FlowMin
                      & ″ ;  CO2 >″ & CO2Max & ″ and < ″ & CO2Min
      nl = ActiveWorkbook.ActiveSheet.UsedRange.Rows.Count:  ′number of data
          rows in file
     Dim cell As Range
     For Each cell In Range(Cells(1, CO2Column), Cells(nl, CO2Column))
      If cell.Value < CO2Min Or cell.Value > CO2Max Then
        Cells(cell.Row, 3).Clear: ′ (column C) : mark for later deletion
      End If
      If Cells(cell.Row, ChamberColumn).Value <> 750 Then
        ′True chamber (non-zero), so check for flow rate as well :
        If Cells(cell.Row, FlowColumn).Value < FlowMin Or Cells(cell.Row,
             FlowColumn).Value > FlowMax Then
          Cells(cell.Row, 3).Clear: ′ (column C) : mark for later deletion
        End If
      End If
    Next
    ′ move invalid rows (=cleared in column C) to the end of the file, using the ′sort′
         function :
    ActiveSheet.UsedRange.Sort    Key1:=Range(″B1″),    Key2:=Range(″C1″),
     Order1:=xlAscending, Header:=xlGuess, OrderCustom:=1, MatchCase:=False,
     Orientation:=xlTopToBottom
    LastValidLine = Range(″C1″).End(xlDown).Row
    Range(Cells(LastValidLine + 1, 1), Cells(nl, 1)).EntireRow.Delete:  ′ delete
     invalid rows (= those that are cleared in column C)
  Application.StatusBar = ″Ready″
End Function
-----------------------------------------------------------
```

```
Function ClearBadChambers_And_Average()
    Dim cell As Range, CurrChamber As Integer, NumberOfDataPoints As Integer,
          FirstRowOfChamber As Integer
    nl = ActiveWorkbook.ActiveSheet.UsedRange.Rows.Count: ′number of data rows in
          file
    Application.StatusBar = ″Discard chambers that have less than 5 valid data points and
          compute average for those that are left″
   ChamberColumn = 4
   CO2Column = 5
   FlowColumn = 6
    CurrChamber = Cells(1, ChamberColumn).Value
    NumberOfDataPoints = 1
    FirstRowOfChamber = 1
    For Each cell In Range(″D2:D″ &
ActiveWorkbook.ActiveSheet.UsedRange.Rows.Count + 1)
        If cell.Value + 0 = CurrChamber Then
            NumberOfDataPoints = NumberOfDataPoints + 1: ′count number of data points
                                                                            for current chamber
        Else: ′new chamber
            If NumberOfDataPoints < 5 Then
                Range(Cells(FirstRowOfChamber, 3), Cells(cell.Row - 1, 3)).Clear: ′
                    (column C) : mark for later deletion
            Else: ′compute averages in first row, then prepare deletion of next rows :
                Cells(FirstRowOfChamber, FlowColumn).Value = WorksheetFunction.
                    Average(Range(Cells(FirstRowOfChamber, FlowColumn), Cells(cell.Row
                              - 1, FlowColumn)))
                Cells(FirstRowOfChamber, CO2Column).Value = WorksheetFunction.
                    Average(Range(Cells(FirstRowOfChamber, CO2Column), Cells(cell.Row
                              - 1, CO2Column)))
                If FirstRowOfChamber + 1 <= cell.Row - 1 Then
                    Range(Cells(FirstRowOfChamber + 1, 3), Cells(cell.Row - 1, 3)).Clear:
                              ′ (column C) : mark other rows of that chamber for later deletion
                End If
```

```
            End If
            CurrChamber = cell.Value: ′update current chamber information
            NumberOfDataPoints = 1
            FirstRowOfChamber = cell.Row
        End If
    Next
    ′ move invalid rows (=cleared in column C) to the end of the file, using the ′sort′ function :
    ActiveSheet.UsedRange.Sort Key1:=Range(″B1″), Key2:=Range(″C1″), Order1:=xlAscending, Header:=xlGuess, OrderCustom:=1, MatchCase:=False, Orientation:=xlTopToBottom
    LastValidLine = Range(″C1″).End(xlDown).Row
    Range(Cells(LastValidLine + 1, 1), Cells(nl, 1)).EntireRow.Delete:  ′ delete invalid rows (= those that are cleared in column C)
    Application.StatusBar = ″Ready″
End Function
------------------------------------------------------------
Function CutByPercentile(LowerFlow As Double, UpperFlow As Double, ColumnSetFlow As Integer, LowerCO2 As Double, UpperCO2 As Double, ColumnSetCO2 As Integer)
  ′ColumnSet 5=co2 6=flow
Dim TopRow As Integer, CurrentChamber As Integer, LowerPoint As Double, UpperPoint As Double
Dim cell As Range, R As Range, cell2 As Range
nl = ActiveWorkbook.ActiveSheet.UsedRange.Rows.Count:  ′number of data rows in file
Application.StatusBar = ″Cut by percentile, flow : low=″ & LowerFlow & ″  up=″ & UpperFlow & ″ ; CO2 : low=″ & LowerCO2 & ″  up=″ & UpperCO2
If LowerFlow < 0 Or LowerFlow > 1 Or UpperFlow < 0 Or UpperFlow > 1 Or LowerCO2 < 0 Or LowerCO2 > 1 Or UpperCO2 < 0 Or UpperCO2 > 1 Then
    MsgBox (″lower and upper must between 0-1″): Exit Function
End If
CurrChamber = Cells(1, ChamberColumn).Value
    TopRow = 1
```

```
For Each cell In Range("D2:D"
 & ActiveWorkbook.ActiveSheet.UsedRange.Rows.Count + 1)
   If cell.Value + 0 <> CurrentChamber Then
      'End of current chamber, so Cut for Percentile / CO2 (reserve for later deletion):
      Set R = Range(Cells(TopRow, ColumnSetCO2), Cells(cell.Row - 1,
            ColumnSetCO2))
      LowerPoint = Excel.WorksheetFunction.Percentile(R, LowerCO2)
      UpperPoint = Excel.WorksheetFunction.Percentile(R, UpperCO2)
      For Each cell2 In R
         If cell2.Value < LowerPoint Or cell2.Value > UpperPoint Then
            Cells(cell2.Row, 3).Clear: ' (column C) : mark for later deletion
         End If
      Next
      'check for chamber number and Cut for Percentile / Flowrate (reserve for later
            deletion) if non-zero (true chamber) :
      If Cells(cell.Row, ChamberColumn).Value <> 750 Then
         Set R = Range(Cells(TopRow, ColumnSetFlow), Cells(cell.Row - 1,
            ColumnSetFlow))
         LowerPoint = Excel.WorksheetFunction.Percentile(R, LowerFlow)
         UpperPoint = Excel.WorksheetFunction.Percentile(R, UpperFlow)
         For Each cell2 In R
            If cell2.Value < LowerPoint Or cell2.Value > UpperPoint Then
               Cells(cell2.Row, 3).Clear: ' (column C) : mark for later deletion
            End If
         Next
      End If
     CurrentChamber = cell.Value: 'update current chamber information
      TopRow = cell.Row
   End If
Next
'move invalid rows (=cleared in column C) to the end of the file, using the 'sort'
     function :
```

