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Temporal and structural relations within bark and trunk in *Hevea brasiliensis* Muell. Arg. (Euphorbiaceae): Physiological maturity index of bark and latex vessels

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Abstract

In Africa and particularly in Côte d'Ivoire, the exploitation of *Hevea brasiliensis* clones having strong vegetative growth based on the criterion of vigour adopted so far has raised physiological and physical problems characterized by a high rate of tapping panel dryness and wind damage. A study was conducted on three clones of *Hevea brasiliensis* (PB 235, GT 1 and PR 107) belonging respectively to classes of fast, moderate and slow vegetative growth, in order to determine the right moment for an exploitation which would minimize those drawbacks. Trunk measurements, bark collecting and histological sections followed by laticifers counting made on rubber trees aged from one to fifteen years, have enabled to describe the process of establishment of bark and laticifers. The intensity of development and thickening of the bark and the rate of laticifer emission are described respectively by distinct hyperbolic and logistic sigmoid functions. However, their temporal evolution is strongly marked by an irreversible decrease of the whole process of formation, whatever the clone, from six years after planting. Furthermore, the density of laticifers per mm^2 switches, whatever the clone studied, from a number greater than 5, the first six years ($5\text{-}8 < \Delta_{lv} < 2$) to 0.35 the next 25 years ($2 < \Delta_{lv} < 0.4$). This evolution which is very significant the first six years varies relatively little the rest of time. These results show six years after planting a major phenologic phenomenon, like a physiological maturity, which occurs within the tree. This study has allowed identifying good indicators for determining the age and/or the time when plantations should be tapped in *Hevea brasiliensis*. These relationships have certain and practical interests insofar as they will allow to determine the maturity of exploitation for plantations which age is unknown by using only a bark gauge to measure bark thickness and a measuring tape to measure the girth.

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Introduction

The animal and/or plant living kingdom is governed by a certain number of relations whom knowledge allows to better exploit the potentialities of that kingdom. Rubber tree is not an exception to that rule, all the more since its yielding mode is particular from the other vegetables from which yielding are fruits, tubers, roots (Templeton, 1969). Moreover, in that species, maturity is not exteriorized; at least, it's not obvious. Rational and optimal exploitation of *Hevea brasiliensis* require so, more than in the major part of its homologous of the plant kingdom, knowledge and control of the setting and functioning of the main organs involved in the processing of cis-polyisoprene, the source of natural rubber production (Jacob *et al*., 1988; Sekha, 1989), the main resource expected from rubber tree cultivation.

Therefore, every part of rubber tree (roots, trunk, branches and leaves) is concerned by these investigations, as laticifers are found in these organs at any age (Meunier, 1912; Bobilioff, 1923; Riches and Gooding, 1952; Dickenson, 1969; Gomez, 1982, 1975; Hébant and Fay, 1980). However, the works of Bobilioff (1923), Gomez (1982), Gomez (1975) and Compagnon (1986) have shown that the trunk of rubber tree (2.50 m above the ground) is the part which has the highest latex extraction yield and which is easier to manage. The presence of latex at any age in the bark of the trunk rises up the problem of the ideal moment to start tapping. This tapping moment put on appearance an important character as Gomez (1975) has shown that laticifers switch from juvenility to old age, via maturity. But as rubber yielding is highly energy and photosynthetate demanding (Le Bras, 1953; Templeton, 1969; Wycherley, 1976), the exploitation of the rubber-producing tissue should be made, without damage on the physiological state of the trees, only at maturity of this tissue and laticifers that it bears. Indeed, the current criterion for first tapping that is, arbitrary and based on vigour (Compagnon, 1986) and which allow, to exploit rubber trees having 50 cm girth at one meter above the ground level has some deficiencies. Fast-growing clones (PB 235, IRCA 18, etc.) show physico-physiological constraints, such as dry tapping panel dryness and wind damage (Premakumari, 1991; CIRAD-CP, 1993, Obouayeba and Boa, 1993; Jacob *et al*., 1994; Dian 1993; Dian *et al*., 1995) more important than those of moderate-growing or slow-growing clones (GT 1, PB 217, PR 107, etc.). The recent works of Obouayeba *et al*., (2000a) shown that fast-growing clones are precociously exploited (tapped). But Templeton (1969), Gohet (1996) have already indicated that precocious exploitation is prejudicial to further productions. Indeed, precocity in tapping provokes a strong reduction in radial vegetative growth during tapping (Obouayeba and Boa, 1993; Obouayeba *et al*., 2002), and a high rate of tapping panel dryness (Dian, 1993), Dian *et al*. (1995) leading to less and less sustained yielding from the 5th exploitation campaign (Templeton, 1969; Ouattara, 1998).

The research of criteria more relevant than the current one becomes then necessary; and is justified. Indeed, Obouayeba *et al*. (2000a,b) have already determined the age, notably the tapping at 6 years after planting as the criterion susceptible to solve this problem (Obouayeba *et al*., 2002). Furthermore, it is not excluded that some relations, between time and structural organs of the trunk can also contribute to elucidate the problem, allowing thus to determine, for an unknown age, the maturity of the bark and/or those organs.

To solve this problem and make the exploitation of rubber tree more efficient, a study on some structural and temporal relations at the level of the bark and trunk of rubber tree has been carried out. The present paper sums up the results of this study which concerned three clones of *Hevea brasiliensis*; PB 235; GT 1 and PR 107, grown on the whole rubber cultivation area of Côte d'Ivoire.

Material and methods

Description of the study region

The study was conducted in 1999 to 2004, in rubber growing region located in, south-eastern (4°75'-5°75'N, 3-6°W), south-western and centre-western (4-5°50'N, 6-9° W) of Côte d'Ivoire (Fig. 1). This region is characterized by the bimodal rainfall regime, with two rainy seasons and the average annual rainfall reaches 1600 mm. The soil is acidic (4 < pH ≤ 5) ferralitic, deep, very desaturated on tertiary sand in the south-eastern and on metamorphic sediments in the south-western and centre-western. This region was previously under rubber tree cultivation for more than 30 years (Fig. 1).

Table 1. Girth (cm) of *Hevea brasiliensis* clones PB 235, GT 1 and PR 107 at 1.70 m height

Clone	Years										
	5	6*	7	8	9	10	11	12	13	14	15
PB 235	51,0	54,5	58,1	62,4	65,3	68,6	70,3	73,0	75,4	78,2	80,9
GT 1	41,9	47,5	50,8	54,1	56,6	59,2	61,9	64,7	67,3	69,4	72,0
PR 107	39,6	46,8	49,9	52,7	55,4	58,0	60,8	63,3	65,8	67,9	70,1

* The clone PB 235 started being tapped at the beginning of 6th year after planting at about 51 cm girth)

Plant material

The study deals with *Hevea brasiliensis* clones belonging to three different vegetative growing classes, PB 235 (Fast-growing), GT 1 (moderate-growing) and PR 107 (Slow-growing) (Obouayeba *et al.*, 2000a). Theses clones were grown in the plantations established in South, West and Centre-West Côte d'Ivoire (Fig. 1).