```
    ActiveSheet.UsedRange.Sort Key1:=Range(″B1″), Key2:=Range(″C1″), Order1:=xlAscending, Header:=xlGuess, OrderCustom:=1, MatchCase:=False, Orientation:=xlTopToBottom
    LastValidLine = Range(″C1″).End(xlDown).Row
    Range(Cells(LastValidLine + 1, 1), Cells(nl, 1)).EntireRow.Delete: ′ delete invalid rows (= those that are cleared in column C)
    Application.StatusBar = ″Ready″
End Function
```

Macro file : ClearDataAverage_et_Temperature_to_ASC

```
----------------------------------------------------------
Sub Make_ASC()
Dim d As Single, StartTime As Double, l As Integer, voie As Integer, t As Double, l_Temperature As Double
′ ThisWorkbook.ActiveSheet.EnableCalculation = False
repdef_Resp$ = ″C:\siemens\19chamber\rawdata1\average″: ′ To be updated according to actual computer configuration
rep_Resp$ = InputBox(″Name of folder containing the respiration data files to be processed :″, ″Folder definition (1)″, repdef_Resp$) & ″\″
repdef_Tpr$ = ″C:\weather\temp_chamber″: ′ To be updated according to actual computer configuration
rep_Tpr$ = InputBox(″Name of folder containing the temperature data files to be processed :″, ″Folder definition (2)″, repdef_Tpr$) & ″\″
′ check consistency between both folders (in Unakorn′s folder organization system):
l = InStr(UCase(rep_Resp$), ″_TO_″): plage$ = UCase(Mid$(rep_Resp$, l - 2, 14))
If InStr(UCase(rep_Tpr$), plage$) = 0 Or Mid$(plage$, 13, 1) <> ″0″ Then
    MsgBox ″Inconsistent or faulty folder names !:″ & Chr(13) & Chr(13) & rep_Resp$ & Chr(13) & rep_Tpr$, vbExclamation
    Stop: End
End If
′ and extract initial and final days to be processed :
InitDay$ = Left$(plage$, 2) & Right$(plage$, 5): FinalDay$ = Mid$(plage$, 7, 2) & Right$(plage$, 5)
```

```
Select Case Mid$(plage$, 10, 3)
    Case ″JAN″: mm$ = ″01″: Add = 0
    Case ″FEB″: mm$ = ″02″: Add = 31
    Case ″MAR″: mm$ = ″03″: Add = 59
    Case ″APR″: mm$ = ″04″: Add = 90
    Case ″MAY″: mm$ = ″05″: Add = 120
    Case ″JUN″: mm$ = ″06″: Add = 151
    Case ″JUL″: mm$ = ″07″: Add = 181
    Case ″AUG″: mm$ = ″08″: Add = 212
    Case ″SEP″: mm$ = ″09″: Add = 243
    Case ″OCT″: mm$ = ″10″: Add = 273
    Case ″NOV″: mm$ = ″11″: Add = 304
    Case ″DEC″: mm$ = ″12″: Add = 334
    Case Else: MsgBox Mid$(plage$, 10, 3) & ″ : Bad month name″, vbExclamation:
Stop: End
End Select
For d = Val(Left$(InitDay$, 2)) To Val(Left$(FinalDay$, 2))
ThaiDay = DateValue(CStr(d) & ″/″ & Mid$(InitDay$, 3, 3) & ″/″ &
CStr(Right(InitDay$, 2)) + 43)
chainecourte = Right$(″0″ & CStr(d), 2) & ″_″ & mm$ & ″_″ & Right$(plage$, 2)
NomTemperature$ = rep_Tpr$ & chainecourte & ″.txt″
ChaineLongue = CStr(d) & ″_″ & CStr(Val(mm$)) & ″_20″ & Right$(plage$, 2)
NomResp$ = rep_Resp$ & ChaineLongue & ″_ClearDat_Average.txt″
′ For d = 37567 To 37572: ′ Initial version for 7 through 12 November, Gregorian
calendar
′ ChaineCourte = Format(d, ″dd_mm_yy″): NomTemperature$ = rep_Tpr$ &
ChaineCourte & ″.txt″
′ ChaineLongue = Format(d, ″d_m_yyyy″): NomResp$ = rep_Resp$ & ChaineLongue &
″_ClearDat_Average.txt″
If Dir(NomResp$) <> ″″ Then
    Workbooks.OpenText FileName:=NomResp$, DataType:=xlDelimited, Comma:=True,
        Space:=False, FieldInfo:=Array(Array(1, 1), Array(2, 3), Array(3, 1), _
        Array(4, 1), Array(5, 1), Array(6, 1))
```

```
    NR = ActiveWorkbook.Name
    nl = ActiveWorkbook.ActiveSheet.UsedRange.Rows.Count: 'number of respiration data
       lines
    'Correct for decimal separator (maybe unnecessary for non-French versions of
Windows/Excel) :
    If Format(-1.1) = "-1,1" Then
        ActiveSheet.Range(Cells(1, 3), Cells(nl, 8)).Replace what:=".", replacement:=",",
          lookat:=xlPart, searchorder:=xlByRows, MatchCase:=False
    End If
    If ActiveWorkbook.ActiveSheet.Range("B1").Value <> ThaiDay Then MsgBox "Day
       Mismatch ! (" & chainecourte & ")": Stop: End
    If Dir(NomTemperature$) <> "" Then
        Workbooks.OpenText FileName:=NomTemperature$, DataType:=xlDelimited,
          Space:=False, Tab:=True, consecutivedelimiter:=False
        NT = ActiveWorkbook.Name
        nlt = ActiveWorkbook.ActiveSheet.UsedRange.Rows.Count: 'number of temperature
          data lines
        'Correct for decimal separator (maybe unnecessary for non-French versions of
          Windows/Excel) :
        If Format(-1.1) = "-1,1" Then
            ActiveSheet.Range(Cells(2, 1), Cells(nlt, 25)).Replace what:=".",
          replacement:=",", lookat:=xlPart, searchorder:=xlByRows, MatchCase:=False
        End If
        'Stop
        If ActiveWorkbook.ActiveSheet.Range("A2").Value <> d + Add Then MsgBox "Day
          Mismatch ! (" & ChaineLongue & ")": Stop: End
        'Stop
        StartTime = ActiveWorkbook.ActiveSheet.Range("B2").Value: StartTime_$ =
          Right(Space$(10) & Str(StartTime), 4)
        StartTime = Val(Left$(StartTime_$, 2)) / 24 + Val(Right$(StartTime_$, 2)) /
          (24 * 60): ' should be 00:00 with a few exceptions
    Else: NT = ""
    End If
```

```
For l = 1 To nl
    'Stop
    ThisWorkbook.ActiveSheet.Cells(l + 7, 1).Value = Left$(chainecourte, 2) & "/" & Mid$(chainecourte, 4, 2) & "/" & Right$(chainecourte, 2)
    'Stop
    t = Workbooks(NR).ActiveSheet.Cells(l, 3).Value: ' current time (as fraction of 1 day)
    ThisWorkbook.ActiveSheet.Cells(l + 7, 2).Value = t
    voie = (Workbooks(NR).ActiveSheet.Cells(l, 4).Value - 750) / 750: If voie > 18 Then voie = 18
    ThisWorkbook.ActiveSheet.Cells(l + 7, 3).Value = voie
    ThisWorkbook.ActiveSheet.Cells(l + 7, 5).Value = 0.007781 * Workbooks(NR).ActiveSheet.Cells(l, 5).Value - 148.758:  'CO2
    ThisWorkbook.ActiveSheet.Cells(l + 7, 4).Value = 0.00032 * Workbooks(NR).ActiveSheet.Cells(l, 6).Value:   'FlowRate
     l_Temperature = 2 + 24 * 60 * (t - StartTime) / 10: ' theoretical line number (in temperature file) for current time (normally fractional)
    If l_Temperature < 2 Or l_Temperature > nlt + 1 Or NT = "" Then
        ThisWorkbook.ActiveSheet.Cells(l + 7, 7).Formula = "=na()"
        ThisWorkbook.ActiveSheet.Cells(l + 7, 8).Formula = "=na()"
    Else
        IL = Int(l_Temperature): DeltaT = l_Temperature - IL
        T1 = (1 - DeltaT) * Workbooks(NT).ActiveSheet.Cells(WorksheetFunction.min(IL, nlt), 3).Value
        T2 = DeltaT * Workbooks(NT).ActiveSheet.Cells(WorksheetFunction.min(IL + 1, nlt), 3).Value
        ThisWorkbook.ActiveSheet.Cells(l + 7, 7).Value = T1 + T2:  ' external temperature
        Select Case voie
            Case 0, 1, 2: nc = voie + 3: ' zero, A1 & A2
            Case 4, 5: nc = voie + 2:  ' B1A< & B1B>
            Case 7: nc = 8:  ' B2A>
            Case 10: nc = 9: ' B2B<
```

```
        Case 12: nc = 10: ' C1A<
        Case 14, 15: nc = voie - 3:  ' C1B< & C2A>
        Case 17: nc = 13:  ' C2B>
        Case Else: nc = 0: ' no temperature available for chamber
      End Select
      If nc = 0 Then
        T3 = 0: T4 = 0
      Else
        T3 = (1 - DeltaT) * Workbooks(NT).ActiveSheet.Cells(IL, nc).Value
        T4 = DeltaT * Workbooks(NT).ActiveSheet.Cells(WorksheetFunction.min(IL
          + 1, nlt), nc).Value
      End If
      ThisWorkbook.ActiveSheet.Cells(l + 7, 8).Value = T3 + T4:  ' temperature
          chambre
    End If
  Next l
'ThisWorkbook.ActiveSheet.EnableCalculation = True
ThisWorkbook.SaveAs FileName:=rep_Resp$ & chainecourte & ".asc",
FileFormat:=xlCSVWindows
ThisWorkbook.ActiveSheet.Range("A8:J1000").EntireRow.Delete
If NT <> "" Then Workbooks(NT).Close savechanges:=False
Workbooks(NR).Close savechanges:=False

End If
'Stop
Next d
End Sub
```

Macro file : Siemens-Resp-Template

```
------------------------------------------------------------
Sub FileCreation()
Dim i As Integer, cell As Range, CurrentLine As Integer, CurrentTime(-1 To 19) As
Double, NewTime As Double, PrevTime As Double, PrevDay As Single
```

```
For i = 1 To 21: ThisWorkbook.Worksheets(i).EnableCalculation = False: Next
CurrentLine = 7
repdef$ = "C:\Data.r\1106": sep$ = "\":  ' To be updated according to actual computer
configuration
rep$ = InputBox("Name of folder containing the daily ASC files:", "Creation of Excel
Respiration Data file", repdef$)
'Stop
'-----------------------------------------------------------
' Appends all ASC files into one single file, named "total.txt":
  fich1$ = Dir(rep$ & sep$ & "*.asc")
If fich1$ <> "" Then
    FileCopy rep$ & sep$ & fich1$, rep$ & sep$ & "total.txt"
    Open rep$ & sep$ & "total.txt" For Append As #1
Else: MsgBox "This folder contains no ASC files!", vbOKOnly, "File creation : ERROR
          !!!": End
End If
fich1$ = Dir()
While fich1$ <> ""
        Open rep$ & sep$ & fich1$ For Input As #2
        For i = 1 To 7: Line Input #2, ligne$: Next: ' ignore header lines
        While Not EOF(2): Line Input #2, ligne$: Print #1, ligne$: Wend
        Close #2
        fich1$ = Dir()
Wend: Close #1
'-----------------------------------------------------------
'Opens the resulting single ASC file as an Excel Worksheet :
Workbooks.OpenText Filename:=rep$ + sep$ + "total.txt", DataType:=xlDelimited,
Tab:=True, comma:=True, Space:=False, _
                              FieldInfo:=Array(Array(1, 4), Array(2, 1), Array(3, 1),
Array(4, 1), Array(5, 1), Array(6, 1), Array(7, 1), Array(8, 1)), _
                              consecutivedelimiter:=False
tl = Workbooks("total.txt").ActiveSheet.UsedRange.Rows.Count
'Stop
```