Table 2. Total number of latex vessels according to the age and clones of *Hevea brasiliensis*

Clones	Years										
	1	2	3	4	5	6	7	8	9	10	11	15
PB 235	1064	1841	3310	-	5161	6866	-	8252	8755	9181	9731	117500
GT 1	773	1550	2737	3734	5038	5845	-	8053	8314	-	8840	10362
PR 107	637	1250	2147	3548	4415	5867	5989	6516	6999	8202	8613	-

Hevea brasiliensis, species belonging to the Euphorbiaceae family (Schultes, 1977, 1987; Carron, 1989) is a superior plant. In the inner part, its trunk consists of wood surrounded with bark. The bark, seat of exploitation, consists of many structures among which latex vessels (laticifers), specialised cells producing latex (Obouayeba *et al.* 2002; Obouayeba *et al.*, 2000b; Premakumari *et al.*, 1991; Gomez, 1982, 1975; Bobilioff, 1923). They are produced by cambium and organised in concentric sleeves around the wood (Gomez, 1982). *Hevea brasiliensis* is the highest rubber-yielding among the ten species of the genus *Hevea* (H. *brasiliensis*, H. *benthamiana*, H. *camargoana* H. *camporum*, H. *guianensis*, H. *microphylla*, H. *nitida*, H. *pauciflora*, H. *rigidifolia*, H. *spruceana*) (Schultes, 1970, 1977; Compagnon, 1986; Webster and Paardekooper, 1989).

Fig. 1. Study localities.

Location of plantations and sampling method of trees and bark collected

The sampling was made by age, 1 to 31 years, and by clone, whatever the rubber growing area in Côte d'Ivoire. The vegetative growth model of Obouayeba *et al.*, (2000a); Girth=Me$^{-b/(age-a)}$, served as standard for the selection of untapped trees aged 1 to 6 years.

In tapped trees aged from 7 to 31 years, a model adjusted according to Gohet (1996) and taking into account the growth reductions associated with tapping (Obouayeba *et al.*, 2002) was employed (Table 1). The girth of tapped and untapped trees was measured at a height of 1.70 m, followed by the harvesting at the same height, of a portion of bark. Two bark samples of 2 cm x 3 cm were collected per tree in diametrically opposite positions (Fig. 2). The sampling was carried out per age on 20 trees, that is, 40 bark samples per year of cultivation.

Table 3. Mean square of regression and residual of the variance analysis of different growth models,

Model	Analytic expression	Mean square	
		Regression	Residual
Johnson-Schumacher	$Y=M\ e^{[-b/(x-a)]}$	$269.9\ 10^6$	$14.5\ 10^6$
Gaussien	$Y = M\ [1-e^{(-((x-a)/b)2)}]$	$223.1\ 10^6$	$13.1\ 10^4$
Gompertz	$Y = M\ e^{[-Exp(-(x-a)/b)]}$	$223.1\ 10^6$	$11.4\ 10^4$
Logistic	$Y = M/[1+e^{(-(x-a)/b)}]$	$222.7\ 10^6$	$24.9\ 10^4$

Operative mode of section, staining, visualization-counting and photograph of bark samples

The bark samples obtained by frozen section technique were preserved in a fixative solution, the F.A.A. (Formalin Acetic Acid). It consists of 10 ml of 40 % formalin, 50 ml of 95° alcohol, 5 ml of glacial acetic acid and 35 ml of distilled water. After fixation, the material (bark) was rinsed in different baths of alcohol for 15 minutes. It was then rehydrated through three baths of tap water. The bark sample is then dissected as follows:

- In cross section planes which make the count possible so as to estimate the total amount of latex vessels in the tree
- In radial longitudinal sections which show latex vessels under the form of vertical beams and enables to determine the number of latex vessels rows from outer bark to cambium.

The sections were made with the freezing microtome (mark OSI) set at 90 to 240 µm thickness. They were stained with Sudan III (oil-red) determined as 0.25 g oil red and 5 ml of acetic acid.

Fig. 2. Collecting a portion of bark for histological study. The picture shows the collecting of a portion of bark, the other portion is obtained in opposite position that is to say at the same height on the other side.

This preparation was boiled and after cooling, we added 100 ml of 90° alcohol. The mixture was set to rest for 24 hours in darkness, and then filtered for use. The sections were selected and placed in distilled water with staining, on glass slide and covered with a glass cover for systematic counting of latex vessels rows and/or preserved in laevulose syrup for photographs. These sections are observed with a phase-contrast optical microscope (mark Zeiss) with zoom 4. The photomicrographs were made in normal light microscope equipped with filters and zoom 4 and 16 for easier views, the final zooms were respectively 16 and 64.

Studied parameters

For each bark sample (size = thickness of bark x 5 mm x 5 mm), the thickness of the bark was measured and expressed in mm (Th_b); the number of latex vessels rings in radial longitudinal section (n_{ls}), the density of latex vessels rings per sample in cross section and the density of latex vessels row per surface unit (Δ_{lv}) (number of latex vessels row/mm^2) was recorded; the total number of laticifers (latex vessels) in a tree at a certain time t (N,) (Table 2) was numbered. N_t, it is approximately assessed as n_{ls} f G (Premakumari and Panikkar, 1992) where, f, is the density of latex vessels per ring and G, the girth in cm.

Data analysis

The software SAS was used for the analysis of variances of different models. The proc-n lin programme was used by iterations in the value determination of parameters a, b and M.

The increment of organs is like vegetative growth, a phenomenon marked by an irreversible increase (Franquin, 1972; Heller, 1990) of volume of organs. Hence the functions and curves describing increments will obey the same criteria of choice of growth models and will be called increment models or functions or curves (Franquin, 1970). The growth model able to describe the increment such as latex vessels' increment is this of growth functions which are constant (Debouche, 1979; Burillon, 1990; Obouayeba *et al*., 2000). However, the model of Mitscherlich is excluded for its non-sigmoid pace (Debouche, 1979), non-representative of latex vessel increments. These latex vessel increments conformed to this of vegetative growth with sigmoid pace (Obouayeba *et al*., 2000). Only four of these models, such as Johnson-Schumacher, Gauss, Gompertz and logistic have been applied to the experimental results (Table 3).

Results

Relation between the inner structural organs (soft part) of the bark and time.