```
′------------------------------------------------------------
′ sort lines according to date & time (first 2 columns):
Range(Cells(8, 1), Cells(tl, 8)).Sort Key1:=Range(″A8″), Order1:=xlAscending,
Key2:=Range(″B8″), Order2:=xlAscending, Orientation:=xlTopToBottom
tl = Workbooks(″total.txt″).ActiveSheet.UsedRange.Rows.Count
′
′Stop
′------------------------------------------------------------
′------------------------------------------------------------
′Correct for decimal separator (maybe unnecessary for non-French versions of
Windows/Excel) :
   If Format(-1.1) = ″-1,1″ Then
      Workbooks(″total.txt″).ActiveSheet.Range(Cells(8, 3), Cells(tl, 8)).Replace
what:=″.″, replacement:=″,″, lookat:=xlPart, searchorder:=xlByRows, MatchCase:=False
  ′For Each cellule In Workbooks(″total.txt″).ActiveSheet.Range(Cells(8, 3), Cells(tl, 8))
      ′If Not IsNumeric(cellule.Value) Then cellule.Value = Val(cellule.Value)
      ′Next
   End If
′Stop
′------------------------------------------------------------
′complete missing individual rows (removed by previous macro data management due to
bad measurement)
′Prev_i = Workbooks(″total.txt″).ActiveSheet.Cells(8, 3).Value - 1:
PrevTime = Workbooks(″total.txt″).ActiveSheet.Cells(8, 2).Value - 3 / (60 * 24):
CurrentLine = 8
While CurrentLine < tl
   NewTime = Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine, 2).Value

   ′Stop
      If Abs(NewTime - PrevTime) > 4.5 / (60 * 24) Then
      PrevDay = Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine - 1, 1).Value
      ′ 4.5 mn = 3 mn (normal time lag between 2 successive rows) + 1.5 mn
(tolerance)
```

```
        If Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine, 1).Value <> PrevDay Then
            ′ new day, so add/remove 24 h (=1 jour):
            PrevTime = PrevTime - 1: ′ and redo time comparison :
            If NewTime - PrevTime > 4.5 / (60 * 24) Then
                Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine, 1).EntireRow.Insert: tl
                    1 + tl
                NewTime = PrevDay + 1 + PrevTime + 3 / (60 * 24): ′ updating (jour +
                    ure)
                Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine, 1).Value =
                    (NewTime)
                NewTime = NewTime - Int(NewTime)
                Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine, 2).Value = NewTime
                i = Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine - 1, 3).Value + 1:
If i = 19 Then i = -1
                Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine, 3).Value = i
                For i = 4 To 8: Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine,
                    Formula = ″=na()″: Next i
            End If
        Else: ′same day
            Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine, 1).EntireRow.Insert:
              tl = tl
            NewTime = PrevTime + 3 / (60 * 24): ′ updating heure
            Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine, 1).Value = PrevDay
            Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine, 2).Value = NewTime
            i = Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine - 1, 3).Value + 1: If i
                    = 19 Then i = -1
            Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine, 3).Value = i
            For i = 4 To 8: Workbooks(″total.txt″).ActiveSheet.Cells(CurrentLine,
                    i).Formula = ″=na()″: Next i
        End If
    End If
    PrevTime = NewTime
    ′Stop
```

```
   CurrentLine = 1 + CurrentLine
Wend
'Stop
'--------------------------------------------------------
'--------------------------------------------------------
' Correct for invalid data :
    '((to check for actual threshold values to be used in current configuration of
        experiment)) :
     ' IRGA threshold values (ppm CO2) :
   'ThresholdLow = -49: ThresholdHigh = 95
   ' For Each cellule In Workbooks("total.txt").ActiveSheet.Range(Cells(8, 5), Cells(tl,
        5))
   '    If cellule.Value > ThresholdHigh Or cellule.Value < ThresholdLow Then
        cellule.FormulaR1C1 = "=na()"
    'Stop
   ' Next
   ' FlowRate threshold values (l per minute) -- don't check for Zero channel:
   'ThresholdLow = 0.2: ThresholdHigh = 1.8
   ' For Each cellule In Workbooks("total.txt").ActiveSheet.Range(Cells(8, 4), Cells(tl,
        4))
   '    If Range(Cells(cellule.Row, 3), Cells(cellule.Row, 3)).Value <> 0 Then
   '        If cellule.Value > ThresholdHigh Or cellule.Value < ThresholdLow Then
                  cellule.FormulaR1C1 = "=na()"
   '    End If
   ' Next
 '-------------------------------------------------------
CurrentLine = 7
CurrentTime(19) = Workbooks("total.txt").ActiveSheet.Range("A8").Value +
Workbooks("total.txt").ActiveSheet.Range("b8").Value - 20 / 480
CurrentTime(1) = CurrentTime(19) + 1 / 480: For i = 2 To 18: CurrentTime(i) =
CurrentTime(i - 1) + 1 / 480: Next
CurrentTime(-1) = CurrentTime(18) + 1 / 480
'Copy DaisyLab File Header to each sheet:
```

```
Workbooks(″total.txt″).ActiveSheet.Range(″A1:A6″).Copy
ThisWorkbook.Activate
Sheets(Array(″A1″, ″A2″, ″B1A>″, ″B1A<″, ″B1B>″, ″B1B<″, ″B2A>″, ″B2A<″, ″B2B>″,
″B2B<″, ″C1A>″, ″C1A<″, ″C1B>″, ″C1B<″, ″C2A>″, ″C2A<″, ″C2B>″, ″C2B<″,
″zer″)).Select
Range(″A8″).Select: ActiveSheet.Paste
Application.CutCopyMode = False
Range(″A1″).Select: Sheets(″A2″).Select: Sheets(″A1″).Activate
Workbooks(″total.txt″).Activate
′-------------------------------------------------------------
′Dispatch original data lines among Excel sheets according to Column C (″voie″), i.e.
Chamber #.:
′Curr_i = 19: CurrTime = 0
For Each cell In Workbooks(″total.txt″).ActiveSheet.Range(″C8:C″ & tl)
    Application.Goto Range((″A″ & cell.Row)), True: ′displays current line of composite
ASCII file (with date:time) under processing
    i = cell.Value: If i = 0 Then i = 19: ′ sheet number = chamber number (exception :
          zero)
    ′ Test integrity of recorded data by DaisyLab software:
    If cell.Value <> Int(cell.Value) Then MsgBox ″′Voie′ data (chamber number) is not
          Integer in File ′total.txt′, line ″ & cell.Row, vbOKOnly + vbExclamation, ″Data
          Record file error″: End
    ′Sheets(i).Select
    NewTime = Workbooks(″total.txt″).ActiveSheet.Range((″A″ & cell.Row)).Value +
Workbooks(″total.txt″).ActiveSheet.Range((″B″ & cell.Row)).Value
    If Abs(NewTime - CurrentTime(i) - 20 / 480) > 1 / 480 Then
         ′2 successive measurements for chamber # i are separated by a time shift other than
          60 minutes (with a tolerance of +- 3 minutes)
        If i = -1 Or (i = 18 And Cells(cell.Row - 1, 3).Value = 18) Or (i = 18 And
          Abs(NewTime - CurrentTime(i) - 21 / 480) < 1 / 480) _
                    Or (i = 18 And Abs(NewTime - CurrentTime(i) - 19 / 480) < 1 /
                              480) Then
            ′do nothing
```

```
  ElseIf i <> 19 Then
    Stop
    MsgBox ″New DaisyLab run started with channel other than Zero″, vbOKOnly +
        vbExclamation, ″Data Record file error″: Stop
  Else: ′ DaisyLab was restarted, so first line is Zero ; duplicate latest Zero line, skip
        line, and update current time in Excel File:
    ThisWorkbook.Sheets(19).Range(″A″ & CurrentLine, ″G″ & CurrentLine).Copy
        Destination:=ThisWorkbook.Sheets(19).Range(″A″ & (CurrentLine + 1))
    ThisWorkbook.Sheets(19).Range(″N″ & CurrentLine + 1, ″O″ & CurrentLine +
        1).Formula = ″=na()″
    CurrentLine = CurrentLine + 2
    ThisWorkbook.Sheets(19).Range(″M9:O9″).Copy
   Destination:=ThisWorkbook.Sheets(19).Range(″M″ & CurrentLine)
    CurrentTime(1) = NewTime - 19 / 480: For i = 2 To 18: CurrentTime(i) =
        CurrentTime(i - 1) + 1 / 480: Next
  End If
Else
  Select Case i
  Case -1: ′do nothing
  Case 19: CurrentLine = CurrentLine + 1: ′ increment line for new 20-channel cycle
    ThisWorkbook.Sheets(i).Range(″M9:O9″).Copy
   Destination:=ThisWorkbook.Sheets(i).Range(″M″ & CurrentLine): ′copy formulas
  Case Else
    ThisWorkbook.Sheets(i).Range(″H9:Q9″).Copy
   Destination:=ThisWorkbook.Sheets(i).Range(″H″ & CurrentLine): ′copy formulas
   (will be overwritten in column H by chamber T°C if available)
  End Select
End If
CurrentTime(i) = NewTime
Select Case i
  Case -1: ′do nothing
  Case 3, 6, 8, 9, 11, 13, 16, 18, 19: ′ these chambers (or zero) have no chamber
   T°C data, so keep estimation formula in column H
```

```
          Workbooks("total.txt").ActiveSheet.Range("A" & cell.Row, "G" &
          cell.Row).Copy Destination:=ThisWorkbook.Sheets(i).Range("A" & CurrentLine)
     Case Else: 'these chambers (or zero) have a chamber T°C data, so copy it (column H)
            Workbooks("total.txt").ActiveSheet.Range("A" & cell.Row, "H" &
          cell.Row).Copy Destination:=ThisWorkbook.Sheets(i).Range("A" & CurrentLine)
    End Select
Next
' remove irrelevant extrapolated temperature data (for 'zero' and chambers that have no
chamber T°C data)
ThisWorkbook.Sheets(19).Range("A" & CurrentLine, "G" & CurrentLine).Copy
Destination:=ThisWorkbook.Sheets(19).Range("A" & (CurrentLine + 1))
ThisWorkbook.Sheets(19).Range("N" & CurrentLine + 1, "O" & CurrentLine +
1).Formula = "=na()"
'Stop
For i = 3 To 18
    Select Case i
        Case 3, 6, 8, 9, 11, 13, 16, 18
            ThisWorkbook.Sheets(i).Range("H8").Formula = "=na()"
        Case Else
            ThisWorkbook.Sheets(i).Range("Q8").Formula = "=na()"
            For Each cell In ThisWorkbook.Sheets(i).Range("H9:H" & CurrentLine)
                If IsEmpty(cell) Then cell.Formula = "=na()"
            Next
    End Select
    For Each cell In ThisWorkbook.Sheets(i).Range("Q9:Q" & CurrentLine)
        If IsEmpty(cell) Then cell.Formula = "=na()"
    Next
Next
For i = 1 To 21: ThisWorkbook.Worksheets(i).EnableCalculation = True: Next
'Stop
ThisWorkbook.SaveAs (rep$ + sep$ + "total.xls")
Workbooks("total.txt").Close (0)
End Sub
```

Macro file : Summary

```
---------------------------------------------------------
Option Explicit
Global jour As Date, jour0 As Date, jour1 As Date, fich As Variant, u_f As Integer, i As Integer, j As Integer, jj As Integer, k As Integer, m As Integer
Global LastLine As Long, l As Long, SummaryLastLine As Long, heure As Single, Q10 As Double, r As Double, rep$, sep$, nom$
Global NumColSimple(1 To 5) As Integer, NumColTriple(1 To 5) As Integer: ' numeros des colonnes sources dans 'xxxx.xls', et des colonnes "24h" in ThisWorkbook
Global Valeurs As Variant, cumul() As Double, NbValid() As Integer
---------------------------------------------------------
Sub DayToDaySynthesis()
' Inventaire des fichiers Respiration à traiter:
jour0 = ThisWorkbook.Sheets(1).Cells(6, 1): 'First day of data in ThisWorkbook
fich = Application.GetOpenFilename(FileFilter:="Excel Workbook (*.xls),*.xls", Title:="Select respiration files (date >= " & Format(jour0, "dd/mm/yy") & ") to process for synthesis (multiple selection allowed)", MultiSelect:=True)
If VarType(fich) = 11 Then End: ' boolean 'False' : user has cancelled
u_f = UBound(fich)
rep$ = CurDir: sep$ = "\"
For j = 1 To 18: ' 18 worksheets / chambers
   ThisWorkbook.Sheets(j).EnableCalculation = False
Next j
'opens successively all individual data files (xxxx.XLS') that have been selected :
For i = 1 To u_f
Workbooks.Open (fich(i))
nom$ = ActiveWorkbook.Name
For j = 1 To 18: ' 18 worksheets / chambers
   SummaryLastLine = ThisWorkbook.Sheets(j).UsedRange.Rows.Count:
   SummaryLastLine = ThisWorkbook.Sheets(j).Cells(SummaryLastLine + 1, 1).End(xlUp).Row
   Valeurs = ThisWorkbook.Sheets(j).Range("d7:av" & SummaryLastLine).Value
```