Model of bark constitution and thickening

The bark thickening of the clones studied is related to their vegetative growth class; for the same age, the thickening varies from one clone to another. The bark thickening modelized data show that the constitution and width growth of bark are a process, not linear, related to time by a hyperbolic relation having an analytical expression:

$Y = f(x) = A X / (B + X)$ where A and B are parameters, Y is the bark width and X the age of rubber trees. For the cones studied, this expression becomes (Fig. 3):

$Th_{b\ PB\ 235}$ = 16.14 age / (9.49 + age)

$Th_{b\ GT\ 1}$ = 14.11 age / (7.76 + age)

$Th_{b\ PR\ 107}$ = 16.99 age / (11.29 + age)

Table 4. Rate of latex vessel establishment in the Hevea clones according to Gausien, Logistic and Gompertz's models

Age	$V_{GAUSSIEN}$			$V_{LOGISTIC}$			$V_{GOMPERTZ}$		
	PB 235	**GT 1**	**PR 107**	**PB 235**	**GT 1**	**PR 107**	**PB 235**	**GT 1**	**PR 107**
1	756	706	620	543	416	436	709	642	556
2	909	923	827	702	596	605	906	913	809
3	1011	1061	961	865	808	794	1041	1096	985
4	1060	1117	1015	1007	1021	970	1101	1160	1052
5	1059	1098	998	1094	1180	1083	1090	1120	1019
6	1016	1018	923	1105	1230	1097	1025	1009	920
7	938	896	808	1036	1153	1005	927	864	787
8	838	753	674	906	977	840	812	713	648
9	725	605	536	745	760	650	694	572	519
10	609	466	408	583	553	474	582	449	406
11	497	345	298	438	383	330	481	348	313
12	394	245	209	320	256	224	392	266	239
13	305	168	141	228	168	148	317	202	180
14	229	111	91	160	108	97	255	152	136
15	168	70	57	112	69	63	204	114	101

The values of bark width permit to notice that at 6 years, the bark width ranges between 5.90 and 6.25 mm while at 15 years, it varies from 9.30 to 9.90 mm. It appears whatever the clone, a quick evolution of the bark until 6 years then a relative slowdown the rest of time. The bark constitution, inner and soft part of the vegetative biomass, has an evolution similar to this one of the vegetative total biomass (bark, wood, root, leaf, etc.), (Bobilioff, 1923; Heller, 1990).

Table 5. Mean square of regression and residual of the variance analysis of different growth models.

Model	Analytic expression	Mean square	
		Regression	Residu
Johnson-Schumacher	$Y = M\ e^{[-b/(x-a)]}$	10653.2	9.5
Gaussien	$Y = M\ [1-e^{(-((x-a)/b)2)}]$	10659.5	7.2
Gompertz	$Y = M\ e^{[-e(-(x-a)/b)]}$	10660.8	6.7
Logistic	$Y = M/[1+e^{(-(x-a)/b)}]$	10666.7	4.4

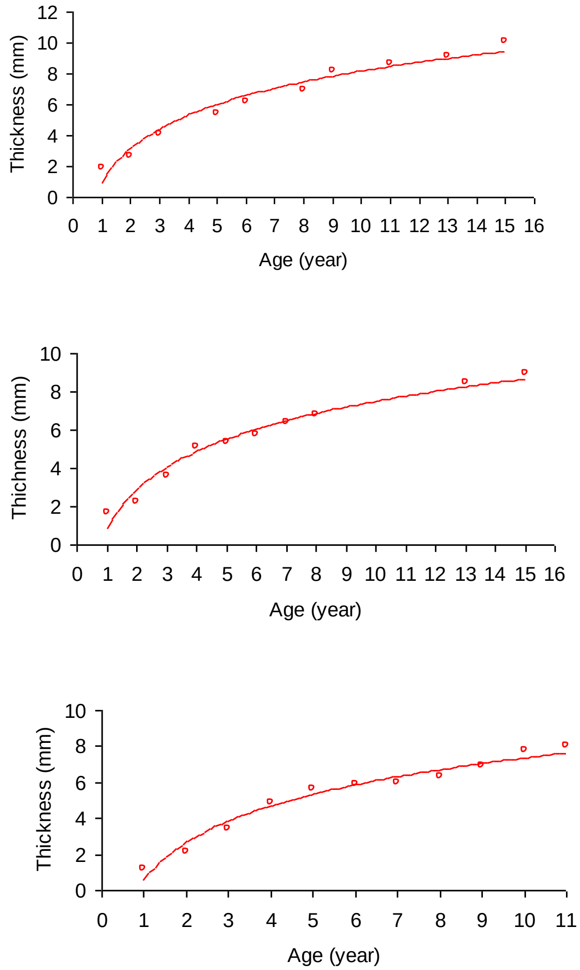

Fig. 3. Hyperbolic relationship between bark thickness and age of Hevea brasiliensis clones PB 235, GT 1 and PR 107.

Model of latex vessel formation and increment

The results of Table 2 showed that, excepted Johnson-Schumacher's model with higher residual error, the functions of Gompertz, Gauss and logistic can describe well the phenomenon of increment of latex vessels. The cumulative number of latex vessels and its increment at the maximal rate of latex vessels formation (Table 4) correspond to half of the total number of latex vessels observed during the study period. This means that the latex vessel increment's model have shown symmetry. Such evolution is that of a logistic function. Moreover, the logistic model allows a good interpretation of phenomenon observed during the development of latex vessels. Its analytical expression is:

$Y = f(X) = M/[1+e^{(-(x-a)/b)}]$

Concerning the 3 clones, from the results of table 2, the analytical expression of the logistic model and the growth of latex vessels is the following (Fig. 4):

PB 235 : $N_t = 11460 / [1 + Th^{-(age - 5.63)/2.58}]$

GT 1 : $N_t = 10598 / [1 + Th^{-(age - 5.89)/2.15}]$

PR 107 : $N_t = 9810 / [1 + Th^{-(age - 5.62)/2.22}]$

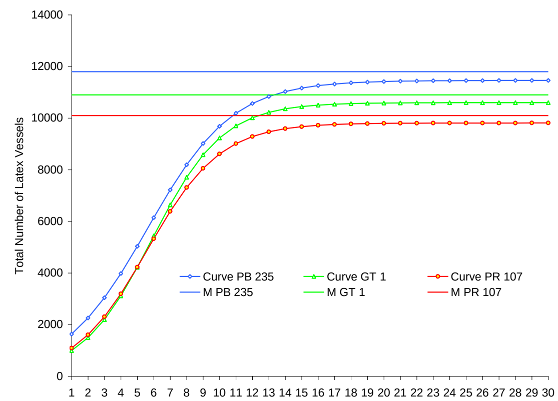

Fig. 4. Relationship between total number of latex vessels and age of Hevea brasiliensis clones PB 235, GT 1 and PR 107.

During the study period, it appears that the clone PB 235 forms, whatever the year, more latex vessels (11460 latex vessels maximum) than the GT 1 (10598 latex vessels maximum) which total number of latex vessels is superior to that of the clone PB 217 (10281 latex vessels maximum) which establishes more latex vessels than those of PR 107 (9810 latex vessels maximum).