```
    Workbooks(nom$).Sheets(j).EnableCalculation = False
    Workbooks(nom$).Worksheets(j).Activate
    LastLine = ActiveSheet.UsedRange.Rows.Count: LastLine = Cells(LastLine + 1,
          1).End(xlUp).Row
′**** COMPUTE DAILY AVERAGES for actual respiration at Actual chamber T°C : ***
    NumColSimple(1) = 8: NumColSimple(3) = 10: NumColTriple(1) = 4:
NumColTriple(3) = 40
    ′compute 3 (day, night, 24h) value pairs (Resp.rate & T°C) ; here night vs. day data
          are already defined in hourly file
        jour1 = Cells(8, 1).Value: ′ initialisation du jour courant
′ initialisation des compteurs : NbValid(k,m) = nb de couples (R, T) pris en compte ;
          m=1: R ou T valide ; m=2: R et T tous 2 valides ;
        ′ cumul(k,m) = cumul des valeurs de chaque variable (m=1: T ; m=2: R) pour les
          couples (R, T) /  R et T tous 2 valides ;
        ′ indices  k :  1 = daytime (8:15 AM to 4:15 PM) ; 2 = nighttime (8:15 PM to
          4:15 AM) ; 3 = ′twilight′ (4:15 to 8:15 AM  &  4:15 to 8:15 PM) :
        ReDim Temperature1(1 To 30) As Variant, Respir1(1 To 30) As Variant,
          Temperature2(1 To 30) As Variant, Respir2(1 To 30) As Variant:
        ReDim Temperature3(1 To 30) As Variant, Respir3(1 To 30) As Variant,
          cumul(1 To 3, 1 To 2) As Double, NbValid(1 To 3, 1 To 2) As Integer
For l = 8 To LastLine
            Application.StatusBar = ″Computing averages for actual T°C : k = ″ & k & ″ ; l =
                    ″ & l
            If VarType(Cells(l, 10)) = 5 Or VarType(Cells(l, 8)) = 5 Then
                ′ at least one of both cell values is valid (type = double, not empty, not error),
                    so go on :
                jour = Cells(l, 1).Value: heure = Cells(l, 2).Value
                If jour < jour1 Then
                    Stop: ′erreur
                Else If jour > jour1 Then
                 ′ new day : for each of the 3 day parts, check whether there are enough values
                              for previous day, and if so, compute average(s) :
```

```
    Call EnterDayCoupledAverages(Respir1, Respir2, Respir3, Temperature1,
              Temperature2, Temperature3)
    jour1 = jour: ' reset compteurs for new day :
    ReDim Temperature1(1 To 30) As Variant, Respir1(1 To 30) As Variant,
              Temperature2(1 To 30) As Variant, Respir2(1 To 30) As
              Variant:
    ReDim Temperature3(1 To 30) As Variant, Respir3(1 To 30) As Variant,
              cumul(1 To 3, 1 To 2) As Double, NbValid(1 To 3, 1 To 2) As
              Integer
End If
' in any case, update arrays :
If heure > 8.25 / 24 And heure < 16.25 / 24 Then
    ' daytime (8:15 AM to 4:15 PM) :
    NbValid(1, 1) = NbValid(1, 1) + 1
    If VarType(Cells(l, 10)) = 5 Then
        Respir1(NbValid(1, 1)) = Cells(l, 10).Value
        If VarType(Cells(l, 8)) = 5 Then
            Temperature1(NbValid(1, 1)) = Cells(l, 8).Value
            NbValid(1, 2) = NbValid(1, 2) + 1: cumul(1, 1) = cumul(1, 1) +
              Cells(l, 8).Value: cumul(1, 2) = cumul(1, 2) + Cells(l,
                        10).Value
        End If
    Else: ' so, VarType(Cells(l, 8) = 5
        Temperature1(NbValid(1, 1)) = Cells(l, 8).Value
    End If
ElseIf heure < 4.25 / 24 Or heure > 20.25 / 24 Then
    ' nighttime (8:15 PM to 4:15 AM) :
    NbValid(2, 1) = NbValid(2, 1) + 1
    If VarType(Cells(l, 10)) = 5 Then
        Respir2(NbValid(2, 1)) = Cells(l, 10).Value
        If VarType(Cells(l, 8)) = 5 Then
            Temperature2(NbValid(2, 1)) = Cells(l, 8).Value
```

```
              NbValid(2, 2) = NbValid(2, 2) + 1: cumul(2, 1) = cumul(2, 1) +
                Cells(l, 8).Value: cumul(2, 2) = cumul(2, 2) + Cells(l,
                10).Value
            End If
          Else: ' so, VarType(Cells(l, 8) = 5
            Temperature2(NbValid(2, 1)) = Cells(l, 8).Value
          End If
        Else: 'twilight' (4:15 to 8:15 AM  &  4:15 to 8:15 PM) :
          NbValid(3, 1) = NbValid(3, 1) + 1
          If VarType(Cells(l, 10)) = 5 Then
            Respir3(NbValid(3, 1)) = Cells(l, 10).Value
            If VarType(Cells(l, 8)) = 5 Then
              Temperature3(NbValid(3, 1)) = Cells(l, 8).Value
              NbValid(3, 2) = NbValid(3, 2) + 1: cumul(3, 1) = cumul(3, 1) +
                Cells(l, 8).Value: cumul(3, 2) = cumul(3, 2) + Cells(l,
                10).Value
            End If
          Else: ' so, VarType(Cells(l, 8) = 5
            Temperature3(NbValid(3, 1)) = Cells(l, 8).Value
          End If
        End If
      End If
    Next l
    Call EnterDayCoupledAverages(Respir1, Respir2, Respir3, Temperature1,
     Temperature2, Temperature3): ' last day in file
'*** COMPUTE DAILY AVERAGES for outside T°C & extrapolated respiration  : ***
  NumColSimple(1) = 7: NumColSimple(2) = 11: NumColSimple(3) = 12
  NumColTriple(1) = 7: NumColTriple(2) = 43: NumColTriple(3) = 46
  For k = 1 To 3: ' 3 (T°out, Resp_T°out, Resp_20°) groups of 3 average values
     (24h, daytime, nighttime) to derive according to time
    jour1 = Cells(8, 1).Value: ' initialisation du jour courant
```

```
    ReDim cumul(1 To 3) As Double, NbValid(1 To 3) As Integer: 'initialisation des
      compteurs : cumul() = valeurs à moyenner ; NbValid() = nb de valeurs prises en
      compte
          ' indices : 1 = daytime (8:15 AM to 4:15 PM) ; 2 = nighttime(8:15 PM
          to 4:15 AM) ; 3 = 'twilight' (4:15 to 8:15 AM  &  4:15 to 8:15 PM)
    For l = 8 To LastLine
        Application.StatusBar = "Computing averages for extrapolated T°C values : k = "
                & k & " ; l = " & l
        If VarType(Cells(l, NumColSimple(k))) = 5 Then
            ' cell value is valid (type = double, not empty, not error), so go on :
            jour = Cells(l, 1).Value: heure = Cells(l, 2).Value
            If jour < jour1 Then
                    Stop: 'erreur
            ElseIf jour = jour1 Then
                If heure > 8.25 / 24 And heure < 16.25 / 24 Then
                    cumul(1) = cumul(1) + Cells(l, NumColSimple(k)).Value:
                          NbValid(1) = NbValid(1) + 1: ' daytime
                ElseIf heure < 4.25 / 24 Or heure > 20.25 / 24 Then
                    cumul(2) = cumul(2) + Cells(l, NumColSimple(k)).Value:
                          NbValid(2) = NbValid(2) + 1: ' nighttime
                Else: cumul(3) = cumul(3) + Cells(l, NumColSimple(k)).Value:
                          NbValid(3) = NbValid(3) + 1: 'twilight'
                End If
            Else: ' new day : check whether there are enough values for previous day, and
                          if so, compute averages :
                Call EnterDayAverages
            End If
        End If
    Next l
    Call EnterDayAverages:    ' last day in file
Next k
'Stop
```

```
'****** COMPUTE DAILY VALUES FOR hour-to-hour Q10 : *************
'in the following, indices 1 & 2 are still for day- & night-time, resp., but index 3 is now
for 24h-based calculation
    NumColSimple(1) = 14: NumColSimple(2) = 15: NumColSimple(3) = 16:
    NumColTriple(1) = 36: NumColTriple(2) = 38: NumColTriple(3) = 34
    For k = 1 To 3:  ' compute 3 (day, night, 24h) value pairs (Q10 and correl.) ; here
      night vs. day data are already defined in hourly file
    jour1 = Cells(8, 1).Value: ' initialisation du jour courant
    ReDim temperature(1 To 30) As Variant, Respir(1 To 30) As Variant: m = 0:
'initialisation des compteurs (to do at each day shift)
    For l = 8 To LastLine
        Application.StatusBar = "Computing Q10 : k = " & k & " ; l = " & l
        If VarType(Cells(l, NumColSimple(k))) = 5 And VarType(Cells(l, 8)) = 5
        Then
            ' both cell values are valid (type = double, not empty, not error), so go on :
            jour = Cells(l, 1).Value
            If jour < jour1 Then
                Stop: 'erreur
            ElseIf jour = jour1 Then
                m = m + 1
            Else: ' new day : check whether there are enough values for previous day, and
                if so, compute Q10 and r :
                If m >= 3 Then
                    If WorksheetFunction.Max(temperature) -
                          WorksheetFunction.Min(temperature) > 0.5 Then
                        Q10 = Exp(10 * WorksheetFunction.Slope(Respir, temperature))
                        r = WorksheetFunction.Correl(Respir, temperature)
                        Valeurs(jour1 - jour0, NumColTriple(k) - 3) = Q10
                        Valeurs(jour1 - jour0, NumColTriple(k) - 2) = r
                    End If
                End If
                jour1 = jour: ' reset for new day :
```

```
                ReDim temperature(1 To 30) As Variant, Respir(1 To 30) As Variant: m
                        = 1
              End If
              ' in any case, update arrays :
              temperature(m) = Cells(l, 8).Value: Respir(m) = Cells(l,
                NumColSimple(k)).Value
            End If
        Next l
        ' last day in file :
        If m >= 3 Then
            Q10 = Exp(10 * WorksheetFunction.Slope(Respir, temperature))
            r = WorksheetFunction.Correl(Respir, temperature)
            Valeurs(jour1 - jour0, NumColTriple(k) - 3) = Q10
            Valeurs(jour1 - jour0, NumColTriple(k) - 2) = r
        End If
    Next k
    Application.StatusBar = "Thinking for a few seconds..."
    ThisWorkbook.Sheets(j).Range("d7:av" & SummaryLastLine).Value = Valeurs
Next j
'****** COMPUTE DAILY AVERAGES for respiration rate differences between chambers
on the same tree : ******
'Workbooks(nom$).Sheets(23).EnableCalculation = False
'Workbooks(nom$).Worksheets(23).Activate
Workbooks(nom$).Sheets("within-tree-diff.").EnableCalculation = False
Workbooks(nom$).Worksheets("within-tree-diff.").Activate
    For j = 19 To 26: ' 8 worksheets in this workbook
  SummaryLastLine = ThisWorkbook.Sheets(j).UsedRange.Rows.Count: SummaryLastLine
= ThisWorkbook.Sheets(j).Cells(SummaryLastLine + 1, 1).End(xlUp).Row
  Valeurs = ThisWorkbook.Sheets(j).Range("d7:am" & SummaryLastLine).Value
  LastLine = ActiveSheet.UsedRange.Rows.Count: LastLine = Cells(LastLine + 1,
        1).End(xlUp).Row
  For jj = 1 To 2:  ' 2 trees
```

```
For k = 1 To 3: ' 3 (ActualResp_T°chamber, Resp_T°out, Resp_20°) groups of 3
   average values (24h, daytime, nighttime) to derive according to time
  NumColSimple(k) = 3 * j + 12 * jj + k - 65 - 12 * (j > 22)
  NumColTriple(k) = 13 + 6 * k + 3 * jj
  jour1 = Cells(8, 1).Value: ' initialisation du jour courant
  ReDim cumul(1 To 3) As Double, NbValid(1 To 3) As Integer: 'initialisation des
        compteurs : cumul() = valeurs à moyenner ; NbValid() = nb de valeurs
        prises en compte
              ' indices :  1 = daytime (8:15 AM to 4:15 PM) ; 2 = nighttime
             (8:15 PM to 4:15 AM) ; 3 = 'twilight' (4:15 to 8:15 AM  &
             4:15 to 8:15 PM)
  For l = 8 To LastLine
    Application.StatusBar = "Computing diff. between paired chambers : tree # " & jj
        & " ; k = " & k & " ; l = " & l
    If VarType(Cells(l, NumColSimple(k))) = 5 Then
      ' cell value is valid (type = double, not empty, not error), so go on :
      jour = Cells(l, 1).Value: heure = Cells(l, 2).Value
      If jour < jour1 Then
          Stop: 'erreur
      ElseIf jour = jour1 Then
        If heure > 8.25 / 24 And heure < 16.25 / 24 Then
          cumul(1) = cumul(1) + Cells(l, NumColSimple(k)).Value:
             NbValid(1) = NbValid(1) + 1: ' daytime
        ElseIf heure < 4.25 / 24 Or heure > 20.25 / 24 Then
          cumul(2) = cumul(2) + Cells(l, NumColSimple(k)).Value:
             NbValid(2) = NbValid(2) + 1: ' nighttime
        Else: cumul(3) = cumul(3) + Cells(l, NumColSimple(k)).Value:
             NbValid(3) = NbValid(3) + 1: 'twilight'
        End If
      Else: ' new day : check whether there are enough values for previous day, and
             if so, compute averages :
        Call EnterDayAverages
      End If
```