Temporal evolution of latex vessels row in radial longitudinal section

In radial longitudinal section, the formation of latex vessels *(n_{ls})* (Fig. 5) is, a linear regression of time, expressed in age, regardless of the clone ($0.8890 < r \leq 0.9844$) (Fig. 6).

Density of latex vessels row and its temporal evolution

The number of latex vessels row, in cross section, enumerated on a unit surface of 1 mm² or density of latex vessels (Fig. 7) is in logarithmic relation with the age expressed in years. The general analytical expression, all clones put together, is presented as follows:

$\Delta_{lv} = a / (1 + \text{age}^{b})$ where a and b are parameters.

For the studied clones, we have the following analytical expressions (Fig. 8):

PB 235 $\Delta_{lv} = 10.43 / (1 + \text{age}^{0.85})$

GT 1 $\Delta_{lv} = 11.73 / (1 + \text{age}^{0.93})$

PR 107 $\Delta_{lv} = 16.84 / (1 + \text{age}^{1.16})$

Table 6. Area (mm2) occupied by a latex vessel according to bark age of Hevea brasiliensis clones PB 235, GT 1 and and PR 107 .

Clone Age	1	2	3	4	5	6	7	8	11	14	15	22
PB 235	0,189	0,259	-	-	-	-	0,533	0,574	0,679	0,776	0,776	0,956
GT 1	0,167	0,212	0,346	0,464	0,465	-	-	0,515	-	0,685	0,704	0,761
PR 107	0,113	0,198	0,321	0,471	0,476	0,528	-	-	0,562	-	-	0,810

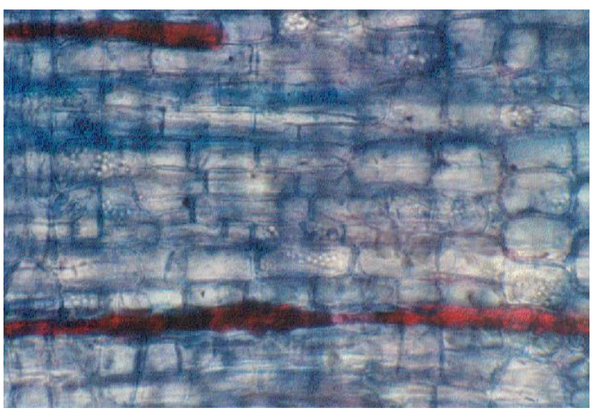

Fig. 5. Radial longitudinal section of bark of Hevea brasiliensis, clone PR 107). We can notice two latex vessels extended (red cells) surrounded by parenchyma (blue cells).

Physiological maturity index of bark and laticifers

Fig. 8 allows noticing that the density of laticifers per mm² shifts from a number superior to 5 in the first years to 0.35 around thirty years. This evolution is strongly marked during the first six years (5-8 < $\boldsymbol{\Delta_{lv}}$ < 2) while it varies slightly during the following 25 years 2< $\boldsymbol{\Delta_{lv}}$ < 0.4. The very low variation of this density on a quarter of century enables considering this variation as negligible. The point from which the variation in density of laticifers is negligible is characteristic. It indicates that the bark and laticifers will have reached an important phenologic stage; physiological maturity. The density of laticifers at this point is a "hinge" density between the two phases mentioned above. This density can be taken as an index of this physiological maturity. Let $I_{\mathbf{blvm}}$ be this index, when Δ_{lv}<2 latex vessels /mm² (Fig. 8) we admit then that this bark and laticifers it contains have reached physiological maturity.

Temporal occupation area of a latex vessel

The temporal distribution of the area (mm²) occupied by a latex vessels (table 4) is a symmetrical logistical function of its age expressed in years. Its general analytical expression is the following $\Psi_{lv} = M/[1 + e^{-(\text{age} - a)/b}]$ where M is the maximum area occupied by a laticifer, a and b are parameters. This relation applied to the three studied clones becomes (Fig. 9):

PB 235 $\Psi_{lv} = 0.92 / [1 + e^{-(\text{age} - 5.47)/4.44}]$

GT 1 $\Psi_{lv} = 0.83 / [1 + e^{-(\text{age} - 5.89)/4.22}]$

PR 107 $\Psi_{lv} = 0.87 / [1 + e^{-(\text{age} - 5.79)/5.23}]$

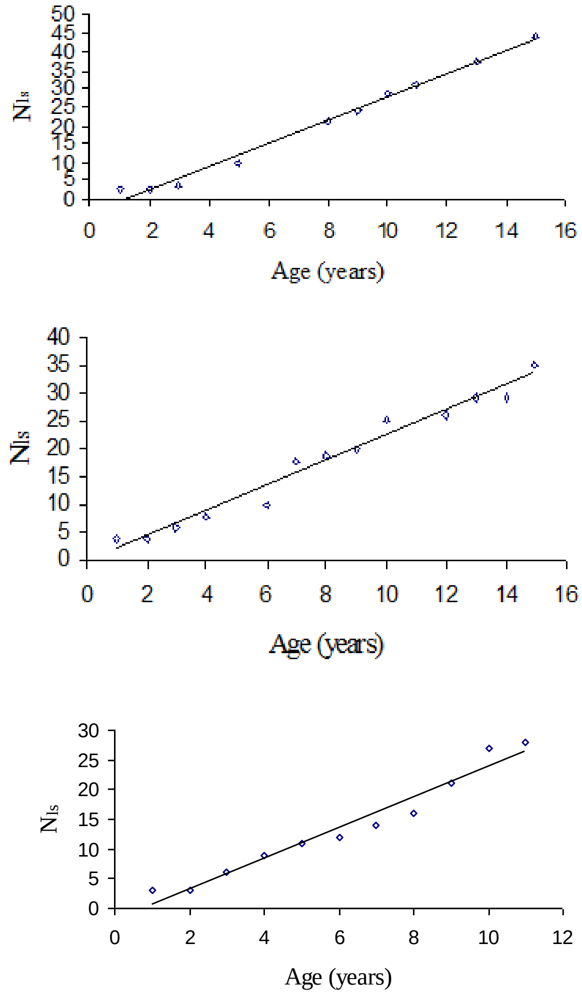

Fig. 6. Linear regression between radial longitudinal section number of latex vessels and age of Hevea brasiliensis. ***Very highly significant. Nls: Number of latex vessels in longitudinal radial section

Occupation speed of the space by a latex vessel

The derivatives of these logistical models or the space occupation speed functions of latex vessels are as such:

PB 235 $\Psi'_{lv} = 0.92\ e^{-(age - 5.47)/4.44} / 4.44\ [1 + e^{-(age - 5.47)/4.44}]^2$

GT 1 $\Psi'_{lv} = 0.83\ e^{-(age - 5.89)/4.22} / 4.22\ [1 + e^{-(age - 5.89)/4.22}]^2$