```
          End If
        Next l
        Call EnterDayAverages:    ' last day in file
    Next k
    'Stop
  Next jj
  Application.StatusBar = "Thinking for a few seconds..."
  ThisWorkbook.Sheets(j).Range("d7:am" & SummaryLastLine).Value = Valeurs
Next j
Workbooks(nom$).Close (0)
Next i
ThisWorkbook.Activate
For j = 1 To 18: ' 18 worksheets - chambers
    ThisWorkbook.Sheets(j).Activate
    ThisWorkbook.Sheets(j).EnableCalculation = True
    Range("J6:AG6").Copy Destination:=Range("J7:J" & SummaryLastLine)
    Range("J7:AG" & SummaryLastLine).SpecialCells(xlCellTypeFormulas,
xlErrors).ClearContents
Next j
For j = 19 To 26: ' 8 worksheets - diff. resp. rates betwen paired chambers
    ThisWorkbook.Sheets(j).Activate
    ThisWorkbook.Sheets(j).EnableCalculation = True
    Range("D6:U6").Copy Destination:=Range("D7:D" & SummaryLastLine)
    Range("D7:U" & SummaryLastLine).SpecialCells(xlCellTypeFormulas,
          xlErrors).ClearContents
Next j
Range("A1").Activate
ThisWorkbook.Sheets(1).Select
ThisWorkbook.Save
Application.StatusBar = "Done ! :-)"
 End Sub
-----------------------------------------------------------------
Function EnterDayAverages()
```

```
        For m = 1 To 2: ' day- and night-time averages are computed only if there are
                at least 3 valid data :
            If NbValid(m) >= 3 Then
                cumul(m) = cumul(m) / NbValid(m): ' replace cumul by average
                Valeurs(jour1 - jour0, NumColTriple(k) + m - 3) = cumul(m)
            End If
        Next
        ' replace 'twilight' cumul by average, then compute grand average, only if there
                are at least 3 valid data for each time range :
        If NbValid(1) >= 3 And NbValid(2) >= 3 And NbValid(3) >= 3 Then
            cumul(3) = cumul(3) / NbValid(3)
            Valeurs(jour1 - jour0, NumColTriple(k) - 3) = (cumul(1) + cumul(2) +
                cumul(3)) / 3
        End If
        If l <= LastLine Then
                ' reset for new day :
                ReDim cumul(3), NbValid(3): jour1 = jour
                If heure > 8.25 / 24 And heure < 16.25 / 24 Then
                    cumul(1) = Cells(l, NumColSimple(k)).Value: NbValid(1) = 1: '
                        daytime
                ElseIf heure < 4.25 / 24 Or heure > 20.25 / 24 Then
                    cumul(2) = Cells(l, NumColSimple(k)).Value: NbValid(2) = 1: '
                        nighttime
                Else: cumul(3) = Cells(l, NumColSimple(k)).Value: NbValid(3) = 1:
                        'twilight'
                End If
        End If
End Function
-----------------------------------------------------------------
Function EnterDayCoupledAverages(Respir1() As Variant, Respir2() As Variant,
Respir3() As Variant, Temperature1() As Variant, Temperature2() As Variant,
Temperature3() As Variant)
    If NbValid(1, 2) >= 3 Then
```

```
        Valeurs(jour1 - jour0, 2) = cumul(1, 1) / NbValid(1, 2)
        Valeurs(jour1 - jour0, 38) = cumul(1, 2) / NbValid(1, 2)
    ElseIf WorksheetFunction.Count(Respir1) >= 3 Then
        Valeurs(jour1 - jour0, 38) = WorksheetFunction.Average(Respir1)
    ElseIf WorksheetFunction.Count(Temperature1) >= 3 Then
        Valeurs(jour1 - jour0, 2) = WorksheetFunction.Average(Temperature1)
    End If
    If NbValid(2, 2) >= 3 Then
        Valeurs(jour1 - jour0, 3) = cumul(2, 1) / NbValid(2, 2)
        Valeurs(jour1 - jour0, 39) = cumul(2, 2) / NbValid(2, 2)
    ElseIf WorksheetFunction.Count(Respir2) >= 3 Then
        Valeurs(jour1 - jour0, 39) = WorksheetFunction.Average(Respir2)
    ElseIf WorksheetFunction.Count(Temperature2) >= 3 Then
        Valeurs(jour1 - jour0, 3) = WorksheetFunction.Average(Temperature2)
    End If
     If NbValid(1, 2) >= 3 And NbValid(2, 2) >= 3 And NbValid(3, 2) >= 3 Then
        Valeurs(jour1 - jour0, 1) = (Valeurs(jour1 - jour0, 2) + Valeurs(jour1 - jour0,
          3) + cumul(3, 1) / NbValid(3, 2)) / 3
        Valeurs(jour1 - jour0, 37) = (Valeurs(jour1 - jour0, 38) + Valeurs(jour1 -
          jour0, 39) + cumul(3, 2) / NbValid(3, 2)) / 3
    ElseIf WorksheetFunction.Count(Respir1) >= 3 And
          WorksheetFunction.Count(Respir2) >= 3 And WorksheetFunction.Count(Respir3)
          >= 3 Then
        Valeurs(jour1 - jour0, 37) = (WorksheetFunction.Average(Respir1) +
          WorksheetFunction.Average(Respir2)+ WorksheetFunction.Average(Respir3)) / 3
    ElseIf WorksheetFunction.Count(Temperature1) >= 3 And
          WorksheetFunction.Count(Temperature2) >= 3 And
          WorksheetFunction.Count(Temperature3) >= 3 Then
        Valeurs(jour1 - jour0, 1) = (WorksheetFunction.Average(Temperature1) +
          WorksheetFunction.Average(Temperature2) +
          WorksheetFunction.Average(Temperature3)) / 3
    End If
End Function
```