PR 107 $\Psi'_{lv} = 0.87\ e^{-(age - 5.79)/5.23} / 5.23\ [1 + e^{-(age - 5.79)/5.23}]^2$

Inflection point

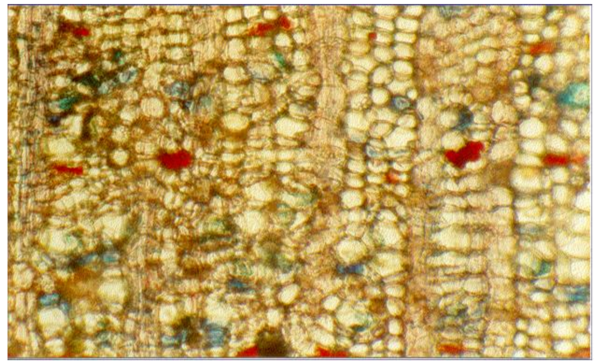

Fig. 7. Latex vessels of 4 years old (coloured in red) clone GT 1. Th_b = 4.9 mm x 5 mm width for 52 LV, that is Δ_{LV} = 2.12/mm², (1 cm) = 0.5 µm.

The inflection point or the point which presents a null value of the speed function derivative is obtained by the following expressions:

PB 235 $\Psi''_{lv} = V'_{lv} = 0 \Leftrightarrow 2 - [1 + e^{-(age - 5.47)/4.44}] = 0$

$\Leftrightarrow e^{-(age - 5.47)/4.44} = 1$

$\Leftrightarrow -(age - 5.47)/4.44 = 0$

$\Leftrightarrow -age + 5.47 = 0$

$\Leftrightarrow -age = 5.47$ years ;

that is 5 years 5 months 19 days

GT 1 $\Psi''_{lv} = V'_{lv} = 0 \Leftrightarrow 2 - [1 + e^{-(age - 5.89)/4.22}] = 0$

$\Leftrightarrow e^{-(age - 5.89)/4.22} = 1$

$\Leftrightarrow -(age - 5.89)/4.22 = 0$

$\Leftrightarrow -age + 5.89 = 0$

$\Leftrightarrow -age = 5.89$ years;

that is 5 years 10 months and 20 days

PR 107 $\Psi''_{lv} = V'_{lv} = 0 \Leftrightarrow 2 - [1 + e^{-(age - 5.79)/5.23}] = 0$

$\Leftrightarrow e^{-(age - 5.79)/5.23} = 1$

$\Leftrightarrow -(age - 5.79)/5.23 = 0$

$\Leftrightarrow -age + 5.79 = 0$

$\Leftrightarrow$ age = 5.79 years; that is 5 years 9 months and 14 days

The inflection point is located between 5 years 5 months and 5 years 11 months whatever the clone.

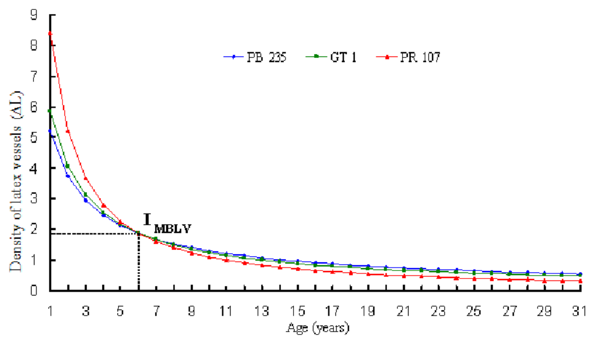

Fig. 8. Temporal evolution of latex vessels density of Hevea brasiliensis clones PR 107, GT 1 and PB 235. IMBLV: Maturity index of bark and latex vessels.

Relations between constituent organs of the trunk of Hevea brasiliensis

Girth of the trunk and bark thickness

The evolution of the trunk girth and bark thickness of *Hevea brasiliensis* allows looking for an eventual relation between these two organs. This relation exists indeed and is not linear because the growth speed of the girth of *Hevea brasiliensis* in time is not constant. Among the growth models described by Debouche 1979, four models (table 3) were tested in order to keep this one that best reflects the relation between girth and thickness. The symmetrical logistic model illustrates well the relation girth-thickness by its low residual value (Table 3). The analytical expressions of the studied clones are (Fig. 10):

$Girth_{PB\ 235} = 100.70 / [1 + e^{-(Th_B - 5.86)/2.16}]$

$Girth_{GT\ 1} = 91.66 / [1 + e^{-(Th_B - 5.72)/1.82}]$

$Girth_{PR\ 107} = 102.28 / [1 + e^{-(Th_B - 6.36)/2.23}]$

The derivatives of these logistical functions, or the increase in girth speed of the trunk in comparison with the thickness of bark, are:

$Girth'_{PB\ 235} = 100.70\ e^{-(Th_b - 5.86)/2.16} / 2.16\ [1 + e^{-(Th_B - 5.86)/2.16}]^2$

$Girth'_{GT\ 1} = 91.66\ e^{-(Th_b - 5.72)/1.82} / 1.82\ [1 + e^{-(Th_B - 5.72)/1.82}]^2$

$Girth'_{PR\ 107} = 102.28\ e^{-(Th_b - 6.36)/2.23} / 2.23\ [1 + e^{-(Th_B - 6.36)/2.23}]^2$

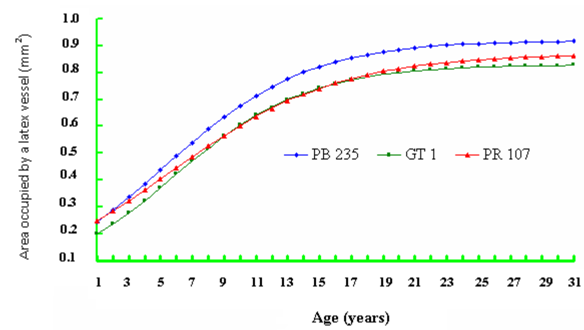

Fig. 9. Symmetrical logistic function of temporal evolution of the area occupied by a latex vessel of *Hevea brasiliensis*, clones PB 235, GT 1 and PR 107.

The symmetrical logistical function presents an inflection point that is obtained when the derivative of the speed function becomes null. For the 3 clones, this means that:

$Girth''_{PB\ 235} = V'_{Girth} = 0 \Leftrightarrow 2 - [1 + e^{-(Th_B - 5.86)/2.16}] = 0$

$\Leftrightarrow e^{-(Th_B - 5.86)/2.16} = 1 - (Th_B - 5.86) / 2.16 = 0$

$\Leftrightarrow - Th_B + 5/86 = 0$

$\Leftrightarrow Th_B = 5.86$ mm

$Girth''_{GT\ 1} = V'_{Girth} = 0 \Leftrightarrow 2 - [1 + e^{-(Th_B - 5.72)/1.82}] = 0$

$\Leftrightarrow e^{-(Th_B - 5.72)/1.82} = 1$

$\Leftrightarrow - (Th_B - 5.72) / 1.82 = 0$

$\Leftrightarrow - Th_B + 5.72 = 0$

$\Leftrightarrow Th_B = 5.72$ mm

$Girth''_{PR\ 107} = V'_{Girth} = 0 \Leftrightarrow 2 - [1 + e^{-(Th_B - 6.36)/2.23}] = 0$

$\Leftrightarrow e^{-(Th_B - 6.36)/2.23} = 1$

$\Leftrightarrow - (Th_B - 6.36) / 2.23 = 0$

$\Leftrightarrow - Th_B + 6.36 = 0$

$\Leftrightarrow -Th_B = 6.36$ mm

These results indicate that the girth growth with this one of a unit of bark thickness becomes null at 5.86, 5.72 and 6.36 mm of bark thickness respectively for the clones PB 235, GT 1 and PR 107.

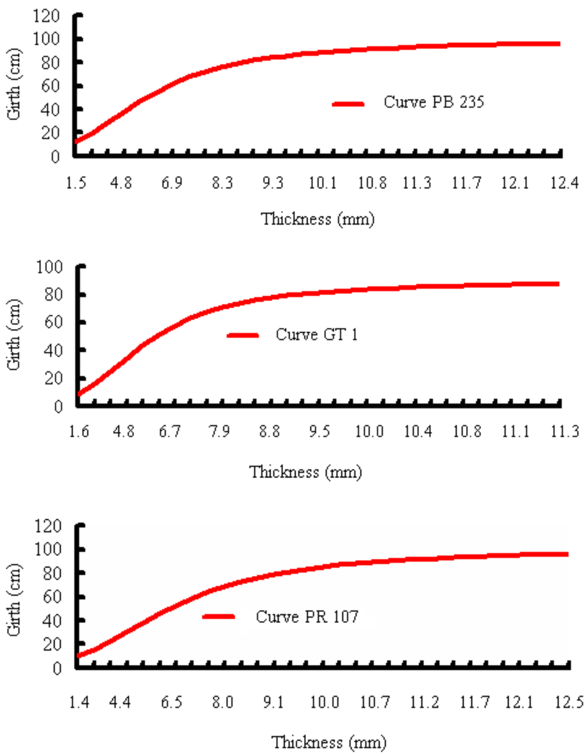

Fig. 10. Logistic model of the relation between girth and bark thickness of *Hevea brasiliensis,* clones PB 235, GT 1 and PR 107.

Girth and total number of latex vessels row

The total number estimated of latex vessels row (N_t) is linearly linked, whatever the clone (PB 235, GT 1, PB 217 or PR 107), to the girth. This relation with high coefficient of correlation ($0.9885 < r \leq 0.9930$) is illustrated in Fig. 11.

Bark thickness and total number of latex vessels row

The total number estimated of latex vessels (N_t) is linearly linked, whatever the clone (PB 235, GT 1, PB 217 or PR 107), to the bark thickness of rubber tree trunk. This link, with high coefficient of correlation ($0.9721 < r \leq 0.9906$), is represented in Fig. 12.

Bark thickness and latex vessels row in radial longitudinal section

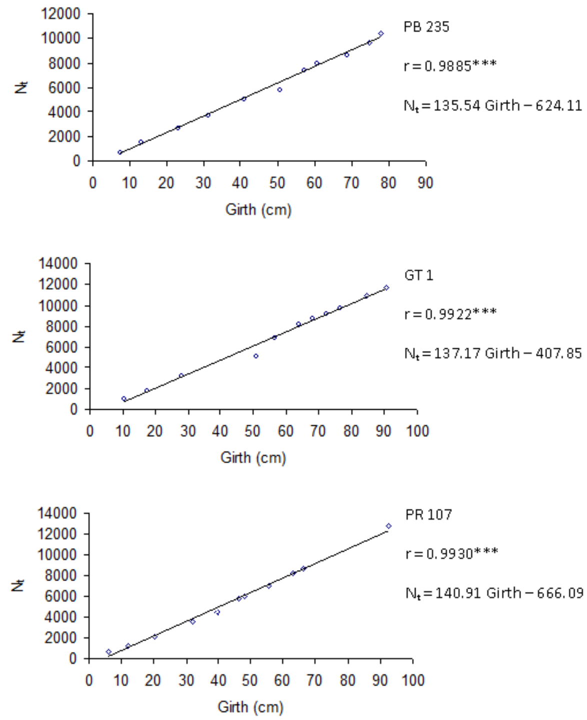

Fig. 11. Linear regression between total number of latex vessels and age.

N_t: Total Number of latex vessels of *Hevea brasiliensis* ; *** Very highly significant.

The number of latex vessels, in radial longitudinal section (n_{ls}), is, without clonal incidence, in a parabolic-type close relation with bark thickness (B_{Th}) ($r \leq 0.968$) (Fig. 13). At the opposite, no relation was evidenced between this histological criterion and the total number of laticifers (N_t).

Discussion

The aim of this study is to contribute to the determination of objective and relevant criteria for tapping *Hevea brasiliensis*. Temporal and inter-structural relations were established between the constituent organs of the bark, seat of tapping and time, on one hand, and the trunk, on the other hand. Some of these relations reveal characteristics which are susceptible to meet the preoccupations, purpose of the study, which identifies a priori the relations that will be analyzed.

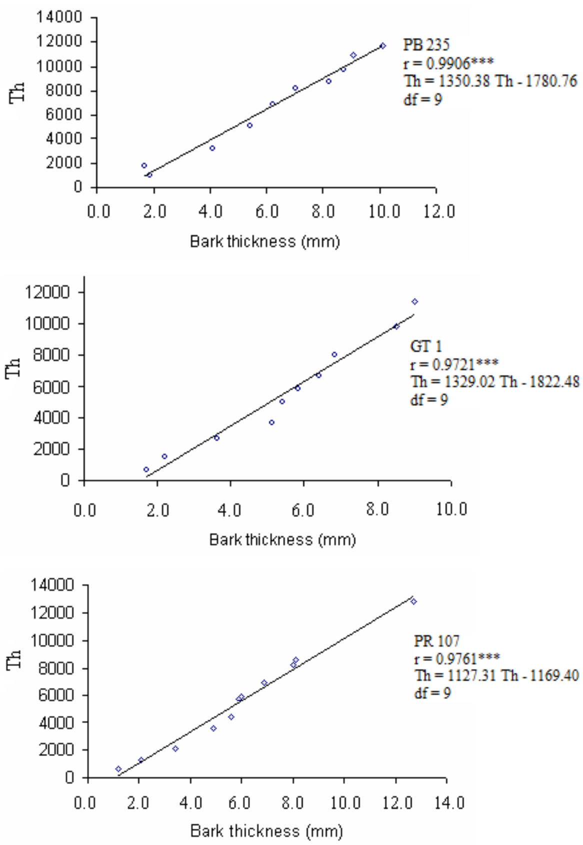

Fig. 12. Linear regression between total number of latex vessels and bark thickness of *Hevea brasiliensis*. Th : Total number of latex vessels; *** Very highly significant.

The process of bark constitution is marked by two successive stages. The first one, very accelerated, occurs within about six years, while the second indicates a slowdown the rest of time from the 7th year (Obouayeba *et al.*, 2000b). This result should be brought closer to this one of temporal increase in the development and growth of latex vessels described by Obouayeba *et al.* (2000b). Indeed, these works show that the establishment process of laticifers follows a symmetrical logistical function which inflection point is located at about 6 years of age, whatever the clone of *Hevea brasiliensis* studied.

Besides, the relation between the girth of the tree and its potential equipment in latex vessels can be exploited in order to decide upon tree tapping. This is all the more interesting since this link has a high coefficient of regression, which proves the reproducibility of the results whatever the conditions of achievement and the place. This relation must also be brought closer to the setting up and growth function of laticifers. In fact, to a girth correspond a number of latex vessels row which, compared to the total quantity of laticifers at the point of modulation of the logistical function of establishment of latex vessels of Obouayeba *et al.* (2000 b), should lead to a decision dealing with tapping of the rubber trees.

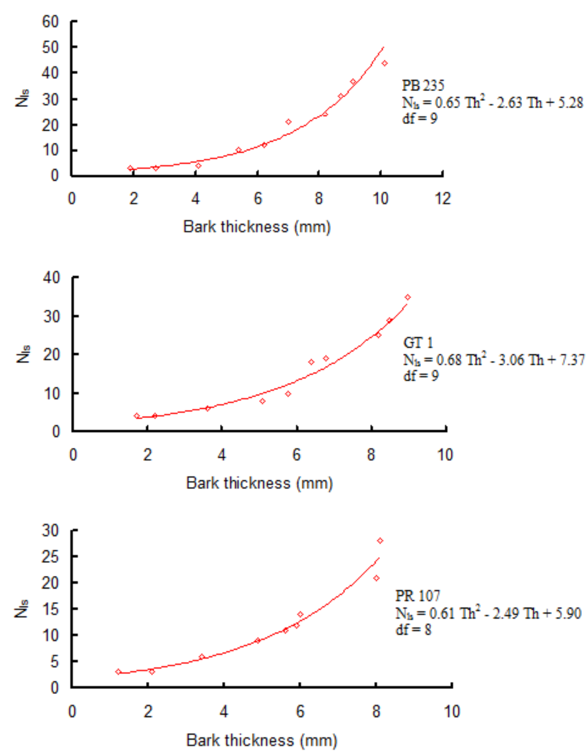

Fig. 13. Parabolic relationship between number of latex vessels in radial longitudinal section and bark thickness of *Hevea brasiliensis*. N_{ls} : Number of latex vessels in radial longitudinal section.

In the same way, the linear regression between the number of latex vessels in radial longitudinal section and the bark age, already determined by Gomez (1982), can lead to the determination of the age of the bark and therefore allow to decide tapping starting period of the rubber trees.

The linear regression between the total number of latex vessels and bark thickness can also allow determining the ideal moment to tap the rubber trees. Indeed, a bark thickness corresponds through this relation to an estimated number of latex vessels. As the formation of laticifers follows a symmetrical logistical time function, this bark thickness can be indirectly linked to an age notably to this one of the inflection point of the logistical function. This inflection point, corresponding to a major phenologic phenomenon, the physiological maturity of bark exploitation (Obouayeba, 2000b) permits by this way to determine the suitable moment for tree tapping.

The number of laticifers per surface unit, notably the density of laticifers by mm^2, is a good indicator (Index) for starting tapping of the trees. This parameter shows a time-related evolution often expressed in years. The aspect of the graph reveals the formation process of the bark and latex vessels. Indeed, Fig. 8 shows that, in the case of the total bark and latex vessels also; the production of laticifers per mm^2 indicates two stages:

"A 1st stage, during which the decrease in the density of laticifers is highly accelerated from 1 to 6 years;

"A 2nd stage, characterized by the levelling of the phenomenon, resulted in very few changes in the density of laticifers from the 7th to the 30th year.

This situation illustrates the fact that the functions of bark and laticifers formation probably get to a crucial, determining point. All this happens as if, suddenly, the plant stops the emission of these major organs. But it's nothing of that, as the functioning of cambium, which originates these formations, is indeterminate (Hallé and Martin 1968), unless it is destroyed. The formation and growth function of Obouayeba *et al.* (2000b) confirms this assertion. In fact, after 6 years, the plant still produces bark and therefore laticifers but it happens that at a given moment the formation is coupled with the destruction of the first formed organs, natural phenomenon of ageing already evoked by several authors: Gomez (1975), Hébant *et al.* (1981), Gomez (1982), Compagnon (1986), Thomas *et al.* (1995), Obouayeba *et al.* (2000b). This precise moment probably corresponds to an equilibrium status between the two functions, giving the impression that nothing more is created. At this stage of the phenomenon, one can consider that the bark (rubber tree) will have reached physiological maturity. In the same way, the latex vessels (intrinsic elements of the bark) acquire simultaneously physiological maturity, since the graphs of latex vessels formation; bark and density of laticifers are similar. Such a conclusion is corroborated by the works of Ho (1975) and mainly Gomez (1975) who had described the different stages of laticifers ageing along time. Moreover, the temporal evolution of the occupation area of a latex vessel is in favour of such a conclusion. In fact, this one is described by a symmetrical logistical function which different inflection points were respectively 5 years 5 months 19 days (PB 235); 5 years 10 months and 20 days (GT 1) and 5 years 9 months and 14 days (PR 107), that is about 6 years.

The synchronous functioning of the setting up of laticifers and bark, which are part of, is all the more normal since these two structures are produced by cambium, at the same time and in a rhythmic way (Hallé and Martin, 1968). The interest of the research on physiological maturity of laticifers resides in the fact that they will have acquired vigour of functioning essential to an extremely demanding activity in photosynthetates necessary to the nutrition of the metabolism of rubber yielding (Le Bras, 1953; Templeton, 1969; Wycherley, 1976). These works therefore clearly evidence that the physiological maturity of rubber tree, for rubber exploitation, is reached at the age of 6 years (Compagnon, 1986; Obouayeba *et al.* (2000a, b)).

Conclusion

The study of the development of the bark and latex vessels shows that despite the fact that the establishment of these two organs is described by distinct hyperbolical and sigmoid functions of logistical type, their evolution is marked by a lot of similarities. These ones characterize a single and same phenologic phenomenon, physiological maturity.

The production mode of laticifers does not differ from that of the total biomass of the studied clones. The formation of laticifers presents an inflection at the abscissa point where the age ranges from 5 years 7 months to 5 years 11 months. The production of latex vessels gradually increases until that maximum point. The emission of laticifers, from that moment, becomes less and less important to reach a stage said of ceiling corresponding to the plateau, analogous to this one observed in the case of vegetative growth. The formation of latex vessels is therefore probably marked by a major phenologic phenomenon, exploitation maturity.

It occurs practically at the same period regardless of the clonal characteristics. The maturity of exploitation of rubber tree is reached at 6 years of age whatever the clone. Moreover, this study enabled locating good indicators of age determination and/or farm exploitation period. These relations present definite and practical interests insofar as they will allow determining the maturity of exploitation of farms having unknown age with only a bark thickness measurement punch and a ribbon meter for the measurement of girth.

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References

Bobilioff W. 1923. Anatomy and Physiology of *Hevea brasiliensis*. Part I Anatomy of *Hevea brasiliensis*. Art. Orell Fussli Zurich, p. 141.

Carron MP, Enjalric L, Lardet L, Deschamps A. 1989. Rubber (*Hevea brasiliensis* Muell. Arg.). In Biotechnology in Agriculture and Forestry, vol. **5**, Trees II (ed. by Y. P. S. Bajaj).

Compagnon P. 1986. Principes de la conduite de l'exploitation. In: Le caoutchouc naturel, Coste R. ed., G.P. Maisonneuve et Larose, Paris, 237-265.

Dian K, Sangaré A, Diopoh JK. 1995. Evidence for specific variation of protein pattern during tapping panel dryness condition development in *Hevea brasiliensis*. Plant Science **105**, 207-216.

Franquin P. 1970. Modèles mathématiques de structures chez les végétaux, II. Relations de structure, Cahiers ORSTOM, série Biologie, **17**, 3-21.

Gohet E. 1996. La production de latex par *Hevea brasiliensis*. Relation 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, France, p. 343.

Gomez JB. 1983. Physiology of Latex (Rubber) Production, Malaysian Rubber Research and Board (MRRDB), Monograph n°**8**, Kuala Lumpur, p. 117.

Gomez JB. 1982. Anatomy of Hevea and its influence on latex production, Malaysian Rubber Research and Board (MRRDB), Monograph n°**7**, Kuala Lumpur, p. 76.

Gomez JB. 1975. Comparative ultracytology of young and mature latex vessels in *Hevea brasiliensis*. Proceedings of International Rubber Conference, Kuala Lumpur October 1975, vol. **2**, 143-163.

Hallé F, Martin R. 1968. Etude de la croissance rythmique chez l'Hévéa (*Hevea brasiliensis Müll.-Arg*. Euphorbiacées-Crotonoïdées). Edition Adansonia, ser. 2, **8 (4),** 475-503.

Hébant C, Devic C, De Fay E. 1981. Organisation fonctionnelle du tissu producteur de l'*Hevea brasiliensis*. Revue Générale des Caoutchoucs et Plastiques **614**, 97-100.

Hébant C, De Fay E. 1980. Functional organisation of the bark of *Hevea brasiliensis* (rubber tree): a structural and histoenzymological study. Zeitschrift für Pflanzenphysiologie **97,** 391-398.

Heller R. 1990. Abrégé de physiologie végétale, Tome 2. Développement, Ed. **4**, Masson, p. 266.

Hénon JM. 1984. Recherche de critères anatomiques de sélection précoce chez *Hevea brasiliensis* Thèse de doctorat 3e cycle en Agronomie Option Phytotechnie, p. 178.

Hénon JM. 1980. Fondements théoriques et pratiques d'une étude histophysiologique de l'appareil producteur (laticifères et éléments associés du système secondaire) de l'*Hevea brasiliensis* Muell. Arg. Mémoire de D.E.A.

Ho CT. 1975. Clonal characters determining the yield of *Hevea brasiliensis*. Proceedings of International Rubber Conference, Kuala Lumpur October 1975, vol. **2**, 27-37.

Le Bras J. 1953. Eléments de Science et de technologie du caoutchouc. Institut Français du Caoutchouc. Société d'éditions techniques coloniales, Paris.

Meunier A. 1912. L'appareil laticifère des caoutchoutiers. Imprimerie Ind. et Fin., Bruxelles, p. 51.

Obouayeba S, Boa D, Aké S, Lacrotte R. 2002. Influence of age and girth at opening on growth and productivity of Hevea. Indian Journal of Natural Rubber Research, **15 (1),** 66-71.

Obouayeba S, Boa D, Aké S. 2000b. Critical age, Bark growth and latex vessel formation as attributes for determination of tapping norms. Indian Journal of Natural Rubber Research, **13(1 & 2),** 38-45.

Obouayeba S, Boa D, Gohet E, Dian K, Ouattara N, Keli J. 2000a. Dynamics of vegetative growth of *Hevea brasiliensis* in the determination of tapping norms. Journal of Rubber Research, **3 (1),** 53-62.

Ouattara N. 1998. Contribution à la détermination d'une norme de mise en saignée d'*Hevea brasiliensis* en Côte d'Ivoire : cas du clone GT 1. Mémoire de fin d'études pour l'obtention du Diplôme d'Agronomie Approfondie (D.A.A). Option production végétale (Agronomie), p. 33.

Premakumari D, Panikkar AON. 1992. Natural rubber: Anatomy and Ultracytology of latex vessels. In: Crop Science, n° **23**, Elsevier (Amsterdam), 67-87.

Schultes RE. 1987. Studies in the Genus *Hevea*. VIII. Notes on Infraspecific variants of *Hevea brasiliensis* (Euphorbiaceae). Economic Botany, **41(2),** 125-147.

Schultes RE. 1977. Wild *Hevea*: an untapped source of germplasm, Journal of the Rubber Research Institute of Sri Lanka, **54**, 227-257.

Schultes RE.1970. The history of taxonomic studies in *Hevea,* Botanical Review **36**, 197-211.

Templeton JK. 1969. Partition of assimilates. Journal of Rubber Research Institute of Malaya **21**, 259-273.

Sekhar AC. 1989. Growth, availability and present status. In: Rubber wood production and utilization. The Rubber Research Institute of India, Kottayam 686 009, Rubber Board, Ministry of Commerce and Supply, Government of India, p. 3-5.

Thomas V, Premakumari D, Reghu CP, Panikkar AON, Saraswathy ACK. 1995. Anatomical and histochemical aspects of bark regeneration in *Hevea brasiliensis*. Annals of Botany Compagny, **75**, 421-426.

Webster CC, Paardekooper EC. 1989. The botany of the rubber tree. In Webster, C. C. And Baulkwill, W. J. (eds) Rubber, 57-84.

Wycherley PR. 1976. Tapping and partition J. Rubb. Res. Inst. Malaysia, **24(4),** 169-194